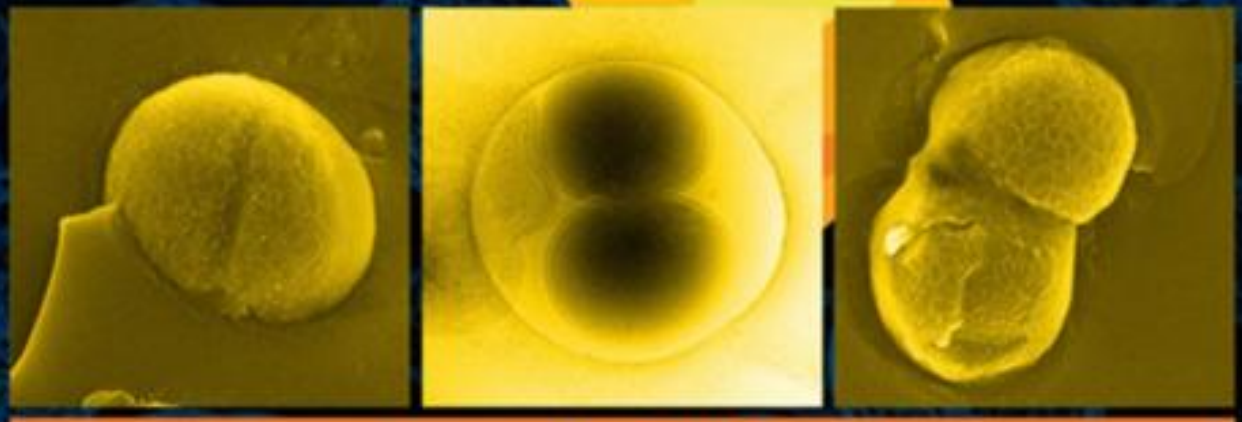
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7th Edition



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Plotkin's VACCINES



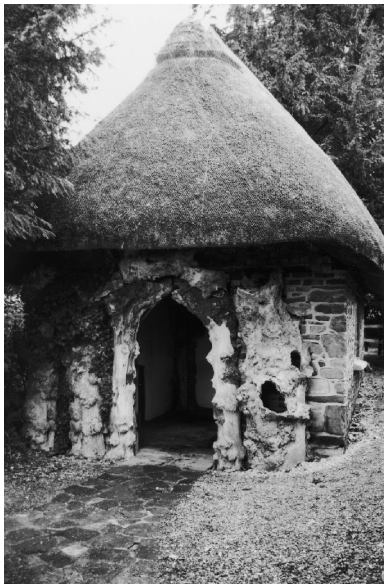
Foreword by Bill Gates

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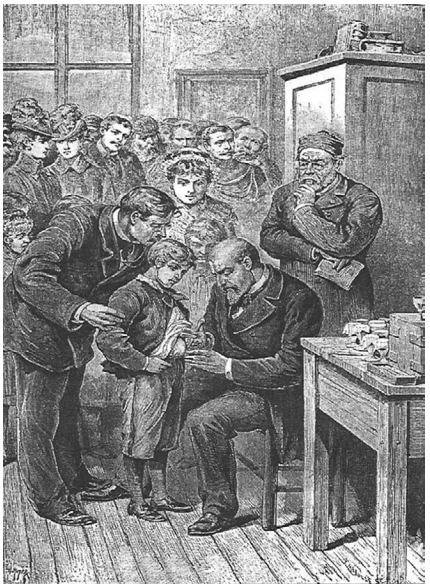
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Detroit Industry, North Wall, 1933 (fresco) by Diego Rivera (1886-1957). *(Courtesy of Detroit Institute of Arts, USA/The Bridgeman Art Library.)*



"Vaccination cottage" near the home of Edward Jenner in Berkeley, England, where he administered smallpox vaccine to thousands of the rural poor. *(Photo by Stanley A. Plotkin.)*



Inoculation of rabies vaccine to young Meister. *Left to right,* Mr. Viala, Joseph Meister, Dr. Grancher, and Mr. Pasteur. "The treatment was supposed to consist simply of an injection under the skin at the bottom of the ribs, with a virus that Mr. Pasteur considered able to preserve the Meister boy from rabies." *(Fr Bournand, Un bienfaiteur de l'humanité, Pasteur sa vie sa œuvre Tolra, Paris 1896.* From Vaccination: a History *by Hervé Bazin. Published by Editions John Libbey Eurotext, 127 Av de la République, 92120 Montrouge, France.)*

Plotkin's Vaccines

SEVENTH EDITION

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The cover shows the impact of the use of meningococcal Group A conjugate vaccine on the incidence of meningococcal disease in the African meningitis belt. The electron micrograph is a picture of *Neisseria meningitidis*, courtesy Ian Feavers, PhD, Head of Bacteriology, NIBSC.

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Foreword

I was born in 1955, the same year that the Salk polio vaccine went into wide distribution. If you talk to anyone old enough to remember the scourge of polio and the terror it engendered, you begin to get a sense of just how remarkable that breakthrough was and how it changed the lives of millions of people for the better.

That event changed the world, and I grew up in an environment where vaccines for polio, diphtheria, whooping cough, and other contagious diseases were simply taken for granted. Today, the risk of polio has been markedly reduced, having dropped from an estimated 350,000 cases and 125 endemic countries in 1988 when eradication began to fewer than 100 cases and three endemic countries (Pakistan, Afghanistan, and Nigeria) in 2015. Yet as long as polio remains endemic anywhere, the risk of exporting virus, more outbreaks, and more disabled people remains. How is it possible that more than a half-century after Salk's vaccine, polio has still not been eradicated?

Some of this is attributable to the biology of the pathogen. But a lack of political will and a failure of health systems to deliver polio vaccines to children at risk have also prevented the eradication of this scourge. We are 99% of the way there and I am optimistic we will succeed, but only if we combine the best of medicine, logistics, education, and community outreach. Similarly, *S. pneumoniae* has been studied for more than a century. Nevertheless, invasive pneumococcal disease kills more than 800,000 children each year. But since 2000, Gavi (The Vaccine Alliance) has been making effective pneumococcal conjugate vaccines available to the world's poorest countries. Between 2008 and 2015, more than 50 Gavi-eligible countries introduced the vaccine via their routine programs at a rapid rate.

There has been significant progress in the case of measles as well. The vaccine for measles was licensed in the 1960s, but its uptake was frustratingly slow in many places. Even 30 years after its introduction, three-quarters of a million children were dying from measles every year. However, since 2000, measles vaccine coverage has substantially increased, and measles deaths have declined by about 80%. This translates into more than 1500 children each day who live instead of die because the measles vaccine is being delivered regularly almost everywhere in the world.

While the greatest focus of vaccination programs has been on children in the first few years of life, some of the most vulnerable children are newborn infants who are too young for vaccines to induce immediate active immunity. There have been increasing efforts to protect these young children from diseases such as pertussis and influenza by vaccinating pregnant women with transplacental transfer of protective antibodies. This new platform is likely to expand as promising candidates to prevent respiratory syncytial virus and other serious pathogens of the neonatal period prove to be safe and effective in clinical trials.

The simple truth is that vaccines save lives.

They are overwhelmingly safe, are remarkably cost effective, and remain the single best tool we have in global health. They protect the human potential that is sapped by rampant sickness in developing countries. As a result, vaccines are one of the best means we have to promote not just global health, but global development. A recent study showed that low- and middle-income countries can see a return of between $16 and $44 for every $1 invested in vaccines. That's smart economics, not just smart science.

If vaccines are this singularly important tool, then the seventh edition of *Plotkin's Vaccines* is a singularly important resource in extending the power of prevention to the largest possible audience worldwide. *Plotkin's Vaccines* is the most respected source of reliable information on the vaccine tools we now have available, summarizing the scientific basis and rationale for vaccine use as well as the directions being taken to expand this power of prevention to other infectious diseases. It is an honor to have been asked to write this introduction to the latest edition.

It is also an honor to be able to speak to the scientists, researchers, and medical and public health professionals who will take up this volume. Your work is critically important. At a time when irresponsible claims about vaccines sometimes garner as much attention as the facts, it is vital to have informed advocates for the power and necessity of comprehensive vaccination worldwide.

As I write this in 2016, it is an exciting time in the field of vaccines. Last year, we witnessed a remarkable event as donors pledged more than $7.5 billion to Gavi, even in the face of a global financial crisis. The generosity of governments, foundations, and private sector entities that participated in this pledging round will substantially expand the ability of poor countries to vaccinate their children. Through expanded vaccine programs, Gavi projects that it will prevent 5 to 6 million deaths with its vaccination programs in 2016 to 2020. On the research front, we are seeing some promising signs in the decades-long search for an AIDS vaccine. Similarly, we are seeing progress with next-generation vaccines for malaria. The need for this in the poorest parts of the world cannot be overstated. The devastating costs of malaria in lives lost, sickness, diminished capacity, and reduced productivity are incalculable.

Pneumococcus and Rotavirus vaccines, with the potential to save millions of lives over time, are increasingly used in poor countries. Significantly, the time lag between vaccine introduction in rich countries and their availability in poor countries has been shortened from more than a decade to only a few years.

The world has seen the introduction of the first-ever vaccine developed specifically for the developing world—a meningococcal A conjugate vaccine that can end a terrifying threat for millions of people who live in Africa's meningitis belt. Over 250 million people have been vaccinated with the vaccine in large-scale campaigns, and now the vaccine is recommended for use in routine immunization programs.

But much remains to be done. We need new vaccines. The work to develop effective vaccines for tuberculosis, malaria, and HIV must continue.

We need better vaccines. The currently licensed BCG vaccine for tuberculosis has been administered 4 billion times over the last 90 years. It is safe and it protects against severe disease in infants. However, its efficacy is quite limited and it does not

protect against adult pulmonary tuberculosis. Other vaccines such as the oral polio vaccine, the rotavirus vaccine, and other orally administrated vaccines are often less effective in children living in extreme poverty.

We need to better understand mechanistic immune markers that correlate with protection to accelerate discovery and introduction of improved vaccines.

We need to secure faster vaccine introduction and adoption of currently approved vaccines. We are still not reaching a significant number of children, resulting in an intolerable number of preventable deaths.

We need affordable and sustainable supplies of vaccines, particularly for developing countries. The high cost of producing vaccines such as the pneumococcal conjugate vaccine and the human papillomavirus (HPV) vaccine has been an obstacle to incorporating these highly effective vaccines into the immunization programs of developing countries with the highest burden of disease. The HPV vaccine is an extremely effective vaccine that prevents cancer in men and women, but its availability is limited, particularly in developing countries. Gavi is playing a major role in making these vaccines available to the poorest countries, but efforts to simplify the manufacturing and regulation of these vaccines can also help ensure a sustainable, affordable supply for everyone.

But we need to do more than just develop these vaccines and make them affordable; we must also focus on driving near-term improvements in the mechanics of delivering these vaccines to the hardest to reach places, to the people who need them the most. This can include advancing innovative solutions for cold chain equipment, expanded use of the controlled-temperature chain, and in some cases, improvements in thermostability.

Vaccines save lives. But that is not their only benefit. When health improves, poor countries can spend more on schools, roads, and other investments that drive growth, which makes them less dependent on aid.

Vaccines deliver all this for, in some cases, just pennies per shot. That is why I say that if you want to save and improve lives around the world, vaccines are a fantastic investment.

We must use every tool at our disposal to continue to develop, improve, and deploy these miracles of science. It is a matter of the most basic human justice that we do all we can to extend these livesaving interventions around the globe.

Bill Gates
Bill Gates is the founder and co-chair of the Bill & Melinda Gates Foundation.

Preface to the First Edition

Since a first attack, which strengthens the host, often provides valuable safeguards for the future, it must be considered desirable, on condition, of course, that it does not produce too much serious damage. Artificially putting the host into a state comparable to that in which it would be if it had been cured of a spontaneous attack of the particular illness is the object of active immunization or vaccination.

Jules Bordet, Traite de l'Immunite dans les Maladies Infectieuses, 1920

A little over a hundred years ago the first vaccines were specifically developed in laboratories. During that time the success of vaccination has been nothing short of phenomenal. Of the major scourges of humankind, malaria and helminthic disease remain without useful vaccines, although they have now been joined by human immunodeficiency virus infection. Of course, there are many endemic diseases, chiefly respiratory, for which vaccines are badly needed, and there is great activity in hundreds of laboratories seeking to develop immunologic prophylaxis against them.

Indeed, ever since the time of Pasteur the medical world and to some extent the lay world have realized that prevention by vaccination is the El Dorado of research in infectious diseases.

Despite this awareness, there have been curiously few books on the subject of vaccines in actual use, as distinct from the basic immunology of vaccination and from the vaccines in developmental stages. In addition, our experience is that physicians and students often know much less about the vaccines they use than about antibiotics and other forms of treatment, although the impact of vaccines on their patients is likely to be just as great. Despite the proven success of vaccines, only in the United States and other developed countries are there well-vaccinated populations. In this country success has been due to the education and resultant practices of pediatricians. Other segments of the medical community, even in this country, seldom use or think of vaccination.

Moreover, vaccination is always under attack by civil libertarians who claim the right to be ill, by religious zealots who believe the will of God includes death and disease, and by a legal system that profits from the failure of most people to understand risk-benefit ratios or public health issues. Short memories also ensure that people do not recall the way it was before vaccination.

To these problems may be added an attitude that pervades the thinking of both physicians and patients: cure is more interesting than prevention. Minute attention is focused on the heart transplant, the surgery of separating Siamese twins, the high technology of intensive care and why not? These are where the drama is perceived to be. And yet no single physician can in his or her career hope to save more than a small fraction of the lives saved by a single vaccine.

We therefore decided to put together a textbook on vaccines and their use, directed at the physicians who employ them, emphasizing licensed products in the United States, but we also included chapters on vaccines far along in development. In a few cases, where we thought the subjects sufficiently important, chapters on vaccines still in the early stages were admitted. Our hope is that this book will serve as a reference source for all who are interested in vaccines in the prevention of disease.

The Greeks had two gods of health: Aesculapius and Hygieia, therapy and prevention, respectively. Medicine in the twentieth century retains those two concepts, and vaccination is a powerful means of prevention. What follows is information on the vaccines that, together with sanitation, make modern society possible, and that if wisely used will continue to bestow on humankind the gift of prevention, which according to proverb is worth far more than cure.

Stanley A. Plotkin, MD
Edward A. Mortimer Jr, MD

Preface to the Seventh Edition

Since the last edition of this book six new vaccines have been licensed in the United States: a live attenuated oral vaccine to protect against cholera; a human papillomavirus (HPV) vaccine containing antigens against an additional five serotypes to enhance protection against HPV-associated cancers; two protein-based meningococcal Group B vaccines, one of which is the product of reverse genetics technology; an adjuvanted influenza vaccine for use in the elderly; and a combination vaccine for use in 4- to 6-year-olds to protect against diphtheria, tetanus, pertussis, and polio. This time interval has also been marked by important global advances in the delivery of previously licensed vaccines against rotavirus diarrhea, cancer caused by HPV, hepatitis A, cholera, and pneumococcal infections. Of particular note has been the progress on global polio eradication. Type 2 polio has been officially declared eradicated, marking in a sense the second human virus ever to have been eradicated through the use of vaccines (the other being smallpox). There is promise that type 3 polio may have been eradicated with the last case detected in 2012, and type 1 polio is at record low levels. In addition, steady progress is being made in exploiting genetic engineering to make new vaccines, and there have been notable, though partial, successes against HIV, dengue, and malaria.

However, challenges remain as new pathogens emerge or reemerge such as the Ebola outbreak in West Africa and more recently the Zika virus in the Western Hemisphere, neither of which have available licensed vaccines. The need to anticipate and rapidly develop vaccines for these and other emerging pathogens is taking on greater importance.

Vaccines remain one of the most effective and economical prevention measures available and the only medical procedure recommended repeatedly for all children and some adults. But vaccines do not save lives; vaccinations save lives. It is critical to ensure that safe and effective vaccines are delivered to all persons for whom they are recommended.

Since the last edition there has been much progress on maternal vaccination. By vaccinating pregnant women, protection is afforded to both the mother and the infant. In fact, universal vaccination of all pregnant women against influenza and pertussis has been implemented in several countries. The potential for administering respiratory syncytial virus and group B streptococcal vaccines to pregnant women to prevent these common diseases in their infants is also being studied. Much progress has been made.

Vaccine availability is also paramount. The advent of new manufacturers in Asia and Latin America has enhanced the availability of vaccines for developing countries, and their growing economic strength has enabled more people than ever to be immunized. Critical to the successful introduction of new vaccines into developing countries has been Gavi, the Vaccine Alliance, with strong advocacy and financial support from the Bill & Melinda Gates Foundation. Nevertheless, the problem of financing for both old and new vaccines remains.

Moreover, with the increase in vaccination throughout the world, resistance to vaccines has also grown as misinformation retailed by the Internet is rife. It is helpful to have the perspective that vaccine hesitancy is as old as Jenner's smallpox vaccine despite the fact that it succeeded in eradicating a terrible disease. Perhaps it is not surprising that prevention by vaccination in healthy people is more difficult for the public to accept than treatment of established disease, but the proverb "better prevention than cure" is still as sound as it ever was.

Finally, a word to our authors and to our readers. We are grateful to our authors for all their work and for their expertise, which allows us to be sure that the information in the book is correct, and we apologize for harassing them to be on time! We are also grateful to our readers, who by their laudatory comments have given us the enthusiasm to manage the editorial tasks. We regret that the references had to move online (available at ExpertConsult.com), but it was necessary to maintain the affordability of the book.

In any case, we hope this edition will be considered the best yet, and that it will be read by all those, young and old, who recognize the value of vaccines.

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Dedication

To Susan, the love of my life, and to my sons Michael and Alec.



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To my wife, Bonnie, who made dreams come true. And to our children, Will and Emily, who make everything worthwhile.



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SECTION 1 General Aspects of Vaccination

1 A Short History of Vaccination

Susan L. Plotkin and Stanley A. Plotkin

Vaccination as a deliberate attempt to protect humans against disease has a short history when measured against the thousands of years that humans have sought to rid themselves of plagues and pestilence. Only in the 20th century did the practice flower into the routine vaccination of large populations. Yet, despite its relative youth, the impact of vaccination on the health of the world's peoples is hard to exaggerate. With the exception of safe water, no other intervention, not even antibiotics, has had such a major effect on mortality reduction and population growth.

Since the first vaccine was introduced by Edward Jenner (Fig. 1.1) in 1798, vaccination has controlled 14 major diseases, at least in parts of the world: smallpox, diphtheria, tetanus, yellow fever, pertussis, *Haemophilus influenzae* type b disease, poliomyelitis, measles, mumps, rubella, typhoid, rabies, rotavirus, and hepatitis B. For smallpox, the dream of eradication has been fulfilled; naturally occurring smallpox has disappeared from the world.[1] Cases of poliomyelitis have been reduced by 99% and this disease also is targeted for eradication. Rubella and congenital rubella syndrome have been officially declared eliminated from the Americas as of 2015.[2] Vaccinations against many other diseases have made major headway. The path to these successes is worth examining.[3–5]

EARLY DEVELOPMENTS

Attempts to "vaccinate" began long before Edward Jenner's smallpox vaccination. While the precise origin of **variolation** remains unknown, it seems to have developed somewhere in Central Asia in the early part of the second millennium and then spread east to China and west to Turkey, Africa, and finally Europe.

In the 7th century, some Indian Buddhists drank snake venom in an attempt to become immune to its effect. They may have been inducing antitoxin-like immunity.[6] In the 16th century, Brahmin Hindus in India practiced a form of variolation by introducing dried pus from smallpox pustules into the skin of a patient.[7] Writings that cite the use of inoculation and variolation in 10th-century China[8–10] make interesting reading but apparently cannot be verified.[11] There is, however, 18th-century documentation of Chinese variolation. *The Golden Mirror of Medicine*, a medical text dated 1742, listed four forms of inoculation against smallpox practiced in China since 1695:

- The nose plugged with powdered scabs laid on cotton wool
- Powdered scabs blown into the nose
- The undergarments of an infected child put on a healthy child for several days
- A piece of cotton smeared with the contents of a vesicle and stuffed into the nose[8,11]

This text, endorsed by the Imperial Court, raised the status of variolation in China, which previously had been considered just a folk remedy. Another Chinese text, published a century before Jenner's work, stated that white cow fleas were used for smallpox prevention.[9] The fleas were ground into powder and made into pills.

Variolation was introduced into England by Lady Mary Wortley Montagu in 1721, after her return from Constantinople, where she lived for 2 years with her husband, the British Ambassador to the Ottoman Empire. Lady Montagu had been disfigured by smallpox earlier in life and her 20-year-old brother had died of the disease. While living in Turkey, she frequently observed variolation and wrote to a friend back home: "The small-pox, so fatal, and so general amongst us, is here entirely harmless, by the invention of engrafting, which is the term they give it…. Every year, thousands undergo this operation…they take the small-pox here by way of diversion, as they take the waters in other countries. There is no example of any one that has died in it…."[12] So impressed was she that she had her own son variolated while still living in Turkey. Dr. Charles Maitland, who performed the procedure on her son in Constantinople, later performed the first variolation in England in 1721 on Lady Montagu's daughter. The treatment was effective, but results were erratic, and 2% to 3% of persons treated died of smallpox contracted from the variolation itself.[13]

The English medical community had previously learned of variolation in 1713 when Emanuel Timoni, MD, a graduate of Oxford University living in Turkey, sent a letter to the Royal Society about variolation. Another physician, Giacomo Pilarino, also reported Turkish variolation to the Royal Society in 1716.[14] The reports did not seem to be significant, and the procedure was not adopted.[15] An earlier hint of variolation is made by the Danish physician Thomas Bartholin of Copenhagen in 1675, who mentioned that there was a "market" in Copenhagen where people would go to buy the poxvirus from enterprising housewives. It is unclear whether these purchases were for the prevention of smallpox in healthy persons or for the treatment of persons already infected.[15–19] Voltaire lauded the variolation of Circassian women to maintain their beauty in his *Lettres Philosophiques* in 1721.

Most intriguing, at the same time that Charles Maitland variolated Lady Montagu's daughter in England (1721), variolation was practiced in America at the instigation of Cotton Mather who first learned of it from his African slave, Onesimus.[15] Mather subsequently read about variolation in volume 29 of the *Philosophical Transactions of the Royal Society of London*, 1714 to 1716, which contained the aforementioned articles by Timoni and Pilarino. There was a smallpox epidemic in Boston at that time, and Mather used the authority and prestige of his position to urge all Boston-area physicians to consider the practice of variolation (letter, June 24, 1721). Physician



Figure 1.1. Edward Jenner. *(Courtesy the Institute of the History of Medicine, The Johns Hopkins University, Baltimore, MD.)*

response was negative, except for Dr. Zabdiel Boylston, who successfully inoculated his own 6-year-old son and two black slaves shortly thereafter. Six weeks later, Dr. Boylston variolated Mather's son, Sammy, and publicized its success. Mather continued to harangue recalcitrant physicians and the public about variolation to the point where a grenade was thrown into his house in utter exasperation![20,21]

Despite the known risks, George Washington was compelled in the 1770s to order that Continental Army recruits undergo variolation against smallpox, to which the Americans were highly susceptible; the great majority of their English enemies were immune from early childhood exposure or from variolation.[22] In the mid-18th century, several treatises were written on inoculation against measles as well; the Scottish physician Francis Home successfully inoculated humans against measles and published his results (1758).[23–25]

In 1774 in Yetminster, England (Dorset County), a cattle breeder named Benjamin Jesty, himself immune to smallpox after contracting cowpox from his herd, deliberately inoculated his wife and two children with cowpox to avoid a smallpox epidemic.[14] This was no spur-of-the-moment idea; like many country farmers in the area, he knew that dairymaids seemed to be protected from smallpox after they had contracted cowpox. He had considered the possibility of deliberately using the inoculation technique with cowpox for quite some time and acted only when there was an imminent threat of a smallpox outbreak. He took his wife and two children to a nearby field where he knew he could find cattle with cowpox. He inoculated all three of them. His experiment succeeded; they were unaffected by the outbreak, and his two sons were still immune 15 years later, when they were deliberately variolated with smallpox.[26–28]

Jesty's story is interesting; although neither a physician nor a scientist, he nevertheless reflected on the evidence of local dairymaids' immunity to smallpox because of prior infection with cowpox and saw the principle involved: inoculation with one moderately harmless disease (cowpox) could provide protection against another far more dangerous disease, smallpox. When Jesty's neighbors learned that his wife had developed inflammation at the site of the inoculation and had to be treated by a physician because he had "vaccinated" her, they vehemently scorned him.[28] Jesty retreated in the face of their disapproval and never attempted to publicize his experiment or to vaccinate anyone else.[27] Truly, Jesty's actions constituted the first known real **vaccination**—the use of cowpox to protect against smallpox.

Some 30 years later, after Jenner had popularized vaccination, and thanks to the intercession of an enthusiastic vaccinator named Rev. Andrew Bell, Jesty was invited to London in 1805 by the Original Vaccine Pock Institute to tell the story of his 1774 "experiment" before the Institute's examiners. At the end of the visit, they issued a public statement in the *Edinburgh Medical & Surgical Journal* recognizing Jesty's cowpox vaccination. They commissioned his portrait as well, which was hung in the Institute.[27,28] This was a vindication of sorts, but he did not share in the significant monetary award that Jenner received. When Jesty died in 1816, his wife made certain that his tombstone recorded for all posterity his central role in the great endeavor (see frontispiece).

Despite Jesty's successful vaccination of his family, Jenner's work with cowpox vaccination still holds title to the first scientific attempt to control an infectious disease on a large scale by means other than transmitting the disease itself.[29]

Cowpox was not a widespread infection. It appeared sporadically in certain rural counties of England. Thus, the local wisdom that persons who contracted cowpox "did not take the smallpox" was not widely known. Jenner knew it because he had been an apothecary apprentice in Chipping Sodbury in 1768, where a milkmaid told him about using cowpox against smallpox. Indeed, he discussed the possible association between the two with John Hunter, with whom he studied in London from 1770 to 1773. For unknown reasons, Jenner did not return to the subject of cowpox/smallpox until 1796. His first manuscript to the Royal Society on vaccination was rejected because his experiment involved only one person, not enough to establish a principle.[20,27] Within 2 years, he expanded his studies and proved that cowpox could be passed directly from one person to another, thereby providing "large-scale" inoculation against smallpox without depending on the sporadic outbreaks of natural cowpox. Jenner self-published his results in *Variolae Vaccinae* in 1798.[29] This publication brought to the attention of the entire medical community the merits of inoculation with the relatively obscure animal disease, cowpox, to prevent one of humankind's deadliest scourges. Fortunately for the world, Jenner had the connections that Jesty did not, and vaccination rapidly replaced variolation. While Jenner did use the term "vaccine," he is not the originator of the term *vaccination*; that honor belongs to his friend Richard Dunning, who used it in 1800.[30,31] By 1810, Jenner realized that immunity against smallpox by vaccination was not lifelong, but he did not know why.[13]

Curiously, the vaccinia virus used in current smallpox vaccine is not the cowpox virus that Jenner used. Vaccinia, cowpox, and variola are all related orthopox viruses, suspected to have been derived from a common ancestor.[32] S. Monkton Copeman[33–35] gave considerable attention to this issue in the Milroy Lectures of 1898, which make fascinating reading. He documents the many ways, times, and methods by which cows were inoculated to keep the supply of cowpox intact, further adding to the confusion of when and how vaccinia replaced cowpox.[33–35] It has been suggested that vaccinia may have originated in a now extinct horsepox.[36] What is clear is that

the exact origin of vaccinia remains unknown, as does how it became substituted for cowpox.

For the first several decades of the 19th century, arm-to-arm transfer was the primary method of human vaccination.[37] Other recognized diseases, such as syphilis and tuberculosis, were known to be occasionally transmitted along with the "cowpox" virus, so a search was underway to find an alternative way to vaccinate and to ensure a steady supply of cowpox vaccine. The concept of "passages" of the immunizing agent (transmission from one human or animal to another) was well known, and in 1836, Edward Ballard[38] argued for choosing new strains of cowpox for vaccination because the old strains were too weak (too attenuated) from so many arm-to-arm passages. He recommended that the lymph (vesicle fluid) be passed back through a calf to regain strength.

According to Ballard, the idea to use animals to propagate cowpox vaccine, as opposed to human arm-to-arm propagation, was first practiced in Naples, Italy, in 1805. Troja used vaccine virus derived from humans to inoculate cows and then used the lymph from the cows' pocks to vaccinate humans. This was referred to as *retrovaccination*. Troja's successor, Galbiati, stated that he used bovine lymph specifically to avoid transmission of other human diseases. By 1842, a third Italian, Negri, gave up the practice of retrovaccination entirely. He started what was called *animal vaccination*, that is, inoculating from one cow to another cow to keep a steady supply of cowpox lymph. But the initial virus source that he inoculated into the cow was from a human! When a natural outbreak of cowpox occurred in Calabria, Negri switched to that source for his lymph, but because he was running a commercial activity, he bought a third "cowpox strain" from London. Its origin was questionable, but Negri used it anyway, from 1858 onward. By mid- century, arm-to-arm vaccination was essentially replaced by animal vaccination but it is easy to understand why the origin of the vaccinia virus is difficult to trace.[35,38]

In 1864, a French physician named Lanoix studied animal vaccination in Naples and brought back to France a calf inoculated by Negri. He and Chambon set up a business in France for production of calf-to-calf lymph vaccine for humans.[15,35,38] Subsequently, the French government became interested and ordered a study of animal vaccination. The lymph used in the experiments was obtained from Negri in Italy, but was really from the London cowpox lymph of questionable origin. By chance, there were two outbreaks of cowpox in France in 1866. Lanoix and Chambon collected cowpox from both, mixed them together, and used the mixture to produce their vaccine.[35,38] From these confused beginnings, animal vaccination spread rapidly throughout the continent.

Following Robert Koch's recommendation, German scientists began to use glycerin to kill bacteria and to preserve the lymph.[39] This generated a ready supply of a stable calf lymph of consistent potency.[13] By the end of the 1890s, the use of glycerinated calves' lymph was standard everywhere, and both arm-to-arm vaccination and unglycerinated animal vaccination were abandoned.

Louis Pasteur and the Age of Vaccination

Louis Pasteur's (Fig. 1.2) work on the attenuation of the chicken cholera bacterium in the late 1870s was the first major advance after Jenner's *Variolae Vaccinae*. Pasteur drew on concepts that had been developing for at least 40 years: attenuation; modification through passage; renewed virulence; and, most important, the need to replace person-to-person (or animal-to-animal) vaccination with something safer, consistent, and less likely to transmit other diseases.[40]

The popular story that Pasteur had a "eureka" moment when he noticed that a chicken cholera culture (*Pasteurella multocida*) left exposed to air during a long holiday period provided immunity when challenged is considerably more complex than originally told. From the moment he obtained the chicken cholera culture from Henry Toussaint in October 1878, Pasteur spoke of wanting to manipulate it to make a vaccine. The culture was virulent, and he killed many chickens trying to keep it alive through passage from chicken to chicken. By January 1879, he found that he could keep the microbe alive in a culture of chicken bouillon. Chickens inoculated from this bouillon culture died, so it had retained the virulence of his original sample. Next, he prepared a bouillon culture using inflamed chicken muscle tissue from the site of inoculation. He noted that this infected muscle tissue culture did not develop normally as the other cultures did; it became acidic. He fed the chickens bread soaked in the first bouillon culture and then fed them bread soaked in the muscle bouillon. They became sick but survived. He then challenged them with virulent organisms and they lived, so he thought he had a vaccine. After the challenge, the chickens sickened again but again survived and eventually returned to health. So by March 1879, Pasteur realized that he had attained "resistance" to disease; he also knew he had not yet found a product that could be safely used as a vaccine. For the next several months, Pasteur subjected the chicken cholera microbe to various conditions, for example, in a vacuum, exposed to air, and various intervals before inoculation. But it was mainly the chickens that had eaten the bread soaked in acidic bouillon that survived.

Pasteur left on vacation at the end of July 1879; he returned in October and again took up his chicken cholera experiments, focusing on the cultures that had become acidic because they seemed to be the ones that conferred immunity. He tinkered with the amount of time the microbe remained in the acidic cultures. On December 18, 1879, he noted that two cultures made from a chicken that died of a pre-vacation inoculation were the only cultures that turned acidic. He focused on them for his vaccine, although he still had many problems with illness in the chickens. By the middle of January 1880, Pasteur realized that the diminution of virulence came from leaving the microbe for a long time in an acid culture.

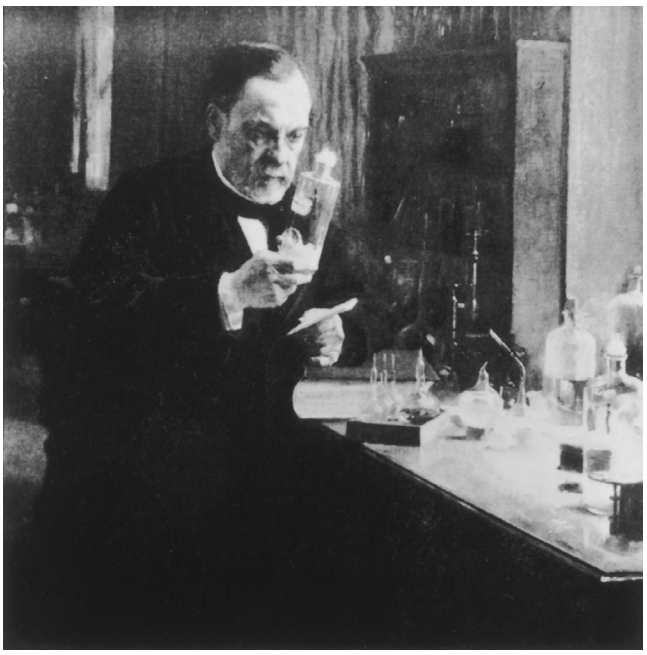


Figure 1.2. Louis Pasteur. *(Courtesy the Pasteur Institute, Paris.)*

Pasteur's definitive test was on January 22, 1880. With highly virulent chicken cholera, he inoculated 19 naïve chickens and 8 that had been previously immunized twice with his acidic cultures. All eight of the previously immunized chickens lived. Most of the naïve candidates did not. So Pasteur's eureka moment was really the result of many months of intensive, deliberate research. Pasteur presented his results at the Académie des Sciences on February 8, 1880, and at the Académie de Médicine on February 10, 1880. He announced that he had achieved successful vaccination against chicken cholera, but he did not reveal his technique. He continued to refine his technique and tried to increase the stability of the final product. Finally, in October 1880, he published at least part of his method for preparation of the vaccine.[20,41] Pasteur's chicken cholera vaccine harkened back to the classic variolation technique, which had used a weakened form of smallpox to inoculate against smallpox. Therefore, the modern concept of vaccination, involving the development of vaccines in the laboratory and using the same agent that caused the disease, was truly introduced with Pasteur's chicken cholera vaccine, 5 years before the famous vaccination of Joseph Meister against rabies. Ironically, the chicken cholera vaccine was never a success; there were frequent vaccine failures. Pasteur was lauded, but the vaccine was eventually discontinued.[20]

Pasteur's research on anthrax began in 1877 and overlapped his work on chicken cholera. Casimir Davaine had seen the anthrax bacillus in 1850, and had postulated it as the cause of anthrax,[42,43] but Koch was the first to obtain pure cultures of anthrax bacillus and to describe its capability to survive indefinitely in the form of spores.[44] He transmitted it to several laboratory animals and proved that there was a causal relationship between this bacillus and the disease anthrax.

Pasteur knew of Davaine's and Koch's work and that of the veterinarian Toussaint (Fig. 1.3).[45] Indeed, he was in a neck-and-neck competition with Toussaint to develop an anthrax vaccine. Toussaint published two articles in July 1880 in the *Comptes Rendus de l'Académie des Sciences* on a live anthrax vaccine that he developed and tested, which induced immunity against anthrax.[20,46–48] He had started his anthrax experiments in May 1880, stating that he was inspired to do so after hearing Pasteur in February 1880 describe his chicken cholera vaccine experiment, although Pasteur had not then revealed his method. Toussaint used the blood of bovines that had died of anthrax, noting that the fluid was full of bacteria, which others thought was extraneous material but Toussaint thought was the causative agent of anthrax. He first attempted to simply "filter" the blood and use it as a vaccine but quickly realized that the bacteria could pass through the filter. He then decided to follow the procedure that Davaine described: to heat the blood for 10 minutes at 55°C or subject the blood to the action of diluted phenol. He used both of these techniques—and a variation on filtering in which he used 12 filters—to produce an attenuated vaccine that he injected in rabbits, sheep, and young dogs. The animals were protected by all three methods, although Toussaint initially thought that the heat-treated was the best.



Figure 1.3. Henry Toussaint.

Toussaint's articles caused quite a stir at the Académie; Pasteur and others challenged their validity and Toussaint was compelled to not only reveal his methods, but also to conduct experiments to prove his claim. On July 28, while still in Toulouse, he took fresh blood from a sheep dying of anthrax and prepared his vaccine in two lots, one with 1% phenol and the other with 1.5%. Both lots were filtered, although by different means. *Neither lot was heat-treated*. He left for Paris with his two lots of "vaccine," where his experiment continued at Vincennes and Alfort under the watchful eyes of several researchers. On August 8, Toussaint inoculated 20 sheep with the first solution (1% and filter papers); 4 of them died very quickly, but the other 16 survived. On August 22, six new sheep were inoculated with material from the second lot (1.5% phenol and rudimentary filtration), and all survived without illness. All 22 sheep were then subjected to challenge with virulent anthrax and all survived. With one injection of a (partially) live vaccine attenuated by filtration and phenol acid, Toussaint had achieved immunity to anthrax![20]

It should be reemphasized that Pasteur did not reveal the method for making chicken cholera vaccine until October 1880. Toussaint's vaccine work was original; it was his own, not Pasteur's. He indeed induced immunity to anthrax, and his was the first anthrax vaccine. Eventually, he received the Prix Vaillant and the Légion d'Honneur for this work.[20,46–49] Toussaint came down with a debilitating neurologic disease in 1881, which prevented him from pursuing his claim as the originator of the first anthrax vaccine. His health continued to decline, and he died in 1890 at age 43 years.[20,46]

The following spring, Pasteur announced the first *public* controlled experiment of anthrax vaccination at Pouilly-le-Fort on May 5,1881.[50] It was initiated by Pasteur in an effort to silence his many critics and to gain recognition for his own anthrax vaccine. Pasteur inoculated 24 sheep, 1 goat, and 6 cows with attenuated anthrax bacilli. On May 17, these same animals were inoculated again with more virulent but still attenuated anthrax bacilli. At the same time, 24 sheep, 1 goat, and 4 cows were kept as control animals and given no inoculations. On May 31, both groups were inoculated with virulent anthrax from spores that Pasteur had kept in his laboratory since 1877.

By the 2nd of June, 21 of the nonvaccinated sheep and the nonvaccinated goat were dead. Two more nonvaccinated sheep died before the spectators' eyes, and the last one died before day's end. All vaccinated sheep, the vaccinated goat, and the six cows remained healthy. (The nonvaccinated cows did not die but showed clear evidence of having contracted anthrax. Their size perhaps had saved them.) At the end of this experiment, the triumphant Pasteur wrote that he had shown

that vaccines could be made that were cultivatable at will by a method that could be generalized, since he had already used the method previously to create a vaccine against chicken cholera. His experiment represented a considerable advancement over Jennerian vaccination, which had not been subjected to the rigors of a controlled experiment.

It has since been documented that Pasteur's results with chicken cholera and anthrax were not as clear-cut as previously thought. Pasteur deliberately withheld critical data (conflicting information on the degree of protection of the vaccine) in his communications to the Académie de Médicine.[51–53] However, this in no way detracts from the significance of his findings, which proved that one could "create" standardized, reproducible vaccines. Pasteur's experiments with chicken cholera and anthrax[41,50] announced to the world that a new, scientific era in vaccination had begun.

By the time the **rabies** vaccine was first administered to humans in 1885,[54] the general public and the scientific community were well aware of the "new vaccination," but only in relation to animals. The reaction when Joseph Meister and Jean Baptiste Jupille became the first humans to be vaccinated against rabies was predictable: outrage. Meister, a 9-year-old boy from Alsace, had been bitten 14 times on the hands and thighs and arrived in Paris some 60 hours after he had been attacked by a rabid dog. The physician working with Pasteur, Dr. Joseph Grancher, was convinced that Meister would die of rabies if left untreated and, therefore, the attempt to vaccinate was justified to save his life. Meister was vaccinated in the same manner that Pasteur was using in his experiments to protect animals (especially dogs) against rabies: with a series of progressively less dried and, therefore, more virulent rabbit spinal cords obtained from rabbits that had died of rabies after having received "fixed virus" injections of rabies virus. A couple of months later, Jean Baptiste Jupille, a 14-year-old from the Jura region of France, arrived 6 days after having been bitten multiple times. (He had fought off a rabid dog that had attacked a number of younger children.) He was given the same course of treatment that Meister received. They both survived.[13]

That Pasteur had deliberately introduced a deadly agent into a human left people aghast. The fact that the rabies virus had been attenuated did not appease the general public or many in the medical community; the cases of rabies that occasionally occurred in subsequent vaccinees were attributed to the vaccine and were viewed as medical murders. Even Émile Roux, one of Pasteur's staunchest allies and a collaborator in the rabies experiments, was appalled at the vaccination of Meister. He thought it was unjustified by the experiments conducted up to that point.

An examination of Pasteur's laboratory notebooks indicates that Roux was right to object.[52] The notebooks tell us that, shortly before vaccinating Meister, in May and June of that same year, Pasteur had seen and recommended vaccination for two other people in local hospitals, each of whom had been admitted with the presumed diagnosis of rabies. The first, an adult, was admitted with an uncertain diagnosis of rabies. The second case was a young girl, 11 years old, who had been bitten by a rabid dog on the lip, remained untreated, and was admitted to a hospital a month later with frank rabies. In both cases, Pasteur used a rabies vaccine made of an emulsion of dessicated spinal cord from a rabid rabbit. Up to this point, he had never published anything about using spinal cords as a vaccine, and in fact, had not yet successfully protected any animal from rabies with such a vaccine. The first patient, the adult whose diagnosis was uncertain, received only one–unauthorized–dose of the vaccine before his doctor forbade the administration of further doses. This patient lived. The young girl, whose rabies diagnosis was not in doubt, received two injections of the same vaccine on her first day in the hospital. When Pasteur and his nephew Adrian Loir arrived the next morning to give the third injection, the young girl died before she could receive it.[52] The death was almost certainly from rabies and not from the two injections she had received. These two cases were never published by Pasteur but were subsequently found in his laboratory notebooks.

Roux left Pasteur's laboratory in protest after Meister's vaccination and did not return until the summer of 1886, after several dozen people who had been bitten by rabid animals had been successfully vaccinated.[13,52,55,56] Ultimately, hundreds were saved from rabies, many more than had died despite the vaccination (presumably from rabies contracted from the bites). Unfortunately, that did not lessen the strenuous opposition to rabies vaccination in humans nor the belief by many that the vaccination itself caused the deaths. After all, only 45 years earlier, once Jenner's vaccination had been accepted, variolation had been made a felony in England for the very same reason: it introduced a deadly live virus into humans (as opposed to cowpox, which was not deadly).[13] Grancher, the physician who administered the rabies vaccine to Meister and many others, was one of Pasteur's staunchest supporters and was invaluable in defending Pasteur before the Académie de Médicine and against recalcitrant physicians.[56] Despite the opposition, and thanks to Grancher and other supporters, Pasteur soon became a worldwide medical hero.

The next major step in vaccine development took place in the United States. It involved a new concept that was equally important: killed vaccines. In 1886, Daniel Elmer Salmon (Fig. 1.4) and Theobald Smith (Fig. 1.5) published their work on a killed hog cholera "virus" vaccine.[57,58] The heated suspension of organisms immunized pigeons against the disease. The vaccine they developed was actually a bacterial vaccine against

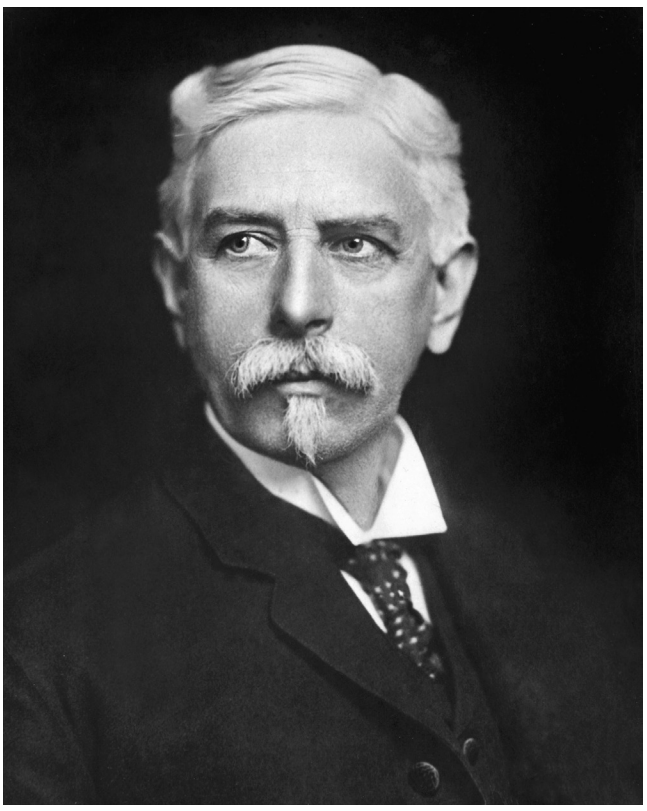


Figure 1.4. Daniel Elmer Salmon. *(Courtesy the American Veterinary Medical Association, Gallery of AVMA Presidents.)*

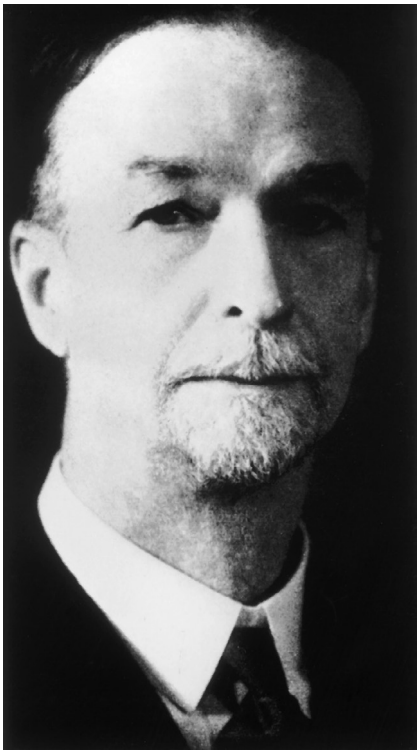


Figure 1.5. Theobald Smith. *(From Cohen B, ed. Chronicles of the Society of American Bacteriologists, 1899–1950. Washington, DC: American Society for Microbiology; 1950:36.)*

a cholera-like salmonellosis,[59] but the term *virus* in the latter half of the 19th century did not have the specific meaning it has today; there was confusion about what pathogens could pass through filters. Their report demonstrated that the ideas of **live and killed vaccines** developed almost simultaneously. This seminal work of Salmon and Smith bore fruit for *humans* 15 years later.

Ironically, their competitors in the development of a killed vaccine were Charles Chamberland and Roux from Pasteur's laboratory, who reported on the same topic in December 1887,[60] some 16 months after Salmon and Smith's original paper. In 1888, Salmon read a paper before the American Association for the Advancement of Science (AAAS) defending their 1886 article and their priority in developing the first killed vaccine.[61] However, the Institut Pasteur had just been established in 1887; Pasteur was at the height of his fame and worldwide prestige thanks to the rabies vaccine. Not surprisingly, Salmon and Smith, working for the U.S. Department of Agriculture, saw their claim lost in the aura surrounding Pasteur and his associates. Thus, even 100 years ago, the Institut Pasteur and the U.S. government were involved in disputations about discovery rights, similar to the late 20th-century controversy about who first isolated the human immune deficiency virus: Luc Montagnier at the Institut Pasteur or Robert Gallo at the National Institutes of Health.

Killed vaccines for typhoid, plague, and cholera followed on the heels of Salmon and Smith's research. Richard Pfeiffer and Wilhelm Kolle in Germany and Almroth Wright in England worked independently on killed **typhoid vaccines.**[62–65] To this day, the debate continues about exactly who inoculated the first human with killed typhoid vaccine. In truth, all three deserve credit because it is now clear that several groups were working on typhoid vaccine at that time.[66]

Shibasaburo Kitasato and Alexandre Yersin, each working independently, discovered the causative bacillus of the **plague** in 1894, *Yersinia pestis* (called *Pasteurella pestis* until 1970).[13,15,67,68] With Albert Calmette and Amédée Borelle, Yersin developed a killed plague vaccine for animals,[69] but it was Waldemar Haffkine who took up the task of developing a vaccine against human plague.[70,71] Haffkine was in India working on cholera vaccine when bubonic plague broke out in Bombay. He switched to studies of plague immunization and was himself the first to be injected with his new killed plague vaccine. More than 8000 people were then vaccinated within a few weeks. For a while, Haffkine was a hero. However, the Mulkowal incident in 1902 when 19 people died from contaminated plague vaccine resulted in Haffkine's removal from his post by the Indian government. The contamination (with tetanus bacillus) does not seem to have been his fault.[15] Nevertheless, his scientific career and reputation were severely damaged; he never fully recovered from the incident and retired early from science at age 55 years. Later, with the wisdom of hindsight, the Indian government renamed the Plague Research Laboratory where he had worked *The Haffkine Institute*. Perhaps as important as his development of the plague vaccine was Haffkine's contribution to the literature on the proper way to conduct controlled field trials.[72]

John Snow had shown that cholera was transmitted by contaminated water in 1848,[73] although he did not know the identity of the contaminant. That answer was supplied by Koch, when he isolated *Vibrio cholerae* as the causal organism in 1883.[74] Early attempts at a **cholera** vaccine were made by Jaime Ferrán, Pasteur's pupil, and by Haffkine. Both used live cultures and both vaccines were rejected because of severe reactions.[13] Kolle developed a heat-killed, human cholera vaccine in 1896.[75,76] He grew the vibrios in agar, suspended them in saline solution, heated them at 50°C for a few minutes (later changed to 56°C for 1 hour), and then added 0.5% phenol.

In parallel with the focused research on vaccines, important work on immunity was being pursued at the end of the 19th century. Elie Metchnikoff, another Pasteur protégé, reported his theory of cellular immunity in 1884.[13,77] He named the cells that ingested and destroyed invading microorganisms and other foreign bodies *phagocytes*. Although he did not understand the role of serum and plasma in immunity at this early date, his work was truly pioneering.

In 1888, Roux and Yersin showed that the **diphtheria** bacillus produced a powerful **toxin**.[13,78] Two years later, Emil von Behring and Kitasato, working in Koch's laboratory in Berlin, followed up on early work by Karl Fraenkel; they showed the presence of powerful **antitoxins** in the serum of animals previously injected with low doses of **tetanus** or diphtheria toxins.[79–81] The antitoxin neutralized diphtheria or tetanus toxin in culture. Further experiments showed that the antitoxin protected animals challenged with the tetanus or diphtheria bacillus. Although he did not use the term, what von Behring had found in the serum of animals previously injected with diphtheria or tetanus toxins were **antibodies**. It was Paul Ehrlich, also working in Koch's laboratory, who first referred to these antitoxins as antibodies—"antikorps."[82]

Progress occurred rapidly after these reports; the first child was treated with diphtheria antitoxin just 1 year later, December 1891. Shortly thereafter, commercial production of diphtheria antitoxin began. von Behring referred to the rabbit serum that contained the antitoxin as "immune serum." Soon, the process of inoculating with the immune serum that contained tetanus or diphtheria antitoxin was referred to for the first time as **immunization**.[15,81]

Ehrlich's receptor theory of immunity, which he referred to as the "side-chain theory," made a strong contribution to

vaccine development. When it was first developed in 1897, the theory was used primarily to explain toxin–antitoxin interactions and subsequently the relationship between antigens and antibodies. It soon became one of the cornerstones of 20th-century immunology.[83] Ehrlich's other major contribution was to point out the difference between active and passive immunity.[13,84]

The last decade of the 19th century produced remarkable advancements from remarkable men. von Behring was awarded the first Nobel Prize in Medicine (1901); Koch received it in 1905, and Ehrlich and Metchnikoff shared the Nobel in 1908.

FIRST HALF OF THE 20TH CENTURY

At the beginning of the 20th century, five human vaccines were in use: Jenner's original smallpox vaccine and Pasteur's rabies vaccine (both containing live virus) and three bacterial vaccines: typhoid, cholera, and plague (all killed). In addition, immunization with diphtheria or tetanus antitoxin was an accepted practice. The 19th century's end also saw the end of arm-to-arm lymph inoculation as a vehicle for smallpox vaccination. This technique was replaced by the use of glycerinated calf lymph in 1898.[13] Most of the fundamental concepts of vaccinology had been introduced by the end of the 19th century; the early 20th century would bring refinements to these theoretical underpinnings. Not until the advent of cell culture 50 years later would the field again become so dramatically fertile (Table 1.1).

Wright proposed mass immunization of British troops with killed typhoid vaccine during the Boer War (1899), but there was opposition because of adverse reactions and he was able to vaccinate only 14,000 volunteers. Opposition ran so high that consignments of vaccine were dumped overboard from transport ships in Southampton. The result was catastrophic: more than 58,000 cases of typhoid and 9000 deaths in the British Army.[63] A bitter battle about the merits of the vaccine was waged in the *British Medical Journal* between Wright and the statistician Karl Pearson. Ultimately, at Wright's insistence, the War Board initiated a broad-based trial that showed the overwhelming effectiveness of the vaccine. Wright was then knighted. By the beginning of World War I in 1914, general typhoid vaccination was conducted in the British Army, although it was still not mandatory.[63,85,86]

During the first few decades of the 20th century the use of "bacterins" as human vaccines came into use. Bacterins consisted of killed bacteria (antigens) that were injected parenterally to produce active immunization. Sometimes they were combined with immune serum to become "serobacterins"—the serum would provide short-term immunity before the killed bacterial antigens kicked in to provide long-term immunity. The concept was an outgrowth of Metchnikoff's theory of phagocytosis and Wright's work on opsonins. It was thought that bacterins worked because opsonins induced by the antigens prepared invading bacteria for phagocytosis. Most bacterins were prepared and sold without clinical trials. In its 1908 catalog, HK Mulford Company, a forerunner to Merck, listed nine bacterins that it sold, including those for gonorrhea, typhoid, pneumococcal disease, and streptococcal disease. Bacterins for humans fell into disuse by the late 1930s as more stringent licensing requirements were imposed by the federal government.[87,88] Bacterins for specific herds are still used as targeted vaccines for animals.

In the early 20th century, the *chemical* inactivation of diphtheria and other bacterial toxins led to the development of the first **toxoids**: diphtheria and tetanus. Here again, Theobald Smith had a significant role. In 1907, he determined that toxoids provided immunity in guinea pigs. In a 1909 report on long-lasting immunity against diphtheria in guinea pigs immunized with toxoid, he suggested that toxoids should be considered for humans.[59,89]

In 1923, Alexander Glenny and Barbara Hopkins showed that diphtheria toxin could be transformed into a toxoid by formalin.[90] The discovery came about when the containers in which the batches of diphtheria toxin were kept were cleaned with formalin (they were too large to be autoclaved). The residual formalin in the vats rendered the batch of toxin so weak that 1000 times the normal dose did not kill the guinea pigs. Although this toxoid was certainly safer than the toxin, it could be administered only in conjunction with antitoxin. In that same year, Gaston Ramon developed a diphtheria toxoid that could be used on its own (i.e., without antitoxin) by adding formalin and incubating the mixture at 37°C for several weeks.[91] Ramon and Christian Zoeller used a tetanus toxoid developed in the same manner for the first human vaccinations against tetanus in 1926.[92,93]

The vaccine against **tuberculosis,** bacille Calmette-Guérin (BCG), was the first live vaccine for humans to be produced since Pasteur's rabies vaccine in 1885. Calmette was a protégé of Emile Roux and founder of the Pasteur Institutes at Lille and in Indochina.[13] In 1906, Calmette and Camille Guérin, a veterinarian, started subculturing a strain of mycobacteria obtained from a bovine, which they perhaps thought was *Mycobacterium tuberculosis* but was in reality *Mycobacterium bovis*. They originally focused on producing a serotherapy, along the lines of von Behring's antidiphtheria serotherapy, but quickly realized they could not inhibit the pathogenicity of the bacillus very easily. That began their pursuit of a vaccine. After 13 years of attenuation by 230 passages in beef bile, potatoes, and glycerol, this strain eventually became the BCG strain. In total, Calmette and Guérin spent more than 20 years trying to understand the mechanism of infection of tuberculosis.[94] Clinical trials in children began in 1921, and the vaccine became available for human use in 1927.[13,95–99] Because the original vaccine strain was sent to numerous laboratories around the world, each of which then produced its own variation of BCG vaccine, standardization has proved difficult. Despite the existence of more than a dozen major BCG vaccine strains that vary widely in strength, BCG remains an effective, if imperfect, vaccine against tuberculosis in children.[100]

In 1931, E.W. Goodpasture introduced the use of the chorioallantoic membrane of the fertile hen's egg as a medium for growing viruses.[55,101] This technique represented a major advance because until then it was thought that human viruses could be grown only in animals such as ferrets and mice. Ferrets were expensive, and mouse brain could produce allergic brain encephalitis. The chick embryo proved to be a cheaper and safer medium for the cultivation of viruses. Earlier, in the second Milroy Lecture of 1898, Copeman described conducting an experiment using hens' eggs—successfully—to grow vaccinia virus for the production of smallpox vaccine.[34,102]

Yellow fever virus was isolated in 1927 by two independent groups: researchers at the Rockefeller Foundation working in Nigeria, who isolated the Asibi strain,[103–105] and researchers at the Pasteur Institute in Senegal, who isolated the French strain.[106,107] The French strain was given to various research groups for study.[107] In 1928, A.W. Sellards at the Harvard Medical School began collaborative research on the French strain with Jean Laigret at the Pasteur Institute in Senegal. Max Theiler, working for Sellards at Harvard, developed an animal model to study the virus.[108] Using passage in mouse brain, others were able to "fix" the neurovirulence of the strain,[109] which then was used as a vaccine. This French strain yellow fever vaccine from Theiler's work at Harvard was a live vaccine derived from mouse brain passage.[110] Sellards and Laigret

TABLE 1.1 Outline of the Development of Human Vaccines (Wherever Possible, Date of Licensure Is Indicated)

Live Attenuated	Killed Whole Organism	Native Protein or Polysaccharide	Genetically Engineered
18TH CENTURY			
Smallpox (1798)			
19TH CENTURY			
Rabies (1885)	Typhoid (1896) Cholera (1896) Plague (1897)		
20TH CENTURY, FIRST HALF			
Tuberculosis (bacille Calmette-Guérin) (1927)	Pertussis (1926)	Diphtheria toxoid (1923)	
Yellow fever (1935)	Influenza (1936) Typhus (1938) Tickborne encephalitis (1937)	Tetanus toxoid (1926)	
20TH CENTURY, SECOND HALF			
Polio (oral) (1963)	Polio (injected) (1955)	Pneumococcus polysaccharide (1977)	Hepatitis B surface antigen recombinant (1986)
Measles (1963)	Rabies (cell culture) (1980)	Meningococcus polysaccharide (1974)	Lyme OspA (1998)[b]
Mumps (1967)	Japanese encephalitis (mouse brain) (1992)[b]	*Haemophilus influenzae* type b polysaccharide (1985)[b]	Cholera (recombinant toxin B) (1993)
Rubella (1969)	Tickborne encephalitis (1981)	Meningococcal conjugate (group C) (1999) U.K.[a]	
Adenovirus (1980)	Hepatitis A (1996)	*H. influenzae* type b conjugate (1987)[a]	
Typhoid (*Salmonella* Ty21a) (1989)	Cholera (WC-rBS) (1991)	Hepatitis B (plasma derived) (1981)	
Varicella (1995)		Typhoid (Vi) polysaccharide (1994)	
Rotavirus reassortants (1999)		Acellular pertussis (1996)	
Cholera (attenuated)[b] (1994)		Anthrax secreted proteins (1970)	
21ST CENTURY			
Cold-adapted influenza (2003)	Japanese encephalitis (2009) (Vero cell)	Pneumococcal conjugates (heptavalent) (2000)[a]	Human papillomavirus recombinant (quadrivalent) (2006)
Rotavirus (attenuated and new reassortants) (2006) Rotavirus (monovalent) (2008) Cholera (oral) (2016)	Cholera (WC only) (2009)	Pneumococcal conjugates (13-valent) (2010)	Human papillomavirus recombinant (bivalent) (2009) Human papillomavirus (9-valent) (2014) Meningococcal type B (fH factor) (2014) Meningococcal type B (reverse vaccinology) (2015)
Zoster (2006)		Meningococcal conjugates (quadrivalent) (2005)[a]	

[a]Capsular polysaccharide conjugated to carrier proteins.
[b]No longer available.

intended to do the human trials on the yellow fever vaccine at the Institut Pasteur in Paris, but Roux, who was then the director, refused to allow the human trials with the murine virus to be conducted. He thought it was too dangerous.[111] Eventually it was used in humans without immune serum by Sellards and Laigret in 1932.[112] However, owing to the strain's passage through mouse brain tissue, the neurovirulence of the French strain did indeed present grave dangers.

Theiler subsequently left Harvard to join the Rockefeller Institute and attempted to develop a more attenuated vaccine, using the Asibi strain. Theiler and Hugo Smith developed the 17D strain from Asibi in fertile hen's eggs chorioallantois per Goodpasture's method. Although the French strain was also highly effective, the 17D strain was both effective and much safer.[55,107,113–115] The French strain certainly saved many lives, especially in French West Africa, where it was used extensively. It remained in production (in modified form) until 1982; however, safety concerns about the use of mouse brain tissue overrode its proven efficacy and 17D won out as the vaccine strain of choice.[107] For this work, Theiler was awarded the Nobel Prize in 1951.

Wilson Smith, Christopher Andrewes, and Patrick Laidlaw isolated human **influenza A** virus in ferrets in 1933.[116] They followed the technique outlined by Richard Shope of the

Rockefeller Institute when he isolated the swine influenza virus in pigs in 1931. Within 5 years, Smith's group and Shope were able to show that swine influenza virus was a surviving virus from the great influenza pandemic of 1918.[117,118] Frank Horsfall, Alice Chenoweth, and colleagues developed a live influenza virus vaccine in mouse lung tissue in 1936.[119,120] Chenoweth claimed that it became inactivated or nonreplicating when it was administered parenterally.[120,121] That same year, 1936, saw the development of two influenza A vaccines grown in embryonated eggs, one (live) by Wilson Smith[122] and the other (killed, whole virus) by Thomas Francis and Thomas Magill.[123,124] Even though these two vaccines were considered safer because they were developed in embryonated eggs, Chenoweth's mouse lung vaccine had contained a higher virus yield and was the first to demonstrate true protection in humans, albeit transient.

In 1937, Anatol Smorodintsev and colleagues in the Soviet Union administered the Wilson Smith strain to humans by the intranasal route, using doses that were lethal when given to mice.[125] This is considered the first *live* human influenza virus vaccine, and although it would not receive a passing grade by today's standards (20% of vaccinees developed febrile influenza), it absolutely demonstrated the role of the virus in the development of influenza.[121,126]

Frank Burnet and D.R. Bull showed in the early 1940s that live attenuated influenza virus could be produced in embryonated eggs but also that the resultant virus mutated rapidly. Therefore, the vaccines that were produced were not consistently attenuated and often produced disease.[127,128] By contrast, the Francis and Magill killed, whole-cell influenza A vaccine did not have the problem of mutated viruses and did not produce disease.

In 1940, Francis and Magill independently isolated **influenza B;** at this point, it was recognized that at least three strains of influenza were circulating at the same time. Francis developed a killed (formaldehyde), trivalent (2As, 1B) vaccine that was mass-produced for the U.S. military in World War II. This conferred a certain "legitimacy" to killed influenza vaccine, as the military did not have to be concerned about "down time" from live vaccine–associated influenza. During this same period, Burnet developed a live aerosolized influenza vaccine, but the influenza season had already begun, so an efficacy trial was inconclusive. By then, the killed vaccine of Francis had been very successful during the war. The Australian government denied Burnet permission to continue trials of a live vaccine as too risky.[121,126,129,130] Except for the Soviet Union, which continued to use live vaccine, killed influenza vaccine became the standard until the 1990s (see subsequent text).

During the 1947 flu season, the influenza vaccine did not protect, definitively proving the concept of antigenic variation of the virus strain from year to year, which had first been proposed by Magill and Francis in 1936. The term itself, "antigenic drift," was introduced by Burnet in 1955 in his *Principles of Animal Virology*.[126]

Many attempts were made to develop vaccines against rickettsiae once Charles Nicolle had discovered in 1909 that they were the cause of **typhus**.[13] The first truly successful typhus vaccine was developed in 1938 by Herald Cox,[131] who used the yolk sac of the chick embryo to grow *Rickettsia rickettsii*. Cox was working on Rocky Mountain spotted fever at the time, but once he found a method to cultivate the rickettsia, killed vaccines for typhus and Q fever quickly followed. There was a heavy demand for the typhus vaccine during World War II.[13,132,133]

Jules Bordet and Octave Gengou first observed the causal agent of **pertussis** in 1900 and cultivated it by 1906.[13,134] Several vaccines were tested in small trials. Thorvald Madsen later carried out the first controlled clinical trials of a pertussis vaccine (i.e., whole killed organisms) on the Faeroe Islands in 1923–1924 and again in 1929.[135,136] During the 1923–1924 epidemic, Madsen reported that the vaccine did not prevent disease but greatly reduced mortality and severity of illness among vaccinated persons. By the 1929 epidemic, the vaccine had been considerably improved but still did not prevent disease.[137] In the 1930s, Pearl Kendrick and Grace Eldering (Figs. 1.6 and 1.7), working for the Michigan Department of



Figure 1.6. Pearl Kendrick. *(From Grand Rapids History & Special Collections, Archives, Grand Rapids Public Library, Grand Rapids, MI.)*



Figure 1.7. Grace Eldering. *(From Grand Rapids History & Special Collections, Archives, Grand Rapids Public Library, Grand Rapids, MI.)*

Health, improved the yield of the Bordet-Gengou growth medium and developed a killed (thimerosal) vaccine that they successfully tested in more than 1500 children. Only 4 of 712 vaccinees developed mild cases of whooping cough. They recruited the help of Eleanor Roosevelt to gain additional funds for further research and by 1940, their vaccine was distributed throughout the United States.[138] The American Academy of Pediatrics approved the vaccine in 1943 and the American Medical Association in 1944. Several whole-cell pertussis vaccines were in use by the late 1940s.[139,140] The first **combination vaccine,** DTP (diphtheria, tetanus, pertussis) became available in 1948.[141]

SECOND HALF OF THE 20TH CENTURY TO THE PRESENT

The latter half of the 20th century can truly be called the Golden Age of vaccine development. This occurred primarily because of the ability to grow viruses in stationary **cell culture**. Hugh and Mary Maitland from Manchester University first developed the flask **tissue culture** technique in 1928.[13,59] They succeeded in growing vaccinia virus in sterile cultures of minced rabbit kidney in rabbit serum and mineral salts—a great accomplishment that proved to be a turning point in virus research. George Gey improved the virus yield of this method by continually rolling the tubes, thus increasing the oxygenation of the cells.[59]

After the Second World War, John Enders, Thomas Weller, and Fred Robbins took up research on cell culture at Boston Children's Hospital. After using tissue cultures of the Maitland type (substituting monkey kidney for Maitland's rabbit kidney), they tried to grow viruses in explanted human cells, using fibroblasts grown from the foreskin and muscle tissue of infants who had died soon after birth. Their first success was to grow Lansing type II poliovirus in human cell culture.[142] The ability to grow human viruses in vitro, in a relatively easy and safe manner in monolayer cell cultures, led to an veritable explosion of creativity in vaccinology that continues unabated (see Table 1.1). Indeed, Enders, Weller, and Robbins received the Nobel Prize in Medicine in 1954 for their seminal work.

Paralytic **polio** became an epidemic problem only in the latter half of the 19th century when epidemics broke out in Western Europe and the United States.[143] After the virus was isolated by Landsteiner and Popper,[144,145] there were attempts to make polio vaccines in the 1930s, especially by John Kolmer and Maurice Brodie.[146–148] These vaccines were poorly and hastily tested, and their use resulted in at least six deaths and numerous cases of vaccine-associated paralytic polio. The backlash against these ill-conceived experiments was swift. At the meeting of the American Public Health Association in St. Louis in November 1935, James Leake, medical director of the U.S. Public Health Service, directly accused Kolmer of murder! Kolmer stopped working on vaccines after this disastrous incident and returned to Temple University where he taught Public Health and Preventive Medicine.[31,146] Polio vaccine development for the most part ground to a halt for the next 15 years.[146]

A rare exception to this hiatus was Hilary Koprowski, who did work on polio vaccine during this period and developed the first live oral polio vaccine (OPV) with an attenuated polio variant virus strain grown in mice, not cell culture. It was tested in humans in 1950.[149]

The logjam preventing further polio vaccine research was broken with the aforementioned report by Enders, Weller, and Robbins in *Science* in 1949.[142] All subsequent research on polio vaccine was based on attenuation or passage in cell culture. In rapid succession thereafter, articles appeared showing that the Lansing strain of polio could be grown in a variety of human and other primate tissues,[150–155] and several laboratories began research on polio vaccines.[146] Thus began **"The Polio Wars."**

The first licensed product developed using the cell culture technique was the trivalent, formalin-inactivated polio vaccine (IPV) of Jonas Salk, licensed in 1955.[156] Salk gained his vaccine experience under his mentor Thomas Francis, with whom he developed the first killed influenza vaccine in 1938, which was used to vaccinate the American troops during the Second World War. The majority of Salk's research on killed polio vaccine and the large clinical trial in 1954 (more than 1.8 million children) were funded by the National Foundation for Infantile Paralysis (March of Dimes), founded by Franklin Roosevelt in 1938 and directed by Basil O'Connor. The Foundation's idea on the trial design did not include a placebo-control; on the contrary, Francis insisted on the need for placebo-control. After a shaky start, the clinical trial was designed by a committee that included both points of view, headed by Thomas Francis, Salk's former mentor, who ultimately directed the trial. Although Salk wanted every child in the trial to receive his vaccine, Francis stood his ground and insisted on use of a placebo. The controversial trial design was called a "dual-control" trial. In 11 states, children in grades 1 through 3 received injections of either the polio vaccine or a placebo. In 33 other states, children in grade 2 received injections of the vaccine and all children from grades 1 through 3 were "observed" for the duration of the polio season. In the end, 420,000 children received Salk's vaccine, 200,000 a placebo, and 1.2 million were "observed."[146,157,158]

The quest for a polio vaccine was highly publicized, adding pressure on everyone involved to hurry up and develop a vaccine. Although Salk's public persona generated a lot of animosity from professional colleagues who saw him as a publicity hound, when the overwhelmingly positive results of Salk's trial were announced on April 12, 1955, with great fanfare, ordinary Americans were ecstatic and Salk became an instant "hero." The inactivated Salk vaccine was immediately licensed, and within days, six companies had been granted rights to manufacture the vaccine.[143,146,159]

This haste was understandable, as the "summer polio season" was about to begin and parents were anxious to vaccinate their children. But it was short sighted. Shortly thereafter, the Cutter Incident occurred,[146] in which cases of paralytic polio were detected in vaccine recipients. Epidemiologic evidence showed that the product of one manufacturer, Cutter Laboratories, was implicated in most of these vaccine-related cases. The nation's entire polio vaccination program was put on hold until a review of manufacturing procedures could determine the source of the problem.[146,160,161] More rigorous safety testing was put in place and an additional filtration process added to remove clumps of partly inactivated virus before the vaccination program resumed. The contaminated vaccine resulted in 260 cases of paralytic polio and 10 deaths in recipients, their families, or community contacts. Only one manufacturer was implicated, although several had experienced problems. Because polio was such a widespread and feared disease, there was no lasting boycott of the vaccination program.[143,146]

Despite the success of Salk's killed vaccine, others continued research to develop *live* attenuated polio vaccines in cell culture: Herald Cox at Lederle, Koprowski at the Wistar Institute, and Albert Sabin at Cincinnati Children's Hospital. Like Jenner and Pasteur, these researchers believed that an infection with live virus would provide more long-lasting immunity and greater resistance to disease.[143] Because Salk's vaccine was already in use, Sabin was unable to get funding in the United States for the required clinical trial to prove the efficacy of a

live polio virus vaccine. Despite the Cold War, and probably because he was born in Russia, Sabin was able to collaborate with Russian scientists to do the enormous clinical trials in the Soviet Union. Based on Dorothy Horstmann's favorable review of the Russian trial results, Sabin's vaccine was licensed in the United States in 1960. Until 1963, both vaccines (Salk's killed and Sabin's live) were used in the United States. In 1964, the Committee on the Control of Infectious Diseases of the American Academy of Pediatrics voted a clear preference for Sabin's live OPV, which was grown in monkey kidney cell culture and was easier to administer (vaccine on a sugar cube). As a result, it came into wide use.[143,162]

For the next 30 years OPV was the primary recommended vaccine in the United States and in many, but not all, European countries (Sweden, Finland, and the Netherlands continued using IPV). Although there were reports of vaccine-associated cases of polio with OPV, Sabin adamantly defended his vaccine and refused to believe that it was capable of causing polio. But by the 1990s, the only cases of polio occurring in the United States and Europe were vaccine-associated, caused by mutation of the live virus and reversion to virulence in OPV. In the year 2000, the United States switched to the exclusive use of IPV.

Thanks to both of these vaccines, polio has been eradicated from the Western Hemisphere, and the World Health Organization (WHO) has targeted polio to be the next disease after smallpox to be effaced from the entire world. In the May 2015 WHO report, the recommendation was made to remove Type 2 polio strain (the strain that causes most vaccine-associated polio) from all OPV vaccine by April 2016 and to begin introduction of IPV wherever possible by that same date.[163]

Many others took advantage of cell culture techniques. Samuel Katz, Milo Milanovic, Enders, and colleagues developed the Edmonston strain of **measles** vaccine, grown in chick embryo cell culture,[164] which was attenuated further by Maurice Hilleman and colleagues[165] and by Anton Schwarz.[166] Hilleman also attenuated the Jeryl Lynn strain of **mumps** virus (obtained from and named after his own daughter Jeryl Lynn) in the hen's egg and obtained licensure in 1967.[167] Rubella virus was isolated in 1962, both by Tom Weller[168] and Parkman and associates.[169] The virus was attenuated by passage in cell culture and by 1970, several strains had been developed and were in use: in monkey kidney by Paul Parkman and Harry Meyer (further passaged in duck embryo cells by Hilleman),[170] in rabbit kidney by Abel Prinzie and Constant Huygelen,[171] and in human fibroblasts by Stanley Plotkin.[172] The latter strain (Wistar-RA27/3), the first vaccine made in human fibroblasts, is the sole rubella vaccine in wide use since 1980 because of its better safety and efficacy. In 2015, the Pan American Health Organization (PAHO) announced that rubella was officially declared eliminated from the Western Hemisphere.[2,173]

In the 1950s, the **adenoviruses** were first recovered from adenoids that had been surgically removed.[174] Until their isolation, a great variety of the illnesses they cause were attributed to other diseases. Formalin-inactivated, whole-virus vaccines were made by Hilleman against types 4 and 7; they were licensed for military use only. In the early 1960s, the seed stock was shown to be contaminated with simian virus 40 (SV40)[175]; when all attempts to eliminate SV40 failed, the vaccine was withdrawn in 1963.[176]

Vaccine studies continued, however, using human embryo kidney (HEK) cells and subsequently human diploid cell strains, thus eliminating the SV40 problem. An enteric-coated vaccine tablet was produced for types 4, 7, and 21 by Chanock and colleagues[177–181]; the vaccine was licensed, but again, only for military use. Production ceased in 1996, primarily because of regulatory issues and lack of interest by the military. Not unexpectedly, within a few years significant outbreaks of adenovirus respiratory diseases reoccurred in military recruits. After several years, the U.S. Army signed a contract with another producer to reformulate the original vaccine.[182] In 2011, the U.S. Food and Drug Administration (FDA) again approved a live oral vaccine for adenovirus types 4 and 7 for military populations ages 17 to 50 years.

The live attenuated Oka strain of **varicella** vaccine was developed in the 1970s by Michiaki Takahashi[183,184] and underwent extensive clinical trials before being licensed in Japan and several European countries.[185,186] After a long and convoluted development, licensure was obtained in the United States in 1995.[187] It is now recommended for all children older than 1 year.[188] As of 2009, there has been a 90% decline in varicella-related hospitalizations.[189–193]

The discovery of the relationship between chickenpox and shingles goes back to the late 19th century. A Hungarian scientist, James Bokey, described cases of chickenpox in persons who had been in contact with someone with shingles.[31] Some 30 years later, in an experiment that certainly could not be done today, a German scientist named Kundratitz proved him right by inoculating children with shingles "pus!" They developed chickenpox.[31,194,195] Thus, the attenuated Oka strain in high concentrations could be used as a zoster vaccine.

In May 2006, the FDA granted a license for Zostavax, a higher-potency, live attenuated Oka virus vaccine against **zoster**.[196] This higher-dose vaccine reduces the incidence of shingles and also reduces the severity of postherpetic neuralgia in those cases of shingles that occur in vaccinated persons.[197–201] Originally recommended for those age 60 years and older, it is now recommended for anyone older than age 50 years. A new zoster vaccine based on a viral glycoprotein may be licensed soon.

Influenza vaccines were first licensed in the United States in 1945[202]; the first recommendation for their inclusion in the pediatric schedule did not occur until 2004. By the 1960s, a **live influenza vaccine** that was safe for adults was achieved, the Alice strain (H_3N_2),[203,204] but it could not be used in children, who became febrile.[205] The annually administered *killed* influenza vaccine, which had consistently proved to be safe and effective but without lasting local or cellular immunity, remained the standard.

Newer vaccine design technologies in the 1990s, including reassortment, reverse genetics, and cold adaptation, have again made it possible to develop *live* attenuated influenza vaccines that confer long-term immunity and obviate the need for injections.

Three attenuated master strains were developed for live influenza vaccines: host-range, temperature-sensitive, and cold-adapted mutants. Only the cold-adapted influenza vaccine developed by Hunein Maassab has been licensed in the United States (2003).[206] Other strains were attempted and abandoned because of inconsistent attenuation, instability, and, occasionally, reversion to virulence.[207] Cold-adapted strains allow the vaccine virus to grow in the relative coolness of a subject's nasal passages (32°C) but not in warmer internal organs, particularly the lungs (37°C).[208]

Live attenuated influenza vaccine (LAIV) is administered by nasal spray and is a good example of the advantages of **reassortant technology**, which is possible with viruses that have segmented genomes. Coinfection of cell culture with wild and attenuated strains allows the mixing of genome segments and identification of viruses containing genetic material from both strains. Each year, new influenza vaccine strains for LAIV are made by reassorting the six internal genes from Maassab's master strains with the genes coding for the hemagglutinin and neuraminidase surface glycoproteins of circulating wild strains of influenza viruses.[207,209] (New vaccine strains for *killed*

influenza vaccine also are produced each year by reassortment or by recombinant technology.) The live vaccine was originally shown to be as effective as the killed vaccine in children and offered longer and broader immunity.[207,210–214] Furthermore, the live vaccine is easy to administer and has shown cross-protection against antigenically drifted wild strains.[215,216] However, recent LAIV strains have shown poor immunogenicity, and the vaccine's utility is currently uncertain.

An orally administered, quadrivalent, live **rotavirus** reassortant vaccine developed by Albert Kapikian and associates, was licensed for use in the United States in September 1998.[217–219] Within 10 months of licensure, cases of intussusception among vaccine recipients were reported to the Vaccine Adverse Event Reporting System.[220–222] Based on epidemiological studies, the Centers for Disease Control (CDC) determined that intussusception occurred with significantly increased frequency within the first 2 weeks after vaccination.[223] The vaccine was formally withdrawn from the market in November 1999.[224]

Other rotavirus vaccines were developed by H. Fred Clark, Paul Offit, and Stanley Plotkin at the Wistar Institute and the Children's Hospital of Philadelphia and by Richard Ward and David Bernstein at Cincinnati Children's Hospital. The Wistar/Children's Hospital of Philadelphia rotavirus vaccine is based on a bovine rotavirus (WC-3), attenuated for humans, which has been reassorted with five human rotavirus RNA segments that code for vp4 or vp7 protein, from different serotypes,[225,226] thus producing a pentavalent oral vaccine, which was licensed for use in the United States in 2006.[227] The Cincinnati group developed a vaccine based on a single human rotavirus attenuated by passage in cell culture that contains serotypes G1 and P1a[8]. It was licensed in the United States in 2008.[228–230]

Because concern about intussusception remained strong, both of these vaccines underwent enormous clinical trials (more than 60,000 were tested in each case) to determine whether an association existed. Both vaccines proved highly efficacious, and intussusception was rare.[231–234] Clinical trials expanding the use of these vaccines to developing countries show protection, although not as high as in developed countries. This has led to a global recommendation for the use of rotavirus vaccine by the WHO.[235–239]

Parenteral killed cholera vaccines did not gain wide acceptance because of limited efficacy, fleeting protection, and side effects and were eventually pulled from the market. Several oral vaccines against cholera and its debilitating diarrhea have been developed: two killed and one live. The **killed cholera vaccine** developed by Jan Holmgren and colleagues, combines the whole cell of *V. cholerae* O1 and subunit B of the cholera toxin (WC-rBS).[240,241] The second killed vaccine contains O1 and O139 strains but no recombinant subunit (WC-only). This makes it easier to administer and less expensive to produce. It is currently used primarily in India and Vietnam. Both killed vaccines are prequalified by the WHO, licensed in many countries, and used to prevent travelers' diarrhea as well as for epidemic control.[240–243]

An attenuated **live oral cholera vaccine**, derived from Inada strain 569B has been developed by Myron Levine and colleagues at the University of Maryland.[244–251] This vaccine requires a cold chain and a buffer. The vaccine was licensed and sold in many countries; it offered rapid onset of protection. For reasons unrelated to the vaccine itself, the manufacture was interrupted (bankruptcy proceedings and company takeovers). Ultimately, the rights to the vaccine were returned to the University of Maryland. In 2015, a biotech company reached an agreement with the University of Maryland to bring the live oral cholera vaccine to FDA licensure. In 2016, the vaccine was approved for use in adults aged 18 to 64 years traveling to cholera-affected areas.[251a]

After the early work on killed **typhoid vaccine**, a variety of heat-phenol–killed or acetone-killed, parenteral, whole-cell typhoid vaccines became available.[252–255] All had high rates of adverse reactions and were never considered quite satisfactory. An important advance was made by René Germanier and E. Fürer when they developed an attenuated strain of the Gal E mutant Ty21a of *Salmonella typhi*.[256] After preliminary vaccine studies in the United States,[257] large trials were conducted successfully in Egypt[258] and Chile.[259,260] Protection rates varied; however, there were few adverse reactions, and oral formulation of this vaccine made it less expensive to produce and distribute.[261,262] Typhoid Vi *polysaccharide*, a killed, purified-component vaccine was developed by Landy, Webster, and colleagues[263–265] and later improved by Wong and associates[266] and John Robbins and J.B. Robbins.[267]

The adaptation of **rabies** virus to human diploid cell culture permitted the development of a potent, whole-virus inactivated rabies vaccine by Koprowski, Tadeusz Wiktor, and associates.[268] This vaccine is much more immunogenic than prior rabies vaccines. Since then, many other cell culture rabies vaccines have been developed, including a vaccinia-recombinant rabies vaccine for veterinary use.[269]

The development of a **Japanese encephalitis** (JE) vaccine was attempted during the Second World War,[270] but later a formalin-inactivated whole-virus vaccine, harvested from mouse brain, was developed in Japan in 1965.[271] It was put into use almost immediately to vaccinate Japanese children, although few data regarding its efficacy had been published.

After two Americans who had traveled in Asia died of JE, the U.S. Department of Defense conducted a vaccine trial in northern Thailand[272] that showed an efficacy of 91%. A bivalent vaccine, also in mouse brain, was developed using the Nakayama-NIH strain (from the original vaccine) and the Beijing-1 strain to provide immunity to strains from different geographic areas.[272–274]

X.Y. Yu and coworkers developed live attenuated and inactivated vaccines against JE, each in primary hamster kidney cells.[275–279] The Chinese live vaccine is widely used in Asia, but hamster kidney cells are not approved by the WHO.[280]

The bivalent, inactivated, mouse-brain–derived vaccine needed improvement. An increase in allergic reactions was noted in the 1990s, several doses were required to maintain immunity, and it was developed in animal nervous tissue (mouse brain).[280] In March 2009, the FDA approved a second-generation JE vaccine for adults that is Vero-cell produced, inactivated, and purified. Ixiaro uses the SA_{14}-14-2 strain of virus that had been used in the Chinese live vaccine.[281] In May 2013, the license was extended to include children older than 2 months to 16 years of age. It is the only licensed JE vaccine in the United States; the previous one, made in mouse brain tissue, ceased production in 2006.[281–284]

In 1979, Maurice Hilleman and Phil Provost grew **hepatitis A** virus (HAV) in cell culture,[285] opening the path for the development of a vaccine. Hilleman, Provost, and coworkers developed the first inactivated HAV vaccine in 1986[286]; however, the cell culture used to produce the HAV antigen was not suitable for use in humans. Formaldehyde-inactivated, whole-virion HAV vaccines grown in human fibroblasts were later developed and licensed in the United States in 1995–1996.[287,288] By 2004, HAV infection rates declined to 1.9 per 100,000 population, the lowest rate ever.[289] Universal HAV vaccination for all children was recommended in 2006.[290]

The first killed **tickborne encephalitis** (TBE) vaccine, produced in mouse brain, was developed in the Soviet Union in 1937, shortly after the virus was identified and the tick vector verified.[291,292] In the 1960s, basing their work on Benda and Danes,[293,294] two separate groups, Levkovich in the Soviet

Union[295] and Kunz in Austria,[296] used chick embryo cell culture to develop less-reactogenic, formalin-inactivated vaccines. A **whole virus inactivated vaccine** was developed by Heinz, Kunz, and Fauma in 1980,[297] and is effective against isolates of the TBE virus that share a homologous envelope glycoprotein.[298–300] Since 1999, this vaccine has undergone several changes in the manufacturing process that have improved its purity, especially a switch from mouse brain protein to chick embryo cells.[301] A second inactivated TBE vaccine was licensed in Germany in 1991.[302] After the collapse of the Soviet Union in 1989 and the opening of Eastern Europe, the geographic range of TBE was shown to be quite large; it is now endemic to most European countries, the Russian Federation, and several Asian countries, including China, Japan, and South Korea.

It is clear that by the late 1970s, another transition was occurring in the development of vaccines. Technology had advanced to the point that scientists could identify many components of the infectious agent, viral or bacterial. A new category of vaccines emerged, based on bacterial proteins, polysaccharides, and protein-conjugated polysaccharides. Live attenuated and killed whole-cell vaccines were still important but they did not apply to all diseases. Subunit vaccines were created where the protective antigen could be identified and isolated. Some whole-cell vaccines were replaced by subunit vaccines.

Whole-cell pertussis vaccine from the 1940s caused a number of adverse reactions, mostly mild, but some more serious. In 1975, when two deaths occurred in children shortly after they received whole-cell pertussis vaccine, the Japanese Ministry of Health suspended its use, although a causal relationship was not established. An astronomical increase in the incidence of pertussis followed: 206 reported cases in 1971 grew to 13,105 cases by 1979. Realizing that it had overreacted, the Ministry of Health reinstated use of the vaccine, but only for children older than 2 years.[303] Similar problems occurred in the United Kingdom, where vaccination rates fell to less than 33% by 1977. Three major epidemics followed, causing more than 100,000 cases of whooping cough and 36 deaths.[304] The adverse reactions associated with whole-cell pertussis vaccine led to the development of a Japanese **acellular pertussis** vaccine by Yuji Sato and Hiroko Sato[303,305,306] that is less reactogenic. Based on two of the main protective antigens of *Bordetella pertussis,* toxin and filamentous hemagglutinin,[303,305,307] it was licensed for use in Japan in 1981. Since then, other acellular pertussis vaccines, containing one to five protective antigens, have been licensed in the United States (1996) and other countries.[308] However, despite routine vaccination, an increase in cases of pertussis since 2005 has led to the reevaluation of the acellular vaccines, which give less-persistent immunity. New formulations are being considered to make the acellular vaccine, as well as the use of more frequent boosters.

Modern work on a human **anthrax** vaccine began in the second half of the 20th century. The vaccine, named anthrax vaccine adsorbed (AVA), contains the secreted protein called "protective antigen" that forms part of the toxin. This was obtained from sterile filtrates of an attenuated, unencapsulated, nonproteolytic strain of *Bacillus anthracis*.[309–311] A randomized field study on a similar anthrax vaccine took place from 1955 to 1959 at four mills that processed raw goat hair destined for the clothing manufacturing industry.[312,313] AVA was licensed for use by the U.S. Army in 2002.

Human anthrax had not been viewed as a serious problem in the late 20th century. Worldwide, there were fewer than 2000 cases annually, mostly cutaneous, in the 1980s and 1990s.[314] The bioterrorism incident in 2001, when highly refined anthrax spores were sent through the U.S. postal system, changed that perception. It was considered prudent to ensure the availability and safety of the supply of anthrax vaccine for the military and the general public.[315] Although the FDA has affirmed that the current vaccine is effective, no matter what the route of infection,[316] research has been accelerated to find a new-generation anthrax vaccine based on protective antigen made by recombinant technology or using newer adjuvants. Modern anthrax vaccines will require fewer injections to attain full immunity.[317,318]

During the 1970s and 1980s, several bacterial vaccines consisting of *purified capsular polysaccharides* were developed.

Meningococcal vaccines had been made in the 1940s but failed to protect.[319] Modern work on humoral immunity to meningococcal disease got underway at the Walter Reed Hospital in 1966, ultimately resulting in *capsular polysaccharide vaccines* against meningococcal serogroups A and C by Malcolm Artenstein,[320] Emil Gotschlich,[321] and associates.[320–324]

By the early 1970s, serogroup C meningococcal polysaccharide vaccine was routinely administered to U.S. Army recruits and had virtually eliminated the disease within the military.[318] However, the vaccine did not provide immunity to children younger than 2 years, and the duration of immunity was uncertain. Additional boosters resulted in *reduced* immune responses.[325,326]

Several serogroup A and/or serogroup C meningococcal *conjugates* were developed, conjugated to diphtheria or tetanus toxoid; they provide longer immunity than the polysaccharide vaccine and immunity to children younger than 2 years.[325–330] The serogroup C conjugate was licensed in the United Kingdom in 1999 and placed in the universal immunization schedule in November of that year.[327] After extensive postlicensure studies in the United Kingdom,[326,328–330] a quadrivalent conjugate vaccine against serogroups A, C, Y, and W135 was licensed in the United States in 2005 and a second one in 2010.[331–334]

It took less than 20 years from the time the **pneumococcus** was first isolated (in 1880, by Pasteur and George Sternberg simultaneously)[335,336] to discover the multiplicity of pneumococcal serotypes and appreciate the complexity of developing a vaccine against it. A killed, whole-cell pneumococcal vaccine was made by Almroth Wright in 1911 and tested in South African goldmine workers, but eventually it was abandoned.[337,338] By the late 1940s, extensive work on capsular polysaccharides, notably by Colin MacLeod, resulted in a multivalent capsular polysaccharide pneumococcal vaccine (four-valent and later six-valent).[339–341] However, antibiotics were so successful that the vaccine fell by the wayside.

The current **pneumococcal polysaccharide** vaccine was developed by Robert Austrian and associates.[342] Austrian and Jerome Gold pointed out that pneumococcal disease continued to be a severe problem despite the use of antibiotics.[343] A modern capsular polysaccharide pneumococcal vaccine was subsequently developed for adults by Austrian, initially with 14 antigens (1977) and later increased to 23 antigens (1983).[336,342]

Although the pneumococcal polysaccharide vaccine was effective in adults, it did not protect children younger than 2 years, among whom more than 80% of invasive pneumococcal disease occurs; protein conjugation technology was applied to develop a vaccine that would protect this important group.[344] A *heptavalent pneumococcal conjugate vaccine* (conjugated to a nontoxic mutant of diphtheria) was produced and found to be safe, efficacious, and immunogenic in children younger than 2 years, who are the children most at risk.[345,346] The vaccine was licensed in the United States in February 2000.[347,348] A 1-year postlicensure follow-up study by Steven Black and associates demonstrated a dramatic reduction in age-specific invasive pneumococcal disease incidence.[349] In addition, the use of the vaccine in children has exerted a

marked reduction in adult disease as the result of herd immunity. In February 2010, a 13-valent pneumococcal conjugate vaccine was licensed. PCV13 is recommended for prevention of pneumococcal disease in infants, elderly adults (>50 years of age), and immunocompromised individuals.[350,351]

The first-generation ***H. influenzae* type b** vaccine was developed by Porter Anderson, David Smith,[352] Rachel Schneerson,[353] and their associates. Richard Pfeiffer isolated this bacterium in 1892, but mistakenly thought he had found the causative agent of influenza. For years, it was known as Pfeiffer's bacillus before receiving its current name, *Haemophilus influenzae*.[117] In the 1920s and 1930s, Margaret Pittman had determined that, of the six different polysaccharides of *H. influenzae*, organisms encapsulated with type b caused the largest proportion of serious disease in children. She identified the composition of the capsule as a polymer of ribosylribitol phosphate, now called polyribosylribitol phosphate (PRP).[354] In the 1970s, several teams began research and efficacy studies on an *H. influenzae* type b vaccine, primarily in Finland and North Carolina.[352,353,355–357] This work ultimately culminated in the 1985 licensure of the PRP vaccine.[358] However, the vaccine was not effective for children younger than 18 months, who are most at risk for bacterial meningitis, and it had limited efficacy in older children. Vaccines against *H. influenzae* type b bacteria advanced rapidly to second and third generations.

After it had been shown that the immunogenicity of a capsular polysaccharide could be increased by binding it to a carrier protein,[359,360] Schneerson and John Robbins linked *H. influenzae* PRP to diphtheria toxoid and developed the **first *conjugate* polysaccharide vaccine,** which was licensed in 1987.[361] This vaccine had improved immunogenicity and efficacy and was licensed in 1987 for children older than 15 months. Younger children still remained at risk, but three more immunogenic conjugates soon followed, using nontoxic diphtheria toxoid (HbOC [haemophilus B oligosaccharide-CRM197]) derived from a mutant strain, an outer membrane protein of *Neisseria meningitidis* (PRP-OMP [polyribosylribitol phosphate–outer membrane protein]), or tetanus toxoid.[358,361]

RECOMBINANT PROTEIN VACCINES

The discovery that the particles of **hepatitis B** surface antigen (HBsAg) found in infected people are immunogenic and protective but noninfectious[362–365] provided the basis for efforts to purify these particles from the blood of chronic carriers. Hilleman and colleagues succeeded in licensing a plasma-derived vaccine in the United States in 1981.[365] Although the vaccine was safe and effective,[366] the AIDS epidemic arrived at about the same time as the vaccine licensure; products derived from human blood were considered potentially dangerous. Despite rigorous safety testing and many inactivation processes to kill any foreign agent in the vaccine, the manufacturer could not overcome the reluctance of the public and physicians to use a product that had even a remote risk of containing the AIDS virus. Also, because the vaccine depended on human serum, sources of antigen were limited.

These obstacles prompted the formulation of the **first recombinant vaccine,** HBsAg recombinant, which was licensed in 1986. This was accomplished by Valenzuela, Medina, Rutter, and others at the University of California, San Francisco, and the University of Washington in Seattle, by cloning the gene for HBsAg in yeast (*Saccharomyces cerevisiae*) and in mammalian cells. HBsAg was produced by the cells and then made into vaccine through adsorption on an alum adjuvant.[367–370] In yeast, the surface antigen aggregated into particles similar to the extensively purified surface region antigen from the plasma-derived vaccine.[371] Initial trials and subsequent studies showed the recombinant vaccine to be as effective as the plasma-derived vaccine.[369,372,373] In addition, because it is derived from a gene, it does not bear the stigma of possible contamination with undetected foreign agents.

Lyme disease was first recognized in the United States in 1975, and, within a quarter century, it became the most frequently diagnosed vector-borne disease in the country.[374–377] Named after the town of Lyme, Connecticut, where it was first recognized, it was referred to as "Lyme arthritis" until 1982, when a constellation of associated illnesses was recognized and the name was changed to Lyme disease.[378] Willy Burgdorfer identified the spirochete causing the disease in the United States in 1982,[379] and, subsequently, it was named *Borrelia burgdorferi*.

Personal protection, spraying, and antibiotics[380–383] did not stem the rising tide of Lyme infection. Two vaccine candidates were put into extensive clinical trials.[384–388] Each was based on a recombinant *Escherichia coli* strain containing the gene for outer surface protein A (OspA) of the American Lyme strain.[375] An OspA vaccine was approved by the FDA in 1999 and recommended for use in persons 15 to 70 years old who lived or worked in endemic areas of infection.[377]

Despite the extensive clinical trials and postlicensure surveillance, the vaccine was not well accepted because of a tepid recommendation from the CDC, the alternative of antibiotic treatment after infection, and the need for booster doses. Equally important, a series of class action and individual lawsuits was brought against the manufacturer claiming that the vaccine caused chronic arthritis and other autoimmune problems, although the evidence for vaccine-induced arthritis is absent.[376] In April 2002, the vaccine was withdrawn from the market owing to lack of demand.[389]

A **recombinant, quadrivalent human papillomavirus** (HPV) vaccine was licensed in the United States in 2006, a landmark event because it represents the second vaccine against a human cancer (hepatitis B vaccine being the first) because it is 95% effective in preventing liver cancer caused by hepatitis B infection. The research to develop the HPV vaccine spanned at least 15 years, two continents, several laboratories, and competing claims of priority. The subunit vaccine is based on the capability of self-assembly of virus-like particles (VLPs) in the L1 (major) capsid protein, which is synthesized in yeast.

Using HPV-16, an Australian group under Ian Frazer published data in 1991 showing the synthesis of L1 and L2 genes and the production of VLPs, but only when both L1 and L2 were synthesized together.[390] They recognized that these VLPs could be a potential source material for a vaccine. One year later, John Schiller, Douglas Lowy, and others, at the National Cancer Institute, using a bovine model, established that the L1 capsid protein gene could self-assemble without the need for L2 and produced very high neutralizing antibodies.[391] In effect, the L1 protein made itself into a structure that mimicked the HPV virion.

In 1993, Schiller's group showed that HPV L1 produced in baculovirus was more efficient at self-assembly than the HPV L1 mutant that had been used by Frazer.[392] Laboratories from Georgetown University and Rochester University also made significant contributions to the science underlying this vaccine.[393,394] A concise summary of these various threads has been published by the National Cancer Institute.[395] One HPV vaccine contains VLPs of HPV types 6, 11, 16, and 18. The type 16 and 18 serotypes are responsible for 70% of cervical cancers in the United States. A second, bivalent HPV vaccine was licensed in the United States in 2009. The quadrivalent vaccine is also effective against genital warts, whereas the bivalent targets only cervical cancer.

When first approved in 2006, the quadrivalent vaccine was for use in females only; by 2009 it became clear that boys also

developed cancers induced by HPV and needed to be vaccinated as well, and approval was extended. A nine-valent recombinant HPV vaccine was approved in 2014 to be administered to females ages 9 to 26 years and males ages 9 to 15 years.

The use of proteins, polysaccharides, and recombinant technology broadened the possibilities for vaccine development immensely but challenges remained. Some diseases, such as ***N. meningitidis serogroup B*** had eluded vaccine development via traditional methods, though not for lack of trying. Meningococcus serogroup B did not have readily obvious protective proteins, polysaccharides or antigens. Its capsular polysaccharide was only weakly immunogenic and was thought to cause an autoimmune response.

In 1995 the first complete **genome sequence** was published (*H. influenzae*) and it immediately changed the game in vaccine research.[396] In the decade that followed, more than 100 other pathogen sequences were completed and hundreds more undertaken. **DNA microarray** of these genomes allows for vastly more rapid identification of proteins that could be possible vaccine candidates.[397]

Meningococcus serogroup B is the first disease where genome sequencing has been successful in identifying such vaccine candidates. This was accomplished with **reverse vaccinology**, a technology first pioneered by Rino Rappuoli and colleagues in Siena.[398,399] As the MC58 strain of meningococcus serogroup B was being sequenced, a computer program was used to predict 600 specific antigens as possible vaccine candidates. Of the 600 antigens, 350 (58%) proved workable and 28 (5%) ultimately induced bactericidal antibodies. The technique identified more viable candidates for a meningococcal serogroup B vaccine than had been considered or tested in the preceding 40 years.[397,400]

DNA array analysis and reverse vaccinology have been further aided by the introduction of **proteomics** which allows the identification of subsets of all proteins that are present at any particular point in the life of the bacteria, thus further shortening the time to finding viable vaccine candidates.[397]

The first meningococcal serogroup B vaccine was licensed in the United States in October 2014; it was based on a previously well-known virulence factor, factor H binding protein, two variants of which are contained in the vaccine.[401] The second meningococcal serogroup B vaccine, containing four proteins selected from the 28 that induce bactericidal antibody, was licensed in January 2015, and is the **first vaccine produced by genomics** (also called **reverse vaccinology**.)[402] Both meningococcal serogroup B vaccines are the first licensed vaccines to be approved under the FDA's FAST TRACK program, which was implemented in 2012.

The majority of vaccines now being developed use new technologies that seem to offer greater safety and more possibilities. The focus on subunit (purified protein or polysaccharide), genetically engineered, or vectored antigens has been greatly enlarged by the addition of powerful new techniques such as DNA array analysis, reverse genetics and proteomics. However, older, classic methods such as attenuation and inactivation of whole virus continue to yield new vaccines, as the zoster vaccine demonstrates.

As we continue in the second decade of the 21st century, the prospect for vaccines is stellar. Since 2000, 13 important vaccines have been licensed in the United States and Europe: a heptavalent pneumococcal conjugate vaccine (2000); a cold-adapted influenza vaccine (2003); a meningococcal quadrivalent conjugated polysaccharide vaccine (2005); a high-potency zoster vaccine (2006); the quadrivalent HPV VLP vaccine (2006); a pentavalent rotavirus reassortant vaccine (2006); an attenuated human strain rotavirus vaccine (2008), a second-generation, Vero-cell–produced, inactivated JE vaccine (2009); a bivalent HPV vaccine (2009); a 13-valent pneumococcal conjugate vaccine (2010); a nine-valent HPV vaccine (2014); and, at long last, two meningococcal serogroup B vaccines (2014, 2015). Three cholera vaccines, two killed and one live, have also been licensed in other parts of the world.

This chapter has chronicled the remarkable impact of vaccination on the health of the world's population. In the 21st century, that impact will continue to increase, as a new generation of scientists create, innovate, and plumb the depths of genetic engineering, genomics and the vast array of technologies they have spawned. Despite the vicissitudes of the natural and the political world, the new technologies augur well that the platinum age of vaccinology is indeed upon us.

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2 Vaccine Immunology

Claire-Anne Siegrist

To generate vaccine-mediated protection is a complex challenge. Currently available vaccines have largely been developed empirically, with little or no understanding of how they activate the immune system. Their early protective efficacy is primarily conferred by the induction of antigen-specific antibodies (Box 2.1). However, there is more to antibody-mediated protection than the peak of vaccine-induced antibody titers. The quality of such antibodies (e.g., their avidity, specificity, or neutralizing capacity) has been identified as a determining factor in efficacy. Long-term protection requires the persistence of vaccine antibodies above protective thresholds and/or the maintenance of immune memory cells capable of rapid and effective reactivation with subsequent microbial exposure. The determinants of immune memory induction, as well as the relative contribution of persisting antibodies and of immune memory to protection against specific diseases, are essential parameters of long-term vaccine efficacy.

The predominant role of B cells in the efficacy of current vaccines should not overshadow the importance of T-cell responses: T cells are essential to the induction of high-affinity antibodies and immune memory, directly contribute to the protection conferred by current vaccines such as bacille Calmette-Guérin (BCG), may play a more critical role than previously anticipated for specific diseases like pertussis, and will be the prime effectors against novel vaccine targets with predominant intracellular localization such as tuberculosis.

New methods have emerged allowing the assessment of a growing number of vaccine-associated immune parameters, including in humans. This development raises new questions about the optimal markers to assess and their correlation with vaccine-induced protection. The identification of mechanistic immune correlates—or at least surrogates—of vaccine efficacy is a major asset for the development of new vaccines or the optimization of immunization strategies using available vaccines. Thus, their determination generates a considerable amount of interest. During the last decade, the increased awareness of the complexity of the immune system and its determinants, including at the host genetic level, indicated that using system biology approaches to assess how various processes and networks interact in response to immunization could prove more illustrative than trying to isolate and characterize a few components of vaccine responses.[1] Delineating the specific molecular signatures of vaccine immunogenicity is beginning to highlight novel correlates of protective immunity and better explain the heterogeneity of vaccine responses in a population. The tailoring of vaccine strategies for specific vulnerable populations, including very young, elderly, and immunosuppressed populations, also largely relies on a better understanding of what supports or limits vaccine efficacy under special circumstances—at the population and individual levels. Lastly, the exponential development of new vaccines raises many questions that are not limited to the targeted diseases and the potential impacts of their prevention, but that address the specific and nonspecific impacts of such vaccines on the immune system and, thus, on health in general. These immune-related concerns have largely spread into the population, and questions related to the immunological safety of vaccines—that is, their capacity for triggering non–antigen-specific responses possibly leading to allergy, autoimmunity, or even premature death—are being raised. Certain "off-targets effects" of vaccines have also been recognized and call for studies to quantify their impact and identify the mechanisms at play. The objective of this chapter is to extract from the complex and rapidly evolving field of immunology the main concepts that are useful to better address these important questions.

HOW DO VACCINES MEDIATE PROTECTION?

Vaccines protect by inducing effector mechanisms (cells or molecules) capable of rapidly controlling replicating pathogens or inactivating their toxic components. Vaccine-induced immune effectors (Table 2.1) are essentially antibodies—produced by B lymphocytes—capable of binding specifically to a toxin or a pathogen.[2] Other potential effectors are cytotoxic $CD8^+$ T lymphocytes that may limit the spread of infectious agents by recognizing and killing infected cells or secreting specific antiviral cytokines and $CD4^+$ T-helper (Th) lymphocytes. These Th cells may contribute to protection through cytokine production and provide support to the generation and maintenance of B and $CD8^+$ T-cell responses. Effector $CD4^+$ Th cells were initially subdivided into T-helper 1 (Th1) or T-helper 2 (Th2) subsets depending on their main cytokine production (interferon-γ or interleukin [IL]-4), respectively. This dichotomy became outdated as Th cells were increasingly shown to include a large number of subsets with distinct cytokine-producing and homing capacities (see Table 2.1).[3] A recently identified critical subset of vaccine-induced $CD4^+$ Th cells are follicular T-helper (Tfh) cells: they are specially equipped and positioned in the lymph nodes to support potent B-cell activation and differentiation into antibody-secreting-cells[4] and were identified as directly controlling antibody responses and mediating adjuvanticity.[5–7] Another important subset are T-helper 17 (Th17) cells which essentially defend against extracellular bacteria that colonize the skin and mucosa, recruiting neutrophils and promoting local inflammation.[8,9] These effectors are controlled by regulatory T cells (Tregs) involved in maintaining immune tolerance.[10] Most antigens and vaccines trigger B- and T-cell responses, such that there is no rationale in opposing vaccines favoring antibody production ("humoral immunity") and T-cell responses ("cellular immunity"). In addition, $CD4^+$ T cells are required for most antibody responses, whereas antibodies exert significant influences on T-cell responses to intracellular pathogens.[11]

What Are the Main Effectors of Vaccine Responses?

The nature of the vaccine exerts a direct influence on the type of immune effectors that are elicited and that mediate protective efficacy (Table 2.2).

Capsular polysaccharides (PSs) elicit B-cell responses in what is classically reported as a T-independent manner.[12] The conjugation of bacterial PS to a protein carrier (e.g., glycoconjugate vaccines) provides foreign peptide antigens that are presented to the immune system and, thus, recruit

BOX 2.1 Main Immunological Definitions

ADJUVANT

Agents that increase the stimulation of the immune system by enhancing antigen presentation (depot formulation, delivery systems) and/or by providing costimulation signals (immunomodulators). Aluminum salts are most often used in today's vaccines.

AFFINITY, AVIDITY

Antibody affinity refers to the tendency of an antibody to bind to a specific epitope at the surface of an antigen; that is, to the strength of the interaction. Avidity is the sum of the epitope-specific affinities for a given antigen. It directly relates to its function.

AFFINITY MATURATION

Processes through which antigen-specific B cells undergo somatic hypermutation and affinity-based selection, resulting in B cells that produce antibodies with increased affinity over germline antibodies.

ANTIBODIES

Proteins of the immunoglobulin family, present on the surface of B lymphocytes, secreted in response to stimulation, that neutralize antigens by binding specifically to their surface.

ANTIGEN-PRESENTING CELLS

Cells that capture antigens by endocytosis or phagocytosis, process them into small peptides, display them at their surface through major histocompatibility complex (MHC) molecules, and provide costimulation signals that act synergistically to activate antigen-specific T cells. Antigen-presenting cells include B cells, macrophages, and dendritic cells, although only dendritic cells are capable of activating naïve T cells.

B LYMPHOCYTES

Cells that originate in the bone marrow, mature in secondary lymphoid tissues, become activated in the spleen/nodes when their surface immunoglobulins bind to an antigen, and differentiate into antibody secreting cells (plasma cells) or memory B cells.

CARRIER PROTEIN

A protein that is used as a template to which polysaccharide moieties are chemically conjugated to generate glycoconjugate vaccines. It is believed that carrier proteins provide antigenic epitopes for recognition by $CD4^+$ T-helper cells, in particular follicular T-helper cells.

$CD4^+$ T-HELPER 1 LYMPHOCYTES

$CD4^+$ T cells that on activation differentiate into cells that mainly secrete interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-β, exerting direct antimicrobial functions (viruses), and essentially providing support to cytotoxic T cells and macrophages.

$CD4^+$ T-HELPER 2 LYMPHOCYTES

$CD4^+$ T cells that on activation differentiate into cells that mainly secrete IL-4, IL-5, IL-6, IL-10, and IL-13, exerting direct antimicrobial functions (parasites) and essentially providing support to B lymphocytes.

$CD4^+$ T-HELPER 17 LYMPHOCYTES

$CD4^+$ T cells that mainly secrete IL-17, IL-21, and IL-22 are implicated in host defense against extracellular bacteria colonizing exposed surfaces (airways, skin, gut).

$CD8^+$ T CELLS

Lymphocytes that specialize in the killing of infected cells, through direct contact or cytokine (IFN-γ, TNF-α) production.

CENTRAL MEMORY T CELLS

Memory T cells traffick through the lymph nodes, ready to proliferate and generate a high number of effector cells in response to specific microbial peptides.

CHEMOKINES

Small secreted proteins that function as chemoattractants, recruiting cells that express the corresponding chemokine receptors at their surface and thus migrating toward higher concentrations of chemokines.

COSTIMULATORY MOLECULES

Molecules that become expressed at the surface antigen-presenting cells on activation and deliver stimulatory signals to other cells, namely T and B cells.

DENDRITIC CELLS

Cells that constantly sample their surroundings for pathogens such as viruses and bacteria, detect dangers, and initiate immune responses. Immature patrolling dendritic cells (DCs) have high endocytic activity and a low T-cell activation potential. Contact with a pathogen induces maturation and the expression of certain cell-surface molecules, greatly enhancing their ability to activate T cells.

EFFECTOR MEMORY T CELLS

Memory T cells patrol through the body to detect specific microbial peptides and are capable of an immediate cytotoxic function in case of recognition.

EXTRAFOLLICULAR REACTION

B-cell differentiation pathways that occur outside of germinal centers in response to protein or polysaccharide antigens. Extrafollicular reaction is rapid, generates B cells that are short-lived (days), and produces low-affinity antibodies without inducing immune memory.

FOLLICULAR DENDRITIC CELLS

Stromal cells in the spleen and nodes that on activation express chemokines (notably CXCL13) to attract activated antigen-specific B and T cells and thus nucleate the germinal center reaction. Follicular DCs provide antiapoptotic signals to germinal center (GC) B cells and support their differentiation into plasma cells or memory B cells.

FOLLICULAR T-HELPER LYMPHOCYTES

$CD4^+$ T cells that on activation migrate toward follicular DCs and provide critical help to germinal center B cells, influencing isotype switching, affinity maturation, and differentiation.

GERMINAL CENTERS

Dynamic structures that develop in the spleen/nodes in response to an antigenic stimulation and dissolve after a few weeks. GCs contain a monoclonal population of antigen-specific B cells that proliferate and differentiate through the support provided by follicular DCs and T-helper cells. Immunoglobulin class-switch recombination, affinity maturation, B-cell selection, and differentiation into plasma cells or memory B cells essentially occur in GCs.

Continued on following page

BOX 2.1 Main Immunological Definitions (Continued)

ISOTYPE SWITCHING

Switch of immunoglobulin (Ig) expression and production from IgM to IgG, IgA, or IgE that occurs during B-cell differentiation through DNA recombination.

MARGINAL ZONE

The area between the red pulp and the white pulp of the spleen. Its major role is to trap particulate antigens from the circulation and present them to lymphocytes.

PATTERN RECOGNITION RECEPTORS

Germline-encoded receptors sensing the presence of infection via the recognition of conserved microbial pathogen-associated molecular patterns and triggering innate immune responses.

REGULATORY T CELLS

T cells that on activation differentiate into cells that express specific cytokines (IL-10, transforming growth factor [TGF]-β/ surface markers) and act to suppress activation of the immune system through various mechanisms, maintaining immune homeostasis and tolerance to self-antigens.

RESIDENT MEMORY T CELLS

Effector memory T cells residing in specific tissues (lungs, gut, skin) and conferring an immediate-early line of defense against viral and bacterial pathogens.

SOMATIC HYPERMUTATION

A process that introduces random mutation in the variable region of the B-cell receptor (i.e., immunoglobulin) locus at an extremely high rate during B-cell proliferation. This mechanism occurs through the influence of the activation-induced cytidine deaminase enzyme and generates antibody diversification.

T LYMPHOCYTES

Cells that originate in the thymus, mature in the periphery, become activated in the spleen/nodes if their T-cell receptors bind to an antigen presented by an MHC molecule and they receive additional costimulation signals driving them to acquire killing (mainly $CD8^+$ T cells) or supporting (mainly $CD4^+$ T cells) functions.

T-INDEPENDENT B-CELL RESPONSES

Differentiation pathway of B cells, mainly elicited by polysaccharides, that takes place in the marginal zone and extrafollicular areas of the spleen/nodes. Its hallmarks are to be rapid (days), while eliciting the transient (months) production of antibodies of low affinity without inducing immune memory.

T-DEPENDENT B-CELL RESPONSES

Differentiation pathway of B cells elicited by protein antigens that recruit T and B cells into GCs of the spleen/nodes. Its hallmarks are to be slow (weeks), while eliciting long-lasting (years) production of antibodies of high affinity and immune memory.

TOLL-LIKE RECEPTORS

A family of 10 receptors (TLR1 to TLR10), present at the surface of many immune cells, that recognize pathogens through conserved microbial patterns and activate innate immunity when detecting danger.

TABLE 2.1 Effector Mechanisms Triggered by Vaccines

- Antibodies prevent or reduce infections by clearing extracellular pathogens through:
  - Binding to the enzymatic active sites of toxins or preventing their diffusion
  - Neutralizing viral replication (e.g., preventing viral binding and entry into cells)
  - Promoting opsonophagocytosis of extracellular bacteria (i.e., enhancing their clearance by macrophages and neutrophils)
  - Activating the complement cascade
- $CD8^+$ T cells do not prevent infection but reduce, control, and clear intracellular pathogens by:
  - Directly killing infected cells (release of perforin, granzyme, etc.)
  - Indirectly killing infected cells through antimicrobial cytokine release
- $CD4^+$ T cells do not prevent infection but participate in the reduction, control, and clearance of extracellular and intracellular pathogens by their homing and cytokine-production capacities. Their main subsets include:
  - Follicular T-helper (Tfh) cells producing mainly interleukin (IL)-21 and providing B-cell help
  - T-helper 1 (Th1) effector cells producing interferon (IFN)-γ, tumor necrosis factor (TNF)-α/TNF-β, IL-2, and mainly involved in protection against intracellular pathogens (viruses, *Mycobacterium tuberculosis*)
  - Th2 effector cells producing IL-4, IL-5, IL-13, and responding to extracellular pathogens (bacteria and helminths)
  - Th9 effector cells producing IL-9 and also responding to extracellular pathogens
  - Th17 effector cells producing IL-17, IL-22, and IL-26 and contributing to mucosal defense (*Streptococcus pneumoniae, Bordetella pertussis*, *Mycobacterium tuberculosis*)

antigen-specific $CD4^+$ Tfh cells in what is referred to as a T-dependent antibody response.[13,14] A hallmark of T-dependent responses, which are also elicited by toxoid, protein, inactivated, or live attenuated viral vaccines (see Table 2.2), is to induce higher-affinity antibodies and immune memory. In addition, live attenuated vaccines usually generate $CD8^+$ cytotoxic T cells. The use of live vaccines/vectors or of specific novel delivery systems seems necessary for the induction of strong $CD8^+$ T-cell responses. Most current vaccines mediate their protective efficacy through the induction of vaccine antibodies, whereas vaccine-induced $CD4^+$ T cells contribute to macrophage activation and control of *Mycobacterium tuberculosis*[15] and prevent varicella-zoster reactivation. In addition, CD8+ T cells are also elicited.[16]

The induction of antigen-specific immune effectors (and/or of immune memory cells) by an immunization process does not imply that these antibodies, cells, or cytokines represent surrogates—or even correlates—of vaccine efficacy. This requires the formal demonstration that vaccine-mediated protection is dependent—in a vaccinated person—on the presence of a given marker such as an antibody titer or a number of antigen-specific cells above a given threshold.[17,18]

TABLE 2.2 Correlates of Vaccine-Induced Immunity

Vaccines	Vaccine Type	Serum IgG	Mucosal IgG	Mucosal IgA	T Cells
Cholera	Killed	++	+		
Cholera	Live, oral	+	++		
Diphtheria toxoid	Toxoid	++	(+)		
Hepatitis A	Killed	+++			
Hepatitis B (HBsAg)	Protein	++			
Hib PS	PS	++	(+)		
Hib glycoconjugates	PS-protein	+++	++		
Influenza	Killed, subunit	++	(+)		
Influenza intranasal	Live attenuated	++	+	+	+ ($CD8^+$)
Japanese encephalitis	Killed	++			
Measles	Live attenuated	+++			+ ($CD8^+$)
Meningococcal PS	PS	++	(+)		
Meningococcal conjugates	PS-protein	+++	++		
Meningococcal group B	Proteins				
Mumps	Live attenuated	++			
Papillomavirus (human)	VLPs	+++	++		
Pertussis, whole cell	Killed	++			+? ($CD4^+$)
Pertussis, acellular	Proteins	++			+? ($CD4^+$)
Pneumococcal PS	PS	++	(+)		
Pneumococcal conjugates	PS-protein	+++	++		
Polio Sabin	Live attenuated	++	++	++	
Polio Salk	Killed	++	+		
Rabies	Killed	++			
Rotavirus	VLPs	(+)	(+)	++	
Rubella	Live attenuated	+++			
Tetanus toxoid	Toxoid	+++			
Tuberculosis (BCG)	Live mycobacteria				++ ($CD4^+$)
Typhoid PS	PS	+	(+)		
Varicella (chickenpox)	Live attenuated	++			+? ($CD4^+$)
Varicella (zoster)	Live attenuated				++ ($CD4^+$)
Yellow fever	Live attenuated	+++			

BCG, bacille Calmette-Guérin; Hib, *Haemophilus influenzae* type b; PS, polysaccharide; VLP, virus-like particle.
Note: This table may not be exhaustive and includes only currently licensed vaccines.

Antigen-specific antibodies have been formally demonstrated as conferring vaccine-induced protection against many diseases[19] (see Table 2.2). Passive protection may result from the physiological transfer of maternal antibodies (e.g., tetanus) or the passive administration of immunoglobulins or vaccine-induced hyperimmune serum (e.g., measles, hepatitis, varicella). Such antibodies may neutralize toxins in the periphery, at their site of production in an infected wound (tetanus), or in the throat (diphtheria). They may reduce binding or adhesion to susceptible cells or receptors and limit viral replication (e.g., polio) or reduce bacterial colonization (glycoconjugate vaccines against encapsulated bacteria) if present at sufficiently high titers on mucosal surfaces.[20] The neutralization of pathogens at mucosal surfaces is mainly achieved by the transudation of vaccine-induced serum immunoglobulin (Ig) G antibodies. Neutralization requires serum IgG antibody concentrations to be of sufficient affinity and abundance to result in "protective" antibody titers in saliva or mucosal secretions. As a rule, such responses are not elicited by PS bacterial vaccines but achieved by glycoconjugate vaccines, which may prevent nasopharyngeal colonization or nonbacteremic pneumonia[21] in addition to invasive diseases.

Under most circumstances, inactivated vaccines do not elicit sufficiently high and sustained antibody titers on mucosal surfaces to prevent local infection. It is only after having infected mucosal surfaces that pathogens encounter vaccine-induced IgG serum antibodies that neutralize viruses, opsonize bacteria, activate the complement cascade (see Table 2.1), and limit their multiplication and spread, preventing tissue damage and, thus, clinical disease. That vaccines fail to induce sterilizing immunity is not an obstacle to successful disease control, although it represents a significant challenge for

the development of specific vaccines against chronic viral infection.

Current vaccines mostly mediate protection through the induction of highly specific IgG serum antibodies (see Table 2.2). Live oral or nasal vaccines, such as rotavirus, oral polio, nasal influenza, or cholera vaccines, induce serum IgA and secretory IgA, which also help limit viral shedding on mucosal surfaces.

Under certain circumstances, however, passive antibody-mediated immunity is inefficient (tuberculosis). There is conclusive evidence that T cells are the main effectors of BCG, even though specific T-cell frequency and cytokine expression profiles do not correlate with protection in BCG-immunized infants,[15,22] or in zoster immunized adults.[23,24] However, there is indirect evidence that vaccine-induced T cells contribute to the protection conferred by other vaccines. $CD4^+$ T cells seem to support the persistence of protection against clinical pertussis in children primed in infancy, after vaccine-induced antibodies have waned,[25–28] and may contribute to the longer vaccine efficacy of whole-cell pertussis vaccines.[29–31] Another example is that of measles immunization in 6-month-old infants in whom antibody responses largely are not initiated because of immune immaturity and/or the residual presence of inhibitory maternal antibodies, but significant interferon (IFN)-γ–producing $CD4^+$ T cells are generated.[32,33] The infants remain susceptible to measles infection but are protected against severe disease and death, presumably because of the viral clearance capacity of their vaccine-induced T-cell effectors. Thus, prevention of infection may be achieved only by vaccine-induced antibodies, whereas disease attenuation and protection against complications may be supported by T cells, even in the absence of specific antibodies. The understanding of vaccine immunology requires appraising how B- and T-cell responses are elicited, supported, maintained, and/or reactivated by vaccine antigens.

FROM INNATE TO ADAPTIVE IMMUNITY ACTIVATION: THE FIRST STEPS AFTER IMMUNIZATION

Novel adjuvants essentially enhance vaccine responses by modulating innate immunity, which shapes adaptive responses.[34–38] Indeed, the induction of antigen-specific B- and T-cell responses requires their activation in the draining lymph nodes by specific antigen-presenting cells (APCs), essentially dendritic cells (DCs) that must be recruited into the reaction. Immature DCs patrol throughout the body. When exposed to pathogens in the tissues or at the site of injection, they undergo brisk maturation, modulate specific surface receptors, and migrate toward secondary lymph nodes, where the induction of T- and B-cell responses occurs. The central role for mature DCs in the induction of vaccine responses reflects their unique capacity to provide antigen-specific, costimulation signals to T cells; these "danger signals" are required to activate naïve T cells.[39] The very first requirement to elicit vaccine responses is to provide sufficient "danger signals" through vaccine antigens and/or adjuvants (Fig. 2.1) to trigger an inflammatory reaction that is mediated by cells of the innate immune system.[34–37]

DCs, monocytes, and neutrophils express sets of receptors directed against evolutionarily conserved pathogen patterns that are not contained in self-antigens and are readily identified as "danger."[40] Through these pattern-recognition receptors, among which Toll-like receptors fulfill an essential role (Table 2.3),[40] these host cells sense the potential danger when they encounter a pathogen and become activated (Fig. 2.2). They modulate the expression of their surface molecules and produce proinflammatory cytokines and chemokines,[34–37] which result in the extravasation and attraction of monocytes, granulocytes, and natural killer cells and the generation of an inflammatory microenvironment (see Fig. 2.1) in which monocytes differentiate into macrophages and immature DCs become activated.[38] This activation modifies the expression of homing receptors at their surface and triggers DC migration toward the draining lymph nodes (see Fig. 2.2). In the absence of danger signals, DCs remain immature: On contact with naïve T cells, T cells do not differentiate into immune effectors but into regulatory $CD4^+$ T cells that maintain immune tolerance.[10]

Live viral vaccines most efficiently trigger the activation of the innate immune system through multiple pathogen-associated signals (such as viral RNA), allowing their recognition by pattern-recognition receptors (see Table 2.3).[41] Following injection, viral particles rapidly disseminate throughout the vascular network and reach their target tissues. This pattern is very similar to that occurring after a natural infection, including the initial mucosal replication stage for vaccines administered through the nasal and oral routes. DCs are activated at multiple sites, migrate toward the corresponding draining lymph nodes, and launch multiple foci of T- and B-cell activation. This sequence provides a second explanation of the generally higher immunogenicity of live versus "nonlive" vaccines (Table 2.4).[42] Another consequence of this early diffusion pattern is that the site and route of injection of live viral vaccines are of minor importance; for example, the immunogenicity and reactogenicity of measles vaccine is similar following intramuscular or subcutaneous injection,[43] and measles vaccine may be administered by aerosol. Live bacterial vaccines, such as BCG, multiply at the site of injection, where they generate a prolonged inflammatory reaction, but also at a distance, with the preponderance for local draining lymph nodes.

Nonlive vaccines, whether containing only proteins, PS, glycoconjugates, or inactivated microorganisms (see Table 2.2), may still contain pathogen-recognition patterns. In the absence of microbial replication, however, vaccine-induced activation remains more limited, in both time and space. Nonlive vaccines essentially activate innate responses at their site of injection (see Fig. 2.1). Their site and route of administration are, thus, more important. The high number of DCs in the dermis allows a marked reduction (e.g., 10-fold) of the antigen dose with intradermal immunization. This advantage of the dermal DC concentration is applied to the prevention of rabies in many countries and could prove useful against additional targets as novel microneedle and needle-free devices become available for intradermal administration.[44] Patrolling DCs are also numerous in well-vascularized muscles, which is the preferred route of injection for nonlive vaccines. They are fewer in adipose tissues, such that subcutaneous injections may be less effective than intramuscular injections under conditions of limited immunogenicity, as demonstrated for adult immunization against hepatitis B.[45] Despite many efforts, immunization through the mucosal route remains limited to a few live vaccines. The extreme difficulty in producing nonlive mucosal vaccines reflects the need to overcome a large number of physical, immunological, and chemical barriers, which requires the use of live vaccines or strong adjuvants. This fact is not trivial, as unfortunately illustrated by the association of a novel adjuvanted inactivated intranasal influenza vaccine with Bell palsy.[46]

Following their activation, DCs migrate toward the local draining lymph nodes, for example, the axillary and inguinal area following deltoid and quadriceps injection, respectively. That primary immune responses to nonlive vaccines are essentially focal and likely contribute to the fact that the

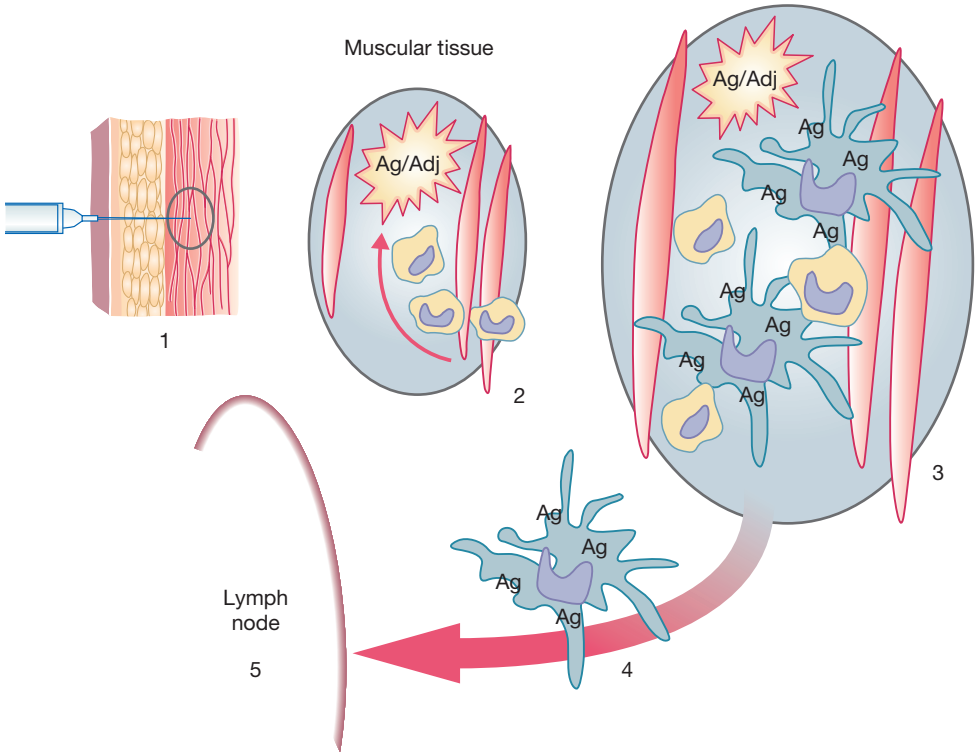


Figure 2.1. Initiation of a vaccine response. Following injection *(1)*, the pathogen-associated patterns contained in vaccine antigens attract dendritic cells, monocytes, and neutrophils that patrol throughout the body *(2)*. Elicitation of sufficient "danger signals" by the vaccine antigens (Ag)/adjuvants (Adj) activates monocytes and dendritic cells *(3)*; the activation changes their surface receptors and induces their migration along lymphatic vessels *(4)*, to the draining lymph nodes *(5)* where the activation of T and B lymphocytes will take place.

TABLE 2.3 Recognition of Vaccine Determinants by Human Pattern-Recognition Receptors

Receptors	Ligands	Demonstrated Ligands in Vaccines
TLR1	Certain bacterial lipoproteins	
TLR2	Peptidoglycan, lipoproteins, glycolipids, lipopolysaccharides	BCG, Hib-OMP, pneumococcal PS
TLR3	Viral double-stranded RNA	Poly I:C (in clinical trial as adjuvant)
TLR4	Bacterial lipopolysaccharides	BCG, pneumococcal PS, HPV-VLPs, AS02, and AS04 adjuvants
TLR5	Bacterial flagellins	Flagellin (in clinical trial as adjuvant)
TLR6	Lipoteichoic acid, lipopeptides	
TLR7	Single-stranded RNA	Yellow fever, live attenuated influenza, whole-cell influenza, TLR7 agonists (in clinical trial as adjuvants)
TLR8	Single-stranded RNA	Yellow fever
TLR9	Unmethylated CpG oligonucleotides	Yellow fever, TLR9 agonists (in clinical trial as adjuvants)
TLR10	Unknown	
NALP3	Multiple	Alum
NOD1, NOD2	Peptidoglycans	Pneumococcal PS

BCG, bacille Calmette-Guérin; CpG, cytosine phosphate guanine; Hib, *Haemophilus influenzae* type b; HPV, human papillomavirus; NALP, Natch domain, Leucine-rich repeat, and PYD-containing protein; NOD, nonobese diabetic; OMP, outer membrane protein; PS, polysaccharide; TLR, Toll-like receptor; VLP, virus-like particle.

simultaneous administration of several distinct vaccines may take place without immune interference if vaccines are administered at distant sites in different limbs draining into distinct lymph node areas. Most nonlive vaccines require their formulation with specific adjuvants to induce danger signals and trigger a sufficient activation of the innate system. The understanding of the mode of action of current and novel adjuvants markedly increased during the last few years, with the long-used aluminum salts revealing some of their secrets.[47] Although the adjuvants currently in use do not trigger the degree of

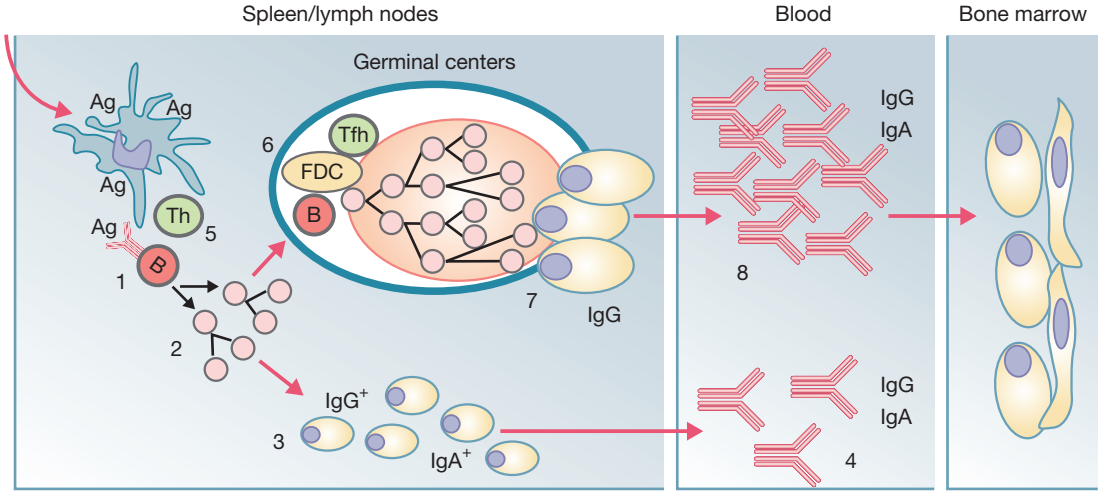


Figure 2.2. Extrafollicular and germinal center responses to protein antigens. In response to a protein antigen reaching lymph nodes or spleen, B cells capable of binding to this antigen with their surface immunoglobulins *(1)* undergo brisk activation. In an extrafollicular reaction *(2)*, B cells rapidly differentiate in plasma cells *(3)* that produce low-affinity antibodies (of the immunoglobulin [Ig] M ± IgG/IgA isotypes) that appear at low levels in the serum within a few days after immunization *(4)*. Antigen-specific T-helper (Th) cells *(5)* that have been activated by antigen-bearing dendritic cells (DCs) trigger some antigen-specific B cells to migrate toward follicular dendritic cells (FDCs) *(6)*, initiating the germinal center (GC) reaction. In GCs, B cells receive additional signals from follicular T cells (Tfh) and undergo massive clonal proliferation; switch from IgM toward IgG, IgA, or IgE; undergo affinity maturation *(7)*; and differentiate into plasma cells secreting large amounts of antigen-specific antibodies *(8)*. At the end of the GC reaction, a few plasma cells exit nodes/spleen and migrate to survival niches mostly located in the bone marrow, where they survive through signals provided by supporting stromal cells.

TABLE 2.4 Determinants of Primary Vaccine Antibody Responses in Healthy People

Determinants	Mechanisms (Presumed)
VACCINE TYPE	
Live vs inactivated	Higher intensity of innate responses through the synergistic activation of several PRRs, higher antigen content following replication, and more prolonged antigen persistence generally result in higher Ab responses to live than to inactivated vaccines.
Protein vs polysaccharide	Recruitment of T-cell help and induction of GCs (i.e., memory induction) results in higher and more prolonged Ab responses to protein or glycoconjugate than to PS vaccines.
Adjuvants	Modulation of antigen delivery and persistence (depot or slow-release formulations) and/or enhancement of Tfh responses (immunomodulator) may support or limit Ab responses.
ANTIGEN NATURE	
Polysaccharide antigens	Failure to induce GCs limits immunogenicity.
Protein antigens	Inclusion of epitopes readily recognized by B cells (B-cell repertoire), inclusion of epitopes readily recognized by Tfh, elicitation of efficient follicular T-cell help, and the capacity of antigen to associate/persist in association with FDCs result in higher Ab responses.
Antigen dose	As a rule, higher Ag doses increase the availability of Ag for B-/T-cell binding and activation and for association with FDCs.
VACCINE SCHEDULE	
Interval between doses	A 3-week minimal interval between primary doses avoids competition between successive waves of primary responses.
Genetic determinants	The capacity of Ag epitopes to associate with a large panel of MHC molecules increases the likelihood of responses in the population. MHC restriction may limit T-cell responses. Gene polymorphisms in molecules critical for B- and T-cell activation/differentiation are likely to affect Ab responses.
Environmental factors	Mostly unidentified
Age at immunization	Early life immune immaturity or age-associated immune senescence

Ab, antibody; Ag, antigen; FDC, follicular dendritic cell; GC, germinal center; MHC, major histocompatibility complex; PRR, pattern-recognition response; PS, polysaccharide; Tfh, follicular T-helper cells.

innate immune activation that is elicited by live vaccines, progress is being made: a single dose of the AS03-adjuvanted influenza H1N1/09 vaccine in healthy children elicited antibody responses similar to those observed in convalescent children[48] and formulating the varicella-zoster-virus IgE protein into the novel AS01b adjuvant system conferred unprecedented vaccine efficacy in the elderly.[24]

VACCINE ANTIBODY RESPONSES

How Are Primary Antibody Responses Elicited?

B cells are essentially activated in the lymph nodes draining the injection site. Vaccine antigens reaching the subcapsular sinus by free-fluid diffusion are taken up by specific subcapsular sinus macrophages and translocated into the B-cell zone. The B cells equipped with surface B-cell receptors[49] capable of binding to the vaccine antigens are activated and migrate to the interface between the B-cell (follicle) and the T-cell zones. There, B cells engage T cells and initiate their proliferation. The cumulative amount of costimulation signals received by B cells determines their fate.[50] Protein antigens (which are taken up and displayed as small peptides on the surface of APCs) activate Tfh cells. This induces a highly efficient B-cell differentiation pathway, through specific structures (germinal centers [GCs]) in which antigen-specific B cells proliferate and differentiate into antibody-secreting plasma cells or memory B cells.[51] Polysaccharide antigens that fail to recruit Tfh cells into the response do not trigger GCs, such that they elicit only short-lived plasma cells resulting in weaker and less durable antibody responses with no immune memory.

T-Dependent Responses to Protein Antigens

The Extrafollicular Reaction. Naïve B cells generated in the bone marrow (BM) reside in lymph nodes until they encounter a protein antigen to which their specific surface IgM receptor binds. Antigen binding initiates B-cell activation and triggers the upregulation of CCR7, a chemokine receptor that drives antigen-specific B cells toward the outer T-cell zone of lymph nodes.[52] At this location, vaccine antigen-specific B cells are exposed to recently (<24 hours) activated DCs and T cells that have upregulated specific surface molecules and, thus, provide B-cell activating signals. This T-cell help rapidly drives B-cell differentiation into Ig-secreting plasma cells that produce low-affinity germline antibodies, in what is called the extrafollicular reaction (see Figs. 2.2 and 2.3).[53]

Ig class-switch recombination from IgM toward IgG, IgA, or IgE occurs during this differentiation of B cells, through the upregulation of the activation-induced deaminase enzyme. Both $CD4^+$ Th1 and Th2 cells exert essential helper functions during the extrafollicular differentiation pathway, and the engagement of their CD40L molecules with CD40 on B cells may skew class-switch recombination into particular Ig classes and subclasses. In rodents, IFN-γ–producing Th1 T cells promote a switch toward IgG_{2a}, whereas Th2 cells essentially support the generation of IgG_1 and IgE (via IL-4) and IgG_{2b} and IgG_3 (via transforming growth factor [TGF]-β).[54] The situation is less clear-cut in humans, where IgG_1 antibodies frequently predominate regardless of the polarization of T-cell help. The extrafollicular reaction is rapid, and IgM and low-level IgG antibodies appear in the blood a few days after primary immunization (see Figs. 2.2 and 2.3). These antibodies are of germline affinity, as there is no hypermutation or selection process during the extrafollicular reaction. This extrafollicular reaction is short-lived, as most cells die by apoptosis within a few days. Consequently, its role in vaccine efficacy is limited to a few months.

The Germinal Center Reaction. Antigen-specific B cells that receive sufficient help from antigen-specific activated Tfh cells proliferate in specialized structures, the GCs, in which they differentiate into plasma cells or memory B cells.[50,55] The induction of GCs is initiated as a few antigen-specific activated B cells upregulate their expression of CXCR5 and migrate toward B-cell follicles, where they are attracted by CXCL13-expressing follicular DCs (FDCs). The FDCs fulfill an essential role in B-cell responses: they attract antigen-specific B and Tfh cells and capture/retain antigen for extended periods. B cells attracted by antigen-bearing FDCs become the founders of GCs (see Fig. 2.2). Receiving additional activation and survival signals from the FDCs and Tfh cells,[56,57] notably through IL-21,[58] B cells undergo massive clonal proliferation—such that each GC is constituted by the progeny of a single antigen-specific B cell. This intense proliferation is associated with two major events: Ig class-switch recombination from IgM toward IgG, IgA, or IgE, and maturation of the affinity of B cells for their specific antigen. This process results in the higher production of antibodies with a higher antigen-binding capacity (see Fig. 2.3).

The maturation of B-cell affinity results from an extensive somatic hypermutation process within the variable-region segments of Ig genes.[50] In a small minority of B cells, the introduction of mutations in their Ig genes increases the affinity of their surface IgG for antigen. This enables these B cells to efficiently compete for binding to the small amounts of vaccine antigens that are associated with the surface of FDCs (see Fig. 2.2). B cells process these vaccine antigens into small peptides that they display at their surface through major histocompatibility complex (MHC) class II molecules. MHC-peptide complexes thus become available for binding by the specific subset of $CD4^+$ Tfh cells.[56,57] These Tfh cells, which express CXCR5, migrate toward CXCL13-expressing FDCs. Differing from Th1 and Th2 cells by their chemokine receptors, transcription factors, surface markers, and interleukins,[56,57] they are uniquely equipped to provide efficient B-cell help through a series of costimulation molecules, including CD40L, ICOS (inducible T-cell costimulator), the IL-10 B-cell growth factor, and IL-21.[56,57] The cellular interactions between antigen-specific GC B cells, antigen-bearing FDCs, and antigen-specific Tfh cells (see Fig. 2.2) result in the proliferation, survival, and selection of B cells that have the highest possible antigen-specific affinity. They also provide the signals required for the subsequent differentiation of GC B cells toward plasma cells secreting large amounts of specific antibodies or toward memory B cells. Tfh cells have thus been identified a major determinant of adult and early life B-cell vaccine responses.[5–7]

The development of this GC reaction requires a couple of weeks, such that hypermutated IgG antibodies to protein vaccine antigens first appear in the blood 10 to 14 days after priming (see Fig. 2.3).[59] Feedback mechanisms terminate GC reactions within 3 to 6 weeks, a period during which a large number of antigen-specific plasma cells will have been generated. It is the magnitude of GC responses, that is, the quality of DC, B-cell, Tfh-cell, and FDC interactions, which controls the intensity of B-cell differentiation into plasma cells and thus the peak of IgG vaccine antibody reached within 4 to 6 weeks after primary immunization (see Fig. 2.3).

T-Independent Responses to Polysaccharide Antigens

Bacterial (*Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Salmonella typhi*) PS antigens released from the injection site reach the marginal zone of the spleen/nodes, an area that is equipped by macrophages exhibiting a unique set of scavenger receptors through the bloodstream. There, PS

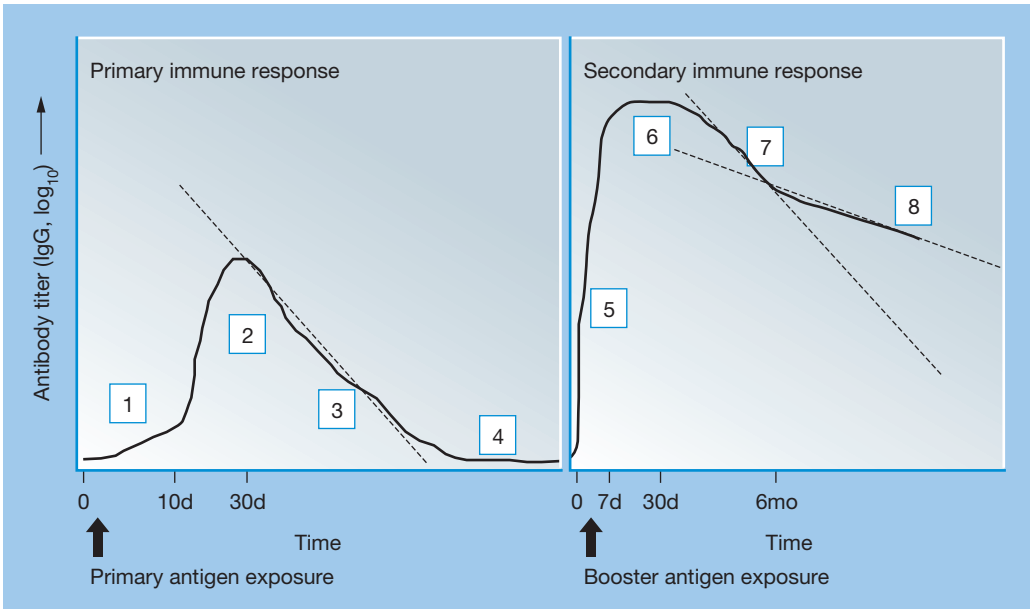


Figure 2.3. Correlation of antibody titers to the various phases of the vaccine response. The initial antigen exposure elicits an extrafollicular response *(1)* that results in the rapid appearance of low IgG antibody titers. As B cells proliferate in germinal centers and differentiate into plasma cells, IgG antibody titers increase up to a peak value *(2)*, usually reached 4 weeks after immunization. The short life span of these plasma cells results in a rapid decline of antibody titers *(3)*, which eventually return to baseline levels *(4)*. In secondary immune responses, booster exposure to antigen reactivates immune memory and results in a rapid (<7 days) increase *(5)* of IgG antibody titer. Short-lived plasma cells maintain peak antibody levels *(6)* during a few weeks—after which serum antibody titers decline initially with the same rapid kinetics as following primary immunization *(7)*. Long-lived plasma cells that have reached survival niches in the bone marrow continue to produce antigen-specific antibodies, which then decline with slower kinetics *(8)*. Note: This generic pattern may not apply to live vaccines triggering long-term IgG antibodies for extended periods.

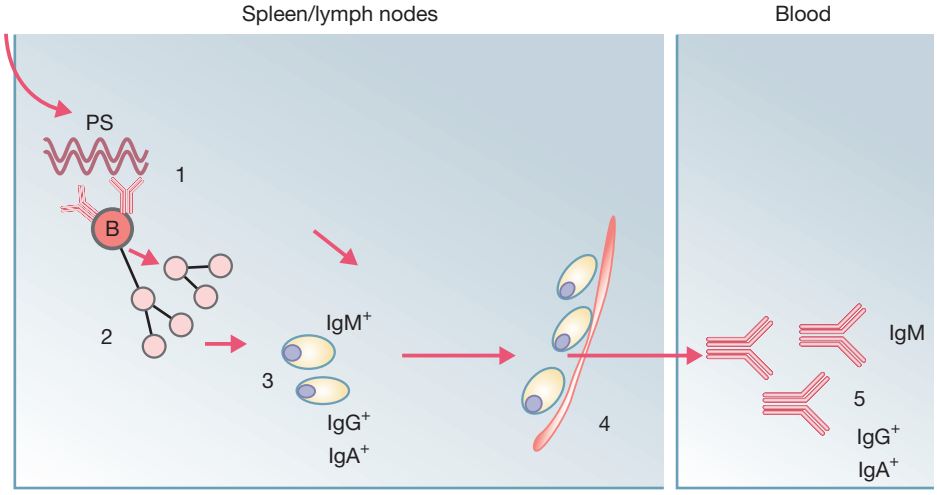


Figure 2.4. Extrafollicular B-cell responses to polysaccharide antigens. B cells use their specific immunoglobulin surface receptors *(1)* to bind to the repetitive structures of polysaccharides reaching the marginal zone of spleen/nodes. In the absence of antigen-specific T-cell help, B cells are activated, proliferate *(2)*, and differentiate in plasma cells *(3)* without undergoing affinity maturation in germinal centers. These plasma cells migrate toward the red pulp of the spleen *(4)*, where they survive for a few weeks/months, secreting low levels of low-affinity immunoglobulin (Ig) M, IgG, or IgA antibodies *(5)*.

bind to marginal zone B cells, and their repetitive structure crosslinks the Ig receptors on the B-cell surface.[53] This activates extrafollicular marginal zone B cells (Fig. 2.4).[53] During the week following immunization, B cells differentiate into plasma cells, undergo some degree of isotype switching from IgM to IgG/IgA, and—in rodents—rapidly produce essentially nonmutated, low-affinity, germline antibodies. Thus, PS vaccines are generally known as triggering T-independent responses characterized by the induction of moderate titers of low-affinity antibodies and the absence of immune memory.

In humans, PS immunization generates the production of intermediate-affinity IgG antibodies bearing some somatic mutations in their variable regions.[60,61] One hypothesis is that PS immunization activates "memory" B cells that have been previously primed by cross-reacting PS bacterial antigens somehow linked to protein moieties—and thus eliciting GC responses.[62] An alternative possibility is that the IgM^+, IgD^+, $CD27^+$ memory B cells that appear in the blood in response to PS immunization may be recirculating splenic marginal zone B cells.[63] This hypothesis is concordant with the fact that bacterial PS vaccines are poorly immunogenic in young children, that is, before the maturation of the splenic marginal zone.[64,65]

After their differentiation in the extrafollicular pathway, PS-specific plasma cells move toward the red pulp of the spleen (see Fig. 2.4) where they persist for some time, before their death by apoptosis and the waning of corresponding antibody responses after a few months. As PS antigens do not induce GCs, bona fide memory B cells are not elicited. Consequently, subsequent reexposure to the same PS results in a repeated primary response that follows the same kinetics in previously primed as in a naïve individual.[66] Revaccination with certain bacterial PS may even induce lower antibody responses than the first immunization, a phenomenon referred to as hyporesponsiveness,[67–69] which is increasingly reported[70–73] and where the molecular and cellular mechanisms include vaccine-induced B cell depletion by apoptosis.[74,75] This phenomenon is time-limited, such that if sufficient time elapses before the administration of a PS vaccine, the B-cell pool would be replenished.

What Are the Determinants of Primary Vaccine Antibody Responses? Numerous determinants modulate the intensity of vaccine-induced GCs and, thus, of peak antibody responses (Table 2.5). The main determinants are the nature of the vaccine antigen and its intrinsic immunogenicity. For example, tetanus toxoid is intrinsically a stronger immunogen than diphtheria toxoid, which becomes more apparent in the face of more limited immunocompetence, such as in preterm infants.[76] Whether this difference reflects a higher capacity of tetanus toxoid to provide antigenic epitopes that bind naïve B cells, the ability to generate cognate Tfh-cell help for B cells, and/or to their association with FDCs is unknown.

The markedly different outcomes of immunization with plain bacterial PS and with protein-conjugated glycoconjugates[67] highlight the differences between the extrafollicular and the GC reactions. It is only when capsular PS are conjugated to a protein carrier driving effective Tfh differentiation that PS-specific B cells are driven toward GC responses, receive optimal cognate help from carrier-specific Tfh cells, and differentiate into higher-affinity antibody-producing cells, longer-lived plasma cells, and/or memory B cells. Protein antigens exhibit markedly distinct carrier properties—regardless of their capacity to induce B- and Th-cell responses.[77,78] That these differences may reflect differences in Tfh induction is a likely hypothesis.[79,80] The limited number of potent carrier proteins implies that an increasing number of conjugate vaccines rely on the same carriers (e.g., CRM_{197}, tetanus or diphtheria toxoids), with the risk of limiting anti-PS responses to individual conjugate vaccines (carrier-mediated epitope suppression) and resulting in vaccine interference.[81,82] This phenomenon may be abrogated by replacing full-length proteins with peptides lacking B-cell epitopes,[83] suggesting that carrier-mediated epitope suppression essentially reflects the competition of carrier- and PS-specific B cells for activation/differentiation signals and factors.

Another determinant of the magnitude of primary vaccine antibody responses (see Table 2.5) is the use of an optimal dose of antigen, which may be determined only experimentally. As a rule, higher doses of nonlive antigens—up to a certain threshold—elicit higher primary antibody responses. This may be particularly useful when immunocompetence is limited, for example, for hepatitis B immunization of patients undergoing dialysis.[84,85] Remarkably, a limiting dose of antigen may restrict primary antibody responses but increase B-cell competition for FDC-associated antigens and, thus, result in a more stringent selection of higher-affinity GC B cells and stronger secondary responses (see subsequent text). Alternatively, adjuvants increasing inflammation at the injection site and, thus, cell recruitment and cell-mediated antigen transport toward lymph nodes, improve antibody responses despite a reduced antigen dose.[86] Little is known about factors that support or limit the affinity maturation process[87,88] which may be modulated by carrier proteins[89] and adjuvants.[90–92]

The nature of the vaccine directly influences the activation of innate immunity and, thus, vaccine responses. The strongest antibody responses are generally elicited by live vaccines that are "naturally adjuvanted," because they activate innate reactions, and, thus, support the induction of adaptive immune effectors in addition to providing a replicating antigen. Nonlive vaccines frequently require formulation with

TABLE 2.5 Determinants of the Duration of Vaccine Antibody Responses in Healthy People

Determinants	Mechanisms (Presumed)
VACCINE TYPE	
Live vs inactivated	Live vaccines generally induce more sustained Ab responses, presumably through Ag persistence within the host.
Polysaccharide antigens	Failure to generate Tfh cells and GCs limits the induction of memory responses and of high-affinity long-lived plasma cells.
VACCINE SCHEDULE	
Interval between primary doses	A minimal interval of 3 weeks between primary doses allows development of successive waves of Ag-specific primary responses without interference.
Interval before boosting	A minimal interval of 4 months between priming and boosting allows affinity maturation of memory B cells and thus higher secondary responses.
Age at immunization	Early life immune immaturity and age-associated immunosenescence limit the induction/persistence of long-lived plasma cells.
Environmental factors	Mostly unidentified.

Ab, antibody; Ag, antigen; GC, germinal center; Tfh, follicular T-helper cells.

adjuvants that enhance and shape vaccine immune responses through a variety of mechanisms.[34–37] The potency of the immune system indeed resides in its highly polymorphic nature, enabling sufficient immunological diversity to overcome a high number of diverse pathogens. This diversity impacts vaccine responses.[93] Probing how host genetic markers may result in variations of vaccine-induced responses is expected to identify gene polymorphisms that predict the likelihood of successful or adverse vaccine outcome, whereas epigenetic studies may help reveal how environmental influences affect innate and adaptive immune responses.[93] This work is still in its infancy, but holds great promise, especially when combined with novel systems vaccinology approaches.[94–96] Immune competence obviously affects vaccine antibody responses, which are limited at the two extremes of life (see subsequent text), and by the presence of acute or chronic diseases, acute or chronic stress, and a variety of factors affecting innate and/or B- and T-cell immunity.

Few nonlive vaccines (e.g., hepatitis A and human papillomavirus [HPV] vaccines) induce high and sustained antibody responses after a single vaccine dose, even in healthy young adults. Primary immunization schedules therefore usually include at least two doses, optimally repeated at a minimal interval of 3 to 4 weeks (longer intervals enhancing rather than reducing the responses) to generate successive waves of primary B-cell and GC responses. These priming doses may occasionally be combined into a single "double" dose, such as for hepatitis A or B and for HPV immunization.[97–101] In any case, vaccine antibodies elicited by primary immunization with nonlive vaccines eventually wane (see Fig. 2.3).

What Controls the Persistence of Vaccine Antibody Responses? Antigen-specific plasma cells elicited in spleen/nodes after immunization have only a short life span, such that vaccine antibodies rapidly decline during the first few weeks and months after immunization. A fraction of plasma cells that differentiated into GCs, however, acquire the capacity to migrate toward long-term survival niches that are mostly located within the BM, from where they may produce vaccine antibodies during extended periods.[102–105]

Some GC-induced plasma cells are attracted toward the BM compartment by cells that provide the signals required for their long-term survival.[50,106–109] In such BM niches, plasma cell survival and antibody production may persist for years. The duration of antibody responses reflects the number and/or quality of long-lived plasma cells generated by immunization[103]: In the absence of subsequent antigen exposure, antibody persistence may be reliably predicted by the antibody titers that are reached 6 to 12 months after immunization, that is, after the end of the short-term plasma cell response (see Fig. 2.3). This is illustrated by the accuracy of mathematical models predicting the kinetics of anti–hepatitis B surface antigen (HBsAg),[110] anti–hepatitis A,[111] or anti-HPV[112,113] antibodies.

A few determinants of the persistence of vaccine antibody responses (see Table 2.5) have been identified. The nature of the vaccine has a crucial role: only live attenuated viral vaccines or virus-like particles induce antibody responses that persist for several decades, if not lifelong, in absence of subsequent antigen exposure and reactivation of immune memory. In contrast, the shortest antibody responses are elicited by PS antigens, which fail to trigger Tfh/GC responses and thus do not elicit high-affinity plasma cells capable of reaching the BM survival niches. Antibody persistence may also be modulated by the use of adjuvants.[114,115] Vaccine schedules also control antibody magnitude and persistence. Closely spaced (1–2 weeks) primary vaccine doses may be administered when a rapid induction of protection is desirable, for example, before travel. However, this raises less-persisting responses than when the same number of vaccine doses are given at longer intervals (1–2 months),[116,117] reflecting the generation of fewer post-GC B cells capable of long-term survival and thus requiring later boosting. Optimal recall and anamnestic responses require longer intervals of at least 3 to 4 months, with longer intervals associated with generally greater responses (see below).

Age at immunization also modulates vaccine antibody persistence, which is shorter at the two extremes of life (see subsequent text). Certain conditions may also limit the persistence of vaccine antibody responses because of enhanced catabolism (as in HIV)[118] or the loss of antibodies in the urinary or digestive tract. The identification of the mechanisms that support or limit the persistence of vaccine antibody responses represents a major challenge.

What Are the Hallmarks of B-Cell Memory Responses? Memory B cells are generated during primary responses to T-dependent vaccines.[50,119] They persist in the absence of antigens but do not produce antibodies (i.e., do not protect), unless reexposure to antigen drives their differentiation into antibody-producing plasma cells. This reactivation is rapid, such that booster responses are characterized by the rapid increase to higher titers of antibodies that have a higher affinity for antigens than do antibodies generated during primary responses (Table 2.6).

Memory B cells are generated in response to T-dependent antigens, during the GC reaction, in parallel to plasma cells (Fig. 2.5).[50,119,120] At their exit of GCs, memory B cells acquire migration properties toward extrafollicular areas of the spleen and nodes. This migration occurs through the bloodstream, in which postimmunization memory B cells are transiently present on their way toward lymphoid organs.

It is essential to understand that memory B cells do not produce antibodies—that is, they do not protect. Their participation in vaccine efficacy requires an antigen-driven reactivation that may occur in response to endemic pathogens, to colonizing or cross-reacting microorganisms ("natural boosters"), or to booster immunization. The activation of memory B cells results in their rapid proliferation and differentiation into plasma cells that produce very large amounts of higher-affinity antibodies.[120] As the affinity of surface Ig from memory B cells is increased, their requirements for reactivation are lower than for naïve B cells: memory B cells may thus be recalled by lower amounts of antigen and without $CD4^+$ T-cell help, although T-cell help supports a second round of GC responses, further magnifying the level/persistence of antibodies.[121] Antigen-specific memory cells generated after primary immunization are much more numerous (and better fit) than naïve B cells initially capable of antigen recognition.[50,119] Thus, the first hallmark of the memory responses (see Table 2.6) is

TABLE 2.6 Hallmarks of Memory B-Cell Responses

Memory B cells:
• Are generated only during T-dependent responses inducing follicular T-helper cells and thus germinal center responses
• Are resting cells that do not produce antibodies
• Undergo affinity maturation during 4–6 months
• Rapidly (days) differentiate into antibody-secreting plasma cells on reexposure to antigen
• Differentiate into plasma cells that produce high(er)-affinity antibodies than do primary plasma cells

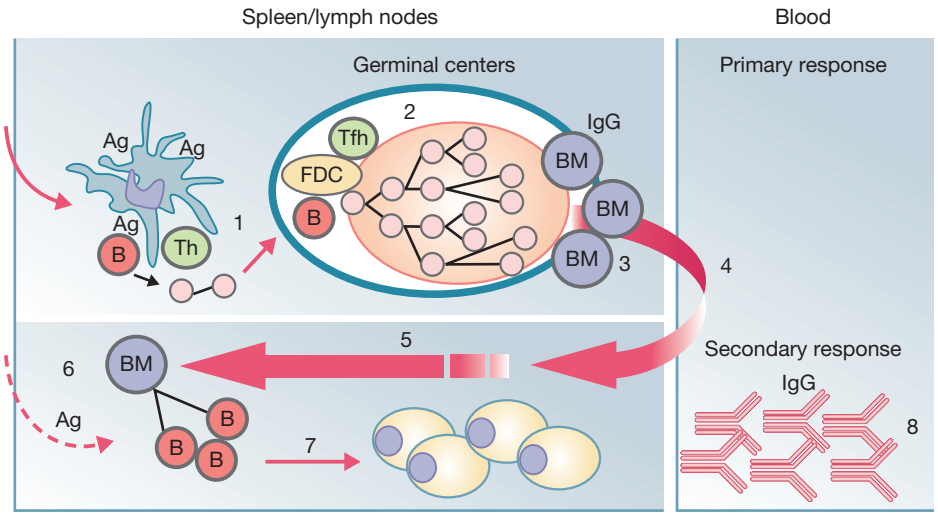


Figure 2.5. Generation of B-cell memory responses. Memory B cells are generated in response to T-dependent antigens *(1)*, during the germinal center (GC) reaction *(2)*, in parallel to plasma cells. At their exit of GCs, these B cells do not differentiate into antibody-secreting plasma cells but into memory B cells *(3)* that transiently migrate through the blood *(4)* toward the extrafollicular areas of spleen and nodes *(5)*. They persist there as resting cells until reexposed to their specific antigens *(6)*. On secondary antigen exposure, memory B cells readily proliferate and differentiate into plasma cells *(7)* secreting large amounts of high-affinity antibodies that may be detected in the serum *(8)* within a few days after boosting. Ag, antigen; BM, bone marrow; FDC, follicular dendritic cell; IgG, immunoglobulin G; Th, T-helper.

to generate significantly higher antibody levels than primary immunization. Should this not be the case, the effective generation or persistence of memory B cells should be questioned.

The reactivation, proliferation, and differentiation of memory B cells occur without requiring the induction and development of GC responses. This process is, thus, much more rapidly completed than that of primary responses. A window of 4 to 7 days after *H. influenzae b* (Hib) PS immunization was reported as sufficient for high levels of PS-specific vaccine antibodies to appear in the blood of previously primed infants.[122] The rapidity with which antigen-specific antibodies appear in the serum is, thus, another hallmark of secondary responses (see Table 2.6). Slower antibody kinetics suggests that memory B-cell induction, persistence, and/or reactivation may have been suboptimal.

Another hallmark of memory B cells is that they display and secrete antibodies with a markedly higher affinity than those produced by primary plasma cells, as a result of somatic hypermutation and selection. The affinity maturation process that is initiated within the GCs extends for several months after the end of the GC reaction. Consequently, vaccine antibodies with higher than baseline avidity (defined as the sum of epitope-specific affinities) for antigen are induced only when sufficient time has elapsed after priming.[123–125] A "classical" prime-boost immunization schedule is, thus, to allow 4 to 6 months to elapse between priming and booster doses, hence the generic "0-1-6 month" (prime-prime-boost) schedule. Secondary antigen exposure (see Table 2.6) thus results in the production of higher-affinity antibodies than primary responses.[126] Notably, this may not be the case when "natural" priming, for example, through cross-reactive bacteria, has occurred prior to immunization.

What Are the Determinants of B-Cell Memory Responses?
The factors that drive the differentiation of antigen-specific GC B cells toward plasma cells or memory B cells are poorly understood.[50] In response to protein antigens, both cell populations are generated in the same GCs, and their differentiation pathway differs only late in the GC reaction. As a rule, factors enhancing plasma cell differentiation and primary antibody responses (such as increasing the antigen dose or using adjuvants) also support the induction of memory B cells (Table 2.7). Postbooster antibody titers are, therefore, higher in people with stronger primary responses. For example, higher postbooster anti-HBsAg responses are observed in people with high (e.g., ≥100 IU/L) rather than intermediate (10–99 IU/L) anti-HBsAg after their primary vaccination.[127,128] This is likely to reflect the induction of a larger pool of memory B cells.

The dose of antigen is also an important determinant of memory B-cell responses (see Table 2.7). At priming, higher antigen doses generally favor the induction of plasma cells, whereas lower doses may preferentially drive the induction of immune memory.[129] Closely spaced primary vaccine doses may be beneficial for early postprimary antibody responses but not for postbooster antibody responses, as illustrated with meningococcal group C glycoconjugates.[130] As a rule, accelerated schedules in which a 4- to 6-month window is not included between priming and boosting result in significantly lower booster responses[125] (see Table 2.7). At the time of boosting, a higher antigen content raises stronger booster responses, presumably by recruiting more memory B cells into the response. This is illustrated by higher antibody responses of children immunized with a higher-antigen-dose pertussis vaccine[131] or primed with a glycoconjugate vaccine and boosted with a higher concentration PS (20–50 μg of PS) when compared with the glycoconjugate (1–3 μg of PS) vaccines.[132,133]

Residual titers of vaccine antibodies present at time of boosting directly influence vaccine antibody responses. As a rule, secondary responses to live attenuated viral vaccines are minimal, since preexisting antibodies neutralize the vaccine virus before in vivo replication. Consequently, even multiple doses of live attenuated vaccines do not have undesirable effects. Responses to nonlive vaccines are also negatively influenced by residual vaccine antibody titers. This may reflect the formation of antigen–antibody complexes that reduce the load of antigen available for B-cell binding and/or antibody-mediated negative feedback mechanisms acting directly on B cells through, for example, fragment c (Fc)

TABLE 2.7 Determinants of Secondary B-Cell Responses

Determinants	Mechanisms (Presumed)
Postprimary antibody titers	As plasma cells and memory responses are generated in parallel in GCs, higher postprimary Ab titers reflect stronger GC reactions and generally predict higher secondary responses.
Residual antibodies at boosting	Neutralization of live viral vaccines; negative feedback mechanisms on nonlive vaccines.
Lower antigen dose at priming	A limited quantity of antigen may induce B-cell differentiation away from PCs and toward memory B cells (?).
Longer intervals before boosting	A minimal interval of 4–6 months is required for optimal affinity maturation of memory B cells.
Higher antigen dose at boosting	A higher availability of antigen may drive higher numbers of memory B cells into differentiation.
ANTIGEN AVAILABILITY	
Exogenous exposure	Exposure to exogenous antigens may reactivate or favor the persistence of memory B cells.
Localization	Memory B cells reactivation requires antigens to reach the draining lymph nodes and not be restricted on mucosal surfaces (HPV, pertussis [?]).
In vivo persistence	Antigen persistence may reactivate or favor the persistence of memory B cells.

Ab, antibody; GC, germinal center; HPV, human papillomavirus; PC, plasma cell.

receptors. Consequently, people with residual antibodies to a given antigen may show only a limited increase of their antibody responses.

The persistence of memory B cells is of utmost importance for long-term vaccine efficacy. Antigen persistence may extend for prolonged periods on the surface of FDCs (see Table 2.7) and contribute to the duration of immune memory.[134] This is likely to contribute to the extended (indefinite?) memory to live attenuated vaccines, recently exemplified by repeated administration of smallpox vaccines decades after priming.[135] Fortunately, memory B cells survive for prolonged periods (e.g., several decades), even in the absence of reexposure to antigen.[136] It has been suggested that memory B cells undergo a certain degree of homeostatic polyclonal activation.[137] Although this does not seem sufficient to maintain antibody responses,[138] it likely contributes to their persistence and the replenishment of BM plasma cells.

The demonstration of the persistence of memory B cells long after vaccine antibodies have eventually disappeared, and of their brisk reactivation on antigen exposure, has direct consequences for immunization programs. First, it implies that an immunization schedule should never be started all over again—but continued where interrupted, regardless of the duration of the interruption. Second, it implies that certain immunization schedules may not need to include booster doses, if the individual is exposed to regular natural boosters. It is intriguing to note, that in the absence of childhood boosters, up to 50% of adolescents or young adults primed against tetanus or hepatitis B in infancy might not raise anamnestic responses, suggesting that infant-induced vaccine memory may not last forever.[139,140]

Immune Memory and Vaccine-Induced Protection: A Race Between Reactivation and Microbial Invasion? All existing vaccines, except T-independent PS, induce immune memory. Nevertheless, vaccine efficacy may be short-term, as illustrated following infant immunization against group C meningococcus.[141] Demonstration of priming—or "boostability"—is therefore not a surrogate marker for long-term vaccine efficacy. This requires identifying the determinants that contribute to—or limit—the persistence of vaccine efficacy. One hypothesis is that this essentially results from the race between the reactivation of immune memory and disease pathogenesis.[142]

It is generally considered that protection by toxoid-based vaccines requires the presence of antitoxin antibodies at time of toxin exposure/production. Persisting immune memory is also not sufficient to protect against *acute* hepatitis B after the waning of vaccine-induced antibodies.[143–145] However, progression to chronic liver disease has not been reported in fully immunized vaccine responders. That immune memory is sufficient to protect against chronic hepatitis B suggests that viral replication and reexposure to HBsAg efficiently drive vaccine-induced memory cells into effector cells before the end of the viral incubation period (4–12 weeks). This process requires enough HBsAg-specific memory B cells to be stimulated, to persist, and to be capable of reactivation even several decades after infant priming. It remains to be defined whether T-cell memory responses contribute to the maintenance of vaccine-induced protection after waning of anti-HBsAg antibodies.

Glycoconjugate vaccines against encapsulated bacteria illustrate the importance of immune memory for vaccine efficacy and some of its limitations. Glycoconjugate priming elicits a bona fide GC reaction, with the induction of high-affinity memory B cells that can be rapidly (4–7 days) recalled on PS immunization.[122] Efficient priming (i.e., induction of immune memory) is readily demonstrated in children primed in infancy.[146,147] However, immune memory can be seen in children with Hib vaccine failure,[148] indicating that their reservoir of memory B cells did not protect them against invasive disease, perhaps through a failure of avidity maturation.[149] The discrepancy between the existence of memory B cells and the lack of protection may again reflect the race against microbial invasion: the time required for production of sufficient levels of circulating antibodies could be too long to interrupt bacterial invasion. Notably, secondary vaccine failures have been relatively rare and primarily observed in countries using an early accelerated infant schedule without a booster dose,[150] the use of diphtheria, tetanus, and acellular pertussis (DTaP)/Hib vaccines with lower Hib immunogenicity is also associated with vaccine failure.[151] Similarly, glycoconjugate vaccines against group C meningococcal disease proved much more efficacious during the first year after infant priming than during the following 3 years.[141] Thus, infant immunization fails to induce sustained protection against group C meningococcus, despite the induction and persistence of immune memory.[152] The requirement for boosters to confer long-term vaccine protection is also well illustrated for pertussis, for which boosters are required to extend protection beyond childhood.[153] An interesting observation is that vaccine-induced memory persists following pertussis immunization—as illustrated by anamnestic responses to a booster dose—but is not sufficient for protection. Yet the incubation period of pertussis exceeds

4 to 7 days. An interesting hypothesis is that as *Bordetella pertussis* bacteria essentially remain on the mucosal surfaces, antigens may fail to efficiently reach the vaccine-induced B and T cells residing in the lymph nodes. For example, the prompt reactivation of immune memory is not sufficient to control polio viral replication in the digestive tract.[154]

Live attenuated viral vaccines (measles, rubella) are considered the prototype inducers of lifelong immunity, although prolonged immunity is also induced by certain nonlive vaccines (hepatitis A, HPV, inactivated poliovirus vaccine, rabies). This derives in part from the induction of sustained antibody responses, which, however, tend to slowly decline in the absence of recurrent exposure,[155] and might eventually result in a growing proportion of seronegative vaccinated young adults, including women of childbearing age. Whether the reactivation of immune memory will be sufficient to curtail the replication process and confer protection against measles, rubella, or varicella, and whether adult booster doses may become needed after microbial control, are essential questions. The resurgence of mumps outbreaks in fully vaccinated young adults may reflect the induction of low numbers of memory B cells[156] and demonstrates that secondary vaccine failure may occur even with live attenuated vaccines.[157] The questions, which are central to sustained vaccine efficacy, are usually unresolved at the time of registration of a new vaccine. For example, to vaccinate young girls against HPV requires reassurance that vaccine protection will extend during several decades. HPV infection of the basal epithelial cells can occur within minutes and is not followed by any antigen exposure to the immune system. Thus, antibody persistence will be required for sustained protection. Remarkably, however, the concentration of vaccine antibodies required to neutralize HPV at the site of entry is so minute[158] and vaccine-induced community-protection so efficient that boosters may indeed not be needed.

Thus, one may expect questions related to the nature (size, type, responsiveness) of the pool of memory cells elicited by various immunization schedules and the relative contribution of long-term antibodies and immune memory to protection to be at the core of many vaccine studies in the next decades.

T-Cell Vaccine Responses

How Do Vaccines Induce CD4⁺ and CD8⁺ T-Cell Responses? The generation of CD4⁺ Th-cell response begins when DCs capture antigen in peripheral tissue and migrate to draining lymph nodes, where T-cell vaccine responses are elicited in parallel to B-cell responses (see Table 2.1). Thus, DCs fulfill a pivotal role in initiating and shaping the immune response to vaccine antigens.

Protein vaccine antigens are taken up by immature DCs activated by local inflammation, which provide the signals required for their migration to draining lymph nodes (see Fig. 2.1). During this migration, DCs mature and their surface expression of molecules changes.[159] Simultaneously, antigens are processed into small fragments and displayed at the cell surface in the grooves of MHC (human leukocyte antigen [HLA] in humans) molecules. As a rule, MHC class I molecules present peptides from antigens that are produced in the cytosol of infected cells, whereas phagocytosed antigens are essentially displayed on MHC class II molecules.[160–163] Thus, mature DCs reaching the T-cell zone of lymph nodes display MHC–peptide complexes and high levels of costimulation molecules at their surface.[164] CD4⁺ T cells recognize antigenic peptides displayed by class II MHC molecules, whereas CD8⁺ T cells bind to class I MHC-peptide complexes (Fig. 2.6).[165] Their recognition is restricted to short peptides (8–11 [CD8⁺] or 10–18 [CD4⁺] amino acids) displayed on specific MHC class I or II molecules, respectively. Antigen-specific T-cell receptors may bind only to specific MHC molecules

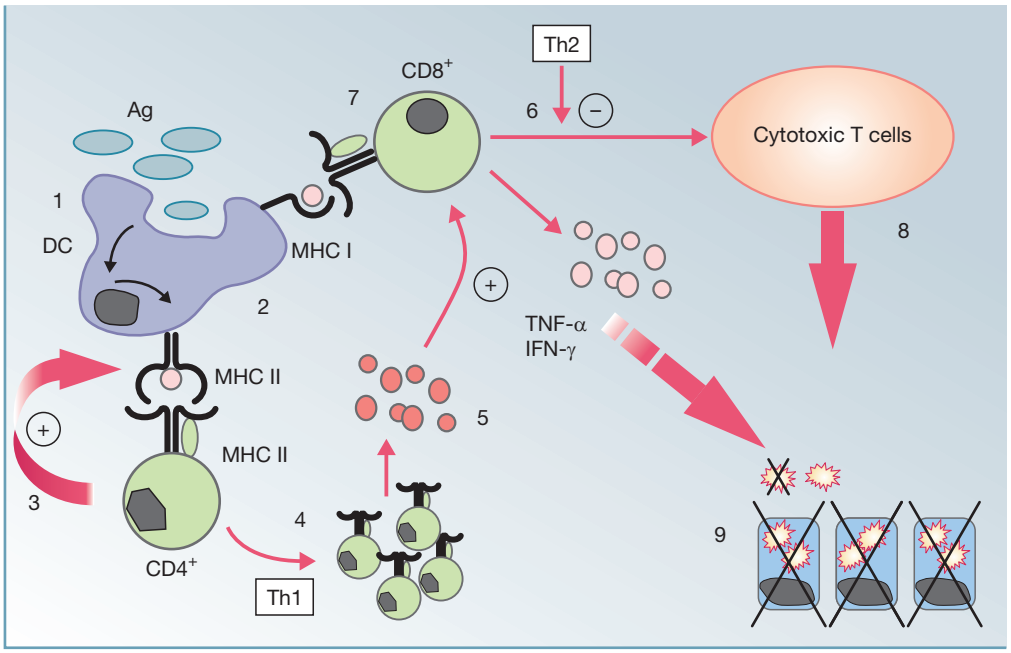


Figure 2.6. Generation of T-cell effector responses. Antigens are phagocytosed by dendritic cells (DCs) *(1)*, processed into small peptides, and displayed at the cell surface in the groove of major histocompatibility complex (MHC) class I and/or class II molecules *(2)*. CD4+ T cells with the appropriate MHC-peptide specificity are activated, provide activation signals to DCs *(3)*, and differentiate in effector cells *(4)* that produce preferentially T helper (Th)1 or Th2 cytokines. Th1 CD4⁺ T cells support *(5)* CD8⁺ T-cell differentiation, which is in contrast inhibited *(6)* by Th2-like cytokines. CD8⁺ T cells recognize MHC class I-peptide complexes *(7)* and differentiate into cytotoxic effector cells *(8)* capable of killing infected cells *(9)* or pathogens. Ag, antigen; IFN, interferon; TNF, tumor necrosis factor.

(e.g., HLA-A2), which differ among individual people and populations. Consequently, T-cell responses are highly variable within a population. These T-cell epitopes may be generated from any region of the vaccine antigens, whether the peptide sequence is located within or at the surface of the protein. This is in contrast with B-cell recognition, which is essentially limited to conformational determinants constituted by amino acids at the antigen surface. This MHC-peptide signal (signal 1) is not sufficient for activation of T cells, which remain anergic or become tolerized in absence of costimulation (signal 2). This ensures that only naïve T cells binding to the surface of activated DCs (i.e., DCs that have sensed danger signals through their Toll-like receptors and responded by a modulation of their surface or secreted molecules) receive the costimulation signals required for their activation.[164]

Activated $CD4^+$ T cells essentially exert supportive functions for DCs, to which they provide signals (CD40L, etc.) resulting in further activation, for B cells (see Fig. 2.2) and for $CD8^+$ cytotoxic T cells (see Fig. 2.6 and Table 2.8). They are elicited by each vaccine type, except plain PS, which are not properly displayed by MHC molecules. Thus, the demonstration of postimmunization $CD4^+$ T-cell responses does not imply a direct role in vaccine efficacy. $CD4^+$ T-cell activation by DCs triggers their differentiation along distinct differentiation pathways.[164,166] By default, DCs essentially trigger the induction of Th2-type $CD4^+$ T cells producing IL-4, IL-5, and IL-13, which are implicated in the defense against extracellular pathogens such as helminths.[167] More potently activated DCs release IL-12p70, which induces the differentiation into Th1 cells that essentially produce IFN-γ and tumor necrosis factor (TNF)-α and, thus, contribute to the elimination of intracellular pathogens directly (cytokine responses) and indirectly through macrophage activation and support to $CD8^+$ T-cell differentiation (see Fig. 2.6).[168] Th1 and Th2 cells support B-cell activation and differentiation during extrafollicular responses, whereas Tfh $CD4^+$ cells provide critical help to GC B cells (see Fig. 2.3).[169] Under certain conditions, activated DCs may also release IL-23, supporting the induction of inflammatory Th17 cells by TGF-β and IL-6.

Numerous factors influence the preferential differentiation of $CD4^+$ T cells toward the Th1, Th2, Tfh, or Th17 pathway.[170] The main determinant of $CD4^+$ T-cell differentiation is the extent and type of DC activation by the innate system,[164] although a recent observation suggests that polarized $CD4^+$ T-cell responses may result from preferential expansion rather than priming.[171] Consequently, DCs are the primary target for specific adjuvants, which may preferentially skew $CD4^+$ responses toward Th1, Th2, or Th17 responses and impact the differentiation of Tfh cells, requiring their careful design and selection.[34–37,39,172]

$CD8^+$ T-cell responses are essentially induced as a result of cross-presentation elicited by infectious, live attenuated vaccines that introduce antigens within the cell cytosol, ensuring their access to MHC class I molecules.[163,173] However, novel delivery systems such as live-vectored vaccines or DNA vaccines delivering antigens directly into the cytosol are now in human trials.[174]

The activation of naïve T cells by vaccine-bearing DCs may also induce their differentiation into Tregs (see Table 2.8), a heterogeneous population with many levels of complexity.[10,175] Vaccine-induced Tregs may use multiple mechanisms to suppress T-cell induction or proliferation: in draining lymph nodes, they may prevent DC maturation, block the priming of effector T cells, or destroy antigen-bearing DCs. These Tregs may be elicited as feedback mechanisms to avoid excessive and, thus, potentially harmful inflammatory responses. By suppressing immune responses, Tregs may limit the efficacy of vaccines, for example, when danger signals are insufficient to elicit immunity, as in chronic infections and cancer.[176–178] Defining the determinants of Treg differentiation may be needed for novel immunization strategies such as therapeutic vaccines. Preclinical studies indicate that adjuvants improving the ratio of antigen-specific effector to Tregs enhance vaccine immunity,[179] opening interesting possibilities.

TABLE 2.8 T-Cell Responses to Vaccines

Type	Mechanisms (Presumed)	Function
$CD4^+$ T-helper cells		
Th1	IFN-γ production	Extrafollicular B-cell help
Th1	Cell contact, IFN-γ	Activation of $CD8^+$ T cells
Th1/Th2	Cell contact, CD40L	Dendritic cell activation
Th2	IL-4, IL-5, IL-13	Extrafollicular B-cell help
Th2	Cell contact, IL-4	Suppression of $CD8^+$ T cells
Th17	IL-17, IL-21, IL-22	Mucosal inflammation
$CD4^+$ follicular T-helper cells		
Tfh1	IFN-γ	Germinal center B-cell help
Tfh2	IL-4, IL-5, IL-13	Germinal center B-cell help
$CD4^+$ regulatory T cells	Multiple mechanisms	Suppression of $CD4^+$/$CD8^+$ responses
$CD8^+$ T cells	IFN-γ, TNF-α	Killing of infected cells
Memory T cells		
Effector memory T cells	Th1/Th2 cytokines, perforin, granzyme	Rapid secondary effectors responses in periphery
Central memory T cells	IL-2, IL-10, CD40L	Delayed activation/proliferation in lymph nodes
Tissue-resident memory T cells	Th1/Th2 cytokines, perforin, granzyme	Tissue localization enabling immediate-early reactivation

IFN, interferon; IL, interleukin; Th, T-helper; TNF, tumor necrosis factor.

What Are the Determinants of Vaccine-Induced T-Cell Memory?

Effector T-cell responses are short-lived, and most (>90%) effector T cells die by apoptosis within a few days. Thus, immune memory is essential to T-cell vaccine efficacy. It is dependent on four main parameters: the frequency of antigen-specific memory T cells, their phenotype, their persistence, and their localization, a recently identified parameter (Table 2.9).[174,180,181] Memory T cells may persist lifelong, even in the absence of antigen exposure and despite their quality and amount being set during the primary immune response.

The frequency of memory T cells directly reflects the magnitude of the initial T-cell expansion and that of its subsequent contraction during which few surviving cells differentiate toward memory T cells. The main determinant of the expansion phase is the level of or duration of antigen stimulation present during priming.[182] This is a major limitation for nonreplicating vaccines, which fail to reach sufficient antigen content and typically require the presence of an adjuvant and/or booster doses. The contraction phase and the transition toward memory cells take place soon after antigen is cleared, which occurs faster for nonreplicating vaccines. Current efforts are, thus, oriented toward the optimization of the primary expansion phase through adjuvants and/or booster administration. As vaccine-induced immunity limits the subsequent "take" of a live vaccine by inducing its rapid neutralization, one attractive approach is the use of distinct vaccines for priming and boosting, as the adenovirus priming–modified vaccinia virus Ankara (MVA) boosting combination currently considered against Ebola virus.[183–186]

The phenotype of memory T cells is also important. Two main types of memory T cells have been identified (see Table 2.8) based on their phenotype and function, central memory cells and effector memory cells.[187] Central memory T cells (Tcm), like naïve T cells, but better equipped, preferentially traffic through lymph nodes and BM and do not exhibit much cytotoxic capacity but have a high proliferative potential. Their role is to recognize antigens transported by activated DCs into lymph nodes and to rapidly undergo massive proliferation and differentiation, generating a delayed but very large wave of effector cells.[188] Effector memory T cells (Tem), closer in phenotype to recently activated T cells, have a high cytotoxic potential that enables them to immediately recognize the pathogen. As they essentially lack lymph nodes homing receptors, it was proposed that Tem recirculate from the blood through nonlymphoid organs, monitoring tissues for the presence of specific microbial peptides.[188] A third type of memory T cells (resident memory T cells [Trm]) was recently recognized as populations of memory T cells which remain settled within specific organs such as the intestine, the lungs, the skin.[189] How Trm cells are induced and maintained in the specific organs is not yet fully deciphered, but as Trm were demonstrated as central for the protection against mucosal infections, novel vaccine strategies against viral (influenza, respiratory syncytial virus [RSV]) or bacterial (pertussis) mucosal pathogens will attempt their induction/maintenance.[190]

Antigen persistence essentially controls the proportion of Tcm and Tem memory cells (see Table 2.9): Tcm cells predominate when antigen is rapidly cleared, whereas Tem/Trm cells become preponderant when antigen persists, such as in chronic infections.[174,180,181] This is a challenge for novel nonreplicating vaccines that should induce and maintain sufficient Tem/Trm cells for immediate clearance in infected tissues. The long-term persistence of memory T cells is well established. Through homeostatic proliferation, memory T cells may persist lifelong, even without antigen exposure.[180,181,191] Studies of the persistence of vaccinia-induced immune memory have confirmed this observation in humans.[192–194]

TABLE 2.9 Determinants of Memory T-Cell Responses

Main Factors	Determinants
Frequency of memory T cells	Magnitude of T-cell expansion (initial antigen load, antigen persistence)
Phenotype of memory T cells	
Effector memory	Induction favored by prolonged antigen persistence
Tissue-resident memory	
Central memory	Induction favored by rapid antigen clearance
Persistence of memory T cells	Supported by interleukin (IL)-15, IL-7

How Specific Are Vaccine Immune Responses?

The specificity of vaccine responses is at the center of many debates. Ideally, one would want vaccine-induced responses to be sufficiently broad to extend protection to nonvaccine strains (e.g., for influenza, rotavirus, *S. pneumoniae*, or HPV vaccines) and sufficiently restricted to not elicit cross-reactions to allergens or self-antigens or other undesirable nonspecific effects. The specificity of vaccine responses has received added interest as a number of studies have also reported both positive and negative "nonspecific" effects of vaccinations in low income countries.[195,196]

As B cells recognize conformational epitopes constituted by distant amino acids, they may bind to antigenic peptides with distinct sequences: It has been estimated that roughly 5% of monoclonal antibodies made against 15 viruses cross-reacted with human proteins.[197] That any viral infection is not followed by the induction or flare of an autoimmune disease highlights the importance of regulatory mechanisms suppressing responses directed against self-antigens. Indeed, the specificity of antibody responses is well controlled. Although polyclonal stimulation has been suggested to activate memory B cells of distinct specificities,[137] this response is not associated with antibody responses. Vaccination with tetanus toxoid was found to expand specific and bystander memory T cells but did not modulate antibody responses to unrelated antigens.[198] Altogether, this indicates that the induction of cross-reactive antibody responses is extremely limited, which may be important in preventing undesirable reactions, but which limits the efficacy of vaccine-induced antibody responses to very few cross-reacting nonvaccine serotypes.[199]

T cells need to recognize only a few amino acids of antigenic peptides displayed by MHC molecules, which offers a much greater potential for cross-reactivity. It has been estimated that each T lymphocyte could potentially bind to a million different peptides.[197] In addition, memory T cells readily respond to homeostatic cytokines, such that bystander memory T cells of distinct antigen specificity may be transiently activated and expand during a flu-like illness or an immunization process.[198,200] However, vaccine-induced exacerbations of autoimmune diseases are very uncommon, probably reflecting the efficacy of regulatory mechanisms limiting the intensity, scope, and duration of the immune responses.[201,202]

The induction of cross-protective T-cell–mediated responses has been repeatedly observed in murine experimental models,

which suggests that cross-reacting viral vaccines could be based on T-cell responses.[203] Yet, convincing examples of heterologous protective immunity in humans are much more limited, including neonatal BCG protects against leprosy,[204] and smallpox vaccine protects against monkeypox.[205] In contrast, the sharing of several T-cell determinants is not sufficient for a single oral polio vaccine strain or influenza strain to confer cross-protection. Consequently, it is tempting to conclude that heterologous protective immunity essentially comes into play for T-cell–mediated rather than for antibody-mediated protective responses. Accordingly, the heterosubtypic immunity conferred by live attenuated influenza vaccines[206,207] could be mediated by T cells and/or by mucosal IgA antibodies.

Nonspecific effects of vaccines are occasionally associated with the fear of immune overload and subsequent enhanced vulnerability to infections, a theory not supported by evidence.[208,209]

In addition to B and T cells, it was recently recognized that innate cells such as natural killer (NK) cells and monocytes acquire a "trained immunity phenotype" upon exposure to certain pathogens and have given support to the idea that vaccines can have off-target effects. The epidemiological studies on this subject have been done mainly by a group working in Guinea-Bissau and their thesis is that live vaccines (including BCG, measles, and oral polio vaccine [OPV]) can reduce mortality caused by respiratory viral infections, whereas killed vaccines, notably diphtheria, tetanus, and pertussis (DTP), can reverse those effects and even increase mortality.[210–213] Data from some other regions are supportive of this theory.[214,215] As most of the epidemiological studies have been nonrandomized studies, this idea has been met with skepticism, particularly as the causes of mortality have been ill-defined. Following a systematic review, the World Health Organization (WHO) Strategic Advisory Group of Experts (SAGE) on immunization concluded that the available data suggest that BCG "has" and measles vaccine "may have" beneficial effects on all-cause mortality, whereas it neither excluded nor confirmed the possibility of beneficial or deleterious nonspecific effects of DTP vaccines on all-cause mortality.[216,217]

Immunologists have now begun to study this issue more comprehensively. Evidence has accrued that BCG strongly stimulates cytokine production and enhances responses to other antigens,[218,219] and NK cells—which can develop memory[220]—are stimulated by BCG to respond to antigens other than mycobacterial.[221] The Danish strain of BCG used in Guinea-Bissau is particularly strong in this respect.[222] Humans given BCG respond with Th1 and Th17 responses and their stimulated monocytes show increased receptor expression.[223,224] Wild measles virus infection in monkeys abolishes immune memory to other antigens,[225,226] making it possible that measles vaccine in addition prevents abolition by the natural virus of the child's ability to respond to other infections.[227] The proposed negative effects of killed vaccines on mortality remains for the moment based only on observation,[228] although nonlive vaccines typically elicit preferential Th2 responses which might hypothetically reduce the Th1 polarization elicited by live vaccines. This subject is one in evolution and a randomized study has begun in Denmark that should shed light on the importance, if any, of nonspecific or off-target effects in a developed country.[228]

Vaccine Responses at the Extremes of Age

The Challenges of Neonatal and Early Life Immunization. According to UNICEF estimates, 4 million infants younger than 6 months die yearly of acute infections.[229] In more developed countries, mortality has been reduced, but infections represent a significant proportion of infant hospitalizations. This disease burden is caused by a limited number of pathogens, such that the availability of a few additional vaccines that would be immunogenic soon after birth would make a huge difference. Early life responses markedly differ from those elicited in mature hosts. The blunting of neonatal immune responses has been regarded for many years as resulting from "neonatal tolerance," reflecting the antigen naïveté of the immune system and, subsequently, its immaturity. Recent work has prompted a change of perspective, leading to the recognition that the neonatal and early life immune system is, in contrast, specifically adapted to the unique challenges of early postnatal life and develops over time through poorly defined but tightly regulated processes.

These specific neonatal features first affect innate responses as pattern-recognition receptors elicit responses biased against the induction of proinflammatory cytokines, which could cause harmful alloimmune reactions against maternal antigens or excess inflammatory reactions.[230,231] In addition, many factors determine the quality and quantity of infant antibody responses: this includes the state of prenatal and postnatal development of the immune system, the type of vaccine and its immunogenicity, the number of doses and their spacing, and the influence of maternal antibodies.[232–234]

Early life immune responses are characterized by age-dependent limitations of the magnitude of responses (Table 2.10). Antibody responses to most PS antigens are not elicited during the first 2 years of life, which is likely to reflect numerous factors, including the slow maturation of the splenic marginal zone,[65,235] limited expression of CD21 on B cells, and limited availability of the complement factors.[236] Although this may be circumvented in part by the use of glycoconjugate vaccines, even the most potent glycoconjugate vaccines elicit markedly lower primary IgG responses in young infants.[237]

Early life antibody responses are directly determined by the prenatal (e.g., gestational age[238]) and the postnatal age at immunization.[236] Accelerated infant vaccine schedules in which three vaccine doses are given at 1-month intervals (2, 3, 4 or 3, 4, 5 months) result in lower immune responses than schedules in which more time elapses between doses (2, 4, 6 months) or between the priming and boosting dose (3, 5, 12 months). However, the magnitude of infant antibody responses to multiple dose schedules reflects the interval between doses, with longer intervals eliciting stronger responses, and the age at which the last vaccine dose is administered. That postnatal immune maturation is required for stronger antibody responses is best demonstrated by comparing antibody responses to single-dose vaccines given to antigen-naïve infants of various ages.[239,240] These studies may be confounded by the persistence of maternal antibodies, which negatively influence infant antibody responses in both epitope and titer specific manners.[241,242] Thus, multivariate analyses of the data for a large number of infants are required to identify the main determinants of vaccine antibody responses.[243]

The induction of B-cell responses is critically dependent on components of the local microenvironment. However, blood is the only accessible compartment in infants and the factors that specifically limit the magnitude of early life antibody responses are difficult to study. Studies in which vaccines routinely administered to human infants were administered at various stages of the postnatal maturation to infant mice indicated that the same limitations of antibody responses are seen in both humans and mice, reflecting similar postnatal constraints.[236] These animal models showed that limitations of antibody responses in early life result from the limited and delayed induction of GCs in which antigen-specific B cells proliferate and differentiate. This was first shown to essentially reflect the delayed development of FDCs required to nucleate

TABLE 2.10 Limitations of Vaccine Responses at the Extremes of Life (Mechanisms Presumed)

In Early Life	
Limited magnitude of Ab responses to PS	Immaturity of marginal zone; low CD21 expression on B cells; limited availability of complement
Limited magnitude of Ab responses to proteins	Limited GC responses (delayed FDC development?); inhibitory influence of maternal antibodies
Short persistence of Ab responses to proteins	Limited establishment of bone marrow plasma cell pool (survival niches?)
Shorter duration of immune memory (?)	Limited GC responses (magnitude of initial memory B-cell pool?)
Limited IFN-γ responses	Suboptimal antigen-presenting cell/T-cell interaction (IL-12, IFN-α)
Limited $CD8^+$ T-cell responses (?)	Insufficient evidence
Influence of maternal antibodies	Inhibition of B-cell but not T-cell responses
In Elderly People	
Limited magnitude of Ab responses to PS	Low reservoir of IgM^+ memory B cells; weaker differentiation into plasma cells
Limited magnitude of Ab responses to proteins	Limited GC responses: suboptimal $CD4^+$ helper responses, suboptimal B-cell activation, limited FDC network development?; changes in B-/T-cell repertoire
Limited quality (affinity, isotype) of antibodies	Limited GC responses; changes in B-/T-cell repertoire
Short persistence of Ab responses to proteins	Limited plasma cell survival?
Limited induction of $CD4^+$/$CD8^+$ responses	Decline in naïve T-cell reservoir (accumulation of effector memory and $CD8^+$ T cell clones)
Limited persistence of $CD4^+$ responses	Limited induction of new effector memory T cells (IL-2, IL-7)

Ab, antibody; FDC, follicular dendritic cell; GC, germinal center; IFN, interferon; Ig, immunoglobulin; IL, interleukin; PS, polysaccharide.

and support GC reactions[244] and subsequently to result from the limited induction of Tfh cells in the draining lymph nodes.[7,245] Direct evidence for a similar mechanism is difficult to obtain[235] in human infants. Efforts are ongoing to identify adjuvants for use in early life. The capacity of the MF59 adjuvant to induce strong Tfh/GC responses in infant mice[7] could also be relevant to the ability of the adjuvant to improve the efficacy of influenza vaccines in young children.[246]

In contrast with this blunting of early life antibody responses, the neonatal immune system readily allows the induction of immune memory, thus reflecting preferential differentiation of early life GC B cells toward memory rather than Ig-producing plasma cells. Neonatal priming may, thus, be used to initiate vaccine responses against hepatitis B or poliomyelitis. Recent work demonstrated that acellular pertussis vaccines may similarly effectively prime neonatal responses, resulting in faster acquisition of infant immunity.[247–249] However, neonatal priming with a combined DTaP vaccine blunted rather than primed subsequent infant pertussis responses,[250] and somewhat reduced Hib and HBsAg responses were also seen following neonatal acellular pertussis priming.[248,251] Thus, vaccine interference issues may be exacerbated in early postnatal life, requiring further studies.[252]

The persistence of immune memory has important implications, especially for infant immunization programs such as for hepatitis B that are intended to protect throughout adult life. The duration of such responses (e.g., the boostability of hepatitis B vaccine antibody responses primed in infancy) extends for at least one decade. However, in the absence of childhood boosters, the boostability of infant-induced immunity may not persist lifelong.[139,140]

Antibody responses elicited before 12 months of age rapidly wane, and antibody titers soon return to near baseline levels,[152,253] which may be associated with a resurgence of vulnerability to infection.[141] This likely reflects the limited survival of antigen-specific plasma cells, as confirmed in infant mice[244] in which early life BM stromal cells provided insufficient survival signals to plasma cells reaching BM niches.[254] Isotype switching and somatic hypermutation (i.e., the affinity maturation of vaccine induced B cells) are already functional in the first year of life,[124,255–257] including in preterm infants.[238] However, several months are required for affinity maturation even in adults,[90] such that high-affinity responses are not observed in very young infants.

Neonatal and infant T-cell responses also differ from those elicited later in life, in particular in the induction of lower IFN-γ[236] and higher Th2 and/or Th17 responses.[258] As examples, IFN-γ responses to OPV are significantly lower in infants than in adults[259]; hepatitis B vaccine induces lower primary IFN-γ responses and higher secondary Th2 responses in early life than in adults[260]; and tetanus-specific IFN-γ $CD4^+$ T-cell responses progressively increase with age.[261] Comparing neonatal and infant priming with acellular pertussis vaccines indicated the preferential induction of Th2 responses on neonatal priming.[262] Whether this results from the fact that neonatal APC responses to Toll-like and other pathogen-associated molecular pattern receptors produce less IFN-α, IFN-γ, and IL-12p70, and more IL-10 than adult cells,[263–265] or result from complex epigenetic controls or the predominance of recent thymic emigrants in neonatal blood,[266] is unknown. The contribution of other factors, such as the predominance of Tregs that are abundant during fetal life[267] and the role of CD71+ immunosuppressive erythroid cells,[268] remains to be defined. Remarkably, adult-like Th1 neonatal responses are notoriously elicited by BCG.[269] Whether neonatal T cells have higher intrinsic requirements for antigen-specific activation require further investigations.

Importantly, the induction of early life B- and T-cell vaccine responses takes place in an environment that may be influenced by the presence of antibodies of maternal origin. IgG antibodies are actively transferred through the placenta, via the FcRn receptor, from the maternal to the fetal circulation.[270] After immunization, maternal antibodies bind to their specific epitopes at the antigen surface, competing with infant B cells and, thus, limiting B-cell activation, proliferation, and differentiation. The inhibitory influence of maternal antibodies on

infant B-cell responses affects all vaccine types, although its influence is more marked for live attenuated viral vaccines that may be neutralized by even minute amounts of passive antibodies.[271] This inhibition is epitope-specific.[272] As a rule, maternal antibodies to carrier proteins (e.g., to tetanus toxoid) blunt infant responses to tetanus toxoid, but not to the PS moiety.[273,274] However, responses to conjugate vaccines may be blunted if anticarrier immunity is required for immunogenicity (e.g., for CRM_{197} conjugates) and maternal antibodies interfere with its induction.[275] Maternal antibodies were reported as inhibiting cotton-rat B-cell responses by interaction with the inhibitory/regulatory FcγRIIB receptor on antigen-specific B cells.[276,277] The extent to which this mechanism accounts for the inhibition of human infant responses remains undefined.

The inhibitory influence of maternal antibodies is dependent on the antibody titer and reflects the ratio of maternal antibodies to vaccine antigen.[90] This was elegantly demonstrated in a study in which Israeli infants were immunized with hepatitis A vaccine at 2, 4, and 6 months.[278] Overall, infant responses were elicited only when maternal antibodies declined to a threshold of 300 to 400 mIU/mL.[278] The maternal antibody titer at which infant responses may be elicited can be defined only experimentally, by comparing antibody responses in infants stratified according to maternal antibody titers at the time of priming. Few vaccines have these precise antibody levels determined by such experimental studies.

The extent and duration of the inhibitory influence of maternal antibodies, therefore, increase with gestational age,[238] for example, with the amount of transferred immunoglobulins, and decline with postnatal age, as maternal antibodies wane.[90] Increasing the dose of vaccine antigen may be sufficient to circumvent the inhibitory influence of maternal antibodies, as illustrated for hepatitis A,[279] measles,[280] and the higher content of pertussis toxin in acellular versus whole-cell pertussis[281] vaccines. However, the higher titers of maternal antibodies elicited by maternal immunization eventually interfere, even with responses to acellular pertussis vaccines.[275,282]

Maternal antibodies usually allow a certain degree of priming (i.e., of induction of memory B cells) through yet undefined mechanisms. As a rule, the blunting of infant antibody responses by maternal antibodies disappears after boosting. Importantly, maternal antibodies do not exert their inhibitory influence on infant T-cell responses, which remain largely unaffected or even enhanced.[283–285] This is best explained by the fate of maternal antibody–vaccine antigen complexes: immune complexes are taken up by macrophages and DCs, dissociate into their acidic phagolysosome compartment, and are processed into small peptides. These peptides are displayed at the surface of APCs and are available for binding by $CD4^+$ and $CD8^+$ T cells.

Thus, the main challenge for further improvement of early life immunization strategies are to identify vaccine formulations and strategies capable of inducing, after one or two early doses, the strong primary antibody responses required against certain early life pathogens—despite the presence of maternal antibodies. Importantly, these formulations/strategies will have to be demonstrated as safe in immunologically immature hosts, adding to the challenges.[286]

Age-Associated Changes in Vaccine Responses. Innate and adaptive antibody and T-cell–mediated cellular immune responses decline with age, which increases the frequency and severity of infections and reduces the protective effects of vaccinations.[287] Aging affects the magnitude and the persistence of antibody responses to protein vaccines,[288,289] as reflected by lower serum antibodies to influenza,[290,291] tetanus, and tick-borne encephalitis (TBE) vaccines.[292] It also affects responses to pneumococcal PS vaccines, although differences in methodological issues have yielded contradictory results.[293] Remarkably, the limitation of antibody responses by aging occurs early: After the age of 20 years, each 10-year period reduced antibody titers elicited by a potent adjuvanted pandemic influenza vaccine in healthy control subjects and immunosuppressed patients by 31%.[294] Limitations of antibody responses in elderly people are also associated with qualitative changes that affect antibody specificity, isotype, and affinity, that is, functional efficacy (see Table 2.10).[295,296]

They result from the influence of a large number of underlying events.[232,297] Responses to PS vaccines are conditioned by a decline in the reservoir of IgM^+ memory B cells that differentiate less efficiently into antibody producing cells, and, thus, limit the IgM responses of aged people.[298] Antibody responses relying on the induction of GCs are also limited,[299] affecting the magnitude of antibody responses and resulting into antibodies of weaker affinities/functional capacities[296] and distribution of subclass antibodies.[300] Numerous factors contribute to limiting the induction of GCs in elderly persons, including factors that are intrinsic to B cells[301] and that affect other cell types, including Tfh cells.[302] For example, studies in aged mice have convincingly demonstrated the existence of age-related changes in FDCs.[303,304] The limited ability of aged subjects to generate high-affinity antibody responses also reflects changes in their antibody repertoire.[304,305]

Age-associated changes in T-cell responses are reflected by a progressive decline in naïve T cells, reflecting declining thymic output. This is associated with a marked accumulation of large $CD8^+$ clones presumably resulting from prior infections. These large T-cell clones (e.g., elicited in response to cytomegalovirus) have reached a state of replicative senescence, and homeostatic mechanisms negatively influence the size of the naïve and effector memory T-cell subsets.[289] In response to influenza immunization, healthy elderly people mount $CD4^+$ responses initially similar to those of young adults but that fail to maintain or expand.[306] This does not reflect a functional impairment of $CD4^+$ T memory cells,[307] but a shift of the T-cell pool from naïve to memory effector $CD4^+$ T cells. The failure to maintain $CD4^+$ responses reflects a lower induction of new Tem cells in relation to lower IL-7 levels.[306,307] Other studies indicated that frail elderly subjects mount blunted and delayed Th1 responses to influenza vaccination, which correlated positively with their reduced total and IgG1 antibody response.[308] Limitations also affect the expansion of infection-driven influenza-specific $CD8^+$ T cells.[308] Strategies to enhance vaccine-induced protection in aging people include the use of higher vaccine doses[309] and/or specific adjuvants. This was recently demonstrated by formulating the IgE glycoprotein of varicella-zoster in the novel AS01E adjuvant.[24] Nevertheless, limitations of effector memory and of GC responses may continue to require the more frequent administration of certain vaccine boosters (e.g., against tetanus or TBE[308]) to compensate for the brevity of B- and T-cell vaccine-induced responses in elderly people.

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3 Correlates of Protection

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The determination of a correlate of protection (CoP) by a vaccine is often critical to its development, as its measurement permits a number of both theoretical and practical extrapolations. Aside from the intellectual interest in identifying the immune response that is protective, knowledge of a CoP permits measurement of consistent lot potency of a vaccine and differentiation of susceptible from immune individuals. If an infection is uncommon or deadly, such that an efficacy trial is not feasible or ethical, a CoP may yet enable licensure of a vaccine, and once established, a CoP will allow bridging from one vaccine preparation to another.

The terminology in this field is confusing, as various definitions have been published, but semantics are important and confusion will be avoided if the definitions used are understood. Table 3.1 gives the terminology used in this chapter, and is crucial to its understanding. Whereas a CoP is simply a variable immune response that is statistically associated with protection, the important distinction is between a mechanistic correlate of protection (mCoP) that is directly responsible for protection and a nonmechanistic correlate of protection (nCoP) that is easy to measure but which may only be a substitute for an mCoP that is unknown or difficult to measure. In addition, a CoP may be absolute, in the sense that there is a threshold value above which protection is certain, or relative in the sense that higher values are quantitatively more protective than lower values but there are occasional failures even at high levels and occasional protection at lower levels. Last, and importantly, there are cocorrelates, meaning that more than one immune function induced by a vaccine correlates with protection in an additive or synergistic way. The reader who wishes to learn more on this subject is referred to previously published articles.[1–6]

The determination of a CoP is sometimes established early in vaccine development, which is the ideal circumstance in that it makes later vaccine development easier, but sometimes a Phase III trial analysis is necessary to identify the CoP. In the latter case, immune responses of vaccine failures are compared with a sample of subjects who did not become infected. Ideally, the immune responses would be measured at the time of exposure to the infection, but most often samples are available only immediately after vaccination. Nevertheless, a useful CoP is often extracted from those data. Other ways in which CoP are inferred include definition of protective levels of passive antibody given parenterally or acquired by an infant from its mother through the placenta, observations made on vaccinated immunodeficient or immunosuppressed individuals exposed to the infection, challenge of vaccinated volunteers by the agent in question, and rarely by extrapolation from challenge of vaccinated animals. Human challenge models have often given critical information about CoP, and should be employed if the challenge is sufficiently attenuated to be ethically acceptable and if a broad range of immune responses are studied. Examples of human challenge that have yielded important information include influenza, cholera, dengue, and cytomegalovirus.

Chapter 2 of this book describes the immunology surrounding vaccination, but the point to bear in mind regarding CoP is that many different functions may serve as a CoP. Serum and mucosal antibodies come in different isotypes and functionality, and T cells, whether $CD4^+$ or $CD8^+$, can act in a variety of ways, including direct action on infected cells, help to B or other T cells, secretion of cytokines, and even down-regulation of immune responses by T regulatory cells. This chapter seeks to simplify immunology in order to identify principles of protection, although the reader should keep in mind that the immune system is complex and redundant.[3] In addition, the reader is directed to chapters on specific vaccines for more extended analysis of each case.

PRINCIPLES OF PROTECTION

Principle 1: Protection Must Be Defined in Relation to Specific Phenomena

Protection against infection may relate to different immune markers than protection against disease. Polio is an example in which paralysis can be prevented by serum antibodies, as the virus must pass from the pharynx or intestines to the central nervous system via blood, whereas infection is prevented by antibodies at the mucosal level, either locally produced immunoglobulin (Ig) A antibodies or diffusion of IgG antibodies onto the mucosal surfaces of the nasopharynx and intestine. Another instructive example is pneumococcal infections. Prevention of bacteremia can be mediated by antibodies in the range of 0.20 to 0.35 µg of antibody as measured by enzyme-linked immunosorbent assay (ELISA). However, prevention of pneumonia, otitis media, and nasopharyngeal carriage may require levels 10 times higher.[7,8]

Principle 2: The Mechanism of Protection by Vaccination Is Not Necessarily the Same Mechanism as Recovery From Infection

The level of antibodies that protect against measles has been defined. A level of 120 mIU of antibody measured by ELISA indicates protection against clinical measles and infection.[9] A level between 200 and 1000 mIU confers protection against measles disease, but not always against infection as indicated by antibody rises; a level of 1000 mIU does provide protection even against subclinical infection. However, vaccinated B-cell–deficient humans do recover from disease, whereas T-cell–deficient humans may suffer continued viremia leading to serious and fatal measles. It has been shown in monkeys that the $CD8^+$ T cells specific for measles are required to suppress viremia.[10]

The poxviruses provide another example. An individual given vaccinia virus requires both B and T cells to overcome the replicating virus and to become immune. However, in a previously vaccinated person only neutralizing antibodies at levels between 1/20 and 1/32 are required for protection, although $CD8^+$ T-cell responses are helpful in the absence of antibodies. Inasmuch as antibody titers decline by 20 years postvaccination, the presence of antipoxvirus T cells enables the vaccinee to have a mild secondary infection in case of exposure.[11–13]

Principle 3: A Large Challenge Dose Can Overcome Immunity

Vaccination against poliomyelitis is usually highly effective in the prevention of paralysis, as stated above, because of the induction of serum antibody. However, gut immunity is imperfect and can be overcome by large infectious doses. A study challenged subjects previously given live vaccine and killed vaccine with two different doses of the oral live vaccine: 800 or 600,000 $TCID_{50}$ (median tissue culture infective dose). The live vaccine recipients were infected by the low-dose challenge 3% of the time and by the high-dose challenge 15% of the time, whereas 30% of the killed vaccine recipients could be infected by a low dose and 70% by a high dose, indicating that high challenge doses can overcome intestinal immunity.[14]

Another challenge study involved an experimental cytomegalovirus vaccine. Seronegative, naturally seropositive, and previously vaccinated subjects were challenged parenterally with 10, 100, or 1000 PFU of a low-passage natural strain of cytomegalovirus. Seronegative subjects were infected with the lowest dose, whereas naturally seropositive subjects were resistant to 10 or 100 PFU; however, some could be infected by 1000 PFU. The vaccine prevented infection by 100 PFU of the challenge virus, but not by 1000 PFU.[15]

TABLE 3.1 Definitions of Terms Used in This Chapter

Term	Definition
Correlate of protection (CoP)	An immune response that is statistically correlated with protection.
Mechanistic correlate of protection (mCoP)	The immune response that is responsible for protection.
Nonmechanistic correlate of protection (nCoP)	An immune response that substitutes for the true immunologic correlate of protection, which may be unknown or not easily measurable.
Absolute correlate	A specific level of response highly correlated with protection; a threshold.

Principle 4: Most Current Vaccines Protect Through Antibodies

Table 3.2 lists the commonly used licensed vaccines and their predominant mechanisms for causing disease. Many viral and bacterial agents reach target organs through viremia or bacteremia; consequently, it is easy to understand that antibodies can prevent that passage. Some agents replicate only on the mucosa, but there, too, the local presence of antibody is preventive. The pathogenesis of toxin-producing bacteria such as diphtheria and tetanus can be restricted by antitoxic antibodies. In the case of rabies, replication occurs in the subcutaneous tissue before attachment to neurons and elicitation of antibodies before that attachment prevents rabies. Only in the cases of zoster and tuberculosis vaccines does it appear that antibodies are not the primary mechanism of control, but that stimulation of specific T-cell subsets correlate with protection.[16] Aside from these theoretical arguments, the fact is that passive administration of antibody works for many infections also preventable by vaccination. Indeed, the efficacy of hepatitis A vaccine was predictable from the fact that induced antibody levels are a thousand times higher than those shown to protect after administration of gamma globulin.[17] As mentioned under Principle 1, higher levels of antibody are necessary to prevent mucosal colonization than disease, as for example, in the case of pharyngeal colonization after *Haemophilus influenzae* type b vaccine.[18]

Principle 5: Correlates May Be Relative

Although high levels of antibodies are more protective than lower levels, after some vaccines breakthroughs may occur at high levels, even if less frequently than at lower levels. Fig. 3.1 shows a retrospective analysis of the relationship between antibody responses to influenza hemagglutinin and protection against disease. At an anti-HA (hemagglutinin) titer of 1/40, generally thought to be an acceptable response, only approximately 50% of vaccine recipients are protected; even at titers four times higher, breakthrough infections occur.[19] Similarly, high levels of pertussis antitoxin after immunization was related to protection both in household exposures and nonhousehold exposures, but mild disease occurred at intermediate levels of antibody. Owing to greater exposure, more

TABLE 3.2 Major Licensed Viral and Bacterial Vaccines for Humans According to the Mechanism of Disease Prevented by the Vaccine

VIRAL		
	Viremia:	Smallpox, yellow fever, measles Mumps, rubella, polio, varicella Hepatitis A, hepatitis B Japanese encephalitis, tickborne encephalitis
	Mucosal replication:	Influenza, rotavirus, human papillomavirus
	Neuronal invasion:	Rabies
	Neuronal reactivation:	Zoster
BACTERIAL		
	Bacteremia:	*Haemophilus influenzae* type b, Meningococcal, Pneumococcal, Typhoid (Vi)
	Mucosal replication:	Pertussis, typhoid (Ty 21a)
	Toxin production:	Diphtheria, tetanus, pertussis Cholera, anthrax
	Macrophage replication:	Tuberculosis

From Plotkin S. Vaccination against the major infectious diseases. C R Acad Sci III. *1999;322(11):943–951.*

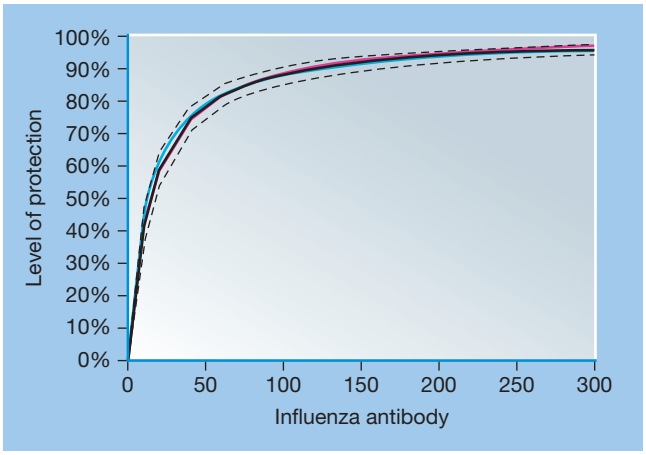


Figure 3.1. Protection against influenza and anti-HA (hemagglutinatinin) antibodies. *(L. Coudeville, personal communication.)*

TABLE 3.3 Pertussis Toxin Antibody as Correlate of Protection Against Pertussis Disease (Modsan Titers)

	Exposure	
Symptoms	**Household**	**Nonhousehold**
Severe	79 U/mL	99 U/mL
Mild	156 U/mL	124 U/mL
None	246 U/mL	155 U/mL

antibody was required to prevent infection within households than outside households (Table 3.3).[20]

Principle 6: Antibodies Must Be Functional

Although neutralization of viruses and killing of bacteria are often mCoP, it has been increasingly realized that there are other important antibody functions. First, Fig. 3.2 shows a case in which neutralization was definitely important, where titers were measured by neutralization or by hemagglutination-inhibition after vaccination with two different strains of rubella vaccines. As can be seen, both strains induced hemagglutination inhibiting antibodies, but RA27/3 stimulated considerably more neutralizing antibodies and proved to be the more protective vaccine.[21]

A study of meningococcal Group C polysaccharide vaccine conducted in Quebec showed the difference in function between antibodies measured by ELISA or by bacterial killing. Adults produced both types of antibodies and were highly protected; children 2 to 5 years old produced more antibodies and had moderate protection; and 1-year-old infants generated ELISA but little bactericidal antibody and had no protection.[22]

A striking example of the importance of antibody function has been reported after an HIV vaccine trial in Thailand. A regimen of priming with a canarypox vector presenting the viral envelope followed by a boost with envelope protein generated little neutralizing antibody, but did generate significant levels of antibody-dependent cellular cytotoxic antibody that potentiates natural killer cell activity. IgG3 antibody-dependent cell-mediated cytotoxicity (ADCC) antibodies directed against the V1-V2 loops of virus were shown to correlate with protection. This illustrated that there are many mechanisms by which antibody may protect.[23–26]

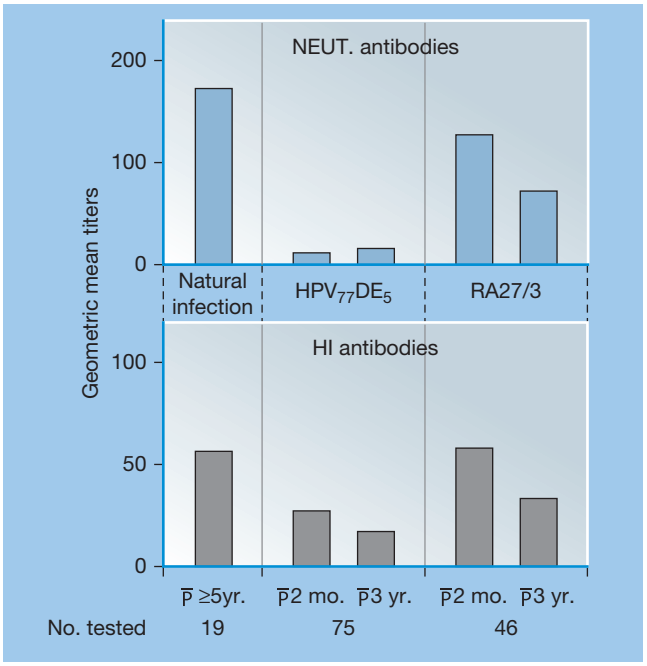


Figure 3.2. Antibody response to rubella vaccines. HI, hemagglutination inhibition; NEUT., neutralization.

Principle 7: T-Cell Responses May Be Correlates

It has usually been assumed that T-cell responses are the important protective correlate for bacille Calmette-Guérin (BCG) vaccine and tuberculosis in general, but it has been difficult to prove that assumption. Interferon-γ secretion has been thought to be important. A study of *Mycobacterium bovis* showed a correlation between interferon-γ–secreting T cells and protective immunity in cows.[27] However, although presumably related to T-cell function, it is uncertain as to which, and the mCoP for tuberculosis vaccine in humans remains elusive.[28–30] On the other hand, malaria is an example where T-cell responses appear to be crucial, and although antibodies to circumsporozoite protein (CSP) are also generated, they do not appear to be a CoP.[31,32] The RTS,S particle containing CSP generates tumor necrosis factor (TNF)-α producing $CD4^+$ cells that appear to predict protection.[33] In contrast, a vaccine based on intravenous injection of irradiated sporozoites depends for efficacy on CSP induction of $CD8^+$ T cells that produce interferon in the liver, where natural sporozoites must first replicate to launch infection.[34]

Although vaccination against chlamydia and tularemia bacteria is still experimental, there is abundant evidence that T-cell responses will be necessary for efficacy.[22,35–37]

There is clearly much still to be learned about T-cell responses as correlates because of the numerous functional classes of these cells. Although $CD8^+$ T-cell cytotoxicity is clearly one of the most important protective functions, $CD4^+$ T cells exist in many varieties with different helper functions for B and T cells that influence protection directly or indirectly through cytokine secretion.[38]

Principle 8: More Than One Factor May Protect as Cocorrelates

A good example of cocorrelates may be derived from studies of influenza. The live, attenuated intranasal vaccine generates both serum antibody and nasal IgA mucosal antibody.[39–41] In

vaccinated children challenged with another dose of the intranasal vaccine, virus shedding occurred at a high rate in vaccinees lacking both responses, at a low rate in vaccinees having both responses, and at an intermediate rate if only one response was present. Thus, the two responses were synergistic, or at least additive. In the case of inactivated influenza vaccine, two separate antibody responses seem important: that against the viral hemagglutinin and that against viral neuraminidase.[42–44] The former block virus entry and the latter block virus exit from the cell. Antibody responses may be poor in the elderly, but granzyme produced by $CD8^+$ cytotoxic T cells are also protective against symptomatic infection by limiting replication.[45–48]

Pertussis vaccines, whether whole cell or acellular, contain multiple components of the bacteria. Toxins and adherence factors are important in pathogenesis, and studies show that whereas antibodies against any of these reduces risk, antibodies against multiple virulence factors reduce risk even more.[49–51] T-helper cell (Th) 1 and Th17 responses also are important for duration of protection.[52–54]

A recent illustration of probable cocorrelates is the chimpanzee adenovirus vectored vaccine against Ebola virus. Whereas monkeys that generate ELISA titers of 2000 or more almost always survive an Ebola challenge, passive antibody did not protect and it appeared that $CD8^+$ T cells are needed for protection, particularly of antibody responses are low.[55–58]

Principle 9: Memory May Be a Mechanistic Correlate of Protection

Hepatitis B vaccine is highly immunogenic at birth, but antibodies are frequently lost later in life. Nevertheless, vaccinees are usually protected because they have an anamnestic antibody response when exposed to the virus.[59–62] Indeed, memory B cells are demonstrable in hepatitis B vaccinees in the absence of serum antibody.[63,64]

An interesting discovery was made recently in simian immunodeficiency virus (SIV)–infected rhesus monkeys. A rhesus cytomegalovirus vector containing various genes of SIV was constructed. The vector induced effector $CD8^+$ T cells directed against the SIV proteins and was able to abort early SIV infections in the monkeys.[65–67] Thus, effector memory of T or B cells is important in diseases for which dissemination occurs early, such as HIV, *H. influenzae* type b and meningococcus, but central memory is important in long incubation period diseases such as hepatitis B that allow for mobilization of an anamnestic response.

Principle 10: There Are Convenient Nonmechanistic Correlates

Zoster is the result of reactivation of varicella virus from dorsal spinal ganglia. The current vaccine consists of live varicella virus in large quantity or the gE protein of the virus. In both cases the vaccines elicit both antibodies and cellular responses. The best CoP is a $CD4^+$ T-cell lymphocyte proliferation index, but there is also a statistical correlation with antibodies to viral glycoproteins.[68,68a,68b] This suggests that the cellular immunity is an mCoP, whereas the antibody is an nCoP, which makes sense if the vaccines maintain the viral latency in the dorsal root ganglia, but it is not impossible that the neutralization of virus by antibody in the skin is also important.

Another example is from rotavirus vaccination. IgG antibodies can prevent infection when given orally or induced by inactivated rotavirus but the replicating live virus vaccines induce mucosal antibody and are effective.[69] However, measurement of mucosal responses is difficult, whereas measurement of serum IgA antibodies is easy. Thus, in the absence of a generally agreed mCoP, serum IgA provides a very useful nCoP.[70–72]

STATISTICAL ISSUES IN DEVELOPING IMMUNE CORRELATES OF PROTECTION

Developing CoPs involves statistical issues in two main areas: (a) development and selection of immunological assays and associated immune response biomarkers meriting study as correlates in preventive vaccine efficacy trials; and (b) statistical evaluation of the selected biomarkers in efficacy trials for their utility as CoPs. For area (a), statistical power for detecting a CoP is eroded for biomarkers with low signal-to-noise ratios[73,74] (e.g., from technical assay measurement error), making the standardized statistical evaluation and optimization of assays within and prior to efficacy trials fundamental for developing CoPs.[25,75–78] Moreover, with systems vaccinology research measuring high-dimensional data on immune responses to vaccination,[79–87] an important area of statistical research is classification of vaccinees into low-dimensional biomarker signature groupings[79,88–90] for assessment as CoPs. From now on we focus on area (b).

In the clinical trials statistical literature, a CoP is an immune response biomarker measured after vaccination that can be used to reliably predict vaccine efficacy (VE) against a clinical end point. Several approaches to defining and statistically evaluating CoPs based on efficacy trials have been proposed.[4,91–103] For CoP assessment, these approaches have been applied to search for thresholds of the biomarker that discriminate vaccinees into disease cases (and hence unprotected) versus undiseased controls (and hence putatively protected),[9,104–108] or to study how protection depends on the distribution of response.[109–111] However, many of these CoP methods do not define protected biomarker subgroups in terms of a causal effect of vaccination, such that an association of the biomarker with disease may not reflect a correlation with VE but rather with pathogen exposure or intrinsic susceptibility to the disease.[4,112–115] Addressing this limitation, the "VE curve" approach directly assesses how VE varies across vaccinated subgroups defined by the level of the biomarker.[4,113–119] With this approach, the most useful CoP has VE of zero for vaccinees with negative/absent immune response and VE near 100% for vaccinees with response above a threshold. A varicella zoster vaccine (VZV) efficacy trial[120] illustrates this approach, where VE to prevent herpes zoster varied widely with fold rise in VZV antibody titers ($P < .001$), with estimated VE of 90% for vaccinees with a 5.26-fold rise compared to 70% and 0% for vaccinees with a 2.39- and zero-fold rise, respectively.[6] These results provide a benchmark for high protective efficacy. As noted above, a useful CoP may or may not be a mechanism of protection.[6]

The major challenge of the VE curve approach is that it requires estimation of how the risk of the clinical end point for a placebo recipient depends on an unmeasured variable—the immune response to the vaccine that the individual would have had, if, counter to fact, the individual had been randomly assigned to receive the vaccine. Consequently, the VE curve statistical methods incorporate techniques for "filling in" the counterfactual immune responses of placebo recipients.[113,114,117,119,121–127] The "baseline immune response predictor" technique develops a model from vaccine recipients predicting individual immune response biomarkers from baseline variables, and applies this model to placebo recipients using these baseline variables. The "closeout placebo vaccination" technique crosses over placebo recipients at the end of study follow-up to receive the vaccine and have the immune response directly measured. Crucially, effective use of these approaches requires storing baseline samples in (almost) all

efficacy trial participants, to make possible measurement of preimmunization predictors of the candidate CoP in most disease cases; unavailability of baseline samples has been one of the greatest limiting factors for CoP assessment in efficacy trials.

We conclude by listing five other statistical issues that are faced in developing CoPs in efficacy trials. First, immune responses are typically only measured in a case-control[128–130] or case-cohort[131–134] subsample, motivating research into optimal sampling design[117,135–139] and analysis[129,130,140] methods.

TABLE 3.4 Correlates of Protection After Vaccination[a]

Vaccine	Immune Function	Protection Level	Type
Adenovirus	Nt Ab	1/4	M
Anthrax	Toxin Nt Ab, anti-PA IgG	1/3000, 10 μg/mL	M
Cholera	Vibrioicidal Ab	?	NM
Dengue	Nt Ab	Variable	M
Diphtheria	Toxin Nt Ab	0.01–0.1 IU/mL	M
Ebola	Ab + CMI	ND	M
H. influenzae conjugate	ELISA Ab[d]	0.15 ng/mL	M
Hepatitis A	ELISA Ab[d]	20 mIU/mL	M
Hepatitis B	ELISA Ab[d]	10 mIU/mL	M
Human papillomavirus	ELISA Ab[d]	Ab, level ND	M
Influenza, inactivated	HI Ab NtAb	1/40 = 50% protection, 1/320 in children 1/40 = 50% protection	M M
Influenza, live	HI Ab, IgA Ab, CMI	ND	M
Japanese encephalitis	Nt Ab	1/10	M
Lyme	ELISA Ab	1400 U/mL	M (in tick)
Malaria	ELISA Ab $CD4^+$ T-cell number	>10 U/mL ?	M M
Measles	ELISA Ab[d]	≥120 miU/mL	M
Meningococcal	Bactericidal Ab	≥1/4	M
Mumps	Nt Ab	ND	M
Pertussis	Ab to PT, Prn, Fim Th1 T cells	ND ND	M M
Pneumococcal, conjugated	ELISA Ab	0.20–0.35 μg/mL	M (invasive disease)
Pneumococcal, polysaccharide	OPA Ab	ND	M
Polio, inactivated	Nt Ab	≥1/8	M
Polio, live	Nt Ab	≥1/8	M
Rabies	Nt Ab	≥0.5 IU	M
Rotavirus	Serum secretory IgA	ND	NM
Rubella	ELISA Ab	≥10–15 IU/mL	M
Smallpox	Nt Ab[c]	≥1/20–1/32	M
Tetanus	Toxin Nt Ab	0.01–0.1 IU/mL	M
Tick-borne encephalitis	Nt Ab	≥1/10	M
Tuberculosis	T-cell responses	ND	
Typhoid	[b]	ND	M
Varicella	GP ELISA[c,d]	≥5 U/mL	M
Yellow fever	Nt Ab	≥0.7 LNI	M
Zoster	$CD4^+$ T cells	ND	M

[a]Also see specific chapters for references.
[b]Depending on the specific vaccine, protection against typhoid is mediated by antibodies to flagella, the Vi capsule, O antigen, as well as cell-mediated immunity.
[c]Cellular responses also important.
[d]Surrogate for neutralizing antibodies.
Ab, antibodies; CMI, cell-mediated immunity; ELISA, enzyme-linked immunosorbent assay; FIM, fimbrial agglutinogens; GP, glycoprotein; HI, hemagglutination inhibition; Ig, immunoglobulin; LNI, log neutralization index; M, mechanistic; MNt, microneutralization; ND, not defined; NM, nonmechanistic; Nt, neutralization; OPA, opsonophagocytic; Prn, pertactin; PT, pertussis toxin.

Second, statistical methods are needed to develop cocorrelates, for example, by estimating VE surfaces across vaccinee subgroups defined by the level of two immune response biomarkers[114,119] or estimating VE curves for a univariable composite biomarker.[126] Moreover, for assessing complex correlates[3] based on high-dimensional immune response biomarkers, supervised machine learning methods are needed to estimate the best models of VE.[88,89,141,142] Third, two types of CoP analyses are defined by measuring the immune response biomarker at a fixed time point shortly after vaccination or at serial time points including just before exposure and infection or disease. Both analyses are important, the former primarily for developing practicable VE-predictive end points and the latter primarily for generating clues about mechanistic CoPs. Fourth, a highly predictive CoP for a particular disease end point, vaccine, and study population may fail for a different end point, vaccine, or study population,[143] indicating that metaanalysis of multiple efficacy trials combined with knowledge of mechanisms of protection is needed for bridging CoPs to new settings.[4,55,94,97,106,115,144,145]

Lastly, for genetically diverse pathogens, the whole discussion above applies to assessing CoPs against particular phenotypes or genotypes of the pathogen (e.g., through pathogen type-specific VE curves), with common application historically to assess serotype- or biotype-specific antibody titers as CoPs against disease with the same type.[146–151] In contemporary efficacy trials, pathogen genomes infecting study participants are sequenced, enabling a statistical sieve analysis that assesses how VE varies with sequence characteristics.[152] Immune response data can inform the sieve analysis by focusing the analysis on putatively protective epitopes such as highly vaccine-reactive peptides or specific reagents that were identified as CoPs.[153,154] Moreover, host characteristics other than immune responses (such as demographics, ecological factors, or genetics such as human leukocyte antigen [HLA] types restricting epitope-specific T-cell responses) may affect VE or type-specific VE, such that the overarching goal of VE curve CoP analysis may be stated as estimation of how VE varies jointly in pathogen sequence characteristics, immune response biomarkers, and any other host characteristics.[3,73,87,155]

COMPLEXITY OF CORRELATES

As Einstein said, "Everything must be made as simple as possible, but not simpler." We have attempted above to reduce this subject to simple principles, but one must recognize that in practice the determination of CoPs is difficult. First, it is necessary to repeat that CoP are not restricted to neutralizing IgG antibody or $CD8^+$ T cell cytotoxicity. Other immunologic responses, such as antibodies that stimulate natural killer cells,[156] Th17 T cells that prevent mucosal infection,[157] and T cells that secrete cytokines are examples of possible CoP.[158]

The CoP for influenza vaccine appears at first to be straightforward: hemagglutination-inhibiting antibody or perhaps neutralizing antibody. However, in children it appears that higher levels of antibody are necessary, perhaps because of the absence of prior influenza infections.[159] In the elderly the situation is even more complex, owing to immunosenescence, such that the presence of cytotoxic T cells may be necessary to protection against serious disease in the presence of a weak antibody response.[46]

A CoP for pertussis vaccine has long been controversial, but the use of acellular vaccines with a variable number of components has been educational. As stated above it appears that high-titered antibodies against pertussis toxin, pertactin, and fimbrial hemagglutinogens each provide some degree of protection and that there is synergy among those antibodies.[52] However, a T-cell response is critical to duration of antibody and to prevention of nasopharyngeal carriage, and Th17 cells are also important based on evidence from mouse and baboon models.[160,161] Thus, B and T cell responses act together for the best efficacy.[162]

The importance of $CD4^+$ T cell immunity to protection by the RTS,S malaria vaccine based on CSP was discussed above. However, protection induced by the liver stage multiple epitope-thrombospondin–related adhesion protein (ME-TRAP) antigen is mediated by $CD8^+$ T cells[163] and the merozoite MSP2 and -3 antigens act by induction of opsonophagocytic antibodies.[164,165]

In line with the doctrine that the immune system is redundant, there are multiple situations in which antibody is the first line of defense and therefore an mCoP, but where cellular immunity provides a backup in the event antibodies are low. Such may be the case for Ebola virus and yellow fever virus.[56,166] For some other agents the two arms of the immune system are synergistic, as in the case of the RTS,S malaria vaccine that induces both types of response against the circumsporozoite antigen of the parasite.[167,168] In other cases, the CoP varies with the type of vaccine. Whereas as described above, the canarypox vector/HIV protein vaccine for HIV depends for its efficacy on an ADCC antibody response,[156] a rhesus cytomegalovirus vectored vaccine that protected monkeys against SIV acts through induction of effector T cells.[66,67] In the case of human cytomegalovirus antibody to surface proteins protect against acquisition by contact,[169] but cellular immunity to internal antigens seems to be key in preventing transplant-associated disease in hematogenous stem cell recipients.[170]

Also still to be defined are CoP relating to mucosal immune responses. Mucosal IgA antibody produced by vaccination has been identified to be important to protection against influenza[39,171] polio,[172] and respiratory syncytial virus,[173] and probably is important for rotavirus vaccine.[70,174] Serum IgG antibody diffusing onto respiratory, gastrointestinal, and genital mucosae undoubtedly also can prevent infection but is poorly characterized.

CONCLUSIONS

The immune system is redundant, but the mCoP after vaccination in most cases is antibody that blocks the agent in the blood. Antibodies have varying functions and the mCoP will vary according to the vaccine. Cellular immunity may synergize with antibody or make up for its lack. Effector T-cell memory may be necessary for short incubation period diseases, whereas central memory with an anamnestic response is sufficient for long incubation period diseases. For some vaccines we do not know the true mCoP but we can use a nCoP that is not protective but is statistically correlated with protection. In general, there is a spectrum of correlates, ranging from pure antibody to the protective antigen in anthrax vaccine to the resurrection of cellular immunity to varicella virus that results from immunization with zoster vaccine.

Table 3.4 gives our best estimates of CoPs for currently licensed vaccines.

References for this chapter are available at ExpertConsult.com.

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4 The Vaccine Industry

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The vaccine industry is composed of companies that are engaged in any of the following activities: research (including that performed in industry and biotech), development, manufacture, or sales, marketing, and distribution of vaccines. They receive their revenue chiefly from sales of vaccine products or expectations thereof. The vaccine industry is relatively small, compared to the pharmaceutical industry, but growing. We estimate that total infectious disease vaccine sales in 2013 were more than $25 billion worldwide and expected to grow to about $35 billion by 2020. Although components of the vaccine industry are found in 50 countries worldwide, the large vaccine companies are primarily U.S.- or European-based and have the dominant share of vaccine business on a revenue basis; but regional companies are gradually growing their market share on a dose basis (Table 4.1).[1]

In the past 20 years, the vaccine business, a former laggard in the pharmaceutical business, has shown remarkable growth powered by new innovative vaccines coupled with superior pricing strategies (Fig. 4.1).[2] Specifically contributing to this spectacular growth were the varicella, hepatitis A, pneumococcal conjugate, shingles, rotavirus, meningococcal conjugate for A, C, Y, W, and human papillomavirus (HPV) vaccines, as well as myriad combination vaccines.

This projected growth may plateau in the early 2020s unless the vaccine industry continues to introduce new innovative products targeting diseases that impact the Western world. Sustaining this growth will be a challenge because of dwindling numbers of high-value vaccine targets for which the biology of protection is well understood (see Table 4.7).

The vaccine business is a capital-intensive business that requires considerable ongoing investment in manufacturing assets, facilities, and people to maintain compliance with ever-increasing regulatory directives. The recent departure of Baxter and Novartis from the vaccine industry is an ominous sign that reflects the continued financial pressure on the remaining four major vaccine makers. Further consolidation of this business is likely. In addition, new alliances will be formed between the big four manufacturers and emerging companies in India, China, and Brazil, to take advantage of increasing immunization rates in those countries as well as growth of their private markets.

The United States has been extraordinarily successful in vaccine research and development (R&D).[3,4] In the past 20 years, most new vaccines approved worldwide were developed in the United States. Approximately 15 new vaccines were approved in the United States between 1995 and 2014.[5,6] Since then, combinations of existing vaccines have been introduced for simplified pediatric vaccination resulting in a wider adoption of acellular pertussis vaccination. A polyvalent pneumococcal conjugate vaccine for infants introduced by Wyeth (now a subsidiary of Pfizer) has been widely adopted and has made Pfizer a major force in the vaccine business. Since 2006, several new vaccines have been licensed, including a combination of measles, mumps, and rubella (MMR) and varicella, as well as new vaccines against rotavirus, herpes zoster, HPV, meningococcus, influenza, and others. The HPV vaccines developed by Merck and GlaxoSmithKline significantly expanded the field of adolescent vaccines and confirmed market acceptance of premium pricing.

In the last 10 years, the vaccine industry in the United States and Europe has considerably improved its reliability as a supplier. Chronic shortages are a thing of the past; this turnaround has primarily been achieved by modernization of vaccine manufacturing and distribution infrastructure supported and funded by the profitability of the vaccine business. The Centers for Disease Control and Prevention (CDC) stockpiling of pediatric vaccines has alleviated some concerns of critical shortages in case of supply interruptions. But the industry's vulnerability because of dependence on single-sourced vaccines continues to be an unresolved concern. The regulators and the industry must proactively develop a solution to this critical challenge and avoid any future public health crisis resulting from vaccine shortages during a prolonged supply interruption.

VACCINE DEVELOPMENT

Vaccine development is difficult, complex, highly risky, and costly, and includes clinical development, process development, and assay development. The risk is high because most vaccine candidates fail in preclinical or early clinical development and less than 1 in 15 vaccine candidates entering Phase II achieves licensure. The high failure rate is the result of a variety of reasons:

1. Not fully understanding the biology of protection.
2. Lack of good animal models to predict vaccine behavior in humans.
3. Unpredictability of human immune system reactions to antigens as it relates to immunogenicity or safety.
4. The unpredictability of the impact of combining multiple components in a vaccine.

Vaccine development requires strong project management systems and controls and requisite skill sets among scientists and engineers. A key strategic document that guides the stakeholders in vaccine development is the "target product profile" (TPP). The TPP summarizes the desired characteristics and features of the product under development, the key attributes of the product that provide competitive advantage, and, finally, a topline roadmap of nonclinical and clinical studies required to evaluate the products efficacy and safety in the target population. A well-defined TPP provides all the stakeholders, including research, process development, manufacturing, clinical, regulatory, and senior management, with a clear statement of the desired outcome of the product development program.

Process development involves making preparations of the test vaccine that satisfy regulatory requirements for clinical testing including clinical lots, preclinical toxicology testing, and analytical assessment, and finally, scale-up methods that lead to a consistent manufacturing process at one-tenth of full scale. Usually three consecutive lots are tested in the clinic for immunogenicity. Assay development involves the definition of specific methods to test the purity of raw materials, stability and potency of the vaccine product, and immunologic and other criteria to predict vaccine efficacy. Go/no-go decisions must be made at each stage of clinical and process development and

must be data driven. Clinical, process, and assay development tasks must be closely integrated. Clinical development involves studies of the effects of vaccines on patients for safety, immunogenicity, and efficacy through a staged process: phase 1, early safety and immunogenicity in small numbers; phase 2, safety, dose ranging, and immunogenicity in 200 to 400 individuals; sometimes phase 2b, nonlicensure, proof-of-concept trials for efficacy; and phase 3, safety and efficacy trials that permit licensure, which generally require thousands of subjects.

"Process" can be broadly divided into two categories: bulk manufacturing and finishing operations. Bulk manufacturing includes cell culture and/or fermentation-based manufacturing followed by a variety of separation processes to purify the vaccine. The finishing operations include formulation with adjuvant/stabilizer followed by vial or syringe filling (including lyophilization in the case of live viral vaccines) followed by labeling, packaging, and controlled storage. Process development may be as costly as clinical development and is critically important to the overall success of a vaccine development program. As development proceeds toward licensure, costs escalate as clinical studies become larger, manufacturing scales up, and facilities must be built. Postlicensure studies of safety and efficacy (phase 4) of vaccines are essential and represent a large additional cost. It is important to note that, unlike pharmaceuticals, vaccines that pass early proof-of-concept studies in humans have a very high probability of achieving licensure.

Clinical activities are more visible than bioprocess development and clearly drive the go/no-go decisions that direct progress. The two are interwoven and each has rate-limiting steps, so they must be done in concert.

The first stage of vaccine development involves acceptance of a candidate from a basic research laboratory and development of a small-scale process and formulation to make material for Phase I study, analytical release assays, preclinical toxicology, immunological assays to evaluate clinical responses, an investigational new drug (IND) filing, and well-designed Phase I/IIa studies.

The second step is to complete the definition of product and process prior to initiation of Phase II dose-ranging studies, which may take a year or more. Product definition includes methods of synthesis/bioprocess steps, number of components, and stability/formulation. Stability, release, and raw material assays must be in place. Immunologic and other assays must be established to support dose-ranging studies, and a regulatory plan for vaccine process and product submissions must be written.

The third step is to define the clinical dose and arrive at the appropriate manufacturing scale, which may take 2 years or more. It results in the identification, manufacture, filling, and release of clinical-grade vaccine—usually in a pilot plant—demonstration of safety and a dose response in a Phase II clinical study; validation of critical assays to support Phase III clinical studies; consistency of lot manufacture (ability to produce three or more consecutive production-scale lots that meet all product specifications based on validated analytical methods); and completion of technology transfer to final site

TABLE 4.1 Market Shares of Vaccine Companies, 2014

Company	Year-End Earnings ($ Billion)[a]	Market Share (%)
GlaxoSmithKline	5.3	19.7
Merck & Co.[b]	6.2	23.4
Novartis	1.5	5.7
Pfizer	4.5	16.8
Sanofi[†]	5.8	21.9
Others	3.4	12.6
Total	**26.7**	**100**

[a]Company 2014 year-end earnings releases from EvaluatePharma (http://www.evaluategroup.com).
[b]Each includes 50% of revenues from Sanofi Pasteur MSD joint venture.

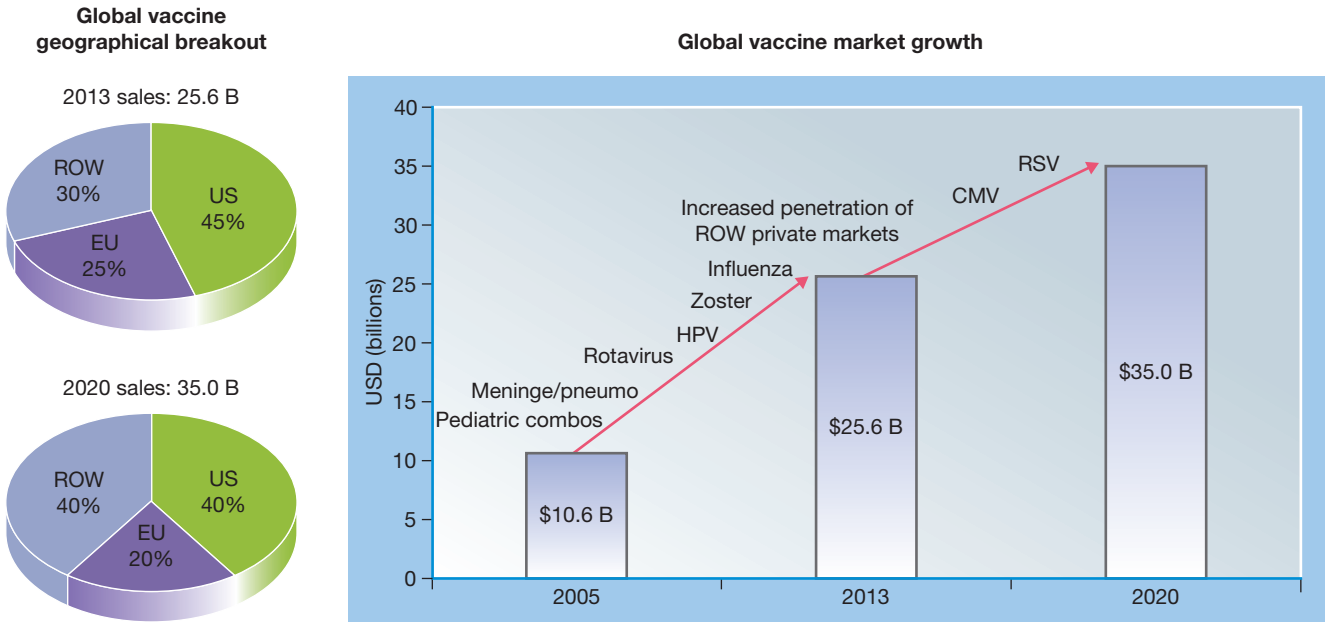


Figure 4.1. Global vaccine market growth. Worldwide projected vaccine business growth from 2005 to 2020. B, billion; EU, European Union; ROW, rest of world; US, United States; USD, U.S. dollars. *(Company earnings releases and presentations, EvaluatePharma research;* http://www.evaluategroup.com.*)*

of manufacture of full-scale lots, including process and analytical procedures. For vaccine targets for which animal studies are not predictive of efficacy in humans, such as HIV, malaria, and tuberculosis (TB), small Phase IIb proof-of-concept studies based on adaptive clinical trial designs may be used to gain confidence before committing significant resources for process development, analytic development, and factory construction.

In general, the analytical and release assays are particularly difficult to develop because, in most cases, vaccines are considered "not well-characterized" biologicals by regulatory agencies. The release assays initially involve functional potency assays such as animal immunogenicity prior to acceptance of more robust and precise in vitro assays that correlate with these functional potency assays. In general, variability of biological assays is a major hurdle in achieving process scale-up and manufacturing consistency.

The fourth stage is the completion of Phase III pivotal clinical studies and corresponding consistency lot studies, which requires 3 to 5 years. Keys to successful Phase III clinical studies are an accurate estimate of sample size based on disease incidence, low dropout rates, precise clinical end point definitions related to future label claims, and rigorous data management to the highest standards. In addition to clinical studies, scale-up and manufacture of consistency lots, including transfer to the facility of all assays, facility validation, demonstration of consistency and real-time stability are needed to support adequate shelf-life claims.

The final stage is Biologics License Application (BLA) preparation, licensure, and vaccine launch, which requires 1.5 to 2 years. Thus the total elapsed time for development is 10 to 15 years, assuming all activities proceed as planned.

Manufacturing plants are very expensive to construct, ranging from $50 million to $300 million depending on the size (dose requirements) and manufacturing complexity, with an additional expenditure of approximately 20% of that cost for cleaning and process validation activities that are now required under the current good manufacturing practices regulations. With few exceptions, each vaccine requires a different plant because of unique manufacturing requirements and the regulatory difficulties associated with changing over to a different product. Some processes are scalable, such as bacterial or yeast fermentation, so that increasing the size of the manufacturing unit (i.e., fermenter) will greatly increase the yield; unit cost will decrease with volume increase. Other manufacturing processes, for example, those dependent on viral growth in embryonated hen eggs or cell lines, are not scalable. Additional plants or modules within plants must be built to increase the throughput, so unit costs do not appreciably decrease with volume increases. Despite the complexity of bulk vaccine manufacturing, 3 to 5 years post–product launch, the fully burdened bulk cost of production for most of the older vaccines declines to as little as $0.50 to $1.00 per dose, and significant elements of product cost are primarily driven by activities related to filling, vialing, and packaging (Table 4.2). Established vaccines with a limited number of suppliers can generate very high profit margins over the product life cycle.

The commitment to build a plant must be made early (4 to 6 years before expected licensure) including a 6- to 12-month finished goods inventory build-up to expedite product to the market. Otherwise a gap of 1 to 5 years between licensure and product launch will occur.

Furthermore, it is far better to produce consistency lots in the final vaccine production factory to demonstrate the ability to manufacture the vaccine reliably and to use those lots in the Phase III efficacy trials. Otherwise, immune studies will be required for "bridging" the product used in the efficacy trial to material manufactured in the commercial factory. This is especially difficult if immune studies are not highly reproducible, as is the case with most cellular immune assays. Such decisions pose large financial risks if the product in development fails and requires access to large amounts of capital, an attribute usually restricted to large pharmaceutical companies.

TABLE 4.2 Vaccine Product Cost

	$/Dose
Bulk[a]	0.20–3.00
Fill/finish[b]	1.00–1.50
Syringe fill (optional)[c]	1.00–2.00
Total cost[d]	2.20–6.50

[a]Bulk range reflects older vaccines such as measles-mumps-rubella (MMR) and hepatitis B, at the low end, to newer vaccines such as shingles and live attenuated influenza at the high end.
[b]Fill/finish range reflects differences in speed, volume, and efficiency of operations.
[c]Syringe-filled product reflects cost of syringe and reduced line efficiency.
[d]Estimated fully burdened manufacturer's cost for U.S.-based operations in 2012.

TABLE 4.3 Vaccine Development Time Lines

Vaccines	Years to Approval
Varicella	25–30
FluMist	25–30
Human papillomavirus[a]	14–16
Rotavirus[a]	14–16
Pediatric combination vaccines	10–12

[a]From filing of first investigational new drug to approval.

Estimates of cost of development of a new drug or vaccine have risen from $231 million in 1991, to $802 million in 2003, to $1 billion in 2010.[7–9] These estimates take into account all costs, including R&D costs of products that fail, postlicensure clinical studies, and improvements in manufacturing processes. Approximately 50% of the cost is for construction; the remainder is the cost of capital interest. These numbers have been debated (others estimate $100 million to $200 million); however, the higher estimates have been validated in two ways. First, the number of new vaccines brought to licensure annually by a company or the industry is very small compared with other products, and correlates with R&D expenditures of $600 million to $800 million for each new product. Thus, if a company spends $100 million annually for vaccine R&D, one might expect one new product every 6 to 8 years, and this appears to hold true. Second, biotechnology companies that are focused on one vaccine and have successfully brought it to market have spent $500 million to $700 million on R&D as exemplified by the development of the live attenuated influenza vaccine by Aviron, now Medimmune. In summary, vaccine development from concept to licensure is a lengthy process as illustrated by timelines for some of the currently licensed vaccines (Table 4.3).

ROLE OF PARTNERS

To understand the predominant role of major pharmaceutical companies in the development of vaccines, one must examine the role of a vaccine development company in relation to its

TABLE 4.4 U.S. Network Partners' Relative Contributions to Vaccine Research and Development

	Research			Development		
	Basic/Related	Targeted	Process	Clinical	Manufacture	Postlicensure Studies
NIH	+++	+++	—	++	—	—
CDC	—	—	—	—	—	++
FDA	—	+	+	+	—	+
DOD	+	+	+	+	—	+
USAID	—	+	—	+	—	—
Large company	+	+++	+++	+++	+++	+++
Small company	+	+++	±	±	±	—
Academia	+++	+++		+++	—	—
NGOs (PDPs)	—	+	±	+++	±	—

CDC, Centers for Disease Control and Prevention; DOD, Department of Defense; FDA, U.S. Food and Drug Administration; NGO, nongovernmental organization; NIH, National Institutes of Health; PDP, product development partnerships; USAID, U.S. Agency for International Development.
Relative contribution: +++, major; ++, intermediate; +, minor; ±, varies by company.
Modified from Marcuse EK, Braiman J, Douglas RG, et al, for the National Vaccine Advisory Committee. United States vaccine research: A delicate fabric of political and private collaboration. Pediatrics. *1997;100:1015–1020.*

partners. The relative contributions of the various partners to the delicate fabric of vaccine R&D is shown in Table 4.4.[10] Several branches of the U.S. government play major roles in vaccine R&D.

The U.S. National Institutes of Health (NIH) is the major funding source via intramural and extramural (largely academic) programs of fundamental research (e.g., gene-based vaccines or T-cell memory studies) and directed research on pathogens (e.g., HIV), which may lead to new vaccine candidates. The NIH, through its vaccine trials network, has increased its role in clinical development domestically and internationally. In addition, the Dale and Betty Bumpers Vaccine Research Center at the NIH was established in 1999 primarily to pursue the development of HIV vaccines.

The Centers for Disease Control and Prevention (CDC) is the primary government agency responsible for epidemiological monitoring of disease trends. The CDC conducts disease surveillance and epidemiological studies to ascertain the prevalence and incidence of specific diseases; this information provides a rationale for prioritizing vaccine development. These studies by the CDC are performed in addition to studies conducted by the vaccine companies, such as Phase IV studies. Through the Advisory Committee on Immunization Practices (ACIP), the CDC recommends usage of vaccines, and is responsible for most of the public purchases (directly through the Vaccines for Children program for approximately 41%, and indirectly through other federal, state, and local government purchases for approximately 16%, together totaling approximately 57% of all childhood vaccines in the United States), thereby playing a major role in determining the demand and potential profit associated with vaccines. Professional organizations such as the American Academy of Pediatrics and the American Academy of Family Physicians also make recommendations for vaccine usage. There is no federal vaccine program for adults, although Medicare does reimburse for influenza and pneumococcal conjugate vaccines. Historically, many adults with private insurance were not covered for immunizations. However, the Affordable Care Act of 2010 requires health plans to cover vaccines recommended by the ACIP prior to September 2009 with no copayments or other cost-sharing requirements when those services are delivered by an in-network provider.

The Department of Defense (DOD) does targeted vaccine R&D to help it perform its mission of protecting deployable personnel and their families against infectious disease threats in the United States and abroad. Thus, the DOD assesses infectious disease risks in specific theaters and establishes prioritization of vaccine targets, especially those not being funded and developed in the private sector.

The U.S. Army Medical Research and Materiel Command (USAMRMC) is a major DOD organization conducting basic and applied medical research programs supporting military operations. The U.S. Army Medical Material Development Activity is its advanced product development agency, which aligns closely with the Walter Reed Army Institute of Research, the U.S. Army Medical Research Institute for Infectious Diseases, and the Naval Medical Research Center in conducting or supporting surveillance studies and vaccine trials. USAMRMC's longstanding overseas laboratories (e.g., in Thailand and Kenya) provide opportunities for the United States to partner with host nations in the development and evaluation of vaccines of shared interest. Some of the more recent efforts have focused on vaccines against malaria, dengue, HIV, norovirus, and Ebola. The Biomedical Advanced Research and Development Authority (BARDA) within the Health and Human Services Department was established in 2006 to facilitate development and purchase of vaccines and other products for public health emergencies. BARDA also manages Project Bioshield for the procurement of advanced medical countermeasures for biological as well as other threats and has successfully developed medical countermeasures against smallpox, anthrax, and botulinum toxin. In addition, BARDA is funding a variety of early stage novel vaccine approaches for pandemic influenza. BARDA essentially is intended to overlap with and close the gap between NIH-funded preclinical or initial Phase I trials and the more advanced Project Bioshield programs that are in late stage Phase III or licensure stages of development.

The U.S. Agency for International Development (USAID) supports limited R&D targeted toward those vaccines that potentially will have the greatest impact on children younger than age 5 years in developing countries. The Center for Biologics Evaluation and Research (CBER), a division of the U.S. Food and Drug Administration (FDA), is responsible for licensing new vaccines. CBER establishes standards for manufacturing processes, facilities, and pre- and postlicensing clinical studies to ensure that licensed vaccines are safe and effective (see Table 4.4). These standards have a profound

impact on the nature and direction of vaccine development and its costs. In addition, CBER maintains a strong research base internally, so it is better positioned to evaluate data from various studies. CBER remains the premier vaccine regulatory agency in the world.

Nongovernmental organizations (NGOs) are playing an increasing role in vaccine research. The Bill and Melinda Gates Foundation supports several organizations including the International AIDS Vaccine Initiative, the Malaria Vaccine Initiative, Aeras (dedicated to developing TB vaccines), and others with significant funding for development of vaccines that would have the greatest impact on diseases of developing countries. In addition, a related organization, Programs for Appropriate Technology in Health (PATH), is a nonprofit group that forges private sector partnerships to develop vaccine technologies suitable for the developing world. These product development partnership organizations (PDPs; essentially not-for-profit biotech companies) bring together specialized knowledge, animal models, immunologic assays, and field sites for vaccine testing as well as early capital investment to reduce the scientific technical risks, opportunity costs, and financial risk to their biotech and large pharma industrial partners. They also provide opportunities for validation of novel vaccine technologies and platforms.

The role of large, full-service vaccine companies (Table 4.5)[12] is predominantly in development. They engage

TABLE 4.5 Vaccine Companies Worldwide

FULL-SCALE COMPANIES WITH LARGE VACCINE FOCUS (~90% WORLD MARKET SHARE)	
France	Sanofi
United Kingdom	GlaxoSmithKline
United States	Merck Pfizer
OTHER FULL-SCALE COMPANIES WITH VACCINE DIVISION	
Australia	CSL (CSL Biotherapies)
United Kingdom	AstraZeneca (MedImmune)
United States	Johnson & Johnson (Crucell)
BIOTECH VACCINE COMPANIES	
Denmark	Bavarian Nordic
France	Vivalis
United States	Dynavax Emergent BioSolutions Genocea Novavax PharmAthene Protein Sciences Vical
REGIONAL COMPANIES	
Argentina	National Administration of Laboratories and Institutes of Health ANLIS Dr. Carlos G. Malbrán Sinergium Biotech S.A.
Bangladesh	Incepta Vaccine Ltd
Brazil	Ataulfo de Paiva Foundation Bio-Manguinhos–Institute of Technology on Immunobiologicals Butantan Institute Ezequiel Dias Foundation (FUNED)
Bulgaria	BB-NCIPD
Canada	InterVax Medicago
China	Beijing Minhai Biotechnology Co., Ltd Beijing Tiatan Biological Products Co., Ltd China National Biotec Group (CNBG) Hualan Biological Engineering Liaoning Cheng Da Biotechnology Co., Ltd (CDBIO) Sinovac Biotech Ltd. Walvax Biotechnology Co., Ltd Xiamen Innovax Biotech Co., Ltd
Cuba	Center for Genetic Engineering and Biotechnology Finlay Institute
Denmark	Statens Serum Institute
Egypt	The Holding Company for Biological Products & Vaccines (VACSERA)
India	Bharat Biotech International Ltd Biological E. Ltd Cadila Pharmaceuticals Ltd Hafkine Bio-Pharmaceutical Corporation Limited Indian Immunologicals Ltd Panacea Biotec Ltd Serum Institute of India Ltd
Indonesia	Bio Farma
Iran	Pasteur Institute of Iran Razi Vaccines
Israel	BiondVax
Italy	Okairos
Japan	Astellas Pharma Denka Seiken Japan BCG Kaketsuken Kitasato Institute Kyoto Biken Takeda
Korea	Boryung Biopharma Cheil Jedant (CJ Pharma) Dong Shin Pharma EuBiologics, Co., Ltd. Green Cross Corporation Korea Vaccine LG Life Sciences Ltd SK Chemicals
Malaysia	Pharm Malaysia
Mexico	Laboratorios de Biologicos y Reactivos de México, S.A. de C.V. (Birmex)
Netherlands	Netherlands Vaccine Institute
Poland	IBSS Biomed
Russia	Immunopreparat Research productive association, Ufa Products Immunologicals and Drugs, Irkustk RIVS, Saint Petersburg
Senegal	Torlak Institute of Immunology and Virology
Serbia	The Biovac Institute

Continued on following page

TABLE 4.5 Vaccine Companies Worldwide *(Continued)*

South Africa	BioNet Asia Co., Ltd
Thailand	The Government Pharmaceutical Organization Queen Saovabha Memorial Institute
Vietnam	Institute of Vaccines and Medical Biologicals (IVAC) The Company of Vaccine and Biological Production No. 1-VABIOTECH
CONTRACT MANUFACTURERS	
Germany	Boehringer Ingelheim IDT
Holland	DSM Biologics
Switzerland	Lonza Biologics
PRODUCT DEVELOPMENT PARTNERSHIPS	
Korea	International Vaccine Institute
United States	Aeras Global TB Vaccine Foundation Dengue Vaccine Initiative International AIDS Vaccine Initiative Malaria Vaccine Initiative Sabin Hookworm Vaccine Initiative

Data from World Health Organization. Influenza vaccine manufacturers. May 13, 2009. Available at http://www.who.int/csr/disease/influenza/Influenza_vaccine_manufacturers2009_05.pdf.

in some limited basic research and significant amounts of targeted research regarding specific organisms, but the preponderance of activity is in clinical and process development. Sufficient personnel and expertise in process development and chemical engineering reside almost exclusively in these companies; there is no other resource for such development. Clinical development that will satisfy FDA standards is also done mostly by the large companies, performed by academia and contract research organizations. Personnel and expertise in clinical research, regulatory affairs, data management, statistics, project management, and all other required disciplines also exist within the large companies. Perhaps most importantly, their management is structured to make rapid go/no-go decisions required to minimize risk and assess efficient vaccine development.

Many smaller organizations, often referred to as biotechnology companies, are engaged in vaccine research. They are often started by university scientists, supported by venture capitalists, and are capable of basic research on a vaccine idea. At this early stage, they usually have limited capacity in process development, manufacturing, and clinical development, and none in distribution, sales, or marketing. If research results are favorable, capacity in process engineering, clinical studies, and manufacturing must be enhanced or obtained by partnering. Because of the large cost of adding new capacities and expertise, many biotech companies in advanced product development will opt to partner with large, full-scale companies.

Although 60 or so small companies claim engagement in vaccine R&D, only about a dozen or so consider it a major activity, and only a very few, such as MedImmune, have made it to the market or close to the market on their own. More have licensed their products or technology platforms to larger companies that have then completed development, yielding new vaccines such as those for hepatitis B and *Haemophilus influenzae* type b. For example, the hepatitis B innovation came from the research laboratories of Chiron Corporation that succeeded in making hepatitis B surface antigen in yeast, and thus enabling Merck and GlaxoSmithKline to commercialize the modern hepatitis B vaccines. In the case of *H. influenzae* type b (Hib), Praxis Biologics and Connaught Laboratories pioneered the development of Hib polysaccharide and conjugate vaccines. These companies were eventually acquired by Sanofi and Wyeth-Lederle, respectively.

The greatest contributions of the biotechnology companies have been the introduction of multiple ideas into early vaccine development, and testing them to determine if they should be rejected or carried forward. These small companies are dependent on several factors for their success:

1. A vibrant basic research environment that allows for creation of new ideas, an environment that exists in well-funded (NIH) academic research programs.
2. A strong venture capital and investment community that views vaccine companies as potentially financially rewarding as other investment opportunities.
3. Strong patent laws providing the intellectual property protection that is essential for commercial success.

FUNDING SOURCES FOR VACCINE RESEARCH AND DEVELOPMENT

Funding sources for vaccine R&D include government, profits from sales of product, risk capital, and charitable foundations. The NIH competes with other federal agencies and programs for taxpayer support, and, in general, has been more successful than most. Similarly, vaccine R&D sponsored through the DOD, FDA, CDC, and USAID is competitive with other public needs as determined by the executive and legislative branches of government. Recent funding for bioterrorism vaccines (anthrax, smallpox) and emerging pathogens (Ebola, West Nile virus, severe acute respiratory syndrome [SARS], Middle East respiratory syndrome [MERS], pandemic influenza) could have long-reaching impact on vaccine research and manufacturing and could potentially create new entrants into the vaccine business.

Risk capital from private investors is the primary source of funds for small companies. Investors are attracted to the potential profits of a new vaccine, a forecast determined in part by sales of current vaccines. Large vaccine companies, which are divisions of much larger pharmaceutical companies, seek a profit by selling products. On average, pharmaceutical companies reinvest approximately 18% of their profits from product sales into R&D, and this proportion applies to vaccine sales as well as other pharmaceutical products (Pharmaceutical Research Manufacturers Association, personal communication, 2001).

Because vaccine companies are subsidiaries of large companies, vaccine R&D and manufacturing must compete with other product areas for resources. Comparisons of the economics of the vaccine industry with the pharmaceutical industry in Europe, and separately in the United States, were performed by the Mercer Consulting Company in 1995 (Fig. 4.2).[13] These studies in the United States showed that the

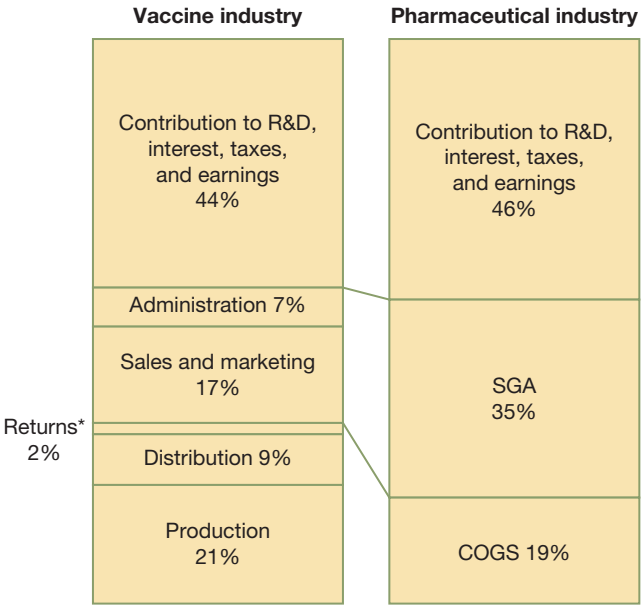


Figure 4.2. Major U.S. vaccine suppliers value-added chain (versus pharmaceutical industry averages). COGS, cost of goods sold; R&D, research and development; SGA, sales, general, and administrative costs. *Negligible returns (products that are sold and subsequently returned for a refund) in the pharma business. *(From Mercer Management Consulting Testimony on vaccine policy before the U.S. House of Representatives Committee on Commerce, June 15, 1995.)*

contributions to R&D, interest, taxes, and earnings after expenses were similar for the two industries (44% vs. 46%, respectively). However, the expenses were quite different. Significantly more was spent on production and distribution (32%, which includes production, distribution, and returns of product) in the vaccine industry compared with the pharmaceutical industry (19%), whereas the pharmaceutical industry spent more than the vaccine industry on sales, marketing, and administrative expenses (35% vs. 24%, respectively).

Consequently, within companies, there is an expectation that sales-to-expense ratios for vaccines will be similar to those of other pharmaceutical products, and that revenues will increase every year. Although some of this increase may be accomplished with sales volume, prices stabilize as vaccine products mature, and increased revenues are no longer possible; hence, the requirement for a steady rollout of new products. However, unlike pharmaceuticals, old vaccines continue to be profitable for a variety of reasons, including:

1. The absence of a regulatory pathway for generic vaccines deters potential entrants from engaging in a complex and expensive approval process.
2. In most cases, access to knowhow, such as proprietary cell lines, virus strains, and internally developed processes, is far more valuable than patent protection.
3. The birth cohort is renewable, providing an ongoing unmet need for vaccines.

As a result, sole-sourced vaccines, manufactured in fully depreciated assets, are profitable for pharmaceutical companies. One such example is the MMR vaccine, which after 40 years still has no competition in the United States. A typical vaccine company will have several vaccine candidates in early development, defined as all R&D through Phase I clinical testing (Table 4.6).[14-17] Those that are most promising in terms of technical feasibility, strong patent protection, and potential market size will be taken forward into development (post–Phase I). In addition, other candidate vaccines might be licensed from small companies. Even in the largest companies, only a few products can be in development at the same time. Thus, go/no-go decisions must be made and market size is a major determinant of the choice between two candidate vaccines, otherwise equal in technical feasibility and likelihood of success (Table 4.7).

This system works extremely well for vaccines with large potential markets in the developed world when technical feasibility is demonstrated. It does not work for vaccines for diseases that exist predominantly in the poorer regions of the world (e.g., TB); it works imperfectly for diseases of the developed world that affect relatively few persons because of geographic restriction (e.g., Lyme disease) or diseases limited to specific risk groups (e.g., cytomegalovirus [CMV] in transplant recipients), and it does not work when technical feasibility has not been demonstrated (e.g., HIV). The last problem has to be solved by a strong basic program in vaccine-related sciences, particularly for HIV, *Staphylococcus aureus*, malaria, and other challenging targets. Niche vaccines for developed-world markets are much more attractive to biotech than to large pharmaceutical companies as evidenced by recent biotech vaccine efforts for West Nile virus, Japanese encephalitis virus, the CMV-transplant indication, and dengue.

To involve large companies in development and manufacturing of vaccines to meet needs such as biodefense or health needs of poorer countries, incentives must be established to convince these companies that they should develop and manufacture such products. Such incentives might take the form of guaranteed purchase of certain volumes of a vaccine if specified standards are met, direct contracting by a government agency, or some other publicly funded mechanism.[18,19] The use of Advanced Market Commitments to create a funding mechanism for vaccines needed in the developing world has been endorsed by the G8 and pilot projects may be starting soon. This will not solve the problem of the high technical risk and opportunity costs associated with such vaccines, but it may contribute to the solution if combined with early investment. Companies may be willing to engage in such work. Indeed, they may already have donated or sold vaccines at very low prices to poorer countries. However, such practices alone will not solve the enormity of the health problems worldwide. Without special incentives, it is unrealistic to expect companies to engage in R&D on diseases that only, or predominantly, affect the poorer regions of the world.[13]

Manufacturers in developing countries (initially in India and China, and more recently in Brazil) are playing an increasing role in meeting these needs. Indeed, they already supply the majority of doses of older vaccines for such countries. As their expertise and capacity in vaccine R&D increases they will perhaps evolve into major participants in supplying new vaccines to the developing world. There are numerous manufacturers in these emerging countries, but a few truly stand out.

India

The vaccine industry has slowly mushroomed in India with several key companies emerging including Bharat Biotech, Biological E., Panacea Biotec, and others, but the largest one is the privately held Serum Institute of India. The Indian vaccine industry has significantly benefited from technology transfer from the West. Despite the industry's success, the available estimates suggest that R&D spending remains relatively low as a percentage of sales.[20]

Serum Institute of India is the world's largest producer of vaccines by number of doses, producing 1.3 billion doses

TABLE 4.6 Pipelines for Vaccine Development in Large, Full-Scale Companies

Sanofi	Merck	GlaxoSmithKline	Pfizer
Drugs or Indications in Phase I Trials			
Streptococcus pneumoniae	Dengue CMV	RSV	*Clostridium difficile*
HSV-2			
Rotavirus			
Drugs or Indications in Phase II Trials			
Rabies	Pneumoconjugate vaccine	*S. pneumoniae*	*Staphylococcus aureus*
Meningitis ACYW conjugate pediatric		Malaria	
TB		Nontypeable *Haemophilus influenzae* TB Hepatitis C	
Drugs or Indications in Phase III Trials			
Clostridium difficile	Ebola	MMR	Meningitis B
Dengue	Herpes zoster inactivated vaccine	Malaria Meningitis groups ACYW Ebola Zoster inactivated vaccine	

Data from company websites.
CMV, cytomegalovirus; HPV, human papillomavirus; HSV-2, herpes simplex virus type 2; RSV, respiratory syncytial virus; TB, tuberculosis.

TABLE 4.7 Vaccine Development Opportunities

Adenoviruses
Clostridium difficile
Chikungunya
Cholera
Cytomegalovirus (CMV)
Dengue
Ebola/Marburg
Enterotoxigenic *Escherichia coli*
Epstein-Barr virus
Herpes simplex viruses 1 and 2
HIV
Hookworm
Improved influenza
Leishmaniasis
Lyme disease
Malaria
Respiratory syncytial virus
Shigellosis
Staphylococcus aureus
Streptococcus A, B
Tuberculosis

a year; its products are used in more than 140 countries. Serum Institute is also one of the largest suppliers of measles-containing vaccines and the diphtheria-tetanus-pertussis (DTP) vaccines to U.N. agencies (UNICEF and Pan American Health Organization [PAHO]). The Institute makes its measles vaccine in MRC-5 cells instead of chick embryos and has productivity estimated at 10- to 20-fold higher than the measles vaccines made by Merck and GlaxoSmithKline. This privately held vaccine company has relentlessly invested in production facilities/infrastructure that surpasses some of the best biotech manufacturing facilities in the United States. So powerful has its growth been that one out of every two children immunized worldwide get at least one vaccine produced by the Serum Institute.

Vaccines recently developed by the Serum Institute are Nasovac (live attenuated trivalent influenza vaccine), MenAfriVac (meningococcal A conjugate vaccine), Pentavac (DTP Hepatitis B-Hib vaccine), and inactivated polio vaccine. The Institute continues to invest in R&D and is currently working on a rotavirus vaccine, a polyvalent meningococcal conjugate vaccine, a pneumococcal conjugate vaccine, and HPV Vaccine, combination vaccines containing acellular pertussis, and others.

China

China ranks as the world's largest vaccine consuming and manufacturing country, with an estimated annual output of 1 billion doses.[21] The original six government-owned regional biological institutes are now part of the China National Biotec Group (CNBG) consolidated under the China National Pharmaceutical Group Corporation (Sinopharm Group Co., Ltd.). CNBG has a large R&D center in Beijing that maximizes the synergies of the six affiliated institutions. Today, CNBG/Sinopharm supplies 85% of the doses of the 14 Chinese National Immunization Program vaccines. China's vaccine manufacturing capabilities are currently intensely focused on supplying their own domestic needs for the pediatric birth cohort of 17 million newborns annually. There are 46 registered vaccine manufacturers in China and 24 licensed vaccines. Several of the manufacturers are members of the Developing Countries Vaccine Manufacturers' Network (DCVMN). In 2013, the World Health Organization prequalified the Chinese-made Japanese encephalitis virus vaccine made by the Chengdu Institute for Biological Products in collaboration with PATH.[22] China became the first country ever

to approve a hepatitis E vaccine, which was developed by Xiamen Innovax Biotech.

Brazil

Brazil has four notable vaccine manufacturing companies. Bio-Manguinhos/Fiocruz is a government-owned entity that supplies the full demand for most vaccines under the Brazilian National Immunization Program (NIP). They also have a R&D collaboration with GlaxoSmithKline for a dengue vaccine. Butantan Institute is another government-owned institution that supplies the full demand for a smaller number of vaccines under the Brazilian NIP. Ataulfo de Paiva Foundation is nonprofit private institution that primarily supplies the BCG vaccine for the Brazilian market. Ezequiel Dias Foundation (FUNED) is a public institution and part of Minas Gerais state. Since 2009, it has supplied the meningococcal conjugate vaccine after transferring the technology from Novartis.

Summary

The Indian vaccine industry is the most advanced among these three developing countries, and is already providing a significant portion of the world's vaccine supply as well as developing new vaccines. China is on the verge of the transition from a domestic-only provider to a vaccine exporter, and is demonstrating solid progress in vaccine innovation. Brazil is approaching the point of supplying its own domestic needs, largely with technology transferred from the developed world. Together, these emerging players from middle-income countries will have increasing influence in the global vaccine industry during the coming years.

PRICING OF VACCINES

Pricing is a critical component of success for large companies and for venture funding of small companies since potential sales determine the desirability of an investment decision. The public expectation is for low vaccine prices, although this has changed somewhat in recent years with the introduction of several new, higher priced vaccines, such as varicella, rotavirus, pneumococcal conjugate vaccine, zoster vaccine, and HPV vaccine (Fig. 4.3). Large companies believe that vaccines should be priced according to value to society such as reduction in health care and related costs, relief from pain and suffering, and/or prevention of death, and that they should be rewarded for taking the enormous risks inherent in early vaccine development. Such prices far exceed manufacturing costs, but are essential to produce the revenue streams that allow vaccines to be competitive for R&D and manufacturing resources within large pharmaceutical companies or that make biotech companies attractive investment opportunities. In general, vaccine prices have declined when more than two companies have competed in a single vaccine market and profitability has fallen sharply. The influenza vaccine market highlights this cyclical ebb and flow of competitors, most recently with the H1N1 outbreak and shortages in 2009 leading to expanded competition and a vaccine surplus, followed by lower prices in 2010.

A vigorous large-company vaccine industry is dependent upon several factors:

1. A rich research environment sponsored largely by the NIH and mostly carried out in academia, as the source for new creative ideas.
2. Strong patent laws and protection of intellectual property.
3. Freedom to price products at fair levels related to value of product to society.
4. Well-implemented immunization practices.

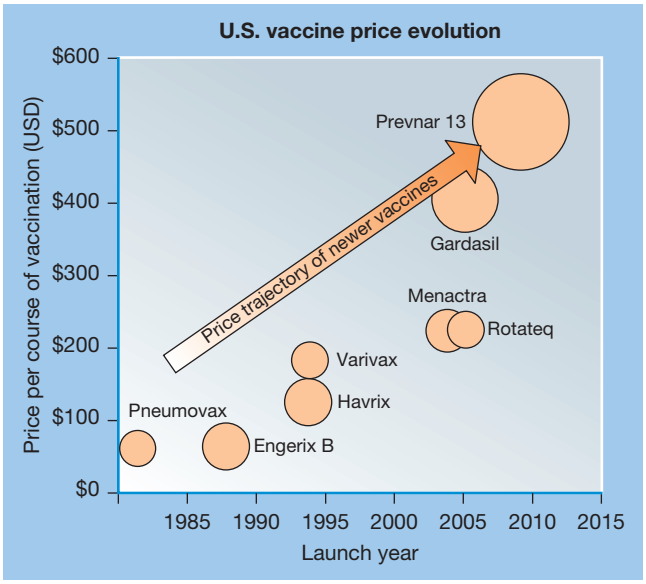


Figure 4.3. U.S. vaccine price evolution. Prices for vaccines are increasing relative to traditionally mandated products. USD, U.S. dollars. *(Data from U.S. CDC, IMS Knowledge Link, and NY Pharma Forum–Global Vaccines Outlook. Courtesy Kevin Fitzpatrick and Nitin Mohan of IMS.)*

Although the first two of these factors have been consistently present in recent years, downward pressure on price is a major threat to current companies and a disincentive to new companies. Freedom to price vaccines is restricted to the private market. Less than 50% of the vaccines for children sold in the United States are sold in the private market; the rest are sold to the federal or state governments at reduced prices. Controls are even greater in Western Europe and Japan, and internationally there is strong downward pressure on prices as one moves from well-developed to less-developed regions of the world.

In addition to the burden of partial price controls, the vaccine industry is subject to intense regulation. It cannot sell products until the vaccine and the facility in which it is manufactured are approved by the FDA or other regulatory authorities; each batch must be released by the appropriate regulatory agency; and the usage, and therefore market size, is largely determined in the United States by the CDC and in Europe by national regulatory authorities. Thus, the vaccine industry does not operate in a free-market environment, and its behavior reflects these constraints.

Vaccine business growth in the future will have three important drivers:

1. New vaccines for CMV, herpes simplex virus (HSV), respiratory syncytial virus (RSV), norovirus, *Clostridium difficile*, enterotoxigenic *Escherichia coli* (ETEC), "improved influenza," and others that will gradually shift the focal point of immunization activities from the pediatric sector to the adolescent and adult sectors.
2. Private market expansion in India and China driven by "high-income family" birth cohorts of 2 million and 6 million, respectively. This birth cohort roughly equals the combined birth cohort of 8 million in the United States and Europe. These high- and even middle-income individuals have shown the desire and ability to pay for vaccines at relatively high prices in relation to their incomes in these and other countries.

3. Public–private partnerships, or PDPs, on emerging pathogens such as pandemic flu, anthrax, SARS, botulism, Ebola, and others, will lead to large-scale manufacturing opportunities for these products. Toward the end of the 2020s, the PDPs for TB, malaria, and HIV are expected to produce effective vaccines for these diseases. A Boston Consulting Group study reports a surprising greater than $600 million per year market for a new TB vaccine (personal communication, 2012). Assuming such vaccines become reality, there is little doubt that the international donor community, working through organizations such as the Global Alliance for Vaccines and Immunization, will provide adequate funds for purchase of effective malaria, HIV, and TB vaccines, all of which are cost-effective, both in terms of cost per life saved and macroeconomic development of poor countries.

Vaccine Market

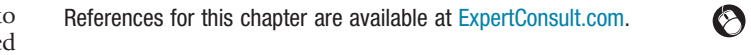
Estimates of the total worldwide vaccine market revenue are $25 billion. The top four Western suppliers (see Table 4.1) account for approximately 85% of these sales; the remainder comes from regional vaccine companies, the largest of which are located in middle-income countries such as India, China, and Brazil (see Table 4.5). The top four companies are slowly losing market share in doses to the DCVMN sourced doses and when polio eradication is achieved their dose share will drop to less than 20% of worldwide dose volume. In the coming years, as the eradication of polio becomes a reality, the developing country manufacturers will phase out their oral polio vaccine production. However, the need for inactivated polio vaccine will grow as developing countries adopt it into their pediatric immunization plans. As the demand for injected polio vaccine grows in developing countries, alternative approaches for local production will be explored, including access to bulk injected polio vaccine, tech transfer by big pharma as a part of their strategic alliances in developing markets, and potential introduction of alternative injected polio vaccine strains such as the Sabin strain. Another key driver will be the expansion of vaccine markets in India, China, and Brazil. Vaccine uptake rates in India, China and Brazil are still low compared with western countries (e.g., India's flu vaccine uptake in 2014 was 1.0 million doses vs. 140 million doses in the United States).[23,24] The immunization rates are also expected to increase in other low-income countries, which will increase vaccine dose requirements substantially. Most of this demand in low-income countries is expected to be met by manufacturers of DCVMN network. As the DCVMN expands its role, one would expect significant downward pressure on vaccine prices.

The delicate balance between innovation, government support, industrial expertise, and market forces has led to the establishment of a robust vaccine industry that will continue into the future. The industry is changing, however, with the growth of new markets in emerging economies and with the pressing need for new vaccines for the developing world. The current efforts of PDPs and public creation of markets in response to this need will be successful if lessons learned from the industrial vaccine effort are incorporated into these government and philanthropically driven expectations.

Acknowledgments

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5 Vaccine Manufacturing

Phillip L. Gomez and James M. Robinson

The vast majority of the more than 1 billion doses of vaccines manufactured worldwide each year are given to perfectly healthy people.[1–4] It is this fact that drives the requirements for vaccines to be among the most rigorously designed, monitored, and compliant products manufactured today. The ability to manufacture these vaccines safely and consistently is built on four competencies:

1. The manufacturing process that defines how the product is made;
2. The compliance of the organization to successfully complete that process;
3. The testing of the product and supporting operations; and
4. The regulatory authorization to release and distribute the product.

This chapter examines how each of these components is established during the development of a new vaccine and how the field of vaccine manufacturing is responding to emerging challenges for increased capacity (e.g., pandemic influenza vaccine), increased safety assurance (e.g., barrier isolator filling), and increasing complexities of manufacture (e.g., conjugate vaccines). All of this must be accomplished while consistently delivering more than 1 billion doses annually at the relatively low cost of similar therapeutic products.

In the United States, vaccines are regulated as biological products. The U.S. Food and Drug Administration's (FDA) Center for Biologics Evaluation and Research (CBER) is responsible for regulating vaccines. Current authority for the regulation of vaccines resides primarily in Section 351 of the Public Health Service Act and specific sections of the Federal Food, Drug and Cosmetic Act.[5,6] Section 351 of the Public Health Service Act gives the federal government the authority to license biological products and the establishments where they are produced.[7] Vaccines undergo a rigorous review of laboratory, nonclinical, and clinical data to ensure safety, efficacy, purity, and potency. Vaccines approved for marketing may also be required to undergo additional studies to further evaluate the vaccine and often to address specific questions about the vaccine's safety, effectiveness, or possible side effects.[8]

In the European Union, animal and human vaccines are regulated by the European Medicines Agency (EMA), whose main responsibility is the promotion of public and animal health. The EMA's Committee on Medicinal Products for Human Use through its Vaccine Working Party has oversight for human vaccines. Vaccines are licensed through a centralized procedure that allows for simultaneous licensure within all countries within the European Union. Human vaccines manufacturing is regulated under a Good Manufacturing Practices (GMP) Directive 200/94/EEC, Annex 16, and Annex 2.

Harmonization of licensing and regulating procedures for vaccines worldwide has obvious benefits in rapidly delivering safe and effective vaccines to the market. Impediments to harmonization include lack of standardized regulatory procedures and mutual recognition of licenses and inspections between countries and worldwide regulatory agencies. Harmonization of regulation continues to progress as joint FDA-EMA establishment inspections programs have become a reality and adherence to International Conference on Harmonisation (ICH) guidance is expected.

New vaccines are subjected to a well-defined regulatory process for approval. The approval process consists of four principal elements:

- Preparation of preclinical materials for proof-of-concept testing in animal models; manufacture of clinical materials according to current GMP; and toxicology analysis in an appropriate animal system.
- Submission of an investigational new drug (IND) application for submission to FDA for review.
- Testing for safety and effectiveness through clinical and further nonclinical studies (Phase I to Phase III clinical studies).
- Submission of all clinical, nonclinical, and manufacturing data to the FDA and EMA in the form of a Biologics License Application (BLA) for final review and licensure.

This chapter outlines the basics of manufacturing a vaccine and a description of some examples of currently licensed products. It then moves to the regulatory requirements for vaccine manufacturing including current GMP compliance, and then discusses the development of new vaccines. The final section examines the great challenges in the field to deliver a product held to an ever-increasing standard of safety while providing sufficient doses at reasonable costs for an ever-increasing number of diseases.

MANUFACTURING BASICS

The manufacture of vaccines is composed of several basic steps that result in the finished product. Table 5.1 summarizes these steps with examples for pathogens that have a licensed vaccine. The first step is the generation of the antigen used to induce an immune response. This step includes the generation of the pathogen itself (for subsequent inactivation or isolation of a subunit) or generation of a recombinant protein derived from the pathogen. Vaccines under development use additional methods that will be discussed later. Viruses are grown in cells, which can be either primary cells, such as chicken fibroblasts (e.g., yellow fever vaccine), or continuous cell lines, such as MRC-5 (e.g., hepatitis A vaccine). Bacterial pathogens are grown in bioreactors using medium developed to optimize the yield of the antigen while maintaining its integrity. Recombinant proteins can be manufactured in bacteria, yeast, or cell culture. The viral and bacterial seed cultures and the cell lines used for viral production are carefully controlled, stored, characterized, and, often, protected. The first step in manufacture is the establishment of a "master cell bank." This is a collection of vialed cells that form the starting material for all future production. It is extensively characterized for performance and the absence of any adventitious agents. From this bank, working cell banks are prepared that are used as the routine starting culture for production lots. The final vaccine is a direct function of its starting materials, and a change in this seed can be as complicated as initiating a new product development altogether.

TABLE 5.1 Examples of Licensed Vaccine Manufacturing Processes

Disease	Trade Name	Generic Name	Cell Culture/Fermentation	Isolation	Purification	Formulation	Preservative
Anthrax	BioThrax	Anthrax Vaccine Adsorbed	Chemically defined protein-free media growing a microaerophilic culture of avirulent, nonencapsulated *Bacillus anthracis*	ND	Sterile filtrate of culture medium	Aluminum hydroxide	Benzethonium and formaldehyde
Haemophilus influenzae	ActHIB	*Haemophilus* b Conjugate Vaccine (Tetanus Toxoid Conjugate)	Grown of *Haemophilus influenzae* type b strain 1482 grown in a semisynthetic medium	Centrifugation	Phenol extraction and alcohol precipitation; Hib polysaccharide conjugated to tetanus toxoid	Lyophilized	None
Hepatitis A	Havrix	Hepatitis A Vaccine, Inactivated	Hepatitis A (strain HM175) propagated in MRC-5 human diploid cells	Cells lysed to form a suspension	Purification by ultrafiltration and gel permeation chromatography followed by formalin inactivation	Adsorbed onto aluminum hydroxide	2-Phenoxy-ethanol
Hepatitis B	Recombivax HB	Hepatitis B Vaccine (recombinant)	Recombinant hepatitis B surface antigen (HBsAg) produced in yeast cells grown in a complex medium of extract of yeast, soy peptone, dextrose, amino acids, and mineral salts	Released from yeast by cell disruption	Series of chemical and physical methods (ND) followed by treatment with formaldehyde	Coprecipitation of HBsAg with amorphous aluminum hydroxyphosphate sulfate	None
Influenza	Fluzone	Inactivated Influenza Virus Vaccine	Propagation on embryonated chicken eggs	Low-speed centrifugation and filtration	Purification/concentration on linear sucrose density gradient using continuous flow centrifugation followed by additional purification by chemical means	Phosphate-buffered saline with gelatin as stabilizer	Thimerosal in some package configurations
Japanese encephalitis	JE-VAX	Japanese Encephalitis Virus Vaccine Inactivated	Intracerebral inoculation of mice	Harvest of brain tissue/ homogenization	Centrifugation, supernatant collection followed by formaldehyde inactivation; further purification by ultracentrifugation through 40% sucrose	Lyophilized	Thimerosal
Measles, mumps, rubella, and varicella	ProQuad	Measles, Mumps, Rubella and Varicella (Oka/ Merck) Virus Vaccine Live	Measles virus propagated in chick embryo cell culture; mumps virus in chick embryo cell culture; rubella virus propagated in WI-38 human diploid lung fibroblasts; varicella virus propagated on MRC-5 cells	ND	ND	Lyophilized	None

Meningococcal	Menactra	Meningococcal (groups A, C, Y, and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine	Meningococcal strains are cultured individually on Mueller-Hinton agar and grown in Watson-Scherp media; *Corynebacterium diphtheriae* grown on modified Mueller and Miller medium	Extraction of polysaccharide from cell	Polysaccharide purified by centrifugation, detergent precipitation, alcohol precipitation, solvent extraction, and diafiltration; diphtheria purified by ammonium sulfate fractionation and diafiltration; conjugate purified by serial diafiltration	Sodium phosphate–buffered isotonic sodium chloride	None
Pneumococcal	Prevnar	Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM197 Protein)	*Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7 F, 9 V, 14, 18 C, 19A, 19 F, and 23 F individually grown on soy peptone broth; *C. diphtheriae* strain containing CRM197 grown in casamino acids and yeast extract–based medium	Polysaccharides isolated by centrifugation; CRM197 ND	Polysaccharides purified by precipitation, ultrafiltration, and column chromatography; CRM197 purified by ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography; conjugation done by reductive amination and the conjugate purified by ultrafiltration and column chromatography	Aluminum hydroxide suspension	None
Polio	IPOL	Poliovirus Vaccine Inactivated	Types 1, 2, and 3 poliovirus individually grown in Vero cells on microcarriers using Eagle MEM modified medium supplemented with newborn calf serum	Clarification (method ND) and concentration	Purification by three chromatography steps: anion exchange, gel filtration, and anion exchange; inactivation by formalin	Medium M-199	2-Phenoxy-ethanol
Rabies	RabAvert	Rabies Vaccine	Rabies virus grown in primary culture of chicken fibroblasts in synthetic cell culture medium with the addition of human albumin, polygeline, and antibiotics	Inactivated with β-propiolactone	Purification by zonal centrifugation in a sucrose density gradient	Stabilized with buffered polygeline and potassium glutamate; lyophilized	None
Streptococcus pneumoniae	Pneumovax	Pneumococcal vaccine polyvalent	ND	ND	ND	Isotonic saline	Phenol
Typhoid fever	Vivotif	Typhoid Vaccine Live Oral Ty21a	Fermentation using medium containing a digest of yeast extract, an acid digest of casein, dextrose, and galactose	Centrifugation	ND	Enteric-coated capsule containing lyophilized product	None
Yellow fever	YF-Vax	Yellow Fever Vaccine	Strain 17D-204 of yellow fever is cultured on living avian leukosis virus-free chicken embryos	Homogenization	Centrifugation	Lyophilized product containing gelatin and sorbitol as stabilizer	None

Data from vaccine package inserts.
ND, not disclosed.

The next step is to release the antigen from the substrate and isolate it from the bulk of the environment used in its growth. This can be isolation of free virus or of secreted proteins from cells or of cells containing the antigen from the spent medium. The next step is purification of the antigen. For vaccines that are composed of recombinant proteins, this step may involve many unit operations of column chromatography and ultrafiltration. For an inactivated viral vaccine, there may simply be inactivation of isolated virus with no further purification. The formulation of the vaccine is designed to maximize the stability of the vaccine while delivering it in a format that allows efficient distribution and preferred clinical delivery of the product. The formulated vaccine may include an adjuvant to enhance the immune response, stabilizers to prolong shelf life, and/or preservatives to allow multidose vials to be delivered.

Formulation consists of combining all components that constitute the final vaccine and uniformly mixing them in a single vessel (Fig. 5.1). Operations are conducted in a highly controlled environment with employees wearing special protective clothing to avoid contamination with adventitious agents. Control monitoring of the environment and critical surfaces is conducted during operations. Quality control (QC) testing at this stage usually consists of safety, potency, purity, sterility, and other assays specific to the product.

During this phase, individual, scrupulously cleaned, depyrogenated, single-dose or multidose containers are filled with vaccine and sealed with sterile stoppers or plungers. If the vaccine is to be lyophilized, the vial stoppers are inserted only partially to allow moisture to escape during the lyophilization process, and the vials are moved to a lyophilization chamber. All vials receive outer caps over the stopper for container closure integrity. To preclude the introduction of extraneous viable and nonviable contamination, all filling operations must take place in a highly controlled environment where people, equipment, and components are introduced into the critical area in a controlled manner. After filling, all containers are inspected using semiautomated or automated equipment designed to detect any minute cosmetic and physical defects. As with the formulation phase of the vaccine manufacturing operation, extensive control and monitoring of the environment and critical surfaces are conducted during operations. QC testing at this stage also consists of safety, potency, purity, sterility, and other assays that may be specific to the product.

Vaccine efficacy can be adversely affected by improper distribution and storage conditions. The sensitivity of vaccines to adverse environmental conditions, particularly temperature extremes, varies depending on their composition. Live attenuated vaccines tend to be more susceptible than inactivated vaccines and toxoids.[1] Vaccines are formulated such that the potency at the end of shelf-life remains above the effective dose demonstrated in human clinical trials. As the product may degrade over the 2 to 3 years of shelf life, the release target potency may be significantly above the specified end-of-shelf-life specification. This "overformulation" can represent a significant production yield loss and cost-of-goods increase for the final product in order to support the necessary lead times to deliver and store the vaccines, especially if they are used as a rotating stockpile to protect against supply interruption or an emergency use that does not materialize. The addition of stabilizers or lyophilization, when feasible, tends to improve the thermal resistance of vaccines. Storage at very low temperatures within the manufacturing supply chain may be used to reduce potency loss during storage.

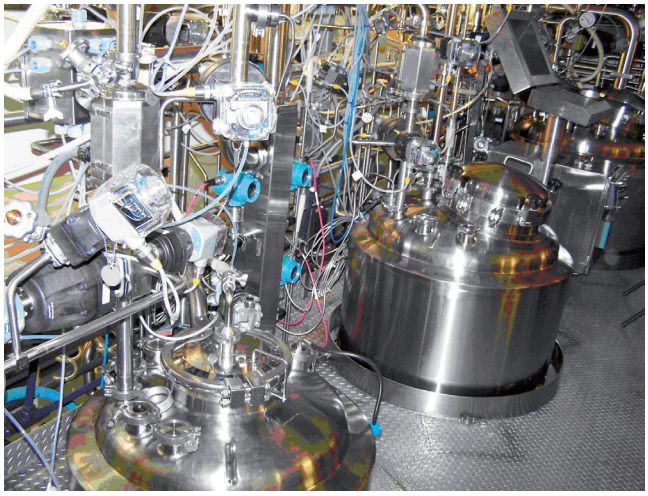


Figure 5.1. Automated vaccine formulation vessels.

Although recommended storage conditions for many vaccines have been detailed,[9] the vaccine manufacturers are responsible for developing data before and after licensing that demonstrate the stability of their vaccines under recommended storage conditions for the claimed shelf life. Generally, these programs provide data in excess of the claimed shelf life (up to 3 years) to support the development of new products intended for clinical use, as well as routine support of currently marketed products, expiration date extension, and supporting distribution conditions.[10,11] Accelerated studies conducted at elevated temperatures are commonly applied to better understand the impact of transient temperature excursions on the vaccine. Manufacturers are required to assure that products under their control are maintained under appropriate conditions so that the identity, strength, quality, and purity of the products are not affected.[12]

Currently, only a limited number of vaccines are required by federal regulation to have specified shipping temperatures.[10] Although most vaccine manufacturers use insulated containers and other precautions for the brief (usually 24 to 72 hours) shipping time, occasional, unanticipated temperature excursions may occur that could have a detrimental impact on the shipped product. Before accepting any vaccine shipment, users should look for any evidence of improper transportation conditions, including excessive transport time and possible adverse ambient temperature conditions.[1]

EXAMPLES OF VACCINE PRODUCTION

Inactivated Virus (Influenza)

Influenza virus vaccine for intramuscular use is a sterile suspension prepared from influenza viruses propagated in chicken embryos. This vaccine is the primary method for preventing influenza and its more severe complications.[13]

Typically, influenza vaccine contains two strains of influenza A viruses (H1N1 and H3N2) and a single influenza B virus. An additional strain of the influenza B virus was added, with the first four-antigen-containing-vaccine licensed in 2012.[14] The two type A viruses are identified by their subtypes of hemagglutinin and neuraminidase. The hemagglutinin and neuraminidase glycoproteins of influenza A virus comprise the major surface proteins and the principal immunizing antigens of the virus. These proteins are inserted into the viral envelopes as spike-line projections in a ratio of approximately 4 : 1.[15]

The trivalent subunit vaccine is the predominant influenza vaccine used today. This vaccine is produced from viral strains that are identified early each year by the World Health Organization, the Centers for Disease Control and Prevention

(CDC), and CBER. For U.S.-licensed manufacturers, the viral strains are normally acquired from CBER or CDC. European strains are typically provided by the National Institute for Biological Standards and Control, and Southern Hemisphere strains by the Therapeutic Goods Administration of Australia. These viral strains are used to prepare cells banks at each manufacturer, which cell banks are ultimately used as the inoculums for vaccine production.

The substrate most commonly used by producers of influenza vaccine is the 11-day-old embryonated chicken egg. A monovalent virus (suspension) is received from CBER or the CDC. The monovalent virus suspension is passed in eggs. The inoculated eggs are incubated for a specific time and temperature regimen under controlled relative humidity and then harvested. In the European Union, the number of passages from the original sample is limited. The harvested allantoic fluids, which contain the live virus, are tested for infectivity, titer, specificity, and sterility. These fluids are then stored wet frozen at extremely low temperatures to maintain the stability of the monovalent seed virus (MSV).[16] This MSV is also certified by CBER.

Once the MSV is introduced into the egg by automated inoculators, the virus is grown at incubated temperatures, and then the allantoic fluid is harvested and purified by high-speed centrifugation on a sucrose gradient or by chromatography. The purified virus is often split using a detergent before final filtration. The virus is inactivated using formaldehyde before or after the primary purification step, depending on the manufacturer. This is repeated for three or four strains of virus, and the individually tested and released inactivated viral concentrates are combined and diluted to final vaccine strength. Fig. 5.2 outlines the overall process.

The inactivated virus vaccine described above is used for the majority of flu vaccine produced and sold today. In recent years, the inactivated influenza vaccine produced on mammalian cell culture has been approved in a number of countries. The process replaces the egg-based virus expansion with a certified cell line; the downstream processes are similar, but focused on removing the host cell protein and DNA to below designated thresholds. A recombinant influenza vaccine, produced in insect cells infected with a recombinant baculovirus to express the hemagglutinin protein has also been approved in the United States.

Recombinant Protein (Hepatitis B)

In July 1986, a recombinant hepatitis B vaccine was licensed in the United States. This vaccine built on the knowledge that heat-inactivated serum containing hepatitis B virus (HBV) and hepatitis B surface antigen (HBsAg) was not infectious, but was immunogenic and partially protective against subsequent exposure to HBV.[17] HBsAg was the component that conferred protection to HBV on immunization.[18] To produce this vaccine, the gene coding for HBsAg, or "S" gene, was inserted into an expression vector that was capable of directing the synthesis of large quantities of HBsAg in *Saccharomyces cerevisiae*. The HBsAg particles expressed by and purified from the yeast cells have been demonstrated to be equivalent to the HBsAg derived from the plasma of the blood of hepatitis B chronic carriers.[17,19,20]

The recombinant *S. cerevisiae* cells expressing HBsAg are grown in stirred tank fermenters. The medium used in this process is a complex fermentation medium that consists of an extract of yeast, soy peptone, dextrose, amino acids, and mineral salts. In-process testing is conducted on the fermentation product to determine the percentage of host cells with the expression construct.[7] At the end of the fermentation process, the HBsAg is harvested by lysing the yeast cells. It is separated by hydrophobic interaction and size-exclusion chromatography. The resulting HBsAg is assembled into 22-nm–diameter lipoprotein particles. The HBsAg is purified to greater than 99% for protein by a series of physical and chemical methods. The purified protein is treated in phosphate buffer with formaldehyde, sterile filtered, and then coprecipitated with alum (potassium aluminum sulfate) to form bulk vaccine adjuvanted with amorphous aluminum hydroxyphosphate sulfate. The vaccine contains no detectable yeast DNA but may contain not more than 1% yeast protein.[7,19,21] In a second recombinant hepatitis B vaccine, the surface antigen expressed in *S. cerevisiae* cells is purified by several physiochemical steps and formulated as a suspension of the antigen absorbed on aluminum hydroxide. The procedures used in its manufacturing result in a product that contains no more than 5% yeast protein. No substances of human origin are used in its manufacture.[20] Vaccines against hepatitis B prepared from recombinant yeast cultures are noninfectious[20] and are free of association with human blood and blood products.[19]

Each lot of hepatitis B vaccine is tested for safety, in mice and guinea pigs, and for sterility.[19] QC product testing for purity and identity includes numerous chemical, biochemical, and physical assays on the final product to assure thorough characterization and lot-to-lot consistency. Quantitative immunoassays using monoclonal antibodies can be used to measure the presence of high levels of key epitopes on the yeast-derived HBsAg. A mouse potency assay is also used to measure the immunogenicity of hepatitis B vaccines. The effective dose capable of seroconverting 50% of the mice (ED_{50}) is calculated.[21]

Hepatitis B vaccines are sterile suspensions for intramuscular injection. The vaccine is supplied in four formulations: pediatric, adolescent/high-risk infant, adult, and dialysis.

All formulations contain approximately 0.5 mg of aluminum (provided as amorphous aluminum hydroxyphosphate sulfate) per milliliter of vaccine.[19] Table 5.2 summarizes the QC testing requirements for the release of recombinant hepatitis B vaccine.

Most vaccines are still released by CBER on a lot-by-lot basis; but for several extensively characterized vaccines, such as hepatitis B and human papillomavirus (HPV) vaccines, which are manufactured using recombinant DNA processes, this requirement has been eliminated.. Their manufacturing process includes significant purification, and they are extensively characterized by their analytical methods. In addition, hepatitis B vaccine had to demonstrate a "track record" of continued safety, purity, and potency to qualify for this exemption.[7,22]

TABLE 5.2 Testing Requirements for the Release of Recombinant Hepatitis B Vaccine

Type of Test	Stage of Production
Plasmid retention	Fermentation production
Purity and identity	Bulk-adsorbed product or nonadsorbed bulk product
Sterility	Final bulk product
Sterility	Final container
General safety	Final container
Pyrogen	Final container
Purity	Final container
Potency	Final container

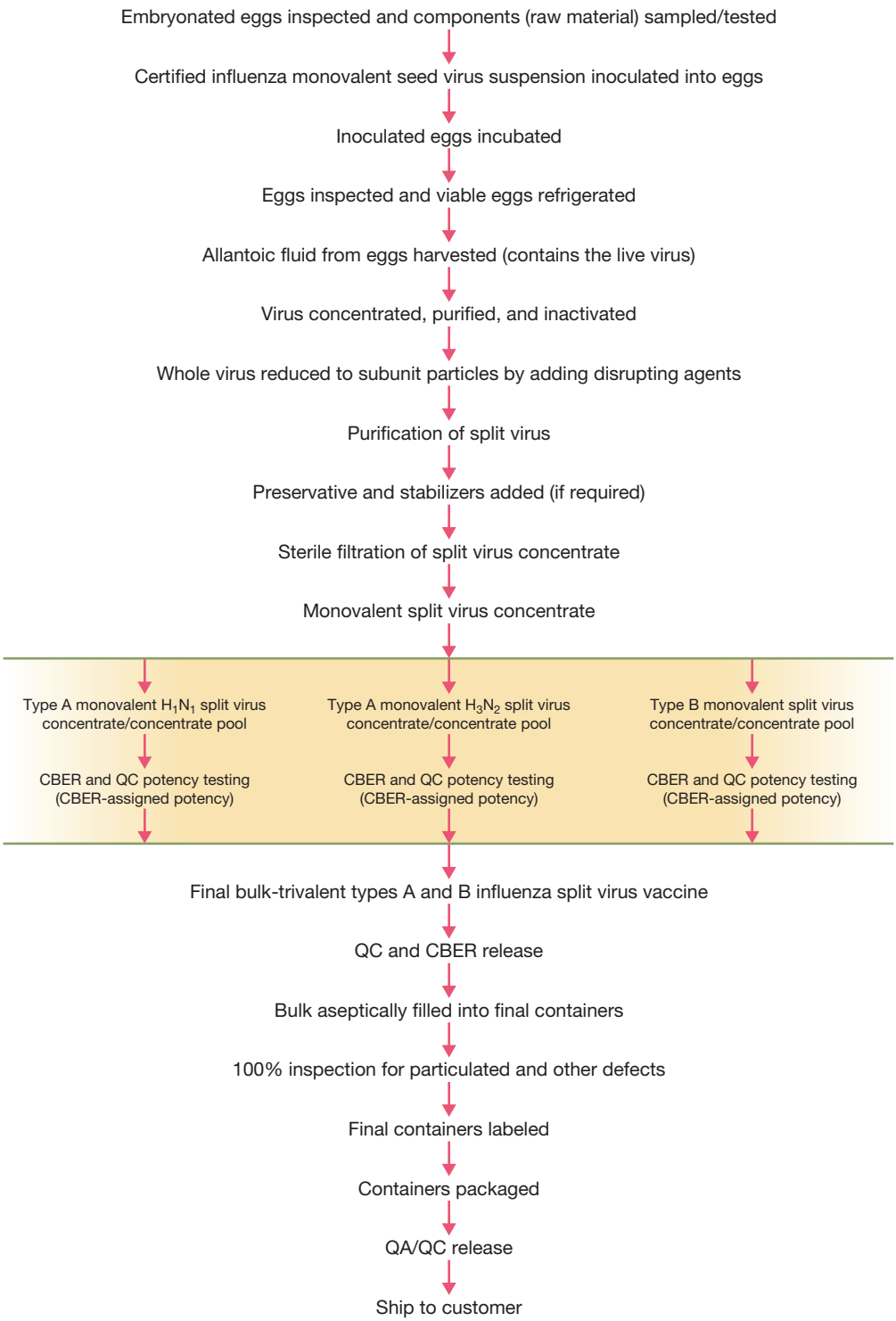


Figure 5.2. Egg-based influenza vaccine manufacturing process flow. CBER, Center for Biologics Evaluation and Research (of the U.S. Food and Drug Administration); QA, quality assurance; QC, quality control.

Conjugate Vaccine (*Haemophilus influenzae* Type B)

The production of *Haemophilus influenzae* type b (Hib) conjugate includes the separate production of capsular polysaccharide from Hib and a carrier protein such as tetanus protein from *Clostridium tetani* (i.e., purified tetanus toxoid), CRM protein from *Corynebacterium diphtheriae,* or outer membrane protein complex of *Neisseria meningitidis*.

The capsular polysaccharide is produced in industrial bioreactors using approved seeds of Hib. A crude intermediate is recovered from fermentation supernatant, using a cationic detergent. The resulting material is harvested by continuous-flow centrifugation. The paste is then resuspended in buffer,

and the polysaccharide is selectively dissociated from disrupted paste by increasing the ionic strength. The polysaccharide is then further purified by phenol extraction, ultrafiltration, and ethanol precipitation. The final material is precipitated with alcohol, dried under vacuum, and stored at −35°C for further processing.

Tetanus protein is prepared in bioreactors using approved seeds of *C. tetani*. The crude toxin is recovered from the culture supernatant by continuous-flow centrifugation and diafiltration. Crude toxin is then purified by a combination of fractional ammonium sulfate precipitation and ultrafiltration. The resulting purified toxin is detoxified using formaldehyde, concentrated by ultrafiltration, and stored at between 2°C and 8°C for further processing.

The industrial conjugation process was initially developed using tetanus toxoid by a team headed by J.B. Robbins at the National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Maryland.[23]

Conjugate preparation is a two-step process that involves: (a) activation of the Hib capsular polysaccharide and (b) conjugation of activated polysaccharide to tetanus protein through a spacer. Activation includes chemical fragmentation of the native polysaccharide to a specified molecular weight target and covalent linkage of adipic acid dihydrazide. The activated polysaccharide is then covalently linked to the purified tetanus protein by carbodiimide-mediated condensation using 1-ethyl-3(3-dimethylaminopropyl)carbodiimide. Purification of the conjugated material is performed to obtain high-molecular-weight conjugate molecules devoid of chemical residues and free protein and polysaccharide. Conjugate bulk is then diluted in an appropriate buffer, filled into unit-dose and/or multidose vials, and lyophilized.

Live Attenuated Vaccine (Measles)

The measles virus, isolated in 1954, is part of the genus *Morbillivirus* in the family Paramyxoviridae. Current vaccines are derived from Edmonston, Moraten, or Schwarz strains. Such vaccines have been on the market since the 1960s and in combination (measles, mumps, rubella [MMR]) since the 1970s. The final vaccine is a live attenuated viral vaccine inducing immunity in more than 90% of recipients.

For one measles vaccine, the manufacture of the vaccine starts with specific pathogen-free embryonated chicken eggs that are incubated several days. The embryos are collected and treated with trypsin to prepare the chick embryo fibroblasts for cell culture. All of the operations are done under strict aseptic conditions, performed by well-trained operators.

Cell culture are grown in roller bottles using fetal calf sera and M199 Hanks media for optimal cell growth. Chick embryo fibroblast cells are further infected by the viral working seed and incubated several days for viral culture. At the end of the viral culture, the cells are disrupted by mechanical lysis to release the virus. The virus is purified by centrifugation and filtration and stored frozen. After release of all QC tests, the vaccine is formulated alone or with mumps and rubella vaccines and lyophilized to obtain the stable product. The vaccine is reconstituted just before use.

Other manufacturers use different cell substrates; for example, the Serum Institute of India uses human diploid cells to manufacture their measles vaccine (see http://www.seruminstitute.com/content/products/product_mvac.htm).

Virus-Like Particle–Based Vaccines

Traditional viral vaccines rely on attenuated virus strains or inactivation of infectious virus. Subunit vaccines based on viral proteins expressed in heterologous systems have been effective for some pathogens, but have often had poor immunogenicity because of incorrect folding or modification.[24] Virus-like particles (VLPs) are designed to mimic the overall structure of virus particles and, thus, preserve the native antigenic conformation of the immunogenic proteins. VLPs have been produced for a wide range of taxonomically and structurally distinct viruses and have unique *potential* advantages in terms of safety and immunogenicity over previous approaches.[1] Attenuation or inactivation of the VLP is not required; this is particularly important as epitopes are commonly modified by inactivation treatments.[25] However, if a viral vector (e.g., baculovirus) is used as the expression system, inactivation may be required if the purification process cannot eliminate residual viral activity.

For a VLP to be a realistic vaccine candidate, it needs to be produced in a safe expression system that is easy to scale up to large-scale production[1] and by an accompanying purification and inactivation process that will maintain native structure and immunogenicity and will meet the requirements of today's global regulatory authorities. A number of expression systems manufacture multimeric VLPs, including the baculovirus expression system (BVES) in Sf9 and High Five cells, *Escherichia coli*, *Aspergillus niger*, Chinese hamster ovary cells, human function liver cells, baby hamster kidney cells, transgenic plants (potato, tobacco, soybean), *S. cerevisiae*, *Pichia pastoris*, human embryonic kidney 293 (HEK293) cells, and lupin callus (a plant-cell production system) with yields ranging from 0.3 to 10 μg/mL or as high as 300 to 500 μg/mL with *E. coli* and HEK293 (purified).[2]

The BVES has proven quite versatile, demonstrating the capability of preparing vaccine candidates for papillomavirus, feline calicivirus, hepatitis E virus, porcine parvovirus, chicken anemia virus, porcine circovirus, SV40 (simian virus 40), poliovirus, bluetongue virus, rotavirus, hepatitis C virus, HIV, simian immunodeficiency virus, feline immunodeficiency virus, Newcastle disease virus, severe acute respiratory syndrome (SARS) coronavirus, Hantaan virus, influenza A virus, and infectious bursal disease virus.[1]

Many pathogenic viruses, such as influenza, HIV, and hepatitis C, are surrounded by an envelope, a membrane that consists of a lipid bilayer derived from the host cell, inserted with virus glycoprotein spikes. These proteins are targets of neutralizing antibodies and are essential components of a vaccine. Owing to inherent properties of the lipid envelope, assembly of VLPs in insect cells for these viral vaccines is a different type of technical challenge to those produced viruses with multiple capsids.[1] For these targets, production of VLPs is a challenging task because the synthesis and assembly of one or more recombinant proteins may be required. This is the case for VLPs of rotavirus, which is an RNA virus with capsids formed by 1860 monomers of four different proteins. In addition, the production of most VLPs requires the simultaneous expression and assembly of several recombinant proteins, which, in the case of RLP, needs to occur in a single host cell.[26] Purification of VLPs also constitutes a particularly challenging task. VLPs are structures of several nanometers in diameter and of molecular weights in the range of 10^6 Da. Also, for guaranteeing the quality of the product, it is not sufficient to demonstrate the absence of contaminant proteins; it is also necessary to show that proteins are correctly assembled into VLPs.

Production of HPV VLPs represents another challenge. The HPV type 16 major 55-kDa capsids protein, L1, when produced in certain recombinant expression systems such as *S. cerevisiae*, can form irregularly shaped VLPs with a broad size distribution. These HPV VLPs are inherently unstable and tend to aggregate in solution. The primary challenge of HPV vaccine

formulation development was the preparation of aqueous HPV VLP solutions that are stable under a variety of purification, processing, and storage conditions. By treating the HPV VLPs through a process of disassembly and reassembly, the stability and in vitro potency of the vaccine are enhanced significantly. In addition, the in vivo immunogenicity of the vaccine was also improved by as much as approximately 10-fold, as shown in mouse potency studies.[27] The disassembly and reassembly of particles may also be important to remove residual proteins from the expression system or host cells used in the production and is a serious processing challenge, particularly for enveloped VLPs.

PRODUCT DEVELOPMENT

Vaccine development involves the process of taking a new antigen or immunogen identified in the research process and developing this substance into a final vaccine that can be evaluated through preclinical and clinical studies to determine the safety and efficacy of the resultant vaccine. During this process, the product's components, in-process materials, final product specifications, and manufacturing process are defined. The manufacturing scale used during development is usually significantly smaller than that used in the final manufacturing process. Phase I and, sometimes, Phase II clinical trial vaccines are typically produced in product development, but it is usually anticipated that at least one of the three or more consistency lots used for Phase III clinical trials will be manufactured at full-scale production volume. The product manufactured during the development phase is manufactured according to current GMP.[28]

INDUSTRY'S RESPONSE TO NEW CHALLENGES

Manufacturing Flexibility in Scale-Up: New Trends in Single Use

Early vaccines were produced in vivo (e.g., infecting calves with cowpox or rabbits with rabies virus; indeed, most influenza virus vaccines are still made in chicken embryos) and used neat or purified with largely glass lab equipment. Later, the animal cells used for viral vaccines were grown in vitro (e.g., roller bottles) and then infected to amplify virus for viral vaccines; later these cells were grown on microcarriers in deep culture to enable high manufacturing volumes and efficiencies. Microbial vaccines have been produced in large (500 to 5000 L) bioreactors at larger scale. These products were processed in stainless steel equipment for purification. Large-scale production was used to increase capacity to meet the increased demand for these lifesaving products. The cost of these facilities was increasingly expensive through the past few decades, and systems were increasingly complex to automate, clean, sterilize, and validate the facilities for manufacturing. Large central manufacturing facilities enabled lower cost of manufacturing; higher volumes were needed to lower the high fixed cost of construction, validation, and operation. The large central facilities were increasingly efficient, but also limited the manufacturing to a limited number of sites in developed countries.

Higher production yields and new manufacturing techniques, and an increasing focus on personalized medicine and niche products (e.g., low-volume products for rare diseases),[29] has allowed a new paradigm of smaller manufacturing facilities and bioreactors. The advent of disposable single-use equipment has eliminated the high cost of cleaning and sterilizing manufacturing equipment, reducing the complexity and cost of manufacturing facilities, and allowing economical manufacturing at smaller scale. Manufacturing systems that were enabled by 100% single-use technology enabled competitive pricing and a significant time advantage in smaller facilities.[30] The promise of the technology is clear and has triggered a significant investment by equipment developers into the single-use space.

The single-use promise is significant; however, there are a number of hurdles as in any new technology format. Glass and stainless steel equipment of historical manufacturing processes is generally inert and do not contribute impurities to the manufacturing process. The polymers used in single-use equipment may have different reactivity with the product (extractables and leachables), have been shown to shed particulate, and have shown a tendency to leak. Many of these concerns are being overcome in time through polymer engineering, and altering production techniques. Next, the ability to eliminate the high cost of cleaning and sterilizing equipment requires that the entire system be single-use. Many processes have not been able to make this conversion because of yield (low yield means high volume; volumes >2000 L are challenging because of weight and hydrostatic pressure), pressure (high O_2 demand, aeration volume, or flow rate), solvent use, and temperature control (heat transfer limitations). Chromatography also has been a challenging process in single-use systems, although there are promising advances and increasing options for prepacked columns.[29]

With the promise and dynamic pace of advancement in single-use systems, manufacturers are faced with a plethora of options and a jigsaw puzzle of parts to connect effectively. As the industry is rather new, the standards necessary to allow interconnectivity of parts are still marginal but gaining attention.[31] Standards are in development for extractables and leachables, particulate classification and management, integrity, supplier evaluation, and interchangeability. Governing and sponsoring agencies include Parental Drug Associate, BioProcess Systems Alliance, Extractables and Leachables Safety Information Exchange, Product Quality Research Institute, International Society of Pharmaceutical Engineers, American Society for Testing Materials, and United States Pharmacopeia, to name a few. Managing standards will be key to an effective use and integration of technologies supporting the vision of 100% single-use, reliable contingency supplies, robust supply chains, and limited surprises in change management.

Promise and Challenges of Distributed Manufacturing

One major advantage of the right-sized, single-use process is the ability to support distributed manufacturing. Today, a vast majority of supply of vaccines comes from a handful of developed countries because of the cost and complexity of the large-scale facilities and the profitability of those markets that support the significant manufacturing and clinical investment of public companies necessary to license and manufacture a vaccine. Once a large facility is built, duplicating it is a significant challenge as the complexity of the support systems essentially makes every facility unique. The advent of low-capital, single-use manufacturing platforms supports "scale-out" of processes built with "off-the-shelf" single-use systems, truly duplicating the original licensed process and minimizing the challenge of providing equivalence of the product from new facilities to the original facility. One can also imagine making clinical materials at modest scale and avoiding the challenge of scale-up for all markets, which could leverage addition of multiple single-use bioreactors instead of a large stainless system as demand grows. The approach supports a "pay-as-you-go" capital approach, avoiding large investments prior to demonstrating proof of concept and has the promise to make

product available more broadly and more quickly after license through global partnerships and distribution of facilities nearer to the market they will serve. It is well aligned with the current developing policies of multiple emerging economies to be self-reliant for essential services like vaccine supply. For example, a 2009 agreement between GlaxoSmithKline and Brazil describes the sale of GlaxoSmithKline pneumococcal conjugate vaccine to Brazil for 8 years, with an agreement to transfer the manufacturing process to Brazil for potential use in future years.[32]

The distributed manufacturing approach has many benefits including limiting global supply shortages from a catastrophic event at a single plant, the ability to customize a product for a region (e.g., strain of organism changing by region), and offsetting some of the cost of manufacturing (potentially paid by government) with tax-generating local jobs. There are some challenges to be considered. Maintaining alignment of the process among multiple facilities can be challenging in biological manufacturing processes. A process can "drift" in any single plant; having multiple manufacturing plants increases the chances that at least one plant will drift from the licensed, proven process. Likewise, control of raw material quality and source, single-use component supply, changes in supplier's materials in components, and similar unintended, unexpected consequences can leave a region without supply until the issue is identified and resolved. Single-use systems are ever improving; keeping the very specific needs of a process may be challenging with multiple small purchasers of the equipment versus a large central organization. Managing the regulatory files of a diverse production approach within a single company or across companies for a single product may also be difficult for regulators.

In spite of the challenges noted, the benefits of the distributed model, the advancement of standards within single-use manufacturers and the solutions that come with the experience of expanded use are sure to make this a reality in time.

Future Challenges in Polio Vaccine Manufacturing

The manufacture of polio vaccines began in 1955, and ultimately resulted in two products that have been widely distributed: the Salk inactivated polio vaccine (IPV) and the Sabin live attenuated polio vaccine (OPV). The OPV is manufactured typically on a cell line with minimal purification postharvest. This combined with the relatively high productivity of doses/liter of capacity results in a lower cost of manufacture. Because the risk of vaccine-associated paralytic poliomyelitis (VAPP) is estimated at 0.42 per million[33] over a three-dose schedule, there is a planned transition to the Salk IPV as the polio comes closer to eradication. To ultimately make this switch, however, will require substantial increases in the capacity to manufacture IPV, and without productivity improvement, it would require substantial increases in the cost of delivering polio vaccines to the target populations.

The production of IPV as described in Table 5.1 shows that the virus must be grown in cell culture, and then purified using three chromatography steps, followed by inactivation. As with any bioprocess, each steps results in loss of material that reduces the overall productivity. Alternatively, OPV is manufactured on cell lines with minimal purification. In addition, the dose of a live viral vaccine is typically much lower than an inactivated one, also helping the productivity of the process. This is reflected in the pricing of these vaccines. UNICEF publishes the prices of vaccine procured, and in 2014 OPV in a 20-dose vial from the Serum Institute of India was $0.14/dose, whereas IPV from Bilthoven Biologicals/Serum Institute of India in a five-dose vial was $1.90/dose.[34]

An additional challenge is the World Health Organization (WHO) guidelines on the use of wild-type polio virus necessary for the manufacture of IPV. Given the potential for an accident in manufacturing that could release wild-type poliovirus into the environment, the WHO has recommended limiting wild-type manufacturing of IPV to geographies with high IPV coverage in the surrounding community (>90% and at least three doses), and in environments with low transmission potential.[35] Currently, most of the vaccines for global vaccination come from India and China, where the suppliers of vaccines have focused on low-cost production and delivery of vaccines, and these geographies are currently not an option for IPV manufacture based on the WHO GAPIII recommendations.

A variety of strategies are currently being developed in the field to address these challenges, including a focus on the manufacturing technologies utilized to manufacture IPV, and opportunities to increase productivity and reduce unit costs. Existing polio vaccine manufacturing processes were developed years ago and use stainless steel fixed infrastructure manufacturing processes. Newer single-use technologies allow lower costs for initial facility design and could be used for polio vaccine manufacturing. An analysis by Lopes and colleagues shows that using newer facility design approaches that include single-use technologies can reduce bulk IPV costs by 40%, predominantly by reducing the capital costs of the facility.[36] Another approach to decreasing the cost is to increase the productivity of the manufacturing process.

Optimization of cell culture conditions and use of alternative cell lines are being evaluated, which suggest much higher titers of wild-type virus that could drive down the costs of the vaccine. Thomassen and colleagues showed that Vero cell culture can be further optimized to increase the production of D-antigen threefold.[37] Crucell has presented data that show its PER.C6 human-derived cell line has productivity 30 times higher than the Vero cells that are currently used to manufacture IPV.[38]

To allow broader manufacture of polio vaccines, an alternative vaccine using the Sabin attenuated strains but then inactivating them is also being pursued (Sabin-IPV [sIPV]). These viruses have shown lower yields in cell culture, but further work is being done to optimize their expression and purification yields.[39,40] This work again is focusing on cell-line selection, optimization, and facility design. Most importantly, the use of sIPV will allow a broader geographic set of potential locations for manufacturing, including India and China where there is substantial manufacturing infrastructure for vaccines focused on low-cost manufacturing.

Just as the initial discovery of polio vaccines required innovation in manufacturing methods to enable their launch, the ultimate eradication of polio will require innovation in manufacturing to enable the full public health impact of the vaccines.

Prime-Boost Vaccines

Given the complexities of developing vaccines for difficult targets like HIV, tuberculosis (TB), and malaria, a new tool has emerged that uses a "prime-boost" strategy for vaccination. The concept uses one vaccine as a prime, followed by the use of an entirely different vaccine for the boost. Most notably, an HIV vaccine efficacy trial in Thailand had a positive result using this approach. The Thai trial primed with a recombinant canarypox vector and followed with a recombinant gp120 protein boost.[41] Neither the canarypox nor the recombinant protein had shown efficacy on its own, but when combined gave a positive result in the trial. This approach has been used

in additional HIV vaccine efficacy testing,[42] TB vaccines,[43] and malaria,[44] among others.

Several challenges arise in the use of prime-boost vaccines that will need to be overcome if they are to be used in future licensed vaccines for distribution. The first is the complexity of multivalent vaccines. To have a successful batch release, prime-boost vaccines, like multivalent vaccines require successful manufacture and release of multiple components. For example, the HIV vaccines developed by the Vaccine Research Center at the National Institutes of Health (NIH) for HIV and tested for efficacy contained six plasmid DNA components in the prime, and five recombinant adenovirus vectors in the boost.[43] This requires the production of 10 separate active pharmaceutical ingredients, which must then be combined into two separate formulations and tested. Failure of any one component will delay both the ability to combine materials and the manufacturing of the final products. Yields of individual components and consequent costs can vary widely, requiring unique manufacturing supply chain designs that provide consistent supply of the projected demands.

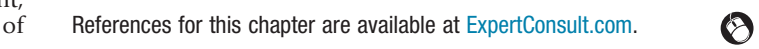
A critical component of assay development for any vaccine is the development of a potency assay for release. With two vaccines required for efficacy testing, defining exactly what the potency requirements are from each component will be important during clinical studies to ensure that future batches for distribution meet these criteria. It may require a prime-boost immunogenicity evaluation in animals for batch release, which has decreased with improved in vitro methods for potency evaluation of vaccines.

Finally, most of the prime-boost approaches often involve multiple organizations or companies. Most vaccine companies focus on a core set of vaccine platform technologies, which allow them to build expertise in process development, assay development, and manufacturing facilities. Nearly all of the vaccines cited for HIV, TB, and malaria use products from separate organizations. Ultimately, if the vaccines prove successful, there needs to be a single regulatory license holder responsible for the distribution of the vaccine, which requires careful collaboration between the different organizations for licensure and life-cycle management of the vaccines.

Most of the prime-boost vaccines being tested are for early clinical trials that are designed to see an early efficacy signal from the vaccine. Given this, there is limited focus on the future manufacturing supply chain and production costs. Even at these early stages of clinical studies, however, it is important to ensure partnerships are clearly defined among vaccine developers, and the clinical studies are designed to demonstrate the true requirement for the prime-boost regime and the characteristics that each component must elicit after immunization. Early cost modeling can help target investments in process development to ensure that a successful signal in efficacy testing can ultimately result in a vaccine that can be successfully manufactured and distributed to the target populations.

A final challenge will be the coordination of the supply chains. As two separate vaccines are required for complete immunizations and potentially different immunization times, deployment and supply of the vaccines have to be synchronized to demand. For routine immunizations this is relatively straightforward; but for any catch-up or mass vaccination campaigns, it requires care management of expiration dates and planning. Vaccine supply shortages are unfortunately not uncommon, and a requirement for multiple vaccines increases the possibility that one might not be continuously available, creating partially immunized and potentially off-schedule immunized individuals. The implications of these scenarios may have to be explored during Phase II dosing studies or postmarketing surveillance.

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6 Evolution of Adjuvants Across the Centuries

Nathalie Garçon and Martin Friede

Adjuvants are substances that are added to vaccine antigens to enhance and modulate the immunogenicity of the antigen. The first adjuvants developed focused on increasing antibody responses, and this has often been sufficient for the vaccines considered. During the last 2 decades, however, it has been realized that simply increasing antibody responses is not always sufficient for candidate vaccines to be effective. It has been observed that adjuvants can be used very effectively to:

- provide a strong priming response in naïve populations, effectively reducing the number of doses required to induce protection.
- increase the duration of the immune response.
- enhance specific arms of the immune response such as cell-mediated immunity (CMI), a critical target for many of the remaining infectious diseases for which we do not have vaccines.
- increase the breadth of the immune response to variable antigens, enabling broader cross-protection.
- enhance the immune response in poorly responsive populations, such as elderly and immunosuppressed populations.
- allow for dose sparing of antigens where antigen supply is limited.

Generally speaking, adjuvants are useful for antigens such as inactivated, subunit, and recombinant proteins, which can lose, during the purification process, some of the immunological information present in the pathogen that are needed to trigger an immune response. They have not yet been required for live attenuated vaccines, which carry the necessary immune-stimulating signals themselves. As discussed later, however, some preliminary research suggests that adjuvants can have an effect on live vaccines as well.

CHANCE AND NECESSITY: THE DISCOVERY OF ADJUVANTS

The use of adjuvants has been known for more than a century, and it is only recently that their mechanism of action was elucidated, in part owing to the progress in microbiology and immunology. The first recorded observation of immune potentiation by "adjuvants" is probably that of Coley, who in 1893 observed that administration of killed bacteria (Coley toxins) could in some cases cure certain forms of cancer. It was only in the 1990s that it was determined that this effect was the result of immune stimulation mediated by bacterial DNA. From there on, the specific oligonucleotide sequences that could stimulate the immune response and enhance it to a coadministered antigen were discovered.

It took another 2 decades to recognize the usefulness of adjuvants to enhance humoral immunity. In 1925, Ramon[1] observed that administering diphtheria toxoid to horses with a variety of substances, including starch, plant extracts, or fish oils, substantially enhanced the antibody response to the toxoid. A year later, Glenny[2] observed a similar effect with aluminum potassium sulfate, or alum. Alum was used thereafter as an adjuvant for numerous human vaccines, and to this day, other aluminum salts, in the form of aluminum oxyhydroxide or hydroxyphosphate, are the most widely used adjuvants in human vaccines. The starch and fish oils shown by Ramon to act as adjuvants have, in recent decades, been tested in vaccines in the form of inulin and squalene, respectively.

During the 80 years following the first use of aluminum salts as adjuvants, a wide variety of substances were tested as adjuvants, but many of them failed to be accepted for human use. In the 1940s, Jules Freund developed a water-in-oil emulsion, the Freund adjuvant, in which the vaccine antigen is emulsified as water droplets in a continuous mineral oil phase, containing killed mycobacterium (Freund complete adjuvant) or not (Freund incomplete adjuvant). The latter was briefly used for a commercial influenza vaccine in the United Kingdom in the 1960s, but was soon withdrawn owing to unacceptable reactogenicity. This, however, led to the development of oil-in-water emulsions, in which oil droplets are present in a continuous aqueous phase.

The first oil-in-water emulsions were based on a nonmetabolizable oil (squalane) and replaced later with metabolizable oils (squalene), as opposed to mineral oils as in the original Freund adjuvant. However, a water-in-oil emulsion similar in structure to the Freund adjuvant has been introduced in a cancer vaccine, using mineral oil with a higher degree of purity that allows for use in human vaccine candidates.

In the 1970s liposomes and virosomes that adsorb or encapsulate antigen were developed. Liposomes consist of lipid layers that form nanospheres or microspheres and can encapsulate or integrate antigens into their membranes. Several licensed vaccines contain virosomes, which are reconstituted empty envelopes of influenza viruses similar in structure to liposomes.

A TURNING POINT: BETTER UNDERSTANDING OF IMMUNOLOGY AND ITS IMPACT ON THE DEVELOPMENT OF ADJUVANTS

For most of the 20th century, adjuvant discovery and development was based on observations and experimentation with no clear immunological knowledge of the mechanism behind the adjuvant effect. This, however, dramatically changed in 1996 with the discovery of the Toll-like receptors (TLR) family in *Drosophila* and their relation to fungal resistance by Lemaitre and colleagues.[3] One year later, in 1997, Janeway[4] identified the link between human TLR4 and its key role in initiating an adaptive immune response, the first necessary step to a long-lasting immune response. The discovery by Poltorak and colleagues[5] that TLR4 functioned as a lipopolysaccharide (LPS)-sensing receptor and, hence, the use of LPSs or their derivatives as adjuvants, brought the final piece to the understanding of the mechanism of actions of TLR agonist molecules.[6]

In the early 1980s, Edgar Ribi[7] established that it was possible to produce a molecule that retained the immune potentiation activity of LPS without the associated toxicity. Approximately 30 years later, in 2009, the molecule monophosphoryl lipid (MPL) A was the first new adjuvant in a vaccine (Cervarix vaccine against human papillomavirus

[HPV]) approved for use by the U.S. Food and Drug Administration.

It is now well understood that the immune system uses pathogen-associated molecular patterns (PAMPs) to activate pathogen-recognition receptors such as TLR, and also a host of other more recently discovered receptors: retinoic-acid–inducible gene-based-I–like receptors (RLRs),[8] and cytosolic nucleotide oligomerization domain (NOD)-like receptors (NLRs).[9,10] These receptors bind various pathogen ligands (ranging from, for example, bacterial cell wall and cell membrane components to bacterial or viral nucleotides, to fungal lipids) to trigger different types of immune responses and, if combined with an antigen, can initiate and enhance specific arms of the immune responses to that antigen. For the purpose of brevity, readers are directed to other sources for a detailed description of how pathogen components stimulate various cytokine pathways and how they direct different arms of the immune response[11]; Fig. 6.1 shows this information schematically. As shown in Fig. 6.1, different TLRs, located on the plasma membrane or intracellularly, respond to different pathogen-derived signals to induce proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, or type 1 interferon, leading to a predominantly T-helper cell 1 (Th1) response. However, certain TLR2 agonists have been reported to activate Th2 responses.[12]

Table 6.1 lists the various agonists that activate the TLRs, as well as the adaptor molecules, also known as TLR agonists, and examples of adjuvants, which function through these receptors.

Based on this understanding, it is possible to recognize today how most of the adjuvants function. This knowledge should allow rapid screening of compound libraries for molecules that bind these receptors and that may have adjuvant activity leading to the rational design of new adjuvants aimed at stimulating specific arms of the immune response. The level of knowledge associated with the mechanism of action of those specific molecules and, as a consequence, the pattern of cytokines induced should also allow an assessment of the impact of an adjuvant on the safety of a vaccine. However, this has not yet led to the discovery of new molecules with defined

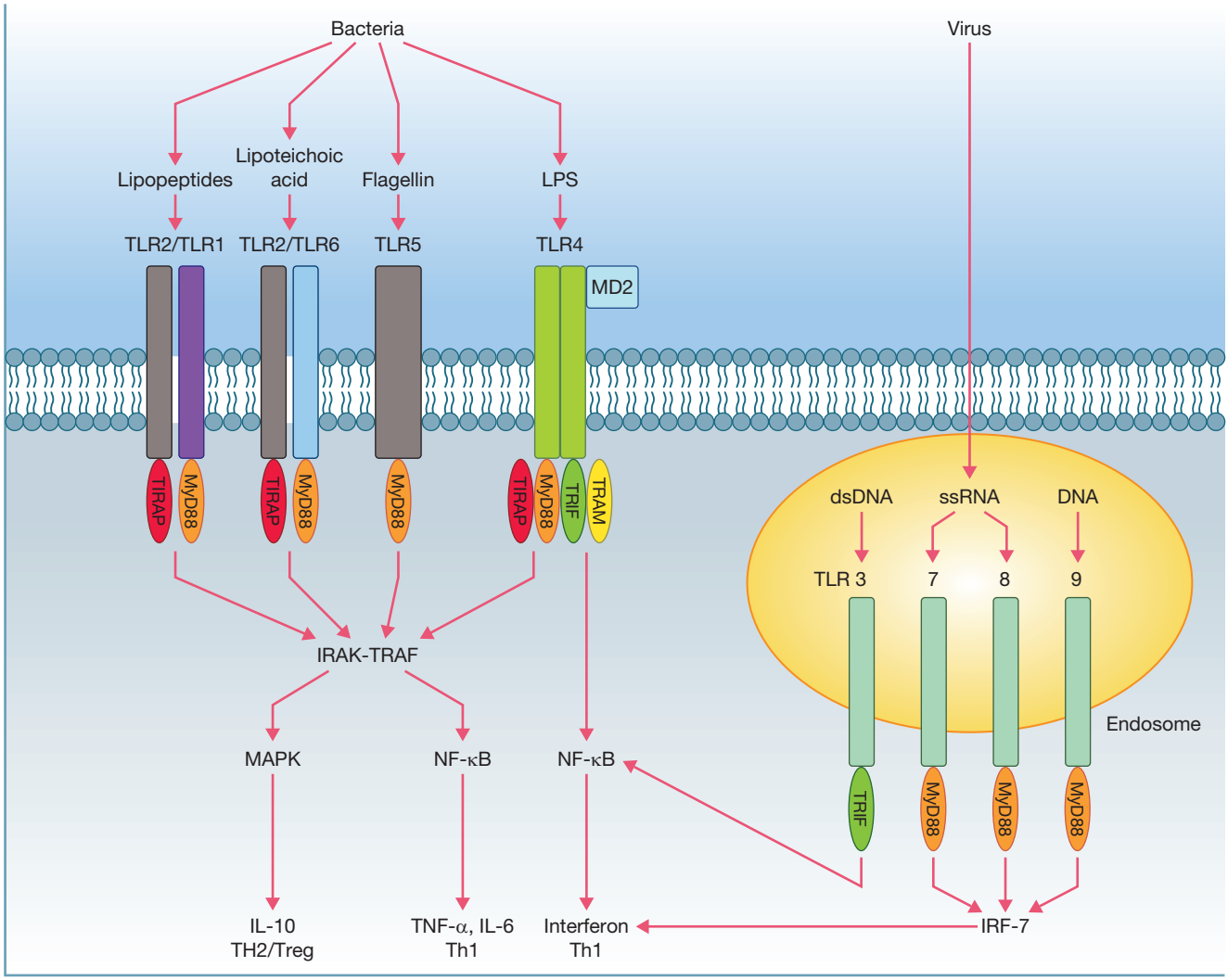


Figure 6.1. Activation of the innate immune system via interaction of bacterial or viral components with specific Toll-like receptors (TLRs), messaging through the myeloid differentiation 88 (MyD88) or TRIF pathway, and subsequent secretion of interleukin (IL)-10, tumor necrosis factor (TNF)-α and IL-6, or type 1 interferon. dsDNA, double-stranded DNA; IRAK, interleukin receptor–associated kinase; IRF, interferon regulatory factor; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; ssRNA, single-stranded RNA; Th, T-helper cell; TRAF, TNF receptor–associated factor; Treg, T-regulatory cell; TRIF, TIR domain–containing adapter-inducing interferon-β.

TABLE 6.1 Agonists That Activate the TLRs, Adaptor Molecules, and Examples of Adjuvants

TLR	Ligand	Ligand Location	Adaptor	Adjuvant
1	Tripalmitoyl-cysteine lipopeptides	Bacterial membranes	MyD88 MAL	
2	Lipopeptides, β-glucan, glycolipids	Bacterial membranes	MyD88 MAL	
3	Double-stranded RNA	Viral RNA	TRIF	Poly(I:C); poly(A:U)
4/MD2	Lipopolysaccharide	Bacterial membranes	MyD88 MAL/TRIF	MPL, GLA, E6020, RC529
5	Flagellin	Bacterial outer surface	MyD88	VaxInnate
6	Dipalmitoyl-cysteine lipopeptides	Bacterial membranes	MyD88 MAL	
7	Single-stranded RNA		MyD88	Imidazoquinolines: imiquimod, loxoribine
8	Single-stranded RNA		MyD88	R848
9	Bacterial DNA, unmethylated CpG DNA sequences, poly(dI:dC)	Bacteria	MyD88	CpG; IC-31 CpG 1018

CpG, cytosine phosphate guanine; GLA, glucopyranosyl lipid adjuvant; MAL, MyD88 adaptor-like; MPL, monophosphoryl lipid A; MyD88, myeloid differentiation 88; TLR, Toll-like receptor; TRIF, TIR domain-containing adaptor-inducing IFN-β.

activities, but in certain cases has enabled a better understanding of the mode of action of specific adjuvants and helped to support their safety profile within a given vaccine.[13] A clear understanding of the pathogenesis of immune-mediated disorders and their triggers is required, however, to ascertain the potential impact of the adjuvant. For several adjuvants the exact mechanism of action remains elusive (such as the saponins, which are described later) or may present multiple modes of action (such as aluminum salts).

This is only the first step in defining the value of a molecule as an adjuvant. Further evaluation of the compound in vivo and its safety profile will define its real potential as an adjuvant for vaccines.[14,15]

DEFINING ADJUVANTS: CLASSIFICATION AND EVALUATION

This section is limited to a review of the types of adjuvants that are incorporated in licensed vaccine formulations or for which there is extensive clinical experience.

A Categorization of Adjuvants Based on Mechanism of Action

The majority of adjuvant reviews during recent decades have tried to classify adjuvants according to their mechanism of action and typically classified adjuvants as *vehicles* or *immunostimulants*. Immunostimulants are substances that act directly on the immune system, such as TLR ligands. Vehicles are thought to act primarily by presenting antigens to the immune system. In this group are various aluminum salts, emulsions, immunostimulatory immune complexes (ISCOMs), and biodegradable microparticles. It is now known that most of the vehicles act directly on the immune system, and antigen presentation may be only a minor component of the adjuvant activity (e.g., see the later discussions of modes of action of aluminum salts ["Aluminum Salt Adjuvants"] and oil-in-water emulsions ["Oil-in-Water Emulsions"]). This classification seems outdated, and it may be preferable to classify adjuvants according to their receptor or, when the receptor is unknown, by their physical or chemical nature.

Currently 10 adjuvants are approved for use in vaccines (three aluminum salts with different counter-ions, four oil-in-water emulsions, aluminium/MPL combination, virosomes, and polyoxidonium). Table 6.2 lists the approved vaccines containing these adjuvants. Numerous other adjuvants are in vaccines that are under development. Noteworthy, AS01, the combination of liposome, MPL and QS21, will most likely be the next new adjuvant present in a licensed vaccine, in this case the malaria RTS,S vaccine. These and their classifications by receptor or physicochemical nature are presented in Table 6.3 and discussed in the following sections. Many more adjuvants are in preclinical development; however, they are too numerous to discuss in this chapter.[16]

Aluminum Salt Adjuvants

Aluminum-containing adjuvants have historically served as immunostimulants in vaccines and continue to be the most widely used adjuvants. Several aluminum compounds are used and are known as aluminum hydroxide, aluminum phosphate, and alum. All three of these commonly used names are scientific misnomers. Although this family of adjuvants has been used the longest, it is only recently that we have begun to understand their mechanism of action and the complexity of formulating them with antigens.

The following sections summarize the structure and properties of different aluminum salts, the mechanisms by which they stimulate the immune response, and the effect of freezing on aluminum-adjuvanted vaccines.

Structure and Properties. Aluminum hydroxide adjuvant is not $Al(OH)_3$, but rather crystalline aluminum oxyhydroxide (AlOOH).[17] This difference is important because crystalline aluminum hydroxide has a low surface area (approximately 20 to 50 m^2/g) and as such is a poor adsorbent. Crystalline aluminum oxyhydroxide has a surface area of approximately 500 m^2/g,[18] which makes it an excellent adsorbent. This high surface area is a result of its morphology. The primary particles are fibers having dimensions of approximately $5 \times 2 \times 200$ nm.

Aluminum oxyhydroxide is a stoichiometric compound. The surface is composed of Al-OH and Al-O-Al groups. The Al-OH surface groups can accept a proton, resulting in a positive surface charge, or donate a proton, resulting in a negative surface charge. As shown in Fig. 6.2, the isoelectric point (IEP) of Al-OH is 11.4. Thus, aluminum oxyhydroxide exhibits a positive surface charge at pH 7.4, which is the pH of interstitial fluid.

TABLE 6.2 Types of Licensed Vaccines Containing Adjuvants

Vaccine	Trade Name[a]	Adjuvant
Diphtheria and tetanus vaccine (DT)	Diphtheria and Tetanus Toxoid Adsorbed USP (1)	Aluminum potassium phosphate
DT acellular pertussis (DTaP)	Tripedia (1)	Aluminum potassium phosphate
Haemophilus influenzae type b (Hib)	Liquid PedvaxHIB (2)	Aluminum hydroxyphosphate sulfate
DTaP + Hib	TriHIBit (1)	Aluminum potassium phosphate
Hepatitis B	Recombivax HB (2)	Aluminum hydroxyphosphate sulfate
Hepatitis B	Engerix-B (3)	Aluminum hydroxide
Hepatitis B + Hib	Comvax (2)	Aluminum hydroxyphosphate sulfate
Hepatitis A	Havrix (3)	Aluminum hydroxide
Hepatitis A	Epaxal (6)	Virosomes
Hepatitis A + hepatitis B	Twinrix (3)	Aluminum hydroxide/phosphate
Pneumococcal conjugate vaccine	Prevnar (4) Synflorix (3)	Aluminum phosphate
Influenza vaccine	FLUAD (4)[b]	MF59
Influenza vaccine	Inflexal V (6)[b]	Virosomes
Pandemic influenza vaccine	Pandemrix	AS03
Pandemic influenza vaccine	Focetria	MF59
Pandemic influenza vaccine	Humenza	AF03
Human papillomavirus (HPV)	Gardasil (2)	Aluminum hydroxyphosphate sulfate
HPV	Cervarix (3)	Aluminum hydroxide + MPL
Hepatitis B	Fendrix (3)[b]	AS04 (MPL + aluminum phosphate)
Hepatitis B	SUPERVAX (7)[b,c]	RC529

MPL, monophosphoryl lipid A.
[a]Manufacturers are as follows: 1, Sanofi Pasteur; 2, Merck; 3, GlaxoSmithKline; 4, Wyeth now Pfizer; 5, Novartis; 6, Crucell; 7, Dynavax Europe.
[b]Licensed in Europe.
[c]Licensed in Argentina.

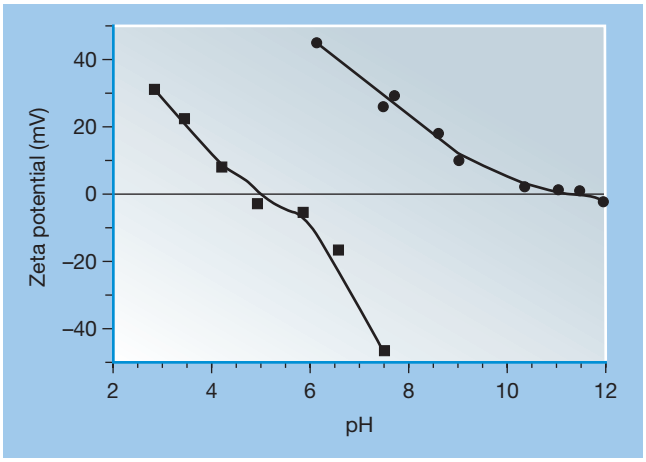


Figure 6.2. Isoelectric points of aluminum hydroxide adjuvant *(right)* and aluminum phosphate adjuvant *(left).* *(From Rinella JVJ, White JL, Hem SL. Effect of pH on the elution of model antigens from aluminum-containing adjuvants.* J Colloid Interface Sci. *1998;205: 161–165.)*

Aluminum phosphate adjuvant is a chemically amorphous aluminum hydroxyphosphate in which some of the hydroxyl groups of aluminum hydroxide are replaced by phosphate groups. The disordered, amorphous state is responsible for the high surface area and high adsorptive capacity.

The surface of aluminum phosphate adjuvant is composed of Al-OH and Al-OPO_3 groups. The IEP varies from 9.4 to 4.5 depending on the degree of phosphate substitution.[19] Commercial aluminum phosphate adjuvants have IEP values in the 4.5 to 5.5 range. In contrast with aluminum oxyhydroxide, commercial aluminum phosphate adjuvants are negatively charged at pH 7.4 (see Fig. 6.2).

Alum, which is water-soluble, is chemically aluminum potassium sulfate, $AlK(SO_4)_2$. The earliest vaccines containing aluminum adjuvants were prepared by in situ precipitation. A solution of alum was mixed with a solution of the antigen dissolved in a phosphate buffer. It is common practice to refer to the adjuvant produced by in situ precipitation as alum. The precipitate is amorphous aluminum hydroxyphosphate and has similar composition and properties as aluminum phosphate adjuvant.[17,20]

The techniques that can be used to characterize aluminum-containing adjuvants have been reviewed by White and Hem.[21]

Effect of Freezing. Vaccines containing aluminum hydroxide adjuvant or aluminum phosphate adjuvant should not be allowed to freeze and should not be used if suspected of having been exposed to freezing temperatures.[22] Freezing may affect the aluminum-containing adjuvant and the adsorbed antigen. Coagulates, which cannot be redispersed by shaking, form when aluminum-containing adjuvants are frozen. Thermostability of vaccines is of increasing importance, and as demonstrated for aluminium salts does not only concern excursion at high temperature, but also at low temperature, such as freezing.

TABLE 6.3 Nonexhaustive List of Adjuvants in Vaccines Under Development or Approved

Class (By MOA)	Component	Adjuvant Name and Other Compounds	Manufacturer	Phase I	Phase II	Phase III	Licensed
		Hiltonol (polylysine)	Oncovir	Cancer			
TLR4	MPL	AS04 (alum)	GSK				HPV, HBV
	MPL	AS02 (emulsion, QS21)	GSK		Cancer		
	MPL	AS01 (liposome, QS21)	GSK			Malaria, zoster	
	MPL	AS15 (liposomes QS21, CpG)	GSK			Cancer	
	MPL	(tyrosine)	ALT			Allergy	
	RC529	RC529 (alum)	GSK				HBV
	GLA	SE-GLA (emulsion)	IDRI	Influenza			
		Stimuvax	Biomira/Oncothyreon			Cancer	
		E6020	Esai				
TLR5	Flagellin	Fusion to influenza hemagglutinin	VaxInnate	Influenza			
TLR7	Imiquimod	Topical		Cancer			
	Imiquimod	Combined with ISA51			Cancer		
TLR9	CpG	1018 ISS	Dynavax		Allergy	HBV	
		CpG 7909	Coley/Pfizer	HBV	Cancer		
		CpG 7909 + alum	NIAID	Malaria			
		AS15 (liposomes, MPL, QS21)	GSK		Cancer		
	dI:dC	IC31 (cationic peptide)	Intercell	Influenza, TB			
Saponins	QS21	AS01 (liposome, MPL)	GSK			Malaria	
	QS21	AS15 (MPL, CpG)	GSK			Cancer	
	QS21	QS21	Universities	HIV, influenza	Cancer		
	Quil fractions	ISCOM (cholesterol)	CSL				
		Iscomatrix (cholesterol)	CSL	HPV, influenza	Cancer		
	GPI-0100			Cancer			
Oil-in-water emulsion	Squalene	MF59	Novartis	HIV	HBV, CMV		Seasonal influenza, pandemic influenza
	Squalene	AF03	Sanofi Pasteur	Influenza			
		SE	IDRI	Influenza			
	Tocopherol	AS03 (squalene)	GSK				Pandemic influenza
	Squalane	CoVaccine (acyl sucrose sulfate)	Protherics	Angiotensin			
			Nobilon	Influenza			
Water-in-oil emulsion	Squalene	ISA 720	Seppic			Malaria, cancer	
		ISA 51	Seppic	Malaria		Cancer	
Polysaccharides	Inulin	Advax (alum)	Vaxine	HBV, influenza			
Cationic liposomes	DDA	CAF (TDM)	SSI	TB			
		JVRS-100 (DNA)	Juvaris	Influenza			
Virosomes			Crucell				HAV, influenza
			Pevione	Malaria			
Polyelectrolytes		Polyoxidonium	Microgen				Influenza

Classified according to adjuvant receptor or adjuvant physicochemical nature (alum-derived adjuvants excluded for clarity).
ALT, alanine aminotransferase; CMV, cytomegalovirus; CpG, cytosine phosphate guanine; DDA, dimethyldioctadecylammonium; GLA, glucopyranosyl lipid adjuvant; GSK, GlaxoSmithKline; HAV, hepatitis A virus; HBV, hepatitis B virus; HPV, human papillomavirus; IDRI, Infectious Disease Research Institute; ISCOM, immunostimulatory immune complex; MOA, mechanism of action; MPL, monophosphoryl lipid A; NIAID, National Institute of Allergy and Infectious Diseases; SE, stable emulsion; TB, tuberculosis; TLR, Toll-like receptor.

Adsorption Mechanisms. The major mechanisms responsible for the adsorption of antigens are electrostatic attraction, hydrophobic forces, and ligand exchange. Electrostatic attraction is probably the most frequently used adsorption mechanism.

Electrostatic attraction can be optimized by determining the IEP of the antigen and then selecting an adjuvant that will have the opposite surface charge at the desired pH. For example, at pH 7.4, aluminum hydroxide adjuvant (IEP = 11.4) adsorbs albumin (IEP = 4.8) but does not adsorb lysozyme (IEP = 11.0). In contrast, aluminum phosphate adjuvant (IEP = 4.0) adsorbs lysozyme but not albumin at pH 7.4.[23]

Care must be taken in selecting a buffer for an aluminum hydroxide adjuvant–containing vaccine. Electrostatic attraction for an acidic antigen may be reduced or reversed if a phosphate buffer is used. Acetate and tromethamine (TRIS) are examples of buffers that do not alter the IEP of aluminum hydroxide adjuvant.[17]

Aluminum hydroxide adjuvant can also be pretreated to lower the IEP to optimize electrostatic adsorption of basic antigens.[24]

Hydrophobic forces can also contribute to the adsorption of antigens by aluminum-containing adjuvants. The contribution of hydrophobic attractive forces can be determined by observing the effect of ethylene glycol on adsorption.[25] Ethylene glycol stabilizes the hydration layer of proteins, which renders hydrophobic interactions thermodynamically unfavorable.

Mechanisms of Action of Aluminum Salt Adjuvants. There is still no consensus regarding the mechanisms by which aluminum-containing adjuvants potentiate the immune response. Several mechanisms are frequently cited to explain how aluminum-containing adjuvants increase antibody production. The depot mechanism was initially thought of as the dominant one; later the promotion of uptake of antigens by antigen-presenting cells (APCs)[26] and, more recently, a direct immune-stimulating mechanism were proposed.

- The depot mechanism postulates that the aluminum-containing adjuvant and the adsorbed antigen remain at the site of injection. The antigen is released slowly to stimulate the production of antibodies. This hypothesis is supported by the observation with some antigens that stronger binding to the aluminum salt crystals can result in higher immune responses. This hypothesis is, however, inconsistent with the observation that alum injection sites can be excised shortly after injection with no impact on immunogenicity.[27]
- It has also been proposed that adsorption of antigen to aluminum-containing adjuvants converts the soluble antigen to a particulate form. APCs take up particulate matter more efficiently by phagocytosis. Thus, antigen, which remains adsorbed, is taken into macrophages and dendritic cells. A dendritic cell culture study[28] revealed that antigens that elute from the aluminum-containing adjuvants are internalized by macropinocytosis, while those that remain adsorbed are internalized by phagocytosis. Antigen internalization by dendritic cells was enhanced when the antigen remained adsorbed to the aluminum-containing adjuvant following administration and when the aggregate size of the adjuvant was smaller than dendritic cells.
- Several groups have identified a role for a direct stimulation of the immune system through innate immune receptors and identified activation of the Natch domain, leucine-rich repeat, and PYD-containing protein (NALP)-3 inflammasome pathway by alum as the mechanism of action.[29–31] The precise mechanism by which alum stimulated NALP3 remained unknown. This has been refined and alum crystals have been shown to interact directly with membrane lipids on the surface of dendritic cells. The resulting lipid sorting triggers signaling cascades, independent of the inflammasome, that promote $CD4^+$ T-cell activation.[32]

It is likely that all three proposed mechanisms contribute to the immunostimulation produced by aluminum-containing adjuvants.

Safety of Aluminum-Containing Vaccines. Aluminum salts as adjuvants have the longest and largest safety track record of all adjuvanted vaccines, with more than 3 billion vaccine doses used during the past 80 years with a positive risk-to-benefit ratio. Focal histologic lesions were observed in patients with diffuse muscular symptoms that included persistent myalgias, arthralgias, and persistent fatigue. In the approximately 130 cases studied, these lesions were identified as macrophagic myofasciitis (MMF).[33] Intracytoplasmic inclusions in the infiltrating macrophages have been identified as containing aluminum by electron microscopy, microanalysis, and atomic adsorption spectroscopy. The presence of aluminum in the deltoid muscle biopsies suggested to Gherardi and colleagues that the source of the aluminum was aluminum hydroxide adjuvant.[34] However, no relationship between the presence of aluminum and MMF and the clinical symptoms has been established. The Vaccine Safety Advisory Committee of the World Health Organization (WHO) reviewed MMF at a meeting in 1999. The committee found that there was no basis for recommending a change in vaccination practices involving vaccine selection, schedule, delivery practices, or information involving aluminum-containing vaccines. The committee recommended that "research studies be undertaken to evaluate the clinical, epidemiological, and basic science aspects of MMF."[35] The U.S. Food and Drug Administration, while recognizing the desirability of new adjuvants, confirmed its support of aluminum salts in vaccines.[36] Research studies undertaken to assess the neurotoxicity of aluminum when it is administered intramuscularly or in a vaccine, showed a difference between the control group and the aluminum-based vaccine tested.[37] A repeat of the experiment, however, did not confirm any differences between the control group and the two vaccines containing aluminum. To date, even though it is established that aluminium salt can be recovered at the injection site months or years after intramuscular injections, no link between the presence of aluminium salt and the MMF syndrome has been clearly established.

Water-in-Oil Emulsions

Water-in-oil emulsions, of which the Freund adjuvant is the best-known example, were included in a commercial influenza vaccine in the United Kingdom in the 1960s. The vaccine was later withdrawn owing to occasional abscesses observed at the site of injection. Initial large-scale clinical studies on 18,000 military recruits conducted in 1953[38,39] resulted in some nodules at the injection sites, which were attributed to impurities (short-chain fatty acids) in the Arlacel-A surfactant. However, when this surfactant was purified, the incidence of cysts was reduced. A 10-year follow-up on these volunteers[40] showed that cyst-like reactions had required hospitalization in 0.1% to 0.6% of the volunteers, but otherwise there were no adverse effects of the vaccine. A subsequent 35-year follow-up[41] demonstrated that not only were there no adverse correlations with different diagnoses, including autoimmune diseases, but also, for some of the disease categories, there was decreased mortality. In contrast with these data from a large clinical trial, studies in rodents in 1972 demonstrated that when male Swiss mice were injected with mineral oil–based

emulsions, the mice developed tumors,[42] and the unacceptability of mineral oil–based emulsions for human use was concluded.

As a result, water-in-oil emulsions based on metabolizable oil were developed using squalene instead of mineral oil. The best-known examples of these are the Montanide adjuvants such as ISA 720 produced by the company Seppic. ISA 720 water-in-oil emulsion has been widely tested in more than 70 clinical trials in which it was often shown to induce immune responses rarely surpassed by other adjuvants. However, as with mineral oil–based emulsions, cysts or sterile abscesses at the site of injection are not infrequent and tend to increase in frequency on boosting.[43] In addition, instability of the antigen in contact with the emulsion was observed.[44] Finally, the difficulty in performing reproducible formulation at the time of administration led to a preformulation that may be incompatible with antigen stability. These challenges suggest that for prophylactic vaccines, researchers should, when possible, avoid using water-in-oil emulsions. For therapeutic vaccines, however, the risk of cysts and the challenges of formulation may be less significant. Such a vaccine (CIMAvax), which contains a mineral oil–based water-in oil-emulsion, Montanide ISA 51, has been licensed in Cuba for non–small cell lung cancer.[45]

Oil-in-Water Emulsions

Oil-in-water emulsion adjuvants were initially developed as an alternative to water-in-oil emulsions; the lower viscosity makes them easier to inject. The first emulsion of this class to be developed for human use was the SAF adjuvant made by Syntex Corporation. This emulsion was based on nonbiodegradable squalane, the catalytically hydrogenated form of squalene, and was designed as a vehicle to carry a synthetic immunostimulant, threonyl muramyl dipeptide (MDP).[46] The SAF adjuvant, while displaying strong adjuvant activity, was too reactogenic for use, partially because of MDP. However, because it was less effective without the immunostimulant, the emulsion was abandoned. Later, Chiron Corporation developed a range of oil-in-water emulsions by replacing squalane with squalene as another vehicle for muramyl derivatives. One of these emulsions (MF59) demonstrated some adjuvant properties and was further evaluated. MF59 and the majority of the later-developed oil-in-water emulsions used squalene, a natural, metabolizable product found in all plant and animal cells where it is a precursor of cholesterol. The commercial source is generally from shark liver, where it is abundant; alternative sources such as phytosqualene are being explored.[47] However, to date, only squalene from shark origin allows for a product with a purity level acceptable for human use.

Despite extensive clinical studies with a wide range of antigens, MF59 was approved only in one vaccine, FLUAD, an influenza vaccine for older adults, and licensed in several European countries from 1997 onward. While there was benefit of the adjuvanted vaccine in terms of antibody response to the influenza hemagglutinin in the target population,[48] the really significant benefit of MF59 and other oil-in-water emulsions became clear during investigations on pandemic influenza vaccines. The emergence of avian H5N1 influenza with occasional human-to-human transmission and the fear that this could become a pandemic led to intensive research in academic and pharmaceutical environments for ways to immunize a largely immunologically naïve population in the context of limited antigen supplies. This was especially critical when it was shown that for an H5N1 pandemic strain, sixfold more antigen was required to induce an immune response to a level equivalent to the seasonal influenza vaccine (90 μg compared with 15 μg).[49] It was shown that MF59 enabled immunization with significantly reduced doses of antigen, down to 7.5 μg, nearly a 12-fold dose reduction.[50]

In parallel to the development of MF59, several other oil-in-water emulsions were developed. For example an oil-in-water emulsion containing α-tocopherol as the immunostimulating compound was formulated by GlaxoSmithKline. This emulsion was tested earlier as part of the initial development of a malaria vaccine, alone (AS03) or in combination with the immunostimulants MPL and QS21 (described later).[51] AS03 demonstrated potent dose-sparing potential for pandemic influenza antigens, allowing for dose sparing down to 3.75 μg.[52]

In response to the need for dose sparing and the demonstrated potential of oil-in-water emulsions, Sanofi Pasteur developed AF03. This adjuvant is also squalene based; however, unlike the other emulsions, which are made by microfluidization of the components, the emulsification of AF03 is achieved without mechanical energy and uses a temperature-induced self-emulsification process (PCT application WO2007080308).

With the outbreak of the H1N1 influenza pandemic, European regulatory authorities approved three oil-in-water emulsions containing pandemic influenza vaccines, with MF59, AS03, and AF03 as adjuvants.

Other emulsions are under development, such as SE (stable emulsion), a squalene-based emulsion, originally developed by researchers at Corixa as a vehicle for MPL and synthetic TLR4 agonists.[53] This emulsion differs from the others in that the emulsifier is a natural phospholipid rather than a surfactant such as Tween-80. SE has been tested in clinical trials in combination with MPL in the context of a *Leishmania* vaccine,[54] as well as in combination with the TLR-4 agonist GLA (glucopyranosyl lipid adjuvant) in a schistosomiasis vaccine,[55] and in an influenza vaccine.[56] CoVaccine is an experimental adjuvant comprising sucrose fatty acid sulfate ester, combined with squalane, in the form of an oil-in-water emulsion. This adjuvant has been reported to allow for dose sparing in the context of influenza vaccines.[57] A single immunization with CoVaccine HT-adjuvanted H5N1 influenza virus vaccine induces protective cellular and humoral immune responses in ferrets and is undergoing clinical evaluation.

Table 6.4 gives an overview of oil-in-water emulsions used as adjuvants in licensed and investigational vaccines.

Adjuvant Effect of Oil-in-Water Emulsions on Naïve Versus Primed Persons. Oil-in-water emulsion adjuvants are highly effective at enhancing immunogenicity and allowing for dose reduction in pandemic influenza vaccines when the vaccinees are naïve. In contrast, the adjuvant effect for seasonal vaccines in healthy adults is quite poor.[58] This suggests that these adjuvants are excellent for priming but do not boost efficiently preexisting immune responses. The benefit for priming is also evident in the studies on the use of MF59-adjuvanted seasonal vaccine in infants, who usually respond poorly to a single administration of seasonal vaccine. These studies show a strong effect of MF59 on immunogenicity[59] and on efficacy of seasonal influenza vaccines, with the effect being strongest in the youngest ages.[60] The adjuvanted vaccine demonstrated 89% efficacy against vaccine-matched strains during two influenza seasons compared with 45% for the nonadjuvanted seasonal influenza vaccines group. On the basis of these data, Canada approved in early 2015 the use of MF59-adjuvanted seasonal influenza vaccine (FLUAD Pediatric) for the pediatric population (6 months to 2 years of age).

The situation is slightly different in the elderly population. Older adults have, in general, been primed to seasonal influenza; however, immunological senescence results in a decreased ability to induce sufficient antibody responses to

TABLE 6.4 Composition of Various Oil-in-Water Emulsions

Name	Components	Regulatory Status
SAF	Squalane; block copolymer; MDP	Abandoned
MF59	Squalene; Tween-80; Span 85	Approved for seasonal influenza for elderly people; approved for pandemic influenza (by the EMA); clinical benefit demonstrated for seasonal influenza in infants
AS03	Tocopherol; squalene; Tween-80	Approved for pandemic influenza (by the EMA)
AF03	Squalene; Tween-80; trometamol	Approved in pandemic influenza (by the EMA)
SE	Squalene; lecithin; block copolymer; glycerol; vitamin E	Clinical evaluation for *Leishmania*, influenza
CoVaccine	Squalane; sucrose fatty acid sulfate; Tween-80	Clinical evaluation for hepatitis

EMA, European Medicines Agency; MDP, muramyl dipeptide; SE, stable emulsion.

conventional influenza vaccines. FLUAD, the MF59-containing seasonal influenza vaccine that has been licensed in numerous countries for the past decade, has been shown to enhance the immune response in this nonresponder population.[61] A similar candidate vaccine using the AS03 oil-in-water emulsion has been tested in elderly adults for impact on influenza symptoms, demonstrating that the adjuvanted vaccine had advantages over the nonadjuvanted vaccine.[62]

Enhancing the Breadth of the Immune Response. Possibly one of the most important breakthroughs in adjuvant research during the last few years was the observation during the development of H5N1 pandemic influenza vaccines that oil-in-water adjuvants not only enhance the immune response and allow for dose reduction, but also enhance diversity and affinity of the antibodies induced.[63] This qualitative and quantitative expansion of the antibody repertoire has tremendous relevance for vaccination against pathogens, which undergo frequent antigenic drifts, such as influenza, as this would reduce the need for a perfect match between the antigen and the circulating pathogen strain.

This observation was first demonstrated in the ferret challenge model with the AS03-adjuvanted H5N1 vaccine through cross-neutralizing antibodies and lethal challenge.[64] The cross-neutralizing antibodies were confirmed later in clinical settings using the same AS03-adjuvanted H5N1 vaccine.[65] Data published with MF59-adjuvanted H5N1 vaccine showed the induction of epitope spreading from HA2 to HA1 hemagglutinin and to neuraminidase, suggesting that this is a common feature of this family of adjuvants. MF59 adjuvant enhances diversity and affinity of antibody-mediated immune response to pandemic influenza vaccines.[66]

Mode of Action. For many years oil-in-water emulsions were classified as vehicles, and it was assumed that the mode of action was primarily through enhanced delivery of the antigen to APCs or to the lymph nodes, even though most antigens do not associate physically to the oil droplets. It has, however, been shown that these emulsions stimulate the immune response indirectly. Using gene microarray analysis, Mosca and colleagues[67] demonstrated that skeletal muscle fibers are the target of MF59, where the adjuvant induces production of PTX3 and JunB, which, in turn, stimulate production of TNF-α, IL-1B, and CCLs (chemokines C-C ligands), resulting in activation of resident APCs and recruitment and activation of circulating APCs. A similar mechanism has also been demonstrated for the α-tocopherol–containing oil-in-water emulsion, AS03. Morel and colleagues[68] showed that similarly to MF59, AS03 induces expression of a range of cytokines, granulocyte recruitment at the injection site, and increased antigen uptake by monocytes and migration to the draining lymph node. In this adjuvant, however, the expression of some of the cytokines, such as IL-6, was modulated by the presence of α-tocopherol, which also enhanced the magnitude of the immune response, suggesting an immunomodulatory action of α-tocopherol independent of the oil emulsion. The same authors also demonstrated that the adjuvant effect is local and that temporal and spatial colocalization of antigen and adjuvant were required; that is, injecting the adjuvant in a site distant to the antigen or at a later time resulted in no adjuvant effect, consistent with a local and short-lived direct impact of the immune system.

Safety of Squalene-Containing Oil-in-Water Emulsions. During the H1N1 pandemic, two influenza vaccines containing an oil-in-water emulsion as adjuvant were approved and distributed in Europe (Pandemrix and Focetria); however, the uptake of these vaccines was hampered by broad media claims of the dangers of squalene. Many of these concerns seem to have arisen from a 2002 report that soldiers returning from the Gulf War with the so-called Gulf War syndrome had antisqualene antibodies that had been induced through receiving vaccines allegedly containing squalene.[69] Although the vaccines in question did not apparently contain squalene, the WHO Global Advisory Committee on Vaccine Safety reviewed all available data, including data from clinical trials with the squalene-containing FLUAD vaccine. Animal studies have also suggested that squalene can induce arthritis.[70] However, in the animal model used, the requirement for a specific breed and the use of a complex protocol irrelevant to the vaccination practice make it difficult to assess the relevance of these data for safety evaluation in humans. The committee concluded that there was no evidence that squalene could induce pathological antisqualene antibodies. Before recommending the use of squalene-containing oil-in-water emulsion for the H1N1 pandemic, the WHO also reviewed all clinical data from more than 35,000 volunteers of all ages and concluded that there were no significant safety concerns.

Thorough safety surveillance by authorities during the 2009–2010 pandemic season showed a positive benefit-to-risk profile for the vaccines. Since August 2010 however, an increased number of cases of narcolepsy has been reported in children and adolescents vaccinated with an AS03-adjuvanted H1N1 pandemic vaccine in three northern European countries. Interim reports of epidemiologic studies conducted in those countries have suggested an increased risk of narcolepsy in some vaccinated persons.[71]

The European Medicines Agency Committee for Medicinal Products for Human Use (CHMP) undertook a thorough

review of all available data as of July 2011, and concluded that the overall benefit-to-risk profile of the AS03-adjuvanted H1N1 vaccine remains positive. The CHMP acknowledged that further research in the areas of genetic and environmental factors in particular need to be explored before definitive conclusions can be drawn. In October 2013, based on the safety and efficacy results obtained for the pandemic H5N1 vaccine, the H5N1/AS03 adjuvanted vaccine received a positive opinion from the Vaccines and Related Biological Products Advisory Committee (VRBPAC) for use in adults older than age 18 years.

Although it is not known how the adjuvanted vaccine could cause narcolepsy, several hypotheses have been proposed, including the potential role of viral proteins such as the nucleoprotein,[72,73] suggesting that it is the presence of influenza viral nucleoprotein in the vaccine, rather than the adjuvant, that may be responsible. There are, however, no data clearly establishing a link between the adjuvant and the onset of the disease.

Other Oil Alternatives for Squalene. Little investigation into alternative metabolizable oils has been reported. As mentioned earlier, squalane was used in the SAF emulsion and is included in the CoVaccine adjuvant; however, it is not clear whether this oil can be metabolized or whether it is eliminated through the skin.[74] Miglyol, a metabolizable semisynthetic mixed triglyceride, has been evaluated as an oil-in-water emulsion adjuvant[75] and was shown to enhance immunity but at lower titers than a nonmetabolizable mineral oil. In the future, synthetic oils may overcome some of the challenges associated with the use of animal-derived squalene.

Toll-Like Receptor Agonists

Although TLRs and their role in triggering the innate immune response were understood only after their ligands were identified as adjuvants, it is useful to classify these adjuvants through their specific receptor.

TLR4 Agonists

Although LPS, a major component of the outer membrane of Gram-negative bacteria, has long been known to be a potent stimulator of the immune system, its use in adjuvants has been curtailed by its toxic effects. Early studies demonstrated that removing the core carbohydrate, generating lipid A, reduced the pyrogenicity without reducing the immune-stimulating activity. Lipid A was still too pyrogenic for use; however, it was shown that formulating lipid A in liposomes reduced the pyrogenicity a further 100- to 1000-fold, and liposomal lipid A formulations were used in clinical trials for candidate malaria vaccines without severe adverse events.[76] In 1984, Ribi[77] demonstrated that a significantly less-toxic molecule could be obtained from LPS through sequential acidic and basic hydrolysis steps. The phosphate from the reducing-end glucosamine of lipid A derived from *Salmonella minnesota* RC595 is removed by mild acid hydrolysis, resulting in a molecule, referred to as monophosphoryl lipid A (MPLA, referring to all other TLR4 agonists) that was significantly less toxic than the lipid A and that still stimulated the immune response.[7] Ribi and colleagues then observed that if this MPLA was subjected to a further mild alkali hydrolysis, which selectively removed the acyl chain from the 3′ position of the disaccharide backbone, the resulting molecule, 3-deacylated MPLA (3d-MPL, or MPL), was even less pyrogenic but still exhibited adjuvant activity. GlaxoSmithKline developed a combination of adjuvants, AS04, in which MPL is formulated with aluminum salt. The adjuvant system AS04 is used in two approved vaccines (Cervarix [HPV vaccine] and Fendrix [hepatitis B vaccine for hemodialyzed patients]). Cervarix is aimed at preventing HPV infection and subsequent development of cervical cancer. Extensive clinical trials demonstrated an acceptable safety profile and efficacy of the vaccine against the vaccine strains during a period of more than 8 years in clinical trial follow-up,[78] as well as against divergent HPV strains not present in the vaccine.[79]

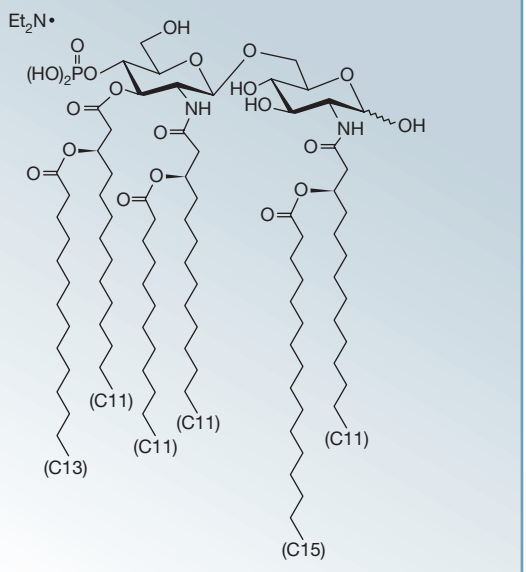
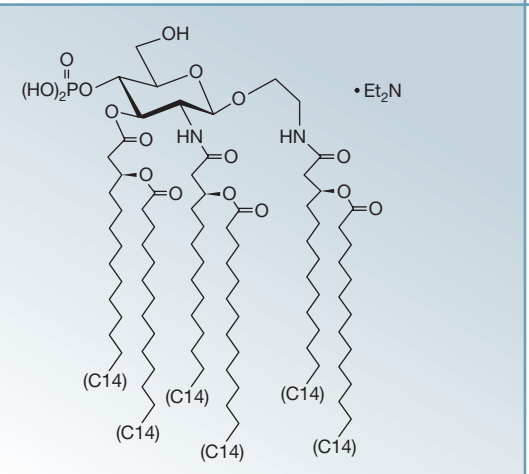
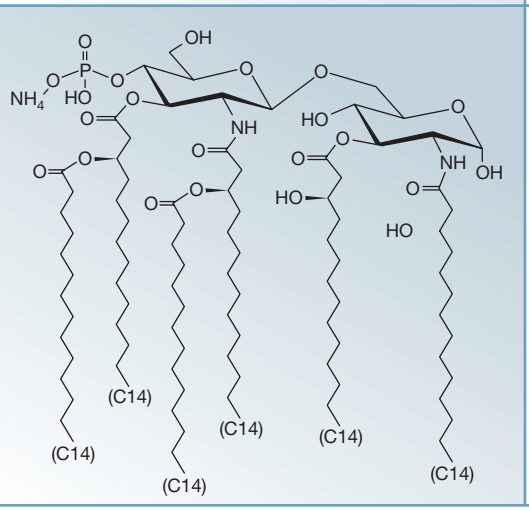
Mechanism of Action of TLR4 Agonists. Immune recognition of TLR4 agonists, such as LPS or MPL, is initiated by extraction of monomers from the aggregates by LPS-binding protein in the serum.[80] Monomeric LPS is transferred from LPS-binding protein to another accessory protein, CD14, which, in turn, transfers LPS to MD2, a secreted glycoprotein that associates with the extracellular domain of TLR4 to form the heterodimeric receptor that is responsible for the physiological recognition of LPS.[81] Lipid A bound to the TLR4–MD2 complex activates two distinct intracellular signaling pathways, which have come to be known by the names of the TLR4-proximal adaptor proteins, myeloid differentiation 88 (MyD88) and TIR domain-containing adaptor-inducing IFN-β (TRIF).[82] The Myd88 pathway leads to the activation of mitogen-activated protein kinase– and nuclear factor-κB–dependent proinflammatory responses, whereas the TRIF pathway activates kinases responsible for type I interferon responses.[83] Fig. 6.1 shows this schematically. To this extent, the TLR4 receptor is unique among the TLRs by its ability to induce two distinct signaling pathways. The activation of these pathways is dependent on the structure of the agonist, where minor changes in the number and length of the acyl chains can have a major effect on pathway activation. MPL has been reported to activate the TLR4-TRAM (TRIF-related adaptor molecule)-TRIF–based signaling pathway and the TLR4-MAL (MyD88 adaptor-like)-MyD88 pathway.[84]

Species Specificity of TLR4 Receptor. One challenge exists, however, in the development of TLR4 agonists for use in humans—the variability in the receptor specificity across species. Human, but not murine, TLR4–MD2 transmits proinflammatory signals in response to hexaacylated but not pentaacylated LPS.[85] Moreover, in humans, but less so in rodents, tetraacylated and pentaacylated forms can inhibit the adjuvant activity. As a result, molecules shown to work in rodents will not necessarily function the same way in humans. One example of this is the OM-174 molecule, a triacylated molecule that functioned in mice,[86] but apparently did not function in humans.

Other TLR4 Agonists. As MPL is isolated from a Gram-negative bacterium, *S. minnesota*, and such extraction can present manufacturing hurdles, many groups started to develop MPL analogs by chemical synthesis. Fig. 6.3 illustrates the structure of MPL and its analogs.

RC529, a member of the aminoalkyl glucopyranosides developed by Corixa scientists to provide a synthetic alternative to MPL, is structurally similar to a hexaacyl MPL, but the reducing terminal glucosamine has been replaced by a nonsaccharide backbone.[87,88] This molecule, formulated with alum, is in a hepatitis B vaccine approved in Argentina (SUPERVAX) that is reported to increase immune response compared with nonadjuvanted vaccine.[89]

Glucopyranosyl lipid adjuvant (GLA) is a synthetic hexaacyl form of MPL, but it is designed on the *Escherichia coli* form of LPS rather than the *S. minnesota* LPS. The molecule has a single acyl chain on the 2′ amine and has an acyl chain on the 3′ hydroxyl.[90] Clinical studies are in progress in different area such as tuberculosis (TB)[91] and influenza.[92]

Structure	Name
	MPL
	RC-529
	GLA

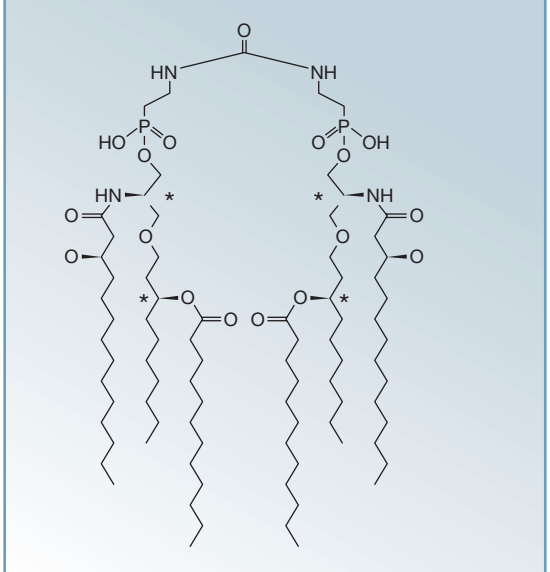
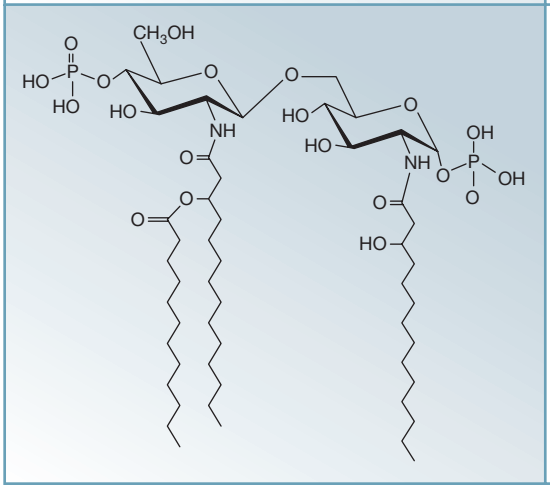
Structure	Name
	E6020
	OM-174

Figure 6.3. Structures of TLR4 agonists in development or in licensed vaccines. GLA, glucopyranosyl lipid adjuvant; MPL, monophosphoryl lipid A.

E6020 is a synthetic TLR4 agonist that is not based on a saccharide backbone, but it is able to stimulate the TLR4–MD2 pathway.[93] E6020 is in Phase I trials.

Butantan, the Brazilian vaccine manufacturer, has initiated a program to produce MPL from the LPS extracted from *Bordetella pertussis*, a side product of the whole-cell pertussis vaccine process. This has been reported to enhance immunity to influenza vaccines.[94] Heptaacyl sulphonyl sucrose, a component of the CoVaccine HT adjuvant, currently in clinical development, bears strong resemblance to the backbone structure of TLR4 agonists and is thought to also act through TLR4.[57] One question remains, however, concerning the ability of synthetic TLR4 agonists to overcome polymorphism associated with the TLR4 receptor.[95] Indeed, it is not clear whether all recipients will respond equally to a given TLR4 agonist molecule and whether a mix of various molecules, as present in the different naturally produced TLR4 agonists, will be necessary to compensate for human diversity.

Formulation Challenges. Ensuring an optimal and reproducible adjuvant effect requires some formulation knowhow. In the currently approved vaccines with TLR4 agonists (Cervarix and Fendrix), the MPL is combined with an aluminum salt, and the combination of the two adjuvants is referred to as AS04. The combination of MPL with an aluminum salt adjuvant demonstrates some of the challenges of using MPL and analogous multiacylated disaccharide TLR4 agonists. Only a well-defined and controlled process can ensure that those molecules, which are insoluble in water, will not clump into aggregates that would lead to difficulty during sterilization by filtration or variability in the adjuvant activity. Various other approaches have been adopted to formulate TLR4 agonists, such as their incorporation into small unilamellar liposomes permitting a stable formulation. However, the activity is dependent on the ratio of lipid to agonist and how the agonist is combined with the liposomes.[96] Other alternatives include the incorporation of the agonists into oil-in-water emulsions or application of thermal or sonic energy to form colloids, which can be further stabilized by the addition of small quantities of lipids. The immune enhancement induced by TLR4 agonists is highly dependent on the physical structures, and determination of the consistency and stability of these requires detailed physicochemical characterization.[97] Successful use of these adjuvants, therefore, requires careful consideration of how to formulate them and how these amphipathic molecules will interact with other components of the vaccine, such as surfactants, aluminum salts, and antigens.

Safety of TLR4 Agonists. MPL, by far the most widely used of the TLR4 agonists, has demonstrated an acceptable safety profile. A metaanalysis of data from 11 clinical trials and more than 74,000 volunteers (two-thirds of whom received the vaccine that contains AS04 and one-third the control) shows no increase in severe adverse events over aluminum alone or hepatitis A vaccine controls.[78] It has been administered to millions of young women worldwide with few reported severe adverse events. This was further supported by the mechanism of action of AS04, which demonstrated that the adjuvant effect is local and that temporal and spatial colocalization of antigen and adjuvant were required; injecting the adjuvant in a site distant to the antigen or several hours after injection of the antigen resulted in no adjuvant effect.[13]

RC529 (see Fig. 6.3), a synthetic TLR4 agonist in an approved hepatitis B vaccine, has a limited history of clinical use, but as for MPL, no severe adverse events are associated with vaccine containing it. It is, however, not certain that other TLR4 agonists will have the same safety profile. Minor changes in the structure of TLR4 agonists can modify the way they activate the MyD88 or TRAM–TRIF pathway, promoting type 1 interferon or proinflammatory responses, and, as described earlier, the difference in receptor specificity between rodent and human TLR4 receptors may prevent these being detected in preclinical models. The acceptability and extensive history of whole-cell pertussis vaccines, which contain residual bacterial LPS, suggests that this is a pathway that can be stimulated without long-term adverse effects.

TLR9 Agonists

Bacterial DNA is recognized by mammalian species as a PAMP and serves as a potent activator in the innate immune system through interaction with the intracellular TLR9. Fragments of bacterial DNA and synthetic single-stranded oligodeoxynucleotides (ODNs) containing unmethlyated cytosine phosphate guanine (CpG) motifs (CpG ODNs) found in bacterial DNA have also been demonstrated to be powerful adjuvants.[98,99] In vivo, however, the lability of phosphodiester bonds results in rapid degradation of DNA oligonucleotides, and it has been found that replacing these labile ester bonds in the oligonucleotide sequences with phosphorothioate bonds stabilizes the oligonucleotides and significantly enhances the activity.[100] Most preclinical and clinical studies with CpG-containing oligonucleotides contained this modification.

Although obtaining pure CpG-containing oligonucleotides is easy, the development of this class of molecules as adjuvants for human vaccines is severely hampered by the fact that the specific hexameric CpG motifs inducing optimal immune enhancement differ between species.[98] Hence, evaluation of efficacy and safety in rodents is not readily translated to humans. Furthermore, the distribution of the TLR9 receptor and the pathways that its activation promotes also differ across species. In humans, CpG motifs are recognized by TLR9 found on natural killer (NK) cells,[101] B cells, and plasmacytoid dendritic cells,[98] but are not recognized by myeloid dendritic cells and monocytes. In contrast, in mice, those cells express TLR9, which recognizes CpG motifs. In both species, CpG motifs trigger B-cell activation and induce directly or indirectly the production of Th1 and proinflammatory cytokines, including IL-1, IL-6, IL-18, TNF-α, and interferon-γ; in some cases, CpG can redirect preestablished Th2 responses toward Th1.[102] CpG ODNs also act as adjuvants for antigens delivered by the mucosal route of administration.[103]

Numerous clinical evaluations of CpG as an adjuvant have been performed, most commonly with a sequence of CpG referred to as 7909, a sequence found to be optimal for human use. CpG has been evaluated with and without alum for malaria vaccines,[104,105] conjugate pneumonia vaccines,[106] and hepatitis B vaccines[107,108] and in HIV-infected patients in whom CpG permits rapid and long-term seroprotection.[107] Furthermore, it has been demonstrated in an elderly target population that when chemically conjugated CpG (1080 sequence) to the HBV (hepatitis B virus) antigen, the seroprotection induced was faster, superior, and more durable than three doses of a licensed comparator HBV vaccine (Engerix-B).[109]

CPG has also been used in cancer vaccines, such as non–small-cell lung cancer or melanoma, but as of this writing, with no significantly positive results in humans.[110] In addition, an improved hepatitis B vaccine containing CpG (Heplisav B), which demonstrated higher and faster immune responses than the current alum adjuvanted vaccine, did not get a positive opinion from VRBPAC (October 2013) because of the too-small size of the intended population safety database and was requested to extend it before potential licensure.

Other TLR9 Agonists. TLR9 receptors were thought for a long time to be specific for unmethylated CpG sequences; however,

it has been shown that poly(dI:dC), a DNA version of the well-known TLR3 agonist poly(I:C), is also a potent adjuvant, acting through the TLR9/MyD88-dependent pathway.[111] This oligonucleotide, in conjunction with a cationic peptide, is being developed as an adjuvant termed IC31 in clinical trials with recombinant TB antigens, and is reported to induce long-lasting T-cell responses to the antigens.[112] The mode of action thought to be independent of IC31 is dependent not only on the poly(dI:dC) TLR9 agonist, but also on the interaction of the oligonucleotide with the coformulated cationic peptide. The complex formed entraps the antigen and acts as a physical depot at the site of injection, resulting in sustained antigen and adjuvant release.[113] As for the examples given with TLR4, this demonstrates how critical formulation parameters are to successful adjuvant development

Other Toll-Like Receptor Ligands

The molecules described in the preceding sections account for the vast majority of late-stage clinical trials and approved vaccines containing adjuvants. However, numerous other TLR agonists are entering or are in early phase clinical trials. These are briefly described in the following paragraphs.

Flagellin is a protein that polymerizes to form the flagella of flagellated bacteria and is recognized by TLR5. This protein has been expressed as a fusion partner to influenza hemagglutinin or influenza M2e, in which it serves as an adjuvant, enhancing the immune response to the influenza antigen as shown in clinical trials.[114–116]

Numerous small molecules have been found to act as agonists of TLR7 and TLR8. The best known of these are the small-molecule nucleoside analogs, imiquimod and resiquimod (R-848),[117,118] that are TLR7 and TLR7/TLR8 ligands, respectively (both produced by 3M). Other well-known agonists include loxoribine[119] and bropirimine.[120] The use of these molecules as adjuvants is complicated by their low molecular weight and rapid removal from the site of injection. Several formulation methods enable these molecules to be used as adjuvants. Aldara cream, a 5% topical preparation of imiquimod that is licensed for treatment of genital warts and basal cell carcinoma, has been tested as an adjuvant for topical administration at the site of subcutaneous or intradermal injection of antigens.[121,122] This has been shown to have some adjuvant activity in human clinical trials for cancer.[123]

Other Adjuvants

Several other adjuvants are in advanced clinical development and do not fall into any of the aforementioned categories. The best known of these are the saponins, for which the mechanism of action is not fully understood. In this category, however, one could also include adjuvants such as virosomes, cationic liposomes, and polyelectrolytes, a class of polymers that includes polyoxidonium (a component of the Grippol influenza vaccine produced in Russia) and polyphosphazenes, which are being evaluated in several clinical trials.

Saponins. Saponins are triterpenoid molecules with a complex sugar backbone extracted from a variety of plants. The most widely used of these extracts is the saponin extract from the South American tree *Quillaja saponaria* Molina, referred to as Quil-A, which has been used as an adjuvant in veterinary vaccines since the 1970s. This mixture of saponins, with varying adjuvant activity and toxicity, was found to be too reactogenic for use in humans. The Quil-A saponins have an exquisite affinity for cholesterol, and when in contact with membranes containing cholesterol, they form a complex creating pores in the membrane. At the site of injection, this results in considerable reactogenicity. However, this affinity for cholesterol has also enabled the development of two adjuvants for human use based on the Quil-A saponins: ISCOMS and AS01.

ISCOMS are complexes of partially purified saponins from Quil-A, combined with cholesterol, to form small porous particles 50 to 60 nm in diameter with a characteristic buckminsterfullerene shape and a high density.[124,125] Originally, the antigen was associated with these structures through entrapment, conjugation, or hydrophobic interaction, processes that were tedious and not readily applicable to large-scale manufacturing. Subsequently, it was shown that association of the antigen with the particle is not required, and simple coadministration with antigen is adequate. As a result, simpler formulations (ISCOMATRIX and ISCOPREP), which can be simply mixed with the antigen, were developed by CSL (Australia).[126,127] These cholesterol–saponin complexes are less reactogenic than the parent saponin, yet maintain a strong adjuvant effect. Clinical trials have shown them to be slightly more reactogenic than placebo or active control; however, the reactogenicity is generally mild and acceptable. No other vaccine-related severe adverse events have been reported.[128]

An alternative approach to reduce the toxicity of Quil-A saponins was taken by Kensil and colleagues,[129] who isolated from the mixture of saponins a pure component, termed QS21, that was less toxic yet retained adjuvant activity. QS21, however, had two drawbacks: It was chemically unstable at even mildly alkaline conditions, and, while less toxic than the parent mixture, it was still reactogenic. It was found that by combining QS21 with liposomes that contained cholesterol, the stability of the molecule was significantly enhanced and the reactogenicity abrogated.[130] The adjuvant activity of this combination could be further enhanced by the addition of the TLR4 agonist MPL, and the resulting combination of adjuvants is termed AS01 by its developers, GlaxoSmithKline. This formulation has been tested in the clinical setting and shown to induce higher CD4 T-cell responses to a plasmodium antigen than the same immunostimulants combined with an oil-in-water emulsion.[131] Further challenge studies demonstrated the higher efficacy induced by this formulation, which led to the selection of AS01 for the Phase III efficacy study of the candidate malaria vaccine.[132,133] This again demonstrates the critical aspect of formulation and the challenges to identifying appropriate formulations for optimal activity of adjuvants. QS21 in its pure form is also being used as an adjuvant for cancer vaccines.[134]

The exact mechanism of action of QS21 has not been fully elucidated. The loss of adjuvant and lytic activity when the molecule is hydrolyzed suggests that membrane lysis has a role.[129] However, the adjuvant activity is also lost when the aldehyde function on the triterpenoid backbone is removed.[135] Several synthetic analogs have been developed that may permit a more detailed analysis of critical components of the molecule and a better understanding of its mechanism of action.[136,137] In addition to the malaria vaccine, AS01 has been used as the adjuvant of choice for a herpes zoster vaccine, based on a recombinant antigen. The zoster vaccine demonstrated a reduced risk of shingles of 97.2% in adults age 50 years and older compared to placebo,[138] and is the first recombinant adjuvanted vaccine to demonstrate such a level of efficacy against disease reactivation in individuals with latent virus.

There is renewed interest in saponin-based adjuvants from other plant sources, particularly from researchers in China and India.

Virosomes. Virosomes are reconstituted liposomes containing viral (typically influenza virus) proteins in the liposomal

membrane and, optionally, with additional antigens incorporated in the liposomal membrane or attached to the membrane.[139] Virosomes have been used for influenza vaccines (licensed in Europe as Inflexal) and as adjuvants for hepatitis A vaccine (licensed in Europe as Epaxal). They are under investigation as adjuvants for numerous other targets.[140]

Polyelectrolytes and Polycations. The influenza vaccine Grippol licensed in Russia contains polyoxidonium,[141] a polyelectrolyte copolymer of N-oxide 1,4-ethylene piperazine and (N-carboxyethyl)-1,4-ethylene piperidium bromide, that has immunostimulatory properties.[142] Even though little has been published about this specific polymer and its use as an adjuvant, an analogous polymer, poly(carboxylatophenoxy) phosphazene (PCPP) has been widely investigated as an adjuvant for vaccines and has been shown to exert adjuvant activity.[143,144]

Polycations such as polyarginine,[145] chitosan,[146,147] and cationic lipids[148] have also been shown to exert adjuvant activity. As for polyelectrolytes, the mode of action is not fully understood but is likely to involve interaction with cell membranes similar to that described for alum.[32] Another cationic adjuvant, CAF01, is a liposome-based adjuvant composed of the cationic quaternary ammonium lipid dimethyldioctadecylammonium (DDA) and the synthetic analog of mycobacterial cord factor; trehalose-dibehenate (TDB). CAF01 was originally developed as a CMI-promoting adjuvant for a subunit vaccine against TB, but the adjuvant has since been demonstrated to promote a diverse immune response resulting in CMI and humoral responses[149,150] and is in clinical evaluation.

Mucosal Adjuvants. There is a strong interest in the vaccine community in administering vaccines via the mucosal route. This route offers the advantages of being easier to administer and not requiring trained health care workers, but also has the potential of inducing mucosal immunity at the portal of entry of many pathogens. While mucosal delivery and induction of mucosal immunity can easily be achieved with live attenuated vaccines (e.g., oral poliovirus vaccines, oral rotavirus vaccines, nasally administered live attenuated influenza vaccines), inducing systemic or mucosal immunity with nonlive antigens is much more difficult. These tend to be nonimmunogenic or poorly immunogenic via the mucosal routes; consequently, numerous adjuvants have been evaluated to enhance the immunogenicity.

The most widely investigated are the bacterial adenosine diphosphate (ADP)-ribosylating exotoxins such as cholera holotoxin and *E. coli* heat-labile enterotoxin.[151] Even though these are potent mucosal adjuvants, their toxicity has, for the most part, precluded their use as adjuvants. Consequently, mutations have been introduced in an attempt to reduce their toxicity while aiming to retain some adjuvant activity.[152] These include adjuvants such as mLT(R192G), dmLT,[153] and LTK63.[154,155] For nasal delivery, these adjuvants have, however, presented unacceptable safety concerns. The native toxin was used as an adjuvant in an intranasal influenza vaccine that was licensed in Switzerland but was associated with occasional severe adverse events in the form of Bell palsy (partial facial paralysis) ascribed to the adjuvant,[156] and similar effects were seen in an experimental vaccine using the LTH63 detoxified mutant.[157] These adjuvants all contain the pentamer B subunit of the toxin, which binds ganglioside GM_1 on nerves and can be transported through retroaxonal transport to the root of the nerve; it is thought that the neurological adverse events may have arisen through such an interaction.[157] An alternative adjuvant, which does not contain the B subunit and, hence, does not bind GM_1 gangliosides, is CTA1-DD, which is currently in development.[158,159] These exotoxins also have been evaluated for oral delivery in several clinical trials; however, their susceptibility to acidic environments requires enteric formulations. The double mutant dmLT is currently being investigated as an adjuvant for oral or sublingual vaccination against enterotoxigenic *E. coli* (ETEC).[160]

FUTURE DIRECTIONS

The past decade has seen an unprecedented surge in understanding how adjuvants work. While the history of adjuvant discovery and development has evolved through serendipity, we are now in a position in which identification, selection, and use of adjuvants can be rationalized. This has opened the door to vaccine candidates against as yet unmet medical needs—vaccines that will not only prevent infectious diseases, but might also treat life-threatening diseases such as cancers or enable management of chronic disorders such as Alzheimer disease. Adjuvants appear also as a potential route to improve on current licensed vaccines that may be less efficient in a specific target population that requires a stronger stimulation on the immune system to develop a protective immune response.

There are a number of factors and unknowns that need to be considered. The most important of these is how to demonstrate the safety of adjuvants. As pointed out several times, there is an underlying concern that is not necessarily based on sound science that adjuvants can induce immune-mediated disorders. The absence of suitable animal models and the differences in receptors and receptor distribution between animals and humans can make it difficult in some cases to demonstrate that this is not the case.

Despite the occurrence of autoimmune reactions in the general population, the fear that adjuvants can induce or exacerbate autoimmune disorders is one that is at the forefront of follow-up and surveillance. Holding clinical development of a vaccine based on a single adverse event demonstrates the challenges of developing vaccines with novel adjuvants. The risk-to-benefit balance associated with the vaccine being developed will always be the guiding principle for ascertaining the value of a given approach. A large safety database to demonstrate rare events in such studies is not always possible, which underlines the difficulty introducing new approaches into the field of vaccines. The paucity of epidemiologic data on such immune-mediated disorders, which are key to establishing the background rate of disease, needs to be addressed so that factual analysis can be undertaken when such events occur.

The safety evaluation of a vaccine encompasses all constituents of the product. It cannot be assumed that an adjuvant that is safe in one vaccine with a given antigen will be safe when added to another vaccine, even if the latter vaccine is safe without adjuvant. A rational approach requires nonclinical toxicology, determination of the mode of action of the adjuvant, an evaluation of differences in receptors and activity in animal and human cells, controlled clinical trials, and postmarketing surveillance.[161]

Also, adequate formulation is critical for the activity of many adjuvants. Yet the knowhow for adjuvant formulation is not widely available, even though predicting how the physicochemical parameters of an adjuvant and its interaction with the antigen affect immune responses is key to its selection. All these points emphasize the criticality of process development, robustness, and reproducibility, and the ability to characterize adjuvants in a relevant and efficient way.

A host of adjuvants are now in clinical development; however, none tested in humans so far has the ability to induce functional $CD8^+$ T-cells to a level that can be seen with live viral vaccines. Live viral vaccines have their limitations, in particular with respect to repeated boosting and their use in

immune-suppressed persons. There is, therefore, a need to pursue research into adjuvants capable of inducing $CD8^+$ T-cell responses.

Finally, because vaccines are built on the combination of antigen(s) and adjuvant(s), the need for relevant and immunogenic antigens should not be overlooked. Because more and more vaccines will require the induction of both humoral immunity and CMI, there is a need for researchers to seek to improve the intrinsic immunogenicity of the antigen and to ensure optimum immune responses if the addition of an adjuvant is required.

It is only through the appropriate combination of antigen and adjuvant, selected on the basis of the targeted disease, relevant protective immune response, and target population, that adjuvants will fulfill their promises and find their place as a relevant and effective tool for improving human health.

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This chapter includes a significant contribution written for the previous edition by our late colleague, Dr. Stanley Hem.

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7 Vaccine Additives and Manufacturing Residuals in Vaccines Licensed in the United States

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In addition to one or more immunogens,* a vaccine may contain any of several added substances such as an adjuvant or a preservative. Residual components from the manufacturing process, in varying amounts, are also present in the vaccine. This chapter addresses the types and amounts of additives that are present in vaccines, the rationale for their inclusion, and the applicable federal regulations. Additionally, residual materials from the manufacturing process that are present in the final formulation of the vaccine, as well as relevant federal regulations regarding these residuals, are discussed. Finally, albeit to a limited extent, several issues and concerns that currently pertain to the use of, or presence of, some of these additives and residuals are examined.

This chapter focuses on vaccines licensed in the United States; vaccines licensed outside the United States may contain the same types of additives and residuals, although the amounts that are present in any given vaccine may differ. For the purposes of this chapter, the term "additives" refers to materials that are added to the immunogen by the manufacturer for a specific purpose. Additives include adjuvants, preservatives (i.e., antimicrobial agents), and stabilizers, as well as materials that are added to affect pH and isotonicity. In addition to additives, vaccines contain residuals that remain from the licensed manufacturing process. The final formulation—immunogen plus additives and residuals—defines the specific vaccine; although not all manufacturing residuals can be identified and quantified, their presence and quantity is assumed to be constant because of the constancy of the manufacturing process. Some information regarding additives and residuals is considered to be a trade secret and thus confidential, and cannot be discussed in this chapter.

Vaccine manufacturing includes in-process and release tests, along with their respective specifications, for the allowable quantity of additives and certain residuals that may be present in the vaccine. These tests and their accompanying specifications are detailed in the product's Biologic License Application (BLA); some of the specifications may be provided in the vaccine's package insert. A manufacturer must report each change in the manufacturing process, including removal or adjustment in the quantity of an additive, to the U.S. Food and Drug Administration (FDA). FDA biologics regulations, found in 21 CFR §610.61, address whether the use of, and quantity of, additives and residuals must be disclosed in vaccine labeling. These regulations state:

> *The following shall appear on the label affixed to each package containing a product …*
>
> *(e) The preservative used and its concentration …*
> *(l) Known sensitizing substances, or reference to an enclosed circular containing appropriate information;*
> *(m) The type and calculated amount of antibiotics added during manufacture;*
> *(n) The inactive ingredients when a safety factor, or reference to an enclosed circular containing appropriate information;*
> *(o) The adjuvant, if present;*
> *(p) The source of the product when a factor in safe administration;*
> *(q) The identity of each microorganism used in manufacture, and, where applicable, the production medium and method of inactivation ….*

VACCINE ADDITIVES

Preservatives

Preservatives are added to vaccine formulations to prevent the growth of bacteria or fungi that may inadvertently be introduced into the vaccine during use. In some cases, preservatives are used during the manufacturing process (e.g., in buffers and column washes) to prevent microbial growth. Improvements in manufacturing technology, however, have decreased this need for the addition of preservatives to control bioburden during the manufacturing process. The Code of Federal Regulations (CFR) requires that, with certain defined exceptions, or with the approval of the Center Director (discussed later), preservatives must be added to multidose vials of vaccines. In the past, tragic consequences followed the use of multidose vials that did not contain a preservative, and served, in part, as the impetus for this requirement (see Wilson[1] for a discussion of incidents related to the lack of preservatives in vaccines). Specifically, 21 CFR §610.15(a) states; "Products in multiple-dose containers shall contain a preservative, except that a preservative need not be added to Yellow Fever Vaccine; Polio-virus Vaccine Live Oral; viral vaccines labeled for use with the jet injector; dried vaccines when the accompanying diluent contains a preservative; or to an Allergenic Product in 50 percent or more volume in volume (v/v) glycerin."

Although the regulation does not specify a quantity, it does require that the preservative used "shall be sufficiently nontoxic so that the amount present in the recommended dose of the product will not be toxic to the recipient, and in the combination used it shall not denature the specific substances in the product to result in a decrease below the minimum acceptable potency within the dating period when stored at the recommended temperature."

The CFR does not, however, provide a definition of a preservative. The definition (i.e., antimicrobial effectiveness) that has been used by the FDA for vaccines and other biologicals is found in the U.S. Pharmacopoeia (USP).[2] This is a functional definition, wherein the final formulation of the vaccine, including the preservative, is challenged with specified quantities of the following organisms: *Candida albicans, Aspergillus brasiliensis, Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. The test sample (preservative-containing vaccine plus the microorganism) is incubated at 20° to 25°C,

*An immunogen is a preparation consisting of all or a portion of a disease-containing organism, or the nucleic acid that encodes one or more of the proteins from that organism, or all or a portion of a human tissue, and it is administered to an individual to induce an immune response to the immunogen for the treatment or prevention of a disease or condition.

and the number of viable microorganisms is determined on days 7, 14, and 28. A preservative is deemed acceptable if the following are achieved:

- Bacteria: a reduction of not less than 1.0 $\log_{10}$ from the initial count at 7 days, and not less than a 3.0 $\log_{10}$ reduction from the initial count after 14 days, and no increase in the 14-day count at 28 days.
- Yeasts and molds: remain at or below the level of the initial inoculum on days 7, 14, and 28.

Note that the antimicrobial agent is not tested by itself; rather, it is the final vaccine formulation that is tested.

Preservatives cannot completely eliminate the risk of bacterial or fungal contamination of vaccines; moreover, they do not address any potential viral contamination. Although it occurs rarely, and not in the recent past, the scientific literature does contain reports[3,4] (see also Wilson[1]) of bacterial contamination of vaccines despite the presence of a preservative, emphasizing the need for meticulous attention to technique when withdrawing vaccines from multiuse vials. At present, the following preservatives are used in U.S.-licensed vaccines: phenol, benzethonium chloride plus formaldehyde, 2-phenoxyethanol, and thimerosal (termed *thiomersal* in some other countries). Recently, the FDA amended the biologics regulations to permit exceptions or alternatives to the regulation for constituent materials (21 CFR §610.15), which includes preservatives and adjuvants. The following section was added to the regulation: "(d) The Director of the Center for Biologics Evaluation and Research (CBER) or the Director of the Center for Drug Evaluation and Research (CDER) may approve an exception or alternative to any requirement in this Section. Requests for such exceptions must be in writing."

The amended regulation could, as an example, allow the use of particular vial adaptors to prevent contamination of products in multidose vials without the use of preservatives. As noted in the final rule,[5] the Director of CBER or the Director of CDER "would not approve an exception or alternative when the data or conditions of use, including the indication and patient population, do not provide a sufficient scientific and regulatory basis for such an approval." The amended regulation took effect in May 2011.

As noted, a preservative "shall not denature the specific substances in the product to result in a decrease below the minimum acceptable potency within the dating period when stored at the recommended temperature." Certain preservatives are not compatible with certain antigens; compatibility must be established. For example, it has been known for a number of years that thimerosal has a deleterious effect on the potency of inactivated poliovirus vaccine (IPV).[6,7] An alternative preservative is necessary for IPV. A preservative that is used in other products, 2-phenoxyethanol,[8] has been found to be compatible with IPV vaccine formulations; it is used as a preservative in both of the currently U.S.-licensed IPV vaccines (IPOL [Sanofi Pasteur SA] and Poliovax [Sanofi Pasteur Ltd.; not currently marketed in the United States]).

Phenol is currently used in three U.S.-licensed vaccines: the polysaccharide vaccines Pneumovax 23 (a 23-valent pneumococcal polysaccharide vaccine manufactured by Merck Sharp & Dohme Corp.) and Typhim Vi (*Salmonella typhi* capsular polysaccharide vaccine manufactured by Sanofi Pasteur SA), and ACAM2000 (the smallpox vaccine from Sanofi Pasteur Biologics Co.); each of these vaccines contains 0.25% phenol as a preservative (phenol is contained in the diluent for ACAM2000). According to the Minimum Requirements of the National Institutes of Health (NIH),[9,10] phenolic compounds (such as phenol or the various creosols) are not permitted as preservatives in diphtheria- and tetanus toxoid–containing products.* This requirement is also reflected in other regulations or requirements, such as those of the World Health Organization (WHO).[11] It has been reported that phenol affects diphtheria toxoid, "so that its immunizing power falls rapidly."[12] Benzethonium chloride with formaldehyde is currently used in only one U.S.-licensed vaccine, anthrax vaccine adsorbed (BioThrax; the preservative is 25 μg/mL benzethonium chloride and 100 μg/mL formaldehyde), manufactured by Emergent BioDefense Operations Lansing LLC.

In recent years, considerable controversy has surrounded the use of thimerosal, an organomercurial, in vaccines. Although allergic responses to thimerosal have been described,[13] a controversy, arising in the late 1990s, centered on the hypothesis that exposure to thimerosal, a derivative of ethyl mercury, may be causally linked to autism and other neurodevelopmental disorders in children. Although there were no clear or definitive data to support a link between thimerosal and neurodevelopmental disorders, the U.S. Public Health Service (PHS), first in July 1999[14] and again in June 2000,[15] in an effort to reduce mercury exposure in children from all sources, recommended that thimerosal be removed from pediatric vaccines as expeditiously as possible. The July 1999 PHS statement was issued jointly with the American Academy of Pediatrics; the June 2000 PHS statement was issued jointly with the American Academy of Pediatricians, the American Academy of Family Physicians, and the Advisory Committee on Immunization Practices (ACIP). Letters from CBER of the FDA, in 1999[16] and again in 2000,[17] to the various vaccine manufacturers noted that the removal of thimerosal from vaccines was merited and requested manufacturers' timelines for thimerosal removal or submission of an explanation as to why thimerosal removal was not currently feasible.

In 2004, the Institute of Medicine (IOM)'s Immunization Safety Review Committee of the National Academy of Science's IOM issued its final report, examining the hypothesis that, inter alia, thimerosal-containing vaccines are causally associated with autism. The committee concluded that the body of evidence favors rejection of a causal relationship between thimerosal-containing vaccines and autism, and that the hypotheses that were generated concerning a biological mechanism for such causality were theoretical only.[18] The European Medicines Agency also noted, as a precautionary measure, "that, although there is no evidence of harm caused by the level of exposure from vaccines, it would be prudent to promote the general use of vaccines without thiomersal and other mercury-containing preservatives."[19] Of note, the WHO continues to recommend the use of vaccines containing thimerosal because the need for multidose preservative-containing vaccines and, thus, the benefit of using such vaccines outweighs the theoretical risk of toxicity.[20] Additionally, the Global Advisory Committee on Vaccine Safety has stated that it remains of the view that there is no evidence supporting a causal association between neurobehavioral disorders and thimerosal-containing vaccines.[21] A more comprehensive update on the thimerosal-autism hypothesis for vaccines may be found in Chapter 82.

At present, with the exception of the inactivated influenza vaccine, all of the U.S.-licensed, routinely recommended pediatric vaccines (hepatitis B, diphtheria–tetanus toxoid–acellular pertussis [DTaP], *Haemophilus influenzae* type b, IPV, pneumococcal conjugate, human papillomavirus [HPV], hepatitis A, rotavirus, measles-mumps-rubella [MMR] and varicella) are thimerosal free or contain only trace amounts (<1 μg

*At the time of the writing of these requirements for diphtheria and tetanus toxoids, U.S. vaccine regulation was the responsibility of the NIH.

TABLE 7.1 Preservatives, Adjuvants, and Inactivation Residues Noted in Labels of Selected U.S.-Licensed Vaccines

Vaccine	Trade Name	Preservative (Amount/ Dose) and μg Hg/Dose[a] (if Noted)	Adjuvant and Aluminum Content Per Dose	Inactivation Residues (Amount Per Dose if Noted)
BACTERIAL VACCINES				
Td (adult)	DECAVAC	None ≤0.3 μg Hg	Alum [$KAl(SO_4)_2$] Al: ≤0.28 mg	Formaldehyde (≤0.1 mg)
	None/MassBiologics	None ≤0.3 μg Hg	$AlPO_4$ Al: ≤0.53 mg	Formaldehyde (<0.1 mg)
DTaP	DAPTACEL	None	$AlPO_4$ Al: 0.33 mg	Formaldehyde (≤0.5 μg) Glutaraldehyde (<50 ng)
	Infanrix	None	$Al(OH)_3$ Al: ≤0.625 mg	Formaldehyde (≤0.1 mg) Glutaraldehyde (%NN)
Tdap	Adacel	None	$AlPO_4$ Al: 0.33 mg	Formaldehyde (≤5 μg) Glutaraldehyde (<50 ng)
VIRAL VACCINES				
Hepatitis A	Havrix 0.5-mL pediatric dose	None	$Al(OH)_3$ Al: 0.25 mg	Formalin (≤0.05 mg)
	VAQTA 0.5-mL pediatric dose	None	Aluminum (mixed salt) Al: 0.225 mg/dose	Formaldehyde (<0.4 μg)
Human papillomavirus	Cervarix	None	AS04 (MPL + $Al(OH)_3$): 0. 5mg Al: 0.17 mg	N/A
	Gardasil 9	None	Aluminum (mixed salt) Al: 0.5 mg/dose	N/A
Influenza	Afluria (2014-2015) (0.5 mL, single dose)	None	None	β-Propiolactone (≤2 ng)
	Afluria (2014-2015) (multidose vial)	Thimerosal 24.5 μg Hg	None	β-Propiolactone (≤2 ng ng)
	Agriflu (2013-2014) (0.5 mL, single dose)	None	None	Formaldehyde (≤10 μg)
	Fluarix (2014-2015) (0.5 mL, single dose)	None	None	Formaldehyde (≤5 μg)
	Fluvirin (2014-2015) (0.5 mL, single dose)	None ≤1 μg Hg	None	β-Propiolactone (≤0.5 μg)
	Fluvirin (2014-2015) (multidose vial)	Thimerosal 25 μg Hg	None	β-Propiolactone (≤0.5 μg)
Japanese encephalitis virus	Ixiaro	None	Al $(OH)_3$ (250 ug) Al: NN	Formaldehyde (≤200 ppm, ≤0.1 mg/0.5 mL dose)
Polio	IPOL	2-Phenoxyethanol (2.5 mg) + formaldehyde (≤0.1 mg)	None	Formaldehyde ≤0.1 mg

DTaP, diphtheria–tetanus toxoid–acellular pertussis; MPL, 3-*O*-desacyl-4′-monophosphoryl lipid A; NN, not noted on label; %NN, amount in final container not noted on label; Td, tetanus-diphtheria vaccine for adolescents and adults; Tdap, tetanus, diphtheria, and pertussis for adolescents and adults.

[a]Dose is 0.5 mL.

of mercury per dose) as a residual from the manufacturing process (Table 7.1). Of the U.S.-licensed trivalent injectable influenza vaccines, six are approved for pediatric use: Fluzone (Sanofi Pasteur Inc.), for use in infants 6 months of age and older; Fluvirin (Seqirus Vaccines, Ltd.), for use in children 4 years of age and older; Afluria (Seqirus Pty Ltd.), for use in children 5 years of age and older; Fluarix (GlaxoSmithKline Biologicals), for use in children 3 years of age and older; FluLaval (ID Biomedical Corporation of Quebec), for use in children 3 years of age and older; and Flucelvax (Seqirus, Inc.), for use in children 4 years of age and older. These six inactivated influenza vaccines are available in either thimerosal-free presentations (Fluzone, Fluarix, Afluria, FluLaval, and Flucelvax) or with trace thimerosal (≤1 μg of mercury per 0.5 mL dose) (Fluvirin). Additionally, there are four U.S.-licensed quadrivalent inactivated influenza vaccines (FluLaval Quadrivalent, Fluzone Quadrivalent, Fluarix Quadrivalent, and Flucelvax Quadrivalent) available for children; the age indications are as above for the trivalent presentations and single-dose presentations of these vaccines contain no thimerosal. FluMist Quadrivalent (MedImmune, LLC) is a live, attenuated quadrivalent influenza vaccine and does not contain thimerosal.

Two Tdap (tetanus, diphtheria, and pertussis for adolescents and adults) vaccines (tetanus, diphtheria, and acellular pertussis vaccines, with the lowercase letters indicating a reduced antigen content for the diphtheria toxoid and one or more of the pertussis antigens) for use in adolescents and adults, Adacel (Sanofi Pasteur Ltd.) for use in persons 10 through 64 years of age, and Boostrix (GlaxoSmithKline Biologicals) for use in persons 10 years of age and older, are licensed in the United States. Neither product contains thimerosal. The quadrivalent (Groups A, C, Y, and W135) meningococcal conjugate vaccines, Menactra (Sanofi Pasteur Inc.) and Menveo (Novartis Vaccines and Diagnostics S.r.l.) do not contain any preservative. MenHibrix (GlaxoSmithKline Biologicals) is a bivalent meningococcal conjugate vaccine

(against serogroups C and Y), additionally containing an *H. influenzae* type b capsular polysaccharide (polyribosyl-ribitol-phosphate) conjugate, and is likewise thimerosal free. Similarly, the two meningococcal serogroup B vaccines BEXSERO (Novartis Vaccines and Diagnostics S.r.l.) and TRUMENBA (Wyeth Pharmaceuticals Inc.) and the HPV vaccines Gardasil 9 (Merck Sharp & Dohme Corp.) and Cervarix (GlaxoSmith-Kline Biologicals) do not contain any preservative.

A diphtheria and tetanus toxoid vaccine (DT) for use in children younger than 7 years of age is available from Sanofi Pasteur Inc. It contains only a trace amount of thimerosal as a manufacturing residual.

Adjuvants

Adjuvants are materials that enhance and direct the immune response (see Chapter 6). Vaccine adjuvants are not licensed separately; rather, the adjuvant is a constituent of the licensed vaccine, and it is the vaccine formulation, in toto, that is tested in clinical trials and is licensed. An adjuvant cannot be added or removed, or its amount in a licensed vaccine changed, without submitting a supplement to the vaccine license and obtaining approval from the FDA. The various aluminum salts (aluminum hydroxide, aluminum phosphate, alum [potassium aluminum sulfate], or mixed aluminum salts) are the most commonly used adjuvants in U.S.-licensed vaccines. One vaccine, Cervarix (GlaxoSmithKline Biologicals), which contains AS04, an adjuvant system composed of an aluminum salt and monophosphoryl lipid A, a detoxified lipopolysaccharide (LPS), has been licensed. In 2013, as part of the U.S. government's national pandemic preparedness initiative, an H5N1 monovalent influenza vaccine, approved for use in persons 18 years of age and older, containing the unique adjuvant, AS03, manufactured by ID Biomedical Corporation of Quebec was licensed. The AS03 adjuvant is an emulsion composed of squalene, DL-α-tocopherol, and polysorbate 80. In 2015, an inactivated influenza vaccine, Fluad (Seqirus Vaccines Ltd.), which contains the squalene-based adjuvant MF59C.1, was licensed.

Despite worldwide use of aluminum salts for more than 50 years, surprisingly little has been known about their mechanism of action as adjuvants (see, e.g., Chapter 6 and Hogenesch[22]). For many years, the prevailing thought was that the aluminum salts functioned as depots for the vaccine immunogens. More recently, it was shown that the aluminum salts also activate inflammasomes, clusters of proteins found inside certain cells. Inflammasomes respond to stresses such as infection or injury by releasing cytokines, which, in turn, stimulate an immune response.[23]

Table 7.1 presents the specific aluminum salt (hydroxide, phosphate, sulfate, or mixed) and the quantity of aluminum that is contained in a number of commonly used vaccines. (The aluminum content that is listed for some vaccines noted in Table 7.1 represents the upper limit of the specification; the vaccine may routinely contain less aluminum.) By regulation (21 CFR §610.15[a]), the aluminum content of a vaccine cannot exceed 0.85 mg of aluminum per dose if the amount is assayed, or 1.14 mg/dose if determined by calculation based on the amount of the aluminum compound that is added. To harmonize with WHO recommendations, this regulation was amended in 1981 to permit up to 1.25 mg of aluminum per dose. However, the higher amount was permitted only "provided that data demonstrating that the amount of aluminum used is safe and necessary to produce the intended effect are submitted to and approved by the Director, Center for Biologics Evaluation and Research" (21 CFR §610.15[a]). The European Pharmacopoeia likewise restricts the aluminum content to 1.25 mg per dose. It should be noted that the above regulation for aluminum content refers to an individual dose of a biological product; thus, for example, for a hypothetical combination vaccine derived from licensed components, the aluminum content for the combination vaccine may still not exceed 0.85 mg.

Recently, the FDA amended the CFR requirements[5] for constituent materials including adjuvants to permit, when justified, exceptions or alternatives such as the use of an increased aluminum content in a vaccine; this change may have a greater impact on certain therapeutic vaccines than on the preventive vaccines.

Concerns have been raised in recent years about the use of aluminum in vaccines and potential adverse outcomes that may be associated with its use at the levels that exist in individual vaccines and through the additive effects of multiple vaccinations. These concerns about the use of aluminum in vaccines prompted a workshop that was sponsored by the National Vaccine Program Office in May 2000. The general use of aluminum salts in vaccines[24] and aluminum toxicokinetics[25] were reviewed during the workshop. In their overall summary of the workshop, Eickhoff and Meyers[26] noted that "based on 70 years of experience, the use of salts of aluminum as adjuvants in vaccines has proven safe and effective." A more recent study by FDA scientists, using updated parameters for toxicokinetic assessments, including current recommended vaccines and aluminum excretion data, concluded that the risk from aluminum exposure from vaccines and the environment to infants was extremely low.[27]

Stabilizers

Various stabilizers are added to vaccines to help protect them from adverse conditions such as the freeze-drying process (for those vaccines that are freeze-dried) or heat. For freeze-dried (lyophilized) preparations of vaccines, it is also necessary to add materials that provide a bulk matrix for the vaccine. The amount of an immunogen that is contained in a vaccine can be extremely small, on the order of tens of micrograms or less. If sufficient amounts of various materials were not added to the vaccine before lyophilization, the vaccine would not be readily observable and would most likely adhere to the vial wall. By way of illustration of vaccine immunogen masses, ActHIB (Sanofi Pasteur SA), a polysaccharide conjugate vaccine that is marketed in a freeze-dried presentation, contains approximately 10 μg of purified polysaccharide conjugated to 24 μg of tetanus toxoid. The immunogen mass for live viral vaccines is even less, on the order of nanograms (approximately 10^3 to 10^5 viral particles per dose). Thus, there is a need to provide a matrix to contain these vaccines during freeze-drying.

The types of material that are added to vaccines as stabilizers include sugars (such as sucrose or lactose), amino acids (such as glycine or the monosodium salt of glutamic acid), and proteins (such as gelatin). Table 7.2 lists the stabilizers that are used for a number of common vaccines.

Added proteins may be of concern for two principal reasons. The first concern arises from the potential for animal- and human-derived protein to contain one or more adventitious agents. The second concern arises from the potential for these proteins to elicit an allergic reaction. The two animal- or human-derived proteins that are currently used as stabilizers in U.S.-licensed vaccines are human serum albumin (HSA) and gelatin. The FDA has, as of this writing, required that, if blood-derived HSA is used in vaccine manufacture, only U.S.-licensed HSA may be used. Additionally, an FDA guidance recommends that the following statement appear in the Warnings section of the package insert for blood-derived HSA-containing products[28]: "This product contains albumin, a

TABLE 7.2 Vaccine Stabilizers, Manufacturing Residuals, and Cell Lines Noted in the Labels of Selected U.S.-Licensed Vaccines

Vaccine	Trade Name	Stabilizers Per Single Dose[a]	Manufacturing Residuals (Except Inactivating Agents) Per Dose[a]	Cell Line
LIVE BACTERIAL VACCINES				
Typhoid	Vivotif (1 capsule)	3.3–34.2 mg Sucrose; 0.2–2.4 mg ascorbic acid; 0.3–3.0 mg amino acid mixture	NN	NA
LIVE VIRAL VACCINES				
Mumps, measles, rubella	MMR-II	1.9 mg Sucrose 14.5 mg hydrolyzed gelatin; 14.5 mg sorbitol; 0.38 mg monosodium L-glutamate; sodium phosphate; ≤0.3 mg recombinant human albumin	25 μg Neomycin; <1 ppm fetal bovine serum albumin; other buffer and media components[b]	Chick embryo cell culture (measles and mumps) WI-38 cells (rubella)
Yellow fever	YF-Vax	Sorbitol and gelatin (%NN)	NN	Chicken embryos
INACTIVATED VIRAL VACCINES				
Polio	IPOL	NN	<5 ng Neomycin; <200 ng streptomycin; <25 ng polymyxin B; <1 ppm calf serum protein	Vero cells
Japanese encephalitis virus	Ixiaro	None added	≤50 ng Bovine serum albumin; ≤100 pg host cell DNA; ≤200 ppm sodium metabisulfite; host cell proteins (≤300 ng/6 μg of protein); ≤0.5 μg protamine sulfate	Vero cells
Hepatitis A	Havrix (1mL dose)	NN	≤5 μg/mL MRC-5 cellular proteins; ≤40 ng/mL neomycin sulfate	MRC-5 cells
	VAQTA (1 mL dose)	70 μg sodium borate/mL	<0.1 μg Nonviral protein/mL; <4×10^{-6} μg DNA/mL; <10^{-4} μg bovine albumin/mL	MRC-5 cells
Rabies	RabAvert	<12 mg polygeline; 1 mg potassium glutamate	<0.3 mg Human serum albumin <3 ng ovalbumin; <1 μg neomycin; <20 ng chlortetracycline <2 ng amphotericin B	Chicken fibroblasts
	Imovax	NN	<100 mg Human serum albumin; <150 μg neomycin sulfate; <20 μg phenol red	MRC-5 cells
RECOMBINANT PROTEIN VIRAL VACCINES				
Human papillomavirus	Gardasil 9	NN	<7 μg Yeast protein	*Saccharomyces cerevisiae*
	Cervarix	NN	<40 ng Insect cell and viral proteins; <150 ng bacterial proteins	*Trichoplusia ni*
Hepatitis B	Engerix-B (1 mL dose)	NN	≤5% Yeast protein	*S. cerevisiae*
	Recombivax HB (1 mL dose)	NN	<1% Yeast protein	*S. cerevisiae*
Influenza	Flublok	NN	≤28.5 μg Baculovirus and insect cell proteins; ≤10 ng baculovirus and insect cell DNA; ≤100 μg Triton X-100	*Spodoptera frugiperda*

Continued on following page

TABLE 7.2 Vaccine Stabilizers, Manufacturing Residuals, and Cell Lines Noted in the Labels of Selected U.S.-Licensed Vaccines *(Continued)*

Vaccine	Trade Name	Stabilizers Per Single Dose[a]	Manufacturing Residuals (Except Inactivating Agents) Per Dose[a]	Cell Line
VIRAL VACCINES				
Influenza (trivalent)	Afluria	NN	≤10 ppm Na taurodeoxycholate; <1 μg ovalbumin; ≤3 ng neomycin sulfate; ≤0.5 ng polymyxin B	Embryonated chicken eggs
	Flucelvax	NN	≤8.4 μg MDCK cell protein; ≤120 μg non-HA protein; ≤10 ng MDCK cell DNA; ≤1125 μg polysorbate 80; ≤13.5 μg cetyltrimethylammonium bromide	Madin-Darby Canine Kidney (MDCK) cells
	Fluarix	NN	≤0.0016 μg Hydrocortisone; ≤0.15 μg gentamicin sulfate; ≤0.05 μg ovalbumin; ≤50 μg sodium deoxycholate	Embryonated chicken eggs
	Fluzone	NN	≤150 μg octylphenol ethoxylate	Embryonated chicken eggs

NA, not applicable; NN, none noted; %NN, percentage not noted in labeling.
[a]Each vaccine dose listed in the Table is 0.5 mL unless otherwise noted.
[b]Amounts of all ingredients not specified in labeling.

derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases. Although there is a theoretical risk for transmission of Creutzfeldt-Jakob disease (CJD), no cases of transmission of CJD or viral disease have ever been identified that were associated with the use of albumin."

For HSA produced as a recombinant DNA protein, it would not be necessary for the albumin to be separately licensed by the FDA nor would the above-quoted warning be included in the package insert (as it is not derived from blood). In August 2005, the FDA approved a license supplement for use of recombinant HSA in MMR-II (Merck Sharp & Dohme Corp.).

Gelatin or processed gelatin is also used as a stabilizer. Gelatin may be bovine or porcine derived. Despite the use of a harsh manufacturing procedure (extremes of heat and pH) in the production of gelatin, there is concern about the presence of the bovine spongiform encephalopathy (BSE) agent in bovine-derived material. Thus, any bovine-derived gelatin that is added to vaccines, or used in the vaccine manufacturing process, must not be sourced from countries reporting BSE *or* from countries that do *not* meet the latest BSE-related standards of the Office International des Epizooties (OIE) (see "Transmissible Spongiform Encephalopathy Agents" later).[29]

A second concern for gelatin relates to allergic responses. Allergic responses to gelatin, although rare, have been described in the medical literature.[30–33] It has been hypothesized that in Japan, use of partially hydrolyzed gelatin, which contained a small amount of high-molecular-weight gelatin, contributed to an increase in the incidence of allergic reactions.[33,34] Nakayama and Aizawa noted that a change to hydrolyzed modified porcine gelatin, together with discontinuation of the use of gelatin-containing DTaP vaccines, may have contributed to a decrease in the incidence of allergic reactions after administration of monovalent measles and mumps vaccine in Japan.[34] A severe allergic reaction to gelatin is a contraindication to receiving gelatin-containing vaccines.

Various buffers (e.g., phosphate buffer) are also used in vaccines to maintain a particular pH range, and salts (e.g., NaCl) may be added to achieve isotonicity.

MANUFACTURING RESIDUALS

In principle, any or all of the materials that are used in the manufacturing process may be present in the final vaccine formulation. For the purposes of this chapter, materials that are present in the final vaccine formulation that derive from the manufacturing process are termed *residuals*. Various steps in the manufacturing process may remove or reduce the amounts of many of these residuals. However, for various vaccines, an acceptable technology to remove these manufacturing residuals may not exist, or there may be no perceived need (e.g., with regard to safety or a potential for an adverse effect on efficacy) for their removal. Additionally, and as a general principle, it is not possible to remove a particular substance completely, nor is it possible to demonstrate that a particular substance has been removed completely. For many substances, the residual amount may be below the limits of detection by current analytic technologies, and may, for practical reasons, be considered absent.

Bacterial and viral inactivation substances must be noted in the package label (21 CFR §610.61[q]). Residual bacterial or cellular culture components, such as antibiotics that are used during manufacture, as well as sensitizing substances (generally proteins), and other inactive ingredients when considered a safety factor, also must be noted in the label (21 CFR §610.61 [l][m][n]). There may be some overlap between these categories; however, they are grouped in this manner for convenience and to aid a discussion of these materials as they are affected by current regulations. Residual bacterial or cell culture components may be included in these categories, but other residuals, such as DNA and endotoxin, are not generally noted in labeling.

Inactivation Residuals

Various agents may be used to inactivate bacteria and viruses or to detoxify bacterial toxins. The goal of these chemical treatments is to inactivate the bacterium or virus or to remove toxic activity while still preserving the antigenicity of the product against the homologous organism or toxin. After inactivation, a virus or bacterium may be processed further to furnish particular antigens. For example, after inactivation, influenza

viruses may be split by various chemical treatments (e.g., detergent) so that more purified inactivated influenza vaccines can be produced.

Formaldehyde has a long and extensive history of use in the preparation of bacterial and viral vaccines. It was used by Ramon in 1923 to detoxify diphtheria, yielding a diphtheria toxoid vaccine termed an *anatoxine*.[35] Requirements for the use of formaldehyde and the permitted residual amount of formaldehyde that is allowed for diphtheria toxoid are provided in the NIH Minimum Requirements.[9] Similar NIH Minimum Requirements exist for tetanus toxoid.[10] These documents note that residual, free formaldehyde in the finished product should not be in excess of 0.02% (i.e., 0.1 mg for a 0.5-mL vaccine dose). Formaldehyde is also used to inactivate viruses (e.g., polio and influenza viruses) when preparing vaccines. Table 7.1 lists the amount of residual formaldehyde that is present or allowed in these and various other U.S.-licensed vaccines; in the diphtheria and tetanus toxoids, the amount does not exceed 0.02%.

Similar requirements for residual formaldehyde exist elsewhere, for example, in Europe, as noted in the European Pharmacopoeia for various vaccines. Concern about the presence of residual formaldehyde in vaccines stems from the known toxic effects of formaldehyde, and from its carcinogenicity potential. The U.S. Environmental Protection Agency (EPA) has established a reference dose (RfD) for formaldehyde through the oral route.[36] The RfD is defined by the EPA as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure or a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime."[37] The RfD for formaldehyde (oral administration) is 0.2 mg/kg of bodyweight per day.[36] The amount of residual formaldehyde in vaccines (which are administered infrequently, not daily) is below this level.

Formaldehyde has been further classified by the EPA as a "probable human carcinogen" (the EPA's B1 classification).[36] The bulk of the carcinogenicity studies on formaldehyde have focused on chronic respiratory exposure because this is the primary route of industrial and routine household exposure. There are fewer data regarding ingested or parenteral exposure to formaldehyde, and the EPA has not developed a risk estimate for either oral or parenteral exposure.[36] Data regarding carcinogenicity studies may be found in documents from the EPA[36] and the International Agency for Research on Cancer.[38]

One point regarding formaldehyde should be made. Formaldehyde is naturally present in the human body as the result of various biochemical processes[38,39] and the steady-state level of formaldehyde in the bloodstream of humans is approximately 2.6 mg/L.[40] The amount of formaldehyde that is naturally, continuously present in the blood of humans, or turned over in a particular day, is markedly in excess of the amount that is present as a vaccine residual. A 2013 FDA-authored study[41] of the risk posed by the residual formaldehyde in vaccines concluded, "In the absence of any known adverse health effects from endogenously produced formaldehyde, which exists in blood and extravascular water at steady-state concentrations that are more than 100-fold higher [than are residual in vaccines], we consider vaccine-related, exogenous formaldehyde to be a miniscule part of the daily formaldehyde turnover by the body, and, therefore, do not find it plausible that vaccine-related formaldehyde represents an unsafe component of infant vaccines."

Inactivating agents other than formaldehyde are used in various U.S.-licensed vaccines and include glutaraldehyde, which is used to inactivate pertussis toxin (PT) in seven acellular pertussis-containing vaccines (Adacel [Sanofi Pasteur Ltd.], Boostrix [GlaxoSmithKline Biologicals], DAPTACEL [Sanofi Pasteur Ltd.], Infanrix, KINRIX, Pediarix [each manufactured by GlaxoSmithKline Biologicals], and Pentacel [Sanofi Pasteur Ltd.]), and β-propiolactone, which is used in the inactivation of three seasonal influenza virus vaccines (Afluria [Seqirus Pty Ltd.] and Fluvirin and Flucelvax [manufactured by Seqirus Vaccines Ltd. and Seqirus, Inc., respectively]), and two rabies vaccines (RabAvert [Novartis Vaccines and Diagnostics GmbH] and Imovax [Sanofi Pasteur SA]).

Residual Cell Culture Materials

Antibiotics

The CFR permits the addition of antibiotics (with the exception of penicillin) in viral vaccine manufacture (21 CFR §610.15[c]). Antibiotics that have been used include streptomycin, polymyxin B, neomycin, and gentamicin. Although a manufacturer need not specifically test for these antibiotics in the final container, the calculated amount of residual antibiotics (based on dilutions of the amount that was added) must be noted on the package label (21 CFR §610.61[m]). Table 7.2 lists the amount of residual antibiotics in several U.S.-licensed vaccines. Although the amended regulation for Constituent Materials[5] (21 CFR §610.15) applies equally to antibiotics, no examples of potential changes for the antibiotic regulation, unlike for preservatives and adjuvants, were presented in the *Federal Register* notice.

SENSITIZING SUBSTANCES

The CFR's biologics labeling regulations (21 CFR §610.61[l]) provide that "known sensitizing substances" should be listed on the product label. Furthermore, 21 CFR §610.15(b) states that "extraneous protein known to be capable of producing allergenic effects in human subjects shall not be added to a final virus medium of cell culture produced vaccines intended for injection." These regulations address the possibility that animal-derived proteins present in the final formulation of a vaccine can cause allergic reactions in some vaccine recipients. (Other sensitizing substances, such as preservatives, are addressed in other sections of these regulations.) Animal-derived materials are used extensively in vaccine manufacturing, particularly in viral cultures. When viral vaccines are grown in embryonated chicken eggs (influenza and yellow fever vaccines) or chick embryo cell culture (measles or mumps virus vaccines), the label will state that residual chicken proteins may be present in the final formulation (see Table 7.2). Although hypersensitivity to any component of the vaccine is a contraindication, the MMR-II and yellow fever vaccine (YF-Vax) package inserts address the vaccination of persons with hypersensitivity to eggs or egg protein.

The two U.S.-licensed hepatitis B vaccines, Engerix-B (GlaxoSmithKline Biologicals) and Recombivax HB (Merck Sharpe & Dohme Corp.), are recombinant DNA–derived proteins and are produced in yeast; their package inserts note that residual yeast protein may be present (Engerix-B contains not more than 5% and Recombivax HB not more than 1% yeast protein). Hepatitis B vaccine is contraindicated in persons with a history of hypersensitivity to yeast; however, the ACIP has noted that "no evidence exists that documents adverse reactions after vaccination of persons with a history of yeast allergy."[42] The HPV vaccines, Gardasil (Merck Sharp & Dohme Corp.) and Gardasil 9 (Merck Sharp & Dohme Corp.), are also manufactured in yeast cells; according to the package inserts, both vaccines contain less than 7 μg of yeast proteins per dose; hypersensitivity to yeast is given as a contraindication to

receiving the vaccine. The other HPV vaccine, Cervarix (GlaxoSmithKline Biologicals), is manufactured in insect cells; levels of insect cell proteins are reduced to less than 40 ng/dose.

It has been reported[43] that anaphylactic reactions to DTaP and Tdap vaccines may be caused by the presence of residual amounts of casein, a milk protein that is used in the culture media for these vaccines; the reactions occurred in children who already had high levels of milk allergy. The authors[43] note, however, that these anaphylactic reactions are exceedingly rare, and that many highly sensitive children with milk allergy tolerate the vaccines. Further studies will be necessary to define whether there is a subpopulation of children with milk allergies who might be at risk for an anaphylactic reaction. Slater and colleagues[44] have, however, noted several potential confounding factors in this analysis.

Vaccine vial stoppers or syringe components (plunger or tip caps) may contain latex, a component of natural rubber; certain persons are sensitive to latex. Vaccine presentations that contain or may contain latex have statements in the Warning and Precautions section of the package insert to the effect that the vaccine may cause allergic reactions in latex-sensitive individuals.

The Food, Drug and Cosmetic Act (Section 502[e][1][A][iii]) states that all inactive ingredients should be noted in labeling; it also states that this requirement is not necessary if trade secret information would be disclosed. The CFR additionally notes that an inactive ingredient should be listed in the labeling if the ingredient's presence is considered a safety factor (21 CFR §610.61[n]). In some cases, even in the absence of any evidence that a particular material might pose a safety factor, manufacturers have elected to disclose the presence of residual materials such as detergents, solvents, and chelating agents (see Table 7.2 for examples of manufacturing residuals). In addition, many manufacturers provide a brief summary of manufacturing methods, including the reagents used in various steps, such as precipitation (ammonium sulfate) or bacterial culture (e.g., an antifoam agent such as polydimethylsiloxane). Many of these substances are removed or markedly reduced in subsequent manufacturing steps.

Bacterial and Cellular Residuals

For bacterial vaccines, manufacturing residuals may include various bacterial cell constituents. Naturally, whole-cell vaccines—such as the previously used whole-cell pertussis vaccine—contain high levels of these components. At present, no parenteral whole-cell bacterial vaccine is in use in the United States; an oral, live attenuated bacterial vaccine, Typhoid Vaccine Live Oral Ty21a (Vivotif, Crucell Switzerland Ltd.), is approved for use.

Vaccines derived from gram-negative bacteria may contain LPS, commonly termed *endotoxin*, a component of the bacterium's outer membrane. Stimulation of the innate immune system by LPS can produce an inflammatory response that can result in fever, shock, and death.[45] Different organisms have different LPS structures, so their respective response potentials differ. Two tests are currently used to detect LPS in vaccines: the rabbit pyrogen test (which is a test for all pyrogens) and the limulus amebocyte lysate (LAL) test. The lysate from the amebocytes of the horseshoe crab, *Limulus polyphemus*, clots in the presence of LPS and forms the basis for this test.[46,47] In cases where pyrogenicity would be attributable to the presence of LPS, the LAL test is more commonly used. The limulus lysate that is used to test for bacterial endotoxin in U.S.-licensed vaccines (and other FDA-regulated biological products) is itself a U.S.-licensed product. The amount of endotoxin remaining in a final vaccine formulation depends on a number of factors, including the purification steps that are used in vaccine production. Although endotoxin testing of antigens derived from gram-negative bacteria is performed during the manufacturing process—and there may be a release specification for this test—the labeling may not contain this information. One of the DTaP vaccine labels (Tripedia [Sanofi Pasteur Inc.], which is no longer available) includes the amount of endotoxin contributed by the inactivated pertussis components (<50 endotoxin units/mL).

Residual bacterial protein may be present in the final vaccine formulation of bacterial vaccines. The consequences of this residual protein can vary, and its presence may be neutral or harmful. For example, it has been recognized for many years that the presence of residual protein may contribute to increased reactogenicity of diphtheria toxoid.[48,49] However, it has also been held that any such protein contributed to the immunogenicity of the vaccine.[50]

Polysaccharide, conjugated polysaccharide, and purified protein vaccines undergo a number of purification steps that reduce the amount of residual bacterial protein. However, these purification steps may not totally eliminate cellular or media protein components. During development of the product, a number of assessments of purity are performed, such as silver staining or polyacrylamide gel electrophoresis (PAGE) gel immunoblotting. After licensure, purity and quality of the vaccine antigen is often assessed by sodium dodecylsulfate (SDS)–PAGE as a release test. However, there is a limit to the sensitivity of these methods, as is illustrated in publications of the National Institute of Allergy and Infectious Disease–sponsored multicenter acellular pertussis trial,[51,52] which show that some children vaccinated with a fourth and fifth dose of Tripedia (DTaP [Sanofi Pasteur Inc.]; a two-component pertussis vaccine containing PT and filamentous hemagglutinin [FHA]), after previous doses of Tripedia, or a fourth dose of Tripedia after a primary series with whole-cell pertussis vaccine, had a booster response to pertactin and fimbriae, suggesting that there was sufficient antigen in Tripedia to stimulate an immune response.

Cell substrates used in viral vaccine manufacture include two diploid cell strains of human origin (MRC-5 and WI-38); a simian-derived, continuous cell line (Vero cells); a dog kidney derived cell line (MDCK [Madin-Darby canine kidney] cells); and chick embryo cells. Several recombinant DNA-derived viral vaccines are manufactured in insect cell lines. Residual protein from these differing cell lines is present to varying degrees in the vaccines produced from them. There is a concern, as noted previously, for residual egg proteins in sensitive individuals.

Residual cellular DNA from normal primary diploid cells or diploid cell strains, as well as from bacterial cells, may be present in the final vaccine formulation, and this DNA is not considered to pose a risk. Residual DNA from continuous cell lines, such as Vero cells, has been considered by a WHO study group.[53] The WHO Expert Committee on Biological Standardization assessed the risk of a transformational event as negligible and concluded that levels of up to 10 ng/dose of injected product are acceptable.[52] This limit was a revision of an earlier, more conservative limit (≤100 pg per parenteral dose) proposed by the 1986 WHO Expert Committee.[54] In revising this limit, the 1997 Expert Committee considered data from human and animal experience. This included data from nonhuman primates showing that milligram amounts of DNA containing an activated oncogene from human tumor cells had not caused a tumor during 10 years of evaluation; consideration that human blood contains substantial amounts of DNA in plasma; and consideration that contaminating DNA in a biological product would probably be in small fragments that are unlikely to encode a functional gene.[53] The committee concluded that continuous-cell-line DNA could be considered

a contaminant rather than a significant risk factor requiring removal to extremely low levels; hence the revised limit of 10 ng/dose.

Manufacturers do not necessarily need to demonstrate that each lot meets this specification through specific testing on each lot; they may be able to validate that the purification process can remove DNA to this level or below. This limit of 10 ng of residual DNA per dose does not apply to products derived from microorganisms, diploid cell strains, or primary animal cells, or to oral vaccines made with continuous cell lines.[53] The U.S.-licensed Japanese encephalitis virus vaccine (Ixiaro, Valneva Scotland Ltd.), IPV vaccine (IPOL, Sanofi Pasteur SA), and the IPV component of the DTaP-HepB-IPV vaccine, Pediarix (GlaxoSmithKline Biologicals) are produced in the continuous Vero cell line. IPOL contains less than 10 pg of DNA per dose.[55] One influenza virus vaccine, Flucelvax (Seqirus Inc.), is produced in MDCK cells, a canine continuous cell line; the vaccine contains less than 10 ng of MDCK DNA per dose.

Adventitious Agents

Use of animal-derived materials, such as gelatin, fetal bovine serum (commonly referred to as fetal calf serum), or primary animal-derived cells, in vaccine manufacture raises concerns about the potential presence of adventitious contaminants. Regulations (21 CFR §610.18) require that cultures used in the manufacture of products be free from extraneous organisms, and that cell lines should be tested for the presence of detectable microbial agents. A 1993 FDA guidance document[56] stated that master cell banks should be tested for adventitious agents and that animal-derived materials should be free from contaminants and adventitious agents, including viruses and the agent of BSE. A final guidance was published in February 2010.[57]

Manufacturers are required to perform such testing as necessary, and to ensure that the certification provided with any raw material is adequate (e.g., documentation that bovine-derived ingredients have been tested for extraneous viruses). Adventitious agent testing, performed to ensure that cell substrates used in vaccine manufacture are free from bacteria, fungi, mycoplasma, and mycobacteria, is described in detail in U.S.[56,57] and international[58,59] guidance documents. The use of polymerase chain reaction–based reverse transcriptase assays to test for adventitious retroviruses is addressed in a CBER letter to manufacturers.[60] The testing that has been carried out for adventitious agents is not described in the product labeling. However, the manufacturing method, including the cell lines and culture media used, are described.

Transmissible Spongiform Encephalopathy Agents

The lack of sensitive, specific, or readily accessible premortem diagnostic tests for transmissible spongiform encephalopathies (TSEs), such as BSE or CJD, limits surveillance and the ability to test for contamination of products for these agents. Diagnosis of a TSE disease is definitively confirmed by postmortem examination of brain tissue. However, even this examination is limited in sensitivity because, at earlier stages of disease, the TSE agent may be present in undetectably low concentrations. Because of these limits on testing, potential contamination of animal-derived materials with TSE agents is controlled through restricted sourcing.[29]

In 2013, the U.S. Department of Agriculture (USDA) published a final rule to base the conditions for importation of products derived from bovines with regard to BSE on the inherent risk of the particular product, as well as the BSE risk status of the region the product came from (9 CFR §92). This rule removed the USDA list of countries where BSE exists or that present an undue risk of BSE, and describes regions of risk generally similar to those classified by the OIE. A proposed rule, published in January 2007, harmonizes requirements for bovine materials for pharmaceutical products with those of USDA-regulated meat and FDA-regulated foods and animal feeds.[61]

SUMMARY

A final vaccine contains materials in addition to the active immunogen. Some of these materials are added by the manufacturer to effect a specific purpose—for example, stabilizers and adjuvants. Others are residual materials from the manufacturing process. Although not all of the results of all final release and in-process testing are contained in the labeling that accompanies a product, the CFR does specify which information should be included. Package inserts also contain information on manufacturing methods and growth conditions.

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8 Passive Immunization

Mark K. Slifka and Ian J. Amanna

Passive immunization, passive immunity, and passive immunotherapy all refer to the transfer of antibodies to an unprotected individual for the prevention or treatment of disease. The first formal demonstration of passive immunization for successfully treating diphtheria and tetanus dates back to animal studies published in *Deutsche Medizinische Wochenschrift (German Medical Journal)* in 1890.[1] The technique was quickly adapted to clinical use and as early as the mid-1890s, diphtheria-specific antitoxin was used successfully in the hospital setting to reduce mortality during diphtheria outbreaks.[2–4] Indeed, in 1901 Emil von Behring was awarded the first Nobel Prize for Physiology or Medicine for the discovery of this important medical intervention.[5] The significance of this clinical advance cannot be overstated; Behring estimated that 45,000 lives were saved each year using diphtheria-specific passive immunotherapy in Germany alone.[6] In the 1890s, the mortality rate of hospitalized cases ranged from 47% to 60%,[7] and the work of Emil von Behring and his colleague, Shibasaburo Kitasato, provided the only hope for diphtheria patients in the preantibiotic era.

According to Behring, the discovery of passive immunization would not have occurred if it were not for his earlier work that focused on characterizing the protective mechanisms of active immunization against diphtheria[5,8] and through the work of his collaborator, Kitasato, on the mechanisms of vaccine-mediated immunity against tetanus.[1] When guinea pigs were infected with *Corynebacterium diphtheriae*, the animals routinely died of the disease. However, when Behring vaccinated animals and they mounted neutralizing antibodies to diphtheria toxin, he found that they were protected from a normally lethal dose of *C. diphtheriae*. To determine if protection was now an intrinsic property of the immune host that could be transferred to a susceptible host, he injected naïve guinea pigs with diphtheria toxin and then successfully treated them with immune serum from vaccinated animals. Likewise, injection of *Clostridium tetani* or purified tetanus toxin was typically lethal, but through a method developed by Paul Ehrlich,[5] animals could eventually become immune to high doses of tetanus toxin by sequentially inoculating them with lower, nonlethal doses of tetanus toxin. Kitasato used this approach to demonstrate that the blood of vaccinated, tetanus-immune rabbits could be transferred to naïve mice and fully protect them from a normally lethal dose of virulent *C. tetani* or from filtered *C. tetani* culture supernatant containing tetanus toxin.[1] Behring and Kitasato may have said it best in the final sentence of their landmark 1890 study, "The result of our experiments remind us forcibly of these words: Blut ist ein ganz besonderer Saft [blood is a very unusual fluid]."[1]

Technology has advanced substantially in the more than 125 years since Behring and Kitasato's first formal demonstration of protective passive immunotherapy.[1] In those early days, it was infeasible to use human immune serum to treat diphtheria, so the first large-scale production of polyclonal diphtheria-immune serum was prepared by vaccinating dairy cows.[5] To this day, commercial antisera used to treat a broad range of toxins are still produced in animals (Table 8.1). Passive immunotherapy with animal-derived antibody preparations should only be used under close medical supervision[9] or the resulting host immune response to the foreign immunoglobulins and serum proteins may trigger serum sickness, urticaria, and/or anaphylaxis following administration. Fortunately, the advent of several innovative technologies that reduce the need for animal-derived antibodies have forged new paths in terms of safety, feasibility, and the protective efficacy afforded by passive immunization. Following the discovery of monoclonal antibody technology,[10,11] further refinements have been made, including use of various display techniques (e.g., phage display, yeast display) to screen large antibody libraries.[12] Other technological advances include the development of chimeric monoclonal antibodies in which the murine antibody is "humanized" by genetically replacing the heavy chain region of the molecule with the human immunoglobulin counterpart and the use of transgenic mice in which the endogenous murine immunoglobulin genes have been replaced by human immunoglobulin genes.[12] This latter approach has the advantage that hybridomas from immunized transgenic mice produce fully human monoclonal antibodies without requiring further genetic modifications. Recently, development of Epstein-Barr virus (EBV)-transformed human memory B cells for the production of monoclonal antibodies has led to yet another surge in the production of new human monoclonal antibodies with rare antigenic specificities to uncommon pathogens and these can be produced directly from immune human subjects.[12,13] Before the era of antibiotics, antibody-based therapy was the only option available for combating many bacterial diseases. Even today, there are only a handful of antiviral drugs available and no therapeutic options exist for most viral diseases. However, new antibody-based therapies are continuing to be developed with the potential to provide protection against a broad array of bacterial and viral pathogens. In this chapter, we describe the role of passive immunity in the protection of the naïve host, discuss the parameters involved with successful immunotherapy, and provide examples of protective efficacy in animal models as well as in human clinical studies.

MATERNAL ANTIBODIES: THE ORIGINAL PASSIVE IMMUNOTHERAPY

Maternal antibodies represent a natural form of passive immunotherapy in which the immunoglobulin (Ig) G repertoire of the mother's preexisting humoral immune response is transferred to the fetus through the placenta. Acquisition of maternal antibodies varies widely among mammalian species.[14] Maternal IgG is transferred in utero to the fetus of humans and monkeys through the placenta with no evidence of postnatal transport, and reaches serum concentrations that are similar between mother and infant. In contrast, there is no prenatal transport of maternal IgG in mink, cows, horses, sheep, goats, and pigs, and although the animals are born with serum that is nearly devoid of IgG, these antibodies are transferred from ingested colostrum into the bloodstream within the first 24 to 48 hours after birth across the gastrointestinal tract. Transmission of maternal IgG in mice, rats, and dogs occurs in utero as well as across the gastrointestinal tract after birth, indicating that they differ from humans and non-human primates as well as being different from mink and the

TABLE 8.1 Licensed U.S. Antibody Products for Passive Immunity to Infectious Diseases or Toxins

Product	Brand Name	Manufacturer	Licensed Indications[a]
STANDARD IMMUNOGLOBULINS (HUMAN)			
Immunoglobulin, intravenous	Bivigam Carimune Flebogamma Gammagard Gammaplex Gamunex-C Octagam Privigen	Biotest Pharmaceuticals CSL Behring Instituto Grifols Baxter BPL Grifols Biotherapeutics Pharmazeutika Produktionsges CSL Behring	Primary humoral immunodeficiency; multifocal motor neuropathy; chronic idiopathic thrombocytopenic purpura; Kawasaki syndrome; chronic inflammatory demyelinating polyneuropathy
Immunoglobulin, subcutaneous	Hizentra Hyqvia Gammagard Vivaglobin	CSL Behring Baxter Baxter CSL Behring	Primary humoral immunodeficiency; multifocal motor neuropathy
Immunoglobulin, intramuscular	GamaSTAN	Grifols Biotherapeutics	Hepatitis A; measles; varicella; rubella
HYPERIMMUNOGLOBULINS (HUMAN)			
Anthrax immunoglobulin intravenous (human)	Anthrasil	Cangene Corporation	Treatment of inhalation anthrax
Botulism immunoglobulin intravenous (human)	BabyBIG	California Department of Health Services	Treatment of infant botulism (type A or type B *Clostridium botulinum*)
Cytomegalovirus immunoglobulin intravenous (human)	CytoGam	CSL Behring	Prophylaxis of cytomegalovirus (CMV) disease associated with organ transplantation
Hepatitis B immunoglobulin intravenous (human)	HepaGam B Nabi-HB	Cangene Corporation Nabi Biopharmaceuticals	Prevention and postexposure prophylaxis for hepatitis B
Rabies immunoglobulin (human)	HyperRab S/D	Grifols Biotherapeutics	Postexposure treatment of rabies, administered in conjunction with the rabies vaccine
Tetanus immunoglobulin (human)	HyperTET S/D	Grifols Biotherapeutics	Prophylactic or therapeutic treatment of tetanus
Vaccinia immunoglobulin intravenous (human)	N/A	Cangene Corporation	Treatment and/or modification of complications resulting from smallpox vaccination
Varicella zoster immunoglobulin (human)	VariZIG	Cangene Corporation	Varicella postexposure prophylaxis in high-risk groups
ANIMAL-DERIVED IMMUNOGLOBULIN PRODUCTS			
Antivenin (*Latrodectus mactans*) (equine)	Black widow spider antivenin	Merck & Co, Inc.	Treatment of bites by the black widow spider (*Latrodectus mactans*)
Botulism antitoxin bivalent (equine) types A and B	N/A	Sanofi Pasteur Ltd	Treatment of botulism (types A or type B)
Botulism antitoxin heptavalent (A, B, C, D, E, F, G)-(equine)	BAT	Cangene Corporation	Treatment of botulism (types A, B, C, D, E, F, or G)
Centruroides (scorpion) immune F(ab')2 (equine) injection	Anascorp	Rare Disease Therapeutics, Inc.	Treatment of scorpion envenomation
Crotalidae immune F(ab')2 (equine)	Anavip	Instituto Bioclon S.A. de C.V.	Treatment of rattlesnake envenomation
Crotalidae polyvalent immune Fab (ovine)	CroFab	Protherics, Inc.	Treatment of rattlesnake and cottonmouth/water moccasin envenomation
Digoxin immune Fab (ovine)	DigiFab	Protherics, Inc.	Treatment of digoxin toxicity or overdose
Diphtheria antitoxin (equine)	DAT	Instituto Butanta[b]	Prophylactic or therapeutic treatment of diphtheria
MONOCLONAL ANTIBODIES			
Palivizumab	Synagis	MedImmune	Prevention of lower respiratory tract disease caused by respiratory syncytial virus (RSV) in high-risk children
Raxibacumab	N/A	Human Genome Sciences/ GlaxoSmithKline	Treatment of inhalation anthrax

N/A, not applicable.
[a]Indications as listed by the manufacturer. Indications have been grouped for each product type.
[b]Distributed by the Centers for Disease Control and Prevention to physicians as an Investigational New Drug.

ungulates. These differences also indicate that care should be taken when choosing an appropriate animal model for studying the role of maternal antibodies against infectious disease as the mechanisms may be more species-specific than typically realized.

In a comprehensive study involving the analysis of antibodies to 16 viruses using samples from 58,500 patients, the relationship between maternal immunity and infant immunity is clear (Fig. 8.1).[15] The prevalence of antibodies to each viral antigen among infants less than 1 month old is remarkably similar to those observed in the 20- to 40-year old adults who represented the main age group of the mothers. For instance, immunity to common childhood diseases such as measles and mumps was comparable between newborns and their mothers. Immunity to less-common viral pathogens, such as influenza B, was relatively low among infants and adults in the cohorts examined in 1971–1972, but higher among those sampled in 1973–1974,1975–1976, and 1977–1978, coinciding with an influenza B epidemic that had occurred in 1974.[15] This shows that the prevalence of maternal antibodies is dynamic and that recent outbreaks involving a specific pathogen will result in a higher frequency of pathogen-immune mothers and a concomitant increase in the number of infants who are likewise bestowed at least transient immunity to that particular microbe. As expected, maternal antibodies wane rapidly during the first 6 months of life and then exposure to pathogens over the following months and years results in an accumulation of different antibody specificities as children reach adulthood (see Fig. 8.1). The overall protective efficacy of maternal antibodies is perhaps most pronounced among children with genetic immunodeficiencies such as severe combined immunodeficiency (SCID), resulting in the lack of functional T and B cells or agammaglobulinemia, in which patients lack functional B cells while still having the ability to mount pathogen-specific T-cell responses. The clinical presentation of SCID is not apparent at birth but relatively uniform diagnosis occurs at a mean of 6.59 months of age,[16] which is also about the age that maternal antibodies have reached their lowest levels[15] (see Fig. 8.1). Likewise, agammaglobulinemic patients also begin to present with symptoms of immunodeficiency around this same age.[17] Maternal antibodies represent an immunological "double-edged sword" in the sense that they are known to interfere with live attenuated virus vaccines such as the MMR (measles, mumps, rubella)[18–20] and rotavirus vaccines,[21,22] whereas direct immunization of mothers in the third trimester of pregnancy can significantly increase protection of infants against common respiratory viruses such as influenza.[23–25] Indeed, maternal vaccination may result in a 45% to 91% reduction in influenza-related hospitalizations among infants younger than 6 months of age.[23–25] Likewise, the importance of maternal vaccination against *Bordetella pertussis* (i.e., whooping cough) was recognized as early as the 1930s to 1940s with studies showing higher antibacterial antibody responses and potential protection from exposure to whooping cough among infants born to vaccinated mothers.[26–29] Recent studies verify these earlier results, demonstrating a 90% to 91% vaccine efficacy against whooping cough among infants younger than 2 months of age who were born to mothers who received pertussis vaccination during pregnancy,[30,31] thus lending further support to the current recommendations for the vaccination of pregnant mothers against *B. pertussis*.[32] The age limit of younger than 2 months was chosen as this is the age at which primary pediatric vaccination is recommended and analysis beyond this age might be confounded by the protective effects of direct vaccination of the child. Nevertheless, the protection afforded by maternally derived IgG against respiratory infections involving viral (e.g., influenza) or bacterial (e.g., *B. pertussis*) pathogens together demonstrate the broad impact that maternal vaccination and the subsequently increased transfer of maternal antibodies can have on the health of young infants.

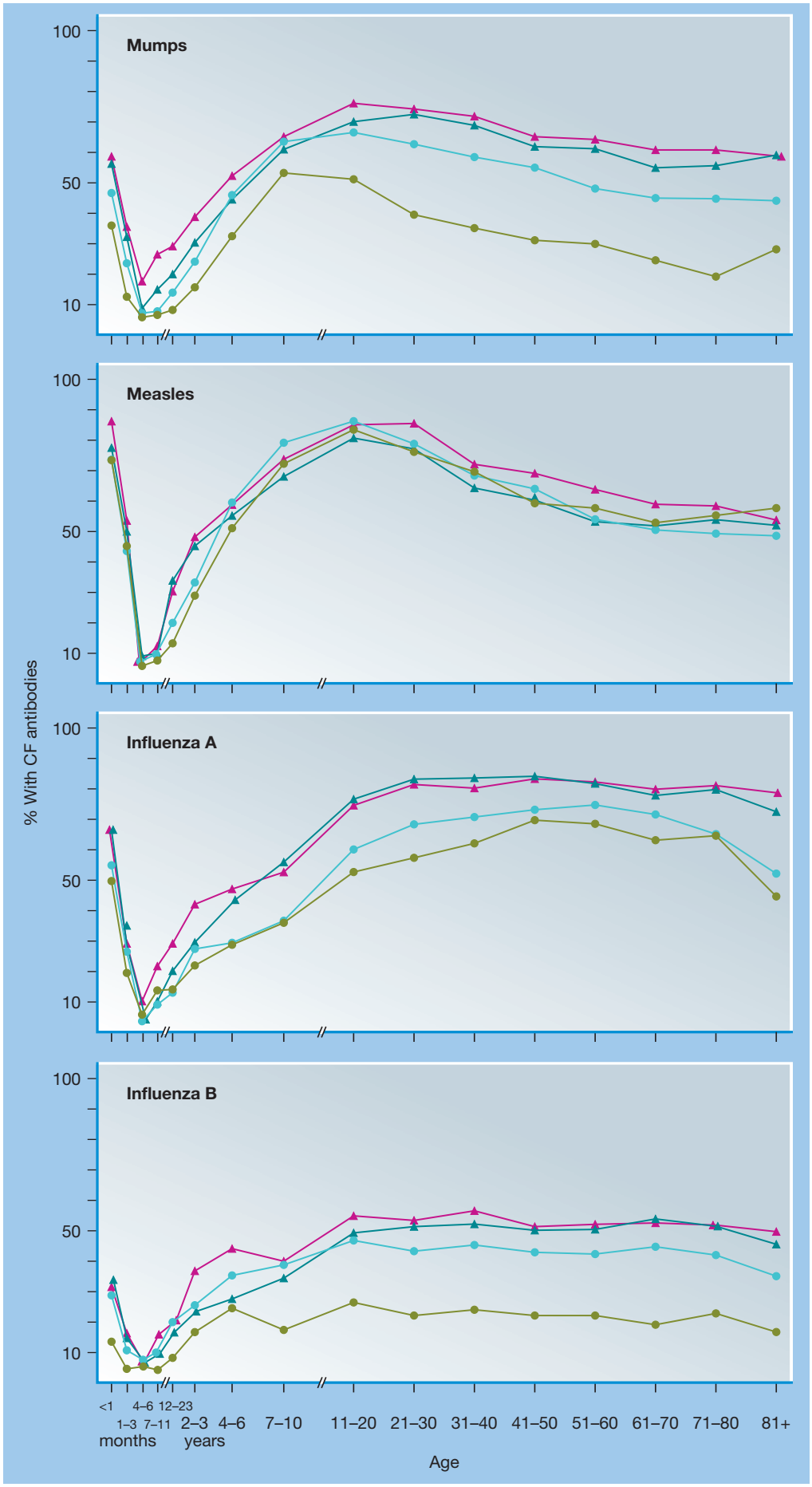
CRITICAL PARAMETERS FOR PASSIVE IMMUNOTHERAPY

Before vaccines and antibiotics revolutionized modern medicine, antibody-based therapies represented the only effective medical treatment for many life-threatening diseases including diphtheria, scarlet fever, bacterial meningitis, and bacterial pneumonia.[33,34] Today, most commercial forms of antibody-based immunotherapy for infectious disease still rely on polyclonal antibodies of human or animal origin, with the notable exceptions of the monoclonal antibodies, palivizumab and raxibacumab (see Table 8.1). The main advantage of using polyclonal antibodies for passive immunotherapy is that this approach will include antibodies to multiple epitope specificities that may work in an additive or synergistic manner with the potential contribution of multiple immunoglobulin isotypes and subclasses that have different biological functions (Table 8.2).[35] On the other hand, there are several potential challenges to using polyclonal antibodies for immunotherapy including low antigen-specific activity, supply limitations (especially for rare diseases), variability between manufacturing lots, and safety as well as quality control issues that are often associated with the use of human blood products. In

TABLE 8.2 Comparison of Polyclonal and Monoclonal Antibody Therapy

	Polyclonal Antibodies	Monoclonal Antibodies
Advantages	Polyvalent specificity Multiple isotypes with different effector functions	High specific activity Standardized potency Unlimited availability Minimal biohazard potential
Disadvantages	Low specific activity Broad variation in potency Limited availability Biohazard risk of human blood products	Monovalent specificity Single isotype Potential to select for escape mutants

Figure 8.1. Age-specific prevalence of antibodies to mumps, measles, influenza A, and influenza B viruses in patients screened with 16 viral antigens during the years 1971–1978. The prevalence curves represent four 2-year periods: 1971–1972 (green line), 1973–1974 (aqua line), 1975–1976 (red line), and 1977–1978 (blue line). CF, complement fixing. *(From Ukkonen P, Hovi T, von Bonsdorff C-H, Saikku P, Penttinen K. Age-specific prevalence of complement-fixing antibodies to sixteen viral antigens: A computer analysis of 58,500 patients covering a period of eight years.* J Med Virol. *1984;13:131–148.)*


Mumps
Measles
Influenza A
Influenza B
100
50
10
% With CF antibodies
<1
1–3
4–6
7–11
12–23
months
2–3
years
4–6
7–10
11–20
21–30
31–40
41–50
51–60
61–70
71–80
81+
Age

contrast, monoclonal antibodies are, by definition, limited to a single epitope specificity but they have several advantages over polyclonal antibodies since they can be manufactured in vitro at large scale, with inherently high specificity and lot consistency (Table 8.2). For example, the combination of 0.7 mg of two tetanus-specific human monoclonal antibodies has the same neutralizing capacity observed with administration of 100 to 170 mg of polyclonal tetanus immunoglobulin.[36] Likewise, administration of 0.023 mg of a vaccinia virus-specific monoclonal antibody provides the same level of protection afforded by 5 mgs of vaccinia immunoglobulin (VIG).[37] Although neutralization escape mutants are a valid concern when using monoclonal antibody therapy,[38,39] this has not yet been a major problem during clinical use of palivizumab for respiratory syncytial virus (RSV). Initially, sequencing of 371 RSV isolates demonstrated that there were no mutations in the neutralizing epitope of the F protein.[40] Subsequent studies identified RSV escape mutants in approximately 5% of 146 breakthrough cases, indicating that selective pressure for escape mutations is still relatively uncommon under current conditions of use.[41] This suggests that monoclonal antibodies can remain effective when used clinically in the long-term, as long as they are specific for a stable epitope for that particular pathogen.

The functional characteristics of the immunoglobulins used for passive immunization is an important consideration in determining protective efficacy in vivo.[35] For example, serum IgG molecules equilibrate into extravascular space whereas IgM is largely confined to intravascular space.[14] IgM molecules also have a short half-life (5 days[14]) and are typically of low affinity, which is why IgM is not an optimal choice for passive immunotherapy. Serum IgA is monomeric and, although it also equilibrates into extravascular space,[14] it has only a 6-day half-life[14] and does not appear to contribute significantly to functional IgA in the lungs of mice.[42,43] Human IgG on the other hand, has an average half-life of approximately 21 days (except IgG_3, which has a 7-day half-life),[14,44] is typically of high affinity, and transudation across mucosal barriers can protect against pathogens that invade through mucosal routes. Interestingly, serum IgG (and serum IgA) responses elicited in response to vaccination against *Neisseria meningitidis* correlate strongly with the levels of antibacterial antibodies present in the saliva at 1 month and 1 year after vaccination,[45] indicating that circulating serum antibodies may be an important contributor to the antibodies released in mucosal secretions. Indeed, after intravenous administration of an HIV-specific monoclonal antibody into rhesus macaques, serum antibody titers of 690 to 725 μg/mL resulted in mucosal antibody titers of 17 to 30 μg/mL in vaginal fluids and provide complete protection against intravaginal challenge with SHIV (chimeric simian immunodeficiency virus expressing HIV envelope).[46] Influenza virus is another mucosal pathogen with strict tropism to the respiratory tract, but influenza-specific serum antibody titers correlate with protection in humans.[47] In mice, the relative roles of influenza-specific polymeric IgA and IgG were compared in terms of antiviral protection in the upper respiratory tract versus the lung after influenza challenge.[42] When polymeric IgA was transferred 4 hours prior to influenza infection, this prevented pathology in the upper respiratory tract but was not effective in the lung, whereas transfer of IgG prevented pathology in the lung, but required higher doses to protect against infection of the upper respiratory tract. The authors concluded that different antibody isotypes may function preferentially at different anatomical sites in vivo. These results are in contrast to experimental influenza infection in humans in which inactivated influenza vaccine-derived IgG is believed to be a major contributor to protection of the nasal compartment.[48] Overall, the ability of IgG to enter nonlymphoid tissues and to penetrate mucosal sites of infection is likely to explain why it is often considered the best immunoglobulin isotype for routine passive immunization and has shown clinical benefit ranging from reduced clinical symptoms to nearly complete protection from lethal infection in a number of infectious disease models (Table 8.3).

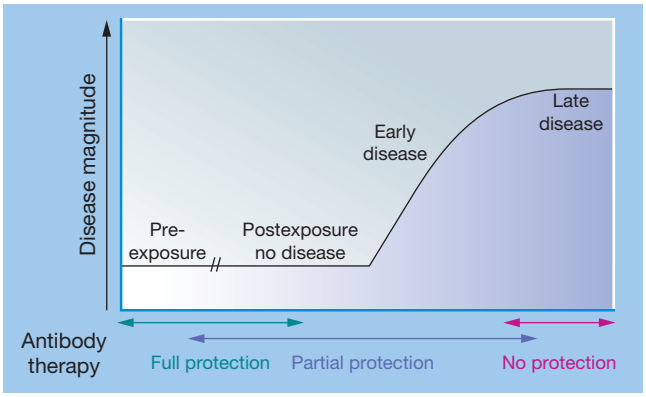


Figure 8.2. Efficacy of passive immunity decreases with disease progression. Full protection from symptomatic disease is best achieved through prophylactic administration of antibody therapy prior to exposure or infection. However, antibody therapy may also be highly effective at early points postexposure, prior to the onset of disease symptoms. Passive immunity is generally less effective when administered after the onset of symptomatic disease, and typically shows little to no clinical benefit once severe late-stage disease has occurred.

Over the last century, it has been well established that high specific antibody titers and early timing of antibody transfer in relation to disease onset are the two most important parameters involved with determining the protective efficacy of passive immunization (Fig. 8.2). In one account of the early days of clinical diphtheria-specific immunotherapy developed by Behring and Ehrlich,[5] initial failures in patients after treatment with weak or unstandardized diphtheria-immune serum brought Ehrlich to describe three points that he believed were important for successful immunotherapy: (a) treatment has to be initiated at the onset of disease; (b) the more the disease has progressed, the higher the serum quantities necessary for cure; and (c) depending on the severity of the case, certain minimal doses can be specified. Later studies confirmed these results: if diphtheria immunotherapy was initiated on the first day of disease, there was 0% mortality ($n = 183$).[49] However, if therapy was delayed to 2, 3, or 4 days after disease onset, then the accompanying diphtheria case-fatality rate subsequently increased to 1.6% ($n = 905$), 4.4% ($n = 632$), and 6.9% ($n = 436$), respectively.[49] These results are similar to those observed during antibiotic-based therapy of bacterial sepsis. In an ideal setting, it is recommended that antibiotics be administered within 1 hour of diagnosis of severe sepsis or septic shock as these drugs provide clinical benefit only if administered early in the course of disease and are generally ineffective during late-stage disease.[50]

The importance of high-dose immunotherapy given at the earliest sign of disease is not unique to bacterial anti-toxin therapy. The same rules apply to preventing or treating viral infections as well. During a measles epidemic in 1931–1932, 72% of exposed individuals ($n = 32$) who received no passive immunization contracted measles. If convalescent serum was administered within 10 days of exposure, then the attack rate was reduced to 16% ($n = 219$) whereas if therapy was not initiated until 12 to 16 days postexposure, approximately 80% of

TABLE 8.3 Efficacy of Passive Immunity for Infectious Diseases[a]

	Animal Models	Clinical Studies
TOXINS		
Anthrax toxin	Prophylaxis[145,146] Treatment[145,146]	Prophylaxis: not available Treatment[147,148]
Botulinum toxin	Prophylaxis[149–151] Treatment[151]	Prophylaxis: not available Treatment[152–155]
Diphtheria toxin	Prophylaxis[156,157] Treatment[157,158]	Prophylaxis[159] Treatment[7,49,160,161]
Ricin toxin	Prophylaxis[162,163] Treatment[163]	Prophylaxis: not available Treatment: not available
Tetanus toxin	Prophylaxis[1,164] Treatment[157,164,165]	Prophylaxis[166] Treatment[166,167]: not supported[168]
BACTERIAL INFECTIONS		
Bordetella pertussis (whooping cough)	Prophylaxis[169,170] Treatment[170,171]	Prophylaxis[30b,31b,172,173] Treatment[173,174]
Borrelia spp. (Lyme disease)	Prophylaxis[175,176] Treatment[175]	Prophylaxis: not available Treatment: not available
Chlamydia trachomatis	Prophylaxis[177–179] Treatment: not available	Prophylaxis: not available Treatment: not available
Clostridium difficile	Prophylaxis[180,181] Treatment[180,181]	Prophylaxis: not available Treatment[182]
Escherichia coli	Prophylaxis[183–185] Treatment[184,185]	Prophylaxis[110–113]: not supported[186] Treatment: not supported[187]
Francisella tularensis (Tularemia)	Prophylaxis[119,120] Treatment[121]	Prophylaxis: not available Treatment[118,188]
Haemophilus influenzae	Prophylaxis[189–191] Treatment[191,192]	Prophylaxis[102] Treatment[99,101]
Mycobacterium tuberculosis (Tuberculosis)	Prophylaxis[124,193] Treatment[124,194,195]	Prophylaxis: not available Treatment[128]: not supported[128]
Neisseria meningitidis (Meningococcal disease)	Prophylaxis[196–199] Treatment[198–200]	Prophylaxis: not available Treatment[33,201]
Pseudomonas aeruginosa	Prophylaxis[202,203] Treatment[202–204]	Prophylaxis: not available Treatment[205,206]: not supported[207]
Salmonella typhi (Typhoid fever)	Prophylaxis[208,209] Treatment[210]	Prophylaxis: not available Treatment[211]
Shigella spp.	Prophylaxis[212b,213b,214] Treatment: not available	Prophylaxis[215] Treatment: not supported[216]
Staphylococcus aureus	Prophylaxis[217–219] Treatment: not supported[218]	Prophylaxis: not supported[220–222] Treatment: not supported[223,224]
Streptococcus agalactiae (Streptococcus group B)	Prophylaxis[225–228] Treatment[225,226]	Prophylaxis[229b,230b] Treatment: not available
Streptococcus pneumoniae (Pneumococcal disease)	Prophylaxis[231–233] Treatment[234–236]	Prophylaxis[237] Treatment[33,236,238]
Streptococcus pyogenes (Streptococcus group A)	Prophylaxis[239–241] Treatment: not available	Prophylaxis[242,243] Treatment[243–248]
Vibrio cholerae (Cholera)	Prophylaxis[249–251,251b] Treatment[250]	Prophylaxis: not available Treatment[252,253]
Yersinia pestis (Plague)	Prophylaxis[254–260] Treatment[254–257,261–263]	Prophylaxis[264] Treatment[257,265]
VIRAL INFECTIONS		
Chikungunya virus	Prophylaxis[266,267] Treatment[266,267]	Prophylaxis: not available Treatment: anecdotal[268c]
Coxsackievirus	Prophylaxis[269] Treatment[270]	Prophylaxis: not available Treatment[271]
Cytomegalovirus	Prophylaxis[272b,273,274] Treatment: not available	Prophylaxis[275–277] Treatment: not available

Continued on following page

TABLE 8.3 Efficacy of Passive Immunity for Infectious Diseases[a] *(Continued)*

	Animal Models	Clinical Studies
Dengue virus	Prophylaxis[278–280] Treatment[278,280]	Prophylaxis: not available Treatment: not available
Ebola virus (Ebola hemorrhagic fever)	Prophylaxis[281] Treatment[281–283]	Prophylaxis: not available Treatment: anecdotal[284]
Epstein-Barr virus	Prophylaxis[285,286] Treatment: not available	Prophylaxis[133] Treatment: not available
Hantavirus (Andes virus and Sin Nombre virus)	Prophylaxis[287] Treatment[287,288]	Prophylaxis: not available Treatment[289]
Hepatitis A virus	Prophylaxis: not available Treatment: not available	Prophylaxis[290–293] Treatment: not available
Hepatitis B virus	Prophylaxis[294] Treatment[294]	Prophylaxis[295] Treatment[296,297]
Hepatitis C virus	Prophylaxis[298] Treatment[298]	Prophylaxis[299] Treatment[300,301]: not supported[301,302]
Hepatitis E virus	Prophylaxis[303] Treatment: not available	Prophylaxis[304] Treatment: not available
Herpes simplex virus	Prophylaxis[92,305,306] Treatment[92,307]	Prophylaxis[308] Treatment[309]
HIV	Prophylaxis[310,311] Treatment[312,313]	Prophylaxis: not available Treatment[140,314,315]: not supported[316,317]
Human papillomavirus	Prophylaxis[318,319] Treatment: not available	Prophylaxis: not available Treatment: not available
Influenza virus	Prophylaxis[92,95,96] Treatment[92,95,96]	Prophylaxis[23–25b] Treatment[97,98,320]
Japanese encephalitis virus	Prophylaxis[321,322] Treatment[321,322]	Prophylaxis: not available Treatment: not available
Junin virus (Argentine hemorrhagic fever)	Prophylaxis[70] Treatment[70–72]	Prophylaxis: not available Treatment[68,69]
Lassa virus (Lassa hemorrhagic fever)	Prophylaxis: not available Treatment[76,77]	Prophylaxis: not available Treatment[78,79]
Machupo virus (Bolivian hemorrhagic fever)	Prophylaxis[323] Treatment[323]	Prophylaxis: not available Treatment: not available
Measles virus	Prophylaxis[324,325] Treatment: not available	Prophylaxis[51,53,80–84] Treatment: not available
Molluscum contagiosum	Prophylaxis: not available Treatment: not available	Prophylaxis: anecdotal[308] Treatment: not available
Monkeypox virus	Prophylaxis[326] Treatment: not available	Prophylaxis: not available Treatment: not available
Mumps virus	Prophylaxis: not available[d] Treatment: not available	Prophylaxis[327–330] Treatment[328]
Parvovirus B19	Prophylaxis: not available Treatment: not available	Prophylaxis: not available Treatment: anecdotal[331,332]
Poliovirus	Prophylaxis[333,334] Treatment[335]	Prophylaxis[336,337] Treatment[54,338–340]
Rabies virus	Prophylaxis[341] Treatment[342–345]	Prophylaxis[346] Treatment: not supported[347]
Respiratory syncytial virus	Prophylaxis[348,349] Treatment[350,351]	Prophylaxis[55–58,60,352] Treatment: not supported[59,61,62]
Rift Valley fever virus	Prophylaxis[353,354] Treatment: not available	Prophylaxis: not available Treatment: not available
Rotavirus	Prophylaxis[104,105,355–358] Treatment[104,358]	Prophylaxis[21b,103,104] Treatment[104–108]
Rubella virus	Prophylaxis: not available Treatment: not available	Prophylaxis[359–362] Treatment[363]

TABLE 8.3 Efficacy of Passive Immunity for Infectious Diseases[a] *(Continued)*

	Animal Models	Clinical Studies
Severe acute respiratory syndrome coronavirus	Prophylaxis[364,365] Treatment[366]	Prophylaxis: not available Treatment[320]
Simian immunodeficiency virus	Prophylaxis[367] Treatment[368,369]: not supported[367]	Not applicable
Simian/human immunodeficiency virus	Prophylaxis[46,370–376] Treatment[141,374]	Not applicable
Tickborne encephalitis virus	Prophylaxis[377,378] Treatment[377]	Prophylaxis[379] Treatment: anecdotal[380]
Vaccinia virus	Prophylaxis[37,381–383] Treatment[37,383,384]	Prophylaxis[385] Treatment[386,387]
Varicella virus	Prophylaxis: not available Treatment: not available	Prophylaxis[388–390] Treatment: not available
Variola (smallpox)	Prophylaxis: not available Treatment: not available	Prophylaxis[64,65] Treatment[65,66]
Venezuelan equine encephalomyelitis virus	Prophylaxis[391] Treatment[391]	Prophylaxis: not available Treatment: not available
West Nile virus	Prophylaxis[392–394] Treatment[392,394,395]	Prophylaxis: not available Treatment: anecdotal[396,397]
Yellow fever virus	Prophylaxis[398–400] Treatment[398,401]	Prophylaxis: anecdotal[401] Treatment: anecdotal[402,403]
PARASITES AND FUNGAL INFECTIONS		
Candida albicans	Prophylaxis[404–407] Treatment[406]	Prophylaxis[308] Treatment: not available
Cryptococcus neoformans	Prophylaxis[408–410] Treatment: not available	Prophylaxis: not available Treatment: not supported[411]
Cryptosporidium parvum	Prophylaxis[412] Treatment[413]: not supported[412]	Prophylaxis: not supported[414] Treatment: not available
Plasmodium spp. (Malaria)	Prophylaxis[415–417] Treatment: not available	Prophylaxis: not available Treatment[418,419]
Toxoplasma gondii (Toxoplasmosis)	Prophylaxis[420–422] Treatment: not available	Prophylaxis: not available Treatment: not available
GENERAL		
Genetic immunodeficiency diseases	Not applicable	Prophylaxis[423–427]
HIV-associated diseases	Not applicable	Prophylaxis[308,428]
Sepsis/septic shock	Not applicable	Treatment[246,426,429–432]

[a]For animal studies, prophylaxis is defined as antibody administration prior to experimental infection and treatment is defined as antibody administration after infection. For clinical studies, prophylaxis is defined as antibody administration prior to disease onset and treatment is defined as antibody administration after disease onset.
[b]Evidence provided through maternal immunization studies.
[c]Anecdotal results are defined as small studies that indicate passive immunization may provide clinical benefit but are too limited in scope to be conclusive.
[d]Not available, although two studies[433,434] demonstrated prophylaxis by direct mixing of mumps virus and antibody prior to inoculation.

contacts subsequently contracted measles (n = 5).[51] In a study published in 1945 involving 1024 cases of measles exposure, 36% of the individuals who received immunotherapy within 0 to 2 days of exposure contracted measles compared to 48% for those whose treatment was delayed to 6 to 8 days postexposure.[52] The dose used in these studies was also critical: 67% of patients who received 0.01 mL/kg of gammaglobulin contracted measles whereas only 16% of patients who received 0.06 mL/kg of gammaglobulin contracted the disease. The titer of virus-specific antibodies will often differ between lots of polyclonal immunoglobulin preparations (see Table 8.2). In another study, when the measles-specific titer of gammaglobulin from different lots decreased from 33 IU/mL to 16 IU/mL, the postexposure incidence of measles increased from 17% to 57% despite either lot being administered within 5 days of exposure.[53] Likewise, the timing of passive immunotherapy is also important for enteric (e.g., polio) and respiratory pathogens (e.g., RSV). An outbreak in 1934 involving 2992 polio patients showed that if convalescent serum was administered within 0 to 2 days of meningitis, then paralysis was reported in 5.4% of patients (n = 2367). If treatment was delayed until 3 to 6 days after meningeal disease onset, 15.5% reported paralysis (n = 536), and if treatment was delayed for more than 6 days, then paralysis was noted in 30.3% of polio patients (n = 89).[54] For RSV, polyclonal RSV-immunoglobulin reduced the incidence of RSV-associated hospitalization by

41% among children with a history of prematurity or bronchopulmonary dysplasia.[55] Prophylactic administration of a neutralizing monoclonal antibody, palivizumab, was shown to significantly improve clinical outcome by reducing RSV-associated hospitalizations of children with congenital heart disease by 45%.[56] Among premature infants or those with bronchopulmonary dysplasia, RSV-associated hospitalizations were reduced by 55%.[57] A third palivizumab study confirmed these results by showing a 70% reduction in hospitalizations among premature infants and infants with chronic lung disease.[58] Another monoclonal antibody, motavizumab,[59] demonstrated a further 26% relative reduction in RSV hospitalizations compared with patients receiving palivizumab-based prophylaxis.[60] In contrast, once RSV infection has been established, the use of palivizumab,[61] motavizumab,[59] or RSV-immunoglobulin[62] shows no clinical benefit, although RSV-immunoglobulin may provide limited protection in the most severe cases.[62]

Passive immunotherapy can be highly successful for severe, even life-threatening human diseases such as smallpox, or hemorrhagic fever caused by arenaviruses including Junin or Lassa fever virus (see Table 8.3). Successful intervention, however, typically requires initiating treatment before or very shortly after symptom onset. When convalescent serum from smallpox survivors was administered to smallpox patients during the late stages of confluent or hemorrhagic smallpox, there was no clinical benefit observed in comparison to untreated controls (80% vs. 72% mortality, respectively).[63] When vaccinia-immune gammaglobulin (VIG) was administered to smallpox contacts prior to disease onset in addition to postexposure vaccination (i.e., standard of care), the number of smallpox cases was reduced by 70% compared to contacts who received postexposure smallpox vaccination alone.[64] Likewise, administration of vaccinia-immune serum of animal origin along with postexposure vaccination resulted in 0 of 13 cases (0%) of smallpox among close contacts compared to 13 of 29 cases (45%) among controls who received smallpox vaccination alone.[65] During a smallpox outbreak in 1941, 3 of 10 patients (30%) died while undergoing standard clinical care.[66] To determine if addition of passive immunotherapy would reduce mortality after smallpox diagnosis, 250 cases of smallpox were treated with convalescent serum or blood, with no smallpox-associated deaths reported (0 of 250). Approximately 75 patients were described as having severe or hemorrhagic smallpox at the time of treatment and yet all survived. This appears to be the result of using convalescent serum obtained at the peak of the humoral immune response shortly after recovery from smallpox and the use of an optimized dosing schedule with higher doses administered to patients with the more severe disease manifestations.[66]

Argentine hemorrhagic fever is caused by infection with the Junin virus and untreated cases result in 15% to 40% mortality.[67–69] Convalescent serum is protective in animal models of Junin infection[70–72] and when administered within 8 days of symptom onset, the mortality rate among human cases drops to 1% to 3%.[68,69] Likewise, in 35% to 50% of hospitalized cases, Lassa fever virus causes severe disease including diffuse capillary leakage and hemorrhagic diathesis.[73] Prophylactic administration of immune serum protects guinea pigs[74,75] and nonhuman primates[76,77] from subsequent lethal challenge, indicating that antibodies play a clear role in protection against this virulent viral pathogen. In one small clinical study, if passive immunotherapy was administered within 0 to 5 days after admission to the hospital, 4 of 4 (100%) patients survived whereas if immunotherapy was initiated 7 to 9 days after hospitalization, 0 of 3 (0%) patients survived.[78] In another study,[79] patients with virologically confirmed Lassa fever who received immune serum within 10 days of hospitalization survived (4 of 4; 100%). However, if treatment was not initiated until more than 10 days after hospitalization, then only 1 of 4 (25%) patients survived, similar to the untreated group in which only 1 of 5 (20%) patients with virologically confirmed Lassa fever survived.

PASSIVE IMMUNITY AGAINST RESPIRATORY AND ENTERIC PATHOGENS

Although passive immunity against toxins and systemic infections such as measles[51,53,80–84] and smallpox[64–66] is well established, the impact of this approach for the prevention or amelioration of disease caused by respiratory and enteric pathogens may not be as well recognized. However, several studies support the role of passive immunity against mucosal pathogens (see Table 8.3), including examples such as influenza (respiratory virus), *Haemophilus influenzae* (respiratory bacterium), rotavirus (enteric virus), and *Escherichia coli* (enteric bacterium). Influenza is a significant cause of morbidity and mortality throughout the world, including both seasonal transmission and pandemic outbreaks.[85–88] The clinical correlation between homotypic influenza immunity and vaccine-associated protection was recognized early in the development of the influenza vaccine.[89–91] Early animal studies confirmed this result, with passive transfer of antibodies (both systemic and mucosal delivery) able to protect naïve animals against subsequent challenge, or provide therapeutic benefit when administered postexposure.[92–94] More recent animal studies with defined monoclonal antibodies continue to support and extend these earlier results.[95,96] Passive immunization against influenza in humans has also been successful. In a comprehensive retrospective metaanalysis of eight passive immunization studies performed during the Spanish Influenza outbreak (1918–1925), a significant 21% decrease in mortality (95% confidence interval [CI], 15–27%; $P < .001$) was observed.[97] Subset analysis of studies that recorded early (treatment initiated within 4 days of pneumonia complications) versus late intervention (>4 days) showed a significant advantage for early treatment, with mortality decreasing from 59% (49 of 83) to 19% (28 of 148) with earlier intervention, consistent with general considerations for effective passive immunity against infectious diseases (see Fig. 8.2). In a recent double-blinded, randomized controlled study during the 2009 influenza pandemic, the use of hyperimmune intravenous immunoglobulin (IVIG) (from recovered convalescent donors) was compared to normal IVIG in the treatment of severe infection in 34 subjects.[98] The hyperimmune treated group ($n = 17$) demonstrated more rapid viral clearance than the control group ($n = 17$), with a greater than 90% drop in viral loads by day 5 posttreatment. In those patients receiving immunoglobulin within 5 days of symptom onset ($n = 22$), all 12 who received hyperimmune IVIG survived (12 of 12), whereas only 60% of patients receiving normal IVIG survived (6/10, $P = .02$).

H. influenzae type b (Hib) is an extracellular gram-negative bacterium that initially infects the host via the respiratory tract and represents another important human pathogen that can be controlled through passive immunization. Several early reports described the use of concentrated rabbit immune serum as a successful adjunct therapy to sulfonamide treatment for patients suffering from Hib meningitis.[99–101] Indeed, a full course of serum therapy (in addition to antibiotics) was able to reduce mortality to 14% (3 of 19) when compared to 78% mortality rate (7 of 9) in those patients only receiving sulfonamides.[101] A more recent study established the prophylactic

use of human immunoglobulin in at-risk populations.[102] Santosham and colleagues administered hyperimmunoglobulin ($n = 353$), or saline placebo ($n = 350$) to infants at 2, 6, and 10 months of age and examined the rates of invasive Hib. For the first 90 days following the passive immunization protocol, none of the treated infants experienced invasive Hib (0% incidence), compared to 7 of 350 placebo-treated children (2.0% incidence, $P = .007$).[102]

Rotavirus represents an enteric viral pathogen wherein protective passive immunotherapy has been demonstrated.[103–108] In one example of postexposure treatment in infants, oral administration of hyperimmune antibody (in addition to standard supportive care) was able to efficiently reduce rotavirus shedding compared to placebo controls; treated patients ($n = 26$) exhibited no evidence of viral shedding by day 8 posttreatment as compared to 25% of controls ($n = 26$).[105] In a separate study, prophylactic passive immunity using orally administered bovine colostrum from immunized animals was tested in a blinded and randomized trial among infant children (3–15 months old) admitted to a hospital, typically for respiratory conditions.[103] Following admission, infants were given a 10-day course of the bovine colostrum or placebo. Infants who received placebo contracted symptomatic rotavirus at a rate of 14% (9 of 65) whereas no symptomatic rotavirus disease was observed in the colostrum-treated infants (0 of 55; $P < .001$). Analysis of rotavirus vaccine failures also indicates that maternally derived antibodies play a role in passive immunity to rotavirus infection. In a study involving 177 vaccinated infants, a strong inverse correlation was observed between maternally derived rotavirus antibodies and the ability of infants to seroconvert following vaccination with a live rotavirus vaccine.[21] This is an important demonstration not only of passive immunity to an enteric pathogen, but also has broader implications on the timing of vaccine administration, especially in developing countries where preexisting immunity is relatively high, and rotavirus vaccine immunogenicity appears impaired.[109]

E. coli is a significant enteric pathogen wherein prophylaxis through passive immunity has been demonstrated in several clinical studies.[110–113] Tacket and colleagues were able to passively protect human subjects against experimentally induced *E. coli* diarrhea with specific bovine antibody.[110] Using heat-inactivated or glutaraldehyde-inactivated *E. coli* for vaccination, pregnant cows were hyperimmunized with a large number of enterotoxigenic O serogroups. Milk collected during the first 10 days of lactation was purified, concentrated, lyophilized, and formulated for oral administration. As a control, a similar preparation was made using rotavirus as the immunizing antigen. Subjects received daily treatment (3 times daily) for 7 days, with *E. coli* challenge administered 3 days into the treatment regimen. Of the 10 subjects who received the *E. coli* antibody prophylaxis, all remained disease-free following challenge, compared with clinical diarrhea in 9 of 10 placebo subjects ($P < .0001$). Using a closely related clinical protocol, Otto and colleagues also demonstrated good efficacy with hyperimmune bovine colostrum tablets.[111] In the first study conducted in this trial, 11 of 15 (73%) of placebo subjects contracted diarrhea following challenge, but this was reduced to only 1 of 15 (7%) in treated subjects ($P = .0005$). In a second study investigating the impact of omitting buffer to the oral prophylaxis, the authors also examined dose sparing. In these studies, the standard dose still conferred significant protection with 3 of 15 (20%) treated subjects contracting diarrhea, compared with 12 of 14 (86%) of controls. Interestingly, if the dose was reduced by one-half then disease incidence increased to 5 of 14 subjects (36%), indicating a key role played by treatment dose in achieving successful passive immunotherapy.

PASSIVE IMMUNIZATION: A PARADIGM SHIFT IN PROGRESS?

With any new scientific advance, there is controversy. In 1890, when Behring demonstrated that immune serum therapy could protect against diphtheria, it went against the current dogma at that time in which the cellular theory of phagocytosis was believed to be the primary mechanism of host protection.[5] There were also skeptics who, as early as 1896, discussed why antibody immunotherapy would not work.[114] However, the science not only prevailed but today a number of passive immunotherapy products are in clinical use (see Table 8.1) and an ever-increasing number of human diseases benefit from the use of this technology (see Table 8.3). Some believe that antibody plays a more important role in protection against cytopathic viruses and extracellular bacteria, but that T cells must be required for protection against infection by noncytopathic viruses and other intracellular pathogens.[115] Although this is partially refuted by the protective efficacy of maternal antibodies and IVIG therapy in SCID patients who do not have functioning T cells, it is important to bear in mind that antibody-mediated protection by passive immunotherapy in immunocompetent individuals does not function in isolation, but instead works best in conjunction with other immune defenses, including host T cells, B cells, natural killer (NK) cells, etc. Although the role of antibody-mediated protection against intracellular bacteria and chronic viral infections was thought to be relatively minor, there are examples in each of these instances in which passive immunity provides substantial clinical benefit.

As noted previously, prior to the advent of antibiotics, passive immunotherapy was the only option for clinical treatment of most bacterial infections including *Francisella tularensis*, a facultative intracellular bacterium that causes tularemia, a severe disease associated with up to 30% mortality in untreated cases.[116,117] When streptomycin became available, a comparative study in 1946 was performed with 542 tularemia patients who received only symptomatic treatment, 832 who received immune equine serum, 60 who received hyperimmune equine serum, and 9 who received streptomycin.[118] The untreated tularemia cases required an average of 3.78 months to recover and only three modes of therapy showed substantial improvement—treatment with immune serum within 9 days of disease onset (2.41 months until recovery), treatment with hyperimmune serum (2.15 months until recovery), and treatment with streptomycin (2.40 months until recovery). Two clinical cases were extensively described, with the following summary: "The clinical responses to each agent [i.e., immune serum, and streptomycin] were similar, prompt amelioration of the symptoms of intoxication–headache, mental dullness or lethargy, sense of prostration and severe malaise; reduction of fever and of the sizes of the buboes, acceleration in the healing of ulcers and in the resolution of pulmonary exudates." In other words, passive immunotherapy appeared in many ways to mimic antibiotic therapy in terms of protective efficacy. However, it was noted that treatment with equine serum caused serum sickness in 51% of the patients and had a more variable outcome than the antibiotic approach, leading to the recommendation that streptomycin would be the agent of choice for future treatment of this disease.[118] With the recent development of polyclonal and monoclonal antibodies that show protective efficacy against tularemia in animal models,[119–121] it may be possible to incorporate both passive immunotherapy and antibiotic treatment into clinical practice not only for tularemia, but for other bacterial diseases, especially in cases in which antibiotic resistance is becoming more widespread.[122,123]

Mycobacterium tuberculosis is another intracellular bacterium that, despite the availability of antibiotics, remains one

of the most common human diseases and it is estimated to infect up to one-third of the world's population.[124] The development of strains of extensively drug-resistant (XDR) tuberculosis (TB),[125] some of which are resistant to all current antibiotic therapies,[126,127] is also a growing concern, especially as there are few antibiotic drugs in the pipeline.[122,123] There is considerable debate over the role of antibodies in controlling TB, with many believing that antibody plays little or no role in protective immunity (reviewed in references 124 and 128). In a comprehensive historical review by Glatman-Freedman and Casadevall,[128] the clinical benefit of antibody-mediated immunotherapy, albeit quite variable, provides evidence to suggest that antibody plays a role in protection against TB. In studies reported by Paquin in 1895, a group of patients with pulmonary TB confirmed by the presence of bacterium in their sputum showed clinical benefit. After 2 months of passive immunotherapy, 82% of patients showed reduced cough, reduction in bacterial load in sputum, clearance of pulmonary infiltrates, reduction in hemoptysis, improved appetite, and weight gain.[128,129] At 6 months after initiating treatment, all the treated patients were alive and more than half were discharged from the hospital. In contrast, more than 30 untreated TB patients from another ward in the hospital had died within 4 months of starting the study. Experimental proof of antibody-mediated protection against TB was also published in 1897 by Fisch.[128,130] After lethal TB challenge of guinea pigs, administration of immune serum was performed on days 4, 7, and 10, with further doses administered every other day for 4 weeks and once a week after that. Fisch reported that 16 of 18 treated animals were alive after 2.5 months (89% survival). If treatment was delayed until day 14 postchallenge, then 2 of 3 (66%) animals survived but showed signs of illness. If no antibody treatment was performed, then 0 of 3 (0%) of the animals survived past day 28. The same approach was used to treat 50 patients with pulmonary TB.[131] All of the 19 patients treated at the earliest stages of disease improved rapidly after passive immunotherapy and were tuberculin negative at the end of the study. Of the 11 patients treated at the "incipient" stage of disease, 36% no longer had bacilli in their sputum and were considered cured and 64% showed substantial improvement in disease symptoms. The 20 patients with advanced TB showed only modest or no improvement after therapy and it was concluded that immune serum was only beneficial in early but not advanced cases of disease.[131]

EBV is a common human pathogen that causes a chronic infection and is a leading cause of posttransplant non-Hodgkin lymphoma resulting from the uncontrolled proliferation of EBV-infected B lymphocytes in patients undergoing immunosuppressive therapies.[132] In a large retrospective study involving 44,828 kidney transplant patients, the effect of prophylactic treatment for cytomegalovirus (CMV) on posttransplant incidence of non-Hodgkin lymphomas was examined.[133] The standardized incidence ratio (SIR) for non-Hodgkin lymphoma was expressed as the number of lymphoma cases per 100,000 persons and calculated after normalizing for age, sex, and geographical origin. The 30,255 patients who did not receive CMV prophylaxis had a SIR = 26.4, which remained unchanged (SIR = 24.2, $P = .62$) among the 12,470 patients who received antiviral drugs (acyclovir or ganciclovir). In striking contrast, the 2103 patients who received anti-CMV immunotherapy showed a complete absence of lymphomas during the first year after transplantation (SIR = 0, $P = .016$ vs. antiviral treatment). The most common anti-CMV immunoglobulin products were shown to contain antibodies against EBV and it is believed that this is the mechanism of action for the protection afforded during the first year posttransplantation.[133] In the subsequent 5 years of follow-up, new cases of lymphoma developed at similar rates among all three groups ($P = .97$). However, because administration of immunoglobulin is typically only performed during the first 4 months after transplantation and antiviral antibody half-life is estimated to be approximately 25 days,[134] it is not surprising that the protective effects of passive immunotherapy were only maintained through the first year. Nevertheless, the inadvertent discovery of the protective role of antibodies in preventing EBV-induced non-Hodgkin lymphoma represents a potential breakthrough in clinical management of this vulnerable patient population.

Despite decades of research aimed at finding a vaccine or a cure for HIV infection, this virus remains a scourge of global proportions. Early attempts at passive immunotherapy using first-generation HIV-specific monoclonal antibodies were not highly effective[135–137] and this approach was not further pursued until a new generation of highly potent and broadly neutralizing antibodies were identified.[138,139] In particular, a recent Phase I clinical trial[140] involving a single administration of a broadly neutralizing antibody, 3BNC117, has renewed interest in the study of passive immunotherapy for HIV prevention and therapeutic intervention. 3BNC117 is an anti-CD4 binding site antibody that neutralizes 195 of 237 HIV strains comprising six different clades and was tested in a dose-escalation study among HIV-positive patients with different levels of viremia. At a dose of 10 or 30 mg/kg, patient viral load was reduced by up to 2.5 $\log_{10}$ (average decline: 1.48 $\log_{10}$) in 10 of 11 individuals. The subject that did not respond to antibody treatment at 10 mg/kg was infected with a resistant strain of HIV. Although the effect of antibody therapy on viremia was mainly transient after a single administration, the viral load remained lower than their preexisting set point in 3 of 10 patients at 56 days and one subject exhibited viremia levels that remained near the limits of detection throughout the 56-day study. It is currently unclear if HIV viremia in these patients will eventually rebound to their original levels. Similar results were observed during antibody-based therapy of SHIV-infected rhesus macaques in which most animals showed a rebound in viral replication after the transferred monoclonal antibodies declined to undetectable levels but a subset of animals maintained virological control in the absence of further infusions.[141] Combinations of antiretroviral drugs are currently the standard of care for treatment of HIV infection and it is unlikely that one dose of a single monoclonal antibody will be sufficient to have a long-term clinical benefit among a broad patient base. However, there is growing optimism that combining a cocktail of potent, broadly neutralizing monoclonal antibodies with antiretroviral drugs and/or agents that activate latent virus reservoirs could theoretically provide long-term reduction in viral load and reduce the rates of transmission.

FUTURE OF PASSIVE IMMUNIZATION

With substantial advances in monoclonal antibody technologies and an increasing appreciation for the role of antibodies in the control of infectious disease, the development of sophisticated new passive immunotherapies is likely to continue at an accelerated pace. Antibiotic resistance among clinically relevant bacteria including multidrug-resistant (MDR) and XDR *M. tuberculosis*, methicillin-resistant *Staphylococcus aureus* (MRSA), and dominant strains of antibiotic-resistant *Salmonella typhi* and other gram-negative bacterial species is a growing concern.[122,125,142–144] This, coupled with the knowledge that fewer new antibiotics are moving through the drug pipeline,[122,123] may further motivate research into the development of antibody-based therapies to overcome these challenges to clinical intervention against microbial disease. One drawback to passive immunization is that antibody half-life in vivo

often provides only transient protection unless repeated administrations are performed. This may change as new technologies that increase the half-life of monoclonal antibodies are employed. For example, the Fc region of an anti-RSV monoclonal antibody, motavizumab, was mutated to increase its binding to the neonatal Fc receptor (FcRn), resulting in serum antibody pharmacokinetics in human subjects that increased from a typical 19- to 34-day half-life to up to a 100-day half-life while still retaining virus-specific neutralizing activity.[144a] Nevertheless, while passive immunization may be sufficient for protection or therapeutic intervention of acute or remittent disease, active immunization through improved vaccine design may still be needed to train the host immune system to maintain long-term levels of protective immunity. Importantly, examples of successful passive immunization approaches may provide a useful framework for developing new and improved vaccines that elicit the most protective antibody responses.

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9 General Immunization Practices

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Recommendations for immunization practices are based on scientific knowledge of vaccine characteristics, biology of immunization, epidemiology of specific diseases, and host characteristics. In addition, experience and judgment of public health officials and specialists in clinical and preventive medicine play a key role in developing recommendations that maximize benefits and minimize risks and costs associated with immunization. General guidelines for immunization practices are based on evidence and expert opinion of benefits and risks of vaccinations as they apply to the current epidemiology of disease and use of vaccines in the United States. However, many of the principles are universal and are applicable to other countries where different public health infrastructures may exist.

VACCINE STORAGE AND HANDLING

Vaccines must be properly shipped, stored, and handled to avoid loss of their biologic activities. Recommended storage and handling requirements for each vaccine are given in each manufacturer's product information.[1] Correct shipping, storage, and handling practices also are published in recommendations of the major vaccine policymaking committees, such as the Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention (CDC), the Committee on Infectious Diseases of the American Academy of Pediatrics (AAP), and the World Health Organization (see Chapter 76).[2–5] Failure to adhere to these requirements can result in loss of vaccine potency, leading to an inadequate immune response in the vaccinee. Visible evidence of altered vaccine integrity may not be present. The state/local health department or the manufacturer should be contacted when questions arise about the correct handling of a vaccine. New vaccines or new formulations of an existing vaccine may have different shipping, storage, and handling requirements. The recommended storage practices for the most commonly used vaccines in the United States are described in the Vaccine Storage and Handling Toolkit.[6]

Refrigerators without freezers and standalone freezers are usually most effective at maintaining the precise temperatures required for vaccine storage and are preferred to combination refrigerator/freezer units. Freezer storage units may be manual defrost or automatic defrost ("frost-free"). Automatic defrost freezers periodically and transiently increase the freezer temperature to reduce the formation of ice. This type of freezer unit is acceptable for storage of vaccines that must be stored in a frozen state (varicella, measles-mumps-rubella-varicella [MMRV], oral polio, zoster).

Exposure to higher or lower temperatures than recommended can damage a vaccine. For example, live virus vaccines, such as oral poliovirus vaccine (OPV), varicella, combination MMRV, and zoster vaccine, are sensitive to temperatures above freezing and should be kept frozen until just before administration. Measles-mumps-rubella (MMR without varicella) vaccine may be stored at either refrigerator or freezer temperature. Rotavirus vaccine and yellow fever vaccine should be stored at refrigerator temperature (2–8°C).[3,7] Some vaccines composed of purified antigens or inactivated microorganisms, such as hepatitis A, hepatitis B, *Haemophilus influenzae* type b (Hib), human papillomavirus (HPV), and inactivated influenza, can lose their potency if frozen and should be kept at refrigerator temperature and never frozen.[3,4] Diluents should not be frozen and may be kept at room or refrigerator temperature. Maintenance of a "cold chain" from vaccine production to use helps ensure vaccine potency at the time of administration. Temperature monitoring and control are important for storage and handling of all vaccines, particularly during transport and field use. Temperatures should be monitored at least twice a day, using a thermometer that records current, maximum, and minimum temperatures. Whereas maintenance of cold and freezing temperatures may be a problem in tropical climates, data suggest that inappropriate freezing of inactivated vaccines is a problem in maintaining vaccine stability in cold and temperate climates. Shipping containers should be sturdy, the correct size for the amount of vaccine to be shipped, and contain a temperature monitor. Appropriate insulation (e.g., panels and boxes of polystyrene, isocyanurate, or polyurethane) and cold source (e.g., bottles with frozen liquid) should be used to maintain the recommended temperature. Dry ice should not be used to transport frozen vaccine, because it might expose the vaccine to temperatures lower than recommended for storage. Loose fillers do not provide reliable temperature insulation.[3]

Vaccines should not be reconstituted until immediately before use. If not administered within the interval recommended by the manufacturer, reconstituted vaccine should be discarded.[5] Only the diluent provided by the manufacturer should be used to reconstitute a lyophilized vaccine. With the exception of OPV, live virus vaccines should not be refrozen after thawing.

Certain vaccines are distributed in multidose vials. When opened, the remaining doses from partially used multidose vials can be administered until the expiration date printed on the vial or vaccine packaging unless otherwise specified by the manufacturer, provided that the vial has been stored correctly and that the vaccine is not contaminated.[5]

VACCINE ADMINISTRATION

Complete and accurate records documenting administration of all vaccines should be maintained by healthcare providers who administer vaccines and vaccine recipients (or their parents). For each immunization, the following information should be recorded: (a) date of vaccination; (b) product administered, manufacturer, lot number, and expiration date; (c) site and route of administration; and (d) name, address, and title of healthcare provider administering the vaccine.

Infection Control and Sterile Injection Technique

Infection resulting from administration of vaccines is unlikely if appropriate precautions are utilized. Hands should be washed with soap and water or cleansed with an alcohol-based waterless antiseptic hand rub before each patient contact to reduce the risk of bacterial contamination and transmission of microorganisms between vaccine recipients and healthcare personnel. In general, use of protective gloves is not necessary when administering vaccines unless the healthcare provider

will have contact with potentially infectious body fluids or has open lesions on the hands.[2,5]

Failure to follow relevant infection control guidelines can result in transmission of bloodborne pathogens or bacterial infection and abscess formation. Contamination of an injection site can occur from bacteria on the skin at the site of injection. To prevent such contamination, skin at the injection site should be prepared with isopropyl alcohol (70%) or another disinfecting agent and allowed to dry before injection. Transmission of pathogens also can occur if needles, syringes, vaccines, or other equipment used to administer vaccines becomes contaminated. To prevent such contamination, syringes and needles must be sterile. A separate needle and syringe should be used for each injection. Disposable needles and syringes should be discarded after a single use in a labeled, puncture-proof container to prevent inadvertent needle-stick injury or reuse. Because recapping and removing a used needle from a syringe can result in injury to the user, needles should not be recapped after use.[5] The needle and syringe should be discarded as a single unit without removing the needle from the syringe. Single-use disposable needles and syringes should not be sterilized and reused.

If only reusable (i.e., nondisposable) needles and syringes are available, they must be thoroughly cleaned and sterilized after each injection to prevent transmission of bloodborne or other pathogens between patients. Reusable syringes are usually glass rather than plastic. Because of its inert characteristics, glass can be cleaned and sterilized more easily than plastic. Because hypodermic needles enter deep tissues, great care must be taken to ensure that all contaminants are removed from the needle and syringe.[8] Liquid germicides alone are insufficient for needle sterilization because of the restricted access of the chemical agent to the lumen of the needle. Strict adherence to the recommended time and temperature for the sterilization procedure used must be observed.

Most vaccines have a similar appearance after they are drawn into a syringe. Cases in which the wrong vaccine was administered often are attributable to the practice of prefilling syringes or drawing doses of a vaccine into multiple syringes before their immediate need.[5] The routine practice of prefilling syringes should be discouraged because of the potential for such administration errors. To prevent errors, vaccine doses should not be drawn into a syringe until immediately before administration. In certain circumstances in which a single vaccine type is used (e.g., in a community influenza vaccination campaign), filling a small number (10 or fewer) of syringes before their immediate use may be considered. Care should be taken to ensure that the cold chain and sterility are maintained until the vaccine is administered. When the syringes are filled, the type of vaccine, lot number, and date of filling must be labeled accurately on each syringe, and the doses should be administered as soon as possible after filling, by the same person who filled the syringes. Manufacturer prefilled-syringes that are activated (i.e., syringe cap removed or needle attached) but unused should be discarded at the end of the clinic day. Likewise, vaccine drawn into syringes by the user (i.e., not by the manufacturer) should be discarded at the end of the clinic day.

Route of Administration

One or more routes of administration (e.g., intramuscular, subcutaneous, intradermal, intranasal, and oral) are recommended for each vaccine and are listed in the manufacturer's product label and in published recommendations of immunization advisory committees (Table 9.1).[2,5] These routes usually are determined during prelicensure vaccine studies and are based on vaccine composition and immunogenicity. Vaccines should be administered in sites where they elicit the desired immune response and where the likelihood of local tissue, neural, or vascular injury is minimal.[2] To avoid unnecessary local and systemic adverse events and to ensure the appropriate immune response, people administering vaccines should not deviate from the recommended route of administration in the product label. A route of administration or anatomic site of injection different from that recommended can result in an inadequate immune response. For example, the immunogenicity of hepatitis B vaccine and rabies vaccine is substantially lower when the gluteal instead of the deltoid vaccination site is used.[9,10] The reduced immunogenicity presumably is a result of inadvertent injection into subcutaneous or deep fatty tissue rather than into muscle.

Deep intramuscular injection generally is recommended for adjuvant-containing vaccines because subcutaneous or intradermal administration can cause marked local irritation, induration, skin discoloration, inflammation, and granuloma formation.[5] However, subcutaneous injection can lessen the risk of local neurovascular injury and is recommended for vaccines that are less reactogenic but immunogenic when administered by this route, such as live virus vaccines. Intradermal administration is preferred for live bacille Calmette-Guérin (BCG) vaccine and one brand of inactivated influenza vaccine.[11]

Aspiration before injection of vaccines or toxoids (i.e., pulling back on the syringe plunger after needle insertion, before injection) is not necessary because there are no large blood vessels at the recommended injection sites. Also, the process of aspiration can be painful, especially for infants.[12]

Subcutaneous Injections

Vaccines recommended for subcutaneous injection usually are administered into the thigh of infants younger than 12 months of age and into the upper, outer triceps area of persons 12 months of age or older. Subcutaneous injections also can be administered into the upper, outer triceps area of an infant. A $\frac{5}{8}$-inch, 23- to 25-gauge needle is recommended in most situations.[2,5] The needle is inserted into the tissues below the dermal layer of the skin. To avoid administering the vaccine into a muscle, the skin and subcutaneous tissue should be held gently between the thumb and fingers to raise these tissues from the muscle layer. The needle is inserted into the resulting skinfold at an approximately 45-degree angle.[5]

Intramuscular Injections

Selection of the site of injection and needle size is based on the volume of vaccine to be administered, the thickness of the overlying subcutaneous tissue, the size of the muscle, and the desired depth below the muscle surface into which the material is to be injected.

The quadriceps muscle mass in the anterolateral thigh is most commonly used for intramuscular injection in infants, whereas the deltoid muscle of the upper arm is the usual recommended site for older children and adults. After a child begins to walk, the upper arm is the preferred site.[13] By this age, the child's deltoid muscle is usually large enough to be used for intramuscular injection. Although the anterolateral thigh is also an acceptable site, intramuscular injection into the thighs of 18-month-old children has been reported to cause transient limping.[14,15] For infants and younger children, if more than two vaccines are injected in a single limb, the thigh is the preferred site because of the greater muscle mass; the injections should be sufficiently separated (i.e., ≥1 inch) so that any local reactions can be differentiated.[13,14]

TABLE 9.1 Dose and Route of Administration for Selected Vaccines

Vaccine	Dose	Route
Diphtheria, tetanus toxoids, and acellular pertussis (DTaP); diphtheria and tetanus toxoids (DT); tetanus and diphtheria toxoids (Td); tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap)	0.5 mL	IM
DTaP–hepatitis B (HepB)–inactivated poliovirus (IPV)	0.5 mL	IM
DTaP-IPV/*Haemophilus influenzae* type b (Hib)	0.5 mL	IM
DTaP-IPV	0.5 mL	IM
Hib	0.5 mL	IM
Hib-MenCY	0.5 mL	IM
Hepatitis A (HepA)	≤18 y: 0.5 mL ≥19 y: 1 mL	IM
HepB	≤19 y: 0.5 mL[a] ≥20 y: 1 mL	IM
HepA-HepB	≥18 y: 1 mL	IM
Live attenuated influenza vaccine (LAIV)	0.2 mL divided dose between nares	Intranasal spray
Inactivated influenza vaccine (IIV)	6–35 mo: 0.25 mL ≥3 y: 0.5 mL	IM
IIV intradermal	18–64 y: 0.1 mL	Intradermal (ID)
Measles, mumps, and rubella (MMR)	0.5 mL	Subcutaneous (SC)
MMR and varicella (MMRV)	0.5 mL	SC
Quadrivalent meningococcal conjugate vaccine (MCV4)	0.5 mL	IM
Quadrivalent meningococcal polysaccharide vaccine (MPSV4)	0.5 mL	SC
Serogroup B meningococcal vaccine	0.5 mL	IM
Pneumococcal conjugate vaccine (PCV)	0.5 mL	IM
Pneumococcal polysaccharide vaccine (PPSV)	0.5 mL	IM or SC
Human papillomavirus (HPV; bivalent vaccine, 2vHPV, quadrivalent vaccine, 4vHPV, or 9-valent vaccine, 9vHPV)	0.5 mL	IM
IPV	0.5 mL	IM or SC
Rotavirus (RV1 or RV5)	(1 or 2 mL)	Oral
Varicella	0.5 mL	SC
Herpes zoster	0.65 mL	SC

[a]Persons age 11–15 y may be administered Recombivax HB (Merck), 1 mL (adult formulation) on a two-dose schedule.
Modified from Immunization Action Coalition: http://www.immunize.org.

Because of the potential risk of injury to the sciatic nerve, the gluteal region is not recommended for routine vaccination.[2,5] This recommendation is based primarily on reported cases of sciatic nerve injury resulting from injection of antimicrobial agents or antiserum into the gluteus.[16,17] No reports of direct nerve injury resulting from gluteal injection of current childhood vaccines have been published.

If injections are given in the gluteal site, care must be taken to avoid nerve injury. The central region of the buttocks should be avoided. The needle should be inserted into the upper, outer quadrant and directed anteriorly (i.e., not caudally or perpendicular to the skin surface). If the gluteal muscle is chosen, injection should be lateral and superior to a line between the posterior superior iliac spine and the greater trochanter or the ventrogluteal site (i.e., the center of the triangle bounded by the anterior superior iliac spine, the tubercle of the iliac crest, and the upper border of the greater trochanter of the femur). These areas are free of major neurovascular structures. Because of the large volume that must be injected and its large muscle mass, the gluteal site often is used for passive immunization with immunoglobulin preparations.[2,5]

A 22- to 25-gauge needle is appropriate for intramuscular administration of most vaccines. The ideal needle length may depend on the vaccination technique.[18] One technique for intramuscular injections consists of gently bunching the muscle in the free hand while the needle is inserted perpendicular to the skin. A second technique consists of using the thumb and index finger to stretch the skin flat over the injection site while inserting the needle perpendicular to the skin and injecting the vaccine.[18,19]

The subcutaneous tissue and muscle layer thickness of the anterolateral thigh and deltoid region have been determined by ultrasonography.[18,20,21] On the basis of the resulting data, a ⅝-inch (16-mm) needle used according to the second technique described above is estimated to be adequate for intramuscular injection in the thigh of infants and toddlers and in the deltoid of toddlers.[18] However, when using the "bunching" technique described above, a ⅞- to 1-inch (22- to 25-mm) needle would be necessary for adequate intramuscular

penetration of the thigh of a 4-month-old infant and of the thigh and deltoid of toddlers and older children.[2,5,18,20] For injection into the thigh of children 3 years through 10 years of age, the needle length must be 1- to 1.25-inches in length.[22]

For adolescents and adults, the ideal needle length for intramuscular injection depends on the weight and sex of the vaccinee. For adolescents the deltoid muscle is preferred; however, the anterolateral thigh may be used, and if used the needle must be 1- to 1.5-inches in length.[23] Poland and colleagues[21] reported that women have a greater deltoid fat pad thickness by ultrasonography and a greater deltoid skinfold thickness than men of an equal body mass index. These authors recommended a 1-inch (25-mm) needle for men for all weight ranges studied (i.e., 59–118 kg); a ⅝- to 1-inch (16–25-mm) needle is recommended for women weighing less than 60 kg, a 1-inch (25-mm) needle is sufficient for women who weigh 60 to 90 kg, and a 1.5-inch (38-mm) needle is recommended for women who weigh more than 90 kg and men who weigh more than 118 kg.[5,21]

Vaccinating Persons With Bleeding Disorders and Persons Receiving Anticoagulant Therapy

Persons with bleeding disorders such as hemophilia and persons receiving anticoagulant therapy can be at increased risk for bleeding after intramuscular injection. If possible vaccination could be scheduled prior to the use of anticoagulant therapy so that the patients' risk of bleeding is not increased by their therapeutic action.

A 23-gauge or smaller needle should be used for the vaccination, and firm pressure without rubbing should be applied to the site for at least 2 minutes following injection. Alternatively, vaccines recommended for intramuscular injection could be administered subcutaneously to persons with a bleeding disorder if the immune response and clinical reaction to these vaccines are expected to be comparable by either route of injection, such as meningococcal conjugate vaccine and hepatitis A vaccine.[2,24,25]

Intradermal Injections

BCG, one influenza vaccine, and smallpox vaccine are the only vaccines in the United States licensed for intradermal administration. Intradermal influenza vaccine is administered near the middle of the upper arm, over the insertion of the deltoid muscle. The intradermal influenza vaccine is administered using a prefilled 1/50-inch microneedle injector system.

Smallpox (vaccinia) vaccine is administered by the intradermal route using multiple punctures with a unique bifurcated needle held perpendicular to the skin (see Chapter 33). A successful vaccination results in a pustular lesion ("Jennerian pustule") at the vaccination site 6 to 8 days after primary vaccination. The skin reaction following revaccination may be less pronounced, with more rapid progression and healing, than that after primary vaccination.[26]

Oral Administration

For vaccines given orally, the vaccine must be swallowed and retained. A dose of oral polio vaccine (OPV) should be repeated immediately if a patient spits out, does not swallow, or regurgitates a dose within 10 minutes after administration.[24] However, readministration of rotavirus vaccine is not recommended if the dose is spat out or regurgitated. No data exist on the benefits or risks associated with readministering a dose of regurgitated rotavirus vaccine. The infant should receive the remaining recommended doses of rotavirus vaccine following the routine schedule (with a 4-week minimum interval between doses).[5,27]

Intranasal Route

Live attenuated influenza vaccine (LAIV) is licensed for healthy nonpregnant persons 2 through 49 years of age and is the only vaccine administered by the intranasal route. The administration device is a nasal sprayer with a dose-divider clip that allows introduction of one 0.1-mL spray into each naris. The tip should be inserted slightly into the naris before administration. Even if the person coughs or sneezes immediately after administration or the dose is expelled any other way, the vaccine dose need not be repeated. Introduction of low levels of vaccine viruses into the environment likely is unavoidable when administering LAIV; however, no cases have been reported of illness or attenuated vaccine virus infections among inadvertently exposed healthcare providers or immunocompromised patients. The risk for acquiring vaccine viruses from the environment is unknown but is likely low; in addition, vaccine viruses are cold-adapted and attenuated and unlikely to cause symptomatic influenza. Severely immunosuppressed persons should not administer LAIV. However, other persons at higher risk for influenza complications can administer LAIV. These include persons with underlying medical conditions placing them at higher risk or who are likely to be at risk, including pregnant women, persons with asthma, and persons 50 years or older.[28]

Needle-Shielding/Needle-Free Devices

Bloodborne diseases (e.g., hepatitides B and C and HIV) are occupational hazards for healthcare providers. In November 2000, to reduce the incidence of needlestick injuries among healthcare providers and the consequent risk for bloodborne diseases acquired from patients, the Needlestick Safety and Prevention Act was signed into law in the United States. The act directed the U.S. Occupational Safety and Health Administration (OSHA) to strengthen its existing bloodborne pathogen standards. Those standards were revised and became effective in April 2001.[29] These federal regulations require the use of engineering and work practice controls to eliminate or minimize employee exposure to bloodborne pathogens.[30] "Engineering controls" mean controls (e.g., sharps disposal containers; self-sheathing needles; and safer medical devices, such as sharps with engineered sharps injury protections and needleless systems) that isolate or remove the bloodborne pathogens hazard from the workplace.[31] Work practice controls mean procedures followed by employers and workers to control hazards in the workplace (e.g., good housekeeping practices; closed transfers/containers/processes; and hygiene procedures).

Needle-shielding or needle-free devices that might satisfy the occupational safety regulations for administering parenteral injections are available in the United States and are listed at multiple websites.[33] Additional information regarding implementation and enforcement of these regulations is available at the OSHA website.[29]

Jet Injectors

Jet injectors (JIs) are needle-free devices that drive liquid medication through a nozzle orifice, creating a narrow stream under high pressure that penetrates skin to deliver a drug or vaccine into intradermal, subcutaneous, or intramuscular tissues.[35,36] JIs have the potential to reduce the frequency of needlestick injuries to healthcare providers and to overcome the improper reuse and other drawbacks of needles and

syringes in economically developing countries.[37,38] JIs have been reported safe and effective in administering different live and inactivated vaccines for viral and bacterial diseases.[39] The immune responses generated are equivalent to, and occasionally greater than, immune responses induced by needle injection. However, local reactions or injury (e.g., redness, induration, pain, blood, ecchymosis, and transient neuropathy at the injection site) can be more frequent when vaccines are delivered by JIs compared with needle injection.[39]

In the 1990s, a new generation of JIs was introduced with disposable cartridges serving as dose chambers and nozzle. With the provision of a new sterile cartridge for each patient and correct use, these devices avoid safety concerns for multiple-use–nozzle devices. These devices should be used in accordance with their labeling for intradermal, subcutaneous, or intramuscular administration. One brand of inactivated influenza vaccine has been approved by the U.S. Food and Drug Administration for use with a JI device.[40,41]

ALLEVIATION OF PAIN AND DISCOMFORT ASSOCIATED WITH VACCINATION

Several methods have been reported to reduce pain and discomfort associated with vaccination injection, but they have not been tested widely.[39] Pretreatment with topical lidocaine-prilocaine emulsion cream or patch can decrease the pain of diphtheria and tetanus toxoids and pertussis (DTP and DTaP) vaccination in infants by causing superficial anesthesia.[42–44] This product does not interfere with the immune response to MMR[43] or to inactivated vaccines.[45]

Evidence does not support use of antipyretics before or at the time of vaccination; however, antipyretics can be used for treatment of fever and local discomfort that might occur following vaccination. Studies of children with previous febrile seizures have not demonstrated antipyretics to be effective in the prevention of febrile seizures.[46]

A topical refrigerant spray can reduce the short-term pain associated with injections and can be as effective as lidocaine-prilocaine cream.[46,47] Oral administration of sweet-tasting fluid just before injection may cause a calming or analgesic effect in some infants.[48] Breastfeeding is a potent analgesic intervention for infants during blood collection and may, by extrapolation, help decrease injection pain during immunization. Distraction techniques such as listening to music or "blowing away pain" also may help children cope with the discomfort associated with vaccination.[49,50] Swaddling and slow lateral swaying also help as comfort measures.[51]

AGES FOR ADMINISTRATION OF IMMUNOBIOLOGICS

Recommendations for the age and timing of vaccination are based on multiple considerations and may vary in different countries. Optimal response to a vaccine depends on a number of factors, including the nature of the vaccine and the age and immune status of the recipient. Recommendations for the age at which vaccines are administered are influenced by age-specific risks for disease and complications, ability of persons of a certain age to respond to the vaccine, and potential interference with the immune response by passively transferred maternal antibody or previously administered antibody-containing blood products. Vaccines usually are recommended for members of the youngest age group at risk for experiencing the disease for whom efficacy and safety have been demonstrated. These principles are demonstrated with the following examples.

The optimal timing for administration of measles vaccine depends on the rate of disappearance of passively acquired maternal antibody and the risk of exposure to measles virus. At birth and through the first 6 months of life, most infants have passive immunity to measles because of transplacentally acquired maternal measles antibodies. These antibodies interfere with the immune response to live virus measles vaccine by limiting vaccine virus replication. In many developing countries, where measles is highly endemic and frequently affects infants, routine measles vaccination is recommended at 9 months of age (see Chapter 21).[52] However, in the United States, where measles is less common and measles among infants is infrequent, measles vaccine is recommended routinely at 12 through 15 months of age, ages at which almost all infants will have no residual maternal antibody.[2,53]

Another example is the recommended age of pertussis vaccination. Early infancy is the time of greatest risk of serious complications from pertussis, but infants who are younger than 1 month of age do not respond as well immunologically to whole-cell pertussis vaccine (and presumably acellular vaccines) as do older infants.[54–56] Initiation of routine immunization with pertussis vaccine in the United States is recommended at 2 months of age.[57,58] This scheduling represents a compromise between factors affecting the immune response and the epidemiology of the disease necessitating early protection against pertussis. Tdap (tetanus, diphtheria, and pertussis) given to pregnant women will stimulate the development of maternal antipertussis antibodies, which will be transferred to the fetus by the placenta, likely providing the newborn with protection against pertussis in early life.[59] Tdap also will protect the mother from pertussis around the time of delivery, making her less likely to become infected and transmit pertussis to her infant.[59] Vaccination too early in life also may affect the immune response to subsequent doses of vaccine. For example, neonatal administration of diphtheria and tetanus toxoids (DT) may result in suppression of antibody responses to subsequent doses of Hib conjugate vaccines covalently linked to forms of those toxoids.[60] When children who receive measles vaccine before the age of 1 year are revaccinated, they develop vaccine-induced immunity against disease but may have a somewhat diminished antibody response compared with children vaccinated initially after their first birthday.[61,62]

In the United States, the recommended childhood, adolescent, and adult immunization schedules are revised annually and are approved by the ACIP, the AAP (for the childhood and adolescent schedule), the American Academy of Family Physicians, the American Congress of Obstetricians and Gynecologists, the American College of Physicians (for the adult schedule), and the American College of Nurse Midwives (for the adult schedule).[63,64] A recent U.S. childhood vaccination schedule is shown in Fig. 9.1.[63] The most current vaccination schedules for children and adults are available from the CDC Vaccines and Immunizations website (http://www.cdc.gov/vaccines/schedules/index.html).[65] Table 9.2 lists the recommended and minimum ages and recommended and minimum acceptable intervals between doses of vaccines used in the United States. Other vaccination schedules are discussed in Chapters 74, 75, and 76.

SPACING OF VACCINE DOSES

Spacing of Multiple Doses of the Same Vaccine

Although administration of one dose of some vaccines may induce a protective antibody response, most vaccines require administration of multiple doses in a primary series for development of immunity. Examples of the former are rubella and yellow fever vaccines; examples of the latter are poliovirus,

hepatitis B, and pertussis vaccines. In addition, periodic revaccination ("booster doses") with certain vaccines may be necessary to maintain immunity. Examples are typhoid vaccines and tetanus and diphtheria toxoids.

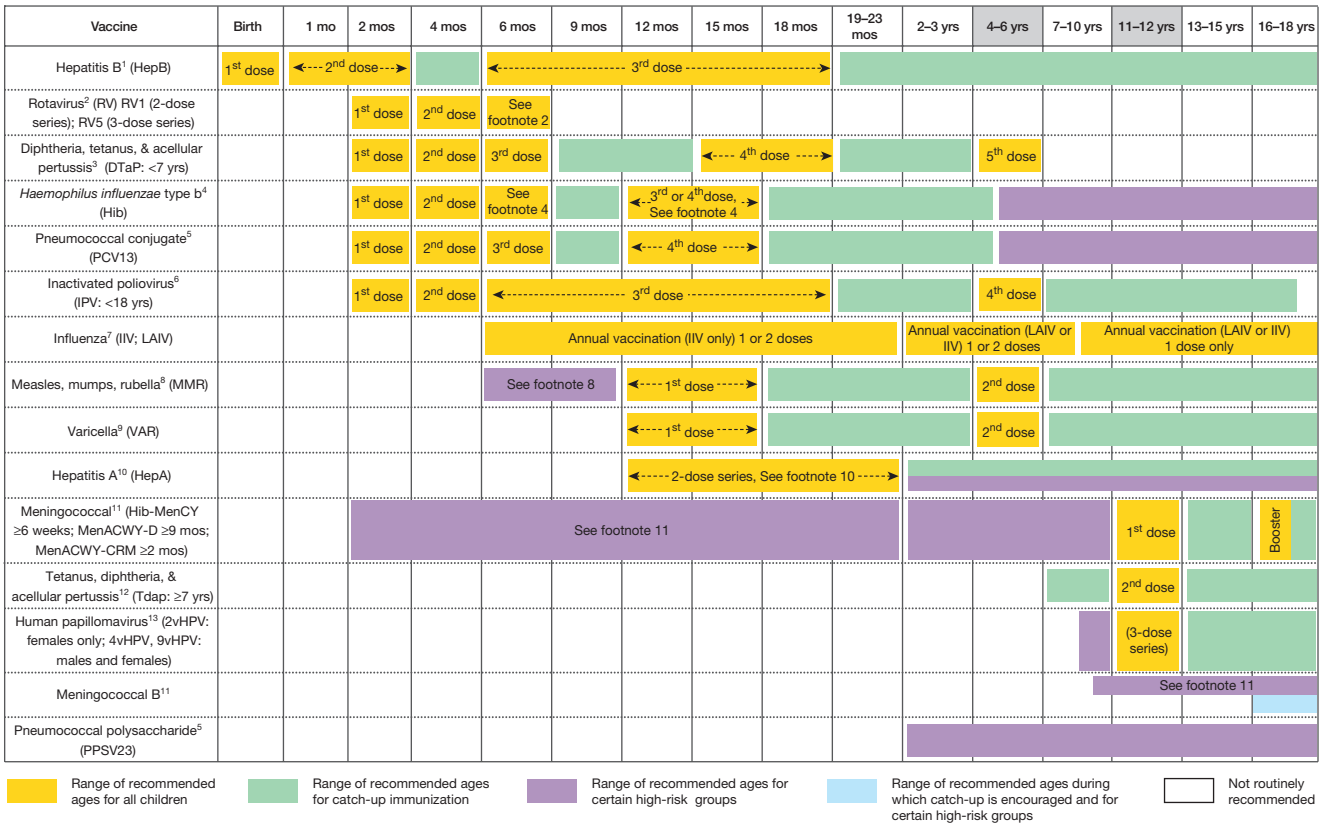
Because of immunologic memory, intervals longer than routinely recommended between doses do not impair the immunologic response to live and inactivated vaccines that require more than one dose to achieve primary immunity. Similarly, delayed administration of recommended booster doses does not adversely affect the antibody response to such doses.[2,5] As a result, interruption of a recommended primary series or an extended interval between booster doses does not necessitate reinitiation of the entire vaccination series. For example, lengthening the interval between two doses of inactivated poliovirus vaccine (IPV) may increase the antibody response to the second dose.[66] In the case of oral typhoid (Ty21a) vaccine, an exception has been proposed. The impact of a lapse in the series of oral typhoid vaccine is not known. However, as a rule of thumb for Ty21a, if fewer than 3 weeks have passed since the last dose, the series may be completed with the missing dose or doses. If more than 3 weeks have passed, the full series should be repeated.

Vaccination providers should strive to adhere as closely as possible to recommended childhood, adolescent, and adult immunization schedules. Clinical studies confirm that recommended ages and intervals between doses of multidose antigens provide optimal protection or have the best evidence of efficacy. Table 9.2 lists recommended intervals between doses of commonly used vaccines.

In certain circumstances, administering doses of a multidose vaccine at shorter than the recommended intervals might be necessary. Examples include when a person is behind schedule and needs to be brought up-to-date as quickly as possible and cases of impending international travel. In these situations, an accelerated schedule with intervals between doses shorter than intervals recommended for routine vaccination can be used.[63] Although the effectiveness of all accelerated

Text continued on p. 106

Recommended immunization schedule for persons aged 0 through 18 years – United States, 2016.
(FOR THOSE WHO FALL BEHIND OR START LATE, SEE THE CATCH-UP SCHEDULE [FIGURE 2]).
These recommendations must be read with the footnotes that follow. For those who fall behind or start late, provide catch-up vaccination at the earliest opportunity as indicated by the green bars in Figure 1. To determine minimum intervals between doses, see the catch-up schedule (Figure 2). School entry and adolescent vaccine age groups are shaded.



This schedule includes recommendations in effect as of January 1, 2016. Any dose not administered at the recommended age should be administered at a subsequent visit, when indicated and feasible. The use of a combination vaccine generally is preferred over separate injections of its equivalent component vaccines. Vaccination providers should consult the relevant Advisory Committee on Immunization Practices (ACIP) statement for detailed recommendations, available online at **http://www.cdc.gov/vaccines/hcp/acip-recs/index.html.** Clinically significant adverse events that follow vaccination should be reported to the Vaccine Adverse Event Reporting System (VAERS) online (**http://www.vaers.hhs.gov**) or by telephone (**800-822-7967**). Suspected cases of vaccine-preventable diseases should be reported to the state or local health department. Additional information, including precautions and contraindications for vaccination, is available from CDC online (**http://www.cdc.gov/vaccines/recs/vac-admin/contraindications.htm**) or by telephone (**800-CDC-INFO** [**800-232-4636**]).

This schedule is approved by the Advisory Committee on Immunization Practices (**http//www.cdc.gov/vaccines/acip**), the American Academy of Pediatrics (**http://www.aap.org**), the American Academy of Family Physicians (**http://www.aafp.org**), and the American College of Obstetricians and Gynecologists (**http://www.acog.org**).

NOTE: The above recommendations must be read along with the footnotes of this schedule.

Figure 9.1. Immunization schedules. Available at www.cdc.gov/vaccines/schedules/hcp/child-adolescent.html. *Continued*

Catch-up immunization schedule for persons aged 4 months through 18 years who start late or who are more than 1 month behind —United States, 2016.

The figure below provides catch-up schedules and minimum intervals between doses for children whose vaccinations have been delayed. A vaccine series does not need to be restarted, regardless of the time that has elapsed between doses. Use the section appropriate for the child's age. Always use this table in conjunction with Figure 1 and the footnotes that follow.

Children age 4 months through 6 years					
Vaccine	Minimum age for dose 1	Minimum interval between doses			
		Dose 1 to Dose 2	Dose 2 to Dose 3	Dose 3 to Dose 4	Dose 4 to Dose 5
Hepatitis B[1]	Birth	4 weeks	**8 weeks** *and* at least 16 weeks after first dose. Minimum age for the final dose is 24 weeks.		
Rotavirus[2]	6 weeks	4 weeks	4 weeks[2]		
Diphtheria, tetanus, and acellular pertussis[3]	6 weeks	4 weeks	4 weeks	6 months	6 months[3]
Haemophilus influenzae type b[4]	6 weeks	**4 weeks** if first dose was administered before the 1st birthday. **8 weeks (as final dose)** if first dose was administered at age 12 through 14 months. **No further doses needed** if first dose was administered at age 15 months or older.	**4 weeks**[4] if current age is younger than 12 months **and** first dose was administered at younger than age 7 months, **and** at least 1 previous dose was PRP-T (ActHib, Pentacel) or unknown. **8 weeks** *and* **age 12 through 59 months (as final dose for healthy children)**[4] if current age is younger than 12 months **and** first dose was administered at age 7 through 11 months (wait until at least 12 months old); **OR** if current age is 12 through 59 months **and** first dose was administered before the 1st birthday, **and** second dose administered at younger than 15 months; **OR** if both doses were PRP-OMP (PedvaxHIB; Comvax) **and** were administered before the 1st birthday (wait until at least 12 months old). **No further doses needed** if previous dose was administered at age 15 months or older.	**8 weeks (as final dose)** This dose only necessary for children age 12 through 59 months who received 3 doses before the 1st birthday.	
Pneumococcal[5]	6 weeks	**4 weeks** if first dose administered before the 1st birthday. **8 weeks (as final dose for healthy children)** if first dose was administered at the 1st birthday or after. **No further doses needed** for healthy children if first dose administered at age 24 months or older.	**4 weeks** if current age is younger than 12 months and previous dose given at <7months old. **8 weeks (as final dose for healthy children)** if previous dose given between 7–11 months (wait until at least 12 months old); **OR** if current age is 12 months or older and at least 1 dose was given before age 12 months. **No further doses needed** for healthy children if previous dose administered at age 24 months or older.	**8 weeks (as final dose)** This dose only necessary for children aged 12 through 59 months who received 3 doses before age 12 months or for children at high risk who received 3 doses at any age.	
Inactivated poliovirus[6]	6 weeks	4 weeks[6]	4 weeks[6]	6 months[6] (minimum age 4 years for final dose).	
Measles, mumps, rubella[8]	12 months	4 weeks			
Varicella[9]	12 months	3 months			
Hepatitis A[10]	12 months	6 months			
Meningococcal[11] (Hib-MenCY >6 weeks; MenACWY-D >9 mos; MenACWY-CRM >2 mos)	6 weeks	8 weeks[11]	See footnote 11	See footnote 11	
Children and adolescents age 7 through 18 years					
Meningococcal[11] (Hib-MenCY >6 weeks; MenACWY-D >9 mos; MenACWY-CRM >2 mos)	Not applicable (N/A)	8 weeks[11]			
Tetanus, diphtheria; tetanus, diphtheria, and acellular pertussis[12]	7 years[12]	4 weeks	**4 weeks** if first dose of DTaP/DT was administered before the 1st birthday. **6 months (as final dose)** if first dose of DTaP/DT or Tdap/Td was administered at or after the 1st birthday.	**6 months** if first dose of DTaP/DT was administered before the 1st birthday.	
Human papillomavirus[13]	9 years	Routine dosing intervals are recommended.[13]			
Hepatitis A[10]	N/A	6 months			
Hepatitis B[1]	N/A	4 weeks	**8 weeks and** at least 16 weeks after first dose.		
Inactivated poliovirus[6]	N/A	4 weeks	4 weeks[6]	6 months[6]	
Meningococcal[11]	N/A	8 weeks[11]			
Measles, mumps, rubella[8]	N/A	4 weeks			
Varicella[9]	N/A	**3 months** if younger than age 13 years. **4 weeks** if age 13 years or older.			

NOTE: The above recommendations must be read along with the footnotes of this schedule.

Figure 9.1. cont'd

Footnotes — Recommended immunization schedule for persons aged 0 through 18 years—United States, 2016
For further guidance on the use of the vaccines mentioned below, see: **http://www.cdc.gov/vaccines/hcp/acip-recs/index.html.**
For vaccine recommendations for persons 19 years of age and older, see the Adult Immunization Schedule **www.cdc.gov/vaccines/schedules/hcp/adult.html**.
Additional information

- For contraindications and precautions to use of a vaccine and for additional information regarding that vaccine, vaccination providers should consult the relevant ACIP statement available online at **http://www.cdc.gov/vaccines/hcp/acip-recs/index.html.**
- For purposes of calculating intervals between doses, 4 weeks = 28 days. Intervals of 4 months or greater are determined by calendar months.
- Vaccine doses administered 4 days or less before the minimum interval are considered valid. Doses of any vaccine administered ≥5 days earlier than the minimum interval or minimum age should not be counted as valid doses and should be repeated as age-appropriate. The repeat dose should be spaced after the invalid dose by the recommended minimum interval. For further details, see *MMWR, General Recommendations on Immunization and Reports* / Vol. 60 / No. 2; Table 1. *Recommended and minimum ages and intervals between vaccine doses available* online at **http://www.cdc.gov/mmwr/pdf/rr/rr6002.pdf**.
- Information on travel vaccine requirements and recommendations is available at **http://wwwnc.cdc.gov/travel/destinations/list**.
- For vaccination of persons with primary and secondary immunodeficiencies, see Table 13, *"Vaccination of persons with primary and secondary immunodeficiencies," in General Recommendations on Immunization* (ACIP), available at **http://www.cdc.gov/mmwr/pdf/rr/rr6002.pdf**.; and American Academy of Pediatrics. "Immunization in Special Clinical Circumstances," in Kimberlin DW, Brady MT, Jackson MA, Long SS eds. *Red Book: 2015 report of the Committee on Infectious Diseases. 30th ed.* Elk Grove Village, IL: American Academy of Pediatrics.

1. Hepatitis B (HepB) vaccine. (Minimum age: birth)
Routine vaccination:
At birth:

- Administer monovalent HepB vaccine to all newborns before hospital discharge.
- For infants born to hepatitis B surface antigen (HBsAg)-positive mothers, administer HepB vaccine and 0.5 mL of hepatitis B immune globulin (HBIG) within 12 hours of birth. These infants should be tested for HBsAg and antibody to HBsAg (anti-HBs) at age 9 through 18 months (preferably at the next well-child visit) or 1 to 2 months after completion of the HepB series if the series was delayed; CDC recently recommended testing occur at age 9 through 12 months; see **http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6439a6.htm**.
- If mother's HBsAg status is unknown, within 12 hours of birth administer HepB vaccine regardless of birth weight. For infants weighing less than 2,000 grams, administer HBIG in addition to HepB vaccine within 12 hours of birth. Determine mother's HBsAg status as soon as possible and, if mother is HBsAg-positive, also administer HBIG for infants weighing 2,000 grams or more as soon as possible, but no later than age 7 days.

Doses following the birth dose:

- The second dose should be administered at age 1 or 2 months. Monovalent HepB vaccine should be used for doses administered before age 6 weeks.
- Infants who did not receive a birth dose should receive 3 doses of a HepB-containing vaccine on a schedule of 0, 1 to 2 months, and 6 months starting as soon as feasible. See Figure 2.
- Administer the second dose 1 to 2 months after the first dose (minimum interval of 4 weeks), administer the third dose at least 8 weeks after the second dose AND at least 16 weeks after the **first** dose. The final (third or fourth) dose in the HepB vaccine series should be administered **no earlier than age 24 weeks**.
- Administration of a total of 4 doses of HepB vaccine is permitted when a combination vaccine containing HepB is administered after the birth dose.

Catch-up vaccination:

- Unvaccinated persons should complete a 3-dose series.
- A 2-dose series (doses separated by at least 4 months) of adult formulation Recombivax HB is licensed for use in children aged 11 through 15 years.
- For other catch-up guidance, see Figure 2.

2. Rotavirus (RV) vaccines. (Minimum age: 6 weeks for both RV1 [Rotarix] and RV5 [RotaTeq])
Routine vaccination:
Administer a series of RV vaccine to all infants as follows:

1. If Rotarix is used, administer a 2-dose series at 2 and 4 months of age.
2. If RotaTeq is used, administer a 3-dose series at ages 2, 4, and 6 months.
3. If any dose in the series was RotaTeq or vaccine product is unknown for any dose in the series, a total of 3 doses of RV vaccine should be administered.

Catch-up vaccination:

- The maximum age for the first dose in the series is 14 weeks, 6 days; vaccination should not be initiated for infants aged 15 weeks, 0 days or older.
- The maximum age for the final dose in the series is 8 months, 0 days.
- For other catch-up guidance, see Figure 2.

3. Diphtheria and tetanus toxoids and acellular pertussis (DTaP) vaccine. (Minimum age: 6 weeks. Exception: DTaP-IPV [Kinrix, Quadracel]: 4 years)
Routine vaccination:

- Administer a 5-dose series of DTaP vaccine at ages 2, 4, 6, 15 through 18 months, and 4 through 6 years. The fourth dose may be administered as early as age 12 months, provided at least 6 months have elapsed since the third dose.
- Inadvertent administration of 4th DTaP dose early: If the fourth dose of DTaP was administered at least 4 months, but less than 6 months, after the third dose of DTaP, it need not be repeated.

Catch-up vaccination:

- The fifth dose of DTaP vaccine is not necessary if the fourth dose was administered at age 4 years or older.
- For other catch-up guidance, see Figure 2.

4. *Haemophilus influenzae* type b (Hib) conjugate vaccine. (Minimum age: 6 weeks for PRP-T [ACTHIB, DTaP-IPV/Hib (Pentacel) and Hib-MenCY (MenHibrix)], PRP-OMP [PedvaxHIB or COMVAX], 12 months for PRP-T [Hiberix])
Routine vaccination:

- Administer a 2- or 3-dose Hib vaccine primary series and a booster dose (dose 3 or 4 depending on vaccine used in primary series) at age 12 through 15 months to complete a full Hib vaccine series.
- The primary series with ActHIB, MenHibrix, or Pentacel consists of 3 doses and should be administered at 2, 4, and 6 months of age. The primary series with PedvaxHib or COMVAX consists of 2 doses and should be administered at 2 and 4 months of age; a dose at age 6 months is not indicated.
- One booster dose (dose 3 or 4 depending on vaccine used in primary series) of any Hib vaccine should be administered at age 12 through 15 months. An exception is Hiberix vaccine. Hiberix should only be used for the booster (final) dose in children aged 12 months through 4 years who have received at least 1 prior dose of Hib-containing vaccine.
- For recommendations on the use of MenHibrix in patients at increased risk for meningococcal disease, please refer to the meningococcal vaccine footnotes and also to *MMWR* February 28, 2014 / 63(RR01);1–13, available at **http://www.cdc.gov/mmwr/PDF/rr/rr6301.pdf**.

Catch-up vaccination:

- If dose 1 was administered at ages 12 through 14 months, administer a second (final) dose at least 8 weeks after dose 1, regardless of Hib vaccine used in the primary series.
- If both doses were PRP-OMP (PedvaxHIB or COMVAX), and were administered before the first birthday, the third (and final) dose should be administered at age 12 through 59 months and at least 8 weeks after the second dose.
- If the first dose was administered at age 7 through 11 months, administer the second dose at least 4 weeks later and a third (and final) dose at age 12 through 15 months or 8 weeks after second dose, whichever is later.
- If first dose is administered before the first birthday and second dose administered at younger than 15 months, a third (and final) dose should be administered 8 weeks later.
- For unvaccinated children aged 15 months or older, administer only 1 dose.
- For other catch-up guidance, see Figure 2. For catch-up guidance related to MenHibrix, please see the meningococcal vaccine footnotes and also *MMWR* February 28, 2014 / 63(RR01);1–13, available at **http://www.cdc.gov/mmwr/PDF/rr/rr6301.pdf**.

Vaccination of persons with high-risk conditions:

- Children aged 12 through 59 months who are at increased risk for Hib disease, including chemotherapy recipients and those with anatomic or functional asplenia (including sickle cell disease), human immunodeficiency virus (HIV) infection, immunoglobulin deficiency, or early component complement deficiency, who have received either no doses or only 1 dose of Hib vaccine before 12 months of age, should receive 2 additional doses of Hib vaccine 8 weeks apart; children who received 2 or more doses of Hib vaccine before 12 months of age should receive 1 additional dose.
- For patients younger than 5 years of age undergoing chemotherapy or radiation treatment who received a Hib vaccine dose(s) within 14 days of starting therapy or during therapy, repeat the dose(s) at least 3 months following therapy completion.

4. *Haemophilus influenzae* type b (Hib) conjugate vaccine. (cont'd)

- Recipients of hematopoietic stem cell transplant (HSCT) should be revaccinated with a 3-dose regimen of Hib vaccine starting 6 to 12 months after successful transplant, regardless of vaccination history; doses should be administered at least 4 weeks apart.
- A single dose of any Hib-containing vaccine should be administered to unimmunized* children and adolescents 15 months of age and older undergoing an elective splenectomy; if possible, vaccine should be administered at least 14 days before procedure.
- Hib vaccine is not routinely recommended for patients 5 years or older. However, 1 dose of Hib vaccine should be administered to unimmunized* persons aged 5 years or older who have anatomic or functional asplenia (including sickle cell disease) and unvaccinated persons 5 through 18 years of age with HIV infection.

* *Patients who have not received a primary series and booster dose or at least 1 dose of Hib vaccine after 14 months of age are considered unimmunized.*

5. Pneumococcal vaccines. (Minimum age: 6 weeks for PCV13, 2 years for PPSV23)
Routine vaccination with PCV13:

- Administer a 4-dose series of PCV13 vaccine at ages 2, 4, and 6 months and at age 12 through 15 months.
- For children aged 14 through 59 months who have received an age-appropriate series of 7-valent PCV (PCV7), administer a single supplemental dose of 13-valent PCV (PCV13).

Catch-up vaccination with PCV13:

- Administer 1 dose of PCV13 to all healthy children aged 24 through 59 months who are not completely vaccinated for their age.
- For other catch-up guidance, see Figure 2.

Vaccination of persons with high-risk conditions with PCV13 and PPSV23:

- All recommended PCV13 doses should be administered prior to PPSV23 vaccination if possible.
- For children 2 through 5 years of age with any of the following conditions: chronic heart disease (particularly cyanotic congenital heart disease and cardiac failure); chronic lung disease (including asthma if treated with high-dose oral corticosteroid therapy); diabetes mellitus; cerebrospinal fluid leak; cochlear implant; sickle cell disease and other hemoglobinopathies; anatomic or functional asplenia; HIV infection; chronic renal failure; nephrotic syndrome; diseases associated with treatment with immunosuppressive drugs or radiation therapy, including malignant neoplasms, leukemias, lymphomas, and Hodgkin disease; solid organ transplantation; or congenital immunodeficiency:
  1. Administer 1 dose of PCV13 if any incomplete schedule of 3 doses of PCV (PCV7 and/or PCV13) were received previously.
  2. Administer 2 doses of PCV13 at least 8 weeks apart if unvaccinated or any incomplete schedule of fewer than 3 doses of PCV (PCV7 and/or PCV13) were received previously.
  3. Administer 1 supplemental dose of PCV13 if 4 doses of PCV7 or other age-appropriate complete PCV7 series was received previously.
  4. The minimum interval between doses of PCV (PCV7 or PCV13) is 8 weeks.
  5. For children with no history of PPSV23 vaccination, administer PPSV23 at least 8 weeks after the most recent dose of PCV13.
- For children aged 6 through 18 years who have cerebrospinal fluid leak; cochlear implant; sickle cell disease and other hemoglobinopathies; anatomic or functional asplenia; congenital or acquired immunodeficiencies; HIV infection; chronic renal failure; nephrotic syndrome; diseases associated with treatment with immunosuppressive drugs or radiation therapy, including malignant neoplasms, leukemias, lymphomas, and Hodgkin disease; generalized malignancy; solid organ transplantation; or multiple myeloma:
  1. If neither PCV13 nor PPSV23 has been received previously, administer 1 dose of PCV13 now and 1 dose of PPSV23 at least 8 weeks later.
  2. If PCV13 has been received previously but PPSV23 has not, administer 1 dose of PPSV23 at least 8 weeks after the most recent dose of PCV13.
  3. If PPSV23 has been received but PCV13 has not, administer 1 dose of PCV13 at least 8 weeks after the most recent dose of PPSV23.
- For children aged 6 through 18 years with chronic heart disease (particularly cyanotic congenital heart disease and cardiac failure), chronic lung disease (including asthma if treated with high-dose oral corticosteroid therapy), diabetes mellitus, alcoholism, or chronic liver disease, who have not received PPSV23, administer 1 dose of PPSV23. If PCV13 has been received previously, then PPSV23 should be administered at least 8 weeks after any prior PCV13 dose.
- A single revaccination with PPSV23 should be administered 5 years after the first dose to children with sickle cell disease or other hemoglobinopathies; anatomic or functional asplenia; congenital or acquired immunodeficiencies; HIV infection; chronic renal failure; nephrotic syndrome; diseases associated with treatment with immunosuppressive drugs or radiation therapy, including malignant neoplasms, leukemias, lymphomas, and Hodgkin disease; generalized malignancy; solid organ transplantation; or multiple myeloma.

6. Inactivated poliovirus vaccine (IPV). (Minimum age: 6 weeks)
Routine vaccination:

- Administer a 4-dose series of IPV at ages 2, 4, 6 through 18 months, and 4 through 6 years. The final dose in the series should be administered on or after the fourth birthday and at least 6 months after the previous dose.

Catch-up vaccination:

- In the first 6 months of life, minimum age and minimum intervals are only recommended if the person is at risk of imminent exposure to circulating poliovirus (i.e., travel to a polio-endemic region or during an outbreak).
- If 4 or more doses are administered before age 4 years, an additional dose should be administered at age 4 through 6 years and at least 6 months after the previous dose.
- A fourth dose is not necessary if the third dose was administered at age 4 years or older and at least 6 months after the previous dose.
- If both OPV and IPV were administered as part of a series, a total of 4 doses should be administered, regardless of the child's current age. If only OPV were administered, and all doses were given prior to 4 years of age, one dose of IPV should be given at 4 years or older, at least 4 weeks after the last OPV dose.
- IPV is not routinely recommended for U.S. residents aged 18 years or older.
- For other catch-up guidance, see Figure 2.

7. Influenza vaccines. (Minimum age: 6 months for inactivated influenza vaccine [IIV], 2 years for live, attenuated influenza vaccine [LAIV])
Routine vaccination:

- Administer influenza vaccine annually to all children beginning at age 6 months. For most healthy, nonpregnant persons aged 2 through 49 years, either LAIV or IIV may be used. However, LAIV should NOT be administered to some persons, including 1) persons who have experienced severe allergic reactions to LAIV, any of its components, or to a previous dose of any other influenza vaccine; 2) children 2 through 17 years receiving aspirin or aspirin-containing products; 3) persons who are allergic to eggs; 4) pregnant women; 5) immunosuppressed persons; 6) children 2 through 4 years of age with asthma or who had wheezing in the past 12 months; or 7) persons who have taken influenza antiviral medications in the previous 48 hours. For all other contraindications and precautions to use of LAIV, see *MMWR* August 7, 2015 / 64(30):818–25 available at **http://www.cdc.gov/mmwr/pdf/wk/mm6430.pdf.**

Figure 9.1. cont'd

Continued

7. **Influenza vaccines. (cont'd)**
For children aged 6 months through 8 years:
- For the 2015–16 season, administer 2 doses (separated by at least 4 weeks) to children who are receiving influenza vaccine for the first time. Some children in this age group who have been vaccinated previously will also need 2 doses. For additional guidance, follow dosing guidelines in the 2015–16 ACIP influenza vaccine recommendations, *MMWR* August 7, 2015 / 64(30):818–25, available at http://www.cdc.gov/mmwr/pdf/wk/mm6430.pdf.
- For the 2016–17 season, follow dosing guidelines in the 2016 ACIP influenza vaccine recommendations.

For persons aged 9 years and older:
- Administer 1 dose.

8. **Measles, mumps, and rubella (MMR) vaccine. (Minimum age: 12 months for routine vaccination)**
Routine vaccination:
- Administer a 2-dose series of MMR vaccine at ages 12 through 15 months and 4 through 6 years. The second dose may be administered before age 4 years, provided at least 4 weeks have elapsed since the first dose.
- Administer 1 dose of MMR vaccine to infants aged 6 through 11 months before departure from the United States for international travel. These children should be revaccinated with 2 doses of MMR vaccine, the first at age 12 through 15 months (12 months if the child remains in an area where disease risk is high), and the second dose at least 4 weeks later.
- Administer 2 doses of MMR vaccine to children aged 12 months and older before departure from the United States for international travel. The first dose should be administered on or after age 12 months and the second dose at least 4 weeks later.

Catch-up vaccination:
- Ensure that all school-aged children and adolescents have had 2 doses of MMR vaccine; the minimum interval between the 2 doses is 4 weeks.

9. **Varicella (VAR) vaccine. (Minimum age: 12 months)**
Routine vaccination:
- Administer a 2-dose series of VAR vaccine at ages 12 through 15 months and 4 through 6 years. The second dose may be administered before age 4 years, provided at least 3 months have elapsed since the first dose. If the second dose was administered at least 4 weeks after the first dose, it can be accepted as valid.

Catch-up vaccination:
- Ensure that all persons aged 7 through 18 years without evidence of immunity (see *MMWR* 2007 / 56 [No. RR-4], available at **http://www.cdc.gov/mmwr/pdf/rr/rr5604.pdf**) have 2 doses of varicella vaccine. For children aged 7 through 12 years, the recommended minimum interval between doses is 3 months (if the second dose was administered at least 4 weeks after the first dose, it can be accepted as valid); for persons aged 13 years and older, the minimum interval between doses is 4 weeks.

10. **Hepatitis A (HepA) vaccine. (Minimum age: 12 months)**
Routine vaccination:
- Initiate the 2-dose HepA vaccine series at 12 through 23 months; separate the 2 doses by 6 to 18 months.
- Children who have received 1 dose of HepA vaccine before age 24 months should receive a second dose 6 to 18 months after the first dose.
- For any person aged 2 years and older who has not already received the HepA vaccine series, 2 doses of HepA vaccine separated by 6 to 18 months may be administered if immunity against hepatitis A virus infection is desired.

Catch-up vaccination:
- The minimum interval between the 2 doses is 6 months.

Special populations:
- Administer 2 doses of HepA vaccine at least 6 months apart to previously unvaccinated persons who live in areas where vaccination programs target older children, or who are at increased risk for infection. This includes persons traveling to or working in countries that have high or intermediate endemicity of infection; men having sex with men; users of injection and non-injection illicit drugs; persons who work with HAV-infected primates or with HAV in a research laboratory; persons with clotting-factor disorders; persons with chronic liver disease; and persons who anticipate close personal contact (e.g., household or regular babysitting) with an international adoptee during the first 60 days after arrival in the United States from a country with high or intermediate endemicity. The first dose should be administered as soon as the adoption is planned, ideally 2 or more weeks before the arrival of the adoptee.

11. **Meningococcal vaccines. (Minimum age: 6 weeks for Hib-MenCY [MenHibrix], 9 months for MenACWY-D [Menactra], 2 months for MenACWY-CRM [Menveo], 10 years for serogroup B meningococcal [MenB] vaccines: MenB-4C [Bexsero] and MenB-FHbp [Trumenba])**
Routine vaccination:
- Administer a single dose of Menactra or Menveo vaccine at age 11 through 12 years, with a booster dose at age 16 years.
- Adolescents aged 11 through 18 years with human immunodeficiency virus (HIV) infection should receive a 2-dose primary series of Menactra or Menveo with at least 8 weeks between doses.
- For children aged 2 months through 18 years with high-risk conditions, see below.

Catch-up vaccination:
- Administer Menactra or Menveo vaccine at age 13 through 18 years if not previously vaccinated.
- If the first dose is administered at age 13 through 15 years, a booster dose should be administered at age 16 through 18 years with a minimum interval of at least 8 weeks between doses.
- If the first dose is administered at age 16 years or older, a booster dose is not needed.
- For other catch-up guidance, see Figure 2.

Clinical discretion:
- Young adults aged 16 through 23 years (preferred age range is 16 through 18 years) may be vaccinated with either a 2-dose series of Bexsero or a 3-dose series of Trumenba vaccine to provide short-term protection against most strains of serogroup B meningococcal disease. The two MenB vaccines are not interchangeable; the same vaccine product must be used for all doses.

Vaccination of persons with high-risk conditions and other persons at increased risk of disease:
Children with anatomic or functional asplenia (including sickle cell disease):
Meningococcal conjugate ACWY vaccines:
1. Menveo
   - *o Children who initiate vaccination at 8 weeks:* Administer doses at 2, 4, 6, and 12 months of age.
   - *o Unvaccinated children who initiate vaccination at 7 through 23 months:* Administer 2 doses, with the second dose at least 12 weeks after the first dose AND after the first birthday.
   - *o Children 24 months and older who have not received a complete series*: Administer 2 primary doses at least 8 weeks apart.
2. MenHibrix
   - *o Children who initiate vaccination at 6 weeks:* Administer doses at 2, 4, 6, and 12 through 15 months of age.
   - *o* If the first dose of MenHibrix is given at or after 12 months of age, a total of 2 doses should be given at least 8 weeks apart to ensure protection against serogroups C and Y meningococcal disease.
3. Menactra
   - *o Children 24 months and older who have not received a complete series:* Administer 2 primary doses at least 8 weeks apart. If Menactra is administered to a child with asplenia (including sickle cell disease), do not administer Menactra until 2 years of age and at least 4 weeks after the completion of all PCV13 doses.

Meningococcal B vaccines:
1. Bexsero or Trumenba
   - *o Persons 10 years or older who have not received a complete series.* Administer a 2-dose series of Bexsero, at least 1 month apart. Or a 3-dose series of Trumenba, with the second dose at least 2 months after the first and the third dose at least 6 months after the first. The two MenB vaccines are not interchangeable; the same vaccine product must be used for all doses.

Children with persistent complement component deficiency (includes persons with inherited or chronic deficiencies in C3, C5-9, properidin, factor D, factor H, or taking eculizumab (Soliris®):
Meningococcal conjugate ACWY vaccines:
1. Menveo
   - *o Children who initiate vaccination at 8 weeks:* Administer doses at 2, 4, 6, and 12 months of age.
   - *o Unvaccinated children who initiate vaccination at 7 through 23 months:* Administer 2 doses, with the second dose at least 12 weeks after the first dose AND after the first birthday.
   - *o Children 24 months and older who have not received a complete series*: Administer 2 primary doses at least 8 weeks apart.
2. MenHibrix
   - *o Children who initiate vaccination 6 weeks:* Administer doses at 2, 4, 6, and 12 through 15 months of age.
   - *o* If the first dose of MenHibrix is given at or after 12 months of age, a total of 2 doses should be given at least 8 weeks apart to ensure protection against serogroups C and Y meningococcal disease.
3. Menactra
   - *o Children 9 through 23 months*: Administer 2 primary doses at least 12 weeks apart.
   - *o Children 24 months and older who have not received a complete series*: Administer 2 primary doses at least 8 weeks apart.

Meningococcal B vaccines:
1. Bexsero or Trumenba
   - *o Persons 10 years or older who have not received a complete series*. Administer a 2-dose series of Bexsero, at least 1 month apart. Or a 3-dose series of Trumenba, with the second dose at least 2 months after the first and the third dose at least 6 months after the first. The two MenB vaccines are not interchangeable; the same vaccine product must be used for all doses.

For children who travel to or reside in countries in which meningococcal disease is hyperendemic or epidemic, including countries in the African meningitis belt or the Hajj
- administer an age-appropriate formulation and series of Menactra or Menveo for protection against serogroups A and W meningococcal disease. Prior receipt of MenHibrix is not sufficient for children traveling to the meningitis belt or the Hajj because it does not contain serogroups A or W.

For children at risk during a community outbreak attributable to a vaccine serogroup
- administer or complete an age- and formulation-appropriate series of MenHibrix, Menactra, or Menveo, Bexsero or Trumenba.

For booster doses among persons with high-risk conditions, refer to *MMWR* 2013 / 62(RR02);1–22, available at **http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6202a1.htm**.
For other catch-up recommendations for these persons, and complete information on use of meningococcal vaccines, including guidance related to vaccination of persons at increased risk of infection, see *MMWR* March 22, 2013 / 62(RR02);1–22, and *MMWR* October 23, 2015 / 64(41); 1171–1176 available at **http://www.cdc.gov/mmwr/pdf/rr/rr6202.pdf, and http://www.cdc.gov/mmwr/pdf/wk/mm6441.pdf.**

12. **Tetanus and diphtheria toxoids and acellular pertussis (Tdap) vaccine. (Minimum age: 10 years for both Boostrix and Adacel)**
Routine vaccination:
- Administer 1 dose of Tdap vaccine to all adolescents aged 11 through 12 years.
- Tdap may be administered regardless of the interval since the last tetanus and diphtheria toxoid-containing vaccine.
- Administer 1 dose of Tdap vaccine to pregnant adolescents during each pregnancy (preferred during 27 through 36 weeks gestation) regardless of time since prior Td or Tdap vaccination.

Catch-up vaccination:
- Persons aged 7 years and older who are not fully immunized with DTaP vaccine should receive Tdap vaccine as 1 (preferably the first) dose in the catch-up series; if additional doses are needed, use Td vaccine. For children 7 through 10 years who receive a dose of Tdap as part of the catch-up series, an adolescent Tdap vaccine dose at age 11 through 12 years should NOT be administered. Td should be administered instead 10 years after the Tdap dose.
- Persons aged 11 through 18 years who have not received Tdap vaccine should receive a dose followed by tetanus and diphtheria toxoids (Td) booster doses every 10 years thereafter.
- Inadvertent doses of DTaP vaccine:
  - – If administered inadvertently to a child aged 7 through 10 years may count as part of the catch-up series. This dose may count as the adolescent Tdap dose, or the child can later receive a Tdap booster dose at age 11 through 12 years.
  - – If administered inadvertently to an adolescent aged 11 through 18 years, the dose should be counted as the adolescent Tdap booster.
  - For other catch-up guidance, see Figure 2.

13. **Human papillomavirus (HPV) vaccines. (Minimum age: 9 years for 2vHPV [Cervarix], 4vHPV [Gardasil] and 9vHPV [Gardasil 9])**
Routine vaccination:
- Administer a 3-dose series of HPV vaccine on a schedule of 0, 1–2, and 6 months to all adolescents aged 11 through 12 years. 9vHPV, 4vHPV or 2vHPV may be used for females, and only 9vHPV or 4vHPV may be used for males.
- The vaccine series may be started at age 9 years.
- Administer the second dose 1 to 2 months after the first dose (minimum interval of 4 weeks); administer the third dose 16 weeks after the second dose (minimum interval of 12 weeks) and 24 weeks after the first dose.
- Administer HPV vaccine beginning at age 9 years to children and youth with any history of sexual abuse or assault who have not initiated or completed the 3-dose series.

Catch-up vaccination:
- Administer the vaccine series to females (2vHPV or 4vHPV or 9vHPV) and males (4vHPV or 9vHPV) at age 13 through 18 years if not previously vaccinated.
- Use recommended routine dosing intervals (see Routine vaccination above) for vaccine series catch-up.

Figure 9.1. cont'd

TABLE 9.2 Recommended and Minimum Ages and Intervals Between Vaccine Doses[a,b,c,d]

Vaccine and Dose Number	Recommended Age for This Dose	Minimum Age for This Dose	Recommended Interval to Next Dose	Minimum Interval to Next Dose
Diphtheria and tetanus toxoids and acellular pertussis (DTaP)-1[e]	2 mo	6 wk	8 wk	4 wk
DTaP-2	4 mo	10 wk	8 wk	4 wk
DTaP-3	6 mo	14 wk	6–12 mo[f]	6 mo[f]
DTaP-4	15–18 mo	15 mo	3 y	6 mo[e]
DTaP-5	4–6 y	4 y	—	—
Haemophilus influenzae type b (Hib)-1[e,g]	2 mo	6 wk	8 wk	4 wk
Hib-2	4 mo	10 wk	8 wk	4 wk
Hib-3[h]	6 mo	14 wk	6–9 mo	8 wk
Hib-4	12–15 mo	12 mo	—	—
Hepatitis A (HepA)-1[e]	12–23 mo	12 mo	6–18 mo	6 mo
HepA-2	≥ 18 mo	18 mo	—	—
Hepatitis B (HepB)-1	Birth	Birth	4 wk–4 mo	4 wk
HepB-2	1–2 mo	4 wk	8 wk–17 mo	8 wk
HepB-3[i]	6–18 mo	24 wk	—	—
Herpes zoster[j]	≥ 60 y	60 y	—	—
Human papillomavirus (HPV)-1[k]	11–12 y	9 y	8 wk	4 wk
HPV-2	11–12 y (+2 mo)	9 y (+4 wk)	4 mo	12 wk[r(l)]
HPV-3[l]	11–12 y (+6 mo)	9 y (+24 wk)	—	—
Inactivated poliovirus (IPV)-1[e]	2 mo	6 wk	8 wk	4 wk
IPV-2	4 mo	10 wk	8wk–14 mo	4 wk
IPV-3	6–18 mo	14 wk	3–5 y	6 mo
IPV-4[m]	4–6 y	4 y	—	—
Influenza, inactivated[n]	≥ 6 mo	6 mo[o]	4 wk	4 wk
Live, attenuated influenza vaccine (LAIV, intranasal)[n]	2–49 y	2 y	4 wk	4 wk
Measles, mumps, and rubella (MMR)-1[p]	12–15 mo	12 mo	3–5 y	4 wk
MMR-2[p]	4–6 y	13 mo	—	—
Pneumococcal conjugate vaccine (PCV)-1[g]	2 mo	6 wk	8 wk	4 wk
PCV-2	4 mo	10 wk	8 wk	4 wk
PCV-3	6 mo	14 wk	6 mo	8 wk
PCV-4	12–15 mo	12 mo	—	—
Pneumococcal polysaccharide vaccine (PPSV)-1	—	2 y	5 y	3 y
PPSV-2[q]	—	7 y	—	—
Quadrivalent meningococcal conjugate vaccine (MCV4)-1[r]	11–12 y	6 wk[s]	4–5 y	8 wk
MCV4–2	16 y	11 y (+8 wk)	—	—
Quadrivalent meningococcal polysaccharide vaccine (MPSV4)[r]	—	2 y	5 y	5 y
MPSV4–2	—	7 y	—	—
Rotavirus-1[t]	2 mo	6 wk	8 wk	4 wk
Rotavirus-2	4 mo	10 wk	8 wk	4 wk
Rotavirus-3[u]	6 mo	14 wk	—	—
Tetanus and diphtheria toxoids (Td)	11–12 y	7 y	10 y	5 y

Continued on following page

TABLE 9.2 Recommended and Minimum Ages and Intervals Between Vaccine Doses[a,b,c,d] *(Continued)*

Vaccine and Dose Number	Recommended Age for This Dose	Minimum Age for This Dose	Recommended Interval to Next Dose	Minimum Interval to Next Dose
Tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap)[v]	≥11 y	7 y	—	—
Varicella-1[p]	12–15 mo	12 mo	3–5 y	12 wk[w]
Varicella-2[p]	4–6 y	15 mo[x]	—	—

[a]Combination vaccines are available. Use of licensed combination vaccines is generally preferred to separate injections of their equivalent component vaccines. When administering combination vaccines, the minimum age for administration is the oldest age for any of the individual components (exception: the minimum age for the first dose of MenHibrix is 6 weeks); the minimum interval between doses is equal to the greatest interval of any of the individual components.

[b]Information on travel vaccines, including typhoid, Japanese encephalitis, and yellow fever, is available at http://www.cdc.gov/travel. Information on other vaccines that are licensed in the United States but not distributed, including anthrax and smallpox, is available at http://www.bt.cdc.gov.

[c]In the recommended interval and the minimum interval columns, "months" refers to calendar months.

[d]Within a number range, a dash refers to "through."

[e]Combination vaccines containing the HepB component are available (see Table 9.1). These vaccines should not be administered to infants <6 weeks because of the other components (i.e., Hib, DTaP, HepA, and IPV).

[f]The minimum recommended interval between DTaP-3 and DTaP-4 is 6 months. However, DTaP-4 need not be repeated if administered at least 4 months after DTaP-3. This is a special grace period of 2 months which can be used if evaluating records retrospectively. An additional 4 days should not be added to this grace period.

[g]For Hib and PCV, children receiving the first dose of vaccine at age ≥7 months require fewer doses to complete the series.

[h]If polyribosylribitol phosphate-meningococcal outer membrane protein conjugate (PRP-OMP; Pedvax-Hib, Merck Vaccine Division) was administered at ages 2 and 4 months, a dose at age 6 months is not necessary.

[i]HepB-3 should be administered at least 8 weeks after HepB-2 and at least 16 weeks after HepB-1 and should not be administered before age 24 weeks.

[j]Herpes zoster vaccine is recommended as a single dose for persons aged ≥60 years.

[k]Bivalent HPV vaccine is approved for females age 9–25 years. Quadrivalent HPV vaccine is approved for males and females age 9–26 years. Nine-valent HPV vaccine is approved for females age 9–26 years, and males age 9–15 years.

[l]The minimum age for HPV-3 is based on the baseline minimum age for the first dose (i.e., 9 years) and the minimum interval of 24 weeks between the first and third dose. Dose 3 need not be repeated if it is administered at least 16 weeks after the first dose and the intervals between dose 1 and dose 2, and dose 2 and dose 3, are maintained at 4 weeks and 12 weeks, respectively.

[m]A fourth dose is not needed if the third dose was administered at ≥4 years and at least 6 months after the previous dose.

[n]One dose of influenza vaccine per season is recommended for most persons. Please see influenza vaccine specific chapter to determine which children age <9 years should receive two doses in a single season.

[o]The minimum age for inactivated influenza vaccine varies by vaccine manufacturer. See package insert for vaccine-specific minimum ages.

[p]Combination MMRV vaccine can be used for children ages 12 months to 12 years.

[q]A second dose of PPSV 5 years after the first dose is recommended for persons age ≤65 years who are at highest risk for serious pneumococcal infection and for persons likely to have a rapid decline in pneumococcal antibody concentration. (Source: CDC. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices [ACIP]. *MMWR Recomm Rep.* 1997;46[RR-8]:1–24).

[r]Revaccination with meningococcal conjugate vaccine is recommended for previously vaccinated persons who remain at high risk for meningococcal disease. (Source: CDC. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for revaccination of persons at prolonged increased risk for meningococcal disease. *MMWR Morb Mortal Wkly Rep*. 2009;58:1042–1043).

[s]MenACWY-D (Menactra) can be given as young as 9 months of age for high-risk persons, MenACWY-CRM (Menveo) can be given as young as 2 months of age for high-risk persons. Hib-MenCY can be given as young as 6 weeks of age for high-risk persons. Hib-MenCY is a four-dose series at ages 2 months, 4 months, 6 months, and 12 to 18 months.

[t]The first dose of rotavirus must be administered at age 6 weeks through 14 weeks and 6 days. The vaccine series should not be started for infants age ≥15 weeks, 0 days. Rotavirus should not be administered to children older than 8 months, 0 days of age regardless of the number of doses received between 6 weeks and 8 months, 0 days of age.

[u]If two doses of Rotarix (GlaxoSmithKline) are administered as age appropriate, a third dose is not necessary.

[v]Only one dose of Tdap is recommended for nonpregnant persons. Subsequent doses should be given as Td. For management of a tetanus-prone wound in persons who have received a primary series of tetanus-toxoid–containing vaccine, the minimum interval after a previous dose of any tetanus-containing vaccine is 5 years.

[w]The minimum interval from varicella-1 to varicella-2 for persons beginning the series at age ≥13 years is 4 weeks. For persons younger than 13 years, a special grace period of 2 months can be applied to the minimum interval, so the minimum interval would also be 4 weeks for this age cohort. However, an additional 4 days should not be added to this grace period for this age cohort.

[x]A special grace period of 3 months, based on expert opinion, can be applied to the minimum age of 15 months when evaluating records retrospectively, which results in an acceptable minimum age of 13 months. An additional 4 days should not be added on to this grace period.

schedules has not been evaluated in clinical trials, immune responses with accelerated intervals are likely to induce adequate protection.[5] Table 9.2 lists accelerated, or minimum, intervals and ages that can be used for scheduling catchup vaccinations.

Administration of doses of a vaccine at intervals less than the minimum intervals or earlier than the minimum age (see Table 9.2) may result in a reduced immune response with diminished vaccine efficacy and should be avoided.[2,5] Multiple doses of some live vaccines are recommended to stimulate an immune response to different types of the same virus, such as poliovirus types 1, 2, and 3, or to induce immunity in persons who failed to mount an immune response to an earlier dose of vaccine, such as measles.[53,67] These multiple doses constitute a primary vaccination series and are not booster doses.

Spacing of Different Vaccines

Table 9.2 includes guidelines for spacing administration of different vaccines.[5] Inactivated vaccines are not known

to interfere with the immune response to other inactivated vaccines or to live vaccines (see below for exceptions). An inactivated vaccine can be administered simultaneously or at any time before or after a different inactivated vaccine or live vaccine.

The possibility that two doses of the same or different live virus vaccines administered within too short an interval may inhibit the immunologic response to the second dose is based on evidence from animal and human studies. Petralli and colleagues[68,69] reported that the immune response to smallpox vaccination was affected by prior administration of live attenuated measles vaccine. Interferon produced in response to the initial dose of measles virus vaccine has been postulated to inhibit replication of vaccinia virus in the subsequent vaccine dose. In a study in two U.S. health maintenance organizations, persons who received varicella vaccine less than 30 days after MMR vaccination had an increased risk of varicella vaccine failure (i.e., varicella disease in a vaccinated person) of 2.5-fold compared with persons who received varicella vaccine before or 30 days or more after MMR.[70] In contrast, Stefano and colleagues[71] determined that the response to yellow fever vaccine is not affected by monovalent measles vaccine administered 1 to 27 days earlier. In general, live virus vaccines administered orally do not interfere with live virus vaccines administered parenterally or orally, although a reduction in the seroconversion rate following the first dose of monovalent rotavirus vaccine has been reported when this dose is administered concurrently with OPV.[72] The effect of nasally administered vaccine on the response to other live vaccines not administered at the same visit is not known.

To minimize the potential risk for interference, parenterally or nasally administered live virus vaccines not administered on the same day should be administered 4 weeks or more apart whenever possible. If parenterally or nasally administered live virus vaccines are separated by less than 4 weeks, the live virus vaccine given second should be readministered 4 or more weeks after the previous dose.[5] Yellow fever vaccine can be administered at any time after single-antigen measles vaccine (single-antigen measles vaccine is not currently available in the United States). Oral and parenteral live virus vaccines can be administered simultaneously or at any interval before or after each other, if indicated.[5]

SIMULTANEOUS ADMINISTRATION OF DIFFERENT VACCINES

Simultaneous administration of all indicated vaccines is an essential component of childhood vaccination programs.[2,5] Simultaneous administration of different vaccines is particularly important when return of the recipient for further vaccination is uncertain, imminent exposure to several vaccine-preventable diseases is expected, or a vaccinee is preparing for international travel on short notice. The Strategic Advisory Group of Experts (SAGE) of the World Health Organization supports multiple vaccine injections in a single visit, and encourages this practice based on the benefits they confer.[73]

Unless specifically licensed for injection in the same syringe, different vaccines administered simultaneously should be injected separately and at different anatomic sites. If both upper and lower limbs must be used for simultaneous administration of different vaccines, the anterolateral thigh is often chosen for intramuscular injections and the triceps region for subcutaneous injections. If more than one injection must be administered in a single limb of an infant or young child, the thigh usually is preferred because of its large muscle mass. The distance separating two injections in the same limb should be sufficient (e.g., 1 inch or more) to minimize the chance of overlapping local reactions.[5,13,14] In general, different vaccines, including live virus products, can be administered simultaneously without reducing their safety and effectiveness (Table 9.3).[74] Studies of cortisol concentration and behavioral responses to vaccination indicate that responses are similar in infants who receive two injections during one visit and infants who receive a single injection, suggesting that a second injection does not increase stress.[76,77]

TABLE 9.3 Guidelines for Spacing of Live and Inactivated Antigens

Antigen Combination	Recommended Minimum Interval Between Doses
Two or more inactivated[a,b]	Can be administered simultaneously or at any interval between doses
Inactivated and live	Can be administered simultaneously or at any interval between doses
Two or more live intranasal or injectable[c]	4-wk minimum interval if not administered simultaneously

[a]The American Academy of Pediatrics suggests a 1-month interval between tetanus toxoid, reduced diphtheria toxoid, and reduced acellular pertussis vaccine and tetravalent meningococcal conjugate vaccine if these vaccines are not administered on the same day.[75]
[b]In persons with functional or anatomic asplenia, MenACWY-D (Menactra) and PCV13 should not be administered simultaneously and should be spaced by 4 weeks. Likewise, for persons with immunosuppressive high-risk conditions for invasive pneumococcal disease, if both PCV13 and PPSV23 are indicated, PCV13 should be administered first and PPSV23 should be administered 8 weeks later. For persons with immunocompetent high-risk conditions for invasive pneumococcal disease and healthy persons aged ≥65 years indicated for both PCV13 and PPSV23, PCV13 should be administered first, and PPSV23 should be administered 1 year later.
[c]Live oral vaccines (e.g., Ty21a typhoid vaccine and rotavirus vaccine) can be administered on the same day or at any interval before or after inactivated or live injectable vaccines.

Increased severity or incidence of adverse reactions has not been observed after simultaneous administration of the most widely used vaccines.[5] Similarly, simultaneous administration of vaccines generally does not cause immunologic interference, with two exceptions. Pneumococcal conjugate vaccine and Menactra brand meningococcal conjugate vaccine should not be administered simultaneously in children with functional or anatomic asplenia, because Menactra interferes with the immune response to the seven-valent pneumococcal conjugate vaccine (and presumably PCV13 as well). Children with functional or anatomic asplenia are at high risk for pneumococcal infection. All doses of pneumococcal conjugate vaccine should be administered first and then a dose of Menactra should be administered 4 weeks after the last dose of pneumococcal conjugate vaccine. Interference is not known to occur with other brands of meningococcal conjugate vaccine and pneumococcal conjugate vaccine. Pneumococcal polysaccharide vaccine may interfere with the immune response to pneumococcal conjugate vaccines. If both doses are indicated, they should not be administered simultaneously. For persons with immunosuppressive high-risk conditions indicated for both PCV13 and PPSV23, PCV13 should be administered first, with PPSV23 administered 8 weeks later. For persons with immunocompetent high-risk conditions for invasive pneumococcal disease or for healthy persons aged 65 years or older indicated for both PCV13 and PPSV23, PCV13 should be administered first, with PPSV23 administered 1 year later. Pneumococcal conjugate vaccine should be administered first, followed by pneumococcal polysaccharide vaccine at least 8 weeks later.[74,77,78]

INTERFERENCE BY IMMUNOGLOBULINS

Passively acquired antibodies can interfere with the immune response to certain vaccines, both live and inactivated, and to toxoids. The result can be the absence of seroconversion or a blunting of the immune response with lower final antibody concentrations in the vaccinee. This potential for interference underlies the recommendation that hepatitis B vaccine and hepatitis B immunoglobulin be administered at separate sites when administered simultaneously. Passively acquired antibody does not affect the immune response to all vaccines.

Interference With Live Virus Vaccines

To elicit an adequate immune response, live vaccine virus must replicate in the recipient. The probable mechanism by which passively acquired immunoglobulin blunts the immune response is neutralization of vaccine virus, resulting in inhibition of viral replication and insufficient antigenic mass.[79] For example, persisting transplacentally acquired maternal measles antibodies inhibit the response to live measles vaccine in infants for as long as 12 months and perhaps longer.[80,81] The age to which inhibition persists has been correlated with concentrations of maternal or cord blood antibodies.[82–84] Rubella vaccine virus may be less susceptible than measles vaccine virus to these transplacentally acquired maternal antibodies.[83,85] The effect of blood and immunoglobulin preparations on the response to mumps and varicella vaccines is unknown, but commercial immunoglobulin preparations contain antibodies to these viruses. The effect of blood and immunoglobulin preparations on the response to live rotavirus and influenza vaccines is unknown.

Intramuscular or intravenous administration of immunoglobulin–containing preparations (e.g., immunoglobulin, hyperimmunoglobulins, intravenous immunoglobulin, and blood) before or simultaneously with certain vaccines also can affect the immune response to live virus vaccines. When partially attenuated Edmonston B measles vaccine, which is no longer available in the United States, was administered concurrently with measles immunoglobulin in an effort to reduce the incidence of adverse events associated with this vaccine, the rate of seroconversion was not affected but the geometric mean titer of serum measles antibody was diminished.[86] In a study of an investigational bacterial polysaccharide immunoglobulin (BPIG), children had a reduced immune response to live measles vaccine for as long as 5 months after receipt of BPIG.[87] The measles antibody seroconversion rate and geometric mean titer were lower among children who received BPIG compared with children who received placebo. Blunting of the immune response to live rubella vaccine also occurred after receipt of BPIG but was less marked and of shorter duration.

Although passively acquired antibodies can interfere with the response to rubella vaccine, the low dose of anti-Rh(D) globulin administered to postpartum women has not been demonstrated to inhibit the immune response to RA27/3 strain rubella vaccine.[88] Parenterally administered immunoglobulin preparations also do not seem to adversely affect the immune response to yellow fever vaccine.[89] Although high concentrations of passively acquired antibodies may reduce the serum antibody response to live poliovirus vaccine, they have little effect on replication of vaccine virus and development of gastrointestinal tract immunity.[55,89–91] Data are insufficient to determine the extent to which passively acquired antibodies interfere with the immune response to other live viral or bacterial vaccines, such as varicella, mumps, LAIV, zoster, and typhoid (Ty21a strain). A humanized mouse monoclonal antibody product (palivizumab) is available for prevention of respiratory syncytial virus infection among infants and young children. This product contains only antibody to respiratory syncytial virus; hence, it will not interfere with immune response to live vaccines.[92]

Interference With Inactivated and Component Vaccines

Interference with current inactivated and component vaccines is less marked than with live vaccines and requires exposure to large doses of passively acquired antibodies.[93] The mechanism by which passively acquired antibodies interfere with the immunologic response to inactivated and toxoid vaccines is not clear. Moderate doses of parenterally administered immunoglobulins have not inhibited development of a protective immune response to DTP, tetanus toxoid, and Hib conjugate vaccines.[94,95] Although the concurrent administration of inactivated hepatitis A vaccine and immunoglobulin can result in lower serum antibody concentrations than if vaccine alone is administered, seroconversion rates have not been diminished.[96,97] Infants with high concentrations of passively acquired maternal antibody to hepatitis A virus had lower serum antibody concentrations after receipt of hepatitis A vaccine but had seroconversion rates similar to those of vaccinated infants without maternal antibodies.[98] Similarly, seroconversion rates to inactivated polio vaccine given on a 2-, 4-, and 6-month schedule were lower among infants who had high maternal antibody levels for all three poliovirus types.[99]

RECOMMENDATIONS FOR SPACING ADMINISTRATION OF VACCINES AND IMMUNOGLOBULINS

Interference of immunoglobulins with the immune response to vaccines is dose-related and more likely to occur and to persist for a longer period after receipt of larger doses of immunoglobulins.[87,100] The recommended interval between administration of immunoglobulin preparations and vaccines is based on whether evidence suggests interference between immunoglobulin and the vaccine, the dose of the immunoglobulin administered, and the expected half-life of immunoglobulin G. Tables 9.4 and 9.5 list the recommended intervals between administration of immunoglobulin preparations and various live and killed vaccines.

In the United States, inactivated and component (subunit) vaccines may be administered simultaneously with or at any time before or after receipt of an immunoglobulin preparation.[2,5] The vaccine and immunoglobulin preparation should be administered at different sites, and the standard recommended doses of the corresponding vaccines should be given. Supplemental doses are not indicated.

Recommendations for administration of live virus vaccines vary on the basis of the aforementioned considerations. After receipt of an immunoglobulin preparation or other blood product, measles vaccine should be deferred during the intervals listed in Table 9.5.[5,91,101] Human blood and immunoglobulin preparations also contain rubella, mumps, and varicella antibodies. High doses of passively acquired antibodies can inhibit the immune response to live rubella vaccine for as long as 3 months.[87,102] The effect of immunoglobulin preparations on the response to live mumps and live varicella vaccines has not been defined. To reduce the possibility of interference, postponement of administration of rubella,

TABLE 9.4 Guidelines for Administering Antibody-Containing Products[a] and Vaccines

Simultaneous Administration (During Same Clinic Day)	
Products	**Recommended Minimum Interval Between Doses**
Antibody-containing products and inactivated antigen[b]	Can be administered simultaneously at different anatomic sites or at any interval between doses
Antibody-containing products and live antigen	Should not be administered simultaneously.[c] If simultaneous administration of measles-containing vaccine or varicella vaccine is unavoidable, administer at different sites and revaccinate or test for seroconversion after the recommended interval (see Table 9.5)

Nonsimultaneous Administration Products		
Administer First	**Administer Second**	**Recommended Minimum Interval Between Doses**
Antibody-containing products	Inactivated antigen	No interval necessary
Inactivated antigen	Antibody-containing products	No interval necessary
Antibody-containing products	Measles, mumps, rubella vaccine, varicella vaccine, and combined measles, mumps, rubella, varicella vaccine	Depends on dose of antibody received[c,d]
Measles, mumps, rubella, varicella vaccine, and combined measles, mumps, rubella, varicella vaccine antigens	Antibody-containing products	2 wk[c]

[a]Blood products containing substantial amounts of immunoglobulin include intramuscular and intravenous immunoglobulin, specific hyperimmunoglobulin (e.g., hepatitis B immunoglobulin, tetanus immunoglobulin, varicella zoster immunoglobulin, and rabies immunoglobulin), whole blood, packed red blood cells, plasma, and platelet products. Pneumococcal polysaccharide and pneumococcal conjugate vaccines should be separated by at least 8 weeks in high-risk immunosuppressed persons and by 1 year in healthy or immunocompetent high-risk persons. In persons with asplenia, pneumococcal conjugate vaccine and Menactra brand meningococcal conjugate vaccine should not be given simultaneously. The series of PCV13 should be administered first, with Menactra administered 4 weeks after the final dose of PCV13.
[b]Hepatitis B immunoglobulin (HBIg) and hepatitis B vaccine need to be administered at separate sites.
[c]Yellow fever vaccine, rotavirus vaccine, oral Ty21a typhoid vaccine, live attenuated influenza vaccine, and zoster vaccine are exceptions to these recommendations. These live attenuated vaccines can be administered at any time before or after or simultaneously with an antibody-containing product.
[d]The duration of interference of antibody-containing products with the immune response to the measles component of measles-containing vaccine, and possibly varicella vaccine, is dose-related (see Table 9.5).

mumps, and varicella vaccines for the intervals indicated in Table 9.5 is prudent.[5]

Immunoglobulin preparations administered too soon after vaccination with MMR or varicella vaccines can interfere with the immune response. If administration of an immunoglobulin preparation becomes necessary less than 2 weeks after receipt of MMR, its component vaccines, or varicella vaccine, readministration of the vaccine is recommended after the appropriate interval listed in Tables 9.4 and 9.5, unless serologic testing indicates an antibody response.[5,53] For example, if whole blood is administered less than 14 days after receipt of varicella vaccine, the vaccine should be readministered at least 6 months after the whole blood unless serologic testing indicates an adequate immune response to the initial dose of varicella vaccine.

Although data are not available on the effect of passive antibody on the response to rotavirus vaccine, the ACIP recommends that live rotavirus vaccine may be administered at any time before, concurrent with, or after administration of any blood product, including antibody-containing products.[27] Because the immune responses to OPV, zoster, and yellow fever vaccines have not been demonstrated to be adversely affected by immunoglobulin preparations, these vaccines can be administered at any time in relation to receipt of immunoglobulin preparations.[89] Live oral typhoid (Ty21a) vaccine also is recommended for administration irrespective of the receipt of immunoglobulin preparations.[5,103] LAIV can be administered at any time before or after receipt of an antibody-containing blood product.[5]

INTERCHANGEABILITY OF VACCINES FROM DIFFERENT MANUFACTURERS

Combination and monovalent vaccines against the same diseases with similar antigens and produced by the same manufacturer are considered interchangeable in most situations.[2,5] However, supporting data on the safety, immunogenicity, and efficacy of using comparable vaccines from different manufacturers for different doses of a vaccination series frequently are limited or unavailable. When the same vaccine cannot be used to complete an immunization series, similar vaccines produced by different manufacturers or produced by the same manufacturer in different countries generally have been considered acceptable to complete the immunization series provided each vaccine is given according to licensed recommendations.

Some diseases have serologic correlates of immunity that can be used to evaluate vaccine interchangeability. For example, in studies in which one or more doses of hepatitis B vaccine produced by one manufacturer were followed by doses from another manufacturer, the immune response was comparable to that resulting from use of a single vaccine type.[104–106] Whereas Hib conjugate vaccines differ in antigen composition, interchangeability of different products has been validated on the basis of the accepted serologic correlate of immunity against Hib invasive disease.[107–109]

Determination of vaccine interchangeability is more difficult for diseases without serologic correlates of immunity. When feasible, acellular pertussis vaccine from the same

TABLE 9.5 Recommended Intervals Between Administration of Antibody-Containing Products and Measles- or Varicella-Containing Vaccine, By-product and Indication for Vaccination

Product/Indication	Dose (Including Milligrams of Immunoglobulin G/kg of Body Weight) and Route[a]	Recommended Interval Before Measles- or Varicella-Containing Vaccine[b] Administration (Mo)
Blood transfusion		
Red blood cells (RBCs), washed	10 mL/kg (negligible) IV	None
RBCs, adenine-saline added	10 mL/kg (10 mg IgG/kg) IV	3
Packed RBCs (hematocrit 65%)[c]	10 mL/kg (60 mg IgG/kg) IV	6
Whole blood (hematocrit 35%–50%)[c]	10 mL/kg (80–100 mg IgG/kg) IV	6
Plasma/platelet products	10 mL/kg (160 mg IgG/kg) IV	7
Botulinum immunoglobulin intravenous (human)	1.5 mL/kg (75 mg IgG/kg) IV	6
Cytomegalovirus intravenous immunoglobulin (IgIV)	150 mg/kg maximum	6
Hepatitis A Ig		
Contact prophylaxis	0.02 mL/kg (3.3 mg IgG/kg) IM	3
International travel, <3 mo stay	0.02 mL/kg (3.3 mg IgG/kg) IM	3
International travel, ≥3 mo stay	0.06 mL/kg (10 mg IgG/kg) IM	3
Hepatitis B Ig	0.06 mL/kg (10 mg IgG/kg) IM	3
IGIV		
Replacement therapy for immune deficiencies[d]	300–400 mg/kg IV[d]	8
Immune thrombocytopenic purpura treatment	400 mg/kg IV	8
Postexposure varicella prophylaxis[e]	400 mg/kg IV	8
Postexposure measles prophylaxis for immunocompromised contacts	400 mg/kg IV	8
Immune thrombocytopenic purpura treatment	1000 mg/kg IV	10
Kawasaki disease	2 g/kg IV	11
Measles prophylaxis Ig, standard (i.e., nonimmunocompromised contact)	0.50 mL/kg (80 mg IgG/kg) IM	6
Monoclonal antibody to respiratory syncytial virus F protein (e.g., Synagis [MedImmune])[f]	15 mg/kg IM	None
Rabies Ig	20 IU/kg (22 mg IgG/kg) IM	4
Tetanus Ig	250 U (10 mg IgG/kg) IM	3
Varicella Ig (VariZIG)	125 U/10 kg (60–200 mg IgG/kg) IM, maximum 625 U	5

[a]This table is not intended for determining the correct indications and dosages for using antibody-containing products. Unvaccinated persons might not be protected fully against measles during the entire recommended interval, and additional doses of Ig or measles vaccine might be indicated after measles exposure. Concentrations of measles antibody in an Ig preparation can vary by manufacturer's lot. Rates of antibody clearance after receipt of an Ig preparation also might vary. Recommended intervals are extrapolated from an estimated half-life of 30 days for passively acquired antibody and an observed interference with the immune response to measles vaccine for 5 months after a dose of 80 mg IgG/kg.
[b]Does not include zoster vaccine. Zoster vaccine may be given with antibody-containing blood products.
[c]Assumes a serum IgG concentration of 16 mg/mL.
[d]Measles vaccination is recommended for children with mild immunosuppression from HIV infection, and varicella vaccination may be considered for children with mild immunosuppression, but both are contraindicated for persons with severe immunosuppression from HIV or any other immunosuppressive disorder.
[e]Licensed VariZIG, similar to licensed varicella-zoster immunoglobulin (VZIG), is a purified human Ig preparation made from plasma containing high levels of antivaricella antibodies (IgG).
[f]Contains antibody only to respiratory syncytial virus.

manufacturer is preferred for the entire primary vaccination series.[58] In the absence of a correlate for *Bordetella pertussis* infection, interchangeability of acellular pertussis vaccines is difficult to assess. Available data from one study indicate that, for the first three doses of the DTaP series, one or two doses of Tripedia (manufactured by Aventis Pasteur) followed by Infanrix (manufactured by GlaxoSmithKline) for the remaining doses(s) is comparable to three doses of Tripedia with regard to immunogenicity, as measured by antibodies to diphtheria, tetanus, and pertussis toxoids and filamentous hemagglutinin.[110] However, in the absence of a clear serologic correlate of protection for pertussis, the relevance of these immunogenicity data for protection against pertussis is unknown. Any DTaP vaccine can be used to complete the DTaP series if the

product(s) administered for earlier doses are unknown or unavailable.[5,58,111]

HYPERSENSITIVITY TO VACCINE COMPONENTS

Types of Reactions

Hypersensitivity reactions after vaccination can be local or systemic and can vary in severity from mild discomfort at the site of vaccination to severe anaphylaxis. Onset can be immediate or delayed. Serious allergic reactions are rare. Whether a specific hypersensitivity reaction is caused by a vaccine component or an unrelated environmental allergen can be difficult to determine. However, symptoms occurring immediately after vaccination that are suggestive of an anaphylactic reaction generally contraindicate further administration of that vaccine to the recipient.[2,5]

Urticaria and anaphylactic reactions have been reported after administration of DTP, DT (diphtheria and tetanus toxoids–pediatric), Td (diphtheria and tetanus toxoids–adult), and tetanus toxoid.[112–114] A severe allergic reaction (e.g., anaphylaxis) to a previous dose of vaccine or to a vaccine component is a contraindication to the respective vaccine. Although immunoglobulin E–type antibodies to tetanus and diphtheria antigens have been identified in some patients with these symptoms, transient urticaria-like rashes are not a contraindication to subsequent vaccination because they are unlikely to be anaphylactic unless they appear within minutes after vaccination.[114–116] A serum sickness–type reaction caused by circulating complexes of vaccine antigen and previously acquired antibody is the probable cause of these reactions, and subsequent vaccination at a 10-year interval is unlikely to result in the necessary ratio of antigen-to-antibody concentration to form immune complexes.[114,117]

Tetanus toxoid is contraindicated in persons who experienced an immediate anaphylactic reaction to tetanus toxoid–containing vaccine, unless the person can be desensitized to the toxoid.[2] Because of the importance of tetanus immunization and the uncertainty about which vaccine component might be the cause of the reaction, the patient may be referred to an allergist for evaluation and possible desensitization.[114,118,119] On occasion, a history of an allergic reaction to tetanus vaccine may refer to a reaction to tetanus antitoxin of equine origin given for tetanus prophylaxis before human-derived tetanus immunoglobulin became available in the 1960s. Before use of tetanus toxoid is discontinued because of an alleged episode of anaphylaxis, skin testing and possible desensitization should be considered.[118,120]

Urticaria also has been reported following pneumococcal conjugate, MMR, varicella, and smallpox vaccines.[121–124]

Immediate or delayed onset of generalized urticaria and angioedema that can progress to respiratory distress and hypotension has been reported after receipt of inactivated mouse brain-derived Japanese encephalitis vaccine.[125–127] The pathogenesis of these reactions is not known. Mouse brain–derived Japanese encephalitis vaccine is no longer available in the United States. A vero cell–derived Japanese encephalitis vaccine is available in the United States for people 2 years of age and older.[128,129]

VACCINE COMPONENTS CAUSING HYPERSENSITIVITY

Proteins

Egg protein is a constituent of vaccines prepared with use of embryonated chicken eggs, such as influenza and yellow fever vaccines. On rare occasions, these vaccines can induce anaphylaxis or other immediate hypersensitivity reactions, and these reactions are sometimes attributed to egg protein antigen.[2,5,130–132] As a result, yellow fever vaccine generally is contraindicated in persons with a history of anaphylactic reactions to egg ingestion unless desensitization has been successfully completed. For example, persons needing yellow fever vaccine who have a history of systemic anaphylaxis-like symptoms after egg ingestion can be skin tested with yellow fever vaccine before vaccination and desensitized if necessary.[130]

While a history of anaphylaxis following influenza vaccine is a contraindication to further doses of influenza vaccine, evidence does not support allergy to egg as a cause of anaphylaxis following influenza vaccine. A history of allergy to eggs is not a contraindication to vaccination with influenza vaccine; however, influenza vaccination should only occur under the supervision of a provider who is able to recognize and manage severe allergic conditions. Measles and mumps vaccines are produced in chick embryo fibroblast cell culture. Persons with hypersensitivity to eggs are at low risk for anaphylactic reactions to these vaccines, and skin testing with vaccine is not predictive of allergic reaction after immunization.[2,133–135] Neither skin testing nor administration of gradually increasing doses of vaccine is required when these vaccines are administered to persons who are allergic to eggs.[2,5,53,101,133]

Live virus vaccines, such as measles, mumps, rubella, yellow fever, and varicella, contain gelatin as a stabilizer. Persons with a history of allergy to gelatin have experienced, on rare occasions, an anaphylactic reaction after vaccination with such a vaccine.[121,124,134–136] Skin testing of persons with a history of systemic anaphylaxis-like symptoms after gelatin ingestion may be useful to identify persons at risk for severe hypersensitivity reactions to vaccination. A regimen for administering particular vaccines to persons with anaphylaxis to components contained in those vaccines has been published.[137] Because gelatin used as a vaccine stabilizer may be of porcine origin, whereas ingested food gelatin may be of bovine origin, absence of a history of allergy to gelatin-containing foods does not eliminate the possibility of a gelatin-mediated reaction to vaccine.

Approximately 6% of persons who receive a booster dose of human diploid rabies vaccine have a serum sickness–type illness.[138,139] This reaction is thought to be caused by sensitization to human albumin that has been altered chemically by a virus-inactivating agent used in the production of the vaccine.[2,140]

Anaphylaxis following recombinant hepatitis B vaccines rarely has been reported and usually is attributed to hypersensitivity to residual yeast protein in the vaccine.[141]

Latex

Latex is liquid sap from the commercial rubber tree. Latex contains naturally occurring impurities (e.g., plant proteins and peptides), which are believed to be responsible for allergic reactions. Latex is processed to form natural rubber latex and dry natural rubber. Natural rubber latex and dry natural rubber might contain the same plant impurities as latex but in lesser amounts. Natural rubber latex is used to produce medical gloves, catheters, and other products, whereas dry natural rubber is used in syringe plungers, vial stoppers, and injection ports on intravascular tubing. Synthetic rubber and synthetic latex also are used in medical gloves, syringe plungers, and vial stoppers, but they do not contain natural rubber or natural latex or the impurities linked to allergic reactions.

The most common type of latex sensitivity is contact-type (type IV) allergy, usually as a result of prolonged contact with

natural rubber latex–containing gloves.[142] Although injection procedure–associated latex allergies among patients with diabetes mellitus have been described,[143–145] allergic reactions, including anaphylaxis, after vaccination procedures are rare. Only one report of an allergic reaction after administering hepatitis B vaccine in a patient with known severe allergy (anaphylaxis) to latex has been published.[146]

If a person reports a severe (anaphylactic) allergy to latex, vaccines supplied in vials or syringes that contain natural rubber should not be administered, unless the benefit of vaccination outweighs the risk of an allergic reaction to the vaccine. For latex allergies other than anaphylactic allergies, such as a history of contact allergy to latex gloves, vaccines supplied in vials or syringes that contain natural rubber latex or dry natural rubber can be administered.[5]

Antimicrobial Agents

Live virus vaccines may contain trace amounts of one or more antimicrobial agents, such as neomycin, streptomycin, and polymyxin B. Vaccine contents are listed in each manufacturer's product label for each vaccine. The most common allergic response to neomycin is a delayed-type (cell-mediated) local contact dermatitis consisting of an erythematous, pruritic papule that occurs 48 to 96 hours after vaccine administration.[2,5,147] Such delayed-type reactions are not contraindications for vaccination.[2,5,147,148] However, persons who have experienced an anaphylactic reaction to neomycin or to another vaccine constituent should not receive vaccines containing that antimicrobial agent.[2,5,149,150] No vaccines licensed in the United States contain penicillin or penicillin derivatives.

Thimerosal

Thimerosal is an organic mercurial compound in use since the 1930s and added to certain immunobiologic products as a preservative. A joint statement issued by the U.S. Public Health Service and the AAP in 1999[151] established the goal of removing thimerosal as soon as possible from vaccines routinely recommended for infants. Although no evidence exists of any harm caused by low concentrations of thimerosal in vaccines and the risk was only theoretical,[152] this goal was established as a precautionary measure.

Since mid-2001, vaccines produced in the United States that are recommended routinely for infants younger than 6 months of age have been manufactured without thimerosal as a preservative and contain no thimerosal or only trace amounts. Thimerosal as a preservative is present in certain other vaccines. Examples are tetanus toxoid, Td, DT, certain formulations of influenza vaccine, and meningococcal polysaccharide vaccine in multidose vials.[153] Formulations of influenza vaccine with a reduced concentration of thimerosal or no thimerosal as a preservative are available in the United States.[153]

Receiving thimerosal-containing vaccines has been postulated to lead to induction of allergy in some persons.[154,155] However, there is limited scientific evidence for this assertion. Hypersensitivity to thimerosal usually consists of local delayed-type hypersensitivity reactions.[156–158] Thimerosal elicits positive delayed-type hypersensitivity patch tests in 1% to 18% of persons tested, but these tests have limited or no clinical relevance.[159,160] Most patients do not experience reactions to thimerosal administered as a component of vaccines, even when patch or intradermal tests for thimerosal indicate hypersensitivity.[156,160] A localized or delayed-type hypersensitivity reaction to thimerosal is not a contraindication to receipt of a vaccine that contains thimerosal.[2,5]

MANAGEMENT OF ACUTE VACCINE ADVERSE REACTIONS

Although rare after vaccination, the immediate onset and life-threatening nature of an anaphylactic reaction require that personnel and facilities providing vaccination be capable of providing initial care for suspected anaphylaxis. Epinephrine and equipment for maintaining an airway should be available for immediate use.

Anaphylaxis usually begins within several minutes of administration of vaccine. Rapid recognition and initiation of treatment are required to prevent possible progression to cardiovascular collapse. If flushing, facial edema, urticaria, itching, swelling of the mouth or throat, wheezing, difficulty breathing, or other signs of anaphylaxis occur, the patient should be placed in a recumbent position with the legs elevated. It is important to note that urticaria might not be present in all cases of anaphylaxis. Aqueous epinephrine (1 : 1000) should be administered intramuscularly and can be repeated within 5 to 15 minutes.[161] A dose of diphenhydramine hydrochloride may shorten the reaction, but it will have little immediate effect. Maintenance of an airway and oxygen administration may be necessary. Arrangements should be made for immediate transfer to an emergency facility for further evaluation and treatment. All patients should be observed for 4 to 24 hours after onset of symptoms.[161]

Syncope (vasovagal or vasodepressor reaction) can occur after vaccination and is most common among adolescents and young adults. In 2005, the Vaccine Adverse Event Reporting System (VAERS) detected a trend of increasing syncope reports that coincided with licensure of three vaccines for adolescents: HPV, quadrivalent meningococcal vaccine (MCV4), and Tdap.[162] Of particular concern among adolescents has been the risk for serious secondary injuries, including skull fracture and cerebral hemorrhage after a fall and subsequent head injury. Of 463 VAERS reports of syncope between January 1, 2005 and July 31, 2007, 41 listed syncope with secondary injury with information on the timing after vaccination, and most of these syncope reports (76%) occurred among adolescents. Among all age groups, 80% of reported syncope episodes occur within 15 minutes of vaccine administration (additional information is available at http://www.cdc.gov/concerns/fainting.html). Providers should take appropriate measures to prevent injuries if a patient becomes weak or dizzy or loses consciousness. Adolescents and adults should be seated or lying down during vaccination. Vaccine providers, particularly when vaccinating adolescents, should consider observing patients (with patients seated or lying down) for 15 minutes after vaccination to decrease the risk for injury should they faint.[162] If syncope develops, patients should be observed until the symptoms resolve.

SPECIAL CONSIDERATIONS

Vaccination of Preterm Infants

The immune response to vaccination is a function of postnatal rather than gestational age.[163–165] Transplacentally acquired maternal antibody is present in lower concentrations and, thus, persists for a shorter interval in preterm infants than in gestationally mature infants.[164,166–168] Because preterm infants have less transplacentally acquired maternal antibody, inhibition of the immune response in preterm infants may be less than that in full-term infants.[164,169]

In most cases, infants born prematurely, regardless of birth weight, should be vaccinated at the same chronological age and according to the same schedule and precautions as full-term infants and children.[5] Birth weight and size are not factors in deciding whether to postpone routine vaccination

of a clinically stable preterm infant,[170–174] except for hepatitis B vaccine. The full recommended dose of each vaccine should be used. Divided or reduced doses are not recommended.[175]

Decreased seroconversion rates might occur among certain preterm infants with birth weights of less than 2000 g after administration of hepatitis B vaccine at birth.[176] However, by chronological age 1 month, all preterm infants, regardless of initial birth weight or gestational age, are likely to respond as adequately as older and larger infants.[177–180] Infants weighing less than 2000 g born to hepatitis B surface antigen (HBsAg)-negative mothers should receive the first dose of the hepatitis B vaccine series at chronological age 1 month or hospital discharge, if hospital discharge occurs when the infant is younger than 1 month of age. Low-birth-weight infants born to HBsAg-positive mothers should receive hepatitis B vaccine within 12 hours after birth. The initial vaccine dose should not be counted toward completion of the hepatitis B series, and three additional doses of hepatitis B vaccine should be administered, beginning when the infant is 1 to 2 months of age. For mothers with unknown HBsAg status, hepatitis B vaccine is recommended within 12 hours of birth, regardless of low-birth-weight status.

In addition to hepatitis B vaccines, hepatitis B immunoglobulin (HBIG) is recommended for infants whose mothers are HBsAg-positive or unknown. If the mother is HBsAg-positive, HBIG should be given within 12 hours of birth. If the mother is HBsAg-unknown, providers should first attempt to determine the mother's status. However, if the infant's birth weight is less than 2000 g, HBIG should be given within 12 hours of birth. If the infant's birth weight is 2000 g or greater, providers need to continue determining the mothers' status but should administer a dose of HBIG as soon as possible but before 7 days passes unless the mother is found to be HBsAg-negative.

Several studies suggest that the incidence of adverse events after vaccination of preterm infants is the same as or lower than that of full-term infants vaccinated at the same chronological age.[168,181,182] A temporal association between receipt of DTP and HibTITER Hib vaccine and a transient increase or recurrence of apnea in premature infants has been reported, although the significance of this finding is unclear.[183] Neither of these vaccines is currently available in the United States.

A preterm infant who is still hospitalized at 2 months of age can receive the vaccines routinely scheduled at that age. However, in countries in which OPV is used, IPV may be considered for hospitalized infants. Because poliovirus vaccine strains are excreted after receipt of OPV, IPV will decrease the risk of transmission of vaccine viruses in the hospital.[67,184] ACIP supports rotavirus vaccination of preterm infants according to the same schedule and precautions as full-term infants and under the following conditions: the infant's chronological age meets the age requirements for rotavirus vaccine (e.g., 6 weeks through 14 weeks and 6 days of age for dose 1), the infant is clinically stable, and the vaccine is administered at the time of discharge from the neonatal intensive care unit (NICU) or nursery or after discharge from the NICU or nursery. Although the lower level of maternal antibody to rotavirus in very preterm infants theoretically could increase the risk for adverse reactions from rotavirus vaccine, ACIP believes the benefits of vaccinating an infant when age-eligible, clinically stable, and no longer in the hospital outweigh the theoretic risks.[27]

BREASTFEEDING AND IMMUNIZATION

Neither inactivated nor live virus vaccines administered to a lactating mother or infant who is breastfeeding have adverse consequences.[2,5] Because inactivated and component vaccines do not multiply in the body, they pose no special risk for lactating women or their infants. Lactating women also may safely receive live virus vaccines, such as MMR, LAIV, OPV, and varicella without interruption of their breastfeeding schedule.[5,53,184,185] Although vaccines that contain attenuated live viruses or bacteria replicate in the vaccine recipient, most live vaccine strains are not known to be secreted in human milk. An exception is rubella vaccine virus, which has been detected in human milk and recovered from the nasopharynx and throat of some breastfed infants after maternal immunization.[186,187] In one study, transient seroconversion to rubella virus without evidence of clinical disease was noted in 25% of the breastfed infants.[186] Breastfed infants who acquired rubella vaccine virus and rubella-specific antibodies from human milk have a normal immune response to rubella vaccine administered at 15 to 18 months of age.[188]

Breastfeeding of infants does not adversely affect their development of a protective immune response and is not a contraindication for any routinely administered vaccine.[2,5] Live oral rotavirus vaccines are less immunogenic and efficacious among children in developing countries compared with middle income and industrialized countries. Higher titers of rotavirus immunoglobulin A and neutralizing activity in human milk consumed by infants at the time of immunization has been proposed as a cause of this reduced immunogenicity.[189] However, a clinical trial found no difference in the proportion of infants who seroconverted to rotavirus vaccine between those who were either breastfeed or not breastfeeding during the 30 minutes prior to and after each vaccine dose was administered.[190] Yellow fever vaccine should be avoided in breastfeeding women. Two cases (one confirmed, one probable) of yellow fever vaccine–associated acute neurotropic disease (YEL-AND) have been detected in infants whose mothers were vaccinated but were not vaccinated themselves. In both infants, vaccine virus was recovered from the cerebrospinal fluid (CSF) of the infant, but the exact mode of transmission was not precisely determined because vaccine was not recovered from human milk. However, when nursing mothers cannot avoid or postpone travel to areas endemic for yellow fever in which risk of acquisition is high, they should be vaccinated.[191] Compared with infants who are formula fed, breastfed infants may have an enhanced immune response to certain oral and parenteral vaccines, such as conjugate Hib vaccine, OPV, and DT.[192–194] However, the significance of such an effect is unclear.

VACCINATION DURING PREGNANCY

Risk for a developing fetus from vaccination of the mother during pregnancy primarily is theoretical. No evidence exists of risk from vaccinating pregnant women with inactivated virus or bacterial vaccines or toxoids.[195,196] Live vaccines pose a theoretical risk to the fetus. Benefits of vaccinating pregnant women usually outweigh potential risks when the likelihood of disease exposure is high, when infection would pose a risk to the mother or fetus, and when the vaccine is unlikely to cause harm.

Recommendations for vaccination during pregnancy can be found in the annual U.S. adult immunization schedule.[64] Pregnant women who are not immunized or only partially immunized against tetanus should complete the primary series.[197] The number of doses needed to complete the primary series should be administered. One of these doses should be Tdap. Tdap is optimally administered at 27 to 36 weeks' gestation. Women for whom the vaccine is indicated but who have not completed the recommended three-dose series during pregnancy should receive follow-up after delivery to ensure the series is completed. Use of pertussis vaccine in pregnant women may lead to transfer of higher levels of pertussis antibody across the placenta to the fetus and results in protection

from pertussis in the first few months of life when the disease is most severe.[198] Therefore, a dose of Tdap is recommended for pregnant women during every pregnancy, preferably during 27 to 36 weeks of gestation.[59]

Women in the second and third trimesters of pregnancy are at increased risk for hospitalization from influenza. Therefore, routine influenza vaccination is recommended for all women who will be pregnant (in any trimester) during influenza season, usually November through March in the United States.[28] Influenza vaccination of a pregnant woman also reduces the risk of medically-attended respiratory tract illness in the newborn.[199]

IPV can be administered to pregnant women who are at risk for exposure to wild-type poliovirus infection.[75,200] Pregnancy is not a contraindication to hepatitis B vaccine. Current vaccines contain noninfectious HBsAg and should cause no risk to the fetus.[201] Hepatitis A, pneumococcal polysaccharide, meningococcal conjugate, and meningococcal polysaccharide vaccines should be considered for women at increased risk for those infections.[202–206]

Pregnant women who must travel to areas where the risk for yellow fever is high should receive yellow fever vaccine because the limited theoretical risk from vaccination is substantially outweighed by the risk for yellow fever infection.[203,207] Pregnancy is a contraindication for smallpox (vaccinia), measles, mumps, rubella, and varicella-containing vaccines. Smallpox (vaccinia) vaccine is the only vaccine known to cause harm to a fetus when administered to a pregnant woman. In addition to the vaccinee herself, smallpox (vaccinia) vaccine should not be administered to a household contact of a pregnant woman. However, in a postevent setting, or smallpox emergency, pregnancy or having a household contact who is pregnant would not be considered contraindications, because of a different risk/benefit consideration. Although of theoretical concern, no cases of congenital rubella or varicella syndrome or abnormalities attributable to fetal infection have been observed among infants born to susceptible women who received rubella or varicella vaccines during pregnancy.[53,208] Because of the importance of protecting women of childbearing age against rubella and varicella, reasonable practices in any vaccination program include asking women if they are pregnant or might become pregnant in the next 4 weeks, not vaccinating women who state that they are pregnant, explaining the theoretical risk for the fetus if MMR or varicella vaccine were administered to a women who is pregnant, and counseling women who are vaccinated not to become pregnant during the 4 weeks after MMR or varicella vaccination.[53,208,209]

Routine pregnancy testing of women of childbearing age before administering a live virus vaccine is not recommended.[53] If vaccination of an unknowingly pregnant woman occurs or if she becomes pregnant within 4 weeks after MMR or varicella vaccination, she should be counseled about the theoretical basis of concern for the fetus; however, MMR or varicella vaccination during pregnancy should not be regarded as a reason to terminate pregnancy.[185,209]

All pregnant women should be evaluated for immunity to rubella and varicella and should be tested for the presence of HBsAg in every pregnancy.[53,185,201,210] Women susceptible to rubella and varicella should be vaccinated immediately after delivery or after the termination of the pregnancy. A woman found to be HBsAg-positive should be followed carefully to ensure that the infant receives HBIG and begins the hepatitis B vaccine series no later than 12 hours after birth and completes the recommended hepatitis B vaccine series on schedule.[201] No known risk exists for the fetus from passive immunization of pregnant women with immunoglobulin preparations.

VACCINATION OF HOUSEHOLD CONTACTS

Administration of live and inactivated vaccines to household members does not present a known hazard to pregnant women. Although transmission of varicella vaccine virus from a 12-month-old infant to his pregnant mother has been reported, no virus was detected in fetal tissue after an elective abortion.[211] Pregnancy of a household contact is not a contraindication to administration of varicella vaccine or any other vaccine in the childhood immunization schedule to a household member.[185,212]

VACCINES RECEIVED OUTSIDE THE UNITED STATES

The ability of a clinician to determine that a person is protected on the basis of country of origin and the person's records alone is limited. Vaccines administered outside the United States generally can be accepted as valid if the schedule was similar to that recommended in the United States (i.e., minimum ages and intervals; see Table 9.2). Only written documentation should be accepted as evidence of previous vaccination. Written records are more likely to predict protection if the vaccines, dates of administration, intervals between doses, and the person's age at the time of vaccination are comparable to U.S. recommendations.[5] Although vaccines with inadequate potency have been produced in other countries,[213,214] most vaccines used worldwide are produced with adequate quality control standards and are potent.

The number of American families adopting children from outside the United States has increased substantially in recent years.[215] Adopted children's birth countries often have immunization schedules that differ from the recommended childhood immunization schedule in the United States. Differences between schedules used in other countries include the vaccines administered, the recommended ages of administration, and the number and timing of doses (see Chapters 74 to 76). The ACIP has published guidelines for assessing and vaccinating international adoptees.[5] Children adopted in the United States from other countries should receive vaccines according to U.S.-recommended schedules.

Data are inconclusive regarding the extent to which an internationally adopted child's immunization record reflects the child's protection. A child's record might indicate administration of MMR vaccine when only single-antigen measles vaccine was administered. A study of children adopted from the People's Republic of China, Russia, and Eastern Europe determined that only 39% (range: 17%–88% by country) of children with documentation of more than three doses of DTP before adoption had protective serologic concentrations of diphtheria and tetanus antitoxin.[216] This finding raised questions about whether the vaccines received were potent or whether the immunization records purporting to show receipt of at least three doses were an accurate reflection of the true number of doses actually received. However, antibody testing was performed by a hemagglutination assay, which may underestimate protection and cannot directly be compared with antitoxin concentration measured by other tests.[217] A second study measured antibody to diphtheria and tetanus toxins among 51 children who had records of having received two or more doses of DTP. Most of the children were from Russia, Eastern Europe, and Asian countries, and 78% had received all vaccine doses in an orphanage; 94% had evidence of protection against diphtheria (enzyme immunoassay [EIA] >0.1 IU/mL). A total of 84% had protection against tetanus (EIA >0.5 IU/mL). Among children without protective tetanus antitoxin concentrations, all except one had records of three

or more doses of vaccine, and most nonprotective concentrations were categorized as indeterminate (EIA 0.05–0.49 IU/mL).[218] Reasons for the discrepant findings in these two studies probably relate to different laboratory methods; the study using a hemagglutination assay might have underestimated the number of children who were protected. Additional studies using standardized methods are needed.

If a question exists regarding whether vaccines administered outside the United States were immunogenic, several approaches may be considered. Repeating the vaccinations as age-appropriate is an acceptable option; this usually is safe and avoids the need to obtain and interpret serologic tests.[5] If avoiding unnecessary injections is desired, judicious use of serologic testing can help determine which immunizations are needed, particularly for DTP/DTaP vaccine. Although no established serologic correlates exist for protection against pertussis, diphtheria and tetanus antitoxin levels may be used as surrogates to assess whether doses listed on the immunization record actually were received or were potent.[5]

Increased rates of moderate to severe local adverse reactions after the fourth and fifth doses of DTP or DTaP have been reported.[108] If a revaccination option is adopted and a severe local reaction occurs, serologic testing for specific immunoglobulin G antibody to tetanus and diphtheria toxins can be performed before administering additional doses. Protective concentrations indicate that further doses are unnecessary, and subsequent vaccination should occur as age-appropriate.

Instead of revaccinating a child with DTaP when there is a question about the validity of a record that indicates receipt of three or more doses of DTP or DTaP, serologic testing for specific immunoglobulin G antibody to diphtheria *and* tetanus toxin can be obtained. If a protective concentration is present, recorded doses can be considered valid, and the vaccination series should be completed as age-appropriate. Indeterminate antibody concentration might indicate immunologic memory but waning antibody. Serologic testing can be repeated after a booster dose if the vaccination provider wants to avoid revaccination with a complete series.[5]

Children 7 years of age or older who are not considered fully vaccinated for pertussis should receive one dose of Tdap vaccine. "Fully vaccinated" means at least five doses of DTaP before the seventh birthday or at least four doses of DTaP before the seventh birthday if the fourth dose is given after the fourth birthday.

Serologic testing for HBsAg is recommended for all international adoptees born in Asia, the Pacific Islands, Africa, and other regions of high or intermediate endemicity.[218] Children determined to be HBsAg-positive should be monitored for development of liver disease.[201] Household members of HBsAg-positive children should be vaccinated. A child whose records indicate receipt of three or more doses of vaccine can be considered protected, and additional doses are not needed if at least one dose was administered at or after 6 months of age. Children who received their last hepatitis B vaccine dose when younger than 6 months of age should receive an additional dose at 6 months of age or older.[5] Children who have received fewer than three doses should complete the series at the recommended intervals and ages (see Table 9.2).

The simplest approach to vaccinating a child with uncertain polio vaccine records is to revaccinate with IPV according to the U.S. Schedule. Adverse events after IPV are rare.

VACCINATION OF PERSONS WITH A PERSONAL OR FAMILY HISTORY OF SEIZURES

Infants and young children with a personal history of seizures or a parent or sibling with a history of seizures are at increased risk for seizures after receipt of whole-cell pertussis, MMR (or monovalent measles) vaccine, or MMRV vaccine.[53,219–221] In most cases, these seizures are brief, self-limited, and associated with fever. Studies have not established a causal association between these seizures and residual seizure disorders or permanent neurologic sequelae.[222,223] Acellular pertussis vaccines are associated less often with fever than are whole-cell pertussis vaccines; whole-cell DTP is no longer available in the United States.

Because neurologic disorders such as epilepsy and degenerative disorders marked by loss of developmental milestones often become manifest during infancy, pertussis vaccination may coincide with onset or recognition of such disorders and cause confusion about the etiologic role of the vaccine. For infants with a personal history of a seizure, delaying pertussis vaccination is recommended until a progressive neurologic disorder is excluded or the cause of the seizure has been established.[57,58] Because measles vaccine is administered at an age when a child's neurologic status is likely to already have been established, deferring measles immunization of a child with a personal history of a seizure is not recommended.[53,184]

Pertussis and measles vaccinations are not contraindicated in persons with a family history of seizures. Even though children with a parent or sibling who has had a seizure are themselves at increased risk for a seizure, the benefits of administering pertussis and measles vaccine to children with a family history of seizures substantially outweigh the small risks because the seizures are usually febrile in origin, generally have a benign outcome, and are not likely to be confused with manifestations of a previously unrecognized neurologic disorder.[53,58,184,222,224] In June 2009, after consideration of the postlicensure data and other evidence, ACIP adopted recommendations regarding use of MMRV vaccine for the first and second doses and identified a personal or family (i.e., sibling or parent) history of seizure as a precaution for use of MMRV vaccine. For the first dose of measles, mumps, rubella, and varicella vaccines at age 12 to 47 months, either MMR vaccine and varicella vaccine or MMRV vaccine may be used. Providers who are considering administering MMRV vaccine should discuss the benefits and risks of both vaccination options with the parents or caregivers. Unless the parent or caregiver expresses a preference for MMRV vaccine, CDC recommends that MMR vaccine and varicella vaccine should be administered for the first dose in this age group. For the second dose of measles, mumps, rubella, and varicella vaccines at any age (15 months to 12 years) and for the first dose at age 48 months or older, use of MMRV vaccine generally is preferred over separate injections of its equivalent component vaccines (i.e., MMR vaccine and varicella vaccine). This recommendation is consistent with ACIP's 2009 provisional general recommendations regarding use of combination vaccines which state that use of a combination vaccine generally is preferred over its equivalent component vaccines.[5]

VACCINATION DURING ACUTE ILLNESS

The decision to administer or delay vaccination because of an intercurrent or recent acute illness depends on evaluation of the etiology of the disease and the severity of symptoms.[2,5] Mild illness, febrile (temperature ≥38°C) or afebrile, is not a contraindication to vaccination. Although one study reported a lower rate of seroconversion to the measles but not to the rubella or mumps components of MMR vaccine in children with evidence of a recent or current upper respiratory tract infection compared with children without this history,[223] a difference in seroconversion to measles vaccine in healthy children compared with children who are ill has not been found in other studies.[81,225–228]

Acute minor illnesses, such as upper respiratory tract infection, diarrhea, and acute otitis media, are common during infancy and childhood.[229] Postponing vaccination in children with minor febrile or afebrile illness constitutes a missed opportunity to protect a child from disease, can contribute to outbreaks of vaccine-preventable disease, and can significantly impede efforts to immunize infants and young children on schedule.[230–233] Every opportunity should be used to provide indicated vaccines and to avoid missed opportunities in persons who may not return for medical care and administration of recommended vaccines.[2,5,234,235] The potential benefit of preventing disease by timely vaccination far outweighs any small possible risk of vaccine failure.

Vaccination usually is deferred in persons who have moderate or severe illness. A person with signs or symptoms of moderate or severe illness at the scheduled time of vaccination should be requested to return as soon as the illness improves so that vaccines can be administered at the recommended ages. Waiting until after a person has recovered from the acute phase of a moderate or severe illness avoids superimposing a reaction to vaccination on the underlying illness or mistakenly attributing a manifestation of the underlying illness to the vaccine.[2,5]

CONTRAINDICATIONS TO AND PRECAUTIONS REGARDING VACCINATION

Vaccine contraindications and precautions are described in the manufacturer's product labeling and in the recommendations for the use of vaccines developed by national advisory committees such as the ACIP and the Committee on Infectious Diseases of the AAP. In the United States, the content of the product label is regulated by the Food and Drug Administration on the basis of specific studies required of the manufacturer to prove the safety and efficacy of a specific product. Most recommendations of vaccine advisory committees are the same as those in the product label. However, differences sometimes exist because of advisory committees' assessments of the risks and benefits of a given recommendation, their goal to make immunization as practical as possible, and their responsibility to develop recommendations for the use of vaccines in circumstances in which specific safety and efficacy data may be limited but for which physicians, nurses, and public health officials need guidance. For example, the manufacturer's product label recommends that women vaccinated with live varicella virus vaccine avoid becoming pregnant for 3 months, whereas the ACIP and AAP advise waiting only 1 month.[1,185,236] Similarly, the AAP and ACIP advise that pregnancy should not be considered a contraindication to hepatitis B vaccination, whereas the manufacturer's product label states that hepatitis B vaccine should only be given to pregnant women if clearly indicated.[1,201,237,238]

A contraindication indicates that a vaccine should not be administered. In contrast, a precaution specifies a situation in which vaccine may be administered if the benefit of vaccination to the individual patient is judged to outweigh the risk.[5] Contraindications and precautions may be generic and apply to all vaccines, or they may be specific to one or more vaccines (Table 9.6). The following two guidelines apply to all vaccines: (a) An anaphylactic reaction to a vaccine or vaccine constituent

TABLE 9.6 Contraindications and Precautions[a] to Commonly Used Vaccines

Vaccine	Contraindications	Precautions
Diphtheria and tetanus toxoids and acellular pertussis (DTaP)	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component Encephalopathy (e.g., coma, decreased level of consciousness, or prolonged seizures), not attributable to another identifiable cause, within 7 days of administration of previous dose of diphtheria toxoid, tetanus toxoid, and pertussis (DTP) or DTaP	Progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, progressive encephalopathy; defer DTaP until neurologic status clarified and stabilized Temperature of ≥40.5°C within 48 h after vaccination with a previous dose of DTP or DTaP Collapse or shock-like state (i.e., hypotonic hyporesponsive episode) within 48 h after receiving a previous dose of DTP/DTaP Seizure ≤3 days after receiving a previous dose of DTP/DTaP Persistent, inconsolable crying lasting ≥3 h within 48 h after receiving a previous dose of DTP/DTaP Guillain-Barré syndrome (GBS) <6 wk after previous dose of tetanus toxoid–containing vaccine History of Arthus-type hypersensitivity reactions after a previous dose of diphtheria-toxoid–containing or tetanus toxoid–containing vaccine; defer vaccination until at least 10 y have elapsed since the last tetanus toxoid–containing vaccine Moderate or severe acute illness with or without fever
Diphtheria and tetanus toxoids (DT), tetanus and diphtheria toxoids (Td)	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component	GBS < 6 wk after previous dose of tetanus toxoid–containing vaccine History of Arthus-type hypersensitivity reactions after a previous dose of diphtheria-toxoid–containing or tetanus toxoid–containing vaccine; defer vaccination until at least 10 y have elapsed since the last tetanus-toxoid–containing vaccine Moderate or severe acute illness with or without fever
Haemophilus influenzae type b (Hib)	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component Age <6 wk	Moderate or severe acute illness with or without fever
Hepatitis A	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component	Moderate or severe acute illness with or without fever

TABLE 9.6 Contraindications and Precautions[a] to Commonly Used Vaccines (*Continued*)

Vaccine	Contraindications	Precautions
Hepatitis B	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component; hypersensitivity to yeast	Infant weight <2000 g[b] Moderate or severe acute illness with or without fever
Human papillomavirus vaccine (2vHPV, 4vHPV, 9vHPV)	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component	Moderate or severe acute illness with or without fever; pregnancy
Inactivated influenza vaccine (IIV, RIV)	Severe allergic reaction (e.g., anaphylaxis) after previous dose of influenza vaccine	GBS <6 wk after a previous dose of influenza vaccine Moderate or severe acute illness with or without fever
Inactivated poliovirus (IPV)	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component	Pregnancy Moderate or severe acute illness with or without fever
Live attenuated influenza vaccine (LAIV)	Severe allergic reaction (e.g., anaphylaxis) after previous dose or to vaccine component, including egg protein Persons aged <2 y or >49 y Those with contraindications listed in the package insert: Children ages 2–17 y who are receiving aspirin or aspirin-containing products; persons who have experienced severe allergic reactions to the vaccine or any of its compounds, or to a previous dose of any influenza vaccine; pregnant women; immunosuppressed persons; persons with a history of egg allergy; children ages 2–4 y who have asthma or who have had a wheezing episode noted in the medical record within the past 12 mo, or for whom parents report that a health care provider stated that they had wheezing or asthma within the last 12 mo Persons who have taken influenza antiviral medications within the previous 48 h[c]	GBS <6 wk after a previous dose of influenza vaccine The safety of LAIV in persons with underlying medical conditions that might predispose them to complications after wild-type influenza infection (e.g., chronic pulmonary, cardiovascular [except isolated hypertension], renal, hepatic, neurologic, hematologic, or metabolic disorders [including diabetes mellitus]) has not been established. These conditions, in addition to asthma in persons age ≥5 y, should be considered precautions for the use of LAIV Moderate or severe acute illness with or without fever
Measles, mumps, and rubella (MMR)[d,e]	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component Pregnancy Known severe immunodeficiency (e.g., from hematologic and solid tumors, receipt of chemotherapy, congenital immunodeficiency, or long-term immunosuppressive therapy[f] or patients with HIV infection who are severely immunocompromised)[e]	Recent (≤11 mo) receipt of antibody-containing blood product (specific interval depends on product)[g] History of thrombocytopenia or thrombocytopenic purpura Need for tuberculin skin testing[h] or interferon gamma release assay (IGRA) testing Moderate or severe acute illness with or without fever
Pneumococcal conjugate vaccine (PCV13)	Severe allergic reaction (e.g., anaphylaxis) after a previous dose of PCV13, or any diphtheria toxoid–containing vaccine or to a component of a vaccine (PCV13 or any diphtheria toxoid–containing vaccine)	Moderate or severe acute illness with or without fever
Pneumococcal polysaccharide vaccine (PPSV)	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component	Moderate or severe acute illness with or without fever
Quadrivalent meningococcal conjugate vaccine (MCV4) and monovalent meningococcal b vaccine	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component	Moderate or severe acute illness with or without fever
Quadrivalent meningococcal polysaccharide vaccine (MPSV4)	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component	Moderate or severe acute illness with or without fever
Rotavirus	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component Severe combined immunodeficiency (SCID) History of intussusception	Altered immunocompetence other than SCID Chronic gastrointestinal disease[i] Spina bifida or bladder exstrophy[i] Moderate or severe acute illness with or without fever

Continued on following page

TABLE 9.6 Contraindications and Precautions[a] to Commonly Used Vaccines (*Continued*)

Vaccine	Contraindications	Precautions
Tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap)	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component Encephalopathy (e.g., coma, decreased level of consciousness, or prolonged seizures), not attributable to another identifiable cause, within 7 days of administration of previous dose of DTP, DTaP, or Tdap	GBS <6 wk after a previous dose of tetanus toxoid–containing vaccine Progressive or unstable neurological disorder, uncontrolled seizures, or progressive encephalopathy until a treatment regimen has been established and the condition has stabilized History of Arthus-type hypersensitivity reactions after a previous dose of diphtheria toxoid-containing or tetanus toxoid–containing vaccine; defer vaccination until at least 10 y have elapsed since the last tetanus toxoid–containing vaccine[j] Moderate or severe acute illness with or without fever
Varicella	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component Known severe immunodeficiency (e.g., from hematologic and solid tumors, receipt of chemotherapy, congenital immunodeficiency, or long-term immunosuppressive therapy[f] or patients with HIV infection who are severely immunocompromised)[e] Pregnancy	Recent (≤11 mo) receipt of antibody-containing blood product (specific interval depends on product)[k] Moderate or severe acute illness with or without fever
Zoster	Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component Known severe immunodeficiency (e.g., from hematologic and solid tumors, receipt of chemotherapy, congenital immunodeficiency, or long-term immunosuppressive therapy or patients with HIV infection who are severely immunocompromised[e,f] Pregnancy	Moderate or severe acute illness with or without fever

[a]Events or conditions listed as precautions should be reviewed carefully. Benefits of and risks for administering a specific vaccine to a person under these circumstances should be considered. If the risk from the vaccine is believed to outweigh the benefit, the vaccine should not be administered. If the benefit of vaccination is believed to outweigh the risk, the vaccine should be administered. Whether and when to administer DTaP to children with proven or suspected underlying neurologic disorders should be decided on a case-by-case basis.

[b]Hepatitis B vaccination should be deferred for infants weighing <2000 g if the mother is documented to be hepatitis B surface antigen (HBsAg)-negative at the time of the infant's birth. Vaccination can commence at chronological age 1 month or at hospital discharge. For infants born to HBsAg-positive women, hepatitis B immunoglobulin and hepatitis B vaccine should be administered within 12 hours after birth, regardless of weight.

[c]Source: Fiore AE, Uyeki TM, Broder K, et al; Centers for Disease Control and Prevention (CDC). Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Recomm Rep*. 2010;59(RR-8):1–62.

[d]HIV-infected children may receive varicella and measles vaccine if $CD4^+$ T-lymphocyte count is >15%. (Source: Adapted from American Academy of Pediatrics. Passive immunization. In: Kimberlin DW, Brady MT, Jackson MA, Long SS, eds. *Red Book: 2015 Report of the Committee on Infectious Diseases*. 30th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2015.

[e]MMR and varicella-containing vaccines can be administered on the same day. If not administered on the same day, these vaccines should be separated by at least 28 days.

[f]Substantially immunosuppressive steroid dose is considered to be ≥2 weeks of daily receipt of 20 mg or 2 mg/kg body weight of prednisone or equivalent.

[g]See Table 9.5 for details.

[h]If active tuberculosis is suspected, MMR should be delayed. Measles vaccination might suppress tuberculin reactivity temporarily. Measles-containing vaccine can be administered on the same day as tuberculin skin testing. If testing cannot be performed until after the day of MMR vaccination, the test should be postponed for ≥4 weeks after the vaccination. If an urgent need exists to skin test, do so with the understanding that reactivity might be reduced by the vaccine.

[i]For details, see Cortese MM, Parashar UD; Centers for Disease Control and Prevention (CDC). Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2009;58(RR-2):1–25.

[j]With the exception of pregnant woman who should be vaccinated during every pregnancy regardless of interval since last tetanus toxoid–containing dose.

[k]Vaccine should be deferred for the appropriate interval if replacement immunoglobulin products are being administered (see Table 9.5).

contraindicates further use of that vaccine or vaccines containing that constituent (see "Vaccine Components Causing Hypersensitivity" above). (b) Moderate or severe acute illness, regardless of the absence or presence of fever, is a precaution to vaccination (see "Vaccination During Acute Illness" above).

Immunosuppression resulting from underlying disease or therapy is a contraindication for receipt of most live vaccines.[5,53,184] MMR vaccine is recommended for HIV-infected persons who are not severely immunosuppressed.[172,209,217,239] Varicella vaccine can be considered for HIV-infected persons not severely immunosuppressed.[185] Rotavirus vaccine is contraindicated in infants diagnosed with severe combined immunodeficiency (SCID).[240,241] Corticosteroid therapy can suppress the immune system of an otherwise healthy person, although the minimal dose and duration of therapy necessary to cause immunosuppression are not well defined. Underlying disease, concurrent therapies, and the frequency and route of administration of corticosteroids also can affect immunosuppression. Steroid therapy does not usually contraindicate administration of live vaccines when given in low to moderate doses administered daily or on alternate days, physiologic

TABLE 9.7 Conditions Commonly Misperceived as Contraindications to Vaccination

Vaccine	Conditions Commonly Misperceived as Contraindications (i.e., Vaccination May Be Administered Under These Conditions)
General for all vaccines, including diphtheria and tetanus toxoids and acellular pertussis (DTaP), pediatric diphtheria and tetanus toxoids (DT), adult tetanus and diphtheria toxoids (Td), adolescent–adult tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap), inactivated poliovirus (IPV), measles, mumps, and rubella (MMR), *Haemophilus influenzae* type b (Hib), hepatitis A, hepatitis B, varicella, rotavirus, pneumococcal conjugate vaccine (PCV), trivalent inactivated influenza vaccine (IIV) live, attenuated influenza vaccine (LAIV), pneumococcal polysaccharide vaccine (PPSV 23), quadrivalent meningococcal conjugate vaccine (MCV4), quadrivalent meningococcal polysaccharide vaccine (MPSV4), serogroup B meningococcal human papillomavirus (HPV) vaccine, and herpes zoster	Mild acute illness with or without fever Mild-to-moderate local reaction (i.e., swelling, redness, soreness); low-grade or moderate fever after previous dose Lack of previous physical examination in well-appearing person Current antimicrobial therapy[a] Convalescent phase of illness Preterm birth (hepatitis B vaccine is an exception in certain circumstances)[b] Recent exposure to an infectious disease History of penicillin allergy, other nonvaccine allergies, relatives with allergies, or receiving allergen extract immunotherapy History of Guillain-Barré syndrome[c]
DTaP	Temperature of <40.5°C, fussiness, or mild drowsiness after a previous dose of diphtheria toxoid, tetanus toxoid, and pertussis (DTP)/DTaP Family history of seizures Family history of sudden infant death syndrome Family history of an adverse event after DTP or DTaP administration Stable neurologic conditions (e.g., cerebral palsy, well-controlled seizures, or developmental delay)
Tdap	History of temperature of ≥40.5°C for <48 h after vaccination with a previous dose of DTP or DTaP History of collapse or shock-like state (i.e., hypotonic hyporesponsive episode) within 48 h after receiving a previous dose of DTP/DTaP History of seizure <3 days after receiving a previous dose of DTP/DTaP History of persistent, inconsolable crying lasting >3 h within 48 h after receiving a previous dose of DTP/DTaP History of extensive limb swelling after DTP/DTaP/Td that is not an Arthus-type reaction Stable neurologic disorder History of brachial neuritis Latex allergy that is not anaphylactic Breastfeeding Immunosuppression
IPV	Previous receipt of ≥1 dose of oral polio vaccine
MMR[d,e]	Positive tuberculin skin test Simultaneous tuberculin skin testing[f] Breastfeeding Pregnancy of recipient's mother or other close or household contact Recipient is female of childbearing age Immunodeficient family member or household contact Asymptomatic or mildly symptomatic HIV infection Allergy to eggs
Hepatitis B	Pregnancy Autoimmune disease (e.g., systemic lupus erythematosus or rheumatoid arthritis)
Varicella	Pregnancy of recipient's mother or other close or household contact Immunodeficient family member or household contact[g] Asymptomatic or mildly symptomatic HIV infection Humoral immunodeficiency (e.g., agammaglobulinemia)
Inactivated influenza vaccine (IIV)	Nonsevere (e.g., contact) allergy to latex, thimerosal, or egg Concurrent administration of Coumadin or aminophylline
LAIV	Healthcare providers who see patients with chronic diseases or altered immunocompetence (an exception is providers for severely immunocompromised patients requiring care in a protected environment) Breastfeeding Contacts of persons with chronic disease or altered immunocompetence (an exception is contacts of severely immunocompromised patients requiring care in a protected environment)
PPSV	History of invasive pneumococcal disease or pneumonia
HPV	Immunosuppression Previous equivocal or abnormal Papanicolaou test Known HPV infection Breastfeeding History of genital warts

Continued on following page

TABLE 9.7 Conditions Commonly Misperceived as Contraindications to Vaccination *(Continued)*

Vaccine	Conditions Commonly Misperceived as Contraindications (i.e., Vaccination May Be Administered Under These Conditions)
Rotavirus	Prematurity Immunosuppressed household contacts Pregnant household contacts
Zoster	Therapy with low-dose methotrexate (≤0.4 mg/kg per week), azathioprine (≤3.0 mg/kg per day), or 6-mercaptopurine (≤1.5 mg/kg per day) for treatment of rheumatoid arthritis, psoriasis, polymyositis, sarcoidosis, inflammatory bowel disease, or other conditions Healthcare providers of patients with chronic diseases or altered immunocompetence Contacts of patients with chronic diseases or altered immunocompetence Unknown or uncertain history of varicella in a U.S.-born person

[a]Antibacterial drugs might interfere with Ty21a oral typhoid vaccine, and certain antiviral drugs might interfere with varicella-containing vaccines and LAIV.

[b]Hepatitis B vaccination should be deferred for infants weighing <2000 g if the mother is documented to be hepatitis B surface antigen (HBsAg)-negative at the time of the infant's birth. Vaccination can commence at chronological age 1 month or at hospital discharge. For infants born to HBsAg-positive women, hepatitis B immunoglobulin and hepatitis B vaccine should be administered within 12 hours after birth, regardless of weight.

[c]An exception is Guillain-Barré syndrome within 6 weeks of a dose of influenza vaccine or tetanus-toxoid–containing vaccine, which are precautions for influenza vaccine and tetanus-toxoid–containing vaccines, respectively.

[d]MMR and varicella vaccines can be administered on the same day. If not administered on the same day, these vaccines should be separated by at least 28 days.

[e]HIV-infected children should receive immunoglobulin after exposure to measles. HIV-infected children can receive varicella and measles vaccine if $CD4^+$ T-lymphocyte count is >15%. (Source: Modified from American Academy of Pediatrics. Passive immunization. In: Kimberlin DW, Brady MT, Jackson MA, Long SS, eds. *Red Book: 2015 Report of the Committee on Infectious Diseases*. 30th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2015.)

[f]Measles vaccination might suppress tuberculin reactivity temporarily. Measles-containing vaccine can be administered on the same day as tuberculin skin testing. If testing cannot be performed until after the day of MMR vaccination, the test should be postponed for at least 4 weeks after the vaccination. If an urgent need exists to skin test, do so with the understanding that reactivity might be reduced by the vaccine.

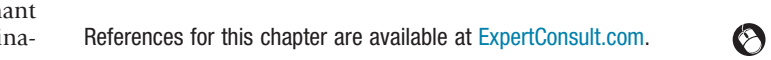
[g]If a vaccinee experiences a presumed vaccine-related rash 7 to 25 days after vaccination, the person should avoid direct contact with immunocompromised persons for the duration of the rash.

maintenance doses, or doses administered topically, by aerosol, or by local (e.g., intraarticular) injection.[5,184] In most cases, persons receiving high doses of systemic corticosteroids (i.e., ≥2 mg/kg per day or 20 mg/day of prednisone or its equivalent) for less than 14 days can receive live vaccines immediately after discontinuation of therapy.[2,5] However, live vaccines usually are not administered to persons who have received high doses of systemic corticosteroids for 14 days or more until at least 1 month after cessation of steroid therapy.[5,184]

Live vaccines usually are contraindicated for pregnant women because of a theoretical risk to the fetus (see "Vaccination During Pregnancy" above). However, the small theoretical risk from administration of a live vaccine to a pregnant woman is sometimes far outweighed by the risk of contracting a disease with serious consequences for mother and fetus (e.g., yellow fever vaccine).

Healthcare providers sometimes inappropriately consider a condition to be a contraindication or precaution to vaccination.[2,5] Withholding vaccine in such situations results in a missed opportunity to administer needed vaccine (Table 9.7).

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SECTION 2

Licensed Vaccines and Vaccines in Development

10 Adenovirus Vaccines

Gregory C. Gray and Dean D. Erdman

HISTORY OF DISEASE

In the 1950s, multiple researchers[1,2] identified new viruses as the cause of acute respiratory disease (ARD), pharyngitis, conjunctivitis, and pneumonitis. Soon afterward, these viruses were recognized to be a related group of viruses and given their present name: adenoviruses.[3]

WHY THE DISEASE IS IMPORTANT

The adenoviruses are often the cause of respiratory illness among U.S. military trainees[4,5] and children.[6–13] In the 1960s, adenoviruses were recognized to infect as many as 80% of trainees, with 20% requiring hospitalization.[5] Median attack rates for trainees ranged from 6 to 16.7/100 per month at the most affected northern and Midwestern U.S. military bases, and from 2.3 to 2.6/100 per month for posts in the South and West.[14] Up to 40% of the trainees in a unit were lost to illness within a 2-week period, and many who were hospitalized had to restart training.[15] Adenoviruses were also recognized to cause as much as 15% of instances of gastroenteritis in infants and children, and up to 10% of instances of pneumonia among hospitalized children.[16–18] Adenovirus morbidity can be severe and lead to death, especially in young children, transplantation recipients, and other immunocompromised patients.

The search for vaccines against adenoviruses was driven by adenovirus morbidity among military trainees, their disruption to military training, and their medical care costs.[15] In 1971, after a series of misstarts, military recruits began routinely receiving live oral enteric-coated vaccines for adenovirus types 4 and 7 (Ad4 and Ad7), which were safe and effective.[19] With few exceptions, adenovirus morbidity was markedly controlled. However, in 1996, the sole manufacturer of these vaccines ceased production. As vaccine stores were depleted, U.S. military trainees again experienced large outbreaks of adenovirus disease, with some deaths reminding public health officials of the impact of adenoviruses.[20–22] In one comprehensive 2006 study, Russell and colleagues documented 98% Ad4 attack rates among 180 susceptible military recruits during 4 weeks of training.[23] The importance of these pathogens has been further emphasized through the recent emergence of multiple novel Ad3, Ad7, and Ad14 strains and the subsequent epidemics and deaths they caused.[24–29]

BACKGROUND

Clinical Description

Human adenoviruses have been classically grouped into seven species (A through G) containing as many as 68 unique types (Ad1 through Ad68) and multiple more genotypes often determined by restriction enzyme digests (Ad7a, Ad7d2, etc). However, the nomenclature and classification system is undergoing change. In 2013, the International Committee of Taxonomy of Viruses added a genus prefix (eg, human adenovirus A has become human mastadenovirus A).[30] A number of investigators have additionally argued that it is time to move away from neutralization and hemagglutination assays in determining serotype and to chiefly use a genetic classification approach.[31] Such virologists have used large DNA sequence comparisons to describe as many as 68 novel adenovirus types,[32,33] although this approach has met with some debate.[34–37]

No matter which adenovirus nomenclature or classification system is agreed upon, it is clear that adenoviruses affect most organ systems. Individual adenovirus types often have different tissue tropisms that lead to specific signs and symptoms (Table 10.1).[39,40]

Endemic Respiratory Adenovirus in Children

Although most children become infected with many of the common adenoviruses early in life, only approximately 50% of these infections result in disease.[39,41,42] Isolation studies have indicated that Ad1, Ad2, Ad5, Ad3, and Ad6 are most prevalent types detected,[39,43] and often manifest by pharyngitis, bronchitis, bronchiolitis, croup, or pneumonia.[43,44] The incidence of adenovirus disease is higher in late winter, spring, and early summer, and both sexes are equally affected.[41,45]

Epidemic Respiratory Adenovirus in Children

Occasionally, epidemics occur in daycare facilities, neonatal intensive care units, chronic care facilities, and orphanages, especially with Ad3 and Ad7.[46–49] In addition to causing outbreaks in closed populations, Ad7 can cause large epidemics affecting children in open communities.[50,51]

Acute Respiratory Disease of Military Recruits

In young adults who live in closed communities such as boarding schools and military recruit camps, adenoviruses may cause epidemics of illnesses similar to influenza, including tracheobronchitis and pneumonia severe enough to require hospitalization.[2,4,52,53] Adenovirus pneumonia may be fatal, especially when it is associated with Ad7 strains.[16,54–56] The incubation period of the disease is 4 to 5 days.[53] Before the use of vaccines in the U.S. military, Ad4 and Ad7 accounted for 90% of all respiratory illnesses among recruits who were hospitalized, whereas Ad3, Ad14, and Ad21 were less frequently observed.[4] At some northern basic training sites, rates of 6 to 8 per 100 trainees per week translated into 600 to 800 ARD hospital admissions per week.[15] Recently, the newly

TABLE 10.1 Clinical Syndromes Associated With Human Adenovirus Infections

Clinical Syndrome	Common Types	Population at Risk
Endemic respiratory	1, 2, 3, 5, 6	Infants, children
Epidemic respiratory	7, 14, 55	Children
Acute respiratory disease	3, 4, 7, 14, 21	Military recruits
Pharyngoconjunctival fever	1, 3, 4, 7, 14	School-age children, young adults
Keratoconjunctivitis	8, 11, 19, 37, 53, 54	All age groups
Hemorrhagic cystitis	11, 34, 35	Immunocompromised patients, children
Gastroenteritis	31, 40, 41, 52	Children, immunocompromised patients
Other syndromes	2, 4, 7, 12,19, 32, 37	Children, adults
Immune deficiency	34, 35, 43–49, 50, 51	Transplantation recipients, persons with AIDS, and immunocompromised patients

There is agreement regarding the classification of adenoviruses 1 to 51, which have been classically typed with neutralization and hemagglutination assays. However, the classification of adenoviruses 52 to 68 remains controversial as these have been chiefly identified through genetic sequencing. In particular, adenovirus 55 is a recombination of 11 and 14.[126,148]

recognized Ad55 (Ad11 and Ad14 recombinant) has been documented to have caused significant ARD morbidity among military trainees in China.[57]

Typical ARD is a febrile disease with symptoms of sore throat, fever, cough, coryza, rhinorrhea, headache, and chest pain.[2,39,58] With extension into the lungs, physical examinations can reveal rales and rhonchi with little evidence of consolidation, and chest radiography often shows patchy interstitial infiltrates, principally in the lower lung fields.[2,53,59] Symptoms last 3 to 10 days.[39] The infection is often self-limited, but occasionally deaths occur.[2,60] Routes of transmission are thought to include direct contact or aerosolized virus inhaled into the lung. The virus has been isolated from the oropharynx more than 2 weeks after exposure.[2,61] In a 2006 report, Russell and colleagues demonstrated viable adenovirus was ubiquitous on surfaces in the military trainee barracks.[23]

Pharyngoconjunctival Fever

This syndrome is characterized by pharyngitis, conjunctivitis, and spiking fever.[62] First described in the 1920s as associated with swimming, the cause has been subsequently linked to insufficient chlorination.[63–65] One or both eyes are affected, and diarrhea, coryza, tonsillar exudates, and lymphadenopathy may be observed. The most frequently identified types have been Ad3 and Ad7, but other types, such as Ad1, Ad4, and Ad14, are also associated with pharyngoconjunctival fever.[62] The disease is associated with summer camps, swimming pools, and lakes; occurs in children and young adults; and often spreads to other family members.[62,66,67] The incubation period is 6 to 9 days, and the virus may be isolated from pool water.[62,65] There is little bacterial superinfection and no permanent damage to the eye.[59]

Epidemic Keratoconjunctivitis

Epidemics of conjunctivitis in adults caused by adenoviruses were first described as "shipyard eye."[68,69] The disease was observed at industrial settings where shipbuilding took place and was probably transmitted because of inadequate infection control practices when workers sought care for chemical irritation and minor trauma from paint and rust chips.[68] The disease has an incubation time of 8 to 10 days and is characterized by conjunctivitis, edema of the eye, pain, photophobia, and lacrimation. Superficial erosions and subepithelial infiltrates of the cornea may occur.[39] Preauricular lymph gland swelling and involvement of cervical and submaxillary lymph glands may be observed.[39] Epidemic keratoconjunctivitis (EKC) is associated with Ad8, Ad19, Ad37, and, rarely, other types.[70–74] In addition, Ad19 and Ad37 have been isolated from the genital tract of young adults with EKC, and the possibility of sexual transmission has been considered.[75,76] Numerous epidemics of EKC have been associated with ophthalmology practices, where spread from contaminated ophthalmic solutions, fingers, and instruments has been implicated.[70,72,74,77,78]

Hemorrhagic Cystitis

Hemorrhagic cystitis syndrome in children is caused by adenovirus infections in 23% to 51% of cases in the United States and Japan.[79] Although the specific route of spread is unknown, Ad11 and Ad21 were the most frequently isolated adenoviruses.[79] Boys were two to three times more often affected than girls. Clinical findings included gross hematuria of 3 days' duration. Dysuria, microscopic hematuria, and urinary frequency lasted a few days longer.[79,80] No viremia or structural abnormalities were found. Adenoviral antigen in exfoliated bladder epithelial cells can be demonstrated by immunofluorescence.[79] Cases of acute hemorrhagic cystitis after renal and bone marrow transplantation are increasingly being reported.[81–83] Ad34 and Ad35, as well as species B adenoviruses, were first isolated from renal transplantation recipients, but neither was associated with symptoms of hemorrhagic cystitis.[84]

Gastroenteritis

Adenoviruses are also recognized to cause watery diarrhea with fever. Ad40 and Ad41 are the most prevalent strains, but Ad50, Ad51, and Ad52 have been detected among the immunocompromised. AdV52 has been associated with one outbreak cluster of acute gastroenteritis.[124] These strains often require special cells lines for culture and they may not demonstrate cytopathic effect.[85] Electron micrography or special assays are often necessary for their study.[85] Numerous outbreaks have been described, and adenoviruses may account for up to 12% of all infant diarrhea.[86–88]

Rare Acute Manifestations

A number of case or outbreak reports document other, more unusual manifestations of human adenovirus infection. These occur most frequently among children. Although the studies

are inconsistent, adenovirus is implicated as a possible fetal pathogen.[89,90] Adenovirus infections among children are associated with sudden infant death,[91] encephalitis, meningoencephalitis,[92,93] cerebral edema,[94] acute flaccid paralysis,[95] pertussis-like syndromes,[96,97] mononucleosis-like syndromes,[98] and neonatal disseminated infections.[99] Among adults, adenovirus infections have been associated with a toxic shock–like syndrome,[100] encephalitis, genital lesions, orchitis, urethritis, and cervicitis.[101] Nosocomial transmission to susceptible healthcare workers and patients has been reported. This is likely related to the long periods of viral shedding among adenovirus-infected immunocompromised hosts, the possible aerosolization of the virus, and fomite transmission in the hospital setting.[40,95,102]

Complications

Adenovirus Infections in Immunocompromised Patients

Adenoviruses have been implicated as opportunistic agents in patients with immune deficiency states, such as patients with AIDS, patients receiving cancer chemotherapy, bone marrow transplant recipients, and patients undergoing solid organ transplantation.[11,12,82,103–106] The patients are prone to pneumonia and disseminated adenoviral infection. They have also developed parotitis[107] and urinary tract disease.[108] A number of the more recently described types, such as Ad43 through Ad51, have been recovered from people with AIDS.[108,109]

A review of 201 bone marrow recipients over a 4-year period indicated that adenovirus infections occurred in 20.9% of patients, with a higher incidence in children than in adults (31.3% vs 13.6%).[110] Ad35 was the most common type identified.[110] In 1992, Hierholzer reported that adenovirus infections in immunocompromised patients with pneumonia were associated with case fatality rates as high as 60%, compared with only 15% among immunocompetent hosts with pneumonia.[9] Radiographs of adenovirus-infected patients often demonstrate patchy interstitial infiltrates, usually in the lower lung fields.[111] Fig. 10.1 shows the chest radiograph of a 20-year-old recipient of an autologous bone marrow transplant for diffuse large cell lymphoma (B-cell type) with systemic Ad11 infection, which was obtained on day 45 posttransplantation. He died on day 80. Adenovirus was recovered from conjunctival, urine, and bronchoalveolar lavage cultures. A case fatality rate of 50% occurred in immunocompromised people with hepatitis and associated adenovirus infections, compared with 10% in similarly infected immunocompetent patients with hepatitis.[9] Positive tests of serum by polymerase chain reaction (PCR) for adenovirus has been reported to predict severe disseminated adenovirus infection in immunocompromised patients.[112] Nephritis and kidney failure caused by adenovirus infections have been reported in adults and children receiving bone marrow transplants.[113,114] A study of 532 hematopoietic stem cell transplant recipients between 1986 and 1997 found a 12% incidence of adenovirus infections, with children being more likely than adults to have a positive culture (23% vs 9%).[115]

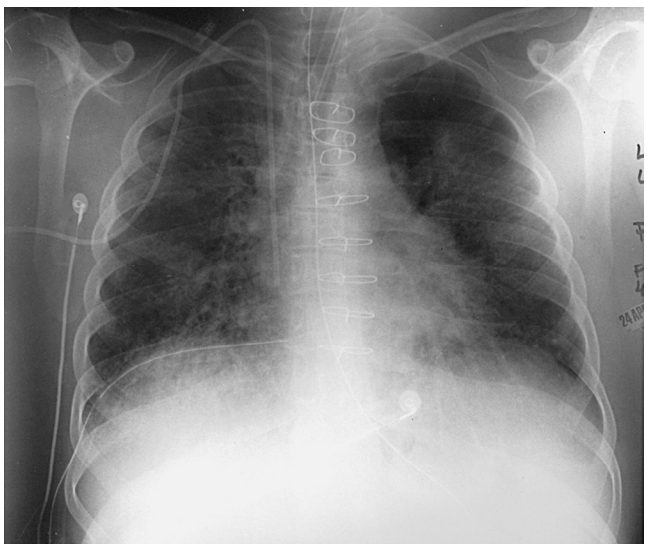


Figure 10.1. Pneumonia caused by adenovirus type 11 in a 20-year-old recipient of an autologous bone marrow transplant. *(Courtesy Stuart Ray, MD, Johns Hopkins University, Baltimore, MD.)*

Chronic Diseases Caused by Adenoviruses

Thanks often to molecular techniques, human adenoviruses have been associated with a number of chronic disease conditions, including chronic airway obstruction,[101] pulmonary dysplasia,[116] myocarditis, and cardiomyopathy.[117] Although the results of studies are mixed, an intriguing series of human and animal studies associate a number of human adenovirus types with obesity.[118–121]

Virology

Human adenoviruses are nonenveloped, double-stranded DNA viruses belonging to the genus Mastadenovirus, family Adenoviridae.[122] They are classified by species (A–G) through hemagglutination properties and by serotype (1–51) with horse or rabbit antisera (Table 10.2).[40,59,123] Different strains within serotypes are further distinguished by whole-genome restriction-enzyme digest patterns. However, as typing serum samples are difficult to acquire and sequencing costs are decreasing, virologists are considering full genome sequencing as a more modern and accurate method to detect unique strains and have described as many as 68 unique adenovirus types.[124–128]

Adenoviruses are considered large viruses, with an estimated diameter of approximately 920 Å.[129] The viruses have an icosahedral capsid shell that is made up of 240 hexon bases, 12 penton bases, and 12 fibers that are associated with the penton bases (Fig. 10.2). Four minor proteins (IIIa, VI, VIII,

TABLE 10.2 Human Adenovirus Species and Types

Adenovirus Species	Types
A	12, 18, 31, 61
B	3, 7, 11, 14, 16, 21, 34, 35, 50, 55, 66
C	1, 2, 5, 6, 57
D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51, 53, 54, 56, 58–60, 63–67
E	4
F	40, 41
G	52

Some information for the recently described adenovirus types is not yet available.

Modified from Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev. *2014;27(3):441–462.*

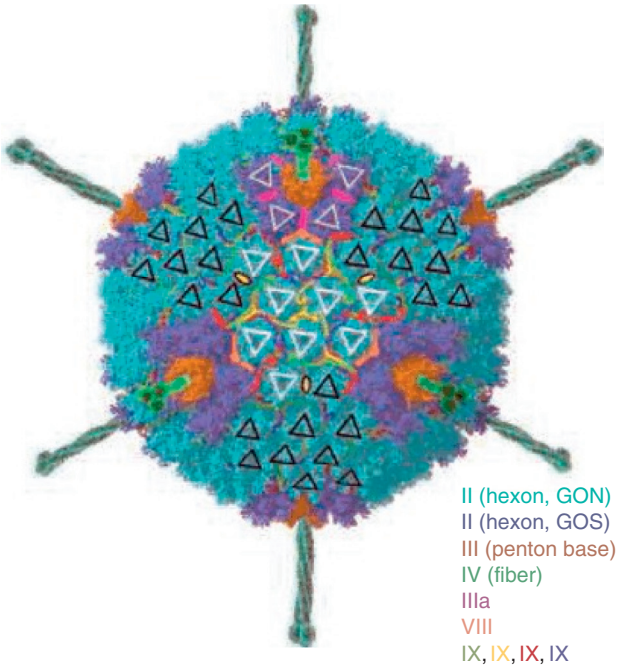


Figure 10.2. Image of adenovirus virion determined by cryoelectron microscopy showing newly understood interactions of the major components (hexon, penton, and fiber proteins) and minor proteins (IIIa, VIII, and IX). The proteins interact in groups of nine (GON) and groups of six (GOS) capsomers. *(Courtesy Hong Zhu, University of California, Los Angeles. Modified from Harrison SC. Looking inside adenovirus.* Science. *2010;329:1026–1027.)*

and IX) add to the complexity of the capsid. The pentons and hexons are each derived from different viral polypeptides.

Adenovirus hexons are both type-specific and species-specific antigens, primarily inducing species-specific complement-fixing antibodies, whereas the pentons are especially active in hemagglutination.[130] The fibers also evoke type-specific antibodies, vary in length among human strains, and are sometimes absent in particular animal strains.[131–134] The genome core of the virus is composed of five more proteins (V, VII, u, Iva2, and terminal protein) and a single molecule of linear, double-stranded DNA of 26 × 106 to 45 × 106 molecular weight. The G + C base compositions of the human virus genomes range from 47% to 60%.[110,122,135,136]

Adenoviruses are unusually stable to physical and chemical agents, as well as adverse pH, and, thus, survive for long periods outside the host, making them available for transmission to others. They can be destroyed by heat at 56°C for 30 minutes, UV irradiation, 0.25% sodium dodecyl sulfate, chlorine at 0.5 μg/mL, and formalin, but are resistant to ether and chloroform.

Adenoviruses replicate in the cell nucleus and tend to be very host-specific. However, there is mounting evidence that humans and animals have exchanged adenoviruses. In 2009, a simian adenovirus epidemic occurred among nonhuman primates at a U.S. research center and subsequently infected the primate caretakers with some evidence of human-to-human transmission.[137,138] A 2011 report documented the recent detection of 45 distinct adenovirus in nonhuman primates most of which mapped phylogenetically very closely to human adenovirus species.[139] In 2012, human infections with a novel avian adenovirus were associated with avian chlamydiosis outbreak in Hong Kong.[140] Finally, it is also important to recognize that Ad4 and Ad52 are thought to have originated from nonhuman primates.[124,141,142] Hence, some virologists have recently reviewed the probability that adenoviruses may be zoonotic.[143]

Some adenovirus types have been determined to be oncogenic in animals and to transform cell lines, but oncogenicity has not been observed in humans. Hybridization or genetic recombination of multiple strains of adenovirus may occur. Sometimes, these recombinant strains or other emergent novel adenovirus strains lead to epidemics. A number of recent examples have been reported in the medical literature.[26,27,29,144–148]

Pathogenesis as It Relates to Prevention

Depending on the route of inoculation, the type of virus, and the immune state of the host, adenoviruses can cause diseases or asymptomatic infections. Respiratory infection is presumed to result from inhalation of aerosolized virus, whereas ocular infection, gastroenteritis, and nosocomial infections may arise from fomites, water, or fecal–oral contact. Reactivation of latent adenovirus is also believed to occur.[53] Some 50% to 80% of tonsils removed surgically may have adenoviruses isolated from the tissue, suggesting that these viruses may remain in a latent state for years.[149,150] Virus has been also isolated from lymphocytes, kidney, blood, cerebrospinal fluid, and most body organs.[9,11,12,54,55,93,151–153] In the lungs, extensive pathology has been found with microscopic necrosis of the tracheal and bronchial epithelium.[39] Acidophilic intranuclear inclusions are seen in bronchial epithelial cells in addition to the basophilic masses of cells surrounded by clear halos, which may indicate aggregations of viral material.[54] A mononuclear infiltrate, rosette formation, and focal necrosis of mucous glands are characteristically seen.

Three types of interaction of adenovirus with infected cells can occur. A lytic infection may take place during which the virus completes an entire replicative cycle.[59] Between 10^5 to 10^6 progeny viruses per cell are produced, of which only 1% to 5% are actually infectious.[59] The second type of interaction is chronic or latent infection, in which small amounts of virus are produced, and an inapparent infection results. Viral shedding from the gastrointestinal tract may occur for years.[39] In fact reactivation of such latent infection likely explains much adenovirus disease in the severely compromised. Correspondingly, monitoring viral loads in the stool of transplant patients has predicted disseminated disease and been useful in guiding antiviral therapy.[154]

In addition to aerosolization, intestinal shedding of respiratory virus is an important factor to consider in the prevention of nosocomial spread in hospitals and chronic care homes.[39,145,155] Persistent infection has been reported in epithelial cells from monkeys.[156] Lymphoid cells are thought to be the reservoir for these persistent infections.[157,158] The third type of interaction is oncogenic transformation, whereby the viral DNA is integrated into the host genome and replicated with the cellular host DNA, but only the early steps in the viral cycle occur and no infectious virions are produced.[159]

The genes from adenoviruses are expressed in the cell nucleus in two phases: "early" (E), which precedes viral DNA replication, and "late."[157] Early genes encode proteins that function to thwart immunosurveillance, especially those from the E3 transcription unit.[157] The late genes primarily encode viral structural proteins. The functions of the E1 proteins include the induction of DNA synthesis in quiescent cells, immortalization of primary cells in cooperation with activated *ras* or with the E1B proteins, transactivation of delayed early genes, induction or repression of several cellular genes, and induction of apoptosis. These proteins modulate the sensitivity of adenovirus-infected cells to tumor necrosis factor (TNF),

a key inflammatory cytokine with antiviral properties.[157] None of the E3 genes are required for adenovirus replication in cultured cells, but several of the E3-coded proteins (10.4 K, 14.5 K, and 14.7 K) inhibit TNF cytolysis.[157,160,161] Because a major function of TNF may be to prevent viral replication, the inhibition of TNF by these viral proteins may be a significant mechanism of pathogenesis.

Another significant E3-coded protein is Gp 19 K. This glycoprotein is located in the endoplasmic reticulum and forms a complex with class I antigens of the major histocompatibility complex (MHC), preventing cells from being killed by cytotoxic T lymphocytes.[157] A cotton rat animal model was used by Ginsberg and colleagues and Ginsberg and Prince to study the pathogenesis of Ad2 and Ad5, which cause pneumonia similar to that seen in humans.[160,162] Two phases of infection were seen; the initial phase, characterized by the infiltration of monocytes and neutrophils, and a later phase associated with the infiltration of lymphocytes. The pathology seemed to reflect the response by host immune defenses to viral infection. The Gp 19 K markedly reduced the transport of the class I MHC to the surface of the infected cells and impeded the attack of cytotoxic T cells.[157,160,161] It is now known that only the early genes are required to induce the complete pathogenesis of adenovirus infection in cotton rats.[161] Although several cytokines, such as TNF-α, interleukin-1, and interleukin-6, were elaborated during the first 2 to 3 days of the infection in the cotton rat model, only TNF-α had a major role in pathogenesis.[161] Steroids almost completely eliminated the pneumonic inflammatory response to infection.[161]

Pathology caused by latent infection with adenoviruses has been linked to chronic obstructive pulmonary disease (COPD).[158,162] Some have suggested that childhood viral diseases represent an independent risk factor for COPD.[163] The adenoviral E1A proteins can stimulate the transcription of many heterologous viral and cellular genes. These proteins have the ability to interact with the DNA binding domains of several cellular transcription factors and activate a wide variety of genes.[164,165] The adenoviral genome has been found to be present in the lungs of more patients with COPD than in control subjects.[166] E1A proteins are expressed in epithelial cells of human lung tissue, and by increasing the expression of several genes important in controlling the inflammatory process, these may contribute to the pathogenesis of COPD. The events described may amplify the airway inflammation associated with cigarette smoking.[161]

The isolation and cloning of a 46-kDa protein adenovirus receptor, which mediates attachment and infection of group B viruses, may facilitate the development of new strategies to limit diseases caused by adenoviruses.[167] The more common receptor is referred to as CAR. This protein has been identified as the receptor for the coxsackie B virus and adenoviruses. Recently, another receptor, desmoglein 2, was identified for Ad3, Ad7, Ad11, and Ad14 strains.[168]

Laboratory Diagnosis

Adenoviral infections generally cannot be diagnosed on clinical grounds alone because the clinical signs and symptoms of these infections are variable and often resemble those caused by other microorganisms.[101] Traditionally, laboratory support and qualified personnel are necessary to accurately diagnose adenoviruses infections. Considerations include specimen type and timing, collection and storage procedures, types of laboratory tests performed, including serological assays, and availability of newer types of diagnostic assays.

Specimen Collection. The optimal specimen for diagnosis of adenovirus infection depends on the clinical presentation and the suspected type.[101] Adenoviruses can be detected in a variety of specimens, including nasal and throat secretions, conjunctival scrapings and swabs, stool, blood, cerebrospinal fluid, and biopsied tissue specimens. Specimens should be collected early in the illness and shipped promptly at 4°C or on dry ice if prompt testing is not possible. Swab and fresh tissue specimens must be transported in appropriate viral transport media[40,58,84,101] whereas fluid samples like urine, stool, and cerebrospinal fluid should not be diluted in transport media. For culture, nonsterile specimens should be treated with antibiotics prior to inoculation. Stool specimens are brought to 10% to 20% suspensions with buffered saline and clarified by low-speed centrifugation. Blood for culture or molecular testing should be collected with an anticoagulant to prevent clotting. Serum separated from clotted blood samples can be used for serological diagnosis (see below).

Cell Culture. Through biological amplification, cell culture offers a sensitive method for adenovirus detection by monitoring for cytopathic effect (CPE). Because adenoviruses are host-specific, isolation of human adenoviruses is most easily accomplished in human cells.[169] Most human adenoviruses have been successfully isolated in cell culture. Several diploid and continuous cell lines, including A549, HeLa, HEp-2, KB, MRC-5 and commercial mixed cell lines with A549 cells, reportedly give good overall recovery and produce typical CPE.[170,171] However, the fastidious enteric adenoviruses Ad40 and Ad41 are an exception. For efficient recovery, most Ad40 and Ad41 strains require the Graham-293 Ad5-transformed secondary HEK (human embryo kidney) cell line for primary isolation.

CPE may develop slowly in monolayers of inoculated cells necessitating several subpassages before becoming visible. Infected cells become rounded, enlarged, and refractile and aggregate into irregular "grape-like" clusters.[40] A 4-week incubation with blind passage is recommended. A rapid procedure for culture identification of adenovirus is the shell vial technique. Here, cell monolayers prepared in glass vials or multiwell plastic culture plates are inoculated with the clinical specimen, centrifuged, and stained after 2 to 3 days incubation with commercial monoclonal antibodies.[172] Cell culture is declining as a routine diagnostic method for detection and identification of human adenoviruses as a result of cost and delays. Antigen and molecular detection techniques have supplanted culture in many laboratories (see below).[169] However, while direct detection methods offer important diagnostic benefits, culture is the only means available for obtaining sufficient virus for immunotyping, assessing virus susceptibility to antivirals, and identifying infectious virus in clinical and environmental specimens.

Direct Visualization of Virus Particles

Electron and immunoelectron microscopy[173] were once commonly used to detect and identify the fastidious adenoviruses in stool specimens from young children, thereby establishing their association with acute gastroenteritis.[40,102,174–177] These methods are still used to localize virus in cells from biopsy and autopsy specimens to confirm disease association and to study disease pathology.

Antigen Assays. Detection of adenovirus antigenic proteins can be accomplished by reacting with virus-specific polyclonal or monoclonal antibodies labeled with various reporter systems. Examples include enzyme immunoassay, immunofluorescence assay, time-resolved fluoroimmunoassay, and latex agglutination.[178–181] These assays can be used to detect adenovirus proteins directly in clinical specimens or to

identify virus isolates. Immunoassays are simple to perform, less costly and provide results more quickly than culture or molecular tests, but are generally less sensitive.[181,182] The immunofluorescence assay is often used in the clinical diagnostic laboratory for rapid identification of adenovirus in cells from unfrozen specimens. Point-of-care antigen immunoassays[181,183] for rapid adenovirus detection and immunohistological staining for adenovirus antigens in biopsy and autopsy specimens are still in common use[182,184]

Molecular Assays. Molecular methods are rapidly replacing classical culture and antigen detection for diagnosis of adenovirus infection. The PCR assay in particular has become a popular diagnostic alternative for these viruses, offering the potential for rapid and sensitive detection and being easily tailored for species-specific and type-specific identification as described below. PCR assays[185] using adenovirus group-specific primers individually[185,186] or combined in assays for multiple human pathogens[187] have proven comparable or better than cell culture or immunodiagnostic methods for detection of adenoviruses in clinical samples. Several commercial multiplex assays for respiratory pathogens including adenovirus have been cleared by the U.S. Food and Drug Administration (FDA) for clinical diagnostic use.[188] Use of quantitative real-time PCR assays have the added benefit of allowing monitoring of adenovirus levels that can be used to predict patient prognosis, manage chemotherapy and monitor efficacy of antiviral therapy.[189]

Adenovirus Typing Assays. Once detected, further identification of adenoviruses to species and type can be accomplished by a variety of immunologic and molecular methods to support outbreak investigations and patient clinical management. For example, adenovirus infections in bone marrow or lung transplant recipients can be rapidly fatal. It is in the patients' interest that healthcare providers determine the species and type of any infecting adenovirus as some are more sensitive to specific antiviral therapy.[190] It is also important to learn if the adenovirus represents a community-acquired strain, a healthcare-acquired strain, a donor-associated strain, or the reactivation of a latent virus. Efforts to further identify adenoviruses have led to the discovery of cross-species infections with simian adenoviruses acquired from nonhuman primate reservoirs.[138,191]

Serum neutralization (SN) using defined hyperimmune animal antisera is the gold standard method used for classifying human adenovirus types.[40] SN targets type-specific neutralizing epitopes on the adenovirus hexon and fiber proteins and to a lesser extent on the penton protein. Hemagglutination inhibition (HI) has also been used of immunotyping adenoviruses. HI targets type-specific epitopes on the adenovirus fiber protein, and when used in combination with SN, can identify hexon/fiber recombinant viruses. Because immunotyping methods require reference antisera that are difficult to obtain and standardize and often difficult to interpret, molecular methods have generally replaced immunotyping for rapid routine adenovirus typing.

Numerous molecular methods for species and type-specific identification of adenoviruses have been described, including conventional[192–194] and real-time PCR[195,196] assays using species-specific and type-specific primers and probes, PCR amplification coupled with restriction endonuclease analysis,[197,198] microarrays,[199] and mass spectrometry[200] and, increasingly, PCR-coupled sequence-based typing strategies that target hypervariable regions of the adenovirus hexon or fiber genes or both.[194,201–208] Several commercial assays have been marketed that use PCR to identify human adenovirus to species.[209–211] As molecular typing strategies often focus only on specific hypervariable regions of the virus genome, potentially important virus strains resulting from recombinations or mutations can be missed. McCarthy and colleagues proposed a molecular typing algorithm that combined examinations of the hexon and fiber genes.[212]

Restriction enzyme analysis (REA) of whole-virus genomes can be used to identify genetic variants of a serotype, aiding epidemiologic studies and outbreak investigations.[213,214] Extensive studies of Ad3, Ad4, and Ad7 using REA have been described[20,215–218] and a number of novel viruses have thus been identified.[26] However, REA is work intensive, dependent on virus isolation to recover genomic DNA and requires expertise to perform and interpret, and is therefore restricted to a few experienced laboratories.

Classical and next generation sequencing technologies are increasingly being used to obtain full genomic sequences of human adenoviruses. Genome sequences are available for all human adenovirus prototype types and sequences of newly identified genomic variants are now routinely placed in public domain providing reference data for adenovirus identification.[126] A new whole genome-based characterization system for adenoviruses is under consideration to replace immunotyping for designating new adenovirus types.[31,36]

Serological Assays. Acute adenovirus infection can also be diagnosed by serological demonstration of a fourfold or greater rise in antibody titer between paired acute-phase and convalescent-phase sera. The first acute specimen should be collected as early as possible after onset of illness and the convalescent specimen should be collected 2 to 4 weeks later. Serologic tests for adenoviruses have included complement fixation (CF), HI, SN, indirect immunofluorescence, and enzyme immunoassay (EIA).[169] The classical CF test measures adenovirus-group specific antibodies, and though once widely used, lacks sensitivity. The EIA also measures adenovirus group-specific antibodies, but is more sensitive than the CF test and is readily automated and therefore more widely used today.[40,101,219] HI and SN assays also are more sensitive than CF, but because they measure type-specific antibodies, they are not suitable for routine diagnosis and most reagents are not commercially available.[40] Although valuable for outbreak investigations and essential for seroprevalence and vaccine efficacy studies, serology is rarely used today for clinical diagnosis because of the inconvenience of serum collection and the 2- to 4-week delay needed to obtain convalescent serum.

Treatment and Prevention With Antimicrobials

Currently, there are no FDA-approved treatment or prophylaxis therapies against adenovirus infections. There are numerous off-label therapeutic case or case series reports, but their results are mixed. There are few, if any, controlled, prospective therapeutic studies.[220] In the 1960s, γ-globulin was studied as prophylaxis against adenovirus infections without good effect.[221] However, Maisch and colleagues found a positive effect of intravenous immunoglobulin therapy in patients with adenovirus-associated inflammatory cardiomyopathy.[222] Some studies suggest that interferon-β may have a beneficial effect on EKC and viral cardiomyopathies.[223,224] More recently, adoptive transfer of adenovirus-specific T-cells has been explored with some success but this is now reserved for immunocompromised patients who do not respond to antiviral therapy.[225]

Off-label antiviral use reports of the antivirals ribavirin, cidofovir, ganciclovir, acyclovir, and vidarabine[220,226] have also been disappointing. Only ribavirin and cidofovir have been used often enough to be worthy of discussion. Nebulized ribavirin was used with some success for two children with pneumonia caused by adenovirus.[227] Intravenous ribavirin has

been reportedly used in immunocompromised patients and in patients with hemorrhagic cystitis and disseminated disease.[219,228–231] Intravenous ribavirin was given to 12 adult blood and marrow transplant recipients without appreciable benefit.[232] The mixed reports may be explained by a recent finding that only human adenoviruses of species C are susceptible to ribavirin.[190] In contrast, all species of human adenovirus appear to be susceptible to cidofovir.[190] Perhaps this finding explains the better results with this antiviral drug. In a retrospective study of 45 bone marrow transplant recipients treated with cidofovir for adenovirus infections, 31 (69%) had successful outcomes; however, in 18 (40%) of the 45 patients, cidofovir-associated toxicity developed.[233]

A new experimental oral antiviral therapy against DNA viruses, brincidofovir (formerly CMX001), shows great promise against adenoviruses.[225,234,235] An analog of cidofovir, it does not have cidofovir's renal toxicity profile. Currently, brincidofovir is being evaluated against DNA viruses including adenoviruses. It has been awarded "Fast Track Designation" by the FDA.

EPIDEMIOLOGY

Incidence and Prevalence Data

Because only approximately 50% of childhood adenovirus infections result in disease, prevalence as detected by antibody studies is high.[42,236,237] Those in species C (Ad1, Ad2, Ad5, and Ad6) are usually endemic and acquired in early childhood, often before 2 years of age.[39] By school age, most children have been exposed to several types of adenoviruses. Infections caused by Ad4, Ad7, Ad14, and Ad21 may occur at a later age. Many of the other types occur sporadically or in epidemics. Many adenovirus infections are subclinical or asymptomatic, especially those in species A and D. Conversely, Ad4, Ad7, and Ad21 usually cause symptomatic respiratory disease.[39]

Most types of adenoviruses are thought to be endemic worldwide.[45] In 1983, Schmitz and colleagues[45] summarized 10 years of adenovirus reports sent to the World Health Organization. They noted an increasing prevalence of Ad7, Ad8, and Ad19 and a declining prevalence of Ad3 and Ad4.[45] Age predilections were highly significant for infants for species A (Ad12, Ad18, and Ad31); for infants and small children for species C (Ad1, Ad2, Ad5, and Ad6); for school children for Ad3; for school children and adults for Ad7; and for adults for Ad4, Ad8, and other types of species B, D, and E.[45] A predilection for males was observed for all types in species B and C and in Ad4 and Ad19.[45]

A summary of 2237 clinical adenovirus submissions to 22 U.S. laboratories during a 25-month period (2004–2006) revealed that Ad3 (prevalence: 34.6%), Ad2 (prevalence: 24.3%), Ad1 (prevalence: 17.7%), and Ad5 (prevalence: 5.3%) explained most illnesses among children. The authors noted an increasing prevalence of Ad21 over time.[177]

Even though most types of adenovirus can be found throughout the world, sometimes marked differences are observed in the geographical distribution of specific genotypes. Of particular note are the distributions of Ad3, Ad4, and Ad7 genotypes, with anecdotal suggestions that some genotypes may be more virulent and have competitive advantage.[238] As yet, no clear markers of increased adenovirus virulence have been identified.

In 2009, Lebeck and colleagues[26] reported on 516 clinical Ad3 isolates from 15 U.S. laboratories with restriction enzyme digests. The most prevalent types were Ad3a2 (36.9%), Ad3a50 (27.1%), Ad3a51, (18.0%), and Ad3a17 (4.6%). Ad3a50 and Ad3a51 were newly described strains that became more prevalent in 2006. Multivariable modeling revealed that children younger than 2 years; persons with chronic disease; and persons infected with Ad3a2, Ad3a50, or multiple or rare strains were at increased risk of severe Ad3 clinical disease. Similarly, beginning in 1986, Chile, Uruguay, and Argentina experienced the emergence of a novel Ad7h strain that supplanted the endemic Ad7c strains.[239,240] Among pediatric patients, the Ad7h strain was associated with longer hospitalizations, higher patient temperatures, greater oxygen requirements, and 55% secondary attack rates among siblings, and it explained 94% of adenovirus mortality.[241,242] Similarly, first detected in 1992 in Israel, Ad7d2 spread to the United States in 1996 and since then has been associated with three of the five documented U.S. civilian outbreaks of Ad7[24] and at least one military outbreak.[20] These Ad7d2 outbreaks led to at least 19 deaths and the suggestion that this new genotype was a more virulent strain. In a 2005 report from Iowa, Ad7d2 had displaced other Ad7 strains.[243]

Most recently, a novel genotype of a seldom-detected Ad14 strain (classified as Ad14p1) emerged in the United States and has spread to other countries causing considerable morbidity and death.[28,146,147,244–249] It differs from the Ad14p prototype strain by markedly greater epithelial cell binding, cell cytotoxicity, plaque morphology, and minor mutations in the fiber knob and E1A genes.[250,251,251a]

High-Risk Groups

As noted, virtually all children have adenovirus infections, and, occasionally, these infections are severe and lead to death. Additionally, adenovirus infections are quite common among the immunocompromised and often associated with significant morbidity. Adenovirus epidemics often occur at intensive care units or long-term care facilities. For instance, in 1999 a novel Ad7b variant caused 84% of the 50 residents (age range: 1–46 years) of a New York long-term care facility to become ill, 26 were hospitalized, and 7 died.[252] In a 2007 national U.S. adenovirus study, the authors found risk factors for severe clinical adenovirus disease included age younger than 7 years, chronic disease, recent transplantation, and infection with Ad5 or Ad21.[177]

Epidemics among military populations, especially when adenovirus vaccines were not available, have been particularly well documented.[4] Before the use of vaccines in the U.S. military, Ad4 and Ad7 accounted for 60% of all ARD among hospitalized recruits. Ad3, Ad14, and Ad21 also occurred, but less commonly.[14] Up to 80% of recruits became infected with adenovirus, while seasoned military personnel experienced lower rates.[4] Such outbreaks have also been reported among military recruits in China, Finland, The Netherlands, The United Kingdom, Turkey, Singapore, and South Korea.[253–261]

Modes of Transmission and Reservoirs of Infection

The disease characteristics of the adenoviruses vary by species and type (see Table 10.1).[39] The reasons for these differences are not well understood. Adenoviruses are transmitted by direct contact, aerosolized virus, the fecal–oral route, and via water. Although recent data suggest that human Ad4 and Ad52 may have simian origins,[124,141] and a novel adenoviruses may have crossed species between humans and animals,[137,262] humans are thought to be by far the primary reservoir for human adenovirus.[263]

Significance as a Health Burden

The most common environment for adenovirus transmission is the home. However, high rates of adenovirus transmission

occur wherever large groups of susceptible people gather. Transmission rates are higher in children's institutions and daycare centers, and in lower socioeconomic groups.[46,47] Enteric adenoviruses may be an important pathogen in the daycare setting.[264] Epidemics associated with Ad3 often occur in association with swimming activities.[66] Ad8 has been associated with transmission in physicians' offices.[70]

Nosocomial outbreaks of adenovirus keratoconjunctivitis have been reported from the accident and emergency department of a major eye hospital in the United Kingdom.[265–267] Nosocomial conjunctivitis, pharyngitis, and pneumonia caused by adenoviruses have been noted in hospital intensive care units.[17,268,269]

With increasing numbers of immunocompromised patients in hospitals, adenovirus infections have become of more frequent cause of severe disease.[9] In addition, reactivation of a latent infection could possibly initiate a nosocomial outbreak. Persons with deficient cell-mediated immunity are at greatest risk for adverse outcomes.[39] Bone marrow transplant recipients are especially susceptible to adenovirus infections.[82,110] The death rate among immunodeficient patients with pneumonia may be as high as 60%.[9]

Chronic diarrhea in patients with AIDS is often a diagnostic problem. A prospective study using extensive diagnostic techniques, such as duodenal, jejunal, and rectal biopsies, demonstrated that 6.5% of the patients had adenovirus infections.[270]

Although the civilian experience has not established a requirement for a vaccine, the epidemic nature and extensive morbidity experienced by military recruits during the 1960s demonstrated an overwhelming need for adenovirus vaccines for military use. One well-studied and typical epidemic at Fort Dix, NJ, exemplified this requirement.[15] A platoon of 48 men was followed up prospectively for their 8 weeks of basic training. Of 92 episodes of respiratory illness, 24 required hospitalization. The documented hospitalization rate for ARD due to adenovirus Ad4 was 5/100 soldiers per week.[15] At large basic training posts, this rate translated to approximately 500 to 800 ARD admissions per week, which had a devastating impact on military hospitals.[15,19] Excess medical costs and the fact that soldiers had to be "recycled" owing to lost training time resulted in significant economic loss to the military. Serious disruptions to training schedules led to administrative attempts to control epidemics, such as sleeping head-to-foot and keeping military units separated (cohorting).[19] The impact of adenovirus infections in the military led to the development of adenovirus vaccines.

PASSIVE IMMUNIZATION

While years ago immunoglobulin therapy was not found to be particularly useful in preventing adenovirus infections among military trainees,[220] modern passive immunization strategies may prove helpful. In particular, recently isolating Ad-specific T cells from stem cell donors have been effective in treating hematopoietic stem cell transplantation recipients who develop severe adenovirus that is nonresponsive to antivirals.[37]

ACTIVE IMMUNIZATION

The first adenovirus vaccine prototype was an injectable bivalent Ad4 and Ad7 vaccine grown in monkey kidney cells and formalin-inactivated.[19] After safety tests, a small trial in 1957 reduced admissions for adenoviruses by 98%, and a large trial of 8238 soldiers was 90% effective in reducing hospitalization.[271,272] A trivalent vaccine, which also included Ad3, was also tested.[273] Large-scale production of vaccine lots led to variation in antigenicity, resulting in a lowering of protection rates, with only a 52% reduction in hospital illnesses caused by adenoviruses.[274] Even this low rate of protection was reported in 1965 to have saved the Army approximately $5 million a year.[274] Adenovirus seed lots were found to be contaminated with simian virus 40 (SV40), a virus oncogenic in animals, and in 1963, the license for the vaccine was rescinded.[15] Although this caused considerable concern at the time, an epidemiologic study of 1959–1961 era U.S. Army male veterans showed no evidence of an increased risk of various cancers that plausibly could be the result of an SV40 exposure.[275]

Use of a live vaccine for ARD caused by Ad7 was investigated, and oral administration was reported to induce high antibody levels in 1960.[276] Couch and colleagues[277] and Chanock and colleagues[278] demonstrated that some adenoviruses infect the gastrointestinal tract but do not produce symptoms in adults. This finding led to the administration of the virus as a vaccine in an enteric-coated capsule, which produced an asymptomatic, intestinal infection and neutralizing antibodies.[277,278] Many safety studies were performed in human volunteers, including the administration of Ad4 and Ad7 vaccines separately and simultaneously.[277] Neutralizing antibodies were elicited, and asymptomatic infections were evidenced by the recovery of virus from rectal specimens. Initial vaccine virus production using HEK cells was later modified to use human diploid fibroblast strains WI-26 and WI-38 because the primary cells were not suitable for large-scale production and there was concern of their contamination with other human pathogens.[278] Because some adenoviruses are oncogenic in animals, much attention was given to safety studies in hamsters and the transformation of cell lines by adenoviruses.[279,280]

Additional field trials for the Ad4 vaccine were conducted at Parris Island, SC, and Great Lakes, IL, indicating that the vaccine was highly protective, safe, and antigenic and showed no evidence of transmission among military trainees. Vaccine use led to reductions in ARD rates by 50%.[281,282] Intrinsic vaccine efficacy was as high as 82%.[282]

Use of the monotypic Ad4 vaccine was followed by the appearance of ARD caused by Ad7 at Fort Dix, NJ.[15] The Army Adenovirus Surveillance Program was initiated to identify the agents of ARD and to assess fluctuations in disease patterns.[14]

Before an Ad7 vaccine could be developed and licensed, numerous studies addressed the oncogenicity potential of Ad7 to humans.[283–286] A trial of the Ad7 vaccine demonstrated safety, infectivity, antigenicity, and lack of communicability similar to that observed for the Ad4 vaccine.[287] Additional trials indicated that when the Ad4 and Ad7 vaccines were given simultaneously, no decrease in immunogenicity occurred, and with mass immunization, there was 95% suppression of Ad7-associated disease.[287–289] Numerous outbreaks of Ad21 in military populations in the United States and Europe led to the initiation of studies using an Ad21 prototype vaccine in safety and immunogenicity trials.[290–294] Trials of Ad1, Ad2, and Ad5 vaccines were also conducted with the goal of protecting children.[295] However, Ad1, Ad2, Ad5, and Ad21 vaccine production was never pursued.

Beginning in 1971, live, enteric-coated, oral vaccines for Ad4 and Ad7 were routinely administered, and adenovirus morbidity was greatly reduced.[19] Ad4 and Ad7 vaccines were licensed by the FDA in 1980 and produced by Wyeth Laboratories. In 1994, the Department of Defense was notified that the sole producer of Ad4 and Ad7 vaccines, Wyeth Laboratories Inc, Marietta, PA, would stop production permanently. Beginning in 1984, Wyeth repeatedly notified the Department of Defense of the need to negotiate a contract that would consider the renovation of the existing vaccine production facility.[296] The contract was not renegotiated.[296] Bulk vaccine production ceased in 1995, and the last vaccine was placed in

tablet form in 1996. Through requests for extensions of expiration dates and use of vaccines only during September through March, rather than year-round, the U.S. Department of Defense attempted to obtain maximum benefit from the remaining supply. By 1999, all vaccine supplies were depleted.

After the loss of the manufacture of the Ad4 and Ad7 vaccines, there were many documented outbreaks of ARD due to adenoviruses in U.S. military training centers. The first recorded epidemic took place during April-May 1995, at Fort Jackson, SC, during a lapse of vaccine administration that occurred because of a logistical error that temporarily interrupted vaccine production.[21] Ad4 was identified as the etiologic agent in unvaccinated soldiers who experienced hospitalization rates of 11.6% during basic training.[21] Numerous additional outbreaks occurred at the other U.S. military camps,[20,22,297] some causing large numbers of hospitalizations[22,297,298] and some spreading to more advanced training camps.[299] Results of a nationwide seroprevalence survey among unimmunized U.S. Army trainees confirmed the lack of protective neutralization antibodies to Ad4 and Ad7, with nearly 90% being susceptible to at least one type.[299,300] Evaluation of the epidemic, clinical, and immunological risk factors for adenovirus infection was performed for a subset of patients, demonstrating that anti-Ad4 immunity was low in new recruits, risk of illness was higher in smokers, and 81% of patients from whom paired serum samples were collected demonstrated a fourfold or higher increase in anti-Ad4 titer after infection.[301]

Gray and colleagues provided a concise overview of population-based surveillance for respiratory disease at four U.S. military training centers as the last of the stores of vaccines were depleted.[302] Between October 1996 and June 1998,53.1% of 1,814 throat cultures from symptomatic trainees yielded adenoviruses.[302] Ad4, Ad7, Ad3, and Ad21 accounted for 57%, 25%, 9%, and 7% of the isolates,[302] respectively (although the prevalence of Ad7 detections has markedly declined in recent years).[303] Russell and colleagues[304] examined an estimated 73,748 clinical cases of adenovirus infection that occurred during the period of 1999 to 2004 among U.S. military trainees at eight training sites. They observed that adenovirus explained from 52.3% to 76.4% of detected febrile respiratory illnesses at these sites.[304] The aggregate number of adenovirus infections among U.S. military trainees has fluctuated with the availability of vaccines and season (Fig. 10.3). In some winter months, more than 2200 preventable clinical infections were documented. Realizing that the detected adenovirus illnesses greatly underestimated true clinical disease, Russell and colleagues[304] posited that the true count of clinical adenovirus disease occurring in these camps was an estimated 45,000 cases per year, 90% of which would be prevented were the adenovirus vaccines available. This realization of preventable morbidity was not lost to public health officials inside and outside the U.S. Department of Defense, with many calling for reacquisition and use of the vaccines.[305–308]

On September 25, 2001, 7 years after Wyeth announced permanent cessation of production of the adenovirus vaccines, two widely publicized adenovirus-associated deaths in nonimmunized recruits,[309] and strongly worded letters from the Institute of Medicine and the Armed Forces Epidemiological Board to expedite contract efforts, induced the Department of Defense to contract for production of adenovirus vaccines.[296,306,310,311] The decision to resume production was based on a continuing low prevalence of antibodies to Ad4 and Ad7 in people coming into the military,[300] the occurrence of documented outbreaks when the vaccines were not given,[20–22,302] and almost 30 years of experience of not having ARD outbreaks when potent adenovirus vaccines were being

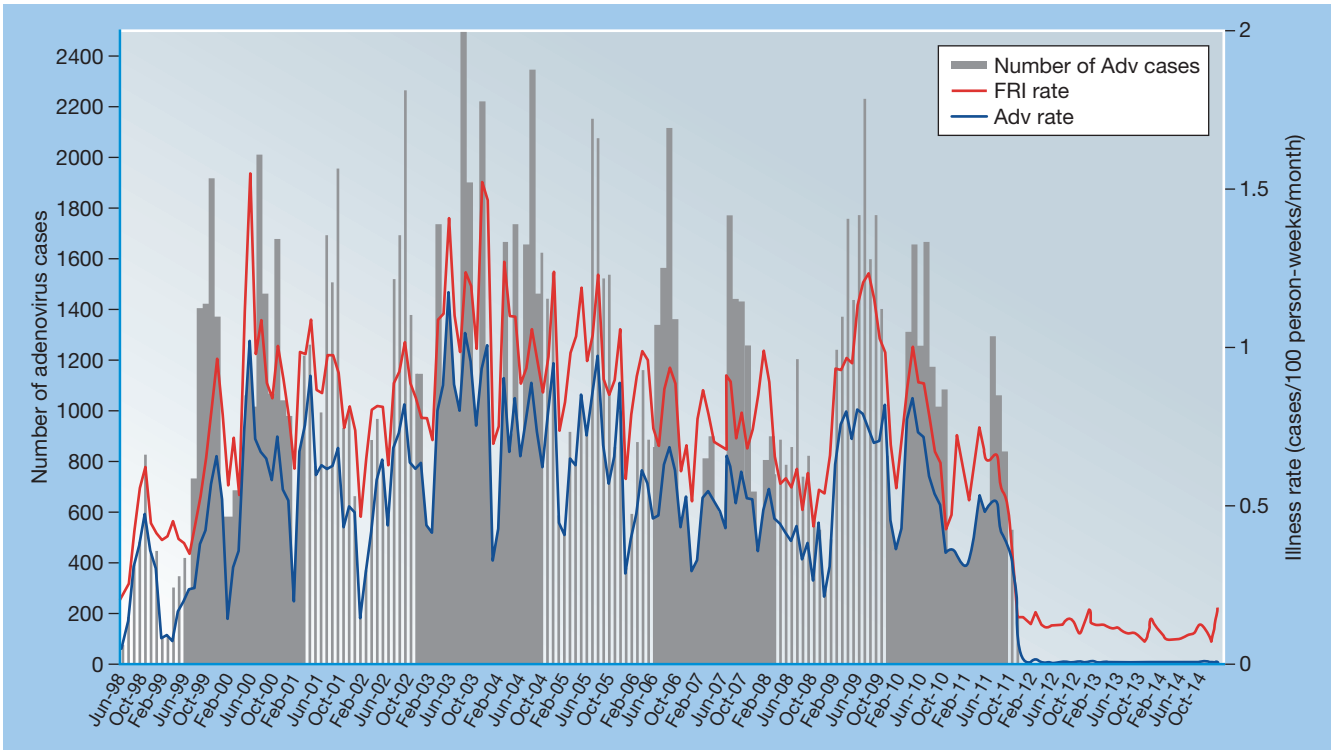


Figure 10.3. Febrile respiratory illness (FRI) and adenovirus (Adv) rates per 100 person-weeks per month with estimated monthly number of adenovirus-associated clinical encounters among trainees at eight U.S. military training sites, June 1998 through December 2014. *(Courtesy Anthony Hawksworth, Naval Health Research Center, San Diego, CA.)*

administered.[19] A genomic analysis of the Wyeth Ad4 and Ad7 vaccine strains show no evidence of attenuation.[312] In 2001 the contract was awarded and seed viruses strains from Wyeth were transferred to Barr Laboratories Inc. (now part of Teva Pharmaceuticals Industries, Inc.).

In the fall of 2004, the Teva Ad4 and Ad7 vaccines were studied in a Phase I double-blind, placebo-controlled trial involving 58 Army medic trainees who had just completed U.S. Army basic training.[313] Among the subjects who had been previously exposed to adenovirus during basic training and before enrollment, 79% and 78% were seropositive to Ad4 or Ad7, respectively. For the trial, 58 initially seronegative subjects were selected. Both vaccines were given to 30, and 28 received placebo tablets. For 32 subjects, final follow-up was permitted at day 180. The participants were closely followed up with cultures, symptom diaries, and clinical examinations during a 56-day period for evidence of vaccine reactions and adenoviral disease. The most commonly reported adverse events in the vaccine recipients were nasal congestion (33%) and dry cough (33%), sore throat (27%), headache (20%), abdominal pain (17%), arthralgia (13%), nausea (13%), and diarrhea (13%). Symptom reporting did not differ between vaccine and placebo groups. Vaccine virus was shed in the stools of 73% and 71% of volunteers seronegative to Ad4 and Ad7, respectively, for as long as 21 days after vaccination. By day 28 after vaccination, 73% of vaccine recipients seroconverted to Ad4, while 63% seroconverted to Ad7. A randomized, double-blind, placebo-controlled Phase II/III trial (NCT00382408) of these vaccines was conducted in 2006–2007 at two military training facilities. Ad4 and Ad7 vaccine or placebo was administered to 4040 U.S. military trainees in a 3 : 1 ratio. As described above the vaccine was found to have excellent efficacy (99.3% for Ad4) and safety profiles similar to the previously manufactured Wyeth vaccine.

In March 2011, after an arduous 10-year vaccine development process, eight likely preventable adenovirus-associated deaths,[314] thousands of hospitalizations, and more than $100 million in funding,[315,316] Teva was awarded FDA licensure for their Ad4 and Ad7 vaccines. The vaccines are indicated for military personnel ages 17 to 50 years of age and contraindicated for persons who are pregnant, who cannot swallow the vaccine tablets without chewing, or who have a severe reaction to the vaccines.

Constituents Including Antibiotics, Preservative, Adjuvants, Etc.

Wyeth vaccine tablets contained live viruses, materials added for the growth and maintenance of viruses and cells, and other pharmaceutical materials. Human-diploid fibroblast cells (strain WI-38) were used for virus preparation, and growth was maintained in Minimal Essential Medium, Eagle solution, antibiotics (neomycin sulfate, gentamicin sulfate, and amphotericin B), fetal bovine serum, and sodium bicarbonate. After harvesting, the viral growth was freed of particulate material by filtration and dried by lyophilization. During drying, additives were used to preserve viability. Before processing into tablets, the virus preparation was diluted with lactose powder.[317]

Using the same Wyeth master seed viruses and a manufacturing process similar to the process Wyeth used, Teva is now producing the Ad4 and Ad7 vaccines. Teva's tablets consist of three layers: a central core containing at least 4.5 $\log_{10}$ tissue culture infective dose ($TCID_{50}$) of lyophilized formulated adenovirus mixed with anhydrous lactose, microcrystalline cellulose, polacrilin potassium, and magnesium stearate, an outer layer of inactive excipients, and a protective enteric coating consisting of cellulose acetate phthalate, alcohol, acetone, and castor oil (Fig. 10.4).[318] The Ad7 vaccine tablet also contains FD&C Yellow #6 aluminum lake dye to give it a distinguishing yellow color.

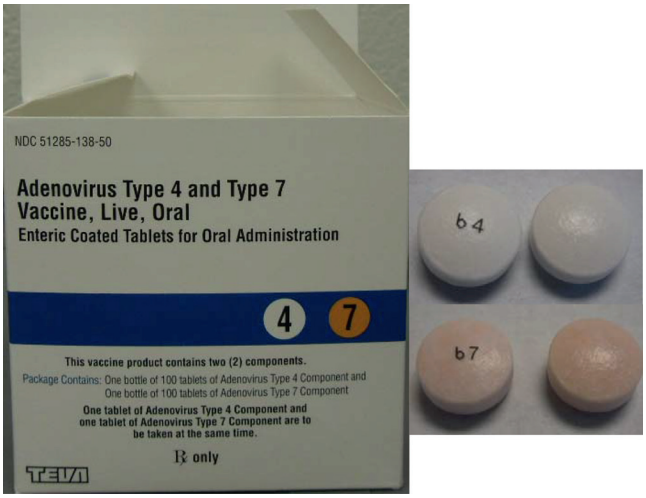


Figure 10.4. Teva Pharmaceuticals Industries Ltd. enteric-coated adenovirus type 4 and type 7 vaccine packaging and tablets. The adenovirus type 7 (b7) tablet is yellow. The enteric coat is designed to resist stomach acid. *(Courtesy John Shaw, Teva Pharmaceuticals, Inc.)*

Preparations Available, Including Combinations

The Teva adenovirus vaccines contain live nonattenuated Ad4 or Ad7 in the form of enteric-coated tablets. They are produced similarly to how the Wyeth vaccines (described above) were produced. The final vaccine is composed of two tablets (one tablet of Ad4 and one tablet of Ad7) designed to pass intact through the stomach and release the live virus in the intestine. Each enteric-coated tablet contains an inner core tablet containing anhydrous lactose, microcrystalline cellulose, polacrilin potassium, magnesium stearate, and live adenovirus, either Type 4 or Type 7, at a potency of no fewer than 32,000 tissue-culture infective doses (4.5 $\log_{10}$ $TCID_{50}$) per tablet. The outer tablet layer contains microcrystalline cellulose, magnesium stearate, and anhydrous lactose, with an enteric coating consisting of cellulose acetate phthalate, alcohol, acetone, and castor oil. The Ad7 tablet also contains FD&C Yellow #6 aluminum lake dye.

The Ad4 vaccine (white tablets) and the Ad7 vaccine (yellow tablets) are packaged in separate bottles and the bottles are combined in a single package.[318] The tablets can be administered simultaneously but have to be swallowed without chewing. Vomiting and diarrhea may interfere with vaccine effectiveness. These vaccines are indicated for military populations shown to be at risk of ARD from the specific adenovirus types represented in the tablets.

The adenovirus vaccines are routinely given to military recruits and U.S. Coast Guard cadets along with numerous other vaccines in the first 8 days after entering training. Type-specific neutralizing antibodies to the adenovirus vaccines are produced in the vaccinated trainees and were associated with protection against ARD. Interference with immune responses when the adenovirus vaccines were given with other vaccines has not been identified.[319]

DOSAGE AND ROUTE: INTRAMUSCULAR, SUBCUTANEOUS, AND ORAL

The initial oral Ad4 vaccine was developed using dried virus contained in enteric-coated capsules and found to be effective in preventing infections.[278] Original trials for Ad7 vaccine were conducted using 0.05 mL of a 1:10 dilution of the Ad7 pool (10^6 $TCID_{50}$) within a hard gelatin capsule and given orally.[277] Time of disintegration within the intestine was assayed roentgenographically using a barium sulfate–containing capsule and varied between 1 and 5 hours.[277] Two small dose ranging studies using vaccine capsules suggested that for both types, nearly 100% seroconversion was achieved by approximately 10^4 $TCID_{50}$ per capsule.[289,313] A 1969 dose-ranging study used three Ad7 doses: $10^{6.8}$ $TCID_{50}$, $10^{4.8}$ $TCID_{50}$, and less than 10 $TCID_{50}$ per capsule.[277,289] There was 100% antibody response with the highest dose, 95% response with the intermediate dose, and 56% response with the lowest dose. Another study evaluated the response when both vaccines were given individually at doses of $10^{5.4}$ $TCID_{50}$ for Ad7 and 10^4 $TCID_{50}$ for Ad4, and simultaneously for both vaccines at the same doses.[287] There was no decrease in the immunogenicity of the Ad4 vaccine when it was given with the Ad7 vaccine.[289] In the mid 1960s, Wyeth Laboratories took over production of the adenovirus vaccines, which were converted to enteric-coated tablets.

Vaccine Stability

The currently licensed Teva vaccines are stored between 2°C and 8°C with a shelf life of 30 months.

IMMUNOGENICITY OF VACCINE

Humoral Responses

Oral adenovirus vaccine recipients routinely shed virus fecally, which can potentially continue for up to 6 weeks.[292,320] Wyeth vaccine recipients developed neutralizing humoral antibody (immunoglobulin [Ig] G, IgM, and IgA).[292] In soldiers who were free of preexisting antibody, an average of 80% to 95% developed a neutralizing antibody level of greater than 1:8, while fewer than 50% demonstrated complement-fixing antibody.[289,321] Neutralizing antibody responses were detected 2 to 3 weeks after vaccination. In general, antibody titers were less than titers achieved after natural infection.[8,282] Local secretory IgA antibody was not induced by the oral vaccine, and reinfection of the respiratory tract was possible, but usually mild or asymptomatic.[322] Since viremia and viruria could occur in patients with febrile disease, invasiveness beyond mucosal surfaces could be important in the pathogenesis of the disease and infection.[323] The serum-neutralizing antibody produced as a result of vaccination may have prevented the typical febrile disease associated with natural infection.[321] Local IgA antibody could be produced experimentally by the intranasal inoculation of an Ad4 vaccine in liquid.[322] A Phase I/II study of the Teva oral Ad4/7 vaccine showed 73% and 63% of immunonaïve subjects seroconverted to Ad4 and Ad7 at 28 days postvaccination.[313] A Phase III study of the same vaccines documented seroconversion at 94.5% (Ad4) and 95% (Ad7).[324]

Cellular Responses

The cellular response to Wyeth Ad4 and Ad7 vaccines has not been well studied. However, the cellular immune responses to some adenovirus-vectored vaccines have been excellent.[325]

Correlates of Protection

A SN assay value of 1:4 against a specific virus is considered as protective.[315] A fourfold rise in titer is considered as evidence of infection.

Special Groups (e.g., Immunosuppressed)

Although some early Ad1, Ad2, Ad4, and Ad5 vaccine trials were conducted among civilians,[320,326,327] the Teva vaccines have not been used in groups other than healthy military trainees.

EFFICACY AND EFFECTIVENESS OF VACCINE

Military recruits who received Ad4 vaccine exhibited increased resistance to respiratory disease caused by this virus.[278,281,328] A number of major well-controlled studies of more than 42,000 soldiers were performed from 1963 to 1966.[329] Use of the vaccine reduced ARD by 50% and adenovirus infection in recruits by more than 90%.

Field trials began in 1969 for the Ad7 vaccine. The trials indicated protection against disease in susceptible persons.[288,289,330] In addition, it was found that the two vaccines (for Ad4 and Ad7) could be administered simultaneously without interference or loss of efficacy.[288,289,331] As a result of using the vaccines at one large Air Force base during a 9-year period, ARD caused by adenovirus disappeared.[332] The adenovirus surveillance program demonstrated that the combination of the two adenovirus vaccines was highly effective in controlling epidemic ARD.[14] From 1971 to 1999, the live enteric-coated Ad4 and Ad7 vaccines were administered to new military recruits and successfully controlled ARD caused by adenoviruses.

The Phase III double-blind, placebo-controlled efficacy trial of the Teva Ad4 and Ad7 vaccines conducted in 2006 found the vaccines to be 99.3% effective in reducing Ad4-caused ARD.[324] Two years after their reintroduction, the Teva vaccines were credited with a 100-fold decline in military trainee adenovirus cases and with the prevention of 1100 to 2700 hospitalizations each year.[303]

Duration of Immunity and Protection, Including Description of Reinfection, If Any

The duration of immunity and persistence of circulating antibody following immunization has not been well studied. The adenovirus vaccines were developed to protect new members of the military (recruits) against Ad4 and Ad7 respiratory disease during the first months of military service. The adenovirus vaccines were very effective in accomplishing that objective, and immunity beyond 56 days has not been studied.

Safety

Common Adverse Events

Four study groups were followed up for inpatient and outpatient episodes of illness during vaccine safety and immunogenicity trials at Lackland Air Force Base, TX, in 1976. In addition to the placebo group, one group received three vaccines simultaneously (Ad4, Ad7, and Ad21), another group received two vaccines simultaneously (Ad4 and Ad7), and the last group received one vaccine only (Ad21).[294] No appreciable differences were noted in the inpatient or outpatient experiences of the different groups.[294]

Studies of the various live adenovirus vaccines have documented fecal and pharyngeal shedding of vaccine virus and

transmission to nonvaccinated study participants.[295,320,321,324,333] Although recent trials of the Teva vaccine demonstrated sparse transmission among military personnel,[324] the possibility of transmission of vaccine virus to other susceptible personnel should be considered for up to 4 weeks after receipt of modern vaccines.

Rare Adverse Events

In 1973, a Navy trainee was hospitalized with fever, malaise, and dyspnea for 11 days after receiving the Ad4 and Ad7 vaccines.[334] The patient died on the 10th hospital day with the diagnosis of Ad7 pneumonia, based on the isolation of the virus by cell culture. However, it was not possible at the time to determine whether his infection was caused by a wild Ad7 before the development of vaccine-induced immunity or to an Ad7 vaccine strain.[334]

Immunocompromised Recipients

As adenoviruses may cause severe disease among immunocompromised persons, when HIV was first discovered, concern grew among U.S. military public health officials that adenovirus vaccine might cause severe disease among HIV-infected military trainees. Subsequently, a study was conducted among HIV-infected and uninfected military trainees. Although significantly fewer HIV-infected subjects responded to the Ad4 vaccine than did soldiers without HIV infection, no clinically apparent adverse reactions were detected.[319] Response to the Ad7 vaccine was difficult to define because many vaccine recipients in both groups had high neutralizing antibody titers at the time of vaccination.[319] More severely immunocompromised persons, who might inadvertently be infected with adenovirus vaccine virus through fecal shedding from "normal" vaccine recipients, have not been studied.

Pregnancy

The live, oral adenovirus vaccines were never subjected to animal reproduction studies because of the lack of a suitable animal model. Therefore, it is not known if these vaccines had the potential to cause fetal damage if given to a pregnant woman or to affect reproductive capacity. Female military trainees are screened with pregnancy tests before they received the adenovirus vaccines. During the recent Teva vaccine trials four of the subjects were found to be pregnant after they had received the Ad4 and Ad7 vaccines.[318] All four subjects delivered healthy babies at 36 to 40 weeks' gestation. Unusual morbidity among pregnant vaccine recipients has not been reported.

Spread to Contacts

Because the virus may be shed fecally for up to 6 weeks, the virus may be spread to family members or very close contacts.[320,326] Hence, vaccine recipients are instructed to maintain good hygiene and to avoid contact with children younger than 7 years of age, the immunocompromised, and pregnant women for 28 days following vaccination.[318] The spread of vaccine virus has not been well studied. In the recent Phase II trial of the Teva vaccines, participants were followed shedding on days 0, 7, 14, 21, 28, and 56. Periodic fecal shedding was noted 7 to 21 days following vaccination.[313]

INDICATIONS FOR VACCINE—WHO AND WHY

Adenovirus vaccines have been indicated for the prevention and control of specific adenovirus-associated ARD in populations with a high risk of exposure, a high level of susceptibility, and a high risk of subsequent infection and disease. Adenovirus vaccines were recommended for use in military populations at risk of developing ARD from adenoviruses. Use of these vaccines was not recommended for other populations. Current and future studies of virus transmission and disease occurrence in pediatric populations, chronic care, and other civilian institutional settings; high-level healthcare organizations; and colleges may identify other potential indications for adenovirus vaccines.

In 1971, the U.S. military began routinely administering both vaccines to males reporting to recruit training centers, but only during the winter months.[15] The incoming recruits were given the vaccines within hours of arrival at a training center to obtain protection as early as possible in the training program. Initially, women were not given the vaccines because ARD outbreaks caused by adenoviruses had never been documented among military women, and there was concern about the possibility of administering the vaccines to women who were pregnant.[19]

The program of administering the vaccines only during the high-risk winter months was directed at control, rather than eradication of ARD caused by adenoviruses.[15] With this schedule, late spring and early fall outbreaks occurred. These outbreaks prompted the U.S. Army and the U.S. Navy to adopt policies of year-round administration of both adenovirus vaccines in 1983.[19] Taking a different course, the U.S. Air Force stopped the administration of adenovirus vaccines at its only recruit training center in Texas in the mid-1980s and adopted a program of surveillance with use of the vaccines only as indicated.[19]

When the military started immunizations to protect against ARD caused by adenoviruses, training programs were segregated by gender. These separate training programs have since been combined, leading to concern that the risk for ARD caused by adenoviruses would be the same for men and women. The U.S. military regulation for immunizations required that, based on risk, Ad4 and Ad7 vaccines be administered simultaneously and only once to Army, Navy, and Marine Corps recruits.[335] In the Air Force and Coast Guard, the vaccines were administered when directed by the appropriate authority.[335] The same regulation described precautions to be taken to avoid unintentional administration of the vaccines during pregnancy and counseling instructions regarding the possibility of pregnancy within 3 months following immunization.[335]

In response to the cessation of adenovirus vaccine production in 1996, the Army, Navy, and Marine Corps modified their policies of year-round vaccine administration to conserve the remaining vaccine for use in higher-risk months only. The modified policies directed that the vaccines be given to arriving military recruits only during the period of September 1 through March 31 until all vaccine stocks were exhausted in 1999.

CONTRAINDICATIONS AND PRECAUTIONS

Teva uses no antibiotics in the virus propagation steps. The vaccine is contraindicated for pregnant women, immunocompromised patients, persons outside the target age range of 17 to 50 years, persons who cannot swallow the tablets whole, or persons who are allergic to the vaccines.

PUBLIC HEALTH CONSIDERATIONS

Epidemiologic Effects of Vaccination, Including Herd and Contact Immunity

In the prevaccine era, ARD from adenoviruses caused significant morbidity at military training centers. These outbreaks

were extremely costly in terms of medical care requirements and time lost from training.[19] The routine administration of the Ad4 and Ad7 vaccines, which began in 1971, resulted in an extremely efficacious, cost-effective, and safe immunization program.[19,336–338] Initially, the vaccines were administered only during the high-risk winter months. The occurrence of spring and fall outbreaks prompted a modification of the program to administration of the vaccines year-round.[19] From 1971 to 1999, except for a period in the 1970s when ineffective vaccines thought to be contaminated with solvent were used,[15] there has never been a reported outbreak of ARD caused by Ad4 or Ad7 in U.S. military units that received the vaccines. Outbreaks of ARD caused by Ad14 and Ad21 have occurred, but these have been sporadic.[19,294,339]

A problem in the administrative system used to procure vaccines for the U.S. military resulted in an adenovirus vaccine production delay that began in the spring of 1994 and lasted until late February 1995.[19] There was only one outbreak of ARD caused by adenoviruses reported in conjunction with that production delay.[21] When Wyeth Laboratories announced in 1994 that it would no longer produce the adenovirus vaccines, many in the U.S. Department of Defense and the different military services asked if the vaccines were still needed. Some thought that improved military barracks with modern heating, ventilation, and air-conditioning systems may have significantly reduced the risk of transmission of the adenoviruses and subsequent disease in susceptible people. The assessment of current risk was hampered by the lack of modern seroprevalence data for Ad4 and Ad7 among new military entrants and the lack of studies of adenovirus carriage, transmission, infection, and disease because the vaccines had been so successful. The U.S. Department of Defense also found it had lost the capability to perform serologic studies for adenovirus infections. Unfortunately, with the loss of the vaccines, adenovirus-associated ARD increased in all uniformed services, including the U.S. Air Force.

Disease Control Strategies

Ad4 and Ad7 vaccines have been the primary means for controlling adenovirus-induced ARD among U.S. military trainees. Secondary control measures have included reducing crowding, aligning trainees' beds head-to-foot, making frequent hand washing a requirement, increasing air exchanges, and keeping contact between different cohorts of recruits to a minimum (cohorting).[340–342] Although interventions like frequent hand washing[340] caused a 45% reduction in upper respiratory tract infections among trainees, the numerous attempted interventions fell far short of the effectiveness of administering adenovirus vaccines in reducing ARD.

Cost-to-Benefit Information

The excess morbidity caused by ARD and associated costs are of significant concern to the U.S. military. A number of cost-to-benefit analyses have been performed each showing significant benefit in using the vaccines.[336–338] Using an estimated cost for receipt of both vaccines at $150 and using the vaccines year-round in approximately 200,000 trainees per year, Radin and colleagues,[303] estimated the U.S. Department of Defense would prevent approximately 6000 to 13,000 clinical adenovirus cases and 1100 to 2700 hospitalizations per year, saving the U.S. government approximately $20 million per year in training and medical costs.

FUTURE VACCINES

Subunit Vaccine

In 1963, soluble viral subunit antigens were found to be highly immunogenic on parenteral administration in animals.[321,343] Crystalline hexon and fiber antigens from Ad5 have been shown to induce neutralizing antibody and protection in human volunteers who were challenged.[344] There has been no additional development of these antigens as potential vaccines. However, adenovirus vaccines consisting of soluble viral subunit antigens would be free of DNA and could alleviate fear of the oncogenic potential of adenoviruses.[321]

Recombinant Vaccines

Advances in molecular biology have permitted the in vivo and in vitro gene transfer into mammalian cells using various adenovirus constructs. Genetically engineered adenoviruses have been altered to carry genes from a wide array of other pathogens including influenza A virus,[345] Ebola virus,[346] and Middle East respiratory syndrome–coronavirus,[347] and to introduce genes as a therapy against various chronic diseases. Adenovirus vaccine construct research is quite dynamic. A detailed description is beyond the scope of this textbook. However, excellent reviews have been published discussing the advantages and drawbacks of recombinant adenovirus vaccine strategies.[348–355]

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11 Anthrax Vaccines*

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Anthrax, a zoonotic disease caused by *Bacillus anthracis*, has three forms: cutaneous, inhalational, and gastrointestinal. Mortality in untreated cutaneous cases is approximately 20%, but less than 1% if antibiotics are promptly given. Inhalational anthrax is almost 100% fatal if untreated, and gastrointestinal cases have an untreated mortality rate of 25% to 75%. Meningitis may be a complication of any of the three forms of disease. Natural cases are primarily associated with industrial, agricultural, or laboratory exposure. The natural disease is not a major global public health problem, although occasional epidemics occur. However, the intentional use of *B. anthracis* as a bioweapon in the fall of 2001 in the United States irrevocably altered our views of public health, not just for anthrax, but also for many other infections with bioterrorism potential.

Historically, anthrax is considered to have been the fifth and sixth plagues described in *Exodus* (circa 1491 BC). Hippocrates described the disease in approximately 300 BC. Europeans recorded epizootics and epidemics in the 16th century. Between 1750 and 1850, the disease in both humans and animals was described in detail, and the organism was characterized. An epidemic of presumed gastrointestinal anthrax occurred in 1770 in Haiti and involved approximately 15,000 people; it was attributed to the ingestion of contaminated beef during a famine.[1]

In the 1870s, Koch cultured *B. anthracis* on artificial media and demonstrated definitively for the first time the microbial etiology of an infectious disease. In 1881, Pasteur attenuated the organism and conducted a successful field test of his attenuated vaccine strain administered for livestock. Greenfield performed similar work at the same time.[2] In the late 1800s and early 1900s, cases of cutaneous and inhalational industrial anthrax involved rag pickers in Germany and woolsorters in England.[3] The term *woolsorters' disease* referred to inhalational anthrax. Because of the large number of reported cases in England, a wool disinfection station was established in Liverpool.[4] All incoming wool and other animal fibers were disinfected using formaldehyde baths before being further processed. Subsequently, the number of cases of anthrax among these workers decreased significantly.

Cases of human anthrax have been reported from almost every country. However, the actual number of cases in the world is at best an estimate. In 1958, Glassman[5] estimated the annual worldwide incidence at 20,000 to 100,000 cases. In the 1980s and 1990s, the total global reported disease decreased to an estimated 2000 cases annually.

Industrial cases occur primarily in European and North American countries, and are associated with the processing of animal materials, such as hair, wool, hides, and bones. Agricultural cases occur primarily in Asian and African countries and result from contact with diseased domesticated animals or their products, such as hair, wool, hides, bones, and carcasses, including meat.

In the United States, the earliest reports of animal anthrax came in the early 1700s from what is now Louisiana. Sporadic animal cases were reported later from almost every state. Areas with more regularly reported animal cases are now called anthrax districts and primarily include the Great Plains states. Human anthrax was first reported from Kentucky in 1824. Human cases subsequently occurred throughout the United States, with the majority reported from industrialized states in the Northeast. However, as the textile industry moved to other parts of the country, human cases arose in the new locations.

Several unusual epidemics have been reported since the late 1970s. The largest epidemic in modern times occurred in Zimbabwe, with approximately 10,000 human cases reported between 1979 and 1985, including approximately 7000 cases occurring in 1979 and 1980.[6–8] Most people had cutaneous lesions, but some gastrointestinal cases also were reported. The source of the infections was infected cattle.

Another unusual epidemic occurred in Sverdlovsk, Russia, part of the Soviet Union, in 1979, after an accidental release of spores from a military microbiology facility. In this epidemic, at least 77 human cases of inhalational anthrax with at least 66 deaths occurred among people exposed to aerosolized *B. anthracis* organisms.[9,10] Some cases also occurred in sheep and cattle grazing as far as 50 km downwind from the facility, possibly as a result of the same release, although natural anthrax outbreaks previously had been reported from the region. In 1995, Iraq admitted to the United Nations that it produced weapons containing anthrax spores and was prepared to launch them during the 1991 Persian Gulf War.[11]

In late September 2001, a Florida man developed inhalational anthrax, the first case in the United States since 1976 and subsequently died.[12] Initially, it was regarded as an isolated case, but it became the first among 11 confirmed inhalational cases and seven confirmed and four suspected cutaneous cases of anthrax reported from Florida, New York, New Jersey, the District of Columbia, and Connecticut.[13–15] Exposure to contaminated mail was the confirmed or apparent source of infection in all these patients.[13–16] Among cutaneous cases, lesions developed on the forearm, neck, chest, or fingers.[17] Of the 11 inhalational cases, the median age was 56 years (range: 43–94 years). The presumed incubation period from exposure to onset of symptoms ranged from 4 to 6 days.[15] Additional details of these cases will be subsequently described. An unusual epidemic of anthrax cases arising secondary to heroin injection began in Europe in 2009 and continues. Since the first report of injectional anthrax in a heroin addict in 2000,[18] there have been more than 70 laboratory confirmed cases with a 37% case fatality rate reported from four European countries, some with unusual clinical syndromes.[19–21] These included cases with compartment syndrome or necrotizing fasciitis and others who presented with acute abdominal pain and signs of peritonitis associated with groin heroin injection. In most cases, the typical lesion consisting of an initial papule progressing to eschar, as described below, was absent.

The incidence of human anthrax in the developed world is extremely low. However, the impetus for the development of an improved human vaccine is the threat of *B. anthracis* used

*Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

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as a biological weapon. This worrisome possibility was given credence by the 1979 Sverdlovsk incident and the 1991 Iraqi experience, and prompted the U.S. Department of Defense to begin anthrax vaccinations for some members of the U.S. Armed Forces on March 10, 1998. The 2001 bioweapon attacks in the United States confirmed fears and heightened interest in the effort to develop new vaccines. The specter of anthrax spores used as a bioweapon against civilian populations on a larger scale than that yet experienced poses potential catastrophic consequences.[22] Given that spores can persist in experimentally infected animals after treatment with antibiotics for well over 30 days,[23–26] the major efforts directed to the management of such an event include early diagnosis and postexposure prophylaxis with antibiotics and vaccination.

BACKGROUND

Clinical Description

There are three primary forms of anthrax: cutaneous, inhalational, and gastrointestinal.[27] Secondary meningitis can occur with all three forms but is most often observed with inhalational anthrax. Rarely, meningitis has been reported without a primary site identified. In the United States, approximately 95% of reported cases of clinical anthrax have been cutaneous and 5% inhalational.

Cutaneous Anthrax

The incubation period for cutaneous anthrax is 1 to 7 days (usually 2–5 days). The lesion is first noted as a small, pruritic papule. Within several days, the papule develops into a vesicle 1 to 2 cm in diameter. Occasionally, the initial papule is surrounded by a ring of vesicles, which coalesce to form a large vesicle. The vesicular fluid is clear or serous colored and contains numerous *B. anthracis* organisms and a paucity of leukocytes. Nonpitting edema and erythema may develop around the lesion. Pain is not present unless there is secondary infection. The vesicle may enlarge to 2 to 3 cm in diameter, sometimes becoming hemorrhagic. Systemic symptoms are usually mild and include malaise and low-grade fever. There may be regional lymphangitis and lymphadenopathy. Approximately 5 to 7 days after the skin lesion, the vesicle ruptures, revealing a straight-edged, depressed ulcer crater that develops a black eschar. During a period of 2 to 3 weeks, the eschar loosens and falls off, usually without leaving a scar. The evolution of the eschar is not affected by antibiotic treatment.

The lesions most often occur on an exposed part of the body, such as the face, neck, or arm. Large, irregularly shaped cutaneous lesions have been reported in some industrial cases after many organisms were rubbed into the skin. Occasionally, extensive ocular involvement occurs, with orbital involvement leading to damage to the lids and ductal system.

More severe cutaneous involvement is referred to as malignant edema, in which multiple bullae surround the site of the initial lesion and extensive local edema, induration, and toxemia are present. At times, the edema may be massive, extending from a primary lesion on the neck to the groin.

Rarely, multiple cutaneous lesions have been reported after multiple inoculations of spores through the skin. Reinfections have been rarely reported, but not confirmed.

Cutaneous anthrax in heroin users, arising mainly from IM injection of heroin, has been associated with varying clinical findings from more typical self-limiting skin lesions to atypical abscesses, cellulitis, compartment syndrome, and necrotizing fasciitis with accompanying systemic toxicity and sepsis. The cases of sepsis may result from coinfection with other organisms in the setting of traumatized tissue.[19–21]

Inhalational Anthrax

In 1 to 5 days after inhaling an infectious dose of *B. anthracis* organisms, nonspecific symptoms develop that include malaise, fatigue, myalgia, slight temperature elevation, and minimal nonproductive cough, usually without symptoms of an upper respiratory infection.[28] There may also be a feeling of precordial tightness. Auscultation of the chest may reveal rhonchi. A slight improvement may occur within 2 to 4 days, but then severe respiratory distress develops suddenly, including dyspnea, cyanosis, stridor, and profuse diaphoresis. In some cases, subcutaneous (SC) edema of the neck and chest may be present. Physical examination reveals a patient with toxic symptoms who has an elevated pulse, respiratory rate, and temperature. Physical examination may reveal signs of a pleural effusion. Widening of the mediastinum on radiographic examination of the chest is frequently seen, as are pleural effusions, that may be hemorrhagic. The leukocyte count may be moderately elevated. Shock may develop terminally, and death usually occurs within 24 hours of respiratory distress. Death likely is caused by lymphatic/vascular obstruction in the mediastinum, with pulmonary hemorrhage and edema associated with large hemorrhagic pleural effusions and toxicity.

The patients treated in the 2001 U.S. outbreak frequently reported chills, prolonged and profound fatigue, nausea or vomiting, and chest discomfort.[15] All had abnormal chest radiographs (Table 11.1),[14] with paratracheal and hilar fullness, and pleural effusions, infiltrates or both. In some patients, the initial findings were subtle. Among all eight patients who had not received antibiotics before diagnosis, *B. anthracis* grew in blood cultures drawn at initial examination. Of the 11 patients, six (55%) survived with aggressive supportive care and multidrug antibiotic regimens.[14,15] All five patients with fulminant signs of illness, with severe respiratory distress or hypotension, or meningitis at initial examination, died despite receiving appropriate intravenous antibiotics and supportive care.[13,14] In all fatal cases, autopsy revealed classical edematous mediastinitis with hemorrhage.

A literature review of articles published from 1900 to 2005, some of which include the patients from the 2001 U.S. outbreak, reports the clinical features of 82 cases of inhalational anthrax from 106 reports.[29] The symptoms and clinical features in the 2001 patients were similar to the other cases but the 2001 patients were more likely to have had therapy initiated during the prodromal phase, to have received multiple antibiotics, and to have had pleural fluid drainage. Additional cases occurred in drumhead makers in 2006[30,31] and 2008.[32]

TABLE 11.1 Initial Clinical Findings in 10 Patients With Bioterrorism-Related Inhalational Anthrax, October to November 2001

CHEST RADIOGRAPHY FINDINGS	
Any abnormality	10/10
Mediastinal widening	7/10
Infiltrates/consolidation	7/10
Pleural effusion	8/10
CHEST COMPUTED TOMOGRAPHY FINDINGS	
Any abnormality	8/8

From Jernigan JA, Stephens DS, Ashford DA, et al., for the Anthrax Bioterrorism Investigation Team. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis. *2001;7:933–944.*

Gastrointestinal Anthrax

Symptoms of gastrointestinal anthrax develop 2 to 5 days after the ingestion of contaminated meat. The initial symptoms of disease consist of nausea, vomiting, anorexia, and fever followed by abdominal pain and diarrhea, which may be bloody. Hematemesis, sometimes severe, may develop. In some cases, the presentation is that of an acute abdomen and has prompted surgical exploration of the abdomen. Physical examination reveals an elevated temperature, pulse, and respiratory rate. Sepsis with toxemia, shock, and death may develop.

Oral–oropharyngeal anthrax occurs when ingested organisms gain entrance to the SC tissues through the oral or oropharyngeal tissue. In these cases, local ulcers, fever, anorexia, cervical or submandibular lymphadenopathy, or edema may develop.

Bacteriology

B. anthracis, the causative agent of anthrax, is a large, Gram-positive, spore-forming, nonmotile bacillus (1.0 to 1.5 × 3 to 10 mm). The organism grows readily on sheep blood agar aerobically and is nonhemolytic under these conditions. The colonies are large, rough, and gray-white in color, with irregular, tapered, curving outgrowths that cause the typical "medusa head" appearance. A loop drawn up through a colony makes the disturbed part of the colony stand upright like whipped egg white. In the presence of high concentrations of carbon dioxide, the organisms form antiphagocytic capsules and two exotoxins, and colonies are noted to be smooth and mucoid. In tissue, the bacteria are encapsulated and appear singly or in chains of two or three bacilli. Bacterial identification is confirmed by the production of toxin, lysis by a specific γ bacteriophage, the presence of a capsule and cell wall polysaccharide confirmed by fluorescent antibody testing, and confirmed virulence when injected in mice and guinea pigs. Polymerase chain reaction (PCR) tests for toxin and capsule genes are also confirmatory. Genetic analyses of different isolates reveal that *B. anthracis* is one of the most monomorphic, homogeneous bacterial species known.[33,34]

The spores are quite resistant to environmental extremes and may survive for decades in soil (see "Epidemiology" below). Recent evidence also suggests that *B. anthracis* infected with lysogenic bacteriophage can survive in soil and colonize earthworm intestines for prolonged periods in the vegetative state and may serve as a source of infectious spores.[35] Viable spores were reported to persist for weeks to months within the lungs of rhesus monkeys after inhalation, while receiving antibiotic treatment. After the cessation of antibiotic treatment, residual spores remain viable and can cause fatal disease.[23–26]

Pathogenesis

The major pathogenic virulence determinants of *B. anthracis* are the capsule and two protein exotoxins, lethal toxin and edema toxin. More recently, additional factors have been shown to contribute to virulence, although to a lesser degree than the capsule and toxins. These other factors include a capsule depolymerase responsible for attachment of the capsule to the cell wall peptidoglycan,[36] a manganese adenosine triphosphate (ATP)-binding cassette transporter,[37] siderophore biosynthesis genes,[38] nitric oxide synthase,[39] a caseinolytic protease component (ClpX),[40] a surface protein that may be involved in cell adhesion (BslA),[41] and a high temperature requirement A gene involved in the bacterial response to stress.[42]

The importance of the capsule was appreciated early in the 20th century when Bail[43] demonstrated that organisms without a capsule were avirulent. However, extensive studies by Sterne[44] and others in the 1930s showed that nonencapsulated strains could induce immunity to anthrax. The strains developed by Sterne[45] have proved remarkably effective as live vaccines for domesticated animals and are used worldwide.

As is true for many bacterial virulence factors, the genes encoding the anthrax capsule are carried on an extrachromosomal 96-kb plasmid (pX02).[46,47] Anthrax strains lacking the capsule plasmid fail to produce capsule and are attenuated.[48] The capsule is a homopolymer of poly-D-glutamic acid and enhances virulence by making the organism resistant to phagocytosis and to lysis by cationic proteins.[49,50] Capsule released from the surface may also interfere with resistance[51] and with maturation of antigen presenting cells induced by peptidoglycan resulting in reduced chemotaxis of these cells to chemokines.[52]

A role for toxins in anthrax pathogenesis was suspected from the earliest studies by Koch,[53] but it was not firmly established until 1954, when Smith and Keppie[54] demonstrated that sterile plasma from experimentally infected guinea pigs was lethal when injected into other animals. Evans and Shoesmith[55] showed that *B. anthracis* culture filtrates produced edema after injection into the skin of rabbits. Additional work in the 1950s and 1960s evaluated the impact of toxins in disease and immunity.[56,57] Since the mid-1980s, many advances have been made in the understanding of the molecular biology of the toxins and their effects on multiple cell types in vivo and in vitro.[58] Nevertheless, their precise role in pathogenesis remains undefined. Anthrax has been characterized as being due to a large bacterium that produces a feeble toxin.[59] Although it is clear that anthrax is an invasive disease and that anthrax lethal toxin, when given intravenously, is relatively impotent compared with other bacterial toxins, the lethal and edema toxins are essential for virulence and thought to be important in the establishment of disease by impairing host defenses.

The anthrax lethal and edema toxins, like many bacterial toxins (e.g., diphtheria, tetanus, botulinum), have a binding domain by which they bind to target cell receptors and an active domain that is responsible for the biochemical and usually enzymatic activity of the toxin. However, the anthrax toxins are unusual in that the binding and active domains exist in two distinct proteins, and the two active domain proteins share the same binding protein. This binding protein is called protective antigen (PA). PA combined with a second protein called lethal factor (LF) constitutes the anthrax lethal toxin, which is lethal when injected into experimental animals.[60,61] The same PA combined with a third protein, edema factor (EF), constitutes the edema toxin, which causes edema when injected into experimental animals[60,61] and can also be lethal for some animals.[62] The edema toxin is responsible for the massive edema that may be present in cases of anthrax, whether in the skin or mediastinum. The 89-kDa EF is a calmodulin-dependent adenylate cyclase that raises intracellular cyclic adenosine monophosphate (AMP) levels.[63] The 85-kDa LF is a zinc metalloprotease that inactivates mitogen-activated protein kinase interfering with multiple host cell signaling pathways.[64,65] Toxicity in macrophages appears to be mediated through activation of caspase-1, requires a specific inflammasome protein, and results in lysosomal permeabilization.[66–68] Consistent with this model, each of the individual proteins alone lacks biologic activity.

The crystal structures of PA,[69] LF,[70] and EF[71] have all been determined. The current model, based on cell culture studies, proposes that PA first binds to a host cell toxin receptor (Fig. 11.1). Two of these receptors have now been identified,[72,73] and additional cell-surface proteins may modulate their activity.[74] PA then is cleaved by a cell-surface protease, releasing a

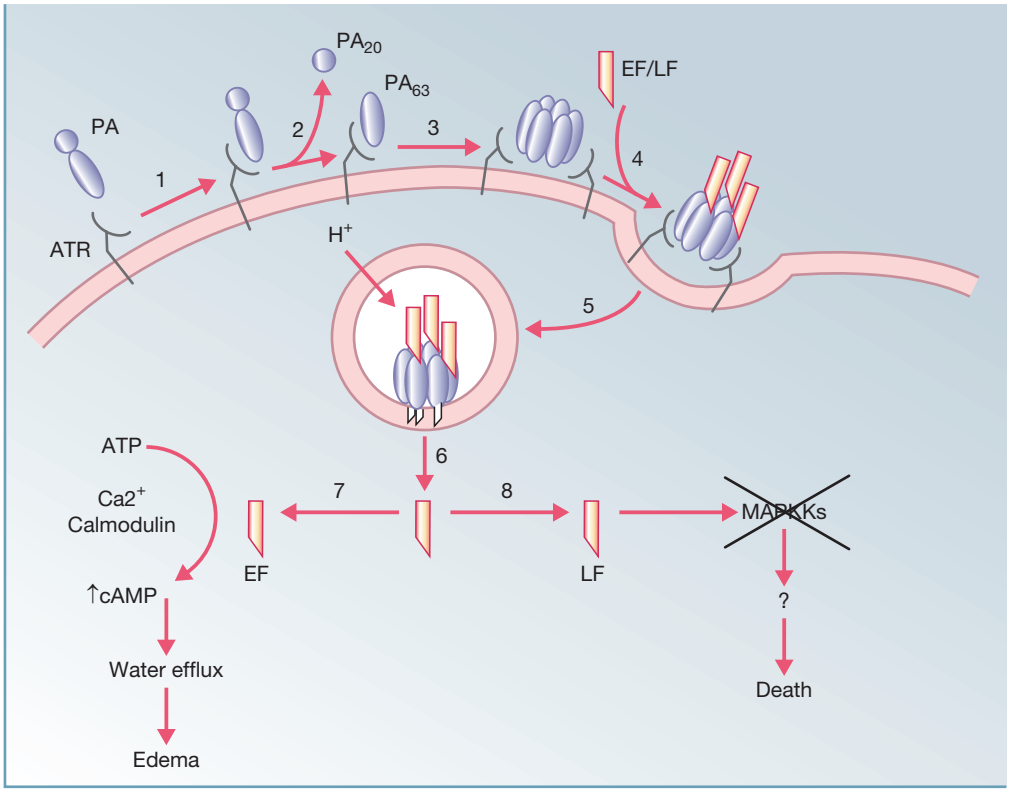


Figure 11.1. Model of anthrax toxin action. Protective antigen (PA) secreted by *Bacillus anthracis* binds to a membrane protein receptor (ATR) *(1)*, two of which have been described. The PA is then cleaved by the protease furin *(2)* with release of a 20-kDa fragment and oligomerization of the remaining 63-kDa monomer *(3)*. The PA_{63} oligomer then binds edema factor (EF) or lethal factor (LF) *(4)*. The complete toxin is then endocytosed *(5)* and trafficked to endosomes. At the low pH of the endosomes, the oligomer inserts into the membrane resulting in translocation of EF/LF to the cytosol *(6)*. EF causes an increase in cyclic adenosine monophosphate (cAMP) *(7)*, while LF cleaves mitogen-activated protein kinases (MAPKKs) *(8)* resulting in host cell damage and death of some cell types. PA can also be cleaved by proteases in the circulation to the 63-kDa monomer and form oligomers with EF/LF which then bind to host cell receptors. *(Modified from Mourez M, Lacy DB, Cunningham K, et al: 2001: a year of major advances in anthrax toxin research.* Trends Microbiol. *2002;10:287–293.)*

20-kDa aminoterminal fragment. The cell-bound 63-kDa carboxyterminal fragment oligomerizes and creates a second binding domain to which either or both of the active proteins (LF or EF) binds. The complex then enters the cell through endocytosis with LF and EF passing through a PA channel induced by the low pH of the endosome, recently visualized by cryoelectron microscopy,[75] to exert their intracellular toxic effects. PA may also be cleaved by proteases in the circulation to form oligomers with LF and EF, which then bind to cell receptors.[58] An alternative pathway has recently been described whereby LF within extracellular vesicles or exosomes can enter and intoxicate cells independently of the PA receptor.[76]

The genes for the toxin production are carried on a second 182-kb plasmid (pX01).[77] Strains deleted of the plasmid coding for the toxin genes remain encapsulated, but are attenuated.[48,77] Of historical significance, it appears that the veterinary vaccine strains produced by Pasteur through passage at high temperature lack the plasmid for the toxin genes, explaining their lack of virulence.[77] It is thought that vaccine preparations consisted of a mixture of organisms, most of which lacked the toxin plasmid together with small numbers of fully virulent organisms that retained the toxin plasmid and were responsible for the vaccine's efficacy. Further work has shown that deleting the PA gene alone eliminates the organism's virulence,[78] thus confirming the central role of PA in the activity of the two toxins and their role in virulence.

Early studies showed that crude toxin preparations or combinations of edema and lethal toxins inhibited neutrophil killing,[79] chemotaxis,[80] or phagocytosis,[49] and that edema toxin inhibits neutrophil phagocytosis[81] and priming of the respiratory burst of neutrophils.[82] At low concentrations, lethal and edema toxins also appear to block the production of proinflammatory cytokines from macrophages,[83,84] directly inhibit macrophage chemotaxis,[85] and interfere with the early protective inflammatory response. At high concentrations, lethal toxin is cytolytic for macrophages from some mouse strains.[86] These mouse studies have shown that the strains with macrophages and neutrophils resistant to lethal toxin, as seen in human cells, are more resistant to infection, with neutrophils more important for resistance than macrophages.[87–89] However, the relevance of these studies in mice with attenuated unencapsulated strains to the mechanism of toxin pathogenesis of human disease remains to be determined. Lethal and edema toxins also have deleterious effects on a multitude of other cells, including lymphocytes and dendritic cells,[90–94] endothelial cells,[95] glucocorticoid receptor–positive cells,[96] and cardiac myocytes,[97] but the physiologically important cell target(s) in the infected host remains to be established. In terms of pathogenesis, the greater importance of lethal toxin versus edema toxin was demonstrated with a mouse model in which an anthrax strain producing the lethal toxin alone retained some virulence, whereas a strain producing only edema toxin was avirulent; the parent strain producing both

toxins was the most virulent.[98] More recent studies show that lethal toxin, but not edema toxin, is essential for virulence in nonhuman primates.[99]

In cutaneous anthrax, infection begins when a spore is introduced through the skin. At the local site, the spore germinates into the vegetative bacillus, which produces the antiphagocytic capsule. The edema and lethal toxins produced by the organism impair leukocyte function and contribute to the distinctive findings of tissue necrosis, edema, and relative lack of leukocytes in the local lesion. If not contained, the bacilli spread to the draining regional lymph nodes, allowing further production of toxins and induction of the typical hemorrhagic, edematous, and necrotic lymphadenitis. From the lymph nodes, the bacteria continue to multiply and enter the bloodstream, producing a systemic infection.

In inhalational anthrax, spores ingested by alveolar phagocytes and lung dendritic cells are transported to the tracheobronchial and mediastinal lymph nodes, where they germinate.[100,101] Local production of toxins by extracellular bacilli leads to the massive hemorrhagic, edematous, and necrotic lymphadenitis and mediastinitis characteristic of this form of the disease. The bacilli then spread to the blood, causing septicemia. In approximately half the cases there may be hemorrhagic meningitis. PA and LF are detectable in the blood early and at high concentrations later,[56,102] with the lethal toxin occurring as a complex of PA and LF at late stages of disease.[103] Capsule is also present at high levels.[102] The site of action and the role of lethal and edema toxins in the mechanism of death from infection remain obscure. Death is a result of respiratory failure with overwhelming bacteremia that is often associated with meningitis and subarachnoid hemorrhage.

Diagnosis

A diagnosis of cutaneous anthrax should be considered after the appearance of a painless, pruritic papule that develops into a 1- to 2-cm vesicle, revealing a black eschar at the base of a shallow ulcer. Examination by Gram stain or culture of the vesicular fluid should confirm the diagnosis, but prior antibiotic therapy quickly renders the infected site culture-negative. Biopsy at the lesion edge, examined by Gram stain, immunohistology, and PCR, may be useful in people who have been treated with antibiotics. The latter two tests are available through the Laboratory Response Network (see below). In addition, there should be a history of exposure to materials contaminated with *B. anthracis*.

The diagnosis of inhalational anthrax is difficult, but should be suspected in cases with a history of exposure to an aerosol containing *B. anthracis*, followed by an initial phase during which symptoms are nonspecific. Once the acute stage develops, a widened mediastinum seen on chest radiograph, often with pleural effusions, should suggest the diagnosis. In untreated cases, culture of blood and pleural effusions will readily establish the diagnosis, and more rapid detection methods, including PCR for the PA gene, immunoassay for PA and capsule antigens, and mass spectrometry for LF or lethal toxin, may all be useful.[102,104] These tests are available through the Laboratory Response Network and state health departments (http://www.cdc.gov/anthrax/labs/labtestingfaq.html). In cases previously treated with antibiotics, these assays for toxin and capsule in blood and pleural fluid, as well as immunohistologic examination of pleural fluid or transbronchial biopsy specimens, are of particular value[14,15,17,105] and may be positive as long as 6 to 8 days after initiation of therapy.[104,106] Because primary pneumonia is not a feature of inhalational anthrax, sputum examinations do not aid diagnosis. The radiographic differential diagnosis should include histoplasmosis, sarcoidosis, tuberculosis, and lymphoma. A computed tomography scan of the chest may be helpful to detect hemorrhagic mediastinal lymphadenopathy and edema, peribronchial thickening, and pleural effusions.

Gastrointestinal anthrax is difficult to diagnose because of its rarity and similarity to other more common severe gastrointestinal diseases. An epidemiologic history of ingesting contaminated meat, particularly in association with other similar outbreak cases, suggests the diagnosis. Microbiologic cultures are not helpful in confirming the diagnosis unless bacteremia is present. The diagnosis of oral-oropharyngeal anthrax can be made from the clinical and physical findings, but the role of bacteriologic cultures in confirming this diagnosis remains unclear.

Treatment and Prevention With Antibiotics

Mild cases of cutaneous anthrax may be treated effectively with oral penicillin, fluoroquinolone, doxycycline, or another antibiotic, depending on antimicrobial susceptibility. If rapid spread or prominent systemic symptoms are present, high-dose parenteral therapy as for inhalational anthrax (see below) should be given until there is a clinical response. Effective therapy reduces the edema and systemic symptoms but does not change the evolution of the skin lesion. In injectional anthrax parenteral antibiotic therapy as for inhalational anthrax (see below) should be initiated and surgical debridement and fasciotomy may be required.

Treatment of inhalational anthrax requires high-dose intravenous therapy (ciprofloxacin or another fluoroquinolone) with at least one or more antibiotics with good central nervous system penetration, for example, meropenem and linezolid, because of the high incidence of meningitis.[14,15,107,108] This approach also applies to gastrointestinal anthrax. Regimens should be altered based on susceptibility testing available at the Laboratory Response Network and state health departments along with the clinical status. The successful treatment of six of the 11 inhalational cases in the 2001 bioweapon attacks suggests that, with rapid antibiotic treatment and modern supportive care, including aggressive management of respiratory distress and pleural effusions, mortality is similar to that of other causes of sepsis.

After exposure to an aerosol of anthrax, postexposure prophylaxis or presymptomatic treatment should include oral antibiotics for 60 days or more, depending on individual circumstances (eg, extent of exposure, vaccination status; see later text).[107,108] Ciprofloxacin or doxycycline are recommended. Other oral antibiotics, including other fluoroquinolones, clindamycin, or amoxicillin or penicillin VK if the strain is susceptible, are alternatives. Levofloxacin is indicated as a second-line drug because of limited safety data beyond 30 days.[108] Amoxicillin is recommended for children and pregnant or lactating women, depending on microbial resistance.[15,107] Preexposure or postexposure vaccination may enable shorter courses of antibiotics (see later text).[107,109] Postexposure vaccination alone would not be expected to protect quickly enough to be effective.[22]

EPIDEMIOLOGY

Several theories explain the ecology of soil contaminated with *B. anthracis* spores. One theory suggests that *B. anthracis* spores can persist for many years in soil rich in nitrogen and organic material with adequate calcium, a pH greater than 6.0, and an ambient temperature greater than 15.5°C. It remains unclear whether there are cycles of germination and replication within the soil or if amplification within mammalian hosts serves to maintain the spore population in the soil.

Animal anthrax results from animals ingesting *B. anthracis* spores by eating contaminated feed or while grazing on pastures. Soil becomes contaminated from contaminated commercial fertilizer, contaminated feed, or diseased animals that contaminate the soil with their secretions before or after death.[110]

The number of reported human anthrax cases in the United States has declined steadily. Between 1916 and 1925, the annual average number of cases was 127; between 1948 and 1957, 44 cases; between 1978 and 1987, 0.9 cases per year; and between 1988 and 2000, 0.25 cases per year. Of the 235 human cases reported in the United States from 1955 to 2000, 20 were fatal (Fig. 11.2). Among these cases, 224 had cutaneous lesions (118 on an arm, 65 on the head or neck, 11 on the trunk, 8 on a leg, and 22 at an unknown site) and 11 were inhalational cases. These cases only include naturally occurring cases and do not include cases related to the 2001 outbreak. As discussed, in 2001, there were a total of 22 cases, 11 inhalational cases that included five deaths, and 11 cutaneous cases associated with contaminated mail. In the years from 2002 to 2014, there have been six cases of anthrax reported in the United States, the last being in 2011.[111] Three of those cases were related to exposure to drumheads made from animal skin; two of these were inhalational anthrax, and one was the first confirmed case of gastrointestinal anthrax reported in the United States. The other cases included two cutaneous cases and one inhalational case associated with travel in four states where anthrax is enzootic.[112]

A new manifestation of anthrax was reported in the United Kingdom in 2009—injectional anthrax. Since then, more than 140 laboratory-confirmed cases of anthrax associated with injecting heroin contaminated with *B. anthracis* have been reported in Europe.[19,21] These cases have demonstrated varying clinical signs and symptoms as well as high mortality rates. It has not been determined whether the heroin itself was contaminated, or *B. anthracis* was present in materials mixed with the heroin.

For naturally occurring anthrax, the basic epidemiologic principles are the same in developing and developed countries. Agricultural anthrax is a more significant problem in developing countries, and industrial anthrax occurs more commonly in developed countries. Industrial anthrax results from exposure of susceptible persons to contaminated animal products including wool, goat hair, hides, or bones. These materials come from animals that were infected with *B. anthracis* before death or contaminated after death (eg, from contaminated soil with which the carcass or animal products came in contact). The wool and hair from infected animals may be clipped from live animals or pulled from carcasses. A hide may be obtained from an animal that has died of anthrax. Bones can be collected from grazing areas on which animals die or from rendering plants that may handle carcasses of animals that have died of anthrax.

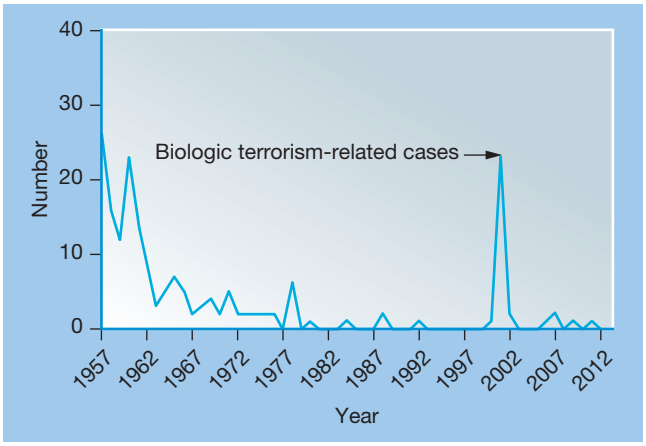


Figure 11.2. Number of both naturally occurring and biological terrorism–related reported cases of anthrax, by year in the United States for the years 1957 to 2012.[111]

Wool and goat hair are processed into yarn used in the textile and carpet industries and in the preparation of other cloth-like materials. Hides are processed into leather goods. Bones are used in preparing bone meal, gelatin, and fertilizer. In industrial cases, cutaneous anthrax results from spores that gain entrance through the skin by entering preexisting wounds or by being rubbed through the skin or on a hair fiber that may penetrate the skin. At times, the processing of goat hair and wool creates infectious aerosols that may result in inhalational anthrax when inhaled. A rendering plant is another source of potential infection.

A case of inhalational anthrax was reported in early 2006 in a 44-year-old male drumhead maker who brought goat skins into the United States from Africa. After cleaning the skins, he developed inhalational anthrax, but with vigorous therapy survived.[27,105] *B. anthracis* was recovered from his work environment. Several additional cases of anthrax associated with drums from Africa have been reported, including one case of disseminated anthrax, another of inhalational anthrax from Europe,[32] and two cutaneous[113] and one presumed gastrointestinal case[114] from the United States.

Cases associated with agricultural settings result from contact with diseased animals or with the products of animals that have died of anthrax. Affected persons are primarily agricultural workers, veterinarians, or people who kill and butcher infected animals or butcher the carcasses of animals that have died of anthrax. This contact results in cutaneous anthrax or, if the infected meat is ingested, gastrointestinal or oral–oropharyngeal anthrax.

Laboratory-associated cases of anthrax are rare. These are essentially all cutaneous, although a few inhalational cases have occurred. Rarely, cases have been reported after contact with contaminated clothing, such as woolen coats or pilots' leather helmets. Table 11.2 presents the sources of infection of the 264 cases reported in the United States from 1955 to 2011. The two vaccine-associated cases of cutaneous agricultural anthrax resulted from the inadvertent injection of live attenuated animal vaccine into the hand of the vaccinator. Two additional vaccine-associated cases of anthrax occurred in persons producing vaccine and were thought to result from contamination of skin lesions with the vaccine strain.[115]

Exposures related to bioterrorist events represent a new category. The anthrax spore attacks in the fall of 2001 resulted in 11 confirmed inhalational cases and seven confirmed and four suspected cutaneous cases reported from Florida, New York, New Jersey, the District of Columbia, and Connecticut.[13–15] Exposure to contaminated mail was the confirmed or apparent source of infection in all patients.[15,16] More than 32,000 people received short courses of prophylactic antibiotics while potential exposures were evaluated,[116] and among the treated people, more than 10,000 continued to receive antibiotics for 60 or more days with or without postexposure vaccination as prophylaxis.[117,118] Exposures likely resulted from opening contaminated letters, from working in buildings with high-speed automated mail-sorting machines, or through direct contact with cross-contaminated pieces of mail or environments contaminated with spores.

PASSIVE IMMUNIZATION

In the era before antibiotics, antisera from convalescent animals were common therapeutic products.[119] One of the first

TABLE 11.2 Sources of Infection in 264 Cases of Human Anthrax in the United States, 1955 to 2011

Source	No. of Cases
INDUSTRIAL	
Goat hair	113
Wool	34
Goat skin	17
Meat	3
Bone	4
Laboratory	1
Animal hide drums	4
Unknown	13
Total	189
AGRICULTURAL	
Animal	42
Vaccine	2
Unknown	8
Total	52
Unknown	1
TERRORISM	
Inhalational	
Working in mail-processing facility	7
Receiving mail (confirmed or presumptive)	4
Cutaneous	
Working in mail processing facility	7
Receiving mail (confirmed or presumptive)	4
Total	22 (18 confirmed and 4 suspected)

was anthrax antiserum, developed in France by Marchoux and in Italy by Sclavo in 1895.[120,121] Although it was used initially for prophylaxis and treatment of anthrax among livestock, Sclavo later used his product to treat cutaneous or septicemic human disease. He reported 10 deaths among 164 treated patients (6% mortality, compared with the Italian case-fatality rate of 24% in untreated cases), although these were not controlled clinical trials. Sclavo injected 30 to 40 mL of antiserum subcutaneously, and repeated it 24 hours later. In severe cases, he also injected 10 mL or more intravenously.

Between the 1910s and 1940s, clinicians in Europe and the Americas treated patients with anthrax antiserum using 25 to 300 mL daily for 5 days, sometimes in combination with arsenicals.[119–130] One patient with severe cutaneous anthrax recovered after receiving 2265 mL of antiserum.[131] No controlled studies were performed to demonstrate efficacy. Anthrax antiserum for therapy of cutaneous anthrax was replaced by therapy with sulfanilamide in 1939, followed by penicillin in 1947 and subsequently by other antibiotics.[129,132]

Equine anthrax antiserum produced by live-spore vaccination has been licensed in China,[133] the Soviet Union, and later Russia for decades, and its use continues, although the frequency of its use is unclear. The Lanzhou Institute of Biological Products in China developed a lyophilized antianthrax $F(ab)_2$ formulation of equine immunoglobulin (Ig) G fragments for human use by intracutaneous, IM, or IV administration, but it is rarely used (Dong Shulin, personal communication, 2002). Several other Fab formulations are in early-stage animal testing.

Experimental evidence indicates that passive immunization with equine antibody produced against attenuated Sterne veterinary vaccine strains or against crude toxins prevents disease in animals when given before or shortly after spore challenge.[23,134] Rhesus monkeys were protected with one or two doses of equine antianthrax spore hyperimmune serum when begun 1 day after low-dose aerosol challenge. Of immune serum–treated animals 45% survived, compared with only 10% of control animals.

Studies by Little and colleagues[135] showed efficacy of anti-PA antiserum prophylaxis against an IM challenge in animals. The anti-PA polyclonal antibody protected against death, and anti-PA monoclonal antibody significantly delayed mortality. Reuveny and colleagues[136] similarly found that passive immunization of guinea pigs with polyclonal anti-PA antisera conferred protection against an intradermal challenge dose of 40 median lethal doses (LD_{50}).

Kobiler and colleagues[137] challenged guinea pigs intranasally with a 25 LD_{50} dose of spores and intraperitoneal administration of rabbit anti-PA serum 24 hours after infection protected 90% of animals, with lesser efficacy seen with anti-Sterne and anti-LF antibodies. Beedham and colleagues[138] demonstrated that mice could be protected against live anthrax challenge with antibody from PA-vaccinated animals, but not with their splenic lymphocytes, supporting the longstanding evidence that antibody is the major mechanism of vaccine-induced immunity.

Although the importance of anthrax toxins in pathogenesis suggests that antiserum to toxins could have a role in treatment, no such products were produced, until interest was rekindled by the anthrax bioweapon attacks in fall 2001.[139] Modern experimental evidence shows that passive immunization with antiserum prevents anthrax in animals when given before or shortly after spore challenge.[140] This includes protecting guinea pigs from intradermal challenge, rhesus monkeys from low-dose aerosol challenge, and rats from parenteral challenge. The need for therapeutics other than antibiotics may be especially great in the case of antibiotic-resistant strains of *B. anthracis*, although there remains no definitive evidence to date of efficacy of antiserum to toxin in humans. In 2007, the U.S. government purchased several thousand therapeutic courses of human polyclonal anthrax immunoglobulin from Cangene Corporation (Winnipeg, Canada). This product was manufactured by fractionating the plasma of volunteers given at least four doses of anthrax vaccine adsorbed (AVA [BioThrax]). The formulation, now known by the trade name Anthrasil, was licensed by the Food and Drug Administration (FDA) in March 2015 for use in combination with appropriate antibacterial drugs. The anthrax immunoglobulin formed part of the successful treatment of a 2006 case of inhalational anthrax in a 44-year-old man exposed via African hides[105] and has been used in treatment of anthrax in drug users in Scotland,[141] but definitive evidence of efficacy in humans is unavailable. Efficacy evidence for licensure relied heavily on animal-challenge data, augmented with cross-species pharmacokinetic data,[142] fulfilling the FDA Animal Rule.

Several human monoclonal anti-PA antibodies have been developed. The U.S. government contracted for several thousand intravenous treatment courses of raxibacumab (ABthrax, Human Genome Sciences, Rockville, MD), a monoclonal anti-PA antibody. This antibody protects rabbits and nonhuman primates from death when given prophylactically against an aerosol spore challenge and when given therapeutically.[143]

The FDA licensed raxibacumab in December 2012 for use in combination with appropriate antibacterial drugs. Even though studies with both polyclonal anthrax immunoglobulin (Anthrasil) and raxibacumab showed increased survival over animals treated with antibiotic alone, the difference was not statistically significant.[108,144] Other monoclonal antibodies are also under development, such as MDX-1303 (or Valortim) developed by Pharmathene.[145] It remains unclear whether polyclonal antibodies that neutralize several epitopes might be preferable to monoclonal antibodies that target only one epitope.[146] If bacteria could be reengineered to modify the single binding epitope of a monoclonal antibody, it might be rendered ineffective. Indeed, it has been reported that modifications to PA can result in lack of effectiveness of a neutralizing monoclonal antibody, whereas a polyclonal antibody was still active.[147]

Mouse and humanized chimpanzee monoclonal antibodies to the other major virulence factor, capsule, have also been shown to be protective in mouse models.[148,149]

ACTIVE IMMUNIZATION

History of Vaccine Development

Although there is great historical interest in Pasteur's first effective live bacterial vaccine, and live attenuated veterinary vaccines are still used, human anthrax vaccines licensed in the United States and Europe consist of purified anthrax proteins, and not live attenuated vaccines. Early human anthrax vaccines (presumably live) were used in the 1910s but found little favor.[121] Then, Sterne developed live attenuated strains in the 1930s, which led to worldwide use for domesticated animals.[45] Russian investigators developed similar vaccines for animal and human use. In 1946, Gladstone[150] identified the PA component of cultures of *B. anthracis* as being an effective vaccine, and only later did subsequent studies identify PA as the cell-binding component of the anthrax toxins. Belton and Strange[134] increased the yields of PA to allow large-scale production, leading to the current British vaccine, anthrax vaccine precipitated. Wright and colleagues[151–153] used similar techniques to develop precursors to the American vaccine.

Protective antigen is the term applied to one of the toxin proteins, which is the plasmid-encoded binding component of the anthrax toxins described previously. The major effective immunogen in culture supernatants is PA, although smaller amounts of LF and EF may be present; their contribution to protective immunity has remained controversial.[56] In older studies, EF enhanced the protective efficacy of PA in some experimental animals.[154,155] These studies are difficult to interpret because the preparations used may not have been pure and free of cross-contamination. Studies using the PA gene cloned into *Bacillus subtilis* demonstrated conclusively that PA alone, in the absence of EF, LF, or other *B. anthracis* proteins, protects animals against experimental infection.[156] Although other experiments showed that purer preparations of PA, free of immunologically detectable LF or EF,[157] or recombinant PA,[158] can protect experimental animals, it remains unknown whether adding EF or LF enhances the efficacy of PA. One study reported that a DNA vaccine with the N-terminal domain of LF alone delayed time to death but did not increase overall survival in rabbits challenged with a virulent strain.[159] Similar results were observed with anti-LF serum in guinea pigs,[137] although antibody to LF[160] and vaccination with inactivated LF or the N-terminal domain[161] protected mice from infection with an attenuated strain. In addition, some protection was observed in a mouse model with EF expressed in an adenovirus vector.[162]

Description of Vaccines

The only human anthrax vaccine licensed in the United States, AVA marketed as BioThrax, is produced by Emergent BioSolutions (Rockville, MD) from sterile filtrates of microaerophilic cultures of an attenuated, unencapsulated, nonproteolytic strain (V770-NP1-R) of *B. anthracis*. The cell-free culture filtrate, containing predominantly PA, is adsorbed to aluminum hydroxide, and the final product contains no more than 2.4 mg of aluminum hydroxide per 0.5-mL dose. Formaldehyde, in a final concentration of no more than 0.02%, and 0.0025% benzethonium chloride are included as preservatives. Current product-content standards require 5 to 20 µg/mL of total protein, of which at least 35% is the 83-kDa PA protein, measured by densitometric analysis on sodium dodecyl sulfate–polyacrylamide gel electrophoresis after pooling 12 sublots.[163] The vaccine is stable for 3 years after a successful potency testing.

Some lots produced in the 1980s appeared to contain small amounts of LF and lesser amounts of EF, as determined by induction of antibody responses in animal recipients,[48,157,164,165] although this has not been reported in the limited observations in human vaccinees.[165] Western blot analysis found no detectable EF. Enzyme-linked immunosorbent assay (ELISA) studies found LF to be present in the range of 10 to 30 ng/mL of fermentation filtrate before adsorption.[163] However, analysis by mouse macrophage cytotoxicity assay suggested that LF was present in a biologically inactive form.[153] Although it is clear that PA by itself is an effective immunogen, it remains unresolved whether the small amounts of LF or EF that may be present in some lots contribute to the vaccine's protective efficacy. A 2008 study of serum samples of AVA vaccinees showed that low levels of antibody to LF and EF were present by Western blot, but these did not contribute to toxin neutralization.[166]

Potency testing of AVA is performed by assessing biologic activity after intradermal challenge of vaccinated guinea pigs with a lethal dose of spores. The vaccine is stored at 2°C to 8°C. In December 2008, the FDA approved a revised recommended schedule for vaccination consisting of 0.5 mL given IM at 0 and 4 weeks and at 6, 12, and 18 months instead of the previous schedule with doses given SC at 0, 2, and 4 weeks followed by doses at 6, 12, and 18 months.[109] With continued exposure, booster doses have traditionally been recommended annually. A study of a simplified dosing schedule revealed that at month 43, anti-PA IgG geometric mean concentrations (GMCs) for three reduced-dosage groups were noninferior to the original eight-dose SC group. At month 43, volunteers receiving doses at 0, 1, 6, and 42 months IM or at 0, 1, 6, 18, and 42 months IM had GMC responses greater than those receiving the original eight-dose SC schedule. Solicited injection-site adverse events occurred at lower proportions in the IM group compared to SC. Route of administration did not influence the occurrence of systemic adverse events. A three-dose IM priming schedule with doses administered at months 0, 1, and 6 elicited long-term immunological responses and robust immunological memory that was efficiently stimulated by a booster vaccination at 42 months.[167]

Anthrax Vaccine Precipitated, a similar vaccine produced by the Health Protection Agency (Porton Down, Salisbury, Wiltshire), was developed in the United Kingdom, first administered to humans in the early 1950s, and licensed there in 1979.[168–173] This vaccine is made by precipitating the sterile cell-free culture filtrate of a derivative of the attenuated, unencapsulated Sterne strain 34 F_2 with aluminum potassium sulfate.[172] LF and EF are present in this vaccine at levels higher than believed to be found in the U.S. vaccine from the 1980s.[168,173] The vaccine contains thimerosal as a preservative.

The British vaccine is administered IM in a regimen of three 0.5-mL doses at 0, 3, and 6 weeks, with a booster dose 6 months after the third dose. Subsequent booster doses are given annually.[165] Other schedules of administration of this vaccine are also being studied.

A vaccine consisting of a suspension of live spores, named STI-1 for the Sanitary-Technical Institute, has been used for humans in the Soviet Union and its subsequent independent republics since 1953.[110,174] This strain, similar to the Sterne strain used in veterinary vaccines, is unencapsulated.[174] Although this vaccine has a reputation for causing substantial side effects, its developers assert that it is reasonably well tolerated and shows some degree of protective efficacy.[174,175] This vaccine, manufactured by the Tbilisi Scientific Research Institute of Vaccines & Serums (Tbilisi, Georgia), the Institute of Microbiology (Kirov [Vyatka], Russian Federation), and perhaps at other locations, is given either by scarification through a 10- to 20-mL drop of vaccine containing 1.3 to 4×10^8 spores or SC.[2,110,172,174,176–178] The initial dose is followed by a second dose 21 days later, with yearly boosters.

Another human live spore vaccine given by scarification has been manufactured by the Lanzhou Institute of Biological Products (Lanzhou, Gansu, People's Republic of China) since the 1960s, based on the avirulent strain A16R.[110,133] A single dose contains 1.6 to 2.4×10^8 colony-forming units. A single booster dose is given 6 to 12 months after the first vaccination (Dong Shulin, personal communication, 2002).

Immunogenicity of Anthrax Vaccine Adsorbed Vaccine Produced in the United States

The results of two studies indicated that immunization with the AVA induced an immune response (as measured by indirect hemagglutination) to PA. In the first study, 83% of vaccinees seroconverted 2 weeks after the first three doses.[179] In the other, 91% seroconverted after receiving two or more doses.[180] The titers fell over time, but 100% of vaccinees responded with an anamnestic response to the annual booster dose. Analysis using a more sensitive ELISA against PA demonstrated that seroconversion rates were between 96% to 100% of vaccinees after the second dose.[181,182]

By using a more sensitive validated ELISA assay, Pittman and colleagues[183] found that one dose of AVA evoked detectable anti-PA IgG antibodies in 60% to 84% of vaccinees. After two doses, 95% to 100% of vaccinees developed anti-PA antibodies. Studies also show that providing a missed 6-month dose after a delay as long as 5 to 7 years did not impair the booster response and it is not necessary to restart the series.[184]

A pilot study comparing SC and IM administration of AVA revealed higher titers to PA when a 4-week interval between the first two doses was compared with a 2-week interval, with fewer injection-site or systemic events with the IM route.[182] As described earlier, based on a larger randomized clinical trial,[185] the FDA changed the licensed schedule of 0.5 mL of vaccine given at 0 and 4 weeks and at 6, 12, and 18 months by the IM route from the old schedule of 0, 2, and 4 weeks and 6, 12, and 18 months given SC. The 2014 Wright study[167] may lead FDA to simplify the schedule further, as has been done by German regulatory counterparts who adopted a booster-vaccination interval of 3 years after a three-dose priming series (0, 1, and 6 months).

Although the change in route from SC to IM resulted in fewer solicited injection-site adverse events, it had no effect on the occurrence of systemic adverse events. Most importantly, the change to the IM route and elimination of the 2-week dose resulted in statistically significantly lower concentrations of anti-PA antibody from week 8 through and including month 6. The antibody titers using the new schedule were noninferior to the old schedule at month 7, after the 6-month dose. However, the percentage of responders with a fourfold rise in titer was similar at 8 weeks and 7 months after the 6-month booster with the two schedules. The significance of the lower immune response up to month 7 remains unclear as the serologic correlation with immunity remains to be determined and will require extrapolation from studies in animal models. However, in those instances where rapid onset of an immune response is desired, as when postexposure vaccination is given together with antibiotics (see later), then the original schedule of doses given SC at 0, 2, and 4 weeks should be considered.[186]

There also seems to be some variability in the response of humans to vaccination with AVA as persons with DRB1-DQA1-DQB1 human leukocyte antigen (HLA) class II haplotypes produce significantly lower levels of anti-PA antibody.[187]

Cellular Responses

Soviet scientists developed a skin-test antigen known as anthraxin in 1957, derived from the edematous fluid of infected animals given an unencapsulated *B. anthracis* strain.[178] Licensed in 1962 in Russia, the skin test product is composed of an undefined heat-stable polysaccharide–protein–nucleic acid complex, without anthrax capsular or toxigenic material.[178,188] A positive skin test after a 0.1-mL intradermal injection is defined as erythema of 8 mm or more with local induration persisting for 48 hours.[168] Anthraxin was useful in identifying cases of anthrax[174,189] and identifying STI-1 vaccine-induced immunity in guinea pigs, sheep, and humans.[174] Experimental data show that guinea pigs vaccinated against anthrax that developed a positive anthraxin skin test were immune to a subsequent parenteral challenge.[178] The positive and negative predictive values of the individual test results have not been published and there is limited experience with the skin-test antigen in humans in Western countries. Thus, its usefulness in predicting immunity in humans remains unknown. Contemporary immunologic studies show that humans develop PA-reactive CD4+ T cells with a T-helper cell type 2 (Th2) cytokine profile[190] as well as long-lived PA-specific memory B-cell responses after vaccination with AVA.[191] Another study examined associations between HLA genotypes, haplotypes, and homozygosity and PA-specific cellular immune responses in healthy subjects following vaccination with AVA. Limited associations were observed between individual HLA alleles or haplotypes and variable lymphocyte proliferative (LP) responses to AVA. Individuals homozygous for any HLA locus demonstrated significantly lower PA-specific LP than subjects heterozygous at all eight loci.[192]

Correlates of Protection

After a naturally acquired infection, antibody to PA develops in 68% to 93% of cases as reported in different series, depending on when samples are drawn.[173,180,181,193] Antibody to LF occurs in 42% to 55% of cases, whereas antibody to EF is less frequently observed.[173,181] Antibody to the anthrax capsule occurs in 67% to 94% of cases.[181,193] This reaction contrasts with that after vaccination, in which no response to capsule is expected because the strain used to produce the vaccine is nonencapsulated. In the 2001 epidemic of inhalational anthrax, antibody to PA was detected in all survivors of confirmed cases.

In experimental animals, there is generally a correlation between immunity and antibody titer to PA after immunization with AVA.[194] In a mouse model using an attenuated strain, systemic rather than mucosal antibody correlated better with

protection against inhalational challenge.[195] However, the live veterinary vaccine provides significantly greater protection against similar challenge doses of anthrax in experimentally infected animals than does the human vaccine, even though it often induces lower levels of antibody to PA,[156,164,165] suggesting other antigens may be involved in protection.

More recent studies using live and protein-based vaccines have demonstrated a strong correlation between antibodies to PA and protective immunity. Barnard and Friedlander[196] showed, for the first time using live vaccines producing varying amounts of PA, that protection was strongly correlated with antibody titers to PA, a finding subsequently confirmed by Cohen and colleagues.[197] A similar correlation of immunity to AVA with antibody to PA, measured by ELISA and toxin neutralization, was observed in a rabbit model of inhalational anthrax.[194] Reuveny and colleagues,[136] using a PA vaccine to protect guinea pigs against an intradermal challenge, found that toxin-neutralizing antibodies correlated better with survival than antibodies measured by ELISA, and similar results were observed in rabbits challenged by the intranasal route.[198] However, studies in nonhuman primates showed that anti-PA IgG was more accurate than toxin-neutralizing antibodies as a correlate of protection against inhalational challenge.[199] Further analysis of the antibody response to different epitopes on PA[200] will increase knowledge of the nature of the protective antibodies as monoclonal antibodies to PA with synergistic, additive or antagonistic effects on neutralization have been described.[201] The overall evidence based on passive immunity with the aforementioned antibodies and protection with PA vaccines confirms that humoral immunity is the predominant mechanism of protection. Vaccine-induced protection seems in some animal models to be a function of the rapidity of the infectious process, the level of antibody present at challenge, and the speed of development of an anamnestic response.[202]

More recent studies sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) and the Centers for Disease Control and Prevention (CDC) to further establish the relationship between antibody response and protection in rabbits (NIAID) and in nonhuman primates (NIAID and CDC) have been conducted. Although the NIAID used second-generation anthrax PA vaccines (see later) and the CDC used the licensed AVA vaccine, studies using both types of vaccines have shown that antibody levels in both species correlate with protection against aerosol challenge. Analysis of multiple studies in rabbits and two nonhuman primate species showed that levels of toxin neutralizing antibodies in one species are predictive of vaccine efficacy in the other species.[203,204] The finding that toxin neutralizing antibody levels can predict cross-species survival supports the extrapolation of data from animals to predict anthrax vaccine efficacy in humans. It is hoped this approach will be suitable for licensure of new anthrax vaccines and/or indications using the FDA pathways for licensure of vaccines that cannot be tested for efficacy in humans.[205]

Efficacy of Vaccine

The protective efficacy of different experimental PA-based vaccines derived from culture filtrates of *B. anthracis* has been clearly demonstrated with the use of various animal models and routes of challenge.[56,171] A comprehensive, peer-reviewed evaluation by the National Academy of Sciences reported that "The committee finds that the available evidence from studies with humans and animals, coupled with reasonable assumptions of analogy, shows that AVA as licensed is an effective vaccine for the protection of humans against anthrax, including inhalational anthrax, caused by all known or plausible engineered strains of *B. anthracis*."[163] The FDA independently affirmed that AVA prevents anthrax regardless of route of exposure.[206]

A controlled field trial was conducted in the late 1950s with a less potent vaccine similar to the currently licensed AVA.[207] This vaccine was composed of an alum-precipitated, cell-free culture supernatant from an attenuated, unencapsulated, nonproteolytic strain of *B. anthracis*. This strain differed slightly from that used to produce the licensed vaccine and was grown under aerobic rather than microaerophilic conditions.[152] The study was conducted in a susceptible population working in four mills in the northeastern United States, where raw imported goat hair contaminated with *B. anthracis* was encountered. The results indicated that vaccination, compared with a placebo, provided 92.5% protection against anthrax, combining the cutaneous and inhalational cases (95% confidence interval [CI], 65%–100%). No isolated assessment of the effectiveness of the vaccine against inhalational anthrax could be made because there were too few cases, although the only inhalational cases observed occurred in nonvaccinated persons.

There have been no controlled clinical trials in humans of the efficacy of the currently licensed U.S. vaccine, although the differences between the AVA vaccine and the PA-based vaccine used in the 1950s study by Brachman and colleagues described above[207] are minor from a regulatory perspective.[163] The AVA vaccine has been tested extensively in animals and protects guinea pigs against IM[164,165] and aerosol[157] challenge. More recent experiments show that this vaccine also protected rhesus monkeys against a lethal aerosol challenge with anthrax spores.[158,208–211] Table 11.3 summarizes inhalational challenge studies in nonhuman primates vaccinated with the licensed human vaccine or a recombinant PA vaccine. Additional studies with various formulations of recombinant PA by different manufacturers have confirmed its efficacy in nonhuman primates (E. Nuzum, personal communication, March 18, 2011).

Duration of Immunity

The duration of immunity induced by vaccination has not been clearly established. In the field trials that evaluated a vaccine similar to the currently licensed AVA, one case of cutaneous anthrax occurred 5 months after receipt of three doses within 4 weeks just before the scheduled 6-month boosting dose.[207] This single observation could indicate that immunity induced by the initial dosing series of AVA may not be long lasting but this was a less-potent vaccine than AVA and complete protection against cutaneous inoculation might not be possible. Ongoing studies of clinical correlates of protection may help clarify this point.[163,185,187]

Postexposure Prophylaxis With Vaccination

Postexposure vaccination by itself is unlikely to be of any acute benefit because of the short incubation period and the rapid course of the disease. Animal studies support this conclusion.[22] However, vaccination combined with antibiotic prophylaxis before onset of clinical illness may offer the best possible protection against inhalational anthrax. This is because of the propensity of anthrax spores to persist in the host for long periods and possibly germinate after antibiotics have been discontinued.[19–22] Vaccination allows an immune response to develop during the period of antibiotic prophylaxis. Thus, postexposure vaccination may shorten the period of antibiotic prophylaxis required for prolonged protection. This is supported by recent studies in nonhuman primates demonstrating that addition of postexposure vaccination to antibiotics resulted in a significant increase in survival

TABLE 11.3 Preexposure Efficacy of Anthrax Vaccines Against Inhalational Challenge in Nonhuman Primates

Reference	Vaccine Product	Vaccination	Challenge Dose	Challenge Strain	Vaccination to Challenge	Vaccinated Animals[a]	Unvaccinated Animals[a]
Wright et al,[152] 1954	Alum-precipitated preparation 138	Two 1-mL SC doses 16 days apart	39,000–82,000 spores	Vollum	16 days	3/4	0/4
			890,000 to 3 million spores	Vollum	34 days	4/4	0/2
Darlow et al,[168] 1956	Alum-precipitated vaccine	Two 1.25-mL SC doses 10 days apart	10–15 LD_{50}	M.36 Vollum	7 days	10/10	0/10
			10–15 LD_{50}	M.36 Vollum	1 y	10/10	0/10
			10–15 LD_{50}	M.36 Vollum	2 y	6/7	1/9
Ivins et al,[208] 1996	Anthrax vaccine adsorbed	Two 0.5-mL IM doses 2 wk apart	255–760 LD_{50}	Ames	8 wk	10/10	0/5
			161–247 LD_{50}	Ames	38 wk	3/3	
			239–535 LD_{50}	Ames	100 wk	7/8	0/2
Pitt et al,[209] 1996	Anthrax vaccine adsorbed	Two 0.5-mL IM doses 4 wk apart	899 ± 62 LD_{50}	Ames	3 mo	9/9	0/2
	rPA 50 μg + $Al(OH)_3$	Two 0.5-mL IM doses 4 wk apart	899 ± 62 LD_{50}	Ames	3 mo	9/10	
Friedlander et al,[210] 1999, and Pitt[b]	Anthrax vaccine adsorbed	Two 0.5-mL IM doses 4 wk apart	133 ± 43 LD_{50}	Ames	3 mo	5/5	0/6
Ivins et al,[158] 1998	Anthrax vaccine adsorbed	One 0.5-mL IM dose	74.4 LD_{50}	Ames	6 wk	10/10	0/3
	rPA 50 μg + various adjuvants	One 0.5-mL IM dose	78–117LD_{50}		6 wk	28/29	
Fellows et al,[211] 2001	Anthrax vaccine adsorbed	Two 0.5-mL IM doses 4 wk apart	398 LD_{50} (Ames equivalent)	ASIL K7978-Namibia	10 wk	10/10	0/2
			1004 LD_{50} (Ames equivalent)	ASIL K9729- Turkey	10 wk	8/10	0/2
Chawla et al,[270] 2009	rPA 25 μg no adjuvant	Two doses IM 4 wk apart	1000 LD_{50}	IVRI	7 wk	6/6	0/6
	rPA 50 μg no adjuvant		1,000 LD_{50}	IVRI	7 wk	6/6	
	rPA 100 μg no adjuvant		1,000 LD_{50}	IVRI	7 wk	6/6	
Food and Drug Administration,[205] 2010	Anthrax vaccine adsorbed	0.5 mL IM 0, 4 wk, 6 mo	200 LD_{50}	Ames	52 mo	8/10	3/13
	Anthrax vaccine adsorbed	0.5 mL 1:5 dilution IM 0, 4 wk, 6 mo	200 LD_{50}	Ames	52 mo	9/9	

IM, intramuscular; LD_{50}, median lethal dose; rPA, recombinant protective antigen; SC, subcutaneous.
[a]Number surviving/number challenged.
[b]Personal communication, M.L.M. Pitt, 2002.

compared with treatment with antibiotics alone.[212] Because the rapid development of an antibody response is particularly critical for postexposure prophylaxis and the new vaccine schedule of 0 and 4 weeks and 6, 12, and 18 months given IM (see earlier) results in a lower anti-PA antibody response at weeks 4 and 8, the FDA recommends using the previously licensed dose schedule and route (0, 2, and 4 weeks given SC) along with antibiotics for a minimum of 60 days until more definitive data are available.[109] Based on initial studies in nonhuman primates,[212] it may be possible to further reduce the recommended duration of antibiotics, but at present, 60 days are recommended. Use of AVA for postexposure prophylaxis along with antibiotics is not licensed at present and will require an investigational new drug protocol or emergency use authorization by the FDA during a public health emergency. Further studies are required to determine the optimal human vaccine dose schedule and duration of antibiotics for licensure for postexposure prophylaxis.

Vaccine Safety

Common Adverse Effects

The licensed AVA vaccine, when first used, gave an incidence of local reactions similar to that of the antecedent alum-precipitated vaccine, although no detailed observations were reported.[153] In an open-label study from 1966 to 1971 with the licensed vaccine given SC, approximately 7000 textile employees, laboratory workers, and other at-risk persons received 15,907 doses.[213] There were 24 reports (0.15% of doses) of severe local reactions (defined as edema or induration >120 mm in diameter or marked limitation of arm motion or axillary node tenderness). There were 150 reports (0.9%) of moderate local reactions (edema or induration 30–120 mm in diameter) and 1373 reports (8.6%) of mild local reactions (erythema only or induration <30 mm in diameter). Four cases of systemic reactions (<0.06%), reported as transient, included fever, chills, nausea, and general body aches. None of these studies used contemporary methods of data collection for signs and symptoms.[214]

The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) assessed the safety of the licensed AVA vaccine as part of a randomized clinical study between 1996 and 1999.[182] A total of 28 volunteers received SC doses according to the licensed schedule. Each volunteer was observed for 30 minutes after administration of AVA and scheduled for follow-up evaluations at 1 to 3 days, 1 week, and 1 month after vaccination. Four volunteers reported seven acute adverse events within 30 minutes after SC administration. These included erythema (3), headache (2), fever (1), and elevated temperature (1). Of these events, one patient reported simultaneous occurrence of headache, fever, and elevated temperature (38.2°C). The most common local reactions were tenderness, erythema, SC nodule, induration, warmth, local pruritus, limited arm motion, and edema. Local reactions occurred more often in women. No abscess or necrosis was observed at the injection site. Systemic reactions included malaise, headache, myalgia, fever, anorexia, respiratory difficulty, and nausea or vomiting (4%). All local and systemic adverse events reported in this study were transient. There was one report of a delayed-type hypersensitivity reaction beginning 3 days after the first dose.

The USAMRIID also analyzed the occupational health records of 1583 workers (1249 men and 334 women) who reported adverse events after receiving 10,722 SC doses of the licensed AVA vaccine from 32 separate vaccine lots.[215] In this group, 273 people received 10 or more doses and 46 people received 20 or more doses. With regard to injection-site reactions, 3.6% of doses were reported to produce redness, induration, itching, or edema. Most people who reported a reaction received subsequent doses without problems, but people who reported an injection-site reaction were more likely to report a local reaction to a later dose. Systemic events of headache, fever, chills, malaise, and muscle or joint aches were reported after 1% of doses. The most common of these were headache (0.4%), malaise (0.4%), and fever (0.1%). Women noted local (i.e., erythema, induration, edema, swollen lymph nodes, lumps) and systemic (i.e., headache, fever, dizziness, hives) events more commonly than did men. Vaccine recipients younger than age 40 years reported adverse events more often than did recipients age 40 years or older.

Two uncontrolled case series used serial self-administered surveys to assess the local and systemic reaction after SC anthrax vaccine administration. Among 601 healthcare workers at an Army hospital in Honolulu,[163,216,217] after the first, second, and third doses more women reported localized itching (56%–68%) than did men (24%–31%); more SC nodules (81%–93%) than did men (56%–65%); more moderate to large injection-site reactions (erythema >10 cm) (40%–51%) than did men (17%–32%); and more swelling of the lower arm (8%–15%) than did men (7%–10%). Overall, nearly 20% of both men and women reported symptoms that they personally judged could be ignored; 15% reported symptoms that affected their activity for a short time, but did not limit their ability to perform duties; 8% reported symptoms that were relieved by self-treatment with nonprescription medication that affected their activity for a short time; and fewer than 2% reported symptoms unrelieved by medication, with their ability to perform duties limited for a short time. From 1.5% to 2.7% of women and from 1.2% to 2.1% of men reported systemic events that limited work performance. leading to limitation of performing duties. Events in both sexes resolved without intervention and without sequelae.

Differential rates of adverse events between the sexes have been reported for other vaccines.[214,218–220] Other studies suggested that female sex, body mass index, and prevaccination serum progesterone levels were associated with an increased risk of adverse events. Elevated prevaccination anti-PA IgG levels in women were also associated with an increased risk of local reactions after anthrax vaccination.[221,222]

The other uncontrolled case series was conducted at a U.S. Army base in South Korea[163,216,223] where 2214 men and 610 women were given AVA vaccine SC. Women reported SC nodules more frequently (50%–62%) than did men (21%–29%); erythema greater than 12 cm in diameter was self-reported by 2% to 4% of women and 0.4% to 1% of men. Women experienced more itching at the local site (20%–37%) than did men (6%–8%), more fever (2%–4%) than did men (1%), more chills (3%–6%) than did men (1%–2%), and more malaise (8%–15%) than did men (4%–7%). Overall, 0% to 1.9% of vaccine recipients reported restricted work activities or limited duty assignments. From 0% to 1.1% of vaccine recipients reported losing 1 or more days of duty and between 0.4% to 1.7% sought medical care for the reaction. Regardless of sex, almost all vaccine recipients reported that events were localized or minor and self-limited and did not lead to impairment of work performance.

As noted, the change in the route of routine administration for AVA from SC to IM in December 2008 resulted in fewer solicited injection-site adverse events but had no effect on the occurrence of systemic adverse events.[185]

Rare Adverse Effects

The best quality evidence evaluating the overall safety of the licensed AVA vaccine comes from database studies conducted

by the Army Medical Surveillance Activity and the Naval Health Research Center (NHRC).[163,224,225] These studies established that anthrax-vaccinated and unvaccinated personnel of either sex are hospitalized and visit outpatient clinics at the same rates overall for each of 14 major categories within the International Classification of Diseases and for each of several specific diagnoses with speculative association with anthrax vaccination. For example, 1 per 35 anthrax-vaccinated people was hospitalized per year, compared with 1 per 28 unvaccinated people hospitalized per year.

A similar cohort study evaluated disability discharge rates, comparing 154,456 anthrax-vaccinated and 562,377 unvaccinated soldiers during 4.25 years of follow-up.[226] Rates of disability evaluations did not differ significantly between the two groups, with an adjusted hazard ratio of 0.96 (95% CI, 0.92–0.99). Subset analyses similarly found no differences between men and women for permanent disability, temporary disability, musculoskeletal disability, or neurologic disability.

Three reports described the long-term health of 99 male laboratory workers who received a variety of licensed and investigational vaccines (volume range: 52–134 mL), including the early and the current formulation of anthrax vaccine.[227–229] The third study included a small control group. Although there were elevations in liver and kidney function test results and white blood cell counts in some of the male vaccine recipients, none developed unusual diseases or unexplained symptoms attributable to repeated doses of multiple vaccines. A 17-year follow-up study comparing 155 multiply vaccinated laboratory workers (142 of whom received anthrax vaccine) and 265 unvaccinated workers in the surrounding community found no differences in clinical side effects attributable to anthrax vaccine.[230]

All reports to the Vaccine Adverse Event Reporting System (VAERS) involving the licensed AVA vaccine for a multiyear interval were evaluated by the Anthrax Vaccine Expert Committee (AVEC), composed of civilian physicians.[231,232] The AVEC evaluated 1857 VAERS reports and additional medical records corresponding to 1793 recipients of the AVA between March 1998 and February 2002. The 1857 adverse event reports could be grouped into three main categories, based on effect on the vaccine recipient's functional status: hospitalization, inability to work for 24 hours or more, and "other." Of the 1857 reports, 64 involved hospitalization. The civilian panel found that 11 of the 64 "very likely/certainly" or "probably" were caused by anthrax vaccine. All 11 involved allergic or inflammatory reactions at the injection site. Another 172 reports involved inability to work for 24 hours or more (but did not involve hospitalization); 94 of the 172 reactions were certainly or probably caused by anthrax vaccine according to the AVEC. These 94 reports primarily described injection-site reactions, various rashes, acute allergic reactions, and viral-like symptoms. In the balance of the 1857 reports, 1621, involved neither hospitalization nor time off work for 24 hours or more. All were reviewed by the AVEC, which found no patterns of unexpected adverse events. Subsequent analyses of VAERS reporting rates did not identify any serious unexpected risk from the vaccine.[233]

Two cases of optic neuritis were reported in soldiers after anthrax vaccination. Optic nerve antibodies were found in one case, but no epitopes were found in common between optic nerve and the anthrax organism.[234] A later database study found no statistically significant elevation in the risk of optic neuritis in AVA vaccinees compared with similar unvaccinated people.[235]

A cohort study involving 4092 active-duty women in the U.S. Army assessed the effect of the licensed AVA vaccine on pregnancy and childbirth.[236] This cohort contrasted 3135 vaccinated women and 957 unvaccinated women, with 39,549 person-months of follow-up. The anthrax-vaccinated and unvaccinated women had an equivalent likelihood of becoming pregnant and of giving birth. A subsequent NHRC assessment found a slight elevation in the rate of birth defects in first trimester–exposed infants (odds ratio = 1.18; 95% CI, 0.997–1.41) compared with infants of women vaccinated outside of the first trimester, but no specific defect occurred at an elevated rate after adjustment for multiple comparisons.[237]

An analysis of men in the military evaluated at an assisted reproductive technology clinic compared 254 anthrax-vaccinated and 791 unvaccinated men and found no differences in semen concentration, sperm motility, sperm morphology, need for intracytoplasmic sperm injection, rate of fertilization of mature oocytes, embryo transfer, or clinical pregnancy.[238]

AVA was the subject of much controversy in the late 1990s and early 2000s, with assertions that the vaccine was associated with a variety of adverse health effects. To provide an objective basis for assessing the vaccine's safety profile, a large number of postmarketing safety studies were conducted. These studies involved cohort studies of acute symptoms,[167,182,183,185,214,215,217,221,223,224,235] hospitalizations,[163,224,239,240] quality of life,[241,242] disability evaluations,[226] periodic physical-examination and clinical-laboratory parameters,[243] and reproductive outcomes,[236,238,244] as well as primary and secondary review of the spontaneous reports to VAERS.[231–233] Several cohort studies span multiple years after immunization.[163,167,185,224,227,230,231,239–242,245–248] Multiple studies used active surveillance, defined as data collected at fixed time points without relying on the passive reporting of symptoms or conditions.[167,182,184,185,217,221,230,241,242] Other studies featured systematic surveillance where data were collected automatically and electronically, without any reporting action required by the clinician or associated with specific vaccines.[163,224,226,235,237,238–240,243,245–248]

After the National Academies of Sciences heard from anthrax vaccinees and comprehensively reviewed the accumulated scientific data, they concluded that anthrax vaccine has an adverse-reaction profile similar to that of other adult vaccines.[163] However, the anthrax immunization program needed improvements in the methods of communication between the Department of Defense and military personnel with regard to overall clinical immunization services. Concern about the value of anthrax immunization among military personnel largely resolved after the anthrax bio-attacks of the fall of 2001 that demonstrated the lethality of anthrax spores.

Indications for Vaccination

Routine preexposure vaccination with doses given IM at 0 and 4 weeks and at 6, 12, and 18 months plus yearly boosters is recommended for industrial workers who handle potentially contaminated animal products, including wool, goat hair, hides, and bones imported from countries in which animal anthrax is reported. These countries are primarily in Asia and Africa but are occasionally in South America and the Caribbean. A veterinarian or agricultural worker who has contact with potentially infected animals should be vaccinated, as should laboratory workers who work with *B. anthracis*.[109] For postexposure prophylaxis the most rapid immune response is generated in unvaccinated persons with three doses of vaccine that should be given SC at 0, 2, and 4 weeks in conjunction with a 60-day course of appropriate antimicrobial agents.[108,109]

Special circumstances that warrant vaccination with anthrax vaccine include a threat of biologic warfare or terrorism. The U.S. Armed Forces began vaccinating designated service members in 1998 to protect against anthrax arising from the

use of *B. anthracis* as a biologic weapon. Between 1998 and 2016, more than 3 million military personnel received more than 13 million doses of AVA.[249]

Contraindications and Precautions

A contraindication to vaccination is a hypersensitivity reaction to the vaccine. This is uncommon, but several persons who have received the initial dose or doses developed moderately severe local reactions with some systemic response. If it is necessary to immunize persons with such responses, pretreatment with antihistamines and nonsteroidal antiinflammatory drugs may be of value, although this has not been evaluated scientifically.[250] Anaphylaxis is extremely rare after vaccination and is a contraindication to further vaccination.[109]

PUBLIC HEALTH CONSIDERATIONS

The use of the anthrax vaccine in industrialized populations had a significant impact on the reduction of natural anthrax cases among industrial workers and is one of the main methods by which industrial anthrax was controlled. Improvements in the industrial environment, with better manufacturing equipment and environmental control, also have helped reduce the industrial risks of naturally occurring anthrax. In addition, replacement of animal products (primarily goat hair) with synthetic fibers has reduced the occurrence of anthrax infection. It is ironic that mail-processing machines replaced wool-processing machines as a source of industrial risk in the 2001 anthrax bioweapon attacks.

Agricultural cases have been reduced by control of the disease in animals through the use of animal vaccines and better disposal of infected carcasses. The routine immunization of animals in areas with continuing cases of animal anthrax and the immunization of appropriate humans exposed agriculturally and industrially to *B. anthracis* will serve to reduce the number of naturally occurring human cases.

FUTURE VACCINES

Current acellular vaccines against anthrax are less than ideal for several reasons. These vaccines are composed of an incompletely defined culture supernatant adsorbed to aluminum hydroxide. There is only partial quantification of the PA content of the vaccine or other constituents, thus the degree of purity is not fully characterized. Standardization is determined by biologic activity in an animal potency test. The schedule currently licensed for the U.S. vaccine is less than optimal, in that five doses are required during 18 months, followed by annual boosters. Clearly, the ideal anthrax vaccine would be more completely defined and able to produce long-lasting immunity within 30 days.[163] Current research to accelerate and enhance the antibody response to AVA is of particular importance in view of the lower immune response seen with the new schedule eliminating the week 2 dose and changing the route from SC to IM. The most advanced effort involves use of the adjuvant cytosine phosphate guanine (CpG). This has been shown in mice, guinea pigs, and nonhuman primates[251,252] and in an initial Phase I trial in humans[253] to enhance the antibody response to AVA, although increased adverse events were seen in humans after the adjuvant. In a follow up Phase I study using lower doses of CpG there was some increase in local reactogenicity, transient lymphopenia, and increased serum C-reactive protein (CRP) levels with CpG. The peak antibody response was higher and occurred 1 week earlier with CpG and further studies are planned to determine the optimal regimen.[254] Additional studies suggest a regimen of 0 and 2 weeks with AVA plus CpG gives a higher peak antibody response than a 0-, 2-, and 4-week regimen with AVA alone.[255] Further understanding of the molecular pathogenesis of anthrax and of the structure of PA and its interaction with LF and EF would likely lead to significant progress in the development of improved vaccines. For example, genetically defined mutations in the receptor-binding domain,[256,257] the protease-sensitive domain,[258] or other parts of the molecule[259,260] may generate a less toxic more stable PA preparation to be used alone or as a complex with EF and/or LF. Similarly, mutations in the edema or lethal toxin may allow evaluation of nontoxic complexes with PA. Evidence in experimental animals suggests that adjuvants other than aluminum may increase the protective efficacy of PA substantially even after a single dose,[261,262] including CpG as described, and new formulations using microcapsules,[263] liposomes,[264] nanoparticles[265,266] or delivery by the transcutaneous,[264,267] intradermal,[268] or intranasal[265,269] route also may be of value.

Several vaccine candidates based on recombinant PA protected nonhuman primates from inhalational challenge.[158,205,270] These vaccines are in advanced stages of development, undergoing Phase I/II human trials.[271–273] The initial small studies revealed the PA vaccine to be well tolerated with reactogenicity similar to or less than that seen with AVA. In one study, the immunogenicity of PA after two doses of 5 to 75 μg was noninferior to AVA while in another study without an AVA group, antibody titers were higher at 50 μg compared to doses of 5 and 25 μg. Although there is evidence that the efficacy of AVA against parenteral challenge in rodents differs with challenge strains,[164,165,173] AVA protected rhesus monkeys against a more rigorous aerosol challenge with all strains tested.[208,211] Nevertheless, there is concern that naturally occurring or genetically engineered strains might circumvent protection seen with a single component PA-based vaccine.

Another approach has been to develop live vaccines for human as live vaccine protects experimental animals better than does the licensed human PA vaccine.[157,164,165,274] The precedent exists for using such a vaccine in humans in Russia and the former Soviet republics. Live attenuated vaccines known to protect experimental animals against anthrax include aromatic compound-dependent, toxigenic, nonencapsulated strains of *B. anthracis*.[274] as well as strains of *B. anthracis*,[196,197,275] *B. subtilis*,[274] *Salmonella*,[276,277] *Lactobacillus*,[278] and vaccinia,[279–281] that have been constructed to contain the cloned PA gene alone or with mutated and inactivated LF and/or EF. A system using killed but metabolically active *B. anthracis* expressing PA and mutated LF and EF protected rabbits against intratracheal challenge with a virulent strain.[282] Finally, other approaches giving protection in animals using PA alone or with LF or EF have included nonreplicating DNA vaccines,[159,283,284] viral replicons,[285] adenovirus vectors,[162,286] bacteriophage,[261] and hepatitis B core particles.[287] However, in the first human trial, a DNA vaccine expressing a mutated PA and mutated LF was poorly immunogenic and showed dose-limiting adverse events.[288]

Attempts to identify antigens other than the PA component of the toxin that contribute to protection are also underway. Spore components[289] and the capsule[290–292] have been shown to offer additional protection in small-animal models, and a capsule vaccine offers some protection in nonhuman primates.[50,293] In more recent studies, a capsule vaccine completely protected nonhuman primates against inhalational anthrax.[294] While some enhancement of protection was observed when PA was combined with spore components, there was no protection when the spore components were used alone,[295–297] and the search for protective spore antigens continues. A recent study showed some protection in mice against challenge with an attenuated strain after vaccination with glyceraldehyde-3-phosphate dehydrogenase or GroEL

isolated from *B. anthracis.*[298,299] In addition, the recent sequencing of the *B. anthracis* genome[300] is anticipated to advance the search for new vaccine candidates using reverse vaccinology to identify surface exposed or secreted antigens.[301]

Although these efforts are in the experimental stage, they may lead to the production of a vaccine that requires fewer doses and provides a more rapid and enhanced immune response and long-lasting immunity In addition, inclusion of other PAs, such as the capsule, with PA, may protect against a broader range of anthrax strains, provide protection of individuals who do not respond optimally to PA, and perhaps increase the efficacy of PA vaccines.

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12 Biodefense and Special Pathogen Vaccines

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This chapter discusses vaccines for military and biodefense research personnel as well as some vaccines that are in development for uncommon or geographically limited diseases. Military personnel have the potential to be exposed to many infectious agents as endemic diseases and in their unnatural form as biological weapons. Increasingly, civilian populations may be targets for terrorist attacks using microorganisms (or their toxins), as was demonstrated by the purposeful dissemination of anthrax spores following ballistic attacks on the World Trade Center and the Pentagon in 2001.

The U.S. Army has had a longstanding program to develop vaccines to combat these threats. Within the U.S. Department of Defense (DOD), the U.S. Army Medical Research and Materiel Command (USAMRMC), located at Fort Detrick, Maryland, is the principal organization responsible for vaccines and other medical countermeasures against biological warfare agents. Within USAMRMC, the unit with direct responsibility for this defensive mission is the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), also at Fort Detrick. Before 1969, when the United States maintained an offensive biological weapons program, the U.S. Army Medical Unit within the Walter Reed Army Institute of Research in Washington, DC, served in this capacity.

Many of the biowarfare vaccines currently available at USAMRIID and elsewhere to protect laboratory researchers studying these agents were conceived at the U.S. Army Medical Unit and were further developed at the now closed National Drug Laboratories (also known as The Salk Institute's Government Services Division [TSI-GSD]). Most of the vaccines developed at Fort Detrick, for a variety of reasons, remain investigational new drugs (INDs). However, all these vaccines have undergone extensive preclinical testing and have progressed to Phase II trials that have continued for many years. Meanwhile, USAMRIID researchers and others continue to apply new technology to develop improved vaccines.

Table 12.1 presents all IND vaccine products of military interest developed at USAMRMC/USAMRIID; most of these vaccines are currently available for administration only through USAMRIID's Special Immunizations Program (SIP).[1] One of the vaccines, the Candid #1 Junin (Argentine hemorrhagic fever [AHF]) vaccine, shown to be efficacious in a Phase III trial[2] in Argentina, has been incorporated into that country's public health program and administered to thousands of individuals. Several other vaccines, including Venezuelan equine encephalitis (VEE) TC-83, eastern equine encephalitis (EEE) vaccine, western equine encephalitis (WEE) vaccine, tularemia live vaccine strain (LVS), Rift Valley fever (RVF) inactivated vaccine, and the whole-cell Q fever vaccine, have also been administered to more than 1000 volunteers, primarily through the SIP. In contrast, the chikungunya (CHIK) vaccine strain 181/clone 25, live attenuated RVF vaccine MP-12, Q fever chloroform-methanol residue (CMR) vaccine, and vaccinia/Hantaan vaccine have been administered to no more than a few hundred volunteers each.

Each of the vaccines in active use in USAMRIID's SIP is administered under an approved human use protocol, and all volunteers provide written informed consent before vaccination. These IND vaccines are administered on a voluntary basis to at-risk laboratory and field workers. Most SIP participants are scientists and technicians from USAMRIID, but scientists from academia, other federal and state agencies, and private drug companies also participate.[1] Extramural scientists who wish to receive one of these vaccines must come to USAMRIID for vaccine administration and safety monitoring. The protocols and consent forms are reviewed by the USAMRIID Scientific Review Committee, the Headquarters USAMRMC Institutional Review Board, and the Office of Research Protections (within USAMRMC Headquarters at Fort Detrick) before submission to the U.S. Food and Drug Administration (FDA). Protocols are conducted in accordance with the Belmont Principles, DOD Instruction 3216.02 ("Protection of Human Subjects and Adherence to Ethical Standards in DOD-Supported Research"), and other applicable DOD and FDA regulations and guidelines. Many of these vaccines have been in use since the 1960s and 1970s, and all are subject to periodic potency and lot-release testing as required by FDA.

Continuous monitoring for safety and immunogenicity is conducted through the IND vaccine managing authority: the U.S. Army Medical Materiel Development Activity and the Non-Clinical Studies Division of USAMRIID. Funding for testing of these products is provided through the Medical Countermeasure Systems, Fort Belvoir, Virginia. To date, all vaccines have demonstrated an acceptable safety profile and reasonable immunogenicity (Table 12.2), except the botulinum toxoid pentavalent (ABCDE) vaccine, which is no longer administered.[3] The most reactogenic product is the live attenuated VEE TC-83 vaccine, which frequently induces a short-term systemic reaction.[4] The inactivated vaccines, except the whole-cell Q fever vaccine, require multiple doses for priming and frequent periodic boosting to maintain acceptable levels of neutralizing antibody. All vaccines have been administered to men and nonpregnant women within a broad range of ages, ethnicities, and races. With the exception of Junin vaccine, efficacy of these products is inferred by the absence of laboratory-acquired infection among recipients. However, because laboratory practices and engineering controls have evolved in concert with use of the vaccines, quantifying efficacy on a continuous basis is difficult.

USAMRIID has been proactive in evaluating, not only the short-term reactogenicity, but also the long-term medical safety of its vaccines and other vaccines administered to at-risk laboratory workers. One study[5] was aimed at detecting any long-term medical effects from repeated injections with multiple vaccines; a second study[6] evaluated volunteers who participated in biomedical research as part of Operation Whitecoat.

The first study consisted of 155 volunteers who had participated in a multiple immunizations program (MIP cohort) and 265 community control volunteers who had not participated in a MIP, matched by age, race, and sex.[5] The majority of study volunteers were male (83%) and older in age (average age: 69.4 years). This study did not link any disease or medical condition to repetitive immunization with multiple antigens or any single antigen. Fatigue was noted

TABLE 12.1 Investigational New Drug, Limited-Use Vaccines in Inventory at USAMRIID: Characteristics and Administration

Name	IND Number	Type	Dosage (mL)	Route	Schedule (days)	Boosters
VEE TC-83 (NDBR 102)	BB-IND 142	Live attenuated	0.5	SC	0	Boost with C84 per titer
VEE C-84 (TSI-GSD 205)	BB-IND 914	Inactivated	0.5	SC	0[a]	Yes; based on titer
WEE (TSI-GSD 210)	BB-IND 2013	Inactivated	0.5	SC	0, 7, 28	Mandatory boost at month 6[b]
EEE (TSI-GSD 104)	BB-IND 266	Inactivated	0.5	SC	0, 28	Mandatory boost at month 6[c]
Chikungunya 181/clone 25 (TSI-GSD 218)	BB-IND 2426	Live attenuated	0.5	SC	0	Not determined
RVF (TSI-GSD 200)	BB-IND 365	Inactivated	1.0	SC	0, 7, 28	Yes; based on titer[d]
RVF MP-12, ZH548 (TSI-GSD 223)[e]	BB-IND 4307	Live attenuated	1.0	IM	0	No
Junin, or Candid #1[e]	BB-IND 2257	Live attenuated	0.5	SC	0	No
Q fever (NDBR 105)[e,f]	BB-IND 610	Inactivated	0.5	SC	0	No
Q fever CMR[e] (TSI-GSD 217)	BB-IND 3516	Inactivated	0.5	SC	0	Not determined
Tularemia LVS (NDBR 101, TSI-GSD 213)	BB-IND 157	Live attenuated	0.06	Scarification	0	No

CMR, chloroform-methanol residue; EEE, eastern equine encephalitis; IM, intramuscular; IND, investigational new drug; LVS, live vaccine strain; RVF, Rift Valley fever; SC, subcutaneous; TSI-GSD, The Salk Institute's Government Services Division; USAMRIID, U.S. Army Medical Research Institute of Infectious Diseases; VEE, Venezuelan equine encephalitis; WEE, western equine encephalitis.

[a]C-84 is given only after TC-83 and titer <1:20.

[b]After month 6, boosts of the WEE vaccine are given as needed per titer.

[c]Boosts of the EEE vaccine before and after month 6 are given as needed per titer (as 0.1 mL intradermally).

[d]Initial responders to RVF (inactivated) vaccine: mandatory boost at month 6, then as needed per titer. Initial nonresponders: boost within 90 days of low titer.

[e]These vaccines are currently not in active use at USAMRIID.

[f]A skin test (using Q fever skin test antigen, MNLBR 110, a dilution of the NDBR 105) is conducted 1 week prior to vaccination with Q fever vaccine (NDBR 105).

TABLE 12.2 Assessment of Efficacy and Safety of Selected Limited-Use Vaccines

			Relative Severity and Frequency of Vaccine-Related Reactions	
Name	Tests of Effectiveness	Effective?	Systemic Severity/Frequency	Local Severity/Frequency
VEE TC-83, live	Reduction in laboratory-associated infections	Yes	+++/+++	+/+
VEE C-84, inactivated	Insufficient data	Unknown	+/+	+/+
WEE, inactivated	Reduction in laboratory-associated infections	Probably	+/++	+/+
EEE, inactivated	Reduction in laboratory-associated infections	Probably	+/++	+/++
Chikungunya, live	Insufficient data	Unknown	+/++	+/++
RVF, inactivated	Reduction in laboratory-associated infections	Yes	+/+	+/+
RVF MP-12, live	Insufficient data	Unknown	+/++	+/+
Junin, live	Formal Phase III field trial	Yes	+/+	+/+
Q fever, inactivated	Reduction in laboratory-associated infections	Probably	+/+++	+/+++
Q fever CMR, inactivated	Insufficient data	Unknown	+/++	+/+++
Tularemia LVS, live	Reduction in laboratory-associated infections	Yes	+/+++	+/+++

EEE, eastern equine encephalitis; LVS, live vaccine strain; RVF, Rift Valley fever; VEE, Venezuelan equine encephalitis; WEE, western equine encephalitis.

Severity of reactions: +, most reactions are mild, <5% are severe; ++, most reactions are mild or moderate, <15% are severe; +++, a majority of reactions are mild or moderate, ≥15% are severe.

Frequency of reactions: +, typically occur after <10% of doses; ++, typically occur after ≥10% but <30% of doses; +++, typically occur after ≥30% of doses.

Table displays an overall, qualitative assessment of the severity and frequency of reactions based on published work[2,56,83,92,311–314] as well as unpublished studies conducted at USAMRIID.

more commonly among subjects who had received multiple immunizations, but this finding was not associated with number of shots, number of antigens, or time in the MIP. Although statistically significant increases and decreases in several clinical laboratory tests were observed, none of these findings appeared to be clinically significant.

The single most important finding of this study was the greater frequency of serum monoclonal proteins observed among MIP subjects compared with control subjects. However, no associations between the presence of monoclonal proteins and specific diseases or medical conditions were seen.[5] The significance and implications of this finding are unclear, warranting further investigation. A larger study with more than 1100 volunteers who participated in the SIP has been completed and should, once the results are analyzed, elucidate these findings further. The results of this study are expected to be published in 2017.

The second study involved men entering military service in the early days of the Cold War and the Vietnam War who expressed a conscientious objection to combat. These men were offered the opportunity to serve as medical research volunteers in Fort Detrick's biological warfare defense program. More than 2000 subjects participated in what became known as Operation Whitecoat. A study to assess the current health status of Operation Whitecoat medical research volunteers who served between 1954 and 1973 was developed as a joint effort between DOD and the Seventh-Day Adventist Church.[6]

Many of the volunteers enrolled in this study had been exposed to an infectious agent, vaccine, or other biological product as part of Operation Whitecoat (study group; n = 358); others had participated in research studies as unexposed control subjects or chose not to participate at all (control group; n = 164). Volunteers completed a self-administered questionnaire regarding their health status, clinical signs and symptoms, reproductive outcomes, and diseases or conditions diagnosed by medical providers. Attempts to assess frequencies of clinical signs and symptoms or diseases by individual vaccine, individual virulent agent, and individual antibiotic/inert substance exposure yielded numbers too small for meaningful analysis for all but recipients of VEE vaccine(s) and tularemia vaccine(s) as well as individuals exposed to virulent *Coxiella burnetii*. Among these exposures, a possible association between developing asthma and receipt of tularemia vaccine(s) (but not VEE vaccine or virulent *C. burnetii*) was observed (13.3% of vaccinees vs 2.4% of control subjects). In this same cohort, an association between receipt of a tularemia vaccine and a greater frequency of chronic headaches was suggested (with 35.6% of vaccinees vs 18.3% of control subjects reporting that headaches were occasionally or more frequently a problem as opposed to never or rarely a problem); however, this association did not reach statistical significance. No adverse impact on the overall health of Operation Whitecoat volunteers could be conclusively attributed to participation in research studies at Fort Detrick.[6] Loma Linda University will continue with intermittent follow-up studies.

LIMITED-USE VACCINES AGAINST VIRAL DISEASES

Alphaviruses

The alphaviruses are a group of mosquito-borne, lipid-enveloped, positive-sense, single-stranded RNA viruses belonging to the family *Togaviridae*. Alphaviruses are responsible for two distinct clinical syndromes: fever, chills, headache, myalgias, vomiting, and encephalitis (e.g., VEE, EEE, and WEE) and fever, rash, and polyarthralgias/arthritis (e.g., CHIK, Ross River virus disease [RRVD, formerly called epidemic polyarthritis], and o'nyong-nyong). Several excellent reviews are available on the classification, epidemiology, and clinical features of these agents.[7–9] Although the DOD has developed vaccines against VEE virus (VEEV), WEE virus (WEEV), EEE virus (EEEV), and CHIK virus (CHIKV), these vaccines remain investigational. Ongoing research seeks to develop improved vaccines against these pathogens.[10] For example, a recent study using replicon technology showed that individual replicon vaccine candidates for VEE and EEE or a combined VEEV/WEEV/EEEV replicon particle vaccine elicited strong neutralizing antibodies and protection against aerosol challenge with VEEV subtype I-AB (Trinidad donkey strain) and EEEV. However, the individual WEEV replicon and the combined VEEV/WEEV/EEEV replicon vaccine elicited low levels of neutralizing antibodies to WEEV and conferred poor protection in macaques.[11] In particular, Wolfe and colleagues[12] argue that an FDA-licensed trivalent VEE/WEE/EEE vaccine—one that overcomes the safety, immunogenicity, and immune interference issues of the existing IND vaccines—is required to meet the requirements of the biodefense community. Such a vaccine also would be of great value to the public health and agricultural communities.

Experience with sequential administration of alphavirus vaccines at USAMRIID has led to a number of interesting observations relating to immunologic interference. Prior immunization with inactivated EEE and/or WEE vaccines decreases one's ability to mount a neutralizing antibody response following receipt of live attenuated VEE TC-83 vaccine. However, no interference is observed when the order of administration is reversed, such that VEE TC-83 vaccine is given first.[13] Similarly, interference occurs when two live attenuated alphavirus vaccines, VEE TC-83 and a CHIK vaccine (CHIK 181/clone 25), are administered sequentially.[14] Volunteers initially vaccinated with VEE TC-83 exhibited poor neutralizing antibody responses to live attenuated CHIK vaccine (46% response rate). Among persons initially inoculated with the CHIK vaccine or placebo who then received the live attenuated VEE TC-83 vaccine, geometric mean antibody titers to VEEV, as measured by 80% plaque reduction neutralization test ($PRNT_{80}$), were uniformly depressed in CHIK vaccine recipients compared with placebo recipients.[14] Interference may be an important consideration for the development of next-generation alphavirus vaccines, and particularly for the development of multiagent alphavirus vaccines.

Venezuelan Equine Encephalitis Virus

The two VEE vaccines available for human use as INDs (vaccines)—the live attenuated product, TC-83, and a formalin-inactivated product, C-84—both derive from the same lineage. The live attenuated TC-83 virus, a subtype I-AB strain, was isolated from a donkey brain in Trinidad and was passaged 13 times in embryonated eggs.[15] The virus was attenuated by 78 passages in fetal guinea pig heart (FGPH) cell cultures, plaque-picked in chick embryo fibroblasts (CEFs), and passaged four additional times in FGPH cell cultures.[16] The VEE TC-83 virus designation is a direct reference to the 83 passages in cell culture. VEE C-84 vaccine is formalin-inactivated and is made from the TC-83 production seed, TC-82, that has undergone one additional passage in CEFs.[17] This C-84 production seed is passaged once more in CEFs to derive the C-84 vaccine, which is inactivated with 0.1% formalin and then freeze-dried. The inactivation procedure is based on that used by Salk and colleagues[18] to inactivate the poliovirus. TC-83 and C-84 contain streptomycin and neomycin, each at a concentration

of 50 μg/mL. Laboratory infections with epizootic VEEV strains closely related to the parent strain have essentially been eliminated since the introduction of these vaccines and improvement in personal protective equipment.

At USAMRIID, immunologically naïve people at risk for exposure to VEE receive a single dose of the live attenuated TC-83 vaccine. Those who seroconvert ($PRNT_{80}$ titer ≥1:20) receive a single booster of C-84 as needed based on their titer ($PRNT_{80}$ <1:20); nonresponders to TC-83 receive a booster with C-84. The response rate to TC-83 alone is 82%.[4] When TC-83 is followed by a single boost with C-84, a combined response rate of well over 90% is observed. Female responders to TC-83 tend to have titers similar to those of male responders, but the frequency of nonresponders tends to be higher among women than men; in one study, the initial response rate among recipients of a single dose of TC-83 was 74% and 85%, respectively, for women and men. The nature of this sex difference is not understood.

Approximately 23% of persons receiving the live attenuated TC-83 sustain adverse reactions, including headache, sore throat, malaise, fatigue, myalgias, arthralgias, chills, and fever—a suite of symptoms similar to those seen following natural VEEV infection but less severe.[4] The local reaction rate is less than 5%. The inactivated vaccine C-84 has a local reaction rate of approximately 5%, but essentially no systemic reactions are associated with its administration. Diabetes mellitus, abortion, and teratogenesis have been associated—epidemiologically and/or in animal studies—with natural wild-type VEEV infection.[19–21] Before pregnancy testing prior to vaccination became available, three cases of spontaneous abortion or stillbirth were temporally related to the administration of TC-83.[22] However, VEEV was not recovered from culture of tissues in either case. (Though they were reported to FDA, these cases were never published as case reports.) Since the advent of pregnancy testing, great care has been used to ensure that women are not pregnant before administration of TC-83. Out of an abundance of caution, persons with a family history of diabetes mellitus are considered ineligible for vaccination with TC-83, despite the lack of evidence for a causal association between diabetes mellitus and VEEV infection or vaccination with TC-83.

The ideal VEE vaccine would have a high seroconversion rate (>95%) and a low reaction rate (<5%). By these standards, TC-83 is reactogenic and has a moderate response rate, as measured by neutralizing antibody. In addition, TC-83 does not protect adequately against distantly related VEEV subtype I-AB variants or the other enzootic VEEV subtypes II through VI. Finally, the manufacturing process for TC-83 requires the manipulation of infectious viral particles in a biosafety level 3 containment laboratory.[10]

A new-generation, live attenuated vaccine candidate, V3526, uses twin site-directed mutagenesis of the full-length complementary DNA clone of the virulent virus RNA.[23] V3526 has two deletion mutations—a lethal deletion at the PE-2 cleavage signal site and a suppressor mutation at site 253 of the E1 glycoprotein—that should prevent reversion to wild-type VEEV. This vaccine candidate also has limited potential for transmission by mosquitoes and elicits cross-protection against different viral strains.[10] After showing promise in preclinical studies,[24] V3526 elicited the development of impressive neutralizing antibody levels in human volunteers during Phase I clinical trials. However, the vaccine was associated with a high frequency of fever and other flu-like symptoms; thus, further development was discontinued.[25,26]

Because of the long history of frequent adverse reactions related to live attenuated VEE vaccines, the manufacturer decided to inactivate V3526 and further develop it as an inactivated vaccine to be used as a priming vaccine that would replace C-84. Testing has shown reduced infectivity by both formalin inactivation (fV3526) and gamma-irradiation (gV3526). For example, both inactivated vaccine candidates showed a loss of neurovirulence after intracerebral inoculation of suckling BALB/c mice, suggesting that the vaccines were completely inactivated. Both fV3526 and gV3526 elicited robust immune responses; furthermore, protection was demonstrated by both vaccine candidates against subcutaneous challenge with VEEV I-AB Trinidad donkey strain following two doses of either vaccine. Recently, researchers have been testing these vaccine candidates, using various adjuvants and routes of administration, against subcutaneous and aerosol challenge.[24,27,28]

In addition to fV3526 and gV3526, other technologies are being evaluated for the production of vaccines against VEE. For example, Sharma and colleagues[29] found that the hydrophobic alkylating compound 1,5-iodonaphthylazide can effectively inactivate virulent VEEV strain V3000. The resulting inactivated vaccine candidate was efficacious in protecting mice from virulent VEEV challenge, and its efficacy was enhanced by the use of adjuvants. Rossi and colleagues[30,31] developed a live attenuated vaccine using an encephalomyocarditis virus internal ribosome entry site (IRES)—a construct that inhibits translation of viral proteins in mosquito cells, thus preventing transmission by the natural VEEV vector. This IRES-based VEE vaccine fully protected mice and macaques from clinical disease after aerosol challenge with virulent VEEV.

DNA vaccines are also under development and, in protective efficacy studies, have shown promise in both mice and guinea pigs.[32] One DNA vaccine candidate has also demonstrated protection against aerosol challenge with wild-type VEEV in nonhuman primates.[33,34] A candidate based on a novel approach to DNA vaccine development, infectious DNA (iDNA), was protective in one BALB/c mouse challenge study.[35]

Western Equine Encephalitis Virus

An inactivated WEE vaccine, TSI-GSD 210, has been used at Fort Detrick since the 1970s to immunize at-risk laboratory personnel. The WEE vaccine is a lyophilized product derived from supernatant fluids of primary CEF cell cultures infected with the attenuated CM4884 strain of WEEV.[36,37] The supernatant fluid is harvested and filtered, the virus is inactivated with formalin, and the final product is lyophilized for storage at −20°C. The vaccine contains 50 μg/mL of neomycin. The primary end point used to measure immunogenicity of the WEE vaccine is the $PRNT_{80}$, with a titer of at least 1:40 considered indicative of a response.

In an analysis of data from 363 volunteers who received 0.5 mL of inactivated TSI-GSD 210 vaccine subcutaneously at days 0, 7, and 28, 151 subjects (41.6%) responded with a $PRNT_{80}$ titer of 1:40 or greater, whereas 212 subjects (58.4%) failed to achieve this neutralizing antibody titer. Of 115 initial nonresponders, 76 (66.1%) converted to responder status after a single booster. Kaplan–Meier plots showed that a regimen consisting of three initial doses and one booster induced a $PRNT_{80}$ titer of at least 1:40 lasting 1.6 years in 50% of initial responders. Local and systemic adverse events are uncommon with this vaccine. Among 363 vaccinees receiving three initial injections of the WEE vaccine, only 5 reported local or systemic reactions (P.R. Pittman, P.H. Gibbs, and T.L. Cannon, unpublished data).

No instances of occupational WEE have been documented among laboratory workers who develop neutralizing antibodies following vaccination. WEE vaccine continues to be administered as part of Phase II clinical trials; however, no efficacy trial has been conducted.

A new lot of the WEE vaccine, Western Equine Encephalitis Vaccine, Inactivated, TSI-GSD 210, Lot 3-1-92, was found to be safe and immunogenic in a recent Phase I clinical trial (Dr. Robert Rivard, USAMRIID, personal communication, 2015). In this study, WEE vaccine was administered in 0.5-mL doses subcutaneously in the upper outer aspect of the triceps in a three-dose primary series (days 0, 7, and 28) with a mandatory boost (day 180). All 10 subjects were classified as responders at day 56. For 4 of 10 subjects, the titers had waned below the acceptable level by month 6. Following the month 6 boost, all 10 subjects developed titers above 1:40, and all remained above this level for at least 1 year.

DNA-based vaccines against WEE have shown promise in challenge models in mice. One such study evaluated a DNA vaccine, pVHX-6, expressing the 26 S structural gene of WEEV strain 71 V-1658. All mice receiving four intraepidermal doses of pVHX-6 survived challenge with a homologous strain, but only 62% and 50% survived challenge with Fleming and CBA87 strains, respectively.[38] Other studies have demonstrated, in a murine model, the efficacy of DNA vaccines expressing the capsid and envelope proteins of WEEV in challenge models. In two studies, a replication-defective human adenovirus serotype-5 (HAd5) was used as a vector for vaccine delivery. In the first study, a HAd5 vector encoding E2 and E1, administered as a single dose, conferred protection against challenge with homologous and heterologous WEEV strains.[39,40] In the second study, a HAd5 vector encoding E1 alone, designated adenovirus (Ad) serotype-5-E1 (Ad5-E1), provided total protection against homologous and heterologous strains of WEEV in mice.[41] A potential drawback of this approach in humans is the widespread prevalence of antibodies against adenovirus.

Eastern Equine Encephalitis Virus

The EEE vaccine (TSI-GSD 104) is a lyophilized product originating in primary CEF cell cultures infected with the attenuated PE-6 strain of EEEV. The seed for the EEE vaccine is passaged twice in adult mice and twice in guinea pigs, then passaged nine times in embryonated eggs, followed by three passages in CEFs.[42] The supernatant fluid, which is harvested and filtered, contains 50 μg each of neomycin and streptomycin and 0.25% w/v (weight per volume) of human serum albumin, USP (U.S. Pharmacopoeia). The virus is then inactivated with 0.05% formalin. When inactivation is completed, the residual formalin is neutralized by treatment with sodium bisulfite. The final product is lyophilized for storage at −20°C.

Among 255 volunteers who received the two-dose primary series of EEE vaccine (administered subcutaneously) between 1992 and 1998, 197 (77.3%) responded with a $PRNT_{80}$ titer of 1:40 or greater. Of initial nonresponders, 66% subsequently seroconverted following receipt of an EEE vaccine booster (which is administered intradermally). Among initial responders whose titers waned over time, 98.6% responded to a booster dose of EEE vaccine. Local and systemic side effects are infrequent, occurring in less than 1% of vaccinees after the primary vaccine series and in 3.7% after the first booster. Kaplan–Meier plots show that two primary doses and one intradermal booster of TSI-GSD 104 provide satisfactory neutralizing anti-EEEV antibodies in 50% of initial responders for up to 2.2 years.[43]

Among recent findings regarding next-generation EEE vaccines, a single subcutaneous dose of an EEE vaccine candidate, attenuated via an IRES, protected 100% of vaccinated mice against intraperitoneal challenge.[44] In addition, an EEEV replicon and a combined VEEV/WEEV/EEEV replicon protected macaques from aerosol challenge.[11]

Chikungunya Virus

CHIKV can cause an acute viral syndrome in humans characterized by fever, rash, and arthritis.[45] Some people, especially those who are human leukocyte antigen (HLA)-B27–positive, may develop long-term joint involvement.[46] Although documented epidemics have occurred since the late 18th century, the virus was first isolated during the 1952–1953 Tanzanian epidemic.[47] During 2004–2006, an epidemic ravaged the Indian Ocean islands east of Madagascar—La Réunion, Mauritius–Seychelles, and Mayotte.[48] More than 2 million people have been affected since the start of the epidemic, which has since spread to the Caribbean and India, with cases also occurring in persons from the United States, Europe, and elsewhere who traveled to the Caribbean.[49–52] In December 2014, Kendrick and colleagues[53] reported on the first 11 cases of CHIK originating in the United States (Florida). Genomic sequencing of six isolates from the Indian Ocean outbreak suggests that the strain responsible for the outbreak is related to East African isolates.[49] In addition, evidence from the sequence data suggests that the CHIKV strain responsible for the Indian Ocean outbreak evolved, during the course of the outbreak, into several distinct variants. One mutation may have allowed the CHIKV to be efficiently transmitted by the most abundant mosquito species on La Réunion (*Aedes albopictus*); this may help explain the noted robustness of the epidemic.[49,52,54]

Several attempts were made, with variable success, to develop an efficacious inactivated vaccine using chick embryo cell cultures, suckling mouse brain, and African green monkey kidney.[55] A live attenuated vaccine was made from the seed of the African green monkey kidney vaccine, using CHIKV strain 15561, which had originally been isolated from an infected patient during a 1962 CHIK epidemic in Thailand.[55,56] The 11th African green monkey kidney passage of CHIKV strain 15561, made at the Walter Reed Army Institute of Research, was transferred to USAMRIID and passaged in Medical Research Council (MRC)–5 cells. After 18 passages in MRC-5 cells, CHIKV 181/clone 25 was selected as a vaccine seed strain based on biomarkers. CHIKV 181/clone 25 was efficacious in challenge models in suckling mice and in nonhuman primates.[55]

A randomized, double-blind, placebo-controlled trial of the CHIK 181/clone 25 vaccine documented a seroconversion rate of 98% among alphavirus-naïve volunteers.[56] One year after immunization, 85% of vaccinees remained seropositive. Injection site and systemic symptoms, including flu-like symptoms, were similar in vaccine and placebo recipients. However, the vaccine was temporally associated with arthralgia in 8% of vaccinees. One volunteer in the vaccine group developed a pruritic, eczema-like rash at the injection site. Interference between the CHIK 181/clone 25 vaccine and the live attenuated VEE TC-83 vaccine is discussed above. This live attenuated CHIK vaccine requires additional Phase II and Phase III clinical testing. Current epidemics in the Caribbean, Indian Ocean, and India offer unique prospects for Phase III clinical testing of this vaccine.

Additional strategies for the development of a safe and efficacious vaccine for CHIK are being pursued. These include chimeric alphavirus vaccine candidates using VEEV attenuated vaccine strain TC-83, a naturally attenuated strain of EEEV, or Sindbis virus as a backbone with the structural protein genes of CHIKV. Wang and colleagues[57] found that each of these chimeras produced robust neutralizing antibody responses, and vaccinated mice were fully protected against disease and viremia after CHIKV challenge. One DNA vaccine candidate expressing a component of the CHIKV envelope glycoprotein produced neutralizing antibodies in mice and macaques.[58] A second DNA vaccine candidate uses a plasmid coding for the CHIKV-capsid,

E1 and E2. When injected into mice, this construct induced broad cellular immunity and produced antibodies, detectable by enzyme-linked immunosorbent assay (ELISA), that recognized native antigen.[59] Another group is evaluating a formalin-inactivated Vero cell–adapted vaccine candidate prepared using a strain from the India epidemic.[60] Most recently, a promising virus-like particle (VLP) vaccine containing the viral envelope has elicited high titers of neutralizing antibodies in nonhuman primates and in human volunteers.[61–63] In addition, a single immunization with a measles virus–vectored CHIK vaccine candidate induced high CHIKV antibody titers and protected mice from a lethal CHIKV challenge.[64]

Ross River Virus

RRVD, or epidemic polyarthritis, was first recognized in Australia in 1928; Ross River virus (RRV), its causative agent, was first isolated in 1963 from a pool of mosquitoes.[65–67] RRVD is essentially limited to Australia, Fiji, and the surrounding islands, including American Samoa and the Cook Islands, where several thousand cases occur per year.[68,69] In humans, polyarthritis may be followed by fever, rash, and lethargy; symptoms generally resolve after 3 to 6 months.[69,70] In 1997, U.S. marines participating in Operation Tandem Thrust in Queensland, Australia, had an infection rate of 1.5%; RRVD developed in nine individuals.[71]

A vaccine candidate was derived from a virus isolated from a human case of classical RRVD using the C6-36 cell line (*A. albopictus*).[72] The candidate underwent four serial passages in MRC-5 human fetal lung cells followed by two passages in Vero cells. Cell cultures contained penicillin (100 U/mL) and streptomycin (100 µg/mL). The virus was inactivated using binary ethyleneimine. In mice, the vaccine induced neutralizing antibodies and conferred protection against viremia after intravenous challenge with live RRV.[73]

More recently, a Vero cell–grown, formalin-inactivated, benzonase-digested (to digest host cell DNA), sucrose gradient–purified vaccine candidate induced neutralizing antibodies in mice.[74] Immunized mice and guinea pigs failed to develop viremia following intravenous challenge with the prototype strain of RRV (T48). In a Phase I/II dose escalation study[75] in 382 healthy, RRV-naïve adults, this vaccine was safe and immunogenic when administered as three immunizations (at days 0 and 21 and month 6) at four dose levels, with or without aluminum hydroxide added as an adjuvant; the adjuvanted 2.5-µg dose stimulated the highest immune response. This group recently completed a Phase III study in 1755 healthy younger adults (ages 16–59 years) and 209 healthy older adults (ages 60 and older).[76] The vaccine was well tolerated. Neutralizing antibody titers (≥1:10) were achieved by 91.5% of the younger adults and 76.0% of the older adults after the third vaccination. The potential for antibody-dependent enhancement of disease, which has been described for RRV in vitro, will need to be considered during the development of vaccines against RRV.[69,77]

Bunyaviruses

The Bunyaviridae are a large family of segmented, lipid-enveloped, negative-stranded RNA viruses. Vaccines against human pathogens representing two of the five genera, RVF virus (*Phlebovirus* genus) and Hantaan virus (*Hantavirus* genus), have been developed at Fort Detrick.

Rift Valley Fever Virus

RVF is a mosquito-borne infection endemic to sub-Saharan Africa and primarily affecting ruminant animals. Under appropriate climatologic conditions, however, explosive epizootics among animals and epidemics in humans occur with considerable morbidity and mortality. In recent years, RVF has demonstrated its ability to spread northward to Egypt and into Yemen and Saudi Arabia on the Asian continent. The consequences of further spread into naïve animal and human populations are potentially devastating.[78] The spread of West Nile virus (WNV) has demonstrated that viruses once bounded by the Atlantic Ocean have the potential to overcome that barrier. Thus, the development of a vaccine or other countermeasures against the RVF virus is important for the protection of human and animal populations in Africa and the Middle East, U.S. military personnel involved in campaigns in those regions, and livestock and people in the United States and elsewhere.

The U.S. Army has developed two vaccines to combat this threat: an inactivated RVF vaccine (TSI-GSD 200) and, more recently, a live attenuated product (RVF MP-12; TSI-GSD 223). The Entebbe strain of RVF, isolated from a mosquito pool in Bwamba County, Uganda, is the source for the inactivated vaccine.[79] The virus was passaged 184 times in adult mice, followed by two passages in fetal rhesus lung (FRhL) cells to form the production seed. Although the original vaccine was produced in primary African green monkey kidney cells, the current vaccine lots are produced in FRhL cells.[80,81] The vaccine is inactivated in 0.05% formalin. Following verification of viral inactivation, the residual formaldehyde is neutralized with sodium bisulfite to less than 0.01%. A study of the immunogenicity and safety of inactivated RVF vaccine in humans during a 12-year period showed that the vaccine was safe and immunogenic when the three-dose primary series and one booster are administered.[82,83] In particular, 540 (90%) of 598 volunteers given three 1.0-mL doses of TSI-GSD 200 subcutaneously on days 0, 7, and 28 responded with a $PRNT_{80}$ titer of 1:40 or greater. Three-fourths of the initial nonresponders developed a $PRNT_{80}$ titer of 1:40 or greater after a single booster. However, approximately 10% of recipients of the inactivated RVF vaccine require repeated boosting. In these individuals, a booster typically results in an adequate titer, which wanes over the next year to <1:40, prompting another booster (P.R. Pittman, unpublished data).

An isolated RVF strain recovered from a nonfatal human case that occurred during the first Egyptian epidemic in 1977 was used to derive the live attenuated RVF vaccine. The virus (ZH548) was passaged twice in suckling mouse brain, then once in FRhL cells. It was then attenuated by 12 serial alternating passages in human lung cell cultures (MRC-5 cells, certified for vaccine use) by previously described methods[84] in the presence or absence of 5-fluorouracil. The resulting RVF MP-12 vaccine is a lyophilized product originating from supernatant fluids harvested from the final mutagenesis passage. The vaccine has undergone extensive safety testing and challenge studies in several animal species: rodents, sheep (including pregnant ewes and naïve neonatal lambs), cattle (including in utero–vaccinated bovids), and monkeys.[85–89] Furthermore, Miller and colleagues[90] showed that RVF MP-12 vaccine, administered to sheep, results in long-lasting immunity and has limited potential to be transmitted to mosquitoes feeding on vaccinated animals.

RVF MP-12 has undergone clinical evaluation in human volunteers at USAMRIID. In a Phase I randomized, double-blind, dose-escalation/route-seeking study, 56 healthy, nonpregnant subjects were randomly selected to receive RVF MP-12 ($10^{4.7}$ plaque-forming units [PFU] subcutaneously, $n = 10$; $10^{3.4}$ PFU intramuscularly, $n = 6$; or $10^{4.4}$ PFU intramuscularly, $n = 27$) or placebo ($n = 13$).[91] Only infrequent and minor side effects were seen among placebo and MP-12 recipients. One volunteer had a titer of 1.3 log by direct

plaquing in cell culture. Six vaccinees had transient low-titer viremia detected by amplification only; all six of these volunteers were from the group receiving $10^{4.4}$ PFU intramuscularly. Neutralizing antibodies (measured by $PRNT_{80}$ titer), as well as RVF-specific immunoglobulin (Ig) M and IgG, were observed in 40 (93%) of 43 vaccine recipients. The highest peak geometric mean antibody titers were observed in the group receiving $10^{4.4}$ PFU intramuscularly. Overall, 28 (82%) of 34 RVF MP-12 recipients available for testing remained seropositive ($PRNT_{80}$ = 1:20) at 1 year following inoculation.[91]

A joint program between the University of Texas Medical Branch and USAMRIID for further development of the RVF MP-12 vaccine has been completed. This study, funded by the National Institute of Allergy and Infectious Diseases, involved the administration of a single intramuscular dose of RVF MP-12 vaccine to 19 naïve male and nonpregnant female subjects. The vaccine was safe and immunogenic in this vaccine trial. A total of 18 (95%) of 19 subjects developed neutralizing antibodies against RVF virus, as determined by a $PRNT_{80}$ titer of 1:20 or more. Results suggested that the vaccine resulted in, at most, only low-level viremia. No virus was detected by direct plaque assay; however, during the first 14 days after vaccination, nine MP-12 isolates were recovered from five subjects with the use of amplification by blind, double passage in Vero cells. No single-nucleotide polymorphisms or reversions were observed in the attenuating mutations of the parent virus.[92]

Another RVF live attenuated vaccine candidate is Clone 13. This vaccine candidate is a plaque-purified clone of RVF virus that contains a large deletion in the small (S) genome segment that disrupts the biological functions of the nonstructural proteins NSs. Clone 13 has proven highly immunogenic in mice, sheep, and goats, although it has demonstrated only moderate immunogenicity in cattle.[93,94]

Other approaches to the development of vaccines against RVF virus, all of which are at the preclinical stage, include vaccines based on viral vectors[95–97]; DNA vaccines with molecular adjuvants[98]; subunit vaccines based on purified proteins[99–103]; and vaccines based on single-cycle replicable vaccine mutants.[104]

Hantaviruses

Hantaviruses causing hemorrhagic fever with renal syndrome (HFRS) have been, and continue to be, significant endemic disease threats to U.S. military forces on the Korean peninsula and throughout Europe.[105] At least 14 distinct viral strains of HFRS-causing viruses are distributed worldwide.[106] Three viral proteins are able to induce protection: two surface glycoproteins, G1 and G2 (also called Gn and Gc), and the N nucleocapsid protein. Neutralizing antibodies are protective, although T-cell responses may also be useful.[107] In addition to HFRS, hantaviruses are responsible for hantavirus pulmonary syndrome.[106,108] The hantavirus causing this syndrome in the southwest United States is called Sin Nombre virus (SNV). Humans become infected with hantaviruses via inhalation of aerosolized rodent excreta.[109] The prototype hantavirus, Hantaan, was first isolated in Korea, and the first vaccine also was developed there to protect against HFRS.[108,110,111] This inactivated vaccine, the ROK84/105 strain, harvested from suckling mouse brains, is concentrated by protamine sulfate precipitation and centrifugation. The concentrate is then exposed to formalin inactivation and purified by ultrafiltration and sucrose gradient ultracentrifugation. Aluminum hydroxide is added to the vaccine as an adjuvant, thimerosal as a preservative, and gelatin as a stabilizer. The final product, produced by Rhein Biotech, reportedly has less than 0.01 ng/mL of myelin basic protein. The recommended regimen for this product, Hantavax, is two doses of 5120 ELISA units (0.5 mL) given 1 month apart by the subcutaneous or intramuscular route.

Little has been published on the vaccine, but the manufacturer reports that tolerance is good, although allergic reactions occur, presumably as a result of mouse brain antigens. A serologic response measurable by indirect fluorescence is seen in nearly all vaccinees. Neutralizing antibodies are usually absent after one dose, but are measured in approximately 75% of subjects after the second dose.[112] They decrease to 16% at 12 months, at which time a booster is recommended. Although placebo-controlled data on the efficacy of the vaccine are not available, a similar vaccine made in North Korea reportedly showed 88% to 100% efficacy, and Hantavax itself has been effective in uncontrolled epidemiologic studies done in South Korea and Yugoslavia.[113,114] Although neutralizing antibody responses were not persistent and not stimulated by a booster dose,[107,115] a case-control study conducted in the Republic of Korea estimated efficacies of 46% for two doses and 75% for three doses, but with wide confidence limits.[116]

Vaccines made in cell culture against hantavirus and Seoul virus strains are licensed in China. The substrate is cell cultures of golden hamster kidney or Mongolian gerbil kidney. The efficacy of these bivalent vaccines is reportedly better than 90%, with fewer reactions than after the use of mouse brain–derived vaccine.[113,114]

Another Korean company has developed a hantavirus vaccine made in Vero cells that protected challenged animals by eliciting strong responses to the G1, G2, and N proteins of the virus.[117]

At USAMRIID, advances in technology fueled the development of bioengineered hantavirus vaccines. Two experimental approaches included recombinant vaccinia-vectored Hantaan virus vaccine carrying envelope and nucleocapsid genes[118,119] and the same genes inserted into bacterial plasmids as DNA vaccines. The recombinant vaccinia-vectored Hantaan virus vaccine was efficacious in the hamster infectivity model; even if preexisting immunity to vaccinia virus was present, it could be overcome by a second intramuscular injection of the vaccine candidate.[118] A double-blind, placebo-controlled clinical trial involving 142 volunteers using two subcutaneous injections 4 weeks apart showed that, for vaccinia-naïve volunteers, neutralizing antibodies to Hantaan virus or vaccinia virus were detected in 72% or 98%, respectively, whereas neutralizing antibodies to Hantaan virus were detected in only 26% of vaccinia-immune volunteers, showing the effect of prior vector-directed immunity.[119] This vaccine has been abandoned.

DNA plasmids coding for the G1 and G2 proteins protected monkeys against challenge with a South American hantavirus and elicited antibodies that exerted passive protection in hamsters.[120] Boosting the monkeys 1 to 2 years later showed that immunological memory had been induced.[121] Alphavirus replicons, baculovirus-produced proteins, and chimeric hepatitis B VLPs are all under study as additional hantavirus vaccine candidates.[113,122,123]

Boudreau and colleagues[124] report on a Phase I clinical trial that evaluated Hantaan virus and Puumala virus M-segment DNA vaccines for preventing HFRS. Each volunteer received either Hantaan DNA vaccine (n = 9), Puumala DNA vaccine (n = 9), or both vaccines (n = 9). Three doses (containing 8 μg DNA/4 mg gold per dose), administered 4 weeks apart, were provided to volunteers by particle-mediated epidermal delivery. The single vaccines elicited neutralizing antibodies to Hantaan virus or Puumala virus, respectively, in 30% and 44% of vaccinees. Neutralizing antibodies to one or

both viruses were detected in 56% of volunteers who received the combined vaccine. In an attempt to increase seroconversion, Hooper and colleagues[125] conducted a Phase I clinical trial of these two DNA vaccines delivered by intramuscular electroporation, similar to the Boudreau study. Volunteers (three groups of nine individuals each) received three doses, 28 days apart, of either the Hantaan DNA vaccine, the Puumala DNA vaccine, or a mixture of both vaccines. Each dose of vaccine contained 2 mg DNA in a total volume of 1 mL saline; the combined vaccine contained 1 mg of each DNA vaccine. Neutralizing antibodies were found in 56% and 78% of volunteers who received the Hantaan or Puumala DNA vaccines, respectively. Results of the combined vaccine showed that 78% of vaccinees developed neutralizing antibodies against Puumala virus. The three volunteers with the highest antibody levels against Puumala also developed neutralizing antibodies against Hantaan. No serious adverse events resulting from the vaccines were noted in either study.

Hooper and colleagues[126,127] also found that DNA vaccines encoding the virus envelope glycoproteins of SNV or Andes virus (both of which cause hantavirus pulmonary syndrome) elicit high-titer neutralizing antibodies in animal models. Hamsters vaccinated with three doses of the SNV DNA vaccine were fully protected from lethal challenge with SNV. Further, a pan-hantavirus vaccine using Andes virus, SNV, Hantaan virus, and Puumala virus plasmids elicited neutralizing antibodies against all four viruses.[126]

West Nile Virus

The spread of the flavivirus WNV from its introduction into New York City in 1999 across the United States has been a dramatic example of an emerging infection. WNV is an arbovirus of birds, for which it is often lethal, and is transmitted by multiple mosquito species. Although the incidence of human infections has recently decreased, WNV frequently causes febrile illness, with the complication of meningoencephalitis in about 1% of infections.[128]

WN-VAX, a formalin-inactivated WNV vaccine derived from a strain isolated in New York City in 1999, has been tested in a number of animal models. This vaccine, administered intraperitoneally in two doses, protected 100% of 4-week-old mice against a lethal challenge with WNV.[129] More recently, Muraki and colleagues[130] found that mice passively immunized with serum from WN-VAX–immunized mice were also protected from lethal WNV infection. When administered to macaques, WN-VAX elicited neutralizing antibodies.[130] Clinical trials on this vaccine are planned.

Several Phase I and Phase II clinical trials have been completed on WNV vaccine candidates. The ChimeriVax West Nile vaccine candidate, containing a yellow fever virus backbone and expressing the premembrane and envelope proteins of WNV, was reported to be safe and induced neutralizing antibodies in a Phase I clinical trial.[131,132] In a Phase II clinical trial, ChimeriVax was well tolerated, and more than 96% of vaccinees seroconverted.[133]

In a Phase I clinical trial, a DNA vaccine candidate elicited neutralizing antibodies against WNV.[134] A recombinant subunit vaccine candidate, consisting of an envelope protein truncated at the C-terminal end and containing 80% of the N-terminal amino acids of the native WNV protein (WN-80E) mixed with adjuvant, produced antibodies detectable by ELISA and neutralizing antibody assays. In the challenge experiment, all control rhesus macaques had detectable viremia for at least 3 days after challenge, whereas none of the vaccinated animals showed viremia.[135] A Phase I trial assessing the safety of this vaccine was recently completed.

Arenaviruses

Several members of the *Arenaviridae* family of segmented, negative-stranded RNA viruses are recognized as causative agents for viral hemorrhagic fever syndromes in humans. To date, efforts to develop protective immunogens against arenaviruses have met with limited success.[136] During the 1980s, the first successful vaccine against an arenavirus, Junin virus, was developed through a collaboration between the government of Argentina and the U.S. Army. Efforts to develop vaccines against other pathogenic arenaviruses have resulted in a number of promising candidates, but none of these has progressed to clinical trials.[137]

Junin Virus

Junin virus is the causative agent of AHF, which is endemic to the pampas of north-central Argentina. Humans become infected with the Junin virus by inhalation of infected rodent secretions and excretions.[138–140] Death occurs in 15% to 30% of untreated patients afflicted with AHF.

For several decades, attempts were made in Argentina to develop an efficacious vaccine against AHF. The resulting inactivated and live attenuated vaccine candidates all failed for various reasons.[136] The product developed at USAMRIID, Candid #1, is a descendant of the prototype XJ strain Junin virus, isolated in guinea pig from a fatal AHF case. Following another passage in the guinea pig, the virus underwent 44 newborn mouse brain passages, then was cloned and passaged 19 times in certified FRhL cells.[136,141,142] Candid #1 proved effective in preventing disease in guinea pigs and rhesus macaques after lethal Junin virus challenge.[140,143,144] In Phase I and Phase II clinical testing in humans, Candid #1 was safe and immunogenic.[145,146] More than 90% of volunteers developed antibodies against Junin virus, although at lower levels than those seen after mild natural infection,[147] and 99% developed a Junin virus–specific cellular immune response. In a pivotal efficacy study, 3255 volunteers were randomized to receive the vaccine and 3245 were randomized to receive a placebo. During the trial, 23 volunteers developed an illness that met the clinical case definition for AHF.[148,149] Of these, 22 had received a placebo and 1 had received the vaccine; vaccine efficacy by intent-to-treat analysis was 95% (95% confidence interval, 82%–99%; $P < .001$). Argentina's National Institute of Human Viral Diseases produced its own Junin vaccine, also called Candid #1, against AHF and tested it in 946 human volunteers to compare its safety and immunogenicity with that of the Candid #1 vaccine produced in the United States and used in previous studies.[150] The vaccine was comparable to the U.S.-produced vaccine in terms of safety and efficacy. No severe adverse events were related to the vaccine. As a result of this study, Argentina's national regulatory authority (ANMAT) licensed the locally produced Candid #1 vaccine.[151,152]

Vaccination with Candid #1 is recommended for persons at risk of occupational (agricultural or laboratory) exposure to the Junin virus. The development of this vaccine represents a successful collaboration between USAMRIID and the Argentine Ministry of Health and Social Action, under the auspices of the United Nations Development Programme and the Pan American Health Organization. Over the years, hundreds of thousands of people in Argentina and other countries have been inoculated, with an excellent record of safety and effectiveness.[153]

Lassa Virus

Another important Arenavirus is Lassa virus, transmitted by rodents in West Africa, where there has been a recent large

epidemic. Several candidate vaccines have shown protection in nonhuman primate models, including reassortants, vesicular stomatitis virus-vectored recombinants, and alphavirus replicons.[153a]

Middle East Respiratory Syndrome Virus

The Middle East respiratory syndrome (MERS) virus—like the severe acute respiratory syndrome (SARS) virus that spread from China and caused a worldwide outbreak in 2002–2003—is a coronavirus.[154,155] The reservoir for MERS is camels, particularly young animals, which acquire a respiratory illness that can be transmitted to humans.[156,157] In addition, human-to-human transmission has been repeatedly observed in Saudi Arabia and in South Korea.[158] All coronaviruses possess a glycoprotein spike that induces neutralizing antibodies; both monoclonal antibodies and vaccines based on the spike have shown good protection in experimental challenge models, including macaques.[159] Development of vaccines for camels and for humans is proceeding.

Novavax has developed 0.2-μm nanoparticles consisting of MERS spike protein which, together with ISCOMATRIX adjuvant, have been shown to induce neutralizing antibodies in mice.[160] German workers have used the MVA vaccinia virus as a vector for the MERS spike protein and induced neutralizing antibodies and protection in a mouse challenge model.[161] The Vaccine Research Center at the National Institutes of Health has developed plasmids and recombinant proteins representing the full-length MERS spike or the S1 domain of the spike. When both antigens were administered to mice or macaques, neutralizing antibodies were induced and animals were protected from challenge with the virus.[162] The further development of these candidate vaccines will depend on epidemiology and market size.

LIMITED-USE VACCINES AGAINST BACTERIAL DISEASES

Q Fever

Q fever is a highly infectious zoonotic disease of humans usually caused by aerosol transmission of *C. burnetii* from infected sheep or goats.[163] The organism was thought to be a *Rickettsia* but is now considered to be related to *Legionella* bacteria. Fever and pneumonia are the most frequent clinical manifestations of Q fever, although hepatitis, endocarditis, and a variety of other complications may develop.[164] Antibiotics are effective but may act slowly, and chronic fatigue syndrome after Q fever has been reported, presumably as a result of cytokine dysregulation.[165]

Mechanisms of immunity to Q fever are complex. Although antibodies are important to clear extracellular organisms, it is sensitization of T-cell lymphocytes to Q fever antigen and secretion of lymphokines that clear intracellular infection and provide immunologic memory.[166]

In the development of a Q fever vaccine, *C. burnetii* was adapted to grow in the chick embryo yolk sac, and an early vaccine was made by formalin inactivation of organisms produced in eggs.[167] This vaccine was effective in humans, including in those subjected to experimental challenge, but it also occasionally resulted in severe local reactions that sometimes progressed to abscess formation. Eventually it was shown that reactions were associated with preexisting immunity to Q fever. Accordingly, the practice of screening prospective vaccinees was adopted—using serology for Q fever antibodies and observation for local induration after inoculation with a skin test antigen made from diluted vaccine.

Advances in *Coxiella* biology and vaccinology led to a new generation of vaccines. For example, evidence showed that only Phase I Q fever organisms, analogous to smooth forms of bacteria, were protective, and that a transition to Phase II (rough) would occur if the organism was passaged too many times in chick embryo. Therefore, Q fever vaccines are based on Phase I organisms only.[168] In addition, researchers recognized that purification to remove chicken protein and lipid and isolation of whole inactivated *Coxiella* by extraction, filtration, or centrifugation would result in a cleaner, less reactogenic, and highly immunogenic product. Two whole-cell vaccines came into use in high-risk subjects: an Australian vaccine made and licensed by Commonwealth Serum Laboratories[166,169] and a vaccine (IND 610) made and tested by the U.S. Army.[170] Both are administered as a single 30-μg subcutaneous injection. The Australian vaccine, Q-Vax, is purified using high concentrations of NaCl to remove nonprotective antigens. The U.S. Army vaccine, Whole-Cell, Inactivated, Q-Fever Vaccine NDBR 105, IND 610, is also extracted with NaCl, then subjected to ethanol–Freon 113 extraction; finally, it is purified further on a $CaHPO_4$ $2H_2O$ (brushite) column. Despite these purification processes, however, skin testing of prospective recipients remains necessary to prevent serious reactions in persons who have experienced prior infection.

Whole-cell vaccines have undergone considerable clinical testing. In Australia, a placebo-controlled trial[171] and an open trial in abattoir workers[172,173] showed efficacy approaching 100% (Table 12.3). From 2002 to 2006, during a vaccination program funded by the Australian government, the incidence of Q fever in Australia declined by more than 50%.[166,174] The U.S. Army vaccine was subjected to a controlled challenge in volunteers; this trial also proved highly successful.[175] Two meta-analyses found that Q-Vax was, respectively, 97% and 83% to 100% effective, but the data were considered to be of only moderate quality.[176,177] A Q fever outbreak, apparently caused by dairy goats, occurred in The Netherlands between 2007 and 2011, affecting more than 4000 individuals.[178] Control measures that included Q-Vax vaccination of individuals at high risk for developing chronic Q fever were

TABLE 12.3 Protection by Q Fever Vaccines

Vaccine	Type of Study	No. of Cases of Q Fever		
		Vaccinated	Unvaccinated	Protection (%)
United States	Random, challenge	2/32	5/6	92
Australian	Random, placebo-controlled	0/98	7/102	100
Australian	Observational	3[a]/2716	52/2012	96–100

[a]Vaccinated during incubation period of Q fever.

Data from Ormsbee RA, Marmion BP. Prevention of Coxiella burnetii *infection: vaccines and guidelines for those at risk. In: Marne TJ, ed.* Q Fever: The Disease, *vol 1. Boca Raton, FL: CRC Press; 1990:225–248.*

effective in containing the outbreak despite limited coverage of the vaccination campaign.[179–181]

To eliminate the problem of reactions, third-generation acellular Q fever vaccines have been prepared in the United States[182,183] and in the former Czechoslovakia.[184] These vaccines, extracted with chloroform–methanol or other lipid solvents to remove lipid A (thought to be the chief offending substance in *C. burnetii*), have not yet undergone sufficient clinical testing to determine whether they are equivalent to whole-cell Q fever vaccine in protection of humans. In addition, it is not yet clear if these vaccines overcome the problem of serious reactions in preexposed individuals.[185,186]

A live attenuated Q fever vaccine produced in chick embryo yolk sac has been developed in Russia, but its safety has been questioned. Other vaccine approaches under investigation are based on the P1 29-kDa protein, a 67-kDa antigen, and DNA plasmids coding for various proteins.[187–189]

Better Q fever vaccines are needed that induce antibodies and T-helper type 1 (Th1) cell responses and yet are well tolerated by individuals with preexisting immunity. Elucidation of the key protective antigens is required.[185,189]

Tularemia

Tularemia, a bacterial bioterrorism threat, can be a significant endemic and epidemic human disease.[190] The causative bacterium of tularemia, *Francisella tularensis*, was isolated in 1912; in 1919, Edward Francis made the association with the human disease then known as "deer fly fever."[191]

Andersson and colleagues[192] describe the transcriptional response in peripheral blood following ulceroglandular tularemia in humans. The authors identified seven genes whose changes in expression predict the early phase of tularemia. In addition to identifying how the host defense develops or is turned off by *F. tularensis* invasion, these data are important in identifying potential diagnostic markers for tularemia infection.

Many of the more than 200 cases of *F. tularensis* documented in the American medical literature resulting from laboratory exposure occurred in laboratory personnel who had received one or more injections of a phenol-killed vaccine and/or acetone-prepared vaccine.[193–196] One of these early inactivated products, the Foshay vaccine, showed incomplete protective efficacy against tularemia organisms introduced by the respiratory and intracutaneous routes.[197,198] Although circulating antibodies could be demonstrated following administration of these vaccines, the antibodies generated were not protective. It was concluded that the protective antigen of *F. tularensis* was destroyed in the inactivation procedures used to prepare the vaccines, although now antibodies alone are considered insufficient.

Partly as a result of the experience with killed vaccines, an effort to develop a live vaccine against *F. tularensis* was undertaken. By avoiding destruction of the protective antigen, live vaccines, which cause actual infection, are thought to produce an immunity closer to that caused by the disease itself, in particular by generating persistent antibodies and CD8-mediated cellular immunity, both of which are required for protection against disease.

Soviet investigators initially developed a live tularemia vaccine in 1942.[199] Ampoules of this "viable" tularemia vaccine were brought to the United States from the Russian Institute of Epidemiology and Microbiology (Gamaleia Institute) by Shope[200] in 1956. From an ampoule of this product, Eigelsbach and Downs[201] derived a vaccine strain, which they designated LVS.

Studies done at Fort Detrick in the early 1960s showed that LVS was protective for mice and guinea pigs after a challenge.[201] This protection was subsequently found to be the result of cell-mediated immunity when passively transferred spleen cells from immunized mice provided protection to nonimmune recipients.[202] Similar immunization studies were done in monkeys, which also demonstrated a significant immune response to vaccination with LVS.[203]

LVS initially was given by scarification to volunteers at Fort Detrick in 1958.[204] The vaccination procedure and adverse events after vaccination were evaluated in 29 subjects.[198] Only a pink scar remained 1 month after vaccination. Transient axillary lymphadenitis was observed in approximately half of the subjects, but none of the 29 men exhibited fever or other systemic reactions following immunization with LVS. All persons showed bacterial agglutinin antibodies; peak titers were observed 29 to 59 days after vaccination and were sustained.[205]

After challenge doses of up to 2500 organisms, a significant protective effect was seen in the volunteers vaccinated with LVS, with only 20% of vaccinated persons showing clinical illness compared with 85% of the unvaccinated control subjects. None of the vaccinated persons developed symptoms severe enough to require treatment. At higher challenge doses, however, immunity was overcome; after challenge with 25,000 organisms, 90% of vaccinated persons showed some symptomatology compared with 85% of the control subjects. However, LVS modulated illness severity such that only 60% of vaccinated volunteers had symptoms severe enough to require treatment, compared with 100% of those in the control group.[205]

The efficacy of the live tularemia vaccine, when administered by aerosol, has also been evaluated.[206,207] In one study,[207] aerosol vaccination with LVS protected 6 of 16 subjects against challenge with the virulent SCHU-S4 strain of *F. tularensis*. All protected subjects had measurable circulating antibodies, while only one of the 10 subjects who developed disease had circulating antibodies.

LVS (also referred to as NDBR 101) has been used since the mid-1960s and has been associated with a significant decline in the rate of laboratory-acquired infections at Fort Detrick.[208] In the absence of firm knowledge concerning the attenuating mutations in LVS and its residual virulence, the vaccine remains investigational and is administered only under protocol and with written informed consent. The lots undergo lot-release and potency tests as required by FDA for IND products.

Efforts are underway to develop new-generation vaccines against tularemia using modern technologies. Lipopolysaccharide from *F. tularensis* gives partial protection through the induction of antibodies, but clearly, cell-mediated responses to other antigens will be necessary for an optimal vaccine against highly virulent strains.[209,210] In addition, aerosol delivery may give better protection than peripheral inoculation.[211] Much work is underway to create new live attenuated mutants[211–217] and novel subunit vaccines.[218] In one promising approach, the oxidant-sensitive *emrA1* mutant of *F. tularensis* LVS elicited a strong humoral immune response in mice without causing adverse effects. Mice vaccinated with a single intranasal dose of the *emrA1* mutant of *F. tularensis* LVS were protected against a lethal respiratory LVS challenge; this vaccine also provided partial protection against lethal challenge with the virulent *F. tularensis* SchuS4 strain.[219]

Brucellosis

Primarily a disease of animals, brucellosis first was described in humans in 1859 as "Mediterranean gastric remittent fever" or "Malta fever."[220] The consumption of unpasteurized goat milk was found to be the source in the Malta epidemic.[182]

Bruce[221] isolated the etiologic agent in 1886 from a fatal case of brucellosis. Initially named *Micrococcus melitensis*, the genus later was named for its discoverer and is currently known as *Brucella melitensis*. *Brucella* species are non–spore-forming, Gram-negative coccobacilli that exist worldwide. Several animal species become infected with *Brucella* species. Humans become infected on contact with infected animals, by consuming unpasteurized infected milk or other dairy products, and by working with the organism under laboratory conditions. Brucellosis manifests with nonspecific flu-like symptoms that are often much more prominent than the gastrointestinal symptoms.[222] The feasibility of infection by aerosol makes *Brucella* a possible agent of biowarfare.

Effective vaccines, both live and killed, are available for animals. Use of these vaccines has led to decreases in human disease where animal infection is enzootic. Nevertheless, an estimated 500,000 annual human cases still occur worldwide.[223] A live vaccine, *Brucella abortus* S19, was extensively used in Russia and gave more than 50% protection, although it was somewhat reactogenic.[224]

Ongoing research seeks to further attenuate live vaccines used in animals and to develop subunit vaccines based on membrane and ribosomal proteins or DNA plasmids coding for those proteins.[225–234] For example, protection in animals has been achieved with an outer membrane protein and its gene,[235,236] with periplasmic protein bp26,[237] by intranasal administration of *Brucella* lipopolysaccharide complexed with *Neisseria meningitidis* outer membrane protein,[238,239] and a recombinant glucokinase protein *Brucella* vaccine candidate.[240]

Pseudomonas

Pseudomonas aeruginosa is a common pathogen in ventilated patients and in those with burns; however, it also causes life-threatening infections in people with cystic fibrosis owing to its production of a thick, mucoid capsule. Although antibodies to the lipopolysaccharide develop in people with cystic fibrosis after infection, they are of low affinity and do not protect. In contrast, artificial immunization with lipopolysaccharide from eight different strains coupled chemically with the *Pseudomonas* exotoxin A elicited high-affinity IgG antibodies.[241,242] Studies of cellular immune responses showed induction of antigen-specific lymphocyte proliferation and a Th1-like cytokine secretion.[243] During a 10-year period, people with cystic fibrosis immunized with O polysaccharide conjugated to toxin A had fewer *Pseudomonas* infections and a tendency toward less colonization with mucoid strains (Herzog C. Personal communication to S. Plotkin, November 26, 2002).[244,245] Importantly, lung function was also better in vaccinees.[244,246,247] In a Phase III trial, patients with cystic fibrosis who received four doses of a bivalent *P. aeruginosa* flagella vaccine were significantly less likely to have one or more acute *P. aeruginosa* infections than were those who received placebo (19.6% vs 30.7% among vaccinees and placebo recipients, respectively).[248] However, additional antigens are expected to provide better protection.[249] A vaccine containing formalin-inactivated whole *Pseudomonas* bacteria has been administered orally to healthy volunteers without adverse reactions and with significant serum IgA responses.[250]

Many other experimental vaccines are under development, including ones based on flagellar antigens, outer membrane proteins such as OprF and OprI, and exotoxin A.[251–254] For example, one DNA vaccine encoding *Pseudomonas* exotoxin A and PcrV has shown promise in mice,[255] and a *P. aeruginosa* hybrid outer membrane protein OprE/I (IC43) vaccine was well tolerated and immunogenic in a recent randomized, placebo-controlled Phase I study.[256] The mucoid exopolysaccharide of *P. aeruginosa* has been conjugated with keyhole limpet hemocyanin and found to elicit opsonophagocytic antibodies in mice.[257] An attenuated salmonella vector expressing the O antigen of *P. aeruginosa* lipopolysaccharide given intranasally protected mice against challenge.[258] Interestingly, Th17 responses, as in the case of other mucosal bacteria, may be necessary for protection,[259] and mucosal routes of administration may improve efficacy.[260–262]

The virulence factor alginate is produced by mucoid forms of *P. aeruginosa*. One strategy in *Pseudomonas* vaccine development is to raise opsonic antibodies against alginate. One group conjugated alginate to the outer membrane vesicle of *N. meningitides*. Immunization of mice with this conjugate provided protection against intranasal challenge with *P. aeruginosa*,[263] thus validating the concept.

Helicobacter pylori

Helicobacter pylori, which colonizes the stomach early in life but exerts its effects in adults, is the cause of gastritis, peptic ulcers, and low-grade gastric lymphomas.[264,265] The mechanisms of disease involve stimulation of Th1, regulatory T, and possibly Th17 cells; antibody and cellular mechanisms seem to be involved in the protection demonstrated in animal models. Clinical trials have tested urease, which is secreted by *H. pylori*; the cagA pathogenicity island; the VacA cytotoxin; and the neutrophil-activating protein.[266] The results in the past have not been promising; more recently, however, an aluminum-adjuvanted combination of the latter three antigens stimulated strong antibody and cellular responses in volunteers and will undergo further clinical development.[267] Other approaches, such as VLPs,[268] epitope-based vaccines,[269–271] alkyl hydroperoxide reductase and mannosylated AhpC,[272] and thiolperoxidase,[273] have shown promise in single- and multicomponent vaccine studies using murine models.

Botulism

Botulism is a neurologic intoxication caused by botulinum neurotoxin, the most poisonous agent known. It is produced by the bacterium *Clostridium botulinum*. Seven toxin types (A through G) have been recognized; all but G cause human disease.[274] Botulinum neurotoxin is a significant biowarfare and bioterrorism threat. The neurotoxin binds to the presynaptic membrane and prevents acetylcholine release, resulting in a symmetric, descending flaccid paralysis with classical bulbar palsies characterized by diplopia, dysphonia, dysarthria, and dysphagia.[275] As paralysis progresses, generalized weakness occurs; if untreated, death occurs from airway obstruction and diaphragmatic muscle paralysis. Interestingly, this toxin has many therapeutic uses as well—notably, but not limited to, treatment of blepharospasm, strabismus, cervical torticollis, and various dystonias.[276–278]

Pentavalent (ABCDE) botulinum toxoid (PBT) was available as a prophylactic countermeasure to botulism from 1959 until November 30, 2011, when the Centers for Disease Control and Prevention stopped providing the vaccine under IND 161 because of reduced potency and increased reactogenicity.[3] Although the old methodology for producing toxoids (i.e., the methods used to produce PBT) can be improved by reducing formalin concentrations and increasing the purity of the botulinum proteins, the most advanced candidates are recombinant nontoxic proteins. The Hc subunits of toxin types A and B were immunogenic in a clinical trial and are proceeding in development.[279–282] Other peptides of the toxin, including the light chain and the N terminal of the heavy chain, are also being tested[283] (Fig. 12.1). A nonreplicating

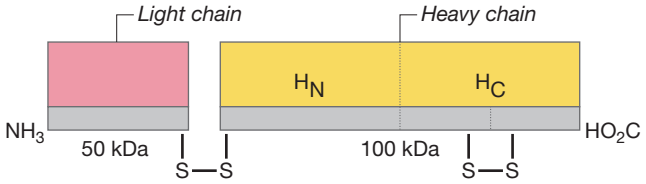


Figure 12.1. Structure of botulinum toxin.

adenovirus-vectored monovalent (C) vaccine, given intranasally or orally, has also been shown to induce serum and secretory antibodies and to protect animals.[284,285] A recombinant bivalent vaccine (rBV A/B) has completed Phase II safety and immunogenicity testing in humans.[286] Inevitably, new vaccines against botulism will have to be licensed through a demonstration of efficacy in animals. Although a titer of antibodies correlating with efficacy is not known for humans, studies in guinea pigs suggest that very low levels (<0.1 U) will be protective.[287]

In March 2013, the FDA approved a heptavalent equine (ABCDEFG) botulism antitoxin to treat individuals with symptoms of botulism after exposure or suspected exposure to botulinum neurotoxin.[288,289] BabyBIG has been available in California to treat infant botulism since 2003.[290] However, no vaccines for prophylactic protection against botulism are currently available in the United States.

Clostridium difficile

Clostridium difficile is an inhabitant of the human gastrointestinal tract that, under certain conditions, produces toxins that cause diarrhea. The precipitating cause of *C. difficile* infection (CDI) seems to be the elimination of other intestinal flora by antibiotic treatment. Incidence of the disease has increased considerably in recent years.[291–294] Antibodies against both of the toxins produced by *C. difficile*, A and B, are needed to prevent illness.[295,296]

Much work is in progress to develop and use monoclonal antibodies against the toxins for prophylaxis and treatment.[297–300] Vaccines against CDI are currently under clinical development at Sanofi Pasteur[301] and Pfizer.[301a] Both candidate *C. difficile* toxoid vaccines are composed of a highly purified formalin or genetically inactivated toxoids together with aluminum adjuvants delivered by intramuscular injection. Early clinical data suggest a relationship between an immune response to toxins A and B with protection against a primary or recurrent case of CDI.[297,302–305] After colonization, a systemic anamnestic response to toxin A, as evidenced by increased serum IgG antitoxin A levels, is associated with asymptomatic carriage of *C. difficile.* Serum IgG antitoxin A levels have also been significantly correlated with IgG levels against toxin B antigens, which were also higher in asymptomatic carriers.[303] In patients with CDI, higher IgG antibodies to toxin A are associated with protection against recurrence.[303] In an initial Phase I study, the candidate vaccine induced IgG antibodies to toxins A and B with resolution of recurrent diarrhea.[304] In a more recent Phase II study, the Sanofi candidate, given in a high dose, elicited functional antibody responses in adult and elderly volunteers for at least 180 days without safety issues.[306]

In addition, work has begun on the use of colonization factors as vaccine antigens. These include a protease (Cwp84) that is an adhesion molecule[307,308] and a cell wall polysaccharide conjugated to the diphtheria toxoid variant CRM.[309] In another approach to vaccine development, Baliban and colleagues[310] created a synthetic DNA vaccine encoding receptor binding domains from *C. difficile* toxin A and toxin B. In mice and nonhuman primates, this vaccine demonstrated robust immunogenicity, and immunized mice were protected from a challenge with *C. difficile* spores from homologous and heterologous strains.

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13 Cancer Vaccines

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THE CONCEPT OF CANCER VACCINES IS ANCHORED IN CLASSICAL VACCINOLOGY

The idea of inducing or enhancing an immune response against cancerous cells is strongly anchored in the concept of prophylactic vaccination against a number of infectious agents, one of the most important and successful achievements of medicine. Adequate preexposure of the immune system to a truncated or surrogate version of a particularly infectious agent reshapes the immune repertoire and enables it to effectively counter a subsequent infectious inoculum. Thus, the immune system is intrinsically endowed with enough plasticity and potency to support an efficient antimicrobial vaccination.

A second line of evidence dating back to Coley[1] proposed the concept of enhancing immunity as a means to cope with cancer. Anecdotal evidence of tumor regression after an infection[2] or exposure to microbial byproducts[3] has been tantalizing enough to encourage study of this approach with increasing effort in the field of "cancer vaccines" during the last two decades.

Nevertheless, even though therapeutic cancer vaccines have proved challenging, with only one vaccine licensed to date[4] out of hundreds of clinical development programs, the field of immune-oncology has expanded greatly based on a deeper understanding of the role of immune system in cancer,[5] successful clinical application of the concept of immune checkpoint blockade,[6] and tantalizing evidence of clinical efficacy of cancer vaccines mediated by tumor antigen-directed T cells.[7]

More specifically, cancer vaccine development has been guided by the paradigm of immunity to viruses.[8] During a successful cell-mediated immune response to a viral infection in a target organ, viral antigens are presented to antigen-reactive T cells by professional antigen-presenting cells (APCs; particularly dendritic cells [DCs]) within secondary lymphoid organs. Activated T cells then proliferate and differentiate to cytotoxic T lymphocytes (CTLs) that secrete cytokines, including interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Activated CTLs enter the circulation and are guided to the site of the viral infection by chemokines and adhesion molecules, where the CTLs kill the virally infected cells by releasing granzymes and perforin or by activating death receptors on the surfaces of the target cells. For example, in the case of Epstein-Barr viral infection[9] the magnitude of the CTL response must be sufficiently strong (on the order of 1 to 10 virus-specific CTLs per 100 CTLs) to clear the infection and must be maintained for a sufficient time (at least weeks to months[10]) to be effective. The former requirement is usually mediated by high-avidity T cells and the latter requirement is fulfilled by the induction of memory T cells.[11] Cancer vaccines should ideally mimic the same steps as those seen with viral infections (Fig. 13.1), except that the ultimate targets are cancer cells instead of virally infected cells. These steps are discussed in more detail as they apply to cancer vaccines.

Although the field of immunotherapy is rapidly expanding and diversifying in oncology, our focus in this chapter remains on "vaccines" that provide exogenous antigens to the immune system to reshape, direct, and empower it to destroy cancerous cells. Nevertheless, we anticipate that various therapeutic platforms in immune-oncology, including both vaccines and immunotherapy, will be used in conjunction with each other, leveraging their strengths and complementing their mechanisms of action leading to more effective and durable protection against the disease process. Thus, based on all evidence to date, vaccines can no longer be seen as a standalone therapeutic platform, but must be seen as a component of a broader, integrated therapeutic armamentarium against cancer that is still under development.

UNDERSTANDING TUMOR IMMUNOBIOLOGY IS KEY TO DESIGNING EFFICACIOUS CANCER VACCINES

Several aspects of tumor immunobiology are very important to appropriately design cancer vaccines. Until recently, the lack of adequate understanding of tumor immunology, especially mechanisms of immune tolerance and evasion, has hampered cancer vaccine development.

Tumor Antigens

Cancer antigens can be recognized by T cells. Like viral antigens, they are peptides derived from degraded proteins that are associated with human leukocyte antigen (HLA) molecules on the surface of APCs.[12] Peptides from intracellular proteins, degraded by the proteasome and bound to HLA class I heavy-chain domains in the endoplasmic reticulum, are recognized by CD8+ T cells. Peptides from "endocytosed" membrane or extracellular proteins are degraded in endosomes where they bind to HLA class II proteins and are subsequently recognized by CD4+ T cells.[12]

In a few instances, cancers are caused by oncogenic virus infections. In these situations, foreign viral proteins can serve as targets for cancer vaccines. More commonly, DNA damage, faulty DNA repair, or aberrant chromosomal rearrangements can lead to mutant self-proteins or new breakpoint regions that are found only in tumor cells. These are called tumor-specific antigens (e.g., m-Ras, bcr-abl) as they are not encountered in noncancerous cells. Most recently these have been termed neoantigens and may or may not play a role in oniogenesis. This is in contrast to the broad category of tumor-associated antigens (TAAs), which also comprise unmodified self proteins that are selectively upregulated or expressed in cancerous cells (e.g., "cancer-testes antigens"). TAAs may also arise through oncogenic signaling processes that increase the expression of proteins or polysaccharides that are otherwise weakly or transiently expressed.[13] Because tumor-specific antigens are novel proteins they are unlikely to be seen by developing T cells in central lymphoid organs (which is more common with tumor-specific antigens), they do not induce tolerance, and they can be recognized by peripheral T cells. More details on tumor antigens are provided below.

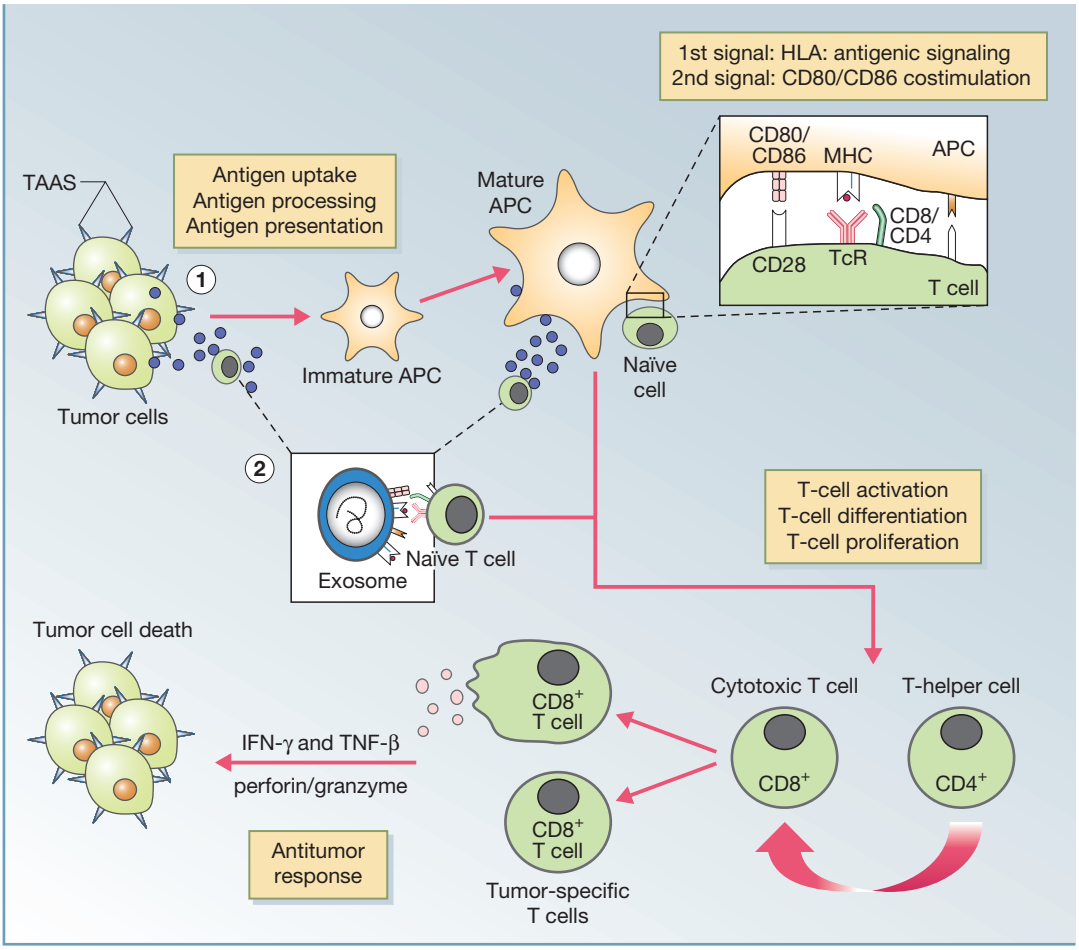


Figure 13.1. Generation of an effective antitumor response. In general, tumor-associated antigens (TAAs) are taken up by immature professional antigen-presenting cells (APCs). The APCs must become activated and process and present TAA epitopes within the binding groove of human leukocyte antigen (HLA) molecules on the surface of APCs. Mechanisms of APC activation are: (1) Immunogenic tumor cell death: Immune modulation (vaccination), radiation, and chemotherapy can cause tumor cell death, which can induce early calreticulin translocation to the cell surface, heat shock protein (HSP)70 and HSP91 release and exposure, late chromatin binding protein high-mobility group box 1 protein (HMGB1), and nucleic acids and their degradation products (such as adenosine triphosphate) release. These danger-associated molecular patterns are recognized by pattern recognition receptors on APCs. (2) Exosome recognition: APC-derived and tumor-derived exosomes activate naïve T cells by displaying HLA molecules, CD80 or CD86, and adhesion molecules. Both of these pathways lead to APC maturation and T-cell activation through T-cell receptor (TCR) recognition of processed epitopes presented on HLA molecules. For activation, T cells require two signals: antigenic peptide/major histocompatibility complex (MHC) (first signal) and costimulation through CD80 and CD86 ligation of CD28 (second signal). Activated T cells then proliferate and differentiate into CD4+ T-helper cells and CD8+ cytotoxic effector T cells. Activated tumor-specific CTLs enter the circulation and are guided to the site of transformation by chemokines and adhesion molecules, where they kill tumor cells by secreting interferon (IFN)-γ and tumor necrosis factor (TNF)-β and by releasing granzymes and perforin.

Antigen Presentation and T-Cell Proliferation

To increase tumor-reactive CTLs to sufficient numbers and to sufficient exposure times to clear tumor cells, it is necessary to provide more than the antigenic signal, or signal 1.[14] Costimulatory, or second signals, in the form of cell surface molecules, cytokines, and chemokines, must also be provided at the same time by APCs. In general, the costimulatory signals required to activate T cells to enter the cell cycle are found on professional APCs, with the most potent being DCs.[15] However, even fibroblasts can stimulate T cells to proliferate in vivo under appropriate circumstances.[16]

Important costimulatory molecules, including the B7-family members, CD80 (B7.1) and CD86 (B7.2) that bind to CD28 on T cells, orchestrate the glycolytic metabolism required by rapidly proliferating cells.[17] Other important costimulatory molecules on APCs deliver signals to antigen-activated T cells, allowing them to survive and progress through the cell cycle. For example, adhesion molecules such as CD54, CD48, and CD58 bind to LFA1, CD2, and CD24 on T cells, respectively, whereas members of the TNF receptor family, CD40 and CD70, bind to CD40L and CD27, respectively.[18]

Tumor-Derived Exosomes

Exosomes are small membranous vesicles that fuse to the plasma membrane, resulting in the release of their vesicular cargo into the extracellular space.[19] B cells and DC-derived exosomes display abundant major histocompatibility complex (MHC) class I and class II molecules, costimulatory molecules CD80 and CD86, and adhesion molecules such as CD54 and CAM-1.[20,21] APC-derived exosomes expressing these ligands mediate antitumor immunity through MHC class II–restricted CD4+ T-cell activation and MHC class I–restricted

CTL activation. Tumor cells also secrete exosomes that have immunostimulatory and immunosuppressive properties depending on the conditions. Tumor-derived exosomes contain abundant TAAs that are processed and presented by DCs to CTLs, initiating tumor-specific immune responses.[22] Tumor-derived exosomes, however, may also promote tumor evasion through initiation of apoptosis of tumor-specific CTL (mediated by exosomes expressing FAS ligand) and through T-cell activation and signaling suppression.[23]

Immunogenic and Nonimmunogenic Cell Death

Cell and tissue death occurs constantly from homeostatic tissue turnover (nonimmunogenic cell death) or through suicide of virus-infected, bacteria-infected, and transformed cells (immunogenic cell death) (see Fig. 13.1).[24] The DCs are responsible for distinguishing between nonimmunogenic and immunogenic cell death and subsequently initiating the appropriate immune response. Pattern recognition receptors on DCs recognize distinct immunogenic damage-associated molecular patterns (DAMPs) that exist on the surface or are released from dying cells.[25] Immunogenic tumor cell death is characterized primarily by early translocation of calreticulin to the cell surface,[26] release and exposure of heat shock protein (HSP) 70 and HSP91,[27] and late release of chromatin-binding protein high-mobility group box 1 protein (HMGB1).[28] Nucleic acids and their degradation products also function as immunogenic DAMPs. DC recognition of these biochemical markers leads to apoptotic body phagocytosis (initiated by calreticulin), DC activation (initiated by HSP70 and HSP91), antigen processing and presenting (initiated by HMGB1). Tumor-specific HMGB1 ligation of Toll-like receptor (TLR) 4 facilitates TAA processing and presentation along with MHC classes I and II and costimulatory molecule expression by inhibiting fusion of phagosomes with lysosomes.[29,30] Similarly, HSPs facilitate DC activation through ligation to CD91. Nucleotides from degraded DNA or RNA act on TLR2, TLR7, TLR8, and TLR9, as well as on intracellular receptors RIG-1, MDA5, and LGP2.[29] Adenosine triphosphate (ATP) and its degraded products activate purinergic P2RX7 receptors.[31] Nucleotide product recognition facilitates DC inflammasome-driven production of inflammatory cytokines such as interleukin (IL)-1, IL-18, and IL-33, which is required for the adequate polarization of IFN-γ–producing CTLs.[32,33]

Chemotherapy-Induced Immunogenic Apoptosis

Chemotherapy indiscriminately kills rapidly dividing tumor cells, including hematopoietic and immune cells, thus producing immunosuppressive side effects. Cancer chemotherapeutics, such as anthracyclines, oxaliplatin, and ionizing irradiation, induce immunogenic cell death despite their immunosuppressive nature. In contrast, agents such as etoposide, mitomycin C, staurosporine, mitochondrial poisons, and endoplasmic reticulum stressors fail to elicit immunogenic cell death.[34] Chemotherapeutic agents that induce immunogenic cell death do so by exposing and releasing calreticulin, HMGB1, HSP70, HSP91, and ATP22, exposing DAMPs, and initiating an effective antitumor response.[31] Nonimmunogenic chemotherapy-induced tumor death occurs if apoptotic cells do not expose DAMPs or if apoptotic cells modify existing DAMPs through caspase activation and reactive oxygen species release and inactivation of HMGB1.[35] Chemotherapy can also cause dying cells to directly release immunosuppressive cytokines such as IL-10 and transforming growth factor (TGF)-β. Production of these cytokines leads to regulatory T cell (Treg) and T helper cell (Th) 2-driven inhibition of inflammation and DC tolerance to dying tumor cells.[22]

T-Cell Differentiation

Entry into the cell cycle allows antigen-activated T cells to differentiate into effector cells. The major pathways of T-cell differentiation are classed as type 1 (Th1/TC1) or type 2 (Th2/TC2).[36] Th17 cells and Tregs have been identified as novel effector T-cell subtypes. Type 1 responses mediate cell-mediated immunity and are thought to be required for successful antitumor[37] and antiviral[38] responses. Type 1 effector cells secrete cytokines such as IFN-γ and TNF-α, express chemokine receptors such as chemokine-related receptor (CXCR) 3 and chemokine receptor (CCR) 5,[39] and kill via perforin and granzyme release.[40] Type 2 immunity is associated with humoral and allergic responses. Type 2 effector cells secrete IL-4, IL-10, IL-5, and IL-13, and express CCRs such as CCR3, CCR4, and CCR8.[41] Th17 cells promote protective inflammatory responses against infectious agents and fulfill an important role in various autoimmune diseases.[42] Th17 secretion of IL-17 mediates proinflammatory antitumor immunity. Antitumor immunity is primarily facilitated by Th1/TC1 polarization, although Th17 cells may have a role.[43]

Maintenance of Effector Cells

A number of different processes are required to ensure that CTL activity is maintained in sufficient numbers and for sufficient duration to mediate an effective antitumor response. Initially, costimulatory molecules control the survival of effector cells. For example, OX40L on APCs promotes the continued activation of CD4+ Th1 cells,[44] whereas 41BBL binding to 41BB promotes the continued activation of CD8+ TC1 cells.[17] In contrast, Th2/TC2 responses are maintained by interactions of CD30 with CD30L on APCs.[45] In the absence of signals from costimulatory molecules, antigen-activated T cells fail to replicate and clear tumor cells. In addition to maintaining effector T-cell survival, costimulatory molecules may also mediate effector T-cell function. For example, OX40L may be required on tumor cells to activate CTLs.[45] Cytokines also maintain the survival of activated and memory T cells and enhance the activity of effector cells.[46] For example, members of the gamma c cytokine family, such as IL-2 and IL-15, can maintain the responses of activated T cells.[47] Many of these cytokines are made by CD4+ helper T cells. In addition to amplifying CTL responses, the cytokines produced by CD4+ T cells recruit other inflammatory cells, such as eosinophils and macrophages, which may help clear tumor cells.[48]

T-Cell Trafficking to Tumors

The complex process of producing large numbers of type 1 effector T cells requires the cytokine and costimulatory molecule milieu of a secondary lymphoid organ.[49,50] To exert their effector functions, the activated T cells must travel from the lymph node to the site of the virally infected or tumor target cells through the bloodstream. A number of molecular families, including lectins and integrins, are involved in recruiting T cells from the bloodstream to tertiary sites. Chemokines are especially important in localizing the appropriate class of effector cells (e.g., CCL2, CXCL12, CXCL16) as well as Treg cells counteracting those (e.g., CCL22).[51,52,53]

ALL HURDLES FACING CANCER VACCINES NEED TO BE SYSTEMATICALLY ADDRESSED

Although the model of antiviral cell-mediated immunity guides the potential development of cancer vaccines, a number of properties intrinsic to the cancer-bearing state may limit the

effectiveness of vaccines. Thus, additional immunomodulatory treatment strategies may need to be added to enhance the efficacy of cancer vaccines.[54]

In essence, there are three major differences between prophylactic vaccination against infectious agents and therapeutic vaccination against cancer that have slowed translation of the latter to effective medicines. First, cancer vaccines are directed at manipulating the immune system in patients who already have a clinically overt disease or asymptomatic tumor burden. In this stage, the endogenous immune system has already been negatively influenced by the tumor biology (immune editing)—and vice versa, tumor cells with increased resistance to immunity have been selected (immune sculpting)—with negative impact on immune system's responsiveness against therapeutic cancer vaccines.[55]

Second, the target to be eliminated often consists of large tissue masses with antigenic heterogeneity and a plethora of immune inhibiting mechanisms, as opposed to small homogenous microbial inocula.

Third, the suboptimal nature of our preclinical models, platform technologies, and limited understanding of best targets and companion biomarkers to guide adequate development of cancer vaccines, is a problem that hampers efforts in this field and turns initial enthusiasm to skepticism.

Mechanistically, the hurdles that limit the efficacy of cancer vaccines can be grouped as below.

T-Cell–Negative Regulation by Antigen-Presenting, Stromal, and Tumor Cells

Just as the cytokines and costimulatory molecules can positively regulate T-cell activation, proliferation, and effector function, other members of cytokine and costimulatory molecule gene families can act as negative regulators (Fig. 13.2). These can be specific to APCs, tumor cells, or shared between those. For example, TGF-β produced by immune cells or tumor cells acts directly on T cells to inhibit their ability to proliferate.[56] As another example, the major effects of IL-10 are immunosuppressive, in part by suppressing active costimulatory molecule and cytokine expression by APCs.[43]

CD80 and CD86 have positive effects on T-cell activation and proliferation when they bind to CD28. However, when they bind to CTLA-4 (CD152), a member of the CD28 gene family inducibly expressed on the surface of activated T cells, strong inhibitory signals limit further T-cell proliferation and activation.[57] CTLA-4 binds CD80 and CD86 with 10- to 20-fold higher affinity than the costimulatory CD28 molecule[58] and CTLA-4 directly inhibits effector T-cell activation through CD28 signaling pathway disruption and by outcompeting CD28 for CD80 and CD86 ligation.[59] Constitutive CTLA-4 expression on Tregs also mediates tumor-specific T-cell suppression.[60]

Programmed cell death ligand 1 (PDL1) (B7-H1 or CD274) and PDL2 (B7-DC or CD272) on APCs or tumor cells inhibit the activation of T cells that express PD1. PD1 ligation inhibits T-cell receptor (TCR) signaling events and T-cell activation[61] and leads to the so-called exhausted T cell. Upregulation of PDL1 on APCs is a feature of chronic viral and other infections and leads to suppression of the immune and inflammatory response often associated with these infections. Intrinsic tumor expression of PDL1 and PDL2 promotes tumor growth by increasing apoptosis of tumor-reactive T cells.[62]

B- and T-lymphocyte attenuator (BTLA) is a costimulatory receptor functionally similar to PD1 and CTLA-4.[15] Herpes virus entry mediator expressed on APCs can inhibit T-cell activation in T cells that express the BTLA receptor.[63]

Similarly, B7-H4 (B7x or B7S1), a member of the B7 family of costimulatory molecules, is a negative regulator of activated tumor-reactive T cells.[64,65] The receptor, which binds to B7-H4, has not yet been identified. Recently, by using ovarian cancer as a model, it has been shown that Tregs in the tumor microenvironment stimulate B7-H4+ tumor-associated macrophages to inhibit T-cell–mediated antitumor immunity via IL-10 and IL-6 expression.[66]

Inducible T-cell costimulator ligand (ICOSL), a member of the B7 family of costimulatory molecules, is predominantly found on professional APCs[18] and peripheral and endothelial cells.[67] ICOSL upregulation during inflammation promotes IL-10 production by T cells.[67] ICOS, which is induced on activated T cells and constitutively expressed on Tregs, negatively regulates antitumor T cells by inhibiting effector T-cell proliferation.[15]

The lymphocyte activation gene (LAG)-3 (CD223), which is morphologically similar to the CD4 coreceptor, negatively regulates T-cell activity through MHC class II binding.[68] LAG-3 ligation inhibits T-cell proliferation and antitumor cytokine production by downregulating expression of the CD3–TCR complex.[69]

Myeloid-Derived Suppressor Cell T-Cell Inhibition

Myeloid-derived suppressor cells (MDSCs) are potent suppressors of antitumor immunity within the tumor environment. MDSCs are a heterogeneous population of cells of myeloid origin consisting of myeloid progenitor cells and immature DCs, macrophages, and granulocytes.[70] MDSCs suppress antitumor immunity through diverse mechanisms that alter the innate and adaptive immune systems (Fig. 13.3). MDSCs inhibit CD8+ and CD4+ T-cell activation through the upregulation of arginase that subsequently depletes arginine (essential for T-cell proliferation).[71] MDSCs, activated by proinflammatory mediators prostaglandin E (PGE)2, IL-1α, IL6, and vascular endothelial growth factor (VEGF), dampen antitumor immunity by skewing it toward a tumor-promoting Th2 response and by downregulating macrophage secretion of antitumor cytokine IL-12.[72] MDSCs also indirectly inhibit T-cell activation by inducing Tregs.[73]

Regulatory T-Cell Immune Suppression

Tregs are important sources of T-suppressor cytokines and inhibitory signals and fall into two broad classes. The first category of Treg cells (Tr1) may be a separate T-cell lineage that develops in the thymus.[74] These Tr1 cells express the glucocorticoid-induced TNF receptor family-related gene (GITR), CTLA-4, PD1, and the high-affinity IL-2 receptor (CD25), and require IL-2 for growth and survival. They also secrete IL-10 and TGF-β, and their gene expression profile is regulated by the transcription factor, forkhead box P3 (FOXP3).[75] The second category of Treg cells (Tr2 cells) develops in response to antigens in the periphery with several types of induced/adaptive Treg cells described. Some induced Tr2 cells are CD4+/CD25+ and express FOXP3-like Tr1 cells. Others, called Th3 cells, secrete only TGF.[76] Various sources of Tregs within the tumor microenvironment exist. The tumor microenvironment is abundant in CCL22 that acts to traffic Tregs via CCR4 (the receptor for CCL22) surface expression.[77] Tumor factors such as VEGF, IL-10, and TGF-β suppress DC differentiation and DC function and activate plasmacytoid DCs[78] and MDSCs that, in turn, stimulate Treg differentiation and expansion.[79] Tregs suppress antitumor immunity by a variety of mechanisms (see Fig. 13.3). Tregs can induce T-cell cycle arrest through B7-H4+ tumor-associated macrophage negative-regulation pathways.[66] Tregs can selectively kill tumor-specific T cells and tumor-specific APCs through perforin- or granzyme B–dependent pathways.[80,81] CTLA-4+ Tregs

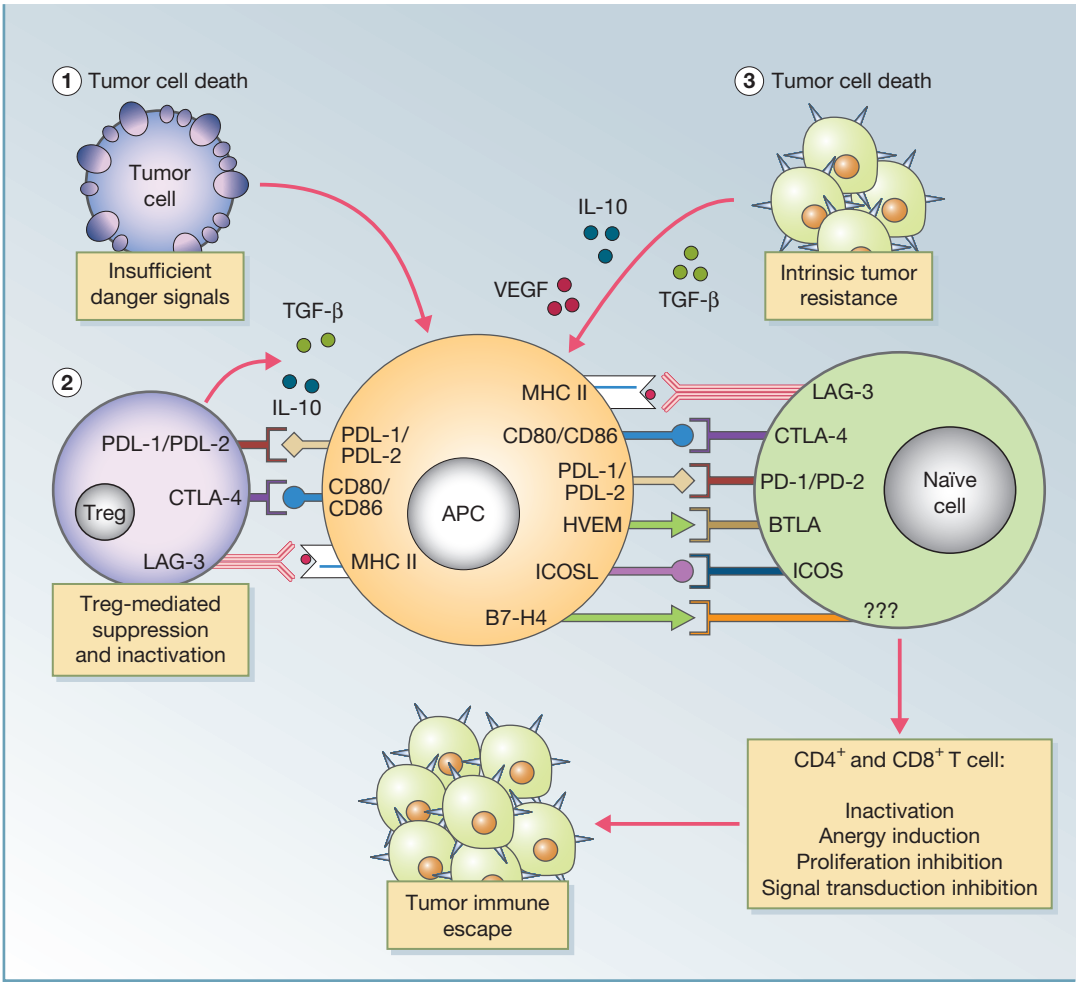


Figure 13.2. Antitumor response suppression by regulatory T cells (Tregs) and antigen-presenting cells (APCs). Under certain conditions, APCs and Tregs can suppress tumor-specific T-cell immunity by a variety of mechanisms. Insufficient APC activation or insufficient danger signals can lead to coinhibitory pathway activation. Tumors can often intrinsically induce expression of cytokines, such as interleukin (IL)-10, transforming growth factor (TGF)-β, and vascular endothelial growth factor (VEGF), that are capable of activating coinhibitory pathways in APCs and Tregs, recruiting additional Tregs to the tumor site, and directly inhibiting cytotoxic effector T-cell function. Treg recruitment to tumor sites suppresses antitumor immunity through a variety of mechanisms. (1) Tregs secrete inhibitory cytokines such as IL-10 and TGF-β, which directly inhibit effector T-cell activation and effector function. (2) Tregs induce T-cell apoptosis through perforin- and granzyme-dependent killing mechanisms. (3) High-affinity CD25 (also known as IL-2 receptor), present on Tregs, deprives tumor-specific T cells of IL-2, a cytokine essential for T-cell survival, leading to cytokine deprivation–mediated death. Negative regulation of T cells diminishes antitumor immunity and can lead to tumor immune escape. (4) Coinhibitory pathways (shown here and described in the text) on APCs and Tregs are responsible for diminishing antitumor immunity by inactivating T cells, anergizing effector T cells, and inhibiting T-cell proliferation.

suppress tumor-specific T-cell activation through induced expression of indoleamine 2,3-dioxygenase (IDO) by APCs, which depletes tryptophan (T-cell activation precursor).[82] Tregs also secrete suppressor cytokines such as IL-10 and TGF-β, which directly inhibit expression of MHC molecules, CD80, CD86, and IL-12 cytokines on APCs.[83] The heterotrimeric IL-2 receptor complex expressed on Tregs has a 100-fold higher affinity for IL-2 than does the dimeric form expressed on conventional T cells. Thus, Tregs can further suppress effector T-cell growth and survival by limiting the available IL-2 in the tumor microenvironment.[84]

Tregs have been found in the blood, lymph nodes, and tumor microenvironments of patients with a number of malignancies and have been shown to limit the activity of antitumor T cells.[52] Selective reduction or elimination of Tregs may enhance immune responsiveness to vaccines. A clinically relevant method to eliminate Tregs involves the use of cytotoxic agents such as cyclophosphamide.[85] CD4+/CD25+ T cells can also be eliminated by CD25 antibodies or Ontak, which consists of a portion of diphtheria toxin coupled to the human IL-2 binding domain, allowing it to bind and kill CD25+ cells.[86] 1-Methyl tryptophan may reduce the suppressive effects of IDO-positive APCs on T cells. CTLA-4 blocking antibodies can reduce inhibitory signaling and increase the proliferation of activated T cells and, potentially, the magnitude of an antitumor response. However, because autoreactive T cells are also activated by this reagent, toxicity from autoimmune disorders is high.[87] In addition, the desired effector T-cell response may be diluted by the activation of other cells. Other methods to increase the number and activity of vaccine-activated tumor-reactive T cells include the use of antibodies against IL-10 or TGF-β.[88]

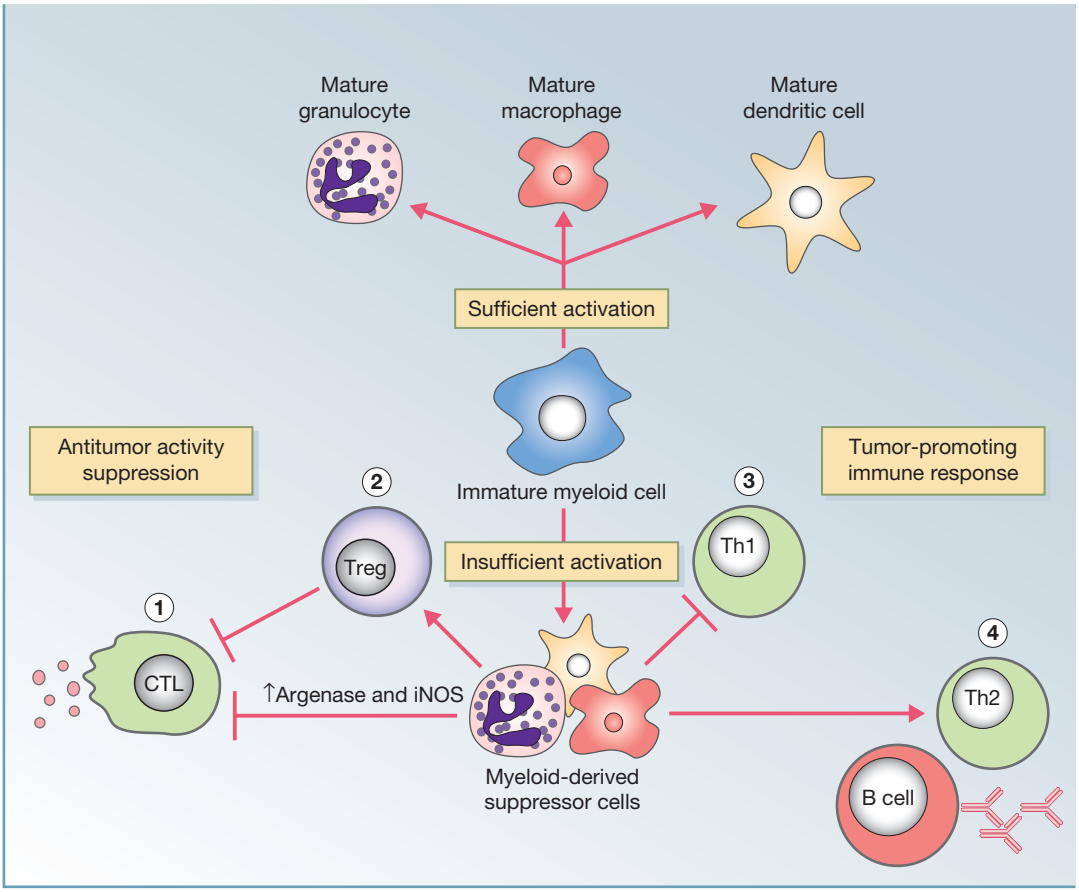


Figure 13.3. Antitumor response suppression by myeloid-derived suppressor cells (MDSCs). Immature myeloid cells that receive sufficient activation differentiate into mature granulocytes, macrophages, and dendritic cells (DCs). However, immature myeloid cells that are not sufficiently activated develop into a heterogeneous population of immature granulocytes, macrophages, and DCs, collectively referred to as MDSCs. MDSCs suppress antitumor immunity through a variety of mechanisms. (1) MDSCs inhibit CD8+ and CD4+ T-cell activation by upregulating arginase, inducing reactive oxygen species (ROS) and peroxynitrite (iNOS) production, and by cysteine sequestering (not shown). (2) MDSCs also indirectly inhibit T-cell activation by activating and recruiting regulatory T cells (Tregs). (3) MDSCs directly promote tumor growth by inhibiting antitumor Th1 cells. (4) MDSCs dampen antitumor immunity by skewing the immune response toward a tumor-promoting Th2 response.

Additional Regulatory Immune Cells

T cells are not the only cells with regulatory function that can suppress adaptive immune responses. CD1-restricted natural killer (NK) T cells with invariant TCRs, nonclassical NK T cells with normal TCRs,[89] T cells,[90] macrophages,[91] and effector B cells[92] also secrete IL-10 and can negatively affect T-cell responses.

Immune-Tolerant T Cells of Low Avidity

As described, tumor antigens are often derived from normal proteins that become accessible to T cells because of increased expression through oncogenic signaling events that drive tumor progression. However, central thymic tolerance mechanisms may result in a TCR repertoire that has only weak affinity for peptides derived from these tumor antigens because they are derived from "self."[93] Clearly, if there are no T cells that can respond to antigens in a vaccine, the vaccine is likely to be ineffective. Adoptive T-cell therapeutic approaches are being developed to overcome this problem.[94] Alternatively, careful selection of tumor antigen vaccine targets is a critical part of any vaccination approach (see "Cancer Vaccine Platform Technologies").

Immune Deviation: Th2 Polarization

Tumor cells often secrete factors such as IL-10, IL-6, and PGE that promote type 2 over type 1 immunity.[56] Because the cytokine milieu at the time of initial encounter with the antigen has a major role in determining the form of the resulting immune response,[36] cancers may, therefore, induce an ineffective (and sometimes tumor-promoting) response. To ensure that a type 1 response ensues, it may be necessary to decrease tumor bulk with cytotoxic chemotherapy to lower the levels of immunosuppressive factors. Alternatively, provision of cytokines (such as IL-12 and IFN-γ) or combinations of multiple cytokines (IRX2)[95] that promote type 1 responses can be used.

Intrinsic Resistance of the Tumor Target to T Cells

The analogy of cancer vaccines and successful viral immunity breaks down almost completely when one considers the nature of the target cell. As intimated in the preceding discussion on chemokines, virally infected target cells do not interact passively with antiviral CTLs. Rather, they cooperate with CTLs

by upregulating the expression of molecules that promote T-cell killing on recognition of antigens. Virally infected cells also secrete cytokines, such as IL-15,[96] that maintain the activity of CTLs and chemokines that guide type 1 effector cells to the region of inflammation.

In contrast, tumors generally arise in response to prolonged cytotoxic insults.[97] To survive, cells become genetically unstable and activate signaling pathways that allow them to grow without regard for social controls.[98] Signaling pathways that are often activated in tumor cells are the antithesis of those that are activated in response to viruses.[56] They include the rat sarcoma viral oncogene homolog (RAS), v-Akt murine thymoma viral oncogene homolog (AKT), and signal transducer and activator of transcription (STAT) proteins, which activate antiapoptotic genes that prevent killing by CTLs and positively regulate the expression of cytokines that inhibit the activity of CTLs, such as IL-10, TGF-β, and VEGF, and chemokines, such as CCL22, that recruit Tregs.[52] STAT3 and STAT5 transcription factors are thought to orchestrate this microenvironment, promoting tumorigenesis and cancer progression by inducing and maintaining a tumor-promoting inflammatory microenvironment.[99]

Suboptimal Immunologic Monitoring

Immunologic monitoring has been long used in both preclinical and clinical studies to evaluate cancer vaccines. However, using such immunologic results has often resulted in faulty decision making, with some vaccines being advanced into further stages of development with modest potential for success.

The immunologic assays developed include enzyme-linked immunospot (ELISPOT) assay, intracellular cytokine staining, and multimer multiparametric flow cytometry assays. The optimal form of immunologic monitoring for cancer vaccines has not been established. Concerns about the assays initially used included issues regarding the reproducibility, sensitivity, standardization, and predictive validation of the assays. There were challenges regarding which assays or combinations of assays were most predictive, and when, where, and which cells to evaluate. Based on these important concerns, leading collaborative networks organized standardization and "harmonization" initiatives for ELISPOT and flow cytometric assays and made recommendations on the types of assays that should be used in cancer immunotherapy/vaccine trials.[87,100] It has been generally agreed that at least two different assays should be performed to confirm an increase in tumor-reactive T cells in response to vaccination as false-positives could be seen when patients are identified as "responders" with only one assay and not another.[101] In addition, steps have been taken to standardize the assay methods and to report in a consistent manner "the minimal information about T-cell assays."[102]

There have been significant advances in various technologic aspects that have improved the type and quality of the antigen-specific immune analyses. These advances include the use of longer peptides spanning the antigen of interest rather than MHC-restricted nonamers and class II binding peptides to obtain a more comprehensive analyses of CD4+ and CD8+ T-cell responses not confined to a single MHC type. In addition, there have been advances in the types of multimers available. These advances offer enhanced sensitivity and improved correlations with functional activity.[103] There have also been advances in the technologies for identifying specific immune subsets, using models that rely on multiple parameters.[104]

Other considerations include the source of T cells (i.e., blood, lymph node, tumor) and methods using either direct ex vivo analysis or in vitro sensitization and culture. This is particularly important as it has been clearly documented that the repertoire of T cells in blood and tumor tissue are not identical,[105] hence overreliance on immune monitoring in blood may lead to misinterpretation of a vaccine's capability to elicit useful immune responses.

Nevertheless, the major issues associated with current immune monitoring remain: (a) standardization; (b) significance from the standpoint of magnitude and profile, particularly in preclinical models that have their own caveats; (c) throughput to also monitor additional T-cell clonotypes recruited to the process through "epitope spreading"; and (d) reliability in clinical studies, as there have been very few benchmarks useful to inform on adequate success criteria for the decision to go, no-go, or optimize a vaccine.

While the above describe major hurdles in front of cancer vaccines, some of them are already addressable but others remain to be elucidated to fully enable the development of this class of vaccines.

CANCER VACCINE PLATFORM TECHNOLOGIES CONTINUE TO UNDERGO SIGNIFICANT DIVERSIFICATION

Based on the preceding discussion, important components required for therapeutic cancer vaccines include the antigen, the adjuvant, and the platform for delivering the antigen and other vaccine components. Immunomodulators to reverse the obstacles that limit immune activation in cancer patients will also be important for maximizing the clinical activity of the vaccine. Adjuvants are not discussed here because they are discussed elsewhere.

The choice of the tumor antigen to be used in a cancer vaccine is a critical. The antigen will determine the specificity of the immune response, influence the overall immunogenicity of the vaccine, and affect the clinical usefulness (efficacy-to-toxicity ratio) of the cancer vaccine.

During the past 15 years, a wide range of different tumor antigens have been identified.[106,107] Most recently, truncated cryptic fragments from TAAs have been identified as tumor specific and highly immunogenic.[108] More commonly, tumor antigens that are associated with certain types of cancers have been evaluated. Tables 13.1 and 13.2 list the major categories and include the cancer testes antigens (CTAs), the differentiation antigens, the overexpressed antigens, and the oncofetal antigens. There also is a class of viral antigens that have been found to be associated with certain malignancies, most notoriously the E6 and E7 proteins of the human papillomavirus.[109] The TAAs have some level of specificity to one or several classes of tumors and are restricted in their expression level in normal tissues and organ systems. Some of these antigens were identified because of their immunogenicity using SEREX (serological expression of complementary DNA expression) or reverse immunology.[110,111] Finally, multiple new technologies, including proteomics, gene array, and immunologic strategies, are being used to identify even more highly expressed, specific, and immunogenic tumor antigens.[112] This search has culminated with the recent discovery of a most exciting class of antigens, that are tumor and patient specific, encompassing strictly tumor specific epitopes resulting from nonsynonymous somatic mutations (their totality representing the cancer mutanome), yielded by genes within the tumor cells.[113,114] Such neoepitopes represent improved targets for vaccines and other immune interventions as they can be "non-self," more immunogenic, and selectively expressed by tumor cells, but not normal tissue. Future strategies encompassing highly personalized vaccines directed at such epitopes are described below.

TABLE 13.1 Categories and Examples of Tumor Antigens

Category of Tumor Antigen	Example
TUMOR SPECIFIC	
Mutated oncogenes	mRas
Immunoglobulin idiotype	Idiotype
Cryptic frameshift sequences	May be tumor-specific
TUMOR ASSOCIATED	
Cancer testis	MAGE-A1, MAGE1, MAGE-A3
Differentiation	gp100, tyrosinase
Oncofetal	CEA
Widely expressed	mP53, 5T4
VIRAL	EBV, HPV

CEA, carcinoembryonic antigen; EBV, Epstein-Barr virus; gp100, glycoprotein 100; HPV, human papillomavirus; MAGE1, melanoma-associated gene 1; MAGE-A3, melanoma-associated gene 3.

TABLE 13.2 Examples of Tumor Types and Antigens

Tumor Type	Example of Tumor Antigen
Colorectal	CEA, KSA, 5T4
Lung	CEA, MAGE-A3, Muc1
Breast	CEA, HER/2-neu, Muc1
Gastric	Gastrin, CEA
Pancreatic	CEA
Melanoma	gp100, Mart1/Melan A, tyrosinase, MAGE-A1, MAGE1, MAGE-A3, NYESO-1
Ovarian cancer	CEA, survivin, NYESO1
Prostate cancer	PSA, PSMA, PAP, 5T4, WT1
Chronic myelogenous leukemia	BCR/ABL, WT1
Lymphoma	Immunoglobulin idiotype

BCR/ABL, breakpoint cluster region/Abelson murine leukemia virus; CEA, carcinoembryonic antigen; gp100, glycoprotein 100; MAGE1, melanoma-associated gene 1; MAGE-A3, melanoma-associated gene 3; NYESO1, New York ESO antigen 1; PAP, prostate acid phosphatase; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; WT1, Wilms tumor antigen 1.

TABLE 13.3 Platforms to Present Antigen

	Peptide	Protein	Carbohydrate	Cellular Tumor	Cellular Dendritic	DNA	Viral
Immune response	CD8+ or CD4+ specific	CD4+	CD4+	CD8+	Mixed	CD8+ and CD4+	CD8+ and CD4+
Antigen specificity	Epitope specific	Antigen specific	Glycosylation specific	Not antigen specific	Potentially broad	Single or multiple defined antigens	Single or multiple defined antigens
Treatment type	Allogeneic	Allogeneic	Allogeneic	Autologous or allogeneic	Autologous	Allogeneic	Allogeneic
Manufacturing	Well defined	Well defined	Well defined	Stability/ characterization	Collection/culture/ characterization	Well defined	Specialized but well defined
Development phase	Phase III complete	Phase III underway	Phase III complete	Phase III complete	Phase III complete; U.S. Food and Drug Administration approved vaccine	Phase I/II	Phase III underway

There are many different platforms that can be used to present the tumor antigen(s) to the immune system (Table 13.3). These platforms can be antigen specific or antigen nonspecific. In the nonspecific approaches, the tumor cell itself is used as an immunogen. In this situation, the tumor cell can be taken from the patient (autologous) or generated from cell lines (allogeneic). To further enhance the immunogenicity, tumor cell vaccines can be genetically modified with immunogenic molecules such as costimulatory molecules, cytokines, or haptens. The antigen-specific vaccines use a particular tumor antigen(s) as the immunogen. These antigens can be any of the tumor-associated or tumor-specific antigens. The tumor antigen(s) can be presented to the immune system in a variety of platforms that may include proteins, carbohydrates, DNA, or viral vectors. In addition, MHC-binding epitopes derived from the proteins can also be presented to the immune system as peptide vaccines.

The choice of platform used to present the antigen to the immune system will influence the characteristics of the immune response. For example, nonamer binding HLA class I peptide immunogens will induce predominantly a CD8+ T-cell immune response and Th1 CD4 type of immune response. On the other hand, an extracellular protein immunogen will undergo endocytosis and be processed through a class II pathway and activate CD4+ T cells and predominantly a Th2 response. Similarly, carbohydrate immunogens will also induce predominantly CD4 and Th2 immune responses. Viral and DC vaccines may generate broader immune responses of CD4 and CD8 types.

Cellular Vaccines—Tumor Cells

Tumor cell–based vaccines were among the earliest cancer vaccines.[115] Tumor cells taken directly from a patient or from allogeneic cell lines were inactivated to enhance the immunogenicity of the tumor cells. This included lysing the cells, radiating the cells, or adding an adjuvant. With tumor cell vaccines, many normal cellular antigens along with tumor antigens were presented to the immune system, with the potential that the large number of normal cellular

immunogens might compete with or dilute out the desired response to the tumor antigens. The potential strength of the approach is that any tumor-specific mutated antigens would be presented to the immune system, particularly with autologous tumor cell vaccine approaches. Both CD4 and CD8 epitopes would be presented to the immune system. Disadvantages of autologous vaccines relate to the logistics of obtaining, processing, manufacturing, and then readministering these autologous vaccine preparations into an individual patient.

Live tumor cells are poorly immunogenic and have been shown to secrete various factors that inhibit various immune responses. Apoptotic or necrotic tumor cells may release factors such as HSPs, HMGB1, uric acid, or ATP (see "Immunogenic and Nonimmunogenic Cell Death") that have various immune-stimulatory properties. There are a number of strategies to kill the tumor cells prior to administration and include freeze-thawing and irradiation. A number of different strategies to genetically modify the tumor cell to make it more immunogenic have also been evaluated.[116,117] These include the introduction of immunogenic haptens, xenogeneic or immunogenic antigens, cytokine molecules, viral oncolysates, and/or costimulatory molecules.

Exosomes are membrane-derived vesicles from multivesicular endosomes that are secreted by many cell types and reflect the protein content of the cells from which they came. Tumor-derived endosomes have been studied as tumor vaccines and, when administered with granulocyte-macrophage colony-stimulating factor (GM-CSF), can activate cytotoxic T cells specific for various tumor antigens. Whole tumor-derived RNA has also been used to pulse DCs, and vaccine-specific T-cell responses have been seen in some patients with melanoma.

Several Phase III trials have been completed with the first generation of tumor cell vaccines (inactivated or cell lysates). These include customized autologous and allogeneic approaches and genetically modified (GM-CSF) tumor cells. In the Phase III trials conducted with various tumor cell vaccines to date, clinical efficacy was not established[118,119] (Table 13.4). However, there has been promising results with the combination of a GM-CSF expressing cellular vaccine (GVAX-Cell Genesis) and ipilimumab, a CTLA-4–blocking antibody that blocks a key immune-inhibiting checkpoint, where a relationship was seen between the induced tumor necrosis and ratio of infiltrating CD8+ T cells over FoxP3 Tregs.[120]

Cellular Vaccines—Dendritic Cells

Technologies to isolate DC precursors or immature DCs from patients and to culture and activate these cells have been developed and optimized.[121,122] These cells can be used to present tumor antigens to the immune system. The type of T-cell activation by DC vaccines is dependent on how the tumor antigens are provided to the DCs. For example, if class I binding peptides are used to pulse these DCs, the immune response will be predominantly a CD8+ T-cell response.[123] Similarly, if tumor antigens are provided through genetic approaches such as plasmid or viral vectors, the tumor antigens will be expressed intracellularly and processed and presented through a class I pathway resulting in a predominantly CD8+ T-cell response. If the tumor antigen is secreted from these genetic expression constructs, the class II pathway may also be used and predominantly CD4+ T-cell responses will be generated. RNA has also been used to provide the tumor antigen, and this will also predominantly use a class I CD8+ T-cell pathway.[123]

There are a number of approaches to load DCs with antigens. Pulsing DCs with autologous tumor cell lysates or tumor cell–derived RNA results in presentation of a wide variety of cellular antigens and the tumor antigens.[123] RNA has certain favorable properties, including the potential for high levels of translation in the cytoplasmic compartment, which can induce CD8+ and CD4+ T-cell responses. One clinical trial showed that vaccines containing multiple peptides can induce a broad immune response to multiple epitopes. As such, a 12-epitope peptide vaccine was shown to be more immunogenic than a four-peptide vaccine.[124] Trials using combinations of class I and class II binding epitopes are underway.

RNA is emerging as an effective way to transduce DCs. This results in effective class I protein processing and presentation.

TABLE 13.4 Vaccines in Phase II or Phase III Development for Prostate Cancer

Vaccine	Company	Type	Tumor-Associated Antigen	Additional Components	Status
Sipuleucel-T or Provenge	Dendreon	Autologous PBMCs specifically activated dendritic cells	PMBCs activated with TAA, PAP	GM-CSF	FDA approved
PROSTVAC-VF	Bavarian Nordic	Primed with vaccina based vector boosted with fowlpox based vector	Both vectors contain the transgene TAA, PSA	Both vectors also contain immune costimulatory transgenes CD80, ICAM-1, and LFA-3. Both viruses are administered with GM-CSF	Phase III
AD5-PSA		In vivo gene therapy mediated by adenovirus	PSA		Phase II
PAP DNA Vaccine		Naked DNA	PAP	Combination therapy with GM-CSF or Sipucel T	Phase II
CV9103	CureVac	TAA mRNAs	PSA, PSCA, PSMA, and STEAP1		Phase IIb
GVAX	Biosante Inc	Two irradiated allogenic prostate cancer cell lines		Cell lines express GM-CSF	Phase III

GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM, intercellular adhesion molecule; LFA, lymphocyte function–associated antigen; mRNA, messenger RNA; PAP, prostate acid phosphatase; PBMC, peripheral blood mononuclear cell; PSA, prostate-stimulating antigen; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; STEAP1, six transmembrane epithelial antigen of the prostate 1; TAA, tumor-associated antigen.

In addition, the expression is transient, and RNA will not integrate into the genome. RNAs can be introduced by lipid-mediated transfection, passive transfection, or, as most recently found, by electroporation. Alternatively, the tumor's array of antigens can be presented by producing DC-tumor cell hybridomas in which the antigen processing and presentation capabilities of the DC are used to process and present tumor-derived proteins.[125] Another approach to provide unique antigens to purified DCs is to pulse DCs with exosomes derived from autologous DCs. These are nanometric vesicles that are released from late endosomes and contain multiple antigens, including tumor-derived antigens.[126] DCs can be loaded with common TAAs using genetic approaches or pulsed with MHC class I or class II epitopes from TAAs.[127] Finally, the activity of DCs can be further enhanced by genetically modifying DCs with molecules that may affect DC migration (chemokines), activation (GM-CSF) or T-cell activation (cytokines).[128]

Preclinical data using DC vaccines demonstrate that specific T-cell responses of the desired functional qualities to induce antitumor activity can be induced. Based on these results a number of different DC-based vaccines have been evaluated in the clinic. Phase II studies in myeloma and lymphoma using patient-specific idiotype antigens[129] and in colorectal cancer using carcinoembryonic antigen peptide targets[130] have demonstrated an expected immunologic response—manifested through induction or elevation of antibody or T-cell responses against the immunizing antigens—and some examples of clinical antitumor responses.

Most recently, a Phase III clinical trial using patient-derived DCs pulsed with a prostatic acid phosphatase–GM-CSF fusion protein (Sipuleucel-T [Dendreon]) in patients with hormone-refractory prostate cancer has demonstrated a small but significant survival advantage for patients who received the vaccine versus the placebo.[131–134] An association between the numbers of CD54 APCs and likelihood of improved survival was seen. This is the first example of a survival advantage using a cancer vaccine and provides clinical proof of concept for cancer vaccines in general. It is somewhat debatable whether this vaccine comprises truly differentiated DCs. Nevertheless, confirmatory trials have duplicated this important result, and, recently, this vaccine has become the first therapeutic vaccine approved by the U.S. Food and Drug Administration (FDA) for use in cancer (castrate-resistant prostate cancer).

Although technologies to purify DCs have become more robust and generalized, the customized patient-specific nature of this approach make broad-scale application of DC approaches a challenge. Cell-based vaccines comprising mostly DCs, are very effective in eliciting both T-helper and CTL responses, but less proficient in inducing B-cell responses.

Peptide Vaccines

The epitopes that are presented on class I or class II MHCs can be predicted and synthesized to use as targets for cancer vaccines.[135] With peptide-based approaches, the MHC type of the patient must be known and peptide immunogens must be specific to a particular MHC type. Most recently, peptides that bind to multiple different MHC subtypes or types have been identified (supertopes).[136] Other improvements to the approach include modifying the sequence of the peptides to increase the strength of binding to the MHCs (anchor modifications)[137] or to the TCR (heteroclitic modifications).[138] These improvements have yielded enhanced immunogenicity in preclinical models, and early trials have suggested that there may be enhanced clinical activity. Because peptide immunogens are highly specific, the breadth of the induced immune response can be enhanced by using multiple peptide epitopes from one or more protein antigens. In addition, combinations of class I and class II binding peptides can be used toward coinduction of CTLs and Th cells acting synergistically in vivo.

Although some immunologic and clinical activity has been seen with peptide immunogens formulated in saline without an adjuvant, most data suggest that peptide immunogens will be more efficacious when formulated in adjuvants such as incomplete Freund adjuvant. However, the overall clinical response rate for a variety of class I binding peptides administered as monotherapy to patients with advanced cancer is very low. Combining peptide-based vaccination with immunomodulators such as IL-2 infusions may result in better T-cell migration to tumor sites and enhanced antitumor activity.[139] Improved antitumor activity has been confirmed in a randomized trial with improved response rates and overall survival in patients receiving a gp100 peptide vaccine with high doses of IL-2 versus high doses of IL-2 alone. A trial comparing the same gp100 vaccine with and without the anti–CTLA-4 monoclonal antibody did not show that clinical measurements were improved by the combination over those seen with the anti–CTLA-4 antibody alone.[140] Together with other evidence demonstrating the induction of durable clinical responses ascribable to anti–CTLA-4 antibody, supported the historical approval of the first immune checkpoint blocking antibody.[141] Enhanced clinical and immune responses may be seen with combinations of multiple peptides over single class I and class II binding peptides. The immunologic activity of peptide vaccines may be enhanced with enhanced adjuvant formulations. Addition of cytosine phosphate guanine (CpG) oligodeoxy nucleotides to the MART-1 immunodominant epitope[142–151] in Montanide emulsion resulted in enhanced T-cell responses with more rapid induction of CD8+ T cells and higher peak responses.[101] Similar immune enhancements, manifested through generation of broad integrated antibody and CD4+ and CD8+ T-cell response, were seen with a recombinant New York esophageal cancer-antigen-1 (NYESO-1) protein with ISCOMATRIX adjuvant[152] or with the addition of RNA adjuvants.[153]

Another promising approach involves use of synthetic long peptide (SLP) vaccines. The use of SLPs, generally polypeptides of greater than 30 amino acids, has several theoretical advantages. They may provide multiple epitopes with the potential to bind to multiple MHC types for presentation to CD4+ and CD8+ T cells. A clinical trial using SLPs has been completed in human papillomavirus (HPV)-related diseases with a high frequency of durable clinical regressions seen in patients with HPV-related vulvar intraepithelial neoplasia.[154]

Promising immune results have been seen with a polypeptide vaccine administered in a novel liposomal formulation (DPX-Survivac [ImmunoVaccine]) in patients with high risk or recurrent ovarian cancer. Polyfunctional T-cell responses were seen in all evaluable patients at the optimal dose.[155] Further evaluation of this vaccine in conjunction with metronomic use of cyclophosphamide in survivin-expressing tumors such as ovarian carcinomas is underway.

Peptide-based immunogens are very immunogenic and simple to manufacture. The major limitation of this approach relates to its HLA restriction, the apparently limited capability to elicit robust immunity, and the need to identify and produce multiple peptides to generate broad immune responses to antigens or to generate Th cell and CTL responses.

Protein-Based Vaccines

Tumor antigen proteins used for vaccination will undergo endocytosis by APCs and be processed and presented through the MHC class II pathway. The processed epitopes will be presented to CD4 cells and, in most situations, promote a Th2-focused immune response with generation of humoral

responses. Formulating the protein in some adjuvants may alter the magnitude of the response and may also help promote a Th1-type response. The advantages of using protein-based vaccines are that they are relatively easy to manufacture and they avoid the MHC requirements of peptides. In fact, the processing and presentation of protein immunogens results in multiple epitopes of the tumor antigen being presented on the patient's MHC class I and class II complexes.

There were positive results from a small randomized clinical trial with a vaccine called Stimuvax (Oncothyreon, EMD Serono Inc.).[156] This is a 25-amino-acid polypeptide vaccine (from the MUC1 tumor antigen) formulated in a liposomal formulation containing monophosphoryl lipid (TLR4 agonist). However a randomized trial of this product in locally advanced stage 3a or 3b lung cancer did not meet a significant clinical end point.

A melanoma-associated gene (MAGE)-A3 full-length protein has been evaluated in melanoma and non–small-cell lung cancer (NSCLC) with different saponin adjuvant formulations. This vaccine has the advantage over idiotype and HSP vaccines in that it is an "off-the-shelf" (allogeneic) vaccine and does not need to be synthesized uniquely for each patient. The choice of adjuvant was critical for the activity of the vaccine, and the MAGE-A3 vaccine with this adjuvant induced CD4+ and specific immune responses in many patients with lung cancer, but the MAGE-A3 vaccine alone was less effective. In a small ($n = 182$) randomized clinical trial in patients with lung cancer, a 33% relative reduction of recurrence risk was seen with vaccine treatment.[157] This vaccine-adjuvant combination was subsequently tested in larger, randomized trials but failed to meet the primary clinical end point.[158]

Two kinds of autologous protein-based cancer vaccines have been comprehensively evaluated. The first is the idiotype protein.[159] The immunoglobulin idiotype is a patient-specific customized autologous approach. Strategies have been developed to isolate patient-specific idiotype protein from B-cell lymphomas or myelomas and include hybridoma technology or polymerase chain reaction–based approaches. The idiotype protein can be purified and conjugated to an immunogenic hapten such as KLH (keyhole limpet hemocyanin) to enhance the immunogenicity of the vaccine. In preclinical models, reproducible humoral responses are induced by these protein immunogens.[160] These responses are protective against subsequent tumor challenge with tumor cells expressing the idiotype protein. Depletion of CD4+ T cells in murine models will reduce the therapeutic effect. The immunogenicity of idiotype protein can be enhanced by several techniques, including conjugation of the idiotype variable region to xenogeneic constant region proteins or vaccination with adjuvants such as GM-CSF or GM-CSF–transduced cells.[161]

Idiotype protein vaccines have been studied in the clinic in patients with B-cell lymphoma or myeloma. The approach is feasible, and idiotype protein can be produced for hundreds of patients by hybridoma technology or more easily by polymerase chain reaction techniques.[162] The vaccines are safe, and few serious adverse events have been documented. Although humoral responses are predominantly induced, cellular responses have also been demonstrated. Immunologic responses are more likely to be seen in patients with low tumor burdens and are correlated with longer remissions. In one study, occult lymphoma cells in the peripheral blood were reduced or eliminated after idiotype vaccination.[163] The impact of vaccination with patient-specific idiotype protein has been evaluated in three randomized clinical trials.[164] In two of these trials, clinical benefit was not seen, but in the third, a significant increase in disease-free survival was seen. In this trial, patients' tumors were significantly debulked with aggressive chemotherapy before vaccination, but most of the patients did not receive rituximab, the current standard of care, as part of this debulking chemotherapy. Thus, additional trials to determine whether this vaccine is active in patients after rituximab-based chemotherapy will be important.

The HSPs have also been evaluated as cancer vaccines.[165] HSPs can be isolated from tumor cells after mild heating of the cells. These HSPs are associated with intracellular proteins and peptides. Although many normal peptides will bind to HSPs, TAA peptides will also bind. It is also possible to make recombinant HSPs that bind to specific tumor antigens. Thus, the HSP-peptides isolated from tumors represent patient-individualized vaccines. The HSPs enter APCs through a specific receptor (CD91 on DCs).[166] These HSPs are then internalized and processed and presented through the proteasomal pathway and generate predominantly a CD8 immune response.[167] HSPs have been evaluated in the clinic in various tumor types and been shown to be safe and immunogenic. However, a Phase III trial evaluating HSP in melanoma did not achieve its clinical end point.[168]

A protein vaccine targeting epidermal growth factor receptor (EGFR) (Rindopepimut [Celldex]) had been studied in glioblastoma where EGFRviii is overexpressed in approximately 30% of these tumors. Rindopepimut consists of the EGFRviii protein conjugated to KLH, with both humoral and cellular responses to EGFR generated after vaccination. In three Phase II trials the median overall survival (OS) was between 20.4 and 21.8 months and the OS at 2 years was between 23 and 33%.[169–171] A Phase III trial is underway. Of patients treated, 85% generated significant titers of antibodies to EGFRviii.

Carbohydrate Vaccines

The pattern of glycosylation on tumor cells is different from normal cells, and often carbohydrate antigens on the surface of tumor cells are upregulated.[172] In addition, autoantibody responses to various glycoproteins are often seen in patients with cancer and may be associated with improved outcome.[173] For this reason, several glycoprotein immunogens have been evaluated as potential TAAs for cancer vaccines. A limitation of carbohydrate TAAs is that there may be low-level expression of carbohydrate targets on some normal tissues.[174] In addition, the tumoral expression of carbohydrate antigens may be highly variable.

In early phase clinical trials, carbohydrate immunogens for breast or colon cancer, including the STN moiety, have been shown to be safe and immunogenic inducing predominantly humoral-based immune responses, particularly immunoglobulin (Ig) M and IgG.[175] A Phase III trial with the Theratope STN cancer vaccine for breast cancer has been completed. In this trial, a small dose of cyclophosphamide was given before the vaccination to reduce or eliminate Tregs.[176] However, a clinically significant vaccine effect was not seen in the Phase III trial.

DNA Vaccines

Tumor antigens can be encoded in plasmid vectors and used as immunogens. Cytokines and costimulatory molecules can also be engineered into plasmids.[177,178] After vaccination, DNA will enter cells in the local vicinity, and the engineered transgenes will be expressed. Tumor antigens will, thus, be processed through the class I pathway and epitopes will be presented predominantly on class I MHCs. If the plasmid is expressed in fibroblasts or local muscle cells, the processed tumor antigens will eventually be cross-presented by professional APCs that phagocytose the dying transfated local cells. In some situations, low-frequency APCs will be directly primed

by the plasmid immunogens. In preclinical models, DNA cancer vaccines have been shown to be effective in generating CD8+ T-cell responses and, in some situations, also CD4+ T-cell responses.[179] These responses can be protective against subsequent challenge with syngeneic tumor cells expressing the TAA.

In the clinic, the experience with DNA vaccines for cancer and other diseases has been less impressive. Immune responses are of lower magnitude and require larger quantities of the plasmid immunogen. Strategies to enhance the immunogenicity of DNA vaccines are being developed and include inclusion of different adjuvants, preparation of different formulations, engineering immunogenic molecules into the vector, and using devices (e.g., gene gun) to enhance delivery of the vaccine.[180] In addition, presenting antigen in a RNA virus to produce large quantities of the antigen locally is also being evaluated (AlphaVax).

Finally, the most promising strategies use DNA vaccines in heterologous prime-boost approaches with other platforms such as viral, protein, or peptide immunogens.[181–183] There have been no completed Phase III trials of DNA vaccines for cancer apart from a recent pivotal trial with a naked DNA expressing sequences encoding HLA-B7 and β_2-microglobulin, aimed to enable an immune response against melanoma that failed to meet its primary end point.[142]

Microbial Vaccines

The TAAs can be engineered into various viral vectors and used as cancer vaccines. Because many viruses are efficient at entering cells and expressing their genetic material, viral vaccines have the advantage of producing relatively high levels of the tumor antigen at the vaccination site. Another potential advantage of viral vaccines is the danger signal that is often provided. This will facilitate the activation of professional APCs. A limitation of many but not all viruses is their potential to replicate and cause various types of illnesses after infection including cytokine storms. Finally, the immunogenicity of the virus can result in antiviral responses that can neutralize the virus, cause unwanted inflammation, and reduce or prevent the effectiveness of subsequent vaccinations or compete with the immune response to the TAA transgene.

Various members of the poxvirus family have been found to be attractive vectors for cancer vaccination.[184,185] The modified vaccinia Akara virus and the Avipox (fowlpox) and canarypox viruses have been shown to have no or limited replication potential in human cells. Thus, there is no or little likelihood of illness being caused by the virus. The poxviruses are large viruses and can be engineered to contain many foreign transgenes. They effectively induce CD4 and CD8 responses.

Significant neutralizing responses to some poxviruses, including MVA and avian pox virus, have not been demonstrated and could potentially be used for chronic vaccination. The major limitations of poxvirus-based approaches relate to the specialized engineering and production technologies that are required for these viruses.

In preclinical models, these vectors have been shown to effectively prime CD8 and/or CD4 responses to the inserted transgene. These responses have been associated with tumor protection in various models. In the clinic, there has been significant experience with fowlpox and canarypox (ALVAC) vaccines for cancer. Both of these vaccines are very safe, with the type of immune response induced dependent on the tumor antigen being studied. With ALVAC vaccines containing carcinoembryonic antigen, predominantly CD8 responses have been documented.[186,187,187a] On the other hand, with ALVAC vaccines containing p53, CD8 responses were not seen and CD4 responses were most common.[188] This and some preclinical studies suggest that there may be a high level of central tolerance at the CD8 level to p53. The immune response induced by these vectors may be augmented by inclusion of costimulatory molecules such as B7 or a triad of costimulatory molecules (TRICOM) in the vaccine.[189] The immune responses may also be augmented by prime-boost immunizations and with adjuvants such as GM-CSF or immune modulators such as interferon.[190] A significant 8.5-month increase in OS was seen in patients with hormone-refractory prostate cancer vaccinated with the Prostvac vaccine (Vaccinia PSA TRICOM and Fowlpox PSA-TRICOM [Bavarian Nordic]). However, there was no increase in progression-free survival with the vaccine.[191] A Phase III trial of this vaccine is now underway in hormone refractory prostate cancer. A Phase III trial with a MVA vector expressing 5T4 (Trovax-Oxford Biomedica) did not meet its clinical end point in patients with renal cancer.[143]

Yeast-based vectors have also been used as therapeutic vaccines in cancer. The Tarmogen platform (GlobImmune) consists of heat inactivated yeast containing an antigen of choice. The yeast is taken up by DCs and presented to the immune system. A Tarmogen vector containing carcinoembryonic antigen has been studied in medullary thyroid cancer and based on favorable Phase I results is now being evaluated in a Phase II trial.[144]

Listeria monocytogenes has also been used as a cancer vaccine vector. The live-attenuated, double-deleted (LADD) platform (Aduro) has been modified to improve its safety profile by deleting two genes that control the virulence of *Listeria* and by engineering a target TAA. A LADD expressing mesothelin has been studied in the clinic in pancreatic cancer where mesothelin is often expressed. Based upon results from a combination Phase I trial with the cellular vaccine GVAX, a randomized controlled trial comparing GVAX with GVAX prime followed by LADD-mesothelin (CRS-207) boost was performed.[145] In this trial, a low dose of cyclophosphamide was administered with GVAX in 90 patients to reduce Treg cells. The safety profile was acceptable and a significant increase in OS was seen in the combination arm (9.7 m vs 4.6 m). Patients with enhanced mesothelin-specific CD8 responses had a longer OS. Similar vaccines containing either Her2, prostate-specific antigen (PSA), and HPV are under development.

Another technology using *Listeria*, Advaxis LM (Advaxis Immunotherapies), uses live attenuated *Listeria* that is engineered with a plasmid containing the fusion protein and the target antigen. APCs take up the *Listeria* and produce the fusion protein and antigen that is then processed and presented to the adaptive immune system. Both innate and adaptive immune responses are stimulated. A Phase I trial using Lm-LLO-E7 (live attenuated *L. monocytogenes* [Lm] that secretes the HPV-16 E7 antigen fused to a nonhemolytic fragment of the Lm protein listeriolysin O [LLO]) administered intravenously targeting HPV in cervical cancer has been reported and although there was a 40% incidence of grade 3 adverse events, the treatment was considered safe and a partial clinical response was seen.[146] A randomized trial of ADXS-HPV with or without cisplatin in 110 patients with recurrent cervical cancer has been completed but not reported and further investigation by the Gynecologic Oncology Group is underway. A combination trial of this *Listeria*-based vaccine with a checkpoint inhibitor and with the IDO inhibitor Epacadostat is also underway.

RNA-Based Vaccines

The application of in vitro transcribed (IVT) messenger RNA (mRNA) as a vaccine platform for cancer has been studied

extensively in preclinical models and has reached the Phase III clinical studies. Two different approaches have been investigated: (a) transfer of mRNA to patient's cells ex vivo and (b) direct delivery of IVT mRNA by injection in skin or lymph nodes. In both of these approaches a DNA template is created and the mRNA is transcribed using a cell free system.[147] The mRNA will contain a start and stop codon, 5′ cap and 3′ poly A regions flanked by untranslated regions. These are usually expressed intracellularly and the transcribed protein is then processed and presented through a MHC class I pathway. Strategies to improve the stability and translatability of the mRNAs have been developed. RNAs have immunostimulatory effects and in general induce immune activation by stimulating pattern recognition receptors whose normal function is to identify and respond to viral RNAs. For cellular delivery, formulations (such as lipid materials) have been developed to protect mRNAs against ribonuclease (RNAse)-mediated degradation. Electroporation is a preferred method of transfer. Intradermal or intranodal administration of naked or protamine-complexed RNA vaccines has also been used. Initial studies with DCs transfected with RNA demonstrated the feasibility, and immunogenicity of mRNA transfected DCs and has led to a Phase III trial, now underway by Argos Therapeutics, in patients with advanced renal cancer. Direct intradermal injection of protamine-complexed IVT mRNA with or without GM-CSF is safe and induces CD8 T-cell responses as well as antibody responses. Curevac is studying this approach in clinical trials of patients with prostate or NSCLC. The mRNA self-adjuvanted vaccine CV9103, which contains PSA, prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), and six transmembrane epithelial antigen of the prostate 1 (STEAP1), was studied in a Phase II trial in patients with hormone refractory prostate cancer.[148] Forty-four patients received up to five intradermal vaccinations in escalating doses and then 32 additional patients were given the vaccine at the recommended Phase II dose. The treatment was well tolerated and immune responses were seen in 26 of 32 patients and responses to multiple antigens were seen in 15 of 33 patients at the recommended dose. The median OS was thought to be favorable at 31.4 months.

Oncolytic Virus Vaccines

Oncolytic viruses are viruses that can selectively replicate in cancerous tissues and not normal tissues.[149,150] They use various oncogenic pathways that are activated for their selective replication including defective interferon pathways (vesicular stomatitis virus [VSV]), deletions of P53 (adenovirus), attenuated PKR (protein kinase regulated by double-stranded RNA) responses (herpes simplex virus [HSV]), deleted thymidine kinase activity *(Vaccinia)*, RAS mutations *(Reovirus)*, and increased expression of nectin-4 (measles virus). Although tumor destruction by replicating virus is an important antitumor mechanism, the induction of a systemic antitumor immune response may be equally as important. Thus strategies to enhance the induction of a systemic immune response are being explored and include engineering immunostimulatory cytokines such as GM-CSF or TAAs and combining administration with other immunomodulators such as checkpoint inhibitors.

There are several oncolytic viruses that look promising based upon initial clinical trials. T-VEC is a HSV1 virus that has been engineered to express GM-CSF (Amgen).[151] The virus is administered through repeated intratumoral injections. In preliminary results from a randomized Phase III trial comparing injections with the virus to injections with GM-CSF alone, the objective response rate was 16% versus 2%, respectively, with trends toward an improved OS. Pexa-Vec (Jennerex) is a *Vaccinia* virus that has also been engineered to express GM-CSF. There is evidence that this virus can replicate in tumor cells after intravenous infusions. In a randomized trial in hepatocellular carcinoma there was a median survival of 14.1 months versus 6.7 months for patients randomized to high-dose Pexa-Vec versus low dose (hazard ratio [HR] 0.39, $P = .02$).[192] In both of these trials there was evidence of viral replication in regressing tumors, so it is difficult to know whether the adaptive immune response played a significant role in the antitumor responses. However evidence of local and distant immunity was seen after T-Vec treatment.[193] Two objective responses of patients with myeloma were seen after treatment with a measles virus (MV-NIS) administered intravenously and there are ongoing trials with this oncolytic virus.[194] Tumor necrosis was seen in two of 21 patients with metastatic melanoma treated with intravenous infusions of the Reovirus Serotype-3-dearing strain (Reolysin [Oncolytics]).[195] Preclinical studies have shown promising results for oncolytic viruses engineered with TAAs and those combined with checkpoint inhibitors or other immunomodulators or cell therapies.

VACCINES IN CLINICAL DEVELOPMENT FOR SPECIFIC CANCERS

A number of vaccines using various platforms are being developed for specific cancers and will be subjected to the usual Phase I to Phase III clinical development pathways through different pharmaceutical companies. The following is a summary of vaccines at more advanced stages of clinical development for common tumor types.

VACCINES IN DEVELOPMENT FOR SPECIFIC TUMOR TYPES

Prostate Cancer

There are many different immunotherapies being tested to treat prostate cancer (see Table 13.4). Leading the way is Sipuleucel-T (Dendreon), an autologous DC vaccine. Recombinant prostatic acid phosphatase (PAP) and GM-CSF are used to activate peripheral blood mononuclear cells (PBMCs), specifically DCs, both ex vivo and in vivo.[196] This product was approved by the FDA after a successful Phase III trial in metastatic castration-resistant prostate cancer. Patients survived 4.1 months longer than those treated with the placebo. In this trial, 73.0% of patients treated with Sipuleucel-T had a T-cell response, compared to 12.1% of patients treated with the placebo.[196] Sipuleucel-T is being tested in Phase II trials in combination with ipilimumab (NCT01804465).

Another promising therapy is PROSTVAC-VF (Bavarian Nordic). This therapy uses two recombinant viruses that have transgenes for the TAA (PSA) and for costimulatory proteins, CD80, intercellular adhesion molecule (ICAM)-1, and lymphocyte function–associated antigen (LFA)-3.[197] Patients are given a *Vaccinia* virus to prime the immune system and a fowlpox virus to boost it. An encouraging Phase II trial showed that 30% of patients treated with PROSTVAC-VF survived 3 years, whereas, only 17% of patients had a 3-year survival when given the placebo.[197] There was also an increase in the median survival by 8.5 months in the vaccine-treated group compared to the control group. PROSTVAC-VF is now being tested in a Phase III trial and in a Phase I trial in combination with ipilimumab (NCT00113984).

Other vaccines being tested in Phase II trials to treat prostate cancer include an adenovirus with a PSA transgene[198] (NCT00583024, NCT00583752), a naked DNA vaccine

that targets the TAA PAP (NCT00849121, NCT01706458), and CV9103 which consists of mRNA for multiple TAAs (NCT00831467, NCT01817738).

Breast Cancer

The protein and peptide vaccines are the most developed platforms for treating breast cancer (Table 13.5). NeuVax (Cancer Insight), a HER-2 derived peptide, E75, was evaluated in a Phase I/II trial when administered with GM-CSF. It had minimal toxicities and showed a marginal increase in 5-year disease-free survival over the control group.[199] A Phase III trial testing NeuVax and GM-CSF is ongoing (NCT0147924) and is also being administered in an ongoing Phase II trial in combination with trastuzumab (NCT01570036).

Theratope (Biomira) consists of carbohydrate antigen, sialyl-Tn, attached to the carrier glycoprotein, KLH protein. This was administered with the adjuvant Enhanzyn in a completed Phase III trial that failed to prolong survival in women with metastatic breast cancer compared to the placebo group[200] (NCT00003638). However, a post hoc analysis revealed a significant increase in time to progression and OS in a stratified group of women who received concomitant endocrine treatment.[201]

Lung Cancer

There were some initial promising results with both a protein and a lipopeptide vaccine in lung cancer.[202] In a Phase III study (NCT00409188), Tecemotide (Stimuvax-EMD Serono), the MUC1 lipopeptide attached to a liposomal delivery system, failed to significantly prolong survival in the overall population of patients with unresectable stage III NSCLC (Table 13.6).[203] However, the group of patients who received concurrent chemoradiotherapy had a prolonged survival of 30.8 months compared to the 20.16 months survival of the placebo group.[203] Another Phase III trial is underway to test patients who have already undergone concurrent chemoradiotherapy (NCT02049151).

The MAGE-A3 antigen is expressed in approximately 35% of patients with NSCLC.[204] MAGE-A3 protein vaccine (GlaxoSmithKline) was delivered with the immune adjuvant AS15 in a Phase II trial and demonstrated trends toward improved outcomes compared to the placebo. However, a Phase III trial was terminated because it failed to meet its primary point of extending disease-free survival (DFS) (NCT00480025).

Another peptide vaccine, GV1001 (GemVax), is a peptide derived from the human telomerase reverse transcriptase and administered with GM-CSF. A Phase II trial showed that the median OS was significantly longer in immune responders than in nonresponders (19 months compared to 3.5 months), and 80% of patients exhibited an immune response.[205] Because of these results, a Phase III trial is in development (NCT01579188).

CIMAvax EGF is a protein vaccine designed to induce a humoral response. The TAA, EGF (epidermal growth factor), is fused with a carrier protein and administered with Montanide ISA 51 adjuvant. In a Phase II study, the OS did not differ significantly from patients given the current standard of care.[206] However, patients who had a good antibody response to the protein survived significantly longer (11.7 months vs 3.6 months).[206]

TABLE 13.5 Vaccines in Phase II or III Development for Breast Cancer

Vaccine	Company	Type	Tumor-Associated Antigen	Additional Components	Status
Nelipepimut-S, E75 or NeuVax	Galena Biopharm	Peptide vaccine	HER2 E75	Administered with GM-CSF	Phase III, Phase II with trastuzumab
Theratope	Biomira	Glycoprotein vaccine	Sialyl-Tn	KLH and Enhanzyn adjuvant	Completed Phase III

TABLE 13.6 Vaccines in Phase II or III Development for Lung Cancer

Vaccine	Company	Type	Tumor Associated Antigen	Additional Components	Status
L-BLP25 or tecemotide	Stimuvax-EMD Serono	Lipoprotein vaccine	MUC1	Monophosphoryl lipid A	Phase III
TG4010	Transgene	Recombinant *Vaccinia* virus	MUC1	Administered with IL-2	Phase IIb
GSK15772932A	GSK	Peptide vaccine	MAGE-A3	Immune adjuvant AS15	Phase III
GV1001	GemVax	Peptide vaccine	Human telomerase reverse transcriptase	Administered with GM-CSF	Phase III
CIMAvax EGF		Peptide vaccine	EGF	Montanide ISA 51 adjuvant	Phase II
Belagenpumatucel-L or Lucanix	NovaRx Corporation	Cancer cell vaccine	TGF-β2		Phase III
Tergenpumatucel-L	NewLink Genetics	Cancer cell vaccine	α-1,3-Galatosyltransferase		Phase II
Racotumomab	Vaxira	Antiidiotype vaccine	Neu-glycolyl–containing gangliosides		Phase III

GM-CSF, granulocyte-macrophage colony-stimulating factor; GSK, GlaxoSmithKline; IL, interleukin; MAGE, melanoma-associated gene; TGF, transforming growth factor.

The TAA MUC1 is also targeted by a recombinant vaccine virus, TG4010 (Transgene). This virus has a transgene for MUC1 and is administered with IL-2. A Phase IIb trial demonstrated a benefit in treating patients with TG4010 with standard chemotherapy over standard chemotherapy alone.[207]

Belagenpumatucel-L (NovaRx), is a cancer cell line vaccine that expresses TGF-β_2. In a Phase II trial, patients who displayed a cellular and humoral immune response survived significantly longer than those who did not (32.5 months vs 11.6 months).[208] A Phase III trial demonstrated an increase in OS in a number of subgroups of patients, including patients who received prior radiation treatment (median survival 28 vs 16 months with placebo)[209] (NCT00676507).

Racotumomab (Vaxira) is an anti-idiotype vaccine that induces an immune response against the TAA neu-glycolyl containing gangliosides. A Phase II study demonstrated a longer median survival over those treated with a placebo (8.23 months vs 6.80 months).[210] A Phase III trial is ongoing (NCT01460472).

Melanoma

gp100 is a peptide vaccine that has been tested in a Phase III trial with IL-2 and the adjuvant, Montanide ISA 51 (Table 13.7). The response rate and median OS was greater with the gp100 vaccine and IL-2 than with IL-2 alone (16% vs 6%, 17.8 months vs 11.1 months, respectively).[211]

Ipilimumab is a fully humanized antibody that targets CTLA-4 and blocks CTLA-mediated T-cell inhibition/anergy. It has gained FDA approval for metastatic melanoma after Phase III trials showed a significant increase in survival in ipilimumab-containing arms. In a trial where ipilimumab was combined with a gp100 peptide vaccine, patients given ipilimumab and placebo had a 28.5% complete response (CR), partial response (PR), or stable disease (SD) compared with 20.1% when ipilimumab was given with gp100 and 11% when gp100 was given alone; the statistically significant differences in objective response and survival between vaccination alone and ipilimumab treatment, supported the approval of this antibody (NCT00094653).[212] Nevertheless, it is unclear why vaccine addition to ipilimumab treatment resulted in a negative trend regarding objective response rate and survival.

GSK2132231A (GlaxoSmithKline) is another peptide vaccine currently being tested in Phase III clinical trials for melanoma (NCT00796445). This consists of a peptide derived from the TAA MAGE-A3 that is administered with Montanide ISA 51. In a Phase II clinical trial, patients treated with GSK2132231A and Montanide ISA 51 showed an increased OS and MAGE-A3 specific antibody titers.[213]

There are multiple allogeneic tumor cell vaccines against melanoma currently being tested in Phase III clinical trials, including the Pol-03A (Polynoma) and CSF470 (Laboratorio Pablo Cassará) vaccines (NCT015465, NCT01729663).

Pancreatic Cancer

The vaccine Algenpantucel-L (NewLink Genetics) is made of allogeneic cancer cell lines that are transduced with α-1,3-galatosyltransferase. In a Phase II trial, the vaccine was administered with the adjuvant gemcitabine plus 5-fluoruracil and the 1-, 2-, and 3-year survival rates were 86%, 51%, and 42% respectively (Table 13.8).[214] This was thought to be superior to that expected based on historical data. A Phase III trial is ongoing (NCT01836432).

GV1001 (GemVax) is the only other vaccine in a Phase III trial to treat pancreatic cancer (NCT00358566, NCT00425360). It is a peptide vaccine derived from the telomerase protein. Two Phase III trials were started based on promising Phase II results. However, one (NCT00425360) was terminated early because of lack of benefit and no survival advantage seen.[215]

Colorectal Cancer

ONCO-VAX (Intracell) is an interesting cellular vaccine that has been studied in a Phase III trial (see Table 13.8). Irradiated autologous tumor cells are returned to the patient with bacille Calmette-Guérin (BCG) adjuvant. A Phase III trial in colon

TABLE 13.7 Vaccines in Phase II or Phase III Development for Melanoma

Vaccine	Company	Type	Tumor-Associated Antigen	Additional Components	Status
gp100 peptide vaccine		Peptide vaccine	gp100	Montanide ISA 51 adjuvant and IL-2	Phase III
GSK2132231A		Peptide vaccine	MAGE-A3	Montanide ISA 51	Phase III
Melanoma TAA pulsed dendritic cells		Adoptive dendritic cell therapy	gp100, MAGE-A1, MAGE-A2, MAGE-A3, MART-1 and tyrosinase		Phase II
Melanoma MHC epitope pulsed dendritic cells		Adoptive dendritic cell therapy	gp100 and tyrosinase		Phase II
Seviprotimut-L or pol-103a	Polynoma	Allogeneic cancer cell vaccine		Bacille Calmette-Guérin adjuvant	Phase III
CSF470	Laboratorio Pablo Cassará	Allogeneic cancer cell vaccine			Phase II/Phase III
Prime boost viral vaccination		Primed with *Vaccinia*-based vector boosted with fowlpox-based vector	Both viruses express NYESO-1		Phase II

IL, interleukin; MAGE, melanoma-associated gene; MART, melanoma antigen recognized by T cell; MHC, major histocompatibility complex; NYESO-1, New York ESO-antigen-1; TAA, tumor-associated antigen.

TABLE 13.8 Vaccines in Phase II or Phase III Development for Other Cancers

Cancer	Vaccine	Company	Type	Tumor-Associated Antigen	Additional Components	Status
Pancreatic cancer	GV1001	GemVax	Peptide vaccine	Human telomerase reverse transcriptase	Concurrent and sequential chemotherapy	Phase III
	Algenpantucel-L	NewLink Genetics	Allogeneic cancer cell vaccine	α-1,3-Galatosyltransferase	Gemcitabine plus 5-fluorouracil	Phase III
	Synthetic RAS peptides		Peptide vaccine	RAS	GM-CSF	Phase I-II
Colon cancer	ONCO-VAX	Vaccinogen	Irradiated autologous tumor cell		Bacille Calmette-Guérin adjuvant	Phase III
	PANVAC		Autologous poxvirus modified dendritic cells	CEA and MUC1	GM-CSF	Phase II
Renal cell carcinoma	AGS-003	Argos Therapeutics	Autologous dendritic cells	Patient-specific tumor RNA	Synthetic truncated CD40 ligand and sunitinib	Phase III
	IMA901	Immatics	Peptide vaccine	9 HLA class I-binding tumor-associated peptides	Cyclophosphamide	Phase III

CEA, carcinoembryonic antigen; GM-CSF, granulocyte-macrophage colony-stimulating factor; HLA, human leukocyte antigen; RAS, rat sarcoma viral oncogene homolog.

cancer found a significant improvement in recurrence-free and overall survival in patients with stage II disease[216] and a Phase IIIb study is currently ongoing to confirm the benefit of this vaccine (NCT02448173).

Renal Cell Carcinoma

AGS-003 (Argos Therapeutics) is a personalized therapy where patient-specific tumor cell RNA is transduced into autologous DCs (see Table 13.8). The patients' DCs are then administered with a synthetic truncated CD40 ligand and sunitinib. The median progression-free survival (PFS) and OS (11.2 and 30.2 months, respectively) in a Phase II trial both exceeded historical data.[217] A Phase III trial for AGS-003 and sunitinib is currently ongoing (NCT01582672).

IMA901 (Immatics) is a combination of nine peptides derived from TAA commonly expressed in renal cell carcinomas. A Phase II study showed that 67% and 54% of patients responded by mounting an immune response to IMA901 and GM-CSF with cyclophosphamide or to IMA901 and GM-CSF alone, respectively.[218] The study showed that IMA901 and GM-CSF reduced the number of Tregs and helped improve OS when the patient had an immune response to the vaccine.[218] A Phase III trial is currently ongoing (NCT01265901).

Advancement to Late-Stage Development Is Not a Success Predictor for Cancer Vaccines

Table 13.9 shows the results of seven promising cancer vaccine trials in which a positive clinical end point was met or approached in the earlier stages of clinical development. Six of the seven trials were randomized.

Despite promising Phase II results, only one vaccine for cancer, sipuleucel-T, has had significantly compelling randomized results to be approved by the FDA. While the initial positive results from other smaller randomized trials predicted successful outcomes, subsequent larger Phase III trials, have not translated to statistically significant positive outcomes for these other vaccines.

These vaccines are characterized by one or more of a number of features that have likely resulted in their successful clinical outcomes in Phase II: (a) clinical development in which the vaccine or vaccine and adjuvant combination have been optimized; (b) combination with an immune modulator; (c) choice of an immunoresponsive patient population; a (d) highly immunogenic vaccine platform; and (e) utilization of surrogate end points to make decisions. In addition, in all these trials the decision to move to Phase III was based on a clinical end point that was supported by biological data. However, the early results may have been misleading as a consequence of nonblinded randomization in some studies, potentially resulting in biases favoring vaccine-dosed cohorts, and the nondefinitive nature of the end points and statistics, such as underpowering, in these exploratory relatively small Phase II trials.

Sipuleucel-T was approved in April 2010 as a therapeutic vaccine for use in castrate-resistant prostate cancer. It is the first cancer vaccine to receive regulatory approval. The pivotal Phase III trial leading to its approval included 512 patients with 2 : 1 randomization ratio and demonstrated a 22% reduction in the risk of death compared with the placebo group ($P = .03$), a 4.1-month improvement in OS (25.8 months in the sipuleucel-T group vs 21.7 months in the placebo group), and a 36-month probability of survival of 31.7% vs 23%. Overall, while statistically significant, the clinical benefit was marginal. The major toxic effects are chills, fever, and headache.[134] This vaccine is manufactured by Dendreon, a Seattle, Washington–based company. This is an autologous approach in which patients undergo three apheresis procedures to obtain sufficient numbers of APCs that are then cultured and incubated with the PAP-GM-CSF fusion protein and subsequently reinfused intravenously into the patient every 2 weeks. Another Phase III trial, called PROTECT, is evaluating the efficacy of sipuleucel-T in patients with prostate cancer with increasing PSA levels after initial local surgery.

The MAGE-A3 vaccine is a recombinant protein vaccine admixed with a proprietary adjuvant. This vaccine has been developed by GlaxoSmithKline. MAGE-A3 is a cancer-testis antigen that is overexpressed in a number of cancers

TABLE 13.9 Phase II Results Do Not Always Predict Phase III Results

Vaccine	Platform Type	Sponsoring Organization	Target Antigen	Tumor Type	Phase II Results	Progression-Free Survival Improvement[a]	Overall Survival Improvement[a]	Stage of Development
Sipuleucel-T	Autologous cellular[134]	Dendreon	Prostatic acid phosphatase	Prostate cancer, castrate resistant	Increased OS by 4 mo (stat sig)—3 trials	2.4 mo in median	4.5 mo in median[b] (25.9 vs 21.4 mo)	FDA approved
Prostvac	Poxvirus[190]	Bavarian Nordic	Prostate-specific antigen	Prostate cancer, castrate resistant	Increased OS by 8.5 mo ($P = .0061$); $n = 82$	Not different	8.5 mo in median[b] (25.1 vs 16.6 mo)	Phase II complete
MAGE-A3	Adjuvanted protein[248]	GlaxoSmithKline	MAGE-A3	NSCLC (stages IB and II)	Increased HR, 66 ($P = .12$); $n = 182$	26% reduction in HR (DFS)	33% reduction in HR	Phase III negative
BLP25	Peptide and immune modulator[156]	EMD Serono	MUC1	NSCLC (stages IIIB and IV)	Increased HR, 73 ($P = .112$); $n = 163$	Not described	4.4 mo in median (17.4 vs 13 mo; greater in IIIB locoregional group)	Phase III negative
HPV-SLP	Long peptide[154]	ISA Pharmaceuticals	HPV-16 and HPV-18 E6 and E7	Vulvar intraepithelial neoplasia	Durable OR 15/20 with corresponding good immune response	Objective response, 79% at 12 mo (not randomized)	Not assessed	Phase III to start
BiovaxID	Autologous protein[164]	Biovest	Immunoglobulin idiotype	Follicular lymphoma	Reductions in MRD in small no. of patients	13.6 mo	Not assessed	Phase III negative
Rindopepimut	Peptide[170]	Celldex	EGFRvIII	Glioblastoma	Promising OS for glioblastoma (21.8 mo) Improved PFS and OS in combination with bevacizumab	Not described	3.2 mo	Phase III negative

DFS, disease-free survival; EGFR, epidermal growth factor receptor; FDA, U.S. Food and Drug Administration; HPV, human papillomavirus; HR, hazard ratio; MAGE, melanoma-associated gene; MRD, minimal residual disease; NSCLC, non–small-cell lung cancer; OR, objective response; OS, overall survival; PFS, progression-free survival; SLP, synthetic long peptide.
[a]Over survival for concurrent control subjects.
[b]Statistically significant.

(melanoma and NSCLC, bladder, head and neck, esophageal, and hepatocellular cancers), but is not expressed appreciably in any normal tissues other than testis and placenta. Thus, a vaccine directed to this antigen should be safe and free of autoimmunity. It is expressed in approximately 35% of patients with NSCLC and in most patients with melanoma. GlaxoSmithKline completed a double-blind, placebo-controlled, randomized Phase II trial in early stage (IB, II) NSCLC. In the trial, 182 patients were treated. The vaccine was well tolerated with mostly injection site–related side effects. An interim Cox regression analysis showed a 33% increased DFS for the group receiving the vaccine (hazard ratio [HR], 0.66; $P = .12$).[157] Based on these results, GlaxoSmithKline initiated a double-blind, placebo-controlled, randomized Phase III trial involving 2300 patients worldwide with stage IB, II, or IIIA NSCLC who had undergone resection and adjuvant chemotherapy, if indicated. However, while some of the biomarker based evaluations of clinical outcomes have been promising,[158] the trial failed to meet its primary end point. There is still a possibility that biomarker identified patient subpopulations will benefit from this approach.

Stimuvax, or L-BLP25, is a vaccine based on a 25-amino-acid repeat found in the broadly expressed tumor antigen MUC1. MUC1 is overexpressed and aberrantly glycosylated in NSCLC. MUC1 is also overexpressed in a variety of other malignancies, including breast, pancreatic, and ovarian cancer. The vaccine is composed of the 25-amino-acid lipopeptide, the adjuvant monophosphoryl lipid A, and three lipids formed into liposomes. The vaccine is administered subcutaneously repetitively, but is preceded by an injection of low-dose cyclophosphamide 3 days before vaccination to reduce Tregs. A Phase IIB trial that included 163 patients randomized to the vaccine or best supportive care was performed. The HR was 0.739 in favor of vaccine-treated patients ($P = .112$).[156] The greatest clinical effect was seen in patients with locoregional stage IIIB disease in whom the HR was 0.524 with a P value of .069. There was no significant toxicity observed. Based on these results, EMD Serono initiated an international randomized Phase III trial comparing L-BLP25 with placebo in patients with stage III NSCLC. However, the vaccine recently failed to achieve its primary end point in this pivotal trial,[203] although patients with earlier-stage disease had better outcomes.

Prostvac-VF is a poxvirus-based vaccine that is being developed for men with castrate-resistant prostate cancer. The vaccine consists of a *Vaccinia* virus and a fowlpox virus, each encoding the prostate TAA, PSA, and the TRICOM costimulatory complex that includes B7.1, LFA-3, and ICAM-1. The TRICOM complex increases the immunogenicity of the vaccine and induces antigen-specific T cells that have higher avidity for the antigen they recognize. The *Vaccinia* vector is used once for priming, and the fowlpox vector is administered repetitively at 2- or 4-week intervals for six administrations. The vaccines are each administered with GM-CSF injected locally. The randomized Phase II trial included 82 patients randomized in a 2:1 ratio to vaccine or placebo injections.[190,191] In this trial, the primary end point was PFS, and there was no difference in this end point between the two arms. However, at 3 years after the study, the Prostvac-VF group had a 25.1-month OS compared with a 16.6-month OS in the placebo group ($P = .0061$). A Phase III trial to confirm these results is being initiated, cosponsored by Bavarian Nordic and BMS, and is ongoing. The results of this trial will take several years, but Prostvac-VF could have several advantages over sipuleucel-T, now approved in the same patient population. Prostvac-VF is not a personalized vaccine and will have manufacturing and likely cost advantages over sipuleucel-T. The sponsor also started Phase II trials combining this vaccine with ipilimumab (CTLA-4 blocking antibody) and enzalutamide (androgen receptor antagonist).

ISA Pharmaceuticals is developing a novel peptide vaccine to the HPV associated tumors. HPV is responsible for a number of genital diseases (genital warts and cervical and vulvar intraepithelial neoplasia) and several cancers, including cervical cancer and some head and neck cancers. The E6 and E7 proteins from certain HPV subtypes are thought to be oncogenic and are often retained in proliferating cancer cells. Panels of SLPs to the HPV E6 and E7 proteins have been tested in patients with HPV-related cellular dysplasia. A high frequency of CD4+ and CD8+ immune responses was documented, and durable regressions in 15 of 20 patients with vulvar intraepithelial neoplasia were seen, including nine complete responses.[154] Patients with the best clinical responses tended to have the best cellular immune responses. Phase III trials with this vaccine are underway.

Rindopepimut (CellDex) is an investigational immunotherapy that targets the tumor-specific oncogene EGFRvIII, a functional and permanently activating mutation of the EGFR, a protein that has been well validated as a target for cancer therapy.[170,219] Rindopepimut is administered via intradermal injection and consists of the EGFRvIII-specific peptide sequence conjugated to the carrier protein KLH. Expression of EGFRvIII correlates with increased tumorigenicity in mouse models and poor long-term survival in clinical studies of glioblastoma patients. EGFRvIII expression may also be associated with tumor stem cells that have been identified in glioblastoma multiformae (GBM). These stem cells contribute to resistance to cytotoxic therapy and tumor recurrence. EGFRvIII is expressed in tumors in approximately 30% of glioblastoma patients. It has not been detected at a significant level in normal tissues; therefore, targeting of this tumor-specific molecule is not likely to impact healthy tissues.

Eighty-five percent of patients in clinical trials evaluating rindopepimut developed significant anti-EGFRvIII antibody titers, which increased with time on study. The majority (67%) of these patients developed titers above 1:12,800. Such immune responses may contribute to the direct destruction of tumor cells expressing EGFRvIII.

Three Phase II trials of rindopepimut have been completed in newly diagnosed EGFRvIII-positive glioblastoma patients with consistent results—ACTIVATE, ACT II, ACT III—and multiple patients continue to be followed for survival. Across all three studies, rindopepimut was generally well tolerated with generation of robust, specific, and durable immune responses. Results from a Phase II study of rindopepimut plus temozolomide in newly diagnosed patients postresection, demonstrated an increase of PFS.[170] However, a recently concluded Phase III randomized trial failed to confirm clinical benefit with temozolomide in patients with newly diagnosed glioblastoma. Rindopepimut and standard adjuvant temozolomide chemotherapy were administered to 65 patients with newly diagnosed EGFRvIII-expressing (EGFRvIII+) glioblastoma after gross total resection and chemoradiation. PFS at 5.5 months (~8.5 months from diagnosis) was 66%. Relative to study entry, median OS was 21.8 months, and 36-month OS was 26%. Extended rindopepimut vaccination (up to 3.5+ years) was well tolerated. Another study evaluating rindopepimut plus bevacizumab versus a control vaccine plus bevacizumab showed encouraging results[219]: for rindopepimut + bevacizumab versus KLH + bevacizumab: progression-free survival at 6 months = 27% (9 of 33) vs 11% (4 of 35); overall response rate (ORR) = 24% (7 of 29) vs 17% (5 of 30). Median OS was also increased: 12 versus 8.8 months in the control group that received bevacizumab alone. Rindopepimut induced robust anti-EGFRvIII antibody titers in 80% of patients. Rapid antibody responses were associated with prolonged OS.

CANCER VACCINES HAVE SUBSTANTIAL POTENTIAL BUT NEED FURTHER OPTIMIZATION AND REPOSITIONING WITHIN THE THERAPEUTIC ARMAMENTARIUM AGAINST CANCER

There have been a number of positive advances in the development of therapeutic vaccines for cancer. Highly specific TAAs have been identified, many of which are immunogenic and capable of inducing immune responses in healthy people and in patients with cancer. With some antigens, there is even evidence of existing antibody or T-cell immune responses that may be further enhanced with vaccination approaches. In addition, a number of promising platforms to deliver tumor antigens to the immune system have been developed with induction of CD4, CD8, or broad CD4 and CD8 immune responses. In addition some vaccines were able to polarize the immune system toward the Th1/CD8 response. T-cell responses approaching those seen in response to viral infections are being generated in some, but not all, patients with some vaccine platforms.

The most promising vaccine technologies and approaches include the following:

- Heterologous prime–boost approaches usually using DNA or viral vectors as priming agents, and distinct viral vectors or recombinant proteins as boosters.[220]
- Long peptides adjuvanted with TLR agonists targeting non-self antigens.[221]
- DC transduced with tumor-derived RNA[222] or other individualized vaccines corresponding to the tumor mutanome, a collection of nonsynonymous mutations in the genome of cancerous cells, corresponding to immunogenic epitopes.[223]
- RNA-based or adjuvanted protein or polypeptide vaccines delivered to APCs within lymphoid organs.[224,225]
- Genetically engineered DCs rendered more potent immunogens than endogenous DCs.[226,227]
- Combinations between vaccines and immune-enhancing agents or checkpoint inhibitors to increase induction of immune effector cells and enable their activity within the tumor environment.

There are opportunities to improve the magnitude and the quality of the response in most patients receiving these vaccines. Strategies to overcome immune-inhibitory pathways induced by the cancer must also be addressed and overcome. These advances, coupled to the improved understanding of the molecular requirements for T-cell activation and the importance of modulating the regulatory T-cell response, are providing rational strategies to enable the T cells primed with therapeutic cancer vaccines to mediate antitumor clinical responses.

Based on developments to date and lessons learned from many vaccines failures, here are some future directions for the research and translation of therapeutic cancer vaccines:

- Understanding the intrinsic limitations of vaccines as single therapy agents, namely the suboptimal capability to elicit clinically efficacious T cells in bulky disease, is a compelling rationale to reposition them. One scenario is to utilize them as single agents in an adjuvant setting, minimal residual disease, or in situ tumors, while considering integrative approaches in later-stage disease, according to the schema described in Fig. 13.4.

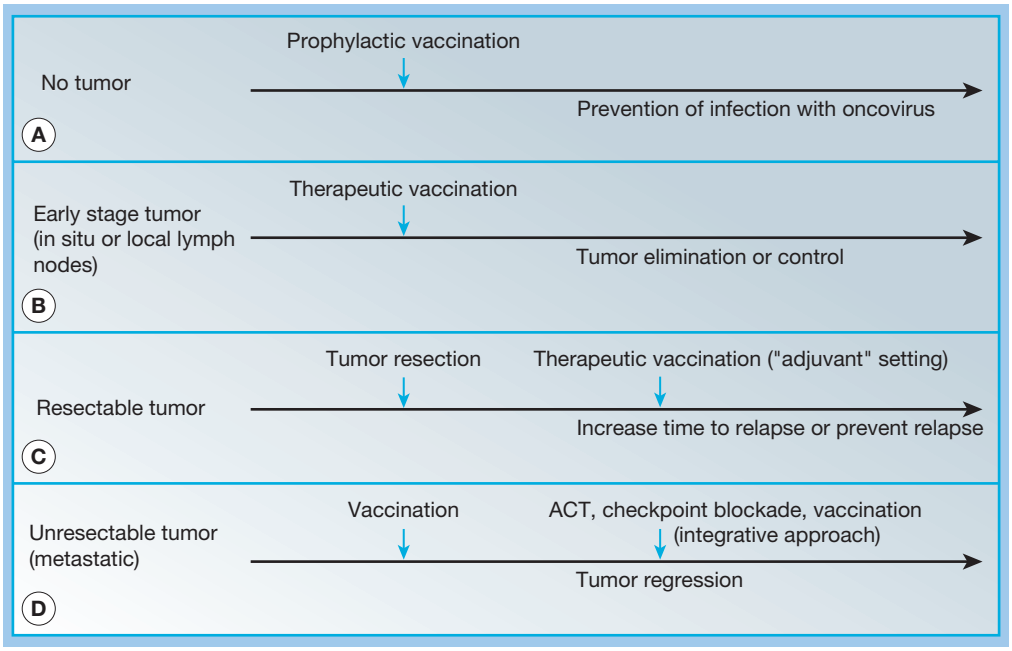


Figure 13.4. Disease stage–dependent applicability of cancer vaccines. Different scenarios where vaccination can be applied to cancer, depending on the disease stage. **A,** Prophylactic vaccination against viruses with oncogenic potential, such as HBV and HPV, could have an indirect but drastic impact on prevalence of liver cancer and cervical or oropharyngeal carcinoma. **B,** Instances where therapeutic vaccination could have an effect by itself are in early stage tumors confined to primary lesions (e.g., CIN, or melanoma with disease confined to locoregional nodes). **C,** The case of resectable tumors, therapeutic vaccination in adjuvant setting could be helpful in eliminating residual cancerous cells. This setting presents an opportunity to generate patient specific epitopes, in a truly individualized manner. **D,** In the case of unresectable, advanced tumors, vaccination could help increase the number of tumor-infiltrating lymphocytes, thus rendering the disease more susceptible to other therapeutic modalities, such as checkpoint blockade. Alternatively, or in addition, vaccines could be used in conjunction with other therapeutic modalities, such as ACT, checkpoint blockade, or other targeted therapies. ACT, adoptive T-cell therapy; CIN, cervical intraepithelial neoplasia; HBV, hepatitis B virus; HPV, human papillomavirus.

- Further optimize for potency and prioritize platform technologies for vaccination developed to date as a joint effort between academia and industry.
- Expand the realm of targets: non-self and neoantigens-viral, immunogenic point mutations, translocations, fusions, alternate reading frames, essentially those categories that correspond to a preserved or functional immune repertoire.
- Biomarker-guided patient stratification and streamlining of clinical development of therapeutic vaccines against cancer.

ENHANCING VACCINE ACTIVITY BY COMBINATION WITH IMMUNE MODULATORS OR OTHER IMMUNOTHERAPIES

During the last several years, Phase III clinical trials using the different vaccine platforms and antigens described have been completed, as described above. Table 13.10 summarizes the results of some of the major trials that have been completed but have not demonstrated evidence of clinical efficacy. As discussed, there are multiple possible reasons for the failure of these trials. These include quality or potency of vaccines; clinical trial design issues, including patient population and extent of disease or number of previous treatments; failure to address cancer-related immune inhibition, and as discussed in the section "All Hurdles Facing Cancer Vaccines Need to be Systematically Addressed," misleading clinical results from small Phase II clinical trials.

As outlined in the section "All Hurdles Facing Cancer Vaccines Need to be Systematically Addressed," effective functional immune responses are limited by various immune-inhibitory pathways in patients with cancer. The major roadblocks are related to suboptimal APC activation, induction of T-cell anergy, induction of T-cell exhaustion, inhibitory cytokines, and induction of Tregs and other inhibitory cells. These pathways may act locally at the tumor site or systemically. The dominant inhibitory pathways may vary from tumor to tumor, stage to stage, and patient to patient. These variations need to be determined for different cancers and different clinical settings.

Clinical strategies could reverse these immune-inhibitory pathways or at least the dominant pathway in a particular clinical setting. This approach would enhance the functionality of T-cell responses induced by cancer vaccination approaches. In the future, it may be possible to obtain patient-specific biomarker profiles of dominant immune-inhibitory pathways to tailor patient-specific immunostimulatory strategies.

A number of immune modulatory treatments are being tested in the clinic and in preclinical models. The National Cancer Institute conducted a workshop to identify and rank the most promising experimental immune modulatory reagents for cancer, such as cytokines and checkpoint blocking agents, that are available for early clinical testing, along with ranking out the most promising antigens.[228,229]

Thus, future cancer vaccine approaches will likely involve a combination of vaccine and immunoadjuvants, immune modulators, and even cell therapies. Vaccines will likely be a critical component of these combinatorial immunotherapies. Agents to optimally activate APCs in combination with the vaccine include TLR agonists, CD40 stimulators, and cytokine combinations. In addition, reagents to reduce Tregs, reverse negative signaling (anti–CTLA-4), or reverse T-cell anergy (anti–PD-1 [programmed death-1]) are examples of immune modulators that are being considered. These approaches, together with optimal patient selection, will be critical for the success of future cancer vaccine approaches.

Vaccines and Checkpoint Inhibitors

Whereas various cancer vaccines may be able to prime immune responses specifically to a cancer, the multiple immune inhibitory mechanisms that cancers may use to avoid immune elimination may prevent meaningful clinical responses, particularly in more advanced disease where these inhibitory mechanisms are even more active. As discussed previously in this chapter, checkpoint inhibition either through CTLA-4– or PD-1–mediated T-cell signaling are important immune inhibitory mechanisms. These pathways can now be addressed and reversed with the checkpoint inhibitor monoclonal antibodies that are under clinical development or approved by the FDA. In summary, both the anti–CTLA-4 monoclonal ipilimumab and the anti-PD-1 monoclonal antibodies pembrolizumab and nivolumab have shown objective tumor responses in patients with metastatic melanoma and lung cancer and have been approved for clinical use for these indications.[230–232] They represent strong candidates for vaccine combination studies.

These checkpoint antibodies work best in tumors where there is some initial evidence of an immune response such as increased numbers of tumor infiltrating lymphocytes and evidence of granzyme and or interferon production. In such situations there is often evidence of increased levels of PDL1 expression on the tumor cells or local APCs. It is postulated that these preexisting T-cell responses are directed against neoantigens generated through tumorigenesis, hence a correlation between mutational load and response to the checkpoints inhibitors. These findings lead to the hypothesis that a therapeutic cancer vaccine and checkpoint inhibitor combination would be an effective therapeutic combination.

Several early phase clinical trials have been performed to assess such immunotherapeutic combinations. In an early trial, ipilimumab was administered after vaccination with the GM-CSF cellular vaccine GVAX.[233] Eleven patients with stage 4 melanoma were treated with ipilimumab infusions every 2 or 3 months starting from 1 to 4 months after GVAX treatment. There were no grade 3 or 4 toxicities. A relationship between therapy induced necrosis and the ratio of intratumoral CD8+/FOXP3+ T cells was discovered, speaking to the detrimental role of Treg cells within the tumor environment. Nine patients with stage 4 ovarian cancer were also similarly evaluated from 1 month to 3 years after treatment. Two cases of grade 3 gastrointestinal toxicities were experienced. Some clinical responses were seen in these patients treated with this combination strategy A similar approach was studied in 30 patients with previously treated pancreatic ductal carcinoma. Patients received ipilimumab every 3 weeks × 4 and then every 3 months alone or with GVAX.[234] Two patients receiving ipilimumab alone had stable disease whereas three patients in the combination treatment group had stable disease. Interestingly, 7 of 15 patients had declines in CA19–9 in the combination arm while no declines were seen in the ipilimumab-alone arm. There was a nonstatistically significant trend toward improved survival in the combination treatment arm. Toxicities were comparable in the two arms.

A Phase III trial of ipilimumab alone, gp100 peptide vaccine alone, or gp100 peptide vaccine and ipilimumab combination was completed in 676 patients with metastatic melanoma[230] that was described in detail in an earlier section of this chapter.

Thirty patients with castration-resistant prostate cancer were treated with the *Vaccinia*-based vaccine Prostvac with escalating doses of ipilimumab. Of the 30 patients, 58% had a decline in PSA.[235] Associations between OS and certain immune cell subsets either before or after therapy were found. There were trends of association between longer OS and certain immune cell subsets before immunotherapy: lower

TABLE 13.10 Summary of Major Phase III Trials That Have Not Shown Clinical Efficacy

Vaccine/Producer	Platform	Antigen	Previous Randomized Phase II?	Disease	Stage	Monotherapy	Immunomodulation
Canvaxin,[249] CancerVax	Cellular	Allogeneic melanoma cell lines		Melanoma	Metastatic; adjuvant	Yes	BCG
Vaccinia melanoma oncolysate[106]	Lysates	Vaccinia and melanoma cell line antigens		Melanoma	II	Yes	BCG
Melacine Ribi[250]	Cellular	Allogeneic melanoma cell lines		Melanoma	Metastatic; adjuvant	Yes	No
OncoVAX, Intracel[251]	Cellular (lysate)	Autologous		Colorectal	II and III	Yes	No
THERATOPE, Biomira[252]	CHO	STn carbohydrate	No	Breast	Metastatic	Yes	Cyclophosphamide
Gastrimmune, Aphton[253]	Peptide-DT conjugate	Gastrin	No	Pancreatic	Metastatic		
Oncophage Antigenics[168]	Protein (heat shock proteins)	Autologous tumor antigens		Renal, melanoma	Renal, nonmetastatic; melanoma, metastatic		
PANVAC, Therion[254]	Viral (poxvirus)	CEA and MUC1	No	Pancreatic	Metastatic	Yes	No
MyVax, Genitope[255]	Protein (anti- idiotype KLH)	Autologous idiotype	No	Pancreatic	Advanced	Yes	No
FavId, Favrille[255]	Protein (anti- idiotype KLH)	Autologous idiotype	No	Non-Hodgkin lymphoma	Advanced	After rituximab	GM-CSF
GVAX, Cell Genesys[256] (autologous)	Cellular (modified with GM-CSF)	Autologous	No	Prostate	Advanced	Yes	No
GVAX, Cell Genesys[256] (allogenic)	Cellular (modified with GM-CSF)	Prostate cell lines	No	Prostate	Advanced	With docetaxel (Taxotere)	No
GMK, Progenics[257]	CHO	GM_2 ganglioside		Melanoma	Adjuvant	Yes	No
TroVax, Oxford BioMedica[34]	Poxvirus	5T4	No	Renal	Advanced	No	No
MAGE-A3 GSK[258]	Protein	MAGE-A3	Yes	Lung	Postsurgery Ib, II, IIIa	Yes	AS15 adjuvant
Stimuvax EMD Serono[203]	Protein	MUC1 (BLP-25)	Yes	Lung	IIb, III	Yes	Cyclophosphamide
Rintega, Celldex (Rindopepimut)[170]	Peptide conjugated to KLH	EGFRvIII	Yes	Glioblastoma multiformae	Recently diagosed	Temozolomide	GM-CSF

BCG, bacille Calmette-Guérin; CEA, carcinoembryonic antigen; CHO, carbohydrate; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSK, GlaxoSmithKline; KLH, keyhole limpet hemocyanin; MAGE, melanoma-associated gene.

PD-1+ Tim-3+ Th cells, higher PD-1− Tim-3+ CTL, and a higher number of CTLA-4− Tregs. They also found that an increase in Tim-3+ NK cells postvaccination versus prevaccination was associated with longer OS.

Based on the scientific rationale and evidence to date, the next steps would be to test combinations of vaccines and licensed checkpoint-blocking agents. Notably, a randomized Phase II trial of combining sipuleucel-T with immediate versus delayed CTLA-4 blockade for prostate cancer has been initiated and is currently accruing (NCT01804465). This is an open-label, randomized, multicenter trial in patients with chemotherapy-naïve metastatic castration-resistant prostate cancer. All patients will be treated with standard vaccination with sipuleucel-T (q2wk × 3). Then, patients will be randomized to one of two arms: an immediate treatment arm comprising ipilimumab q3wk × 4 started 1 day following the final dose of vaccine or a delayed treatment arm comprising ipilimumab q3wk × 4 started 3 weeks following the last dose of vaccine. Following this ipilimumab treatment, patients will then be followed monthly for 3 months and then quarterly until disease progression.

A Phase Ib trial with the herpes virus oncolytic vaccine T-VEC and ipilimumab has been reported in 19 patients with stage IIIb or stage IV melanoma.[236] Grade 3/4 toxicities occurred in six (32%) of the patients. Two patients experienced grade 3/4 autoimmune toxicities commonly seen in patients with ipilimumab, consisting of colitis, hypophysitis, adrenal insufficiency, and elevated amylase and lipase. The objective response rate was 56%, including 33% complete responses. This objective response rate is higher than the objective response rate seen with T-VEC alone (16%) from a historical control trial. There was an increase in CD8+ T cells seen in tumor sites after combination therapy. The results of the randomized trial leading to regulatory approval of T-VEC have been published in the *Journal of Clinical Oncology*[151]: among 436 patients randomly assigned, ORR was significantly higher with T-VEC (16.3%; 95% confidence interval [CI], 12.1% to 20.5%) than GM-CSF (2.1%; 95% CI, 0% to 4.5%; odds ratio, 8.9; $P < .001$). ORR was also higher in the T-VEC arm (26.4%; 95% CI, 21.4% to 31.5% vs 5.7%; 95% CI, 1.9% to 9.5%). Median OS was 23.3 months (95% CI, 19.5 to 29.6 months) with T-VEC and 18.9 months (95% CI, 16.0 to 23.7 months) with GM-CSF (HR, 0.79; 95% CI, 0.62 to 1.00; $P = .051$). T-VEC efficacy was most pronounced in patients with stage IIIB, IIIC, or IVM1a disease and in patients with treatment-naïve disease. These results build a momentum behind combination approaches to further elevate the clinical response rate and durability of clinical benefit. A randomized trial of T-VEC with pembrolizumab is now underway for patients with advanced melanoma.

Some of these trials have been, or are being performed with, anti-CTLA-4 combinations. Given the improved toxicity and clinical activity of the anti–PD-1s and anti-PDL1s it is likely that combination studies with such agents are of increased interest.

Nivolumab, a human IgG_4-blocking antibody against the T-cell PD-1 checkpoint protein, has activity against metastatic melanoma and has been tested in combination with peptide vaccines in melanoma patients. In a Phase I study,[237,238] patients with unresectable stage III or IV melanoma who were ipilimumab naïve and had experienced progression after at least one prior therapy or experienced progression after prior ipilimumab received nivolumab at 1, 3, or 10 mg/kg every 2 weeks for 24 weeks, then every 12 weeks for up to 2 years, with or without a multipeptide vaccine against well characterized antigens such as MART-1 (melanoma antigen recognized by T cell-1) and NYESO-1. While the combination of nivolumab with vaccine was well tolerated and safe at all doses, the objective response rate for both ipilimumab-refractory and ipilimumab-naïve patients was 25%.

The same group investigated nivolumab plus peptide vaccine as adjuvant therapy in resected stage IIIC and stage IV melanoma patients.[239] HLA-A*0201–positive patients with HMB-45–, NYESO-1–, and/or MART-1–positive resected tumors received nivolumab (1 mg/kg, 3 mg/kg, or 10 mg/kg IV) with a multipeptide vaccine (gp100, MART-1, and NYESO-1 with Montanide ISA 51 VG) every 2 weeks for 12 doses followed by nivolumab maintenance every 12 weeks for eight doses. Thirty-three patients were enrolled. Nivolumab with vaccine was well tolerated as adjuvant therapy and demonstrated immunologic activity with improved survival in patients with high-risk resected melanoma. More specifically, the estimated median relapse-free survival was 47.1 months. Increases of tetramer-specific CD8+ T-cell populations were observed with treatment. These results could support future randomized trials.

Another trial evaluates a *Listeria*-based vaccine (ADXS31–142) alone and in combination with pembrolizumab (MK-3475) in patients with prostate cancer (NCT02325557). ADS31–142 is aimed to elicit an immune response against PSA. Part A will be dose-determining of ADXS31–142 monotherapy and part B will be dose-determining of ADXS31–142 and pembrolizumab (MK-3475) in combination.

As this field is expanding rapidly, combining checkpoint inhibitors with new-generation vaccines that can generate potent and broad tumor-specific immune responses should be an increasingly effective strategy.

Vaccines and Cellular Therapy

Among the promising immunotherapies, cell therapies represent one of the most exciting opportunities as they are applicable to advanced cancer. The last decade witnessed a considerable progress in discovery and development of methods to regenerate the immune repertoire in patients with advanced cancer. Adoptive T-cell therapy has shown striking tumor regressions in the clinic. The major types of cell therapies include (a) tumor-infiltrating lymphocytes (TILs) retrieved from tumor lesions and expanded ex vivo, outside the confinement of an immune-inhibiting environment; (b) TCR-engineered T cells; and (c) chimeric antigen receptor–engineered T cells, respectively, that integrate a novel receptor specificity onto an existing T-cell repertoire.[240,241]

Whichever methodology is used for T-cell repertoire regeneration/reshaping (TILs) or reengineering (chimeric antigen receptors and TCRs), an incoming T-cell product still faces a considerably hostile environment within the tumor environment. There is reduced or lack of antigen expressed by professional APCs otherwise needed to effectively stimulate the cells toward achieving optimal in vivo activation, expansion, differentiation, and migration to tumor sites. In addition, there is a generally immunosuppressive environment as a result of preexisting immune-inhibiting cells such as Tregs and MDSCs. The infused T cells must compete with endogenous T cells for vital growth factors such as IL-15 and IL-7. And, finally, as discussed above, the infused cells still are modulated by a plethora of immune-inhibiting mechanisms that act in concert within the tumor environment per se, including those reactively deployed by immunologically active T cells as part of natural immune homeostasis such as PD-1–PDL1 axis. While reactivation of adoptively transferred T cells is not expected to overcome all these barriers, it would render the cells more numerous and potent through increasing their capability to mediate tumor cell killing.

Thus it is very compelling to consider an integrative approach through which methods to regenerate, reshape, or

reengineer the T-cell repertoire are accompanied by cognate vaccines designed to fully tap into the potential of adoptive therapy in patients with advanced cancers (Fig. 13.5). Under one possible scenario, tumor specimen analysis for TAA expression could lead to selection of one or multiple common target antigens to be pursued through adoptive transfer of cognate TCR-engineered T cells, followed by vaccination leading to effective in vivo stimulation of the adoptively transferred T cells. Such vaccination might be done with a live microbial vector (pox virus vector, attenuated *Listeria*, etc) expressing the antigen of interest, with DCs adequately expanded and differentiated ex vivo, pulsed or genetically engineered to present the cognate epitope(s), or with adjuvanted recombinant proteins.

Another scenario is represented by truly individualized approaches, especially when generic cancer target antigen expression is modest or lacking. Advances in gene sequencing (exome analysis, RNA sequencing) afford rapid sequencing of tumor material. Through bioinformatics tools, one can predict, prioritize and select sequences that encompass putatively immunogenic epitopes and use them to assemble minigenes[242,243] that could be effectively integrated into a given vaccine platform such as DC or live virus–based vaccines. The area of neoantigen based vaccination is rapidly evolving based on progress in understanding the true nature and specificity of TILs and the potential for using tumor-specific and patient-specific epitopes as vaccines. Recent studies raise the possibility that the "mutanome," the totality of nonsynonymous tumor-specific mutations, comprise a large percentage of potentially or actually immunogenic epitopes, especially recognizable by CD4+ T cells.[244] Surprisingly, elements of the MHC class II–restricted mutanome were potently immunogenic and protective against tumor, deploying epitope-spreading mechanisms. These were paralleled by coinduction of MHC class I–restricted immunity, resulting in a broad-based T-cell response against the tumor. While this analysis is more advanced in preclinical models, it raises the intriguing possibility that the mutanome is shaping the immune response away from MHC class I–restricted epitopes and that sequencing coupled with bioinformatics approach and a feasible vaccine platform, such as an RNA-based technology, could pave the way toward an individualized vaccination strategy in cancer.

As identification of neoantigens in each patient is now possible owing to exome sequencing or related approaches, construction of patient-specific vaccines is becoming feasible, and has a higher chance of working when combined with PD-1 blockade and/or T-cell therapy. This approach is now

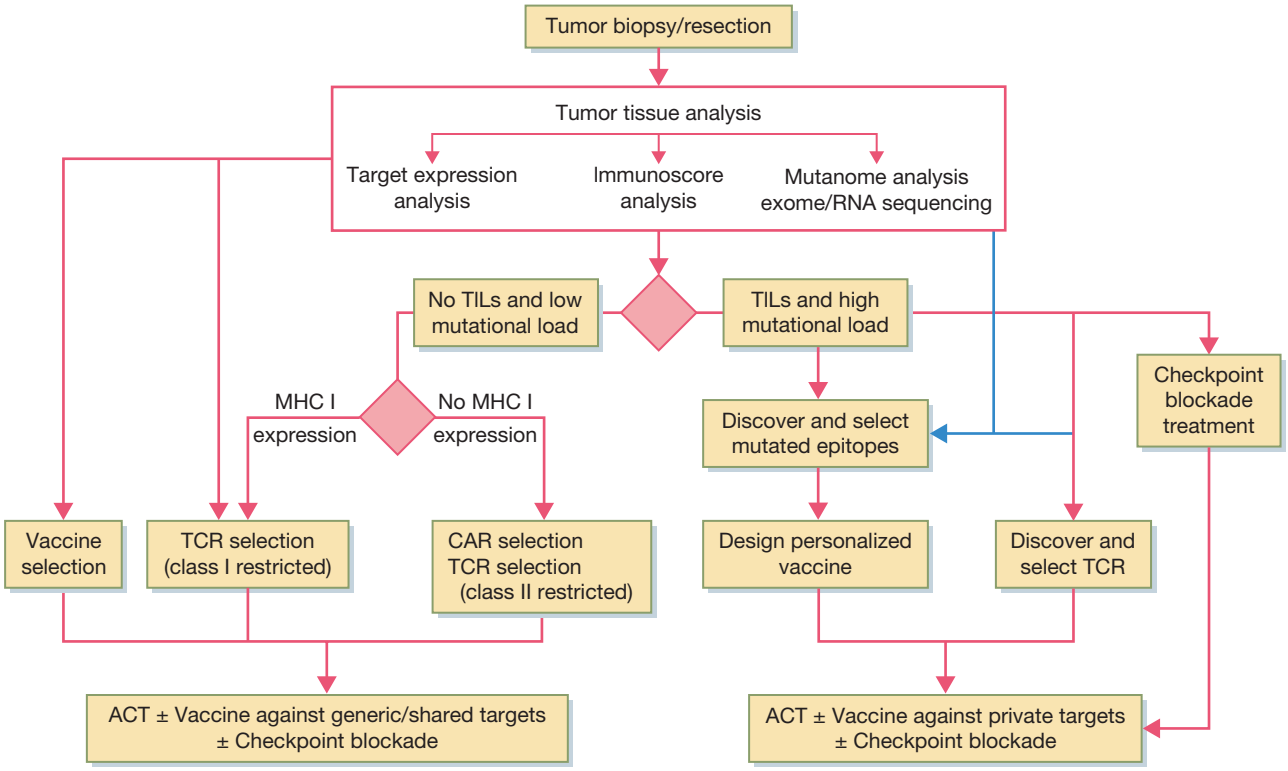


Figure 13.5. An integrative perspective combining vaccination with other therapeutic modalities to achieve durable control of cancer. Novel techniques can be applied to tumor tissue to define the mutanome (the totality of nonsynonymous mutations) by exome sequencing or RNA sequencing, in conjunction with the immunoscore represented by the degree of tumor infiltration with T cells, evaluated by immunohistochemistry. Tumors with high mutational load, which are quite frequently associated with increased TIL infiltrates, would be prime candidates for checkpoint blockade therapy such as CTLA-4 and PD-1 binding antibodies. This category of tumors is also conducive to discovering patient and tumor specific epitopes, cognate TCRs, and/or TILs, resulting in a tool box of highly individualized medicine that could be used as backup or in conjunction with checkpoint blockade to achieve a more durable control of disease. In contrast, tumors with low mutational load would be candidates for immunotherapies directed against generic antigens, prioritized based on tumor antigen expression analysis. Such immunotherapies could comprise TCR or CAR-engineered T cells, as well as vaccines against such generic targets, which could be used in conjunction or separately. ACT, adoptive T-cell therapy; CAR, chimeric antigen receptor; MHC, major histocompatibility; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

pursued by novel companies such as Neon Therapeutics. Thus, two novel investigator-sponsored clinical studies, in melanoma and glioma, are testing patient-specific neoantigens in the form of peptides, adjuvanted with the TLR agonist, stabilized polyI:C (clinical trials NCT01970358; NCT02287428).

Similarly, another novel company BioNTech AG is advancing an approach based on mRNA-expressing neoantigens directly injected into lymph nodes. They have begun enrolment in RNA-based individualized vaccine trial for melanoma, glioblastoma (http://www.gapvac.eu/), pediatric acute lymphoblastic leukemia, hepatocellular carcinoma (http://www.hepavac.eu/), and triple-negative breast tumor (http://merit-consortium.eu/) patients.[245] Trials integrating all these therapeutic modalities—T-cell transfer, vaccination, and checkpoint blockade—are still awaited.

An interesting perspective tapping into the endogenous TAAs is afforded by the concept of "in situ" vaccination, demonstrated by the licensure of a herpes virus–based immune intervention (T-VEC) with the capability to effectively mobilize the systemic immunity against remote tumor lesions.[151] An optimization of this mechanism through use of potent and targeted immune modulatory compounds or approaches, could obviate the need to use conventional vaccines that engage tumor-specific epitopes.

Irrespectively of the generic or individualized nature of this approach, preclinical results to date strongly support the concept of integrating adoptive T-cell therapy with vaccination.[238,246,247] Individualized or generic vaccines could be used in conjunction with adoptive T-cell therapy with TILs or TCR-engineered T cells directed against one of the TIL cognate epitopes discovered through systematic evaluation of the intratumoral immune repertoire. In addition to substantially increasing the antitumoral response, vaccination could obviate the need to use preconditioning, a strategy that enables the expansion of adoptively transferred T cells' expansion by lymphodepleting the endogenous lymphocytes.

Finally, within this T-cell therapy/vaccination integrative paradigm, one could position vaccination in a variety of ways, either just before or shortly after adoptive T-cell therapy to elicit T-cell activation and expansion, or at various intervals after T-cell therapy (weeks, months, or even longer) to increase the persistence and prolong activity of residual antitumor T cells.

However, although more tools necessary to harness the immune system to fight cancer are becoming available, a number of significant challenges remain. We need to carefully combine cancer vaccines with new immune modulatory tools in well-thought-out strategies to generate specific functional T-cell responses. These combination therapies will need to be carefully optimized in early phase clinical trials. The field needs to continue to develop and validate immunologic biomarkers and use these to optimize new combination approaches. In addition, we must use the experience obtained during the last decade with vaccine trials and carefully select patient populations with the greatest likelihood of success, including patients in early stage adjuvant clinical trials for evaluation of vaccines alone, or combinatorial approaches for later stage disease.

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14 Cholera Vaccines

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HISTORY OF DISEASE

Cholera is a rapidly dehydrating, watery diarrheal disease caused by intestinal infection with the bacterium *Vibrio cholerae* serogroups O1 and O139. Cholera has probably existed on the Indian subcontinent for thousands of years. Ancient manuscripts describe the physical symptoms of what we now recognize as cholera gravis: rapid-onset vomiting, abdominal pain, explosive diarrhea, dehydration, and death. Cholera is one of the dreaded epidemic and pandemic diseases that have had the power to alter history. The disease has the unusual ability to rapidly infect large numbers of people, to spread internationally, and to kill a high proportion of those affected. Before the development of effective rehydration therapy with intravenous and oral fluids, cholera epidemics were associated with case-fatality rates that exceeded 40% and led to tens of thousands of deaths. John Snow is credited with understanding the importance of water as a key vehicle for the spread of the disease,[1] but it was not until the early 1880s that Robert Koch isolated and defined the causative agent from the fecal specimen of a patient with the disease.[2,3]

WHY THE DISEASE IS IMPORTANT

Cholera has been thought to exist on the Indian subcontinent since antiquity. From 1817 to 1923, six pandemics occurred worldwide. Earlier pandemics are known or presumed to have been caused by classical *V.* cholerae O1, while the seventh pandemic is ascribed to the El Tor biotype, which has replaced the classical biotype.[4] Disease caused by *V. cholerae* O1 or O139 continues to be a serious threat to human health, particularly in the developing countries of Asia, Africa, and, recently, the Caribbean. Although formerly it was a major problem in South and Central America, relatively few cases have been reported in this region since 1998.[5] Since the beginning of the seventh pandemic, cholera has become endemic in many parts of Africa, as well as in Haiti, causing large, recurrent, and deadly epidemics. In addition to the threat posed to residents of these regions, the disease is also a risk to travelers to these areas. Indeed, the cases of cholera reported to occur in developed countries are almost always acquired during foreign travel.[6,7]

CLINICAL DESCRIPTION AND COMPLICATIONS

Severe cholera, or cholera gravis, is characterized by acute diarrhea and, usually, vomiting, which lead rapidly (within 4 to 18 hours) to moderate or profound dehydration. Typically, a previously healthy person is suddenly stricken with watery diarrhea and copious vomiting. Ten or more voluminous stools may be passed within a few hours, at first liquid in consistency and then becoming like rice water. The complications from cholera arise from the loss of fluid volume and electrolytes, especially sodium, potassium, and bicarbonate, in the stool and vomitus. These losses can result in hypovolemia, metabolic acidosis, and potassium deficiency.[8] Secondary complications may arise from the hypovolemia or from inadequate or inappropriate fluid and electrolyte replacement. Such complications can include renal failure, hypokalemia, arterial occlusions (especially in the elderly), pulmonary edema, and, in pregnant women, premature delivery or abortion. Most patients experience a decrease in blood glucose levels during treatment, but some young children experience profound hypoglycemia and seizures.[9]

Not all patients with *V. cholerae* infections experience the severe cholera gravis syndrome. In fact, most infected people are asymptomatic or have only mild diarrhea. Past studies have estimated the case-to-infection ratio (number of symptomatic cases per number of people infected) to range from 1 in 3, to 1 in 100, depending on the geographic region, biotype, phase of epidemic, and size of inoculum.[10] The nature of the epidemic appears to affect the case-to-infection ratio, because explosive epidemics tend to have more severe cases, probably because higher inoculum sizes result from the large number of infected persons whose stools contaminate drinking water sources.

BACTERIOLOGY

Formerly, epidemic cholera was caused only by toxigenic strains of *V. cholerae* serogroup O1. However, in 1992, another serogroup, O139, emerged as a cause of epidemic cholera in Asia, with a clinical syndrome and epidemiologic spread identical to infections caused by serogroup O1.[11–13] This new O139 strain was derived from the El Tor biotype of O1 strains and was characterized by the presence of a capsule and a modified lipopolysaccharide (LPS).[14] Thus, there are two serogroups of *V. cholerae* that can cause epidemic cholera, O1 and O139. The new serogroup is especially important for vaccine development because immunity to serogroup O1 *Vibrio* does not confer immunity to serogroup O139.[15] Since its first recognition in Bangladesh and India, *V. cholerae* O139 has spread to other countries in Asia but not outside this region. The incidence of cholera caused by O139 strains waned after 1992, but in 2002 there was a resurgence of this serogroup in Bangladesh.[16] In the Indian subcontinent, the serogroup continues to be isolated, though rather uncommonly, with variations in the proportion of cases caused by this strain depending on the season and location.[17]

There are other serogroups of *V. cholerae* that can cause sporadic cases of diarrhea, or may cause local or systemic infections, but they do not cause epidemic disease. These are called non-O1–non-O139 *V. cholerae* (formerly called nonagglutinating [NAG] vibrios because they did not agglutinate with the O1 antiserum). Some strains of O1 or O139 *V. cholerae*, especially those found in the environment, do not produce cholera toxin. These also do not cause epidemic disease. Thus the epidemic strains always belong to either O1 or O139 serogroups and also produce cholera toxin. The serogroup O1 *V. cholerae* is subdivided into two serotypes based on specific antigens in the O antigen: Ogawa and Inaba. *V. cholerae* O1 is also divided into two biotypes based on biochemical reactions and other phenotypic features: classical and El Tor. More recently, genetic markers for the classical and El Tor strains have been identified. Classical strains, which appear to cause more severe disease than El Tor strains, have largely disappeared in recent years, with the last case detected in Bangladesh in the mid-1990s. The current seventh pandemic strain is an El Tor biotype, and it includes both Ogawa and Inaba serotypes.

Variants of El Tor strains that share phenotypic characteristics of both El Tor and classical biotypes were originally described in Bangladesh,[18] and they are termed *hybrid biotype strains*. Newer molecular markers have also revealed the emergence of cholera strains with phenotypic characteristics that classify them as El Tor, but that elaborate classical-type enterotoxin. These new strains, termed *El Tor variants,* have been found to have displaced typical El Tor cholera in Bangladesh since 2002. Subsequently, they have been found in Kolkata, India, and in Beira, Mozambique, where they have also displaced typical El Tor cholera, and elsewhere in Asia, in Africa, and most recently in Haiti.[19–23] It has been asserted that variant El Tor strains, like classical biotype strains, are associated with more severe disease than that caused by the earlier El Tor strains.[24]

Phage typing was formerly used to further classify strains, but molecular methods are now used.[25] Multilocus enzyme electrophoresis can distinguish between classical and El Tor strains[26] and has grouped the toxigenic El Tor biotype strains into four major clonal groups, or electrophoretic types (ETs), representing broad geographic areas. These include the Australian clone (ET1), the U.S. Gulf Coast clone (ET2), the seventh pandemic clone (ET3), and the Latin American clone (ET4).[27] In addition, a standardized ribotyping scheme for *V. cholerae* O1 and for O139 can distinguish seven different ribotypes.[28,29] It has also been proposed that the biotyping of El Tor be refined to account for the newly emergent hybrid biotype and El Tor variant strains.[30] Recently, using advanced genomic sequencing, it has been possible to study in detail the molecular evolution and global spread of cholera in at least three distinct waves during the current seventh pandemic.[31] Although invaluable for molecular epidemiology, these methods and improved classifications are not necessary in the clinical laboratory.

PATHOGENESIS AS IT RELATES TO PREVENTION

Crucial steps in the pathogenesis of cholera include colonization of the small intestinal mucosa and the elaboration of the enterotoxin (cholera toxin [CT]) (Fig. 14.1). Colonization depends on production of pilus structures such as the toxin-coregulated pilus (TCP).[32] Bacterial motility allows the bacteria to penetrate the mucous lining of the mucosa and come into close association with the epithelium. Colonization of the small intestine depends on three critical virulence factors: TCP, hemagglutinin/protease, and the bacterium's single flagellum.[33–35] Lee and colleagues presented a thorough discussion of the temporal expression of the virulence factors of *V. cholerae* and their role in pathogenesis.[36]

If the bacteria colonize the mucosa in sufficient numbers, they are able to secrete quantities of CT close to the mucosa and thereby cause disease. CT, a protein with a molecular weight of 84,000, has a subunit structure consisting of a central active subunit (A subunit) and a surrounding pentameric binding subunit (B subunit).[37,38] The A subunit is responsible for the physiologic and toxic activity of the toxin, and the B subunit for the characteristic tight binding of toxin to GM_1 ganglioside receptors on the intestinal epithelial cell surface.[39] The CT genes *(ctxAB)* are carried on a lysogenic filamentous phage of *V. cholerae,* called CTXPhi. CTXPhi can transfer between toxigenic and nontoxigenic strains of *V. cholerae*. CTXPhi integrates into the bacterial chromosome and forms stable lysogens.[40] After attachment to the surface of the cells via the B subunit, the A subunit (or, more specifically, its released A1 polypeptide) of CT stimulates the enzyme adenylate cyclase, which initiates a cascade of biochemical events that lead to hypersecretion of chloride and bicarbonate with concomitant water loss following the resulting ionic gradient, far in excess of the absorptive capacity of the gut.[41] The excess fluid is thus passed out as diarrheal fluid.

In cholera, the activity of CT is limited to the intestinal mucosa because CT is not systemically absorbed. Antibacterial and antitoxic intestinal secretory antibodies, primarily secretory immunoglobulin (Ig) A, act synergistically to prevent colonization by cholera vibrios and binding of CT, thereby preventing disease.[42] The predominant antigen inducing protective antibacterial immunity is LPS.[43] The data are contradictory about whether immune responses to TCP contribute to protective immunity.[44–46] Protection against CT is directed toward its B subunit, not only protecting against cholera but also cross-protecting against diarrhea caused by heat-labile toxin (LT) producing enterotoxigenic *Escherichia coli* (ETEC).[47]

MODES OF TRANSMISSION

Transmission occurs through consumption of contaminated water or food and is often the result of combined contamination. Contamination may be fecal or from a natural marine reservoir of the organism. Contaminated water, for example, is often used to rinse fresh food, and thus the food becomes contaminated, serving as the vehicle of transmission. Contaminated food, if kept at room temperature, supports the growth of *V. cholerae* and may lead to common-source outbreaks.[48,49]

DIAGNOSIS

The diagnosis of cholera can be suspected clinically but is confirmed by culturing the organism from a fecal culture. *V. cholerae* is preserved well in routine fecal transport media, although Cary-Blair medium is preferred. Primary bacteriologic plates should include selective media such as thiosulfate-citrate-bile salts-sucrose or taurocholate-tellurite-gelatin agar.[50,51] Bacteriologic confirmation of initial cases in a region is essential because of the epidemic implications, but during major epidemics, laboratory confirmation is not needed except on a sample basis. Rapid immunologic diagnostic tests, using immunochromatographic dipsticks, have been developed for both O1 and O139 *V. cholerae* strains.[52,53] These tests have acceptable sensitivity, but have suffered from poor specificity, although specificity of these tests appears to increase after a 6-hour enrichment period of samples in alkaline peptide water. These tests have found greatest application as point of care diagnostics, especially in epidemic contexts where sensitivity and speed are greatly valued. Newer molecular methods, such as polymerase chain reaction (PCR) are increasingly being developed, but still are not practical for most situations where a reliable cholera diagnostic test result is needed quickly.

Clinical management of the individual case does not depend on laboratory confirmation because treatment is aimed at rehydration and only secondarily toward eradicating the bacteria. Acute and convalescent sera can be tested for vibriocidal or agglutinating antibodies to confirm the diagnosis of cholera caused by serogroup O1; a fourfold rise in titer is diagnostic of recent cholera infection.[54] A similar assay is possible for serogroup O139, but it is more difficult because the capsule found on this strain makes it less sensitive to complement-mediated killing, and the assay is performed in only a few research laboratories. The O139 vibriocidal assay generally uses as the target strain a mutant strain lacking the capsule. Antitoxin antibodies are also stimulated during infection and can be measured; however, this measurement is not completely specific because other vibrios, as well as ETEC, can produce an immunologically similar cholera-like toxin and stimulate antitoxin responses. In general, acute and convalescent sera are not needed to diagnose an individual patient; however, the measurement of serum titers can be helpful during epidemiologic evaluations.

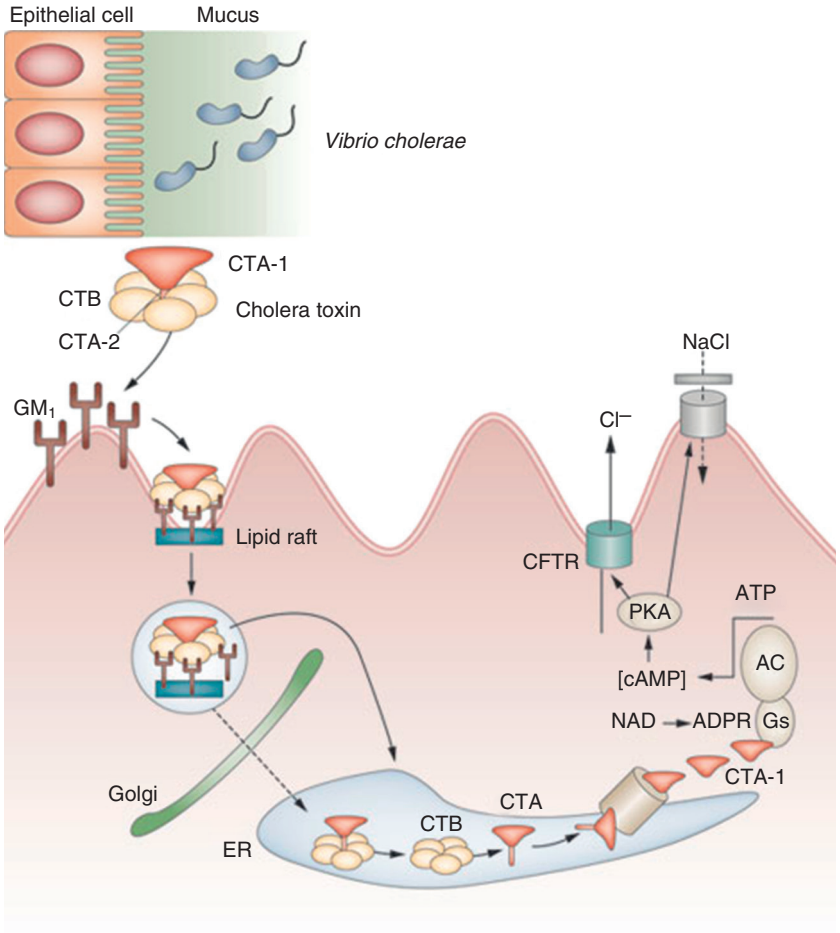


Figure 14.1. Cholera pathogenesis and cholera toxin action. After ingestion, *Vibrio cholerae* colonizes the small intestine and secretes cholera toxin, which has a doughnut-like structure with a central enzymatic toxic active A (A1+A2) subunit associated with pentameric B subunits (B5). After binding to GM_1 ganglioside receptors, mainly localized in lipid rafts on the cell surface, the toxin is endocytosed and travels to the ER via a retrograde pathway which—dependent on cell type—may or may not involve passage through the Golgi. In the ER, the A subunit dissociates from the B subunits and through translocation via the ER degradosome pathway, A1 can reach the cytosol where it can rapidly refold. It binds to ADP-ribosylates Gs, stimulating the AC complex to produce increased cellular levels of cAMP, leading to activation of PKA, phosphorylation of the major chloride channel, CFTR, and secretion of chloride (Cl^-) and water. Cholera toxin-induced chloride (and bicarbonate) secretion is especially pronounced from intestinal crypt cells, while in villus cells the increased cAMP levels instead mainly inhibits the normal uptake of NaCl and water.[14] AC, adenylate cyclase; ADP, adenosine diphosphate; ADPR, ADP ribose; ATP, adenosine triphosphate (ATP); cAMP, cyclic adenosine monophosphate; CTA, cholera toxin A; CTB, cholera toxin B; CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; Gs, guanosine triphosphate (GTP)-binding protein, Gs; NAD, nicotinamide adenine dinucleotide; PKA, protein kinase A. *(From Clemens J, Shin S, Sur D, et al. New-generation vaccines against cholera.* Nat Rev Gastroenterol Hepatol. *2011;8:701–710.)*

CASE MANAGEMENT

Appropriate case management of diarrhea using intravenous and oral rehydration solutions has reduced deaths from diarrhea by approximately 3 million per year over the last 20 years.[55] Oral rehydration salt solution should be considered as the first treatment for cholera, although intravenous fluids with a polyelectrolyte solution (e.g., Ringer lactate) are needed in cases of severe dehydration or shock.[56] The most important elements of therapy are the rapid replacement of fluids to correct the dehydration, base administration to correct the acidosis, and provision of potassium to correct the potassium deficiency. With proper treatment, no patient should die of cholera, but if treatment is delayed, given too slowly, or inappropriately, case-fatality rates exceed 5%, even today.

In severe cholera cases, an effective antibiotic can be used to reduce the volume of diarrhea and the length of time the organism is excreted in the stool. Doxycycline or tetracycline is the antibiotic of choice for sensitive strains, but resistance to this class of drug may occur.[56] Unlike many other enteric organisms, the predominant strains often lose their resistance, so antibiotic resistance is a characteristic that varies from time to time and from place to place.[57,58] Thus, one must monitor antibiotic resistance during cholera epidemics and choose another antibiotic when necessary. Other clinically effective antibiotics include ciprofloxacin, cotrimoxazole, erythromycin, azithromycin, chloramphenicol, and furazolidone.

EPIDEMIOLOGY

Incidence

Endemic cholera denotes the repeated occurrence of cholera in a population over time, without the need for exogenous reintroduction of the pathogen. Practically speaking, the World Health Organization (WHO) defines a cholera-endemic

population as one that has experienced cholera in at least 3 of the past 5 years.[59] In such populations, cholera incidence may still vary considerably from year to year. For example, in Matlab, Bangladesh, the annual incidence of treated cholera episodes varied from 0.2 cases to 5.1 cases per 1000 population over a 15-year period.[60] In cholera-endemic populations, the incidence of cholera is typically greatest in children younger than 5 years, presumably because of lower levels of preexisting natural immunity to cholera in this young age group, reflecting fewer past exposures to cholera vibrios.[61] It had been thought that infants and young toddlers were at low risk in these populations because of the protective effects of breastfeeding,[62,63] but recent studies in Kolkata, India, and in North Jakarta, Indonesia, have shown a very high incidence in this age group.[64]

In contrast to endemic cholera, the term *epidemic cholera* refers to cholera that requires exogenous introduction of cholera vibrios into a population and is not recurrent in time and place. In such populations, which are typically immunologically naïve to cholera because of a lack of past exposures, incidence rates of cholera are usually age independent, and the clinical spectrum of cholera illnesses tends to be more severe. This is well illustrated by the recent outbreak of cholera in Haiti, where high rates have been observed in adults and case-fatality rates have been high. Although useful conceptually, the terms *endemic cholera* and *epidemic cholera* represent two ends of the epidemiologic spectrum, and, in practice, large outbreaks termed epidemics may occur in populations with endemic cholera.

Risk Groups

Cases of cholera occur in both children and adults. In addition to the consumption of specific high-risk foods, other risk factors for cholera include low socioeconomic status, which correlates with the use of impure water, poor sanitation, and poverty. Resettlement camps of internally or externally displaced persons or refugees also pose a key risk for cholera, with approximately 75% of such camps in Africa having an outbreak at some point.[65] Similarly, populations are commonly at risk for cholera outbreaks following natural disasters, as illustrated by the recent major cholera epidemic in Haiti following a severe earthquake and a hurricane in close succession.[66]

Beyond these environmental risk factors, several host factors influence susceptibility to cholera, including being non-breastfed for infants and young toddlers and gastric hypochlorhydria.[62,63,67–70] The protection infants receive from breastfeeding may result from antibodies in the breast milk, but it may also result from less exposure to contaminated food and liquids. Individuals of blood type O are more likely to develop severe illness, although this increased risk appears to apply to El Tor but not to classical cholera.[71–76] A study in Mozambique implicated HIV coinfection as a risk factor for cholera, raising the possibility of the convergence of the ongoing pandemics of these two infections.[77]

Whether or not pregnancy is a risk factor for cholera is not clear, but cholera in pregnancy has been linked with high rates of abortions, stillbirths, premature childbirths, and maternal deaths.[78] Fetal loss rates from cholera cases reaching up to 50% have been reported, for which the degree of dehydration and hypovolemia are the strongest factors associated with fetal loss.[79]

Reservoirs of Infection

Although *V. cholerae* is generally thought to be spread by the fecal–oral route, it can also persist in environmental waters without continued contamination with human feces. During epidemics, environmental waters may become heavily contaminated with *V. cholerae* from infected human feces, and this contamination further spreads the epidemic. Once the surface waters become contaminated, an environmental reservoir may maintain the bacterium without further fecal contamination if appropriate conditions of salinity and temperature exist, as illustrated by the persistent environmental reservoir for *V. cholerae* O1 off the coast of Louisiana in the Gulf of Mexico.[80] As demonstrated in the United States, the environmental reservoir can lead to additional sporadic primary cases from contaminated seafood, but, if sanitation is adequate, secondary cases do not occur. If sanitation is poor, the sporadic cases may be followed by additional secondary cases and may lead to another epidemic through amplification of the inoculum and further contamination of the waters.

In the environment, the *Vibrio* organisms associate with certain plankton (known as copepods), chitinous shelled animals (e.g., crabs), and vegetation.[81–86] These associations appear to be crucial for the long-term survival of *Vibrio* organisms, and they may also enhance horizontal gene transmission among vibrios.[87] Importantly, chitin induces natural competence in *V. cholerae,* enabling bacterial uptake of extracellular DNA from the environment, thereby facilitating genetic transformation.[88] Furthermore, the association with chitinous shells increases the risk of transmission via consumption of crabs, shrimp, and other shellfish, many of which are eaten only partially cooked. In some cultures, raw fish may also become a vehicle of transmission. Unfortunately, even if the fish are caught in uncontaminated waters, they may become contaminated when washed in harbor water as the catch is brought to market, and cooked food may become recontaminated in the kitchen with utensils exposed to uncooked food.

Significance as a Public Health Problem

A recent analysis of the annual global disease burden of cholera estimated that there are approximately 2.8 million cholera cases and 91,500 cholera deaths in endemic countries, and 87,000 cases and 2500 deaths in cholera epidemics.[89] Approximately half of the deaths occur in Africa. More than 1 million cases occurred in Latin America during the first 3 years of the explosive 1991 epidemic, causing approximately 9000 deaths. However, the number of reported cases greatly diminished after 1998, and the last reported cholera death occurred in 2001.[5,90]

Cholera is often underreported to WHO. For example, the actual rates of cholera in Bangladesh, which does not report cholera to WHO, have generally exceeded two per 1000 population annually, suggesting that approximately 300,000 cases occur in this country alone.[91] Bearing in mind these limitations in reporting, the worldwide occurrence of massive and prolonged outbreaks seems to be increasing. Since 2005, such outbreaks, each with a total of more than 10,000 cases, were reported in Afghanistan, Angola, Ethiopia, Haiti, Iraq, Guinea Bissau, Kenya, Nigeria, Somalia, South Africa, South Sudan, and Zimbabwe.[92] The outbreak in Haiti, which began in October 2010, has been responsible as of this writing for approximately 730,000 reported cases and 9000 deaths.[93]

In addition to the endemic and epidemic cases occurring in developing countries, sporadic cases occur along the U.S. Gulf Coast and are often associated with undercooked shellfish, especially crabs.[94] After the U.S. Gulf Coast hurricanes of 2005, two cases of toxigenic *V. cholerae* infection that occurred in a Louisiana couple were attributed to consumption of undercooked or contaminated seafood.[95] Most other cases in the United States and other developed countries arise as a consequence of travel to endemic areas.[96]

PASSIVE IMMUNIZATION

Passive immunization against cholera, although theoretically possible, is not used and would not be practical as a public health tool.

ACTIVE IMMUNIZATION

History of Vaccine Development

The development of vaccines against cholera dates to shortly after the discovery of the bacterial etiology of cholera by Robert Koch in the late 19th century. In the late 20th century, a modern vaccine against cholera was one of the first to exploit the new tools of molecular biology. Yet despite these efforts, cholera remains an important public health problem in much of the world. Until recently, vaccines against cholera have generally not been used to control endemic or epidemic disease. This is in part because interest in using limited resources to improve water quality and sanitation is greater than in using resources to vaccinate populations.[97]

Preparations of parenteral killed whole-cell cholera vaccine were produced shortly after Koch's discovery. In 1884 in Spain, Ferran produced a killed bacterial vaccine and inoculated thousands of people in an area experiencing an epidemic at the time. Of those inoculated, 1.3% came down with cholera, compared with 7.7% of those who were not vaccinated.[98]

Shortly after Ferran, Haffkine began working on a cholera vaccine. His work was stimulated by the cholera epidemics in his native Russia; however, unable to return to his homeland, he went to India, where he began giving vaccines to large numbers of persons living in the Delhi and Kolkata areas. He became convinced of the success of his vaccine in 1894 when none of the 116 immunized persons in a Calcutta slum developed cholera, whereas nine cases were reported among the 84 unimmunized.[100] The popularity of the vaccine grew during the early part of the 1900s as the cholera problem continued and no effective therapy existed. Notable is the account of Russell, who carried out large-scale trials of parenteral cholera vaccines in the 1920s.[101] When vaccine efficacy was assessed in a trial in which more than 8000 persons received two doses and 17,000 received one dose of the parenteral vaccine, and 25,000 persons were not immunized, the vaccines were associated with a protective efficacy of approximately 80% during a 3-month follow-up period. Similarly, in further studies involving up to 3 million persons in uncontrolled trials in India, the injectable vaccine appeared to show excellent efficacy. As well, there were numerous anecdotal reports concerning the effectiveness of the parenteral vaccine.

A killed oral cholera vaccine (OCV) was also tested by Russell in the 1920s in India. In retrospect, this was an especially interesting vaccine because of developments in the 1980s with another killed OCV (described later). The first oral vaccine, termed *Bezredka's bilivaccine* because it was developed at the Pasteur Institute by Bezredka and contained ox bile in addition to killed *V. cholerae* O1, was tested in India along with the parenteral vaccine described earlier. The oral vaccine provided protection that was approximately equal (82%) to that of the injectable vaccine and appeared to be highly protective against cholera-related deaths. Possibly because of the bile, the vaccine also caused diarrhea in some persons, and thus it was not acceptable to potential recipients who feared that the vaccine teams were in fact spreading cholera. Unfortunately, this apparent success with an OCV was not pursued for more than 50 years.

Because of the apparent efficacy of vaccines from these early but poorly designed studies, the panic that accompanied cholera epidemics, and the lack of consistently effective treatment, the parenteral vaccines became widely used. For expatriates, such as colonists living in areas that were endemic for cholera, this was probably wise at the time, especially in the absence of safe water and refrigeration. The requirement to receive booster doses every 6 months was not a serious constraint for these expatriates, because cholera was an ever-present risk, and the vaccine probably prevented many cases during this early colonial period. With the accompanying panic stimulated by cholera epidemics, many countries began requiring proof of vaccination for travelers crossing international boundaries, in the mistaken belief that vaccination would prevent the international traveler from spreading the bacterium between countries.

The parenteral killed whole-cell cholera vaccine ultimately fell from favor for several reasons. First, during the 1960s, several controlled studies from East Pakistan (now Bangladesh), India, the Philippines, and Indonesia showed that the vaccine had only limited efficacy (approximately 50%) and that protection was brief in duration.[100,101] Some vaccine preparations were associated with higher efficacy, but these tended to be associated with higher rates of side effects. Side effects were similar to those of killed whole-cell typhoid vaccines, although perhaps somewhat less severe.[101] More important, however, was that the injectable vaccine had to be given frequently (every 6 to 12 months) to maintain clinically significant protection. Resources for intensive vaccination programs were not available in areas endemic for cholera and, if mobilized, would have taken resources from other more effective interventions. Killed parenteral whole-cell vaccines are no longer recommended by the WHO, and the manufacture and sale of these vaccines in the United States have been discontinued.

Despite the demise of conventional, parenterally administered killed whole-cell cholera vaccines, interest in cholera vaccines continued, but it shifted to the development of orally administered vaccines. The oral approach was motivated by several observations. Persons who have been infected with *V. cholerae* develop a protective immune response. This conclusion is based on both volunteers who were challenged 5 years after an original experimental challenge with virulent *V. cholerae* O1 and on analysis of cohorts from endemic areas of Bangladesh.[102,103] There is some evidence that classical strains may induce a more solid immunity than El Tor strains, although this is confounded by differences in serotype in which Inaba appears to better immunize than Ogawa.[103] Also, with the recognition that immune protection against cholera is the result of intestinal secretory antibacterial and antitoxic antibodies came the observation that oral administration of antigens is the most efficient method of eliciting this mucosal immunity.[104–106] In addition to this biological rationale, oral vaccines also have the major advantages of being easy to administer, not requiring physicians or nurses, and being free of the risk of needle-borne infections. Today's licensed OCVs consist either of killed organisms and antigens, or of live, genetically attenuated strains.

Immunization With Modern, Licensed Oral Vaccines

In recent years, three oral vaccines, two killed and one live, have been developed and licensed in various countries (Table 14.1). A killed vaccine known as Dukoral consists of *V. cholerae* O1 killed whole cells combined with the B subunit of CT (CTB); another vaccine consisting only of killed whole cells, but of both the O1 and O139 serogroups, goes by the trade names Shanchol in India, mORCVAX in Vietnam, and Euvichol in Korea. The live vaccine, formerly produced as Orochol (also known as Mutacol in the Americas) and recently

TABLE 14.1 Comparison of Killed WC-rCTB Vaccine, Live CVD103-HgR Vaccine, and Reformulated Killed WC-Only Licensed Vaccines

Characteristic	WC-rCTB	CVD103-HgR	Reformulated WC-Only
Commercial name	Dukoral	Vaxchora (formerly produced as Orochol and Mutacol)	Vietnam: mORCVAX India: Shanchol Korea: Euvichol
Vaccine type/composition	Killed whole cells of *Vibrio cholerae* O1 (Inaba and Ogawa, classical and El Tor) plus recombinant cholera toxin B subunit (monovalent)	Live attenuated *V. cholerae* O1 classical Inaba strain 596B (monovalent)	Killed whole cells of *V. cholerae* O1 (Inaba and Ogawa, classical and El Tor) and O139 (bivalent)
Regimen	2 doses given 7 to 14 days apart (3 doses for children 2 to 5 y)	1 dose	2 doses given at least 14 days apart
Age range for vaccination	≥2 y	18–64 y	Vietnam: ≥2 y India and Korea: ≥1 y
Duration of protection	2 y (6 mo for children 2 to 5 y)	3 mo (established only in North American volunteers)	At least 5 y
Booster dose requirements	Every 2 y (every 6 mo for children 2 to 5 y)	Unknown	Every 3 to 5 y
Requirement for oral buffer	Yes	Yes	No
Storage temperature	2° to 8°C	–25° to –15°C	2° to 8°C
Shelf life	3 y	18 mo	2 y
International acceptance	WHO prequalified	Not prequalified by WHO	WHO prequalified
Price to the public sector per dose	Will depend on production volume (2008 negotiated price to WHO: ~$5.25/dose for small quantity of 250,000 doses)	Vaccine price not currently available	Vietnam: ~$0.75/dose India: $1.85 per dose Korea: Unknown

WC, whole cell; WC-rCTB, whole cell–recombinant cholera toxin B.
Modified from Abramson JS, Chair; Ad-hoc Cholera Vaccine Working Group. Background Paper on the Integration of Oral Cholera Vaccines into Global Cholera Control Programmes. Presented to the WHO Strategic Advisory Group of Experts (SAGE) in October 2009. Final draft available at: http://www.who.int/immunization/sage/1_Background_Paper_Cholera_Vaccines_FINALdraft_13_oct_v2.pdf.

licensed as Vaxchora, is derived from a strain of *V. cholerae* O1 from which the active moiety of the CT gene *ctxA* is deleted. These are summarized in the following paragraphs.

Killed Whole-Cell Vaccine Plus Cholera Toxin B Subunit

Constituents of the Vaccine. This vaccine contains a mixture of recombinant CTB plus killed strains of *V. cholerae* O1 representing both serotypes (Inaba and Ogawa) and both biotypes (El Tor and classical). Two of the strains are heat-killed (classical Inaba strain Cairo 48 and classical Ogawa strain Cairo 50) to better express the LPS antigens, and two are formalin killed (El Tor Inaba strain Phil 6973 and classical Ogawa strain Cairo 50) to better preserve the protein antigens. The CTB is produced by a genetically engineered *V. cholerae* strain that hyperproduces this antigen. The vaccine is free of the cholera toxin A (CTA) subunit and its toxicity. The recombinant CTB retains its ability to bind to the GM_1 ganglioside of the cell membrane. Because the heat-LT of *E. coli* cross-reacts with CT, this antigen provides cross-protection against diarrhea caused by LT-ETEC.[47] As a killed vaccine, whole cell–recombinant cholera toxin B (WC-rCTB) has no potential for genetic reversion to virulence. CTB is sensitive to stomach acid, so the vaccine is given with a buffer. The protective whole-cell antigens, and especially LPS, are not acid sensitive.

The vaccine is prepared as a liquid formulation, and it comes as a whitish, 3-mL suspension in a single-dose glass vial. Each dose of the vaccine consists of a total dose of 10^{11} killed bacteria with 1 mg of recombinant CTB. The vaccine vial comes with a sachet of buffer consisting of sodium hydrogen carbonate, citric acid, sodium carbonate, saccharin, sodium citrate, and raspberry flavor. The buffer is supplied as white effervescent granules and is to be dissolved in a glass of water, to which the vaccine is added and then taken by mouth.

Manufacture of the Vaccine. Batches of *V. cholerae* 01 strains are grown in large fermenters. The harvested bacteria are then killed with either formalin or heat, and the different batches are combined to make a mixture of the batches in equal concentrations of bacteria. All fermentation, harvesting, and mixing, and all transfers between these steps are performed in a closed, steam-sterilizable system to facilitate processing and to eliminate the risk of contamination. The CTB is prepared from a strain of *V. cholerae* O1 that is genetically engineered to produce this antigen, but it lacks the genes for producing cholera A subunit. After fermentation, CTB is purified and then added to the whole-cell vaccine.

Producer. WC-rCTB (Dukoral) is produced by SBL Vaccin AB (now owned by Vaneva) and is packaged in a two-dose package with two unit-dose vials of vaccine and two sachets with buffer. WC-rCTB is registered in more than 60 countries, but it remains experimental in the United States. It has been prequalified by WHO for purchase by United Nations agencies.

Dosage and Route. For adults, the vaccine is given as two oral doses, coadministered with liquid oral buffer, with an interval of 1 to 2 weeks. Children 2 to 6 years of age should receive three doses with an interval of 1 to 2 weeks between doses. For persons at continued risk for cholera, booster doses should be given every 6 months to children 6 years and younger, and every 2 years to older persons.

Availability in Combinations. The vaccine is not available in combination with any other vaccine.

Vaccine Stability. The vaccine should be kept refrigerated (2° to 8°C), and it should not be frozen.

Immunogenicity and Immune Correlates of Protection. Vaccination with WC-rCTB stimulates the development of both vibriocidal and antitoxin antibodies in the serum, and it also stimulates IgA intestinal antibodies.[105,106] Titers of serum vibriocidal antibodies are lower and less frequent with the killed oral vaccine than with the live oral vaccine (*vide infra*), but the local intestinal immune response is comparable to that seen with naturally occurring cholera.[105] Rises in serum IgG and IgA antitoxin titers as measured by enzyme-linked immunosorbent assay (ELISA) are seen in most vaccinees. CTB stimulates interferon-γ in mucosal tissues after immunization, although it is not known if this relates to protection.[106,107]

As noted earlier, immune protection appears to be mostly attributable to local intestinal IgA antibody against the bacterial cells, although antibody to the toxin also contributes to protection.[104,105,108] Neither a serologic correlate nor a serological surrogate of protection exists. The most commonly used measure of immune responses to cholera vaccines is the serum vibriocidal antibody titer. Seroepidemiologic studies done in Bangladesh demonstrated that titers of these antibodies rise with age, as expected with the cumulative exposure to cholera in this setting. Also, the risk of cholera was shown to be inversely related to the height of serum vibriocidal titers in this setting.[109,110]

However, serum vibriocidal antibodies are not surrogates or correlates of protection, as illustrated by the observation that the previously used whole-cell injectable vaccines stimulated high vibriocidal titers, but these titers did not correlate with long-lasting protection.[100] Moreover, the serum vibriocidal responses to oral whole cell–recombinant B subunit (WC-rBS) are short-lived, lasting only several months, with clinical protection persisting far beyond.[106] Although serum vibriocidal antibodies are not suitable as an immunologic surrogate or correlate of protection, their measurement is nevertheless useful for comparing serologic responses among killed oral vaccines, or among these vaccines when coadministered with different buffers.

After immunization with WC-rCTB, there is a serum vibriocidal response, but it is low in magnitude. WC-rCTB vaccine has typically elicited approximately twofold rises in serum anti-Ogawa and anti-Inaba vibriocidal antibodies in cholera-endemic populations, but higher-fold responses in cholera-naïve populations.[111,112] Little information is available on serum vibriocidal antibody responses to WC-rCTB vaccine in persons infected with HIV, although there is a suggestion that intestinal secretory responses are relatively well preserved except in patients with advanced HIV infections.[113]

Excretion and Transmissibility. As a killed vaccine, WC-rCTB is neither excreted nor transmissible.

Duration of Immunity and Protection. The efficacy of whole cell–cholera toxin B subunit (WC-CTB; in which the B subunit was produced by chemical extraction rather than the currently used recombinant technology) was established in a large field trial in Bangladesh in 1985 (Table 14.2).[114–116] The study enrolled 89,596 persons ages 2 to 15 years and women older than age 15 years; participants were randomized to receive three doses, at 6-week intervals, of one of three preparations: the WC-CTB, a WC-only vaccine with the same cellular constituents as WC-CTB but without CTB, and a killed *E. coli* placebo. Of these, 62,285 persons took all three scheduled doses. In this trial, overall protection by WC-CTB was 85% during the first 4 to 6 months of follow-up, 62% during the first year, 57% during the second year, and negligible thereafter. Vaccination protected adults and children older than 5 years for 3 years, and younger children for approximately 6 months. The efficacy of two doses was similar to that for the complete three-dose regimen, and cross-protection against LT-ETEC diarrhea was conferred to WC-CTB vaccinees.[46] However, efficacy was lower against El Tor cholera than against classical cholera.[116]

The efficacy of WC-rCTB was confirmed in a study in the Peruvian military in which 1426 subjects were randomized to receive two doses of vaccine or placebo 1 to 2 weeks apart.[117] After vaccination, of the 16 cases of cholera detected, 14 occurred in the placebo group (protective efficacy of 86%). In another study in Peru performed over a 2-year period, surveillance was carried out using both active (household) and passive (hospital) surveillance. During the first year, after two doses of vaccine there were few hospital cases and nearly all cases were mild, being detected through active surveillance. During this first year there was no evidence of protection, although methodological issues may have explained an apparent lack of efficacy.[118] A booster dose was given a year after the first immunizations, and during the subsequent year the cholera epidemic was more severe, with most cases being detected in the hospital. During this second year, the vaccine did show high-grade protection.[119]

TABLE 14.2 Protection by Oral B Subunit, Killed Whole Cell (WC-CTB), and Killed Whole Cell–Only (WC) Vaccines in the 1985 Bangladesh Field Trial[a,b]

	Protective Efficacy by Age Group					
	WC-CTB			WC		
Follow-up Period	All Ages	2–5 Years	>5 Years	All Ages	2–5 Years	>5 Years
4 to 6 months	85% (62%)[c]	100%	76%	58% (14%)	35%	71%
First year	62% (50%)	31%	78%	53% (38%)	31%	67%
Second year	57% (42%)	24%	61%	57% (42%)	24%	73%
Third year	17% (–15%)	2%	40%	43% (19%)	2%	62%

[a]Overall 3-year cumulative 3-year protective efficacy of each vaccine was approximately 50%.
[b]Three doses were given at ≥6-week intervals.
[c]Lower boundary of 95% confidence interval for probable error.
From Clemens J, Sack D, Harris J, et al. Field trial of oral cholera vaccines in Bangladesh: results from three-year follow-up. Lancet. *1990;335:270–273.*

Mass vaccination with WC-rCTB was feasible and effective in preventing cholera in Beira, Mozambique, in a population with a high prevalence of HIV infection.[120] In this port city, all healthy, nonpregnant residents of an impoverished neighborhood were invited to receive WC-rCTB vaccine before the anticipated annual cholera outbreak. More than 14,000 persons (57% of the targeted population) received at least one dose of a two-dose regimen. Surveillance for diarrhea was maintained for 1 year after vaccination. In a case-control study, vaccination provided 78% protection against cholera in the year after vaccination and 89% protection against cholera with severe dehydration. Interestingly, all cholera strains isolated during surveillance in this study were El Tor variants that produce classical biotype CT.[120]

Postexposure Prophylaxis. There is no indication for postexposure vaccine prophylaxis.

Safety. WC-rCTB has been well tolerated and is considered safe in persons age 2 years and older.[111,121] Gastrointestinal side effects may occur, but at a rate similar to that observed among placebo recipients. A small, controlled observational study suggested that WC-rCTB inadvertently administered to pregnant women was not associated with adverse pregnancy outcomes.[122] Small studies have found no safety concerns when the vaccine is administered to HIV-infected adults, although a transient increase in HIV viremia after vaccination was noted in one study.[123,124] More than 10 million doses have been administered since licensure, and no important safety concerns have been raised during postlicensure surveillance.

Indications. WC-rCTB is indicated for persons age 2 years and older who are at risk for cholera. The WHO recommends that the vaccine be used in areas where cholera is endemic, and that it be considered for use in areas at risk for cholera outbreaks (preemptive vaccination), as well as in those experiencing such outbreaks (reactive vaccination).[59]

Contraindications and Precautions. The only absolute contraindication to receiving WC-rCTB vaccine is hypersensitivity to a constituent of the vaccine or its excipients. Administration to pregnant or lactating women is guided by assessment of medical risk and benefit.

Reformulated Killed Whole Cell–Only Bivalent Vaccine

The large-scale field trial of killed OCVs in Bangladesh demonstrated that a three-dose regimen of an O1 serogroup vaccine consisting only of killed whole cells without CTB conferred approximately 50% protection that was sustained during 3 years of follow-up.[116] Because this vaccine does not require coadministration with oral buffer and is simple and inexpensive to produce, in the 1980s the Vietnamese government, with assistance from Sweden, embarked on a program to produce an oral killed WC-only vaccine for use in its health programs.[125] A monovalent (O1) WC-only vaccine was licensed as a two-dose vaccine in Vietnam as ORCVAX in 1997. This vaccine was subsequently made bivalent (O1 and O139).[126] Between 1998 and 2009, more than 20 million doses were administered in Vietnamese programs.

In 2004, the International Vaccine Institute in Korea, in cooperation with the Vietnamese producer VaBiotech, made several major modifications to the bivalent ORCVAX to bring the vaccine into compliance with WHO standards for inactivated OCV, including replacing a high CT-producing O1 strain with two alternative O1 strains, doubling the LPS content of the vaccine, development of a production process compliant with current good manufacturing practices (GMP), and introduction of improved quality control and lot release assays.[127] After successful Phase II trials of this vaccine in Vietnam and India, and a large-scale Phase III trial in India, this reformulated WC-only vaccine was licensed in 2009 by Shantha Biotechnics as Shanchol in India (and later licensed as mORCVAX in Vietnam and as Euvichol in Korea).

Constituents of the Vaccine. Each dose of the reformulated WC-only vaccine contains 600 (arbitrary) ELISA units of LPS of formalin-killed *V. cholerae* O1 El Tor Inaba (strain Phil 6973); 300 ELISA units of LPS of heat-killed *V. cholerae* O1 classical Ogawa (strain Cairo 50); 300 ELISA units of LPS of formalin-killed *V. cholerae* O1 classical Ogawa (strain Cairo 50); 300 ELISA units of LPS of heat-killed *V. cholerae* O1 classical Ogawa (strain Cairo 48); and 600 ELISA units of LPS of formalin-killed *V. cholerae* O139 (strain 4260B). Each batch of the vaccine is verified to have no detectable CT by a GM_1 ganglioside ELISA with a lower limit of detection of 1 ng/mL. The vaccine is prepared as a liquid formulation and comes as a whitish, 1.5-mL suspension. Shanchol is available in single-dose vials, mORCVAX is available in five-dose vials, and Euvichol in single-dose vials. As a killed vaccine, this vaccine has no potential for genetic reversion to virulence. The WC antigens, and especially LPS, are not acid sensitive, and thus the vaccine does not need to be coadministered with buffer.

Manufacture of the Vaccine. *V. cholerae* serogroups O1 and O139 are grown in large fermenters. The cultures are harvested and the bacterial cells inactivated with either formalin or heat. The cells are then concentrated and washed to form monovalent bulks. The final vaccine is then formulated by mixing each of the required monovalent bulks to their final specified concentration of LPS antigen in phosphate-buffered saline; thimerosal is added as a preservative. All processes are performed under aseptic conditions in compliance with GMP requirements to ensure a product that meets stringent quality standards.

Producers and Trade Names. The vaccine is licensed in India as Shanchol by Shantha Biotechnics (Hyderabad), in Vietnam as mORCVAX by VaBiotech (Hanoi), and in Korea as Euvichol by Eubiologics (Seoul).

Dosage and Route. The vaccine is given as two oral doses, with a 2-week interdose interval, to all persons age 1 year and older. The label does not specify the timing of booster doses, although clinical trial data show protection for at least 5 years after the primary regimen.

Availability in Combinations. The vaccine is not available in combination with any other vaccine.

Vaccine Stability. The vaccine should be kept refrigerated (2° to 8°C), and it should not be frozen. It has a shelf life of 2 years.

Immunogenicity and Immune Correlates of Protection. As for WC-rBS vaccine measurement of serum vibriocidal antibodies forms the basis of evaluating the immunogenicity of this oral killed whole cell–only vaccine, although, as noted earlier, these antibodies are not immunologic surrogates or correlates of vaccine protection. Because the vaccine is bivalent, measurement of anti-O139, as well as anti-O1 antibodies, is done. However, in contrast to serum anti-O1 vibriocidal antibodies, which have been found in the field to correlate with the risk of cholera in endemic populations, there is currently no assay for measuring serum anti-O139 vibriocidal antibodies that has been shown to correlate with the risk of O139 cholera.[128]

In cholera-endemic settings, serum anti-O1 vibriocidal responses to a two-dose regimen of the reformulated WC-only vaccine were more robust than those earlier seen for WC-rCTB vaccine. In Kolkata, India, during a large-scale field trial among children and adults, geometric mean rises in serum anti-O1 vibriocidal antibodies after a two-dose regimen were approximately sixfold, as opposed to the approximately twofold rises seen in a large-scale field trial of a three-dose regimen of WC-CTB in Bangladesh.[114] Serum anti-O139 vibriocidal antibody responses to the reformulated WC-only vaccine are much lower, generally twofold or lower.[129] As for WC-rCTB vaccine, serum anti-O1 vibriocidal responses to the reformulated WC-only vaccine are higher in populations that are naïve with respect to past cholera exposure than in cholera-endemic populations. A recent study in Haiti showed that HIV-infected individuals developed somewhat lower but still appreciable serum vibriocidal antibody responses, compared with those in HIV-uninfected individuals, and that, among HIV-infected persons, the magnitude of these responses varied inversely with CD4 lymphocyte counts.[130]

With no rise in seroconversion rates following the second dose of Shanchol relative to those after a first dose in cholera-endemic populations,[131] efforts to evaluate the efficacy of a single-dose regimen are in progress, as a single-dose regimen would be of great value in controlling cholera epidemics. Questioning the value of such a regimen for persons without preexisting immunity to cholera, however, are studies in humans given Dukoral, showing that intestinal IgA antibacterial and antitoxin antibody responses increase after the second dose of vaccine, relative to those after a first dose, when given to unprimed individuals.[132] As well, a rising serum vibriocidal antibody response to a second dose of Shanchol was observed in evaluations in Haiti and Ethiopia.[133,134]

Excretion and Transmissibility. As a killed vaccine, WC-only is neither excreted nor transmissible.

Duration of Immunity and Protection. The safety and efficacy of a two-dose regimen of the reformulated WC-only vaccine was studied in a large-scale, randomized, placebo-controlled trial that enrolled 69,328 persons ages 1 year and older in the urban slums of Kolkata, India (Table 14.3).[135] No adverse effects occurred more frequently in vaccinees than in placebo recipients. During surveillance, only El Tor cholera was detected, and all isolates were El Tor variants that produced classical biotype CT. The vaccine exhibited a 5-year cumulative efficacy of 65%, with a lower efficacy noted in children ages 1 to 5 years old (42%). While serum vibriocidal antibody titers waned after 1 year,[136] cumulative efficacy was maintained for at least 5 years in the overall trial population, and currently no formal recommendations on a boosting regimen exist. Serum vibriocidal antibody responses to a two-dose booster regimen dose 5 years after the primary doses among participants in the original Phase III field trial were shown to be comparable to those observed after a primary series.[137] Also, similar immune responses have been demonstrated for a standard two-dose regimen with a 2-week interdose interval and a two-dose regimen given with a 4-week interval in Kolkata, suggesting the possibility for increased flexibility of the dose schedule when offered as part of a targeted vaccination program.[138] As no O139 serogroup cholera cases were detected in the Kolkata efficacy trial, vaccine efficacy against O139 cholera was not evaluable. Serum anti-O1 vibriocidal antibody levels declined markedly at 1 year of follow-up in this field trial, despite clinical protection against cholera that persisted for at least 5 years.

TABLE 14.3 Protective Efficacy of the Reformulated Bivalent Killed Whole Cell–Only Oral Cholera Vaccine (Shanchol) in the Kolkata Field Trial[a]

Follow-Up Period	Protective Efficacy by Age Group[b]			
	All Ages	1–5 Years	5–15 Years	≥15 Years
Year 1	45% (–19%, 74%)[c]	17%	81%	66%
Year 2	76% (47%, 89%)	81%	92%	62%
Year 3	66% (40%, 81%)	37%	89%	64%
Year 4	58% (29%, 75%)	48%	89%	86%
Year 5	80% (40%, 93%)	–10%	89%	86%

[a]Two doses given at 2- to 6-week intervals.
[b]Overall cumulative 5-year protective efficacy was 65%.
[c]95% confidence interval for probable error in parentheses.
From Bhattacharya SK, Sur D, Ali M, et al. 5-Year efficacy of a bivalent killed whole-cell oral cholera vaccine in Kolkata, India: a cluster-randomised, double-blind, placebo-controlled trial. Lancet Infect Dis. *2013;13:1050–1056.*

Postexposure Prophylaxis. There is no indication for postexposure vaccine prophylaxis.

Safety. Adverse events occurred with no greater frequency in recipients of the reformulated WC-only vaccine and placebo in the Phase II trials done in Vietnam and India, and in the large Phase III trial conducted in India.[129,135,139] The vaccine is considered safe in persons age 1 year and older. There are no specific data demonstrating safety in pregnancy and lactation, nor for HIV-infected persons, although it is not expected that the safety profile of this vaccine in these groups would differ from WC-rCTB vaccine.[130]

Indications. The vaccine is indicated for persons age 1 year and older who are at risk for cholera. The 2010 WHO recommendation for cholera vaccines, outlined earlier, also applies to this vaccine.[59]

Contraindications and Precautions. The only absolute contraindication to receiving reformulated WC-only vaccine is hypersensitivity to a constituent of the vaccine or its excipients. Administration to pregnant women may be considered after careful evaluation of the benefits and risks.

Live Oral Vaccine (CVD103-HgR)

Live attenuated oral vaccines have been considered attractive because of the possibility that one dose might be sufficient to induce a maximal immune response. Attempts have been made to develop such vaccines by creating genetically attenuated mutants that do not produce cholera holotoxin and are not virulent. If CTB is expressed, such a mutant may stimulate antibacterial and anti-CTB immunity in a manner similar to that of natural infection but without the symptoms associated with naturally acquired *V. cholerae* infection. CVD103-HgR is one such live attenuated strain.[140,141] The strain, derived from classical Inaba strain 569B, has been engineered to express CTB but not the enzymatically active A subunit, and it carries mercury resistance to serve as a marker of the vaccine strain. CVD103-HgR is safe when given in dosages up to 5×10^9 per dose. The safety profile of this vaccine is probably partly the result of the relatively poor colonization of the human intestine. Because the vaccine strain is live, the viability of the bacteria must be preserved while it is in storage, requiring maintenance of a cold chain, and the bacteria must be

protected from stomach acid, which is accomplished by coadministering the vaccine with a buffer.

Constituents of the Vaccine. CVD103-HgR, sold under the trade name Vaxchora, is presented as two packets. One contains the bacterial lyophilate mixed with excipients including sucrose, lactose, sodium dichloride, ascorbic acid, and casein hydrolysate, and the other contains a neutralized sodium bicarbonate buffer (2.16–2.41 g of sodium bicarbonate, ascorbic acid, and lactose). The vaccine is reconstituted by mixing the contents of both in 100 mL of purified water. Each dose of vaccine contains 4×10^8 to 2×10^9 colony-forming units (CFU) of CVD103-HgR.

Manufacture of the Vaccine. Vaxchora is produced by fermentation and harvesting by tangential flow filtration to preserve cell appendages. The bacterial concentrates are mixed with stabilizers, lyophilized, freeze-dried, and then milled into a fine powder and stored at −25° to −15°C.

Producers and Trade Names. Berna Biotech (Berne, Switzerland), and later Crucell, licensed and commercialized CVD103-HgR under the trade names Orochol (10^8 CFU per dose) for use in developed countries and Orochol E (10^9 CFU per dose) for use in developing countries. The vaccine is registered in multiple countries, but production was suspended in 2004. Recently, the U.S. manufacturer PAXVAX recommercialized this vaccine and licensed it in the United States under the trade name Vaxchora.

Dosage and Route. Vaxchora is given as a single oral dose along with a buffer salt to neutralize stomach acid. The effective dosage of live organisms for persons not living in cholera-affected countries is 4×10^8 to 2×10^9.

Availability in Combinations. The vaccine is not available in combination with any other vaccine.

Vaccine Stability. Vaxchora contains freeze-dried live bacteria; a cold chain (−25° to −15°C) is needed to preserve the vaccine's potency up to the point of reconstitution. The vaccine is administered within 15 minutes after reconstitution.

Immunogenicity and Immune Correlates of Protection. After vaccination with this live oral vaccine, the serum vibriocidal antibody response is the best measure of successful stimulation of antibacterial immunity, although, as noted earlier, serum vibriocidal antibodies are not an immune correlate or surrogate of vaccine protection. High titers of serum vibriocidal antibodies, as well as anti-LPS antibodies, are stimulated in most vaccinees given CVD103-HgR. Intestinal IgA antibodies are also produced against the LPS antigen. People who have not been previously exposed to cholera (naïve volunteers) respond well (fourfold or greater rise in vibriocidal antibody titers) to a dosage of 10^8 organisms, whereas volunteers from endemic areas require a higher dosage of 10^9 organisms to stimulate an equivalent response.[141–146] Serum antitoxin responses are seen, but the rises in titer are less consistent and of lower magnitude than for the antibodies directed against the bacterial cell wall antigens.[142] Serum vibriocidal responses have been noted to be lower in HIV-seropositive patients than in HIV-seronegative patients.[147] Prelicensure trials of Vaxchora have demonstrated a similar safety and immunogenicity profile to previously licensed versions of CVD103-HgR.[148,149]

Excretion and Transmissibility. In nonendemic populations, a minority of recipients of CVD103-HgR excrete the vaccine organism in feces.[150] Excretion is less common in persons residing in cholera-endemic settings.[151] A study in urban Indonesia showed that children who received CVD103-HgR did not transmit the vaccine organism to their siblings.[152]

Duration of Immunity and Protection. In a double-blind, placebo-controlled challenge study of efficacy done in the United States, 134 volunteers received a single oral dose of Vaxchora or placebo.[143] The vaccine was well tolerated. Ten days and 3 months later, subgroups of volunteers underwent challenge with virulent, frozen *V. cholerae* N16961, an O1 El Tor strain that was developed as a standard challenge strain and has been completely sequenced.[153,154] Efficacy against moderate to severe diarrhea was 90% at 10 days and 80% at 3 months. Other cholera challenge studies with earlier versions of CVD103-HgR demonstrated that protection begins as early as 8 days after vaccination and lasts for at least 6 months.[146]

In a randomized, placebo-controlled Phase III trial in Indonesia, among approximately 67,000 persons who received a single dose of Orochol or placebo, vaccine efficacy was 14%, a disparity with earlier North American volunteer challenge study results that remains to be convincingly explained.[155] Orochol E was also given in a mass immunization program during an outbreak of cholera in Pohnpei Island, Micronesia; a retrospective cohort study found that the vaccine conferred 79% protection.[156] In view of the observational design of this latter study and the disparity with the findings of the randomized, double-blind Indonesia trial, these findings require further confirmation.

Postexposure Prophylaxis. There is no indication for postexposure vaccine prophylaxis.

Safety. CVD103-HgR in its different license formulations has been well tolerated and is recognized as safe in persons aged 2 years and older. Monitored side effects, including abdominal pain, diarrhea, nausea, vomiting, fever, headache, and rash, were not notable different in vaccinees than in placebo recipients in prelicensure trials.[141,143–146,157,158] A small study by Perry and coworkers indicated that CVD103-HgR is safe in HIV-positive patients, although the subjects in this study were not severely immunodeficient.[147] Data are not available to address safety in pregnancy.

Indications. Based on data from North American volunteer challenge studies, Vaxchora is indicated in travelers ages 18 to 64 years visiting countries with active cholera transmission. Vaxchora has not been used in populations with endemic cholera.

Contraindications. The package insert for Vaxchora lists as contraindications: a history of severe allergic reactions to any ingredient of the product or to previous receipt of any cholera vaccine and receipt of oral or systemic antibiotics in the previous 14 days. Vaxchora should be given at least 10 days before beginning antimalarial prophylaxis with chloroquine.[142]

PUBLIC HEALTH CONSIDERATIONS

Epidemiologic Setting

For the public health use of modern cholera vaccines in developing countries, it is important to distinguish between endemic and epidemic cholera. For the control of epidemic cholera, selective vaccination of populations at a definably high risk of an epidemic, or reactive vaccination shortly after

the onset of the epidemic, can be considered.[159] Because populations experiencing epidemic cholera often have limited background natural immunity to cholera, vaccines must be effective in immunologically naïve persons, and they should target all age groups, as the risk of epidemic cholera tends to be age independent.[159]

For endemic cholera, vaccines should be able to immunize in the face of the background natural immunity to cholera that develops in recurrently exposed populations. Vaccination may target preschool and school-age children rather than adults in view of the higher risk of cholera in younger age groups in endemic settings. Long-term protection is more critical than early onset of protection after initiation of dosing for vaccines against endemic cholera. In contrast, early onset of protection after the first dose of vaccine would be of greater importance for vaccines used reactively in epidemic situations, and duration of protection would be of lesser importance.[153]

Vaccine Impact

Several studies of the preventive impact of OCVs under realistic public health conditions have been conducted. In a reactive vaccination program launched to control a large epidemic of cholera in Guinea, Shanchol vaccine, the vaccine currently stockpiled by WHO *(vide infra)* demonstrated 86% protection over 6 months against cholera.[160] When administered in programs to control stable, endemic cholera, Shanchol was demonstrated to confer 69% protection in rural India[161] and 53% protection in urban Bangladesh.[162,163]

Vaccine herd protection, protection of nonvaccinees and enhanced protection of vaccinees as a consequence of their proximity to neighboring vaccinees, can greatly augment the protective performance of vaccines given to populations. Data generated in field efficacy trials in Bangladesh with WC-rCTB and WC-only vaccines and in India with WC-only vaccine demonstrated vaccine herd protection.[164,165] This effect was attributed to a vaccine-induced reduction in transmission of cholera vibrios among the targeted population. In addition, in one trial killed OCVs afforded protection to unimmunized children of immunized mothers, most likely because of interruption of transmission from mother to child.[166] One mechanism for these observations is that these killed OCVs may reduce the load of fecally shed cholera vibrios from infected persons.[167]

An implication of these findings on vaccine herd protection is that killed OCVs may exert a major effect in the control of cholera, even in areas of modest coverage. Mathematical models based on the Bangladesh analyses suggest that the occurrence of cholera in a hyperendemic area of rural Bangladesh could be nearly extinguished at levels of vaccine coverage of approximately 60% with a vaccine having the features of the killed WC-only OCV Shanchol (Fig. 14.2).[168]

Nonetheless, vaccination should be considered as only one of several interventions to prevent cholera. Also critical are

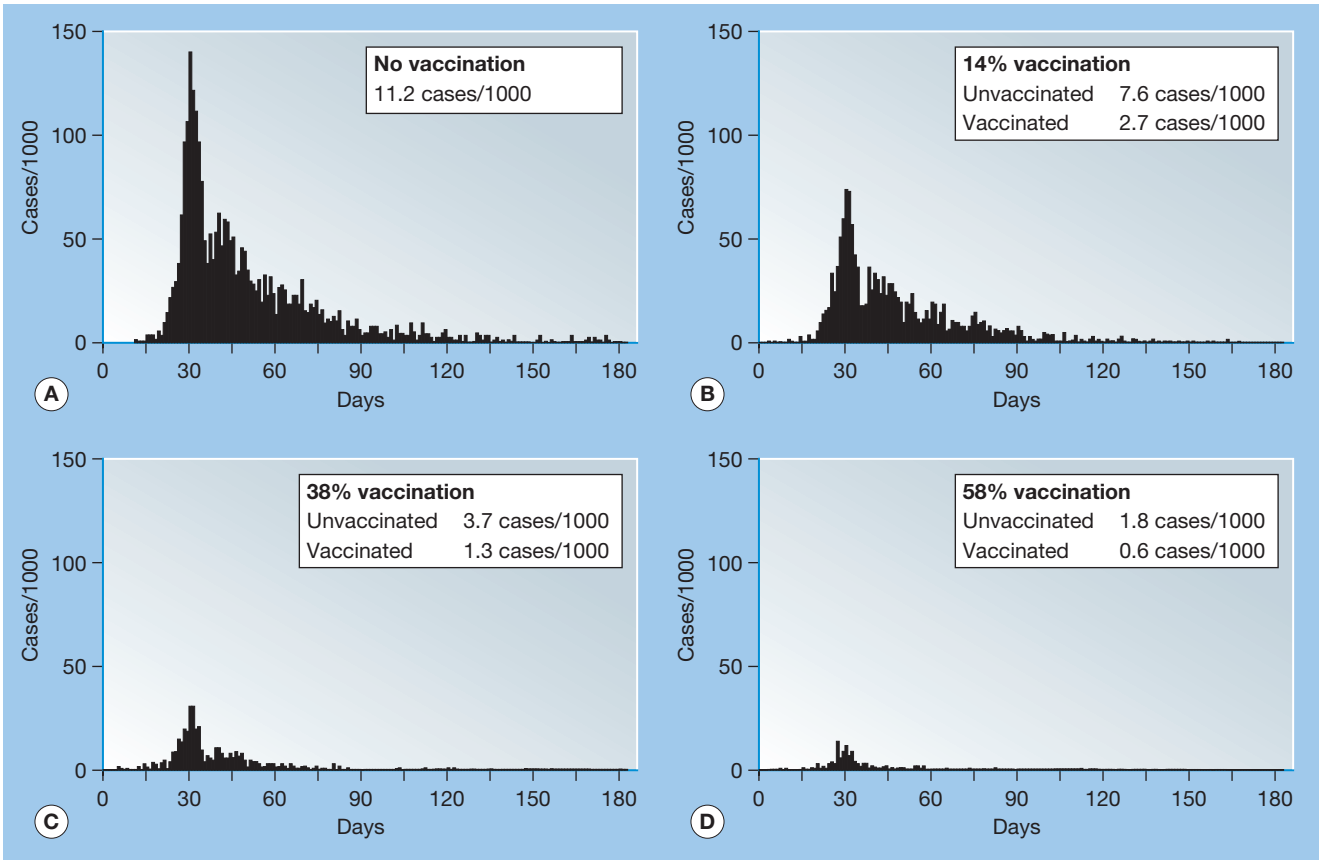


Figure 14.2. Simulated number of cholera cases per 1000 over a 180-day period in the study population in Matlab, Bangladesh, for a single stochastic realization. Mathematical modeling analyses suggest that the occurrence of cholera in a hyperendemic area of rural Bangladesh may be nearly extinguished at levels of vaccine coverage of approximately 60%. **A,** No vaccination; **B,** 14% vaccination coverage of women and children; **C,** 38% vaccination coverage; **D,** 58% vaccination coverage. *(From Longini Jr IM, Nizam A, Ali M, et al. Controlling endemic cholera with oral vaccines.* PLoS Med. *2007;4:e336.)*

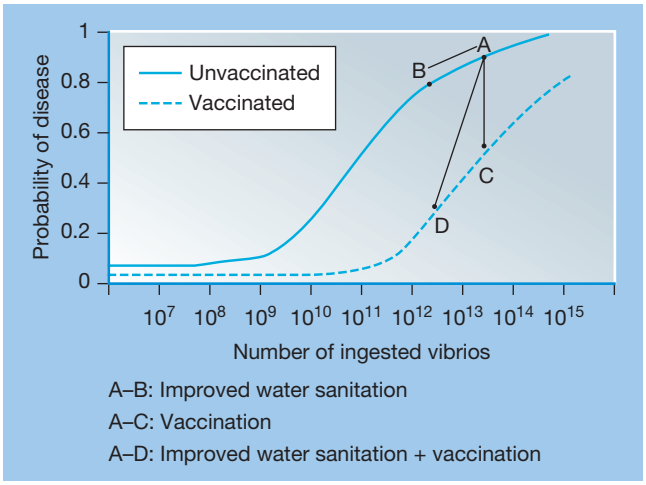


Figure 14.3. Hypothetical impact of oral cholera vaccine complemented by improvements in water quality, hygiene, and sanitation (WASH) on the risk of cholera. Graphic illustration showing relationship of ingested inoculum of cholera organisms to the probability of diarrhea after ingestion. The top curve corresponds to an unvaccinated individual and the bottom curve to a vaccinated individual. The top curve roughly describes the lower risk of symptomatic cholera after WASH interventions, which act to decrease the frequency and/or dose of ingestion of cholera vibrios (movement from state A to state B). The bottom curve reflects the same for vaccinated individuals (movement from state C to state D). The effect of oral cholera vaccines (OCVs) should be to decrease the probability of becoming ill at any (or at least most) ingested doses (movement from state A to state C). Because of these relationships the combined effects of WASH interventions and OCVs should produce a greater preventive effect than either intervention alone (movement from state A to state D). *(From Clemens J, Holmgren J. When, how, and where can oral cholera vaccines be used to interrupt cholera outbreaks?* Curr Top Microbiol Immunol. *2014;379:231–258.)*

complementary control measures to improve water quality, sanitation, and hygiene. There is a theoretical basis to consider these measures as synergistic with vaccination in preventing cholera (Fig. 14.3).

Feasibility and Cost Effectiveness

Logistical feasibility and programmatic acceptability must be considered in decisions regarding the public health use of OCVs. When given in campaigns, two-dose regimens of killed OCVs have been feasibly delivered in endemic settings in Bangladesh, India, Vietnam, and Mozambique.[162,163,169–171] Also, two-dose regimens of WC-rCTB vaccine have been feasibly delivered in refugee camps and during complex emergencies in Uganda and Indonesia.[156,159,172] Because WC-only vaccine is less expensive and does not require the coadministration of buffer, vaccine can be delivered in a faster and less cumbersome manner, as demonstrated in recent preventive and reactive campaigns in Bangladesh, India, Haiti, South Sudan, and Guinea.[66,162,173] The live OCV CVD103-HgR was feasibly delivered in a reactive vaccine effort to contain a cholera epidemic in Micronesia.[156] A published cost-effectiveness analysis of the use of OCVs, which accounted for both the direct and herd vaccine protective effects of Shanchol and used local data from the field on cholera incidence and costs, found the vaccine to be very cost-effective or cost-effective by WHO standards in Kolkata, India; Beira, Mozambique; Matlab, Bangladesh; and Jakarta, Indonesia, depending on the age group targeted.[174] In addition, a recent systematic investment analysis found the vaccine to be very cost-effective in more than 30 countries projected to be early OCV adopters.[175]

Travelers

The rationale for recommending cholera vaccine for travelers and military personnel is similar to that for several other travelers' vaccines—prevention of an unlikely but potentially severe illness. Because OCVs are safe and easily administered, many travelers are likely to welcome the vaccines, especially versions that are also capable of preventing some cases of ETEC diarrhea.[47,121] The Canadian Committee to Advise on Tropical Medicine and Travel has indicated that vaccination with the killed WC-rCTB vaccine, Dukoral, for traveler's diarrhea is of limited value but may be of use for selected, short-term, high-risk travelers older than age 2 years.[176] The U.S. Advisory Committee on Immunization Practices recommends the live oral cholera vaccine, Vaxchora, for adults aged 18 to 64 years traveling to areas with active cholera transmission.

World Health Organization Oral Cholera Vaccine Stockpile

The WHO issued a revised recommendation in 2010 stating that OCVs should be used in areas where cholera is endemic, and should be considered for use in areas at risk for cholera outbreaks (preemptive vaccination), as well as in those experiencing such outbreaks (reactive vaccination).[59] Currently, only Vietnam uses an OCV, produced locally, given in the past in response to epidemics of cholera.[177] Until recently, safe and effective OCVs have failed to be embraced as public health tools for developing countries. Reasons included vaccine expense, moderate levels of vaccine protection, the logistic challenges associated with storage and administration, as well as concerns that vaccine delivery might interfere with other cholera control efforts. In 2011, the 64th World Health Assembly called for an integrated comprehensive strategy, recommending the use of OCV "where appropriate, in conjunction with other recommended prevention and control methods."[178] In 2013, a 2-million dose OCV stockpile was created by WHO as a tool to help control cholera epidemics. Later in the year, the Gavi alliance announced the decision to invest U.S. $115 million over 5 years to fund and expand the stockpile to 20 million doses by 2018 with hopes of breaking the current cycle of low vaccine demand leading to low vaccine supply. The stockpile, which must deploy a WHO prequalified OCV, currently deploys Shanchol because of its relatively low cost and its ease of administration. At the time of this writing, approximately 3.5 million doses of the stockpiled vaccine Shanchol have been used in outbreaks in the African, South East Asian, and Western Pacific regions, and Haiti.[179] Reported levels of protective effectiveness of the vaccine in these settings have ranged from 63% to 87%.[160,180]

FUTURE VACCINES

New Killed Whole Cell–Only Cholera Vaccines

As noted earlier, current killed whole cell–based vaccines consist of a combination of several different inactivated *V. cholerae* strains, some killed by heat, others by formalin. Fermentation of several strains for each vaccine dose is a source of the cost of this vaccine. To circumvent this manufacturing hurdle, workers at the University of Goteborg have genetically engineered an El Tor strain that expresses both Inaba and Ogawa antigens. This Hikojima-like strain is formalin-inactivated, and induces promising serum anti-LPS and vibriocidal antibody

responses in mice.[181] If safe and protective in humans, this single strain vaccine would enable much-less-expensive manufacturing and substantial reductions in vaccine cost.

Live Oral Vaccines Against O1 Serogroup Cholera

Several live oral attenuated vaccine strains against O1 serogroup cholera are under development. Among these is Peru 15, derived by genetically attenuating an O1 El Tor Inaba strain isolated in Peru in 1991. The attenuating mutations include deletion of the entire CT cassette, including flanking recombination sites, and inactivation of the *recA* gene by inserting the coding region for CTB under control of a heat-shock promoter, thereby making the vaccine incapable of incorporating homologous DNA by *recA*-mediated recombination. The strain is also motility deficient. The vaccine was proven safe and highly efficacious in a challenge study of U.S. volunteers, where five of 12 placebo recipients (42%) and none of 24 vaccinees (0%) had moderate or severe diarrhea.[182,183] Placebo-controlled trials conducted among Bangladeshi adults, children, and infants found a single-dose (10^8 CFU) regimen to be safe and immunogenic.[184,185] Cuban investigators have developed *V. cholerae* O1 vaccine strain 638, attenuated by deletion of the CTXPhi prophage and inactivation of the hemagglutinin/protease coding sequences *(hapA)* in an El Tor Ogawa strain. This vaccine was well tolerated in healthy Cuban volunteers, and, in a randomized, double-blind, placebo-controlled trial, strain 638 conferred 100% protective efficacy in a small group of Cuban volunteers who underwent experimental challenge with El Tor *V. cholerae* 01 a month after vaccination.[186] Large-scale production of this vaccine strain has been initiated.[187]

Chinese investigators have constructed a vaccine candidate, designated IEM 108, that consists of an El Tor Ogawa strain naturally deficient in CTXPhi and that contains a *ctxB* gene and an *rstR* gene; the latter precludes acquisition of CTXPhi by the vaccine.[188] Genes *ctxB* and *rstR* are stably maintained by a balanced lethal system based on the gene *thyA*. This strain was immunogenic in rabbits and conferred full protection against wild-type challenges in rabbits.[189]

Investigators in India have developed a vaccine candidate from a clinical nontoxigenic strain of El Tor biotype *V. cholerae* O1, serotype Inaba, which is devoid of the CTX prophage. Through a series of genetic manipulations, *ctxB*, which encodes CTB, was introduced into a cryptic hemolysin locus of the strain. A gene for ampicillin resistance was also introduced as a marker. The resulting strain, termed VA1.3, was found to be safe and immunogenic as a single dose of 10^9 CFU in adult volunteers in India.[190] It has been reformulated and subsequently renamed as VA 1.4.

Live Oral Vaccines Against O139 Serogroup Cholera

Two live oral vaccines against O139 cholera have been tested in volunteers. One O139 vaccine strain, named Bengal 15, has genetic mutations similar to Peru 15, but was produced from a strain of the O139 serogroup. Early trials of freshly harvested vaccine organisms found the vaccine to be safe and efficacious in small numbers of U.S. volunteers.[191] CVD112, another live attenuated strain against O139 cholera, has also been shown to be safe and efficacious in U.S. volunteers.[192] Building on the success of O1 vaccine strain 638, Cuban investigators have constructed an analogous vaccine candidate with an O139 strain. The prototype strain includes a spontaneous deletion in CTXPhi and deletions in *hapA* and *thyA*.[193] This vaccine candidate will undergo clinical studies.

Parenteral Vaccines

A detoxified LPS-protein conjugate vaccine for cholera for parenteral use has been developed and tested in Phase I human studies. This vaccine consists of *V. cholerae* O1 Inaba LPS bound to CT. In volunteers, this vaccine stimulated serum vibriocidal antibodies that persisted longer than those elicited by a killed whole-cell parenteral vaccine, and it stimulated anti-CT antibodies.[194] Other groups have used this strategy to develop a parenteral conjugate vaccine against either O1 or O139 cholera, coupling the *V. cholerae* LPS plus core to tetanus toxoid or a diphtheria toxin mutant. After multiple doses in mice, these vaccine candidates elicited anticapsular polysaccharide and anti-LPS antibodies with vibriocidal activity and were protective in the neonatal mouse model of cholera infection.[195–197]

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15 Combination Vaccines

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The combining of multiple antigens into a single vaccine is not a new concept; combination vaccines have long been a bedrock of immunization programs. Since the early 1990s, we have transitioned from a world in which DTP (diphtheria, tetanus, pertussis) and MMR (measles, mumps, rubella) represented essentially the only combination vaccines in use to one in which even developing countries are routinely using modern combination vaccines providing protection against DTP, hepatitis B, *Haemophilus influenzae* type b, polio, pneumococcus, and various meningococcal serogroups. The field of combination vaccines has matured to the point where a large number of combination vaccines are available with relatively few in development. These efforts, undertaken by many manufacturers and research groups worldwide, have been driven by the recognition that the continual increase in the number of effective childhood vaccines posed substantial economic and logistic difficulties. Providing these vaccines as separate injections requires multiple needlesticks, leading to distressed parents, providers, and vaccinees. Scheduling additional vaccination visits to reduce the number of injections per visit increases costs, burdens staff, and jeopardizes the entire immunization program by increasing the likelihood of missed vaccinations. The shipping, handling, storage, and accountability of a plethora of vaccines are burdensome and expensive and increase the possibility of error. These issues have stimulated continuing efforts to provide new combination vaccines. However, the development and evaluation of combination vaccines can pose numerous issues, as discussed further in this chapter and reviewed elsewhere.[1,2]

The combination vaccines in common use before the 1990s included diphtheria and tetanus toxoids, available alone (DT or Td) or combined with whole-cell (DTwP) pertussis vaccine; inactivated (IPV) or live oral (OPV) trivalent poliovirus vaccine; and measles and rubella vaccine, available alone (MR) or with mumps vaccine (MMR).

The first combination vaccine licensed in the United States was trivalent influenza vaccine, approved in November 1945, and the second was a hexavalent polysaccharide pneumococcal vaccine licensed in 1947.[3] Although developed in 1943, DTwP was not licensed until March 1948. IPV was licensed in 1955. Although the individual OPV serotypes were licensed from 1961 to 1962, efforts to overcome interference seen with simultaneous administration of three attenuated live polioviruses delayed the licensure of trivalent OPV until June 1963. MMR and MR were licensed in April 1971, and quadrivalent polysaccharide meningococcal vaccine in 1978. Only brief mention is made in this chapter of these traditional combination vaccines, which are discussed in their respective chapters.

Most modern pediatric combination vaccines begin with a DTwP or DT/acellular pertussis (DTaP) vaccine and add such antigens as IPV, conjugate *H. influenzae* type b (Hib) and hepatitis B (HepB). As development efforts for the DTaP-based combinations have matured, some manufacturers have turned their efforts toward developing so-called second-shot or companion combinations designed to be given in coordination with a DTaP-based combination, incorporating, for example, conjugate meningococcal antigens. A third developmental stream has been directed toward combination vaccines targeted principally at travelers, typically based on HepB or hepatitis A (HepA) components.

Our focus in this chapter is on the most relevant current combination vaccines, which merge products such as IPV, HepB, Hib, or meningococcal vaccines with each other or with one or more of the aforementioned traditional combination vaccines. Readers interested in combination vaccines of historical interest (e.g., yellow fever/smallpox) or combinations based on components no longer available (e.g., Acel-Imune, PRP-D [polyribosylribitol phosphate-diphtheria toxoid conjugate]) are referred to the 2003 edition of this text.[4]

TERMINOLOGY

The vaccine industry has undergone dramatic consolidation during the past 20 years; long-established companies and new biotechnology startups have been acquired or merged. These changes render nomenclature problematic. For vaccines currently marketed, we will use the name of the current manufacturer, even when describing studies conducted by a predecessor company. For products not presently marketed, we will use the name of the company that produced them, even if that company now is owned or operated under a successor name. To further assist readers, Table 44.6 lists most current major vaccine manufacturers, along with the names of predecessor, component, or acquired companies.

As Box 15.1 shows, the use in this chapter of a virgule to coordinate the names of two vaccines (e.g., DTwP/IPV) indicates a combination of the two vaccines; a plus sign (e.g., DTwP + IPV) indicates concurrent but separate administration. When discussing a specific combination vaccine, the use of a single virgule indicates that the vaccine is supplied with the component following the virgule premixed with the component preceding the virgule; the use of a double virgule (e.g., DTaP//Hib) indicates that the component following the double virgule is reconstituted using the liquid components that precede the double virgule.

Because so many different combination vaccines are available within some classes (e.g., DTaP/Hib) and simple, unambiguous generic names do not exist for the various products, trade names are used whenever possible to refer to specific combination vaccines.

Table 15.1 outlines the current status of the newer combination vaccines, and Table 15.2 provides further details concerning those that are available.

PRINCIPLES OF COMBINED VACCINES

Simultaneous Vaccines

A combination vaccine consists of two or more separate immunogens that have been physically combined in a single preparation. This concept differs from that of simultaneous vaccines, which, although administered concurrently, are physically separate (i.e., injected at separate sites or given by

Text continued on p. 204

BOX 15.1 Key to Typography

Symbol	Meaning
Plus sign (+)	The vaccines linked by the plus sign are administered at separate sites during a single vaccination session. Example: **DTaP** + **Hib**, two separate vaccines given separately but at the same visit.
Single virgule (/)	The vaccines linked by a single virgule are premixed from the manufacturer and are administered in a single injection. Example: **DTaP/Hib**; the two vaccines are shipped by the manufacturer mixed together in a single container.
Double virgule (//)	The liquid vaccine(s) preceding the double virgule are used to reconstitute the dry vaccine following the double virgule. Example: **DTaP//Hib**; the manufacturer ships together a container of liquid DTaP and a container of powdered Hib, with the DTaP used to reconstitute the Hib and the resulting mixture administered as a single injection.

DTaP, diphtheria, tetanus toxoids, and acellular pertussis; Hib, *Haemophilus influenzae* type b.

TABLE 15.1 Combination Vaccines Currently Available or Under Development[a]

	Combination Vendors			
Vaccines Combined[b]	**In European Union**	**In U.S.**	**In Other Countries**	**Combination in Development**
Td/IPV	SPMSD, SP		SP	
DTwP/Hib	GSK		GSK, SII	
DTwP/HepB			Bio Farma, Biological E, SII	
DTwP/HepB/Hib			Bio Farma, Biological E, J&J, GSK, LG Life Sciences, Panacea, SII, SP	
DTwP/HepB/Hib/IPV				Biological E/GSK, SII, SP
DTaP/IPV	GSK, SP, SPMSD, SSI	GSK	GSK, SP	
Tdap/IPV	GSK, SP, SPMSD		GSK, SP	
DTaP/Hib	GSK		GSK	
DTaP/IPV/Hib	GSK, SP, SPMSD, SSI	SP	GSK, SP	
DTaP/IPV/HepB	GSK	GSK	GSK	
DTaP/IPV/HepB/Hib	GSK, SP, SPMSD	MCM	GSK, SP	
HepB/HepA	GSK	GSK	GSK	
HepA/Typhoid	GSK, SPMSD		GSK, SP	
MMRV	GSK, SPMSD	Merck	GSK, Merck	
MnC/Hib	GSK			
MnCY/Hib		GSK		
PhtD/PD/dPly				GSK

Note: While this text was in press, Merck and Sanofi Pasteur announced that their European joint venture, SPMSD, was being dissolved; in the future, products marketed by SPMSD would instead be marketed by their respective manufacturers.

aP, acellular pertussis vaccine (infant formulation); ap, acellular pertussis vaccine (adolescent/adult formulation); D, diphtheria toxoid vaccine (infant formulation); d, diphtheria toxoid vaccine (adolescent/adult formulation); GSK, GlaxoSmithKline; HepA, hepatitis A vaccine; HepB, hepatitis B vaccine; Hib, conjugate *Haemophilus influenzae* type b vaccine; IPV, enhanced inactivated trivalent poliovirus vaccine; J&J, Johnson and Johnson; MCM, Merck Connaught Merieux (joint venture of Merck and Sanofi Pasteur); MMRV, measles, mumps, rubella, and varicella vaccine; Mn, meningococcal conjugate vaccine (included serotypes are shown by following letter[s], e.g., MnC, MnCY); PhtD/PD/dPly, pneumococcal histidine triad protein D, nontypeable *H. influenzae*, and detoxified pneumolysin; SII, Serum Institute of India; SP, Sanofi Pasteur; SPMSD, Sanofi Pasteur MSD; SSI, Statens Seruminstitut; T, tetanus toxoid vaccine; wP, whole-cell pertussis vaccine. See Table 44.6 for information on prior names of pharmaceutical companies.

[a]Products combining only multiple serotypes of a single pathogen are excluded, as are DT (diphtheria and tetanus toxoids–pediatric), Td (diphtheria and tetanus toxoids–adult), DTP (diphtheria, tetanus, pertussis), DTaP (diphtheria, tetanus toxoids, and acellular pertussis), Tdap (tetanus, diphtheria, and pertussis), OPV (oral polio vaccine), IPV (inactivated polio vaccine), and MMR (measles, mumps, rubella). Only those manufacturers who distribute their products internationally are listed; other manufacturers may produce some products (e.g., DTP/IPV) for local or regional use. Some products represent components derived from, or joint efforts of, more than one manufacturer; in such cases, their principal distributor is shown.

[b]No discrimination is made between products distributed in combined form and those distributed in separate containers, for combination at the time of use.

TABLE 15.2 Combination Vaccines Currently Available in Canada, European Union, or United States[a]

	Combination	Trade Name	Source	Each 0.5-mL Dose (After Reconstitution, if Applicable) Is Formulated to Contain (See Listing by Country, Below, for Specific Information Regarding Composition)
	HepB/HepA	*Ambirix*	GSK	
	Tdap/IPV	*Boostrix IPV*	GSK	
	Tdap/IPV	*Boostrix Polio*	GSK	
	DTaP/IPV	*DiTeKiPol*	SSI	
	DTaP/IPV/Hib	*DiTeKiPol/Act-Hib*	SSI	
	HepA/Typhoid	*Hepatyrix*	GSK	
	DTaP/IPV/HepB//Hib	*Hexacima*	SPMSD	
	DTaP/IPV/HepB//Hib	*Hexaxim*	SPMSD	
	DTaP/IPV/HepB//Hib	*Hexyon*	SPMSD	
	DTaP/IPV/HepB//Hib	*Infanrix hexa*	GSK	
	DTaP/Hib	*Infanrix Hib*	GSK	
	DTaP/IPV	*Infanrix IPV*	GSK	
	DTaP/IPV/Hib	*Infanrix IPV Hib*	GSK	
	DTaP/IPV/HepB	*Infanrix Penta*	GSK	
	DTaP/IPV/Hib	*Infanrix Quinta*	GSK	
	DTaP/IPV	*Infanrix Tetra*	GSK	
	DTaP/IPV	*KINRIX*	GSK	
	MnCY/Hib	*MenHibrix*	GSK	
	MnC/Hib	*Menitorix*	GSK	
	DTaP/IPV/Hib	*Pediacel*	SPMSD	
	DTaP/IPV/HepB	*Pediarix*	GSK	
	DTaP/IPV//Hib	*Pentacel*	SP	
	DTaP/IPV/Hib	*Pentavac*	SPMSD	
	DTaP/IPV/Hib	*Pentaxim*	SPMSD	
	MMRV	*Priorix Tetra*	GSK	
	MMRV	*ProQuad*	Merck	
	DTaP/IPV	*Quadracel*	SP	
	DTwP/Hib	*QuattVaxem*	Novartis	
	Tdap/IPV	*Repevax*	SPMSD	
	Td/IPV	*Revaxis*	SPMSD	
	Td/IPV	*Td Polio Adsorbed*	SP	
	DTaP/IPV	*Tetravac*	SPMSD	
	HepB/HepA	*Twinrix*	GSK	
	HepB/HepA	*Twinrix Adult*	GSK	
	HepB/HepA	*Twinrix Junior*	GSK	
	HepB/HepA	*Twinrix pediatric*	GSK	
	HepA/Typhoid	*Tyavax*	SP	
	HepA/Typhoid	*ViATIM*	SP	
	HepA/Typhoid	*Vivaxim*	SP	
CANADA				
	Td/IPV	*Td Polio Adsorbed*	SP	2 Lf DT, 5 Lf TT, poliomyelitis vaccine types 1 (Mahoney, 40D), 2 (MEF-1, 8D) and 3 (Saukett, 32D), 1.5 mg $AlPO_4$, trace polymyxin B and neomycin, 27 ppm formaldehyde, and 0.5% 2-PE
	DTaP/IPV	*Quadracel*	SP	20 μg PT, 20 μg FHA, 3 μg PRN, 5 μg FIM types 2 and 3, 15 Lf DT, 5 Lf TT, poliomyelitis vaccine types 1 (Mahoney, 40D), 2 (MEF-1, 8D) and 3 (Saukett, 32D), 0.6% ± 0.1% (v/v) 2-PE, 1.5 mg $AlPO_4$, trace polymyxin B and neomycin

TABLE 15.2 Combination Vaccines Currently Available in Canada, European Union, or United States[a] *(Continued)*

Combination	Trade Name	Source	Each 0.5-mL Dose (After Reconstitution, if Applicable) Is Formulated to Contain (See Listing by Country, Below, for Specific Information Regarding Composition)
	Infanrix IPV	GSK	At least 30 IU DT, at least 40 IU TT, 25 μg PT, 25 μg FHA, 8 μg PRN, poliomyelitis vaccine types 1 (Mahoney, 40D), 2 (MEF-1, 8D), and 3 (Saukett, 32D); 0.5 mg $Al(OH)_3$, 2.5 mg 2-PE
DTaP/IPV/Hib	*Pediacel*	SP	20 μg PT, 20 μg FHA, 3 μg PRN, 5 μg FIM types 2 and 3, 15 Lf DT, 5 Lf TT, poliomyelitis vaccine types 1 (Mahoney, 40D), 2 (MEF-1, 8D) and 3 (Saukett, 32D), 10 μg PRP conjugated to 20 μg TT, 0.6% ± 0.1% (v/v) 2-PE, 1.5 mg $AlPO_4$; trace streptomycin, polymyxin B, and neomycin may be present
	Infanrix IPV Hib	GSK	25 Lf (30 IU) DT, 10 Lf (40 IU) TT, 25 μg PT, 25 μg FHA, 8 μg PRN, 40 D of type 1 poliovirus, 8 D type 2 poliovirus, 32 D type 3 poliovirus, 10 μg of adsorbed purified PRP covalently bound to 20–40 μg TT per 0.5 mL dose; aluminum adjuvant (as aluminum salts), residual formaldehyde, polysorbate (Tween) 20 and 80, M199, glycine, neomycin sulfate, and polymyxin B sulfate
DTaP/IPV/HepB/Hib	*Infanrix hexa*	GSK	25 Lf (30 IU) DT, 10 Lf (40 IU) TT, 25 μg PT, 25 μg FHA, 8 μg PRN, 10 μg HBsAg, 40 D of type 1 poliovirus, 8 D type 2 poliovirus, 32 D type 3 poliovirus, 10 μg of adsorbed purified PRP covalently bound to 20–40 μg TT per 0.5 mL dose; aluminum adjuvant (as aluminum salts), residual formaldehyde, polysorbate (Tween) 20 and 80, M199, glycine, neomycin sulfate, and polymyxin B sulfate
Tdap/IPV	*Boostrix Polio*	GSK	Not less than 2.5 Lf, or 2 IU of DT; not less than 5 Lf (20 IU) of TT; 8 μg of PT, 8 μg of FHA, 2.5 μg of pertactin (69 kDa outer membrane protein), 40 DU of type 1 poliovirus, 8 DU type 2 polio virus, and 32 DU type 3 polio virus; aluminum adjuvant (as aluminum salts), sodium chloride, water for injection, and medium 199. Formaldehyde, neomycin, and polymyxin are present as traces
HepA/Typhoid	*Vivaxim*	SP	A **1-mL dose** contains 25 μg of the Vi polysaccharide of *Salmonella typhi* and 160 antigen units of inactivated HA viral antigen
HepB/HepA	*Twinrix Junior*	GSK	Not less than 360 EU inactivated HepA virus and 10 μg recombinant HBsAg protein; 0.025 mg $Al(OH)_3$, 0.2 mg $AlPO_4$, 2.5 mg 2-PE
	Twinrix Adult	GSK	A **1-mL dose** contains not less than 720 EU inactivated HA virus and 20 μg recombinant HBsAg protein; 0.05 mg $Al(OH)_3$, 0.4 mg $AlPO_4$, 5.0 mg 2-PE
MMRV	*Priorix Tetra*	GSK	$\geq 10^{3.0}$ $CCID_{50}$ live attenuated measles virus (Schwarz strain); $\geq 10^{4.4}$ $CCID_{50}$ live attenuated mumps virus (RIT 4385 strain, derived from Jeryl Lynn strain); $\geq 10^{3.0}$ $CCID_{50}$ live, attenuated rubella virus (Wistar RA 27/3 strain); $\geq 10^{3.3}$ PFU live attenuated varicella virus (OKA strain); amino acids, lactose, mannitol, sorbitol, neomycin sulfate residue, water for injection
	ProQuad (refrigerated version)	Merck	≥3.00 $\log_{10}$ $TCID_{50}$ of measles virus; 4.30 $\log_{10}$ $TCID_{50}$ of mumps virus; 3.00 $\log_{10}$ $TCID_{50}$ of rubella virus; ≥3.99 $\log_{10}$ PFU of Oka/Merck varicella virus, 20 mg sucrose, 11 mg hydrolyzed gelatin, 2.5 mg urea; 2.3 mg NaCl, 16 mg sorbitol, 0.38 mg monosodium L-glutamate, 0.14 mg sodium phosphate, 0.25 mg human albumin, 0.13 mg sodium bicarbonate, 94 μg potassium phosphate, 58 μg of potassium chloride; residual components of MRC-5 cells including DNA and protein; 5 μg neomycin, bovine serum albumin (0.5 μg), and other buffer and media ingredients
EUROPEAN UNION			
Td/IPV	*Revaxis*	SPMSD	At least 2 IU DT, 20 IU TT, and poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU) and 3 (Saukett, 32 DU); $Al(OH)_3$, 2-PE
DTwP/Hib	*QuattVaxem*	Novartis	At least 30 IU DT, 60 IU TT, 2 IU wP, 10 μg PRP conjugated to 25 μg CRM_{197}, 1.36 mg $AlPO_4$, 0.05 mg thimerosal
DTaP/IPV	*DiTeKiPol*	SSI	At least 30 IU (25 Lf) DT, at least 40 IU (7 Lf) TT, 40 μg PT, and at least 60% of the following poliovirus amounts: type 1 (Brunhilde), 40 DU; type 2 (MEF-1), 8 DU; type 3 (Saukett), 32 DU; 1 mg aluminum (as hydroxide), 0.3 mg sodium dihydrogen phosphate dihydrate, <25 μg formaldehyde, <0.2 μg neomycin, trace phenolsulfonphthalein
	Infanrix IPV, Infanrix Tetra	GSK	At least 30 IU DT, at least 40 IU TT, 25 μg PT, 25 μg FHA, 8 μg PRN, poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU), and 3 (Saukett, 32 DU); 0.5 mg $Al(OH)_3$, 2.5 mg 2-PE

Continued on following page

TABLE 15.2 Combination Vaccines Currently Available in Canada, European Union, or United States[a] *(Continued)*

Combination	Trade Name	Source	Each 0.5-mL Dose (After Reconstitution, if Applicable) Is Formulated to Contain (See Listing by Country, Below, for Specific Information Regarding Composition)
	Tetravac	SPMSD	At least 30 IU DT, at least 40 IU TT, 25 μg PT, 25 μg FHA, poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU), and 3 (Saukett, 32 DU); 0.3 mg $Al(OH)_3$, 2.5 μL 2-PE, 12.5 μg formaldehyde
Tdap/IPV	*Boostrix IPV* *Boostrix Polio*	GSK	2 Lf DT, 5 Lf TT, 8 μg PT, 8 μg FHA, 2.5 μg PRN, poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU) and 3 (Saukett, 32 DU), 0.6% ± 0.1% (v/v) 2-PE, 0.39 mg Al (as $Al(OH)_3$ and $AlPO_4$), trace formaldehyde, polymyxin B and neomycin
	Repevax	SPMSD	2 Lf DT, 5 Lf TT, 2.5 μg PT, 5 μg FHA, 3 μg PRN, 5 μg FIM types 2 and 3, poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU) and 3 (Saukett, 32 DU), 0.6% ± 0.1% (v/v) 2-PE, 1.5 mg $AlPO_4$, trace polymyxin B and neomycin
DTaP/Hib	*Infanrix Hib*	GSK	At least 30 IU DT, at least 40 IU TT, 25 μg PT, 25 μg FHA, 8 μg PRN, and 10 μg PRP conjugated to TT; 0.5 mg $Al(OH)_3$, 2.5 mg 2-PE
DTaP/IPV/Hib	*DiTeKiPol/Act-Hib*	SSI	At least 30 IU (25 Lf) DT, at least 40 IU (7 Lf) TT, 40 μg PT, at least 60% of the following poliovirus amounts: type 1 (Brunhilde), 40 DU; type 2 (MEF-1), 8 DU; type 3 (Saukett), 32 DU, and 10 μg *Haemophilus influenzae* type b polysaccharide conjugated to TT; 1 mg aluminum (as hydroxide), 0.3 mg sodium dihydrogen phosphate dihydrate, <25 μg formaldehyde, <0.2 μg neomycin, trace phenolsulfonphthalein, trometamol
	Infanrix IPV Hib, *Infanrix Quinta*	GSK	At least 30 IU DT, at least 40 IU TT, 25 μg PT, 25 μg FHA, 8 μg PRN, poliomyelitis vaccine types 1 (Mahoney, 40D), 2 (MEF-1, 8D), and 3 (Saukett, 32D), and 10 μg PRP conjugated to TT; 0.5 mg $Al(OH)_3$, 2.5 mg 2-PE
	Pediacel	SPMSD	20 μg PT, 20 μg FHA, 3 μg PRN, 5 μg FIM types 2 and 3, 15 Lf DT, 5 Lf TT, poliomyelitis vaccine types 1 (Mahoney, 40D), 2 (MEF-1, 8D) and 3 (Saukett, 32D), 10 μg PRP conjugated to 20 μg TT, 0.6% ± 0.1% (v/v) 2-PE, 1.5 mg $AlPO_4$; trace streptomycin, polymyxin B, and neomycin may be present
	Pentavac, *Pentaxim*	SPMSD	At least 30 IU DT, at least 40 IU TT, 25 μg PT, 25 μg FHA, poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU), and 3 (Saukett, 32 DU), and 10 μg *H. influenzae* type b polysaccharide conjugated to TT; 0.3 mg $Al(OH)_3$, 2.5 μL 2-PE, 12.5 μg formaldehyde
DTaP/IPV/HepB	*Infanrix Penta*	GSK	At least 30 IU DT, at least 40 IU TT, 25 μg PT, 25 μg FHA, 8 μg PRN, poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU), and 3 (Saukett, 32 DU), and 10 μg recombinant HBsAg protein; 0.5 mg $Al(OH)_3$, 0.2 mg $AlPO_4$, 2.5 mg 2-PE
DTaP/IPV/HepB//Hib	*Infanrix hexa*	GSK	At least 30 IU DT, at least 40 IU TT, 25 μg PT, 25 μg FHA, 8 μg PRN, poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU), and 3 (Saukett, 32 DU), 10 μg recombinant HBsAg protein, and 10 μg PRP conjugated to TT; 0.5 mg $Al(OH)_3$, 0.32 mg $AlPO_4$, 2.5 mg 2-PE
	Hexyon, Hexaxim, *Hexacima*	SPMSD	Pertussis (FHA 25 μg; PT 25 μg), DT >20 IU, TT ≥40 IU, *H. influenzae* type b polysaccharide 12 μg conjugated to tetanus protein 22–36 μg, poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU), and 3 (Saukett, 32 DU), 10 μg recombinant hepatitis B antigen, 0.6 mg Al^{3+} as hydrated aluminum hydroxide, Disodium hydrogen phosphate, dihydrogen potassium phosphate, trometamol, saccharose, essential amino acids including L-phenylalanine, and traces of glutaraldehyde, formaldehyde, neomycin, streptomycin, and polymyxin B which are used during the manufacturing process
HepB/HepA	*Twinrix pediatric*	GSK	Not less than 360 EU inactivated HA virus and 10 μg recombinant HBsAg protein; 0.025 mg $Al(OH)_3$, 0.2 mg $AlPO_4$, 2.5 mg 2-PE
	Ambirix (2-dose pediatric series)	GSK	A **1-mL dose** contains not less than 720 EU inactivated HA virus and 20 μg recombinant HBsAg protein; 0.05 mg $Al(OH)_3$, 0.4 mg $AlPO_4$, 5.0 mg 2-PE
	Twinrix adult	GSK	A **1-mL dose** contains not less than 720 EU inactivated HA virus and 20 μg recombinant HBsAg protein; 0.05 mg $Al(OH)_3$, 0.4 mg $AlPO_4$, 5.0 mg 2-PE
HepA/Typhoid	*Hepatyrix*	GSK	A **1-mL dose** contains 25 μg of the Vi polysaccharide of *Salmonella typhi* and not less than 1440 ELISA units of inactivated HA viral antigen; 0.5 mg $Al(OH)_3$, 5.0 mg 2-PE

TABLE 15.2 Combination Vaccines Currently Available in Canada, European Union, or United States[a] *(Continued)*

Combination	Trade Name	Source	Each 0.5-mL Dose (After Reconstitution, if Applicable) Is Formulated to Contain (See Listing by Country, Below, for Specific Information Regarding Composition)
	ViATIM, Vivaxim, Tyavax	SP	A **1-mL dose** contains 25 μg of the Vi polysaccharide of *S. typhi* and 160 antigen units of inactivated HA viral antigen
MnC/Hib	*Menitorix*	GSK	5 μg *Neisseria meningitidis* serogroup C (strain C11) polysaccharide conjugated to 5 μg TT; 5 μg PRP conjugated to 12.5 μg TT
MMRV	*Priorix Tetra*	GSK	$\geq 10^{3.0}$ $CCID_{50}$ live attenuated measles virus (Schwarz strain); $\geq 10^{4.4}$ $CCID_{50}$ live attenuated mumps virus (RIT 4385 strain, derived from Jeryl Lynn strain); $\geq 10^{3.0}$ $CCID_{50}$ live, attenuated rubella virus (Wistar RA 27/3 strain); $\geq 10^{3.3}$ PFU live attenuated varicella virus (OKA strain); amino acids, lactose, mannitol, sorbitol, neomycin sulfate residue, water for injection
	ProQuad (Refrigerated version)	SPMSD	≥3.00 $\log_{10}$ $TCID_{50}$ of measles virus; 4.30 $\log_{10}$ $TCID_{50}$ of mumps virus; 3.00 $\log_{10}$ $TCID_{50}$ of rubella virus; ≥3.99 $\log_{10}$ PFU of Oka/Merck varicella virus, 20 mg sucrose, 11 mg hydrolyzed gelatin, 2.5 mg urea; 2.3 mg NaCl, 16 mg sorbitol, 0.38 mg monosodium L-glutamate, 0.14 mg sodium phosphate, 0.25 mg human albumin, 0.13 mg sodium bicarbonate, 94 μg potassium phosphate, 58 μg of potassium chloride; residual components of MRC-5 cells including DNA and protein; 5 μg neomycin, bovine serum albumin (0.5 μg), and other buffer and media ingredients
UNITED STATES			
DTaP/IPV	*Kinrix*	GSK	25 Lf DT, 10 Lf TT, 25 μg PT, 25 μg FHA, 8 μg PRN, poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU), and 3 (Saukett, 32 DU), ≤0.6 mg aluminum, ≤100 μg formaldehyde, ≤100 μg polysorbate 80, ≤0.05 ng neomycin, ≤0.01 ng polymyxin B
	Quadracel	SP	15 Lf DT, 5 Lf TT, 20 μg PT, 20 μg FHA, 3 μg PRN, 5 μg FIM, 40 DU type 1 (Mahoney), 8 DU type 2 (MEF-1), 32 DU type 3 (Saukett), 1.5 mg aluminum phosphate (0.33 mg aluminum) as the adjuvant, polysorbate 80 (approximately 10 ppm by calculation), ≤5 μg residual formaldehyde, <50 ng residual glutaraldehyde, ≤50 ng residual bovine serum albumin, 3.3 mg (0.6% v/v) 2-phenoxyethanol (not as a preservative), <4 pg of neomycin, and <4 pg polymyxin B sulfate
DTaP/IPV/HepB	*Pediarix*	GSK	25 Lf DT, 10 Lf TT, 25 μg PT, 25 μg FHA, 8 μg PRN, poliomyelitis vaccine types 1 (Mahoney, 40 DU), 2 (MEF-1, 8 DU), and 3 (Saukett, 32 DU), and 10 μg of recombinant HBsAg protein; 0.5 mg $Al(OH)_3$, 0.2 mg $AlPO_4$, 2.5 mg 2-phenoxyethanol
DTaP/IPV//Hib	*Pentacel*	SP	15 Lf DT, 5 Lf TT, 20 μg PT, 20 μg FHA, 3 μg PRN, 5 μg FIM, 40 DU type 1 (Mahoney), 8 DU type 2 (MEF-1), 32 DU type 3 (Saukett), 10 μg PRP conjugated to 24 μg TT, 1.5 mg aluminum phosphate (0.33 mg aluminum), polysorbate 80 (approximately 10 ppm by calculation), ≤5 μg residual formaldehyde, <50 ng residual glutaraldehyde, ≤50 ng residual bovine serum albumin, 3.3 mg (0.6% v/v) 2-phenoxyethanol (not as a preservative), <4 pg of neomycin, and <4 pg polymyxin B sulfate
DTaP/IPV/HepB/Hib	*TBD*	SP	15 Lf DT, 5 Lf TT, 20 μg PT, 20 μg FHA, 3 μg PRN, 5 μg FIM, 29 DU type 1 (Mahoney), 7 DU Type 2 (MEF-1), 26 DU Type 3 (Saukett), 3 μg PRP covalently bound to 50 μg OMPC, 10 μg of recombinant HBsAg protein, aluminum salts as adjuvant (a total of 319 μg aluminum), ≤100 μg residual formaldehyde, ≤50 ng residual glutaraldehyde, ≤50 ng residual bovine serum albumin, <5 ng of neomycin, <200 ng streptomycin sulfate, <25 ng polymyxin B sulfate, ≤0.125 μg ammonium thiocyanate, and ≤0.1 μg yeast protein (maximum 1% relative to HBsAg protein)
HepB/HepA	*Twinrix*	GSK	A **1-mL dose** contains not less than 720 EU of inactivated HA virus and 20 μg of recombinant HBsAg protein, 0.45 mg aluminum (as hydroxide and phosphate), 5 mg 2-PE, and trace thimerosal (<1 μg mercury), formalin (not more than 0.1 mg), MRC-5 proteins (not more than 2.5 μg), amino acids, yeast proteins, neomycin, and polysorbate 20

Continued on following page

TABLE 15.2 Combination Vaccines Currently Available in Canada, European Union, or United States[a] *(Continued)*

Combination	Trade Name	Source	Each 0.5-mL Dose (After Reconstitution, if Applicable) Is Formulated to Contain (See Listing by Country, Below, for Specific Information Regarding Composition)
MnCY/Hib	*MenHibrix*	GSK	5 μg of purified *Neisseria meningitidis* C capsular polysaccharide conjugated to approximately 5 μg of tetanus toxoid, 5 μg of purified *N. meningitidis* Y capsular polysaccharide conjugated to approximately 6.5 μg of tetanus toxoid, and 2.5 μg of purified *H. influenzae* b capsular polysaccharide conjugated to approximately 6.25 μg of tetanus toxoid, 96.8 μg of Tris (trometamol)-HCl, 12.6 mg of sucrose, and ≤0.72 μg of residual formaldehyde
MMRV	*ProQuad* (frozen version)	Merck	≥3.00 $\log_{10}$ $TCID_{50}$ of measles virus; 4.30 $\log_{10}$ $TCID_{50}$ of mumps virus; 3.00 $\log_{10}$ $TCID_{50}$ of rubella virus; ≥3.99 $\log_{10}$ PFU of Oka/Merck varicella virus, ≤21 mg sucrose, 11 mg hydrolyzed gelatin, 2.4 mg NaCl, 1.8 mg sorbitol, 0.4 mg monosodium L-glutamate, 0.34 mg sodium phosphate dibasic, 0.31 mg human albumin, 0.17 mg sodium bicarbonate, 72 μg potassium phosphate monobasic, 60 μg of potassium chloride; 36 μg potassium phosphate dibasic; residual components of MRC-5 cells including DNA and protein; <16 μg neomycin; bovine serum albumin (0.5 μg); and other buffer and media ingredients

Note: While this text was in press, Merck and Sanofi Pasteur announced that their European joint venture, SPMSD, was being dissolved; in the future, products marketed by SPMSD would instead be marketed by their respective manufacturers.

aP, acellular pertussis vaccine (infant formulation); ap, acellular pertussis vaccine (adolescent/adult formulation); D, diphtheria toxoid vaccine (infant formulation); d, diphtheria toxoid vaccine (adolescent/adult formulation); DU, D-antigen units; ELISA, enzyme-linked immunosorbent assay; EU, enzyme-linked immunosorbent assay (ELISA) units; FHA, filamentous hemagglutinin; FIM, fimbrial proteins; GSK, GlaxoSmithKline; HBsAg, hepatitis B surface antigen; HepA, hepatitis A vaccine; HepB, hepatitis B vaccine; Hib, conjugate *Haemophilus influenzae* type b vaccine; IPV, enhanced inactivated trivalent poliovirus vaccine; IU, international units; Lf, limit of flocculation; MMRV, measles-mumps-rubella-varicella; PCVn, n-valent pneumococcal conjugate vaccine; PFU, plaque-forming units; ppm, parts per million; PRN, pertactin; PRP, polyribosylribitol phosphate; PT, pertussis toxoid; SP, Sanofi Pasteur; SPMSD, Sanofi Pasteur MSD; SSI, Statens Seruminstitut; T, tetanus toxoid vaccine; TCID50, median tissue culture infective dose; v/v, volume in volume; wP, whole-cell pertussis vaccine. See Table 44.6 for information on prior names of pharmaceutical companies.

[a]Products combining only multiple serotypes of a single pathogen are excluded, as are DT (diphtheria and tetanus toxoids–pediatric), Td (diphtheria and tetanus toxoids–adult), DTP (diphtheria, tetanus, pertussis), DTaP (diphtheria, tetanus toxoids, and acellular pertussis), Tdap (tetanus, diphtheria, and pertussis), OPV (oral polio vaccine), IPV (inactivated polio vaccine), MMR (measles, mumps, rubella), and MMRV (measles-mumps-rubella-varicella). Excludes products that are licensed but not distributed. Only those manufacturers who distribute their products internationally are listed; other manufacturers may produce some products (e.g., DTP/IPV) for local or regional use. No discrimination is made between products distributed in combined form and those distributed in separate containers, for combination at the time of use.

separate routes). Although some studies have shown altered immune responses to various vaccines when they are given concurrently with other vaccines but at separate sites, there is no evidence that the efficacy of any vaccine recommended for routine use in childhood is materially altered by concomitant administration with any other vaccines recommended for administration at the same age.[5] Similarly, adverse events after concomitant administration of multiple vaccines generally are increased only modestly, if at all, compared with events after the administration of the most reactogenic vaccine alone. Thus, we will not review the many studies that have evaluated simultaneous administration, except to the extent that they provide reference data to which results from combined vaccines can be compared.

Combination Vaccines and the Immune System

Antibodies recognize conformationally determined epitopes on protein or polysaccharide antigens. Modification of an antigen's B-cell epitopes during vaccine preparation may reduce the ability of vaccine-induced antibody to bind to the pathogen. Consequently, the techniques used to produce an antigen may have important implications for the immunogenicity (and, presumably, the efficacy) of a vaccine containing that antigen.[6] This principle is illustrated by the results from the Multicenter Acellular Pertussis Trial, which compared 13 acellular pertussis (aP) vaccines and found that levels of antibody to pertussis toxin correlated poorly with the quantity of toxoided pertussis toxin present in the vaccines.[7] For example, one of the acellular vaccines contained a genetically inactivated pertussis toxin produced by recombinant technology. This vaccine produced markedly higher antibody responses per microgram than did the remainder of the evaluated vaccines, whose pertussis toxin components were inactivated chemically rather than genetically.[7]

Carrier-Induced Epitopic Modification

Theories regarding the human immune response and vaccine studies in animals and humans suggest that simultaneous exposure to multiple conjugate antigens might result in enhanced or diminished immune responses.[8–12]

The phenomenon of carrier-induced epitope-specific modification (suppression or enhancement) of the immune response is one in which antibody responses to haptens presented on a carrier are inhibited or augmented by prior immunization with the specific carrier. Studies in animals show that the dose, route, choice of carrier protein, and presence of adjuvant all contribute to determining whether epitopic suppression or enhancement of the immune response occurs. Suppression more frequently occurs when large amounts of carrier protein are used for priming and high anticarrier antibody titers are achieved.[13] Concurrent administration of two conjugate vaccines using the same carrier also may lead to interference. For example, a study among infants given a combination vaccine containing Hib capsular polysaccharide (polyribosylribitol phosphate [PRP]) conjugated to tetanus toxoid (PRP-T) plus a quadrivalent pneumococcal conjugate

vaccine (PCV) conjugated to tetanus or diphtheria toxoid found reduced Hib antibodies among the infants whose vaccine was conjugated to tetanus toxoid rather than diphtheria toxoid.[14] Another study showed markedly reduced antibody responses to tetanus-conjugated serotypes in a PCV11 when administered concomitantly with a DTaP2/IPV//PRP-T combination vaccine.[15]

These data make it clear that the effect of prior or concomitant administration of proteins used in conjugate vaccines is unpredictable and must be evaluated for each vaccine combination, and the data have prompted evaluation of alternative protein carriers (e.g., protein D from nontypeable *H. influenzae*, as used in a PCV; see below and Chapter 46).

Another interesting example of interference was observed in studies of infants randomized to receive aP3 vaccine or HepB vaccine at birth; all subsequently received DTaP3/IPV/HepB//PRP-T vaccine at 2, 4, 6, and 12 to 23 months. Robust pertussis antibody responses were seen in all, but those given aP at birth had substantially lower responses to both HepB and Hib after the primary series and after the toddler booster, as well as lower diphtheria antibody levels preceding and following the booster.[16,17]

Other Issues Affecting Immune Responses

Chemical or physical interactions among the vaccine components being combined can result in an alteration of the immune response to vaccine.[13] Adjuvants such as aluminum hydroxide and aluminum phosphate bind to inactivated vaccines by noncovalent ionic binding. The combination of one vaccine that is generally administered with adjuvant with another vaccine that is not administered with adjuvant may lead to displacement of the adjuvant and reduced immunogenicity of the first vaccine. Furthermore, the adjuvant might combine with the second antigen and thereby alter the immune response to the second vaccine as well.

Buffers, stabilizers, excipients, and similar components included in one vaccine may interfere with the components of another vaccine (e.g., thimerosal can destroy the potency of IPV). Although such vaccines cannot be shipped mixed together in a single vial, distribution of the two vaccines in a dual-chambered syringe circumvents this problem.[18,19]

When combining different live attenuated viral vaccine strains, competition between the viruses is the most frequently observed problem. It can be circumvented by increasing the number of vaccine doses (e.g., live attenuated poliovirus vaccine) or modifying the concentrations of the individual viral strains in the vaccine (e.g., MMR and MMRV [measles-mumps-rubella-varicella]).[20]

The interpretation of antibody responses requires correlation of antibody responses with protection from disease. For discussions of correlates of protection, please refer to Chapter 3 and to earlier editions[20] of the present chapter.

Other Impediments to the Development of Combination Vaccines

Combination vaccines can present difficult issues with respect to investment of the funds necessary for clinical development. Typically, at the moment of commercial introduction of a new combination vaccine, its component vaccines already are available and would continue to be used instead of the combination, should the combination's price exceed the additional amount buyers are willing to pay for the convenience of the combination. Thus, the price of the combination is effectively capped, and its costs of development must be expected to be recoverable within that cap or the combination will not be developed. Moreover, systems that base compensation on the number of separate vaccines administered create perverse incentives to avoid combination vaccines.

Patent and other proprietary issues also complicate the generation of combination vaccines. Vaccine manufacturers cannot market vaccines that contain antigens they do not own or license. The best possible combination vaccine might be one that incorporated components from two or more manufacturers, but, absent agreement between the companies, this combination will not become available. Although the dramatic consolidation in recent years among vaccine manufacturers has eased this problem, as have cross-licensing agreements, it has not fully disappeared.

Combining multiple antigens into one injection requires demonstration in clinical trials that the combination will not materially reduce the safety or immunogenicity of the component vaccines (and, in some instances, that efficacy is retained).[21–23] Two strategies are commonly followed. One strategy involves comparing the new vaccine with a previously approved combination vaccine that differs by lacking only a single component that has been added to the new combination. This approach has generally been followed in the European Union, which has approved a full range of combinations, each adding one further antigen. When this approach is followed, the sponsor might have several predecessor combinations to choose from; for example, either DTaP/IPV + Hib or DTaP/Hib + IPV might serve as the control group for a study of a DTaP/IPV/Hib vaccine. In such cases, one almost always finds that the choice made was the one with the lowest risk of failure (e.g., comparing with DTaP/Hib + IPV rather than DTaP/IPV + Hib avoids the risk of revealing once again a reduced Hib response in the combination as compared with separately administered Hib). In the other approach, the new combination is evaluated against each of its major building blocks given alone. For example, a DTaP/IPV/Hib combination vaccine might be compared with concurrent but separate injections of DTaP, trivalent IPV, and Hib vaccines. This has been the approach in the United States, where separate injections have represented the standard of care. However, in 2015 the U.S. Food and Drug Administration (FDA) approved a DTaP/IPV/Hib/HepB vaccine that was evaluated following the European approach.

Another advantage of a study that incorporates a previously evaluated combination vaccine is that it may allow comparison of the current results with those obtained in other arms of an earlier study. This bridging technique was used in the Swedish aP efficacy trials to compare the results from each trial.[24–26] The methodological risks of this approach can be reduced through coordinated efforts to enhance the comparability of serologic and reaction data gathered for similar vaccines in independent studies.

Multicenter studies allow larger enrollments—and thus more arms—than are possible at a single institution and, with the increasing sample-size demands of regulatory agencies, have become the norm. A multicenter trial that is well designed with standardized protocols can be an effective means of evaluating multiple vaccines or multicomponent products, as was shown by the Multicenter Acellular Pertussis Trial.[7] The Swedish and Italian DTaP efficacy trials, with their coordinated protocols and control vaccines, are other pertinent examples.[24,27]

Licensure of Combination Vaccines

To be licensed, vaccines must be demonstrated to be safe and effective. For example, U.S. law states that a combination

pharmaceutical product may be licensed when "combining of the active ingredients does not decrease the purity, potency, safety, or effectiveness of any of the individual active components" (21 CFR 601.25[d][4]). Most licensing authorities have similar criteria for the evaluation of these parameters.

Evaluating Effectiveness

It is uncommon to require an efficacy trial for a vaccine that combines components previously proven efficacious; instead, demonstration of adequate immunogenicity is required. Commonly, the sponsor licenses combination products by conducting noninferiority trials, which provide statistical assurance that the investigational product is not inferior by more than a predefined margin (e.g., no more than a 10% difference in seroprotection rates) as compared with the standard products. A product that meets noninferiority criteria might be superior or might be equivalent, but is not inferior to the standard (or, at least, no more inferior than the prespecified margin).

In any study of finite size, the results represent a point estimate within a definable confidence interval. For typical outcomes of interest in studies of manageable size (e.g., 300–1,000 participants), the 95% confidence interval can easily span a 10% range (for such measures as seroprotection or fever rates) or a 1.5:1 ratio (for such measures as geometric mean antibody titers [GMTs]). Thus, although it might seem that the noninferiority margin should be set to zero so that the possibility of inferiority is completely excluded, doing so would have the undesirable effect of rejecting new products that are truly equivalent or even superior.

An FDA Guidance Document[28] regarding combination vaccines states that "If antibody concentrations induced by the combination vaccine are lower than those induced by the component vaccines, a 'protective' antibody level might still be attained." Although appropriate, this principle can prove difficult to apply in practice because of uncertainty regarding such factors as the level of circulating antibody necessary to confer protection; the minimum antibody concentration (if any) necessary for protection of a person for whom immune memory has been established; whether there is a minimum seroconversion rate required for population protection; and whether achieving antibody concentrations that substantially exceed the minimum necessary to confer personal protection provides any further benefit (e.g., by suppressing colonization and thereby reducing transmission or by assuring prolonged protection).

In the face of these difficulties, the FDA has generally been unwilling to proceed with licensure of combination products whose immune performances fail statistical noninferiority, pointing out that the option always exists to conduct an efficacy or effectiveness trial of the combination and thereby escape the need for noninferiority with respect to a reference vaccination regimen. Unfortunately, for many antigens of interest (including DTP, Hib, and HepB), it is no longer possible to conduct such efficacy trials: There simply is no place left in the world where the disease in question is sufficiently prevalent, an effective vaccine is not available and recommended, and conduct of a large randomized trial is logistically possible.

Evaluating Safety

As a general rule, systemic adverse events are increased only modestly, if at all, after concurrent administration of multiple vaccines compared with events after the administration of the most reactogenic vaccine alone. Local adverse events often are somewhat more common and more severe at the site of injection of the combination, but this increase usually is offset by the avoidance of adverse events that would have been caused by the eliminated separate injections. So far, no combination vaccine has elicited a new type of reaction not previously seen with its separate components.

Recent years have seen an increase in sample-size requirements for safety testing from licensing authorities in general and from the FDA in particular. In addition, many authorities expect some (or most) of the safety data to be obtained among subjects from their own countries. Both trends slow licensure without necessarily providing commensurate benefits. There is little evidence that populations differ materially in the nature or rates of vaccine-associated adverse events, and large increases in sample size require substantial increases in resources while yielding only small increases in statistical power. These issues particularly impact combination vaccines, which commonly do not provide new antigens but merely a more convenient presentation and whose uptake therefore is sensitive to price, as discussed earlier.

Question of Multiple Antigens and Immune Overload

Despite being repeatedly debunked, the assertion continues to circulate—particularly on the Internet—that the normal infant immune system is capable of becoming "overloaded" and that vaccination with multiple antigens can induce such overload. There is no evidence to support such an assertion and much evidence to refute it.[29–32] Indeed, it may be true that the infant immune system requires fairly intensive challenge to develop normally and that insufficient stimulation leads to an increased risk of autoimmune disorders.[33,34]

A concern that infant immune systems may be overwhelmed by simultaneous exposure to several antigens is difficult to entertain when one considers the thousands of antigens to which a newborn is naturally exposed in the first few months of life. Moreover, although more vaccines are administered today, far fewer antigens are delivered than in the past, when DTwP and vaccinia were used routinely.[30]

The Institute of Medicine reviewed this issue and concluded that the evidence favored rejection of a causal relationship between multiple immunizations and an increased risk of type 1 diabetes or heterologous infections and was inadequate to accept or reject a causal relationship with allergic disease, particularly asthma.[35]

Postmarketing Surveillance

Unexpected events occurring soon after vaccination, especially if severe enough to require medical attention, should always be reported. In the United States, the National Childhood Vaccine Injury Act requires healthcare providers and manufacturers to report serious adverse events after vaccination to the Vaccine Adverse Event Reporting System (VAERS),[36] established to provide a single system for collection and analysis of reports of all adverse events associated with vaccines. Because the VAERS reports can be submitted by any concerned person, whether parent, provider, recipient, or observer; are spontaneous and passively collected; and do not provide the ability to calculate reliable population-based rates, they are useful for generating hypotheses but not for testing them. Increasingly, postmarketing surveillance is being facilitated by the use of large databases linking information sources such as pharmacies, healthcare providers, hospitals, and commercial healthcare organizations.[37–40] Integrated data information systems maintained by some governments for their publicly financed

medical care or hospitalization programs also have been used for this purpose.[41,42]

COMBINATIONS BASED ON DTWP (OR ITS COMPONENTS)

As noted earlier, DTwP was developed in 1943 and licensed in the United States in 1948. Its component antigens had long been available separately: the first pertussis vaccine (see Chapter 44) was licensed to the Massachusetts Public Health Biological Laboratories in 1914; mixtures of diphtheria toxin and antitoxin came into use the same year; alum-precipitated diphtheria toxoid (see Chapter 19) was licensed in 1926; and adsorbed tetanus toxoid (see Chapter 58) was licensed in 1937.[3] Whole-cell pertussis vaccine is a potent adjuvant, and the combining of the three antigens in DTwP actually improved the immunogenicity of the toxoids compared with separate administration.[43,44] Adsorption of the vaccines with aluminum further improved immunogenicity while decreasing the severity of adverse reactions associated with pertussis vaccine.[45,46]

Advances in tissue culture techniques permitted the development of trivalent IPV (see Chapter 48), which underwent extensive field trials in 1954. These trials found 90% efficacy against poliovirus types 2 and 3 but only approximately 70% efficacy against type 1.[47] Investigation revealed that the preservative thimerosal inactivated poliovirus, with greater impact on type 1 than types 2 or 3; in response, other preservatives were substituted. Further improvements in production techniques in the late 1970s led to enhanced-potency IPV, which provides substantially higher immunogenicity.[48–50] There is no evidence of interference between the inactivated vaccine strains themselves, unlike the situation with trivalent OPV (see Chapter 49).

DTwP-based combination vaccines have disappeared from North America and most of Europe, but have become a mainstay of public vaccination programs in other parts of the world. The DTwP-based combinations in international distribution that are most widely used are those from Bio Farma (DTwP/HepB and DTwP/HepB/Hib), GlaxoSmithKline (DTwP/Hib and DTwP/HepB/Hib), Johnson & Johnson (Crucell; DTwP/HepB and DTwP/HepB/Hib), Panacea (DTwP/HepB and DTwP/HepB/Hib), Sanofi Pasteur (DTwP/Hib and DTwP/IPV/Hib), and Serum Institute of India (SII; DTwP/HepB, which can be used to reconstitute SII Hib vaccine). The changes since the last edition in the preceding list and in Tables 15.1 and 15.2 reflect the continued growth of local Asian producers, who now are the principle suppliers of DTwP-based vaccines (particularly the pentavalent DTwP/HepB/Hib vaccines now widely used in Expanded Programme for Immunization [EPI] programs) to the international markets and various donor or World Health Organization (WHO) agencies. At the same time, these new entrants to the global marketplace have faced growing pains; between 2010 and 2012, vaccines from Bharat Biotech, Panacea, and Shantha lost WHO prequalification status for quality issues (DTwP/HepB/Hib vaccines from Panacea and Shantha subsequently regained prequalification status).[51,52]

With the growing likelihood of replacement of OPV with IPV, IPV-containing combination vaccines hold enormous attraction for EPI program countries. Yet, numerous problems remain, including: all currently available combination vaccines containing IPV are DTaP-based, posing issues of per-dose cost and number of doses needed per child; IPV remains the most expensive single component, with a cost to WHO comparable per dose to that of DTwP/HepB/Hib; manufacturing capability of IPV based on wild-type poliovirus has resided only with the European manufacturers; EPI programs require multidose vials, but as noted, thimerosal is incompatible with IPV; and, stability of the Hib component requires either reconstitution just prior to use, or, for most DTwP vaccines, redevelopment using a different aluminum adjuvant.[53] These barriers are being addressed in several ways: SII has acquired from The Netherlands government Bilthoven Biologicals, a manufacturer of IPV and a developer of Sabin-strain IPV (sIPV) vaccines; GlaxoSmithKline and Biological E have formed a joint venture to develop a DTwP/IPV/HepB/Hib vaccine; Shantha, now owned by Sanofi Pasteur, has gained access to their IPV; and sIPVs are increasingly becoming available.[53,54] Still, it is unlikely that DTwP/IPV/HepB/Hib vaccines will soon become available.

In general, combinations of DTwP with IPV, HepB, and/or Hib do not demonstrate meaningful interference among their components and do not result in adverse reactions that materially exceed, in frequency or severity, those seen with the same DTwP vaccine given alone. Summary information regarding the various classes of DTwP combination vaccines is provided subsequently. Readers interested in additional details are referred to earlier editions of this text.

DTwP/IPV, DT/IPV, and Td/IPV

Clinically important interference among the diphtheria, tetanus, whole-cell pertussis (wP), and IPV components has not been commonly demonstrated for currently available combinations incorporating those antigens (Table 15.3).[18,19,55–76] In the studies in which combination recipients showed reduced antibody responses to diphtheria and tetanus, all or nearly all nevertheless achieved seroprotective levels. Similarly, pertussis and poliovirus seroconversion rates and absolute antibody levels remained high, even with combined vaccine; the clinical importance of any reduction in mean antibody levels, for polio or pertussis, is unknown.

DTwP/Hib (With or Without IPV)

Many studies have shown that administering DTwP and Hib in combination results in reduced mean PRP antibody levels compared with giving the same components separately (see Table 15.3). However, even in studies with statistically significant reductions,[18,55,56] antibody levels still were high (albeit not as high as with separate administration) and at least 90% of children (typically, >95%) developed greater than 1 μg/mL of antibody to PRP. Thus, reduced immunogenicity of Hib when given in combination with DTwP is most often of no clinical importance. Gold and colleagues[58] demonstrated reduced levels of Hib, tetanus, and some pertussis antibodies following DTwP/IPV/Hib compared to separate vaccines, but those results may have been specific to that combination.

Surveillance data provide further reassurance that use of the combined DTwP//PRP-T does not reduce efficacy in comparison with DTwP and PRP-T administered separately. In Chile, surveillance for pertussis in matched areas that used DTwP alone or DTwP//PRP-T found no significant difference in the rates of pertussis in the two areas.[77] In the area using DTwP//PRP-T, efficacy against invasive Hib disease was more than 90%. Surveillance in Canada found a continued low rate of invasive Hib disease after the licensure and widespread use of DTwP/PRP-T in that country, with no change in the extremely low rates of vaccine failure.[78,79] Similarly, there was no increase in invasive Hib disease in the United States after the 1993 licensure of DTwP//PRP-T and a similar product, DTwP/HbOC (PRP-*Hib* oligosaccharide conjugate; Tetramune, Wyeth Lederle Vaccines & Pediatrics).[80]

None of the combinations that include DTwP and any conjugate Hib vaccine is associated with materially increased

TABLE 15.3 Studies Comparing Combined or Simultaneous Administration of DTwP/IPV, DTwP/Hib, and DTwP/IPV/Hib Vaccines for Primary Immunization of Infants

			Ratio of Antibody Levels With Combined Vaccine to Levels With Separate Vaccines[a]								
									Polioviruses		
Place	Ages (mo)	Vaccines	PRP	D	T	PT	FHA	AGG	1	2	3
Chile[55,56]	2, 4, 6	DTwPf//PRP-T_1, DTwPf + PRP-T_1	0.43[b]	1.00	0.85	0.97	1.07	0.62[c]			
Vancouver[57]	2, 4, 6	DTwPc//PRP-T_1 (Lot 1), DTwPc + PRP-T_1	1.16	1.33	1.00	0.80	1.00	0.88			
Vancouver[57]	2, 4, 6	DTwPc//PRP-T_1 (Lot 2), DTwPc + PRP-T_1	1.03	1.33	0.78	0.80	0.75	0.68[b]			
Vancouver[58]	2, 4, 6	DTwPc/IPV//PRP-T_1, DTwPc/IPV + PRP-T_1	0.75	1.02	0.66	0.78	0.9	0.79			
Chile[59]	2, 4, 6	DTwPu//PRP-T_1, DTwPu + PRP-T_1	0.70	1.32[b]	0.78	1.10	0.86	1.41			
U.S.[60]	2, 4, 6	DTwPu//PRP-T_1, DTwPu + PRP-T_1	0.88	1.09	0.94	1.01	1.00				
U.S.[61]	2, 4, 6	DTwPu//PRP-T_1, DTwPu + PRP-T_1	1.60	1.14	0.96	1.44	0.69		1.55	1.38	0.79
UK[62]	2, 3, 4	DTwPe//PRP-T_1, DTwPe + PRP-T_1	0.75	1.01	1.83[b]	1.68	1.11	1.12			
Chile[63]	2, 4, 6	DTwPf//PRP-T_1, DTwPf				1.12	0.94	0.96			
Israel[64]	2, 4, 6	DTwPf/IPV//PRP-T_1, DTwPf/IPV		0.86	0.65[b]			0.70[b]	1.01	0.78	1.29
Gambia[65]	2, 3, 4	DTwPf//PRP-T_1, DTwPf + PRP-T_1	0.96	1.11	1.16	0.91	0.77	[d]			
UK[66]	2, 3, 4	DTwPf//PRP-T_1, DTwPf + PRP-T_1 [e]	0.73								
Belgium[18]	3, 4, 5	DTwPf//PRP-T_1, DTwPf + PRP-T_1	0.25[b]	0.78	0.79			0.70			
Belgium[18]	3, 4, 5	DTwPf//PRP-T_1 (DCS), DTwPf + PRP-T_1	0.61[b]	1.08	0.99			1.07			
Chile[18]	3, 4.5, 6	DTwPf//PRP-T_1, DTwPf + PRP-T_1	0.37[b]	0.84	1.01			0.99			
Chile[18]	3, 4.5, 6	DTwPf//PRP-T_1 (DCS), DTwPf + PRP-T_1	0.79[b]	0.89	0.97			1.01			
France[19]	2, 3, 4	DTwPf/IPV//PRP-T_1, DTwPf/IPV + PRP-T_1	0.64					1.14	1.03	0.95	0.82
France[19]	2, 3, 4	DTwPf/IPV//PRP-T_1 (DCS), DTwPf/IPV + PRP-T_1	1.41[b]					0.74[b]	0.79	0.88	0.72
Brazil[67]	2, 4, 6	DTwPf//PRP-T_1, DTwPf + PRP-T_1	0.62	0.48	0.15[b]	0.83					
Brazil[67]	2, 4, 6	DTwPf/IPV//PRP-T_1, DTwPf + PRP-T_1	0.79	0.33	0.11[b]	0.75					
U.S.[68]	2, 4, 6	DTwPu/PRP (unconjugated), DTwPu						1.41			
Finland[69]	3, 4, 6	DTwPn/PRP-D, DTwPn		1.17	0.99						
Gambia[70]	2, 3, 4	DTwPu//PRP-OMP, DTwPu + PRP-OMP	1.03	0.80	0.71			0.88			
U.S.[71,72]	2, 4, 6	DTwPv/PRP-HbOC, DTwPv + PRP-HbOC	1.51[b]	1.78[b]	1.82[b]			2.22[b]			
U.S.[73]	2, 4, 6	DTwPu/PRP-HbOC, DTwPu + PRP-HbOC	[f]	[f]	[f]			[f]			
UK[62]	2, 3, 4	DTwPe//PRP-HbOC, DTwPe + PRP-HbOC	1.30	0.93	1.48[b]	1.06	1.39	1.10			
UK[74]	2, 3, 4	DTwPe//PRP-T_2, DTwPe + PRP-T_2	0.51[b]	1.73[b]	0.75	1.20	0.97	0.78			
Spain[75]	2, 3, 4	DTwPg/PRP-CRM_{197}, DTwPg + PRP-CRM_{197}	1.86	1.26	1.16	0.88	[g]				
India[76]	EPI	DTwPs/PRP-T_3, DTwPf//PRP-T_1	0.98	1.07	1.08	0.90[h]					

AGG, pertussis agglutinins; D, diphtheria toxin; DCS, dual-chamber syringe; DTwP, diphtheria and tetanus toxoids and whole cell pertussis vaccine; DTwPc, DTwP produced by Connaught Laboratories (Canada); DTwPe, DTwP produced by Wellcome Laboratories (England); DTwPf, DTwP produced by Sanofi Pasteur (France); DTwPg, DTwP produced by Chiron Vaccines, Marburg, Germany; DTwPn, DTwP produced by National Public Health Institute of Finland; DTwPs, DTwP produced by Serum Institute of India; DTwPu, DTwP produced by Connaught Laboratories (US); DTwPv, DTwP produced by Wyeth Laboratories; EPI, Expanded Program on Immunization vaccination schedule (6, 10, 14 weeks); EU, enzyme-linked immunosorbent assay (ELISA) units; FHA, filamentous hemagglutinin; IPV, inactivated poliovirus vaccine; Ig, immunoglobulin; OPV, oral poliovirus vaccine; PRP, polyribosylribitol phosphate; PRP-D, PRP-diphtheria toxoid conjugate vaccine; PRP-HbOC, PRP-diphtheria CRM_{197} protein conjugate vaccine; PRP-OMP, PRP-meningococcal outer membrane protein conjugate vaccine; PRP-T_1 (*ActHIB*, AvP) and PRP-T_2 (*Hiberix*, GlaxoSmithKline), PRP-tetanus toxoid protein conjugate vaccine (lyophilized and reconstituted at time of use with diluent or DTwP, unless indicated as DCS); PRP-T_3, PRP-tetanus toxoid protein conjugate vaccine produced by Serum Institute of India; PT, pertussis toxin; T, tetanus toxin.

[a]A ratio <1 indicates that mean antibody levels were lower with the combined vaccine than with separate injections; a ratio >1, that levels were higher with combined than separate injections. A blank cell indicates that the comparison was not possible or is not available.

[b]Difference significant at $P \leq .05$.

[c]*P*-value not available. However, the rate of seroconversion (AGG ≥320) was significantly lower ($P < .05$) in the combined group (79%, vs 92%).

[d]Agglutinin titers were not determined. However, ratios for antibody to pertactin and fimbrial antigens were 0.55 ($P < .05$) and 0.74, respectively.

[e]Combined vaccine group was compared to UK historical controls, who received the same PRP-T on the same schedule, but a different DTwP.

[f]Serologic assays were performed only for the DTwP/PRP-HbOC group (PRP, 8.20 μg/mL; D, 0.92 IU/mL; T, 7.52 units/mL; AGG, 110.1/dilution), and were said to be "comparable to values reported…in other series."

[g]Pertactin, 0.56.

[h]Pertussis IgG.

adverse reactions compared with DTwP alone. Typically, injection site reactions were slightly greater with the combinations than with the DTwP alone, but they were less than the aggregate of local reactions seen when separate vaccines were given at separate injection sites.

DTwP/HepB (With or Without Hib)

A number of studies[81–107] have evaluated combination vaccines that incorporate DTwP, HepB, and Hib components (Table 15.4). In general, the addition of HepB to DTwP resulted in significantly increased mean HepB antibody levels and unchanged DTwP responses; the further addition of Hib to the combination resulted in no consistent changes in antibody responses.

DTwP/HepB with or without Hib has been introduced in an increasing number of countries, many of them developing countries that use the Expanded Programme on Immunization (EPI) 6–10–14-week schedule for their public vaccination program.

Crucell (now Johnson & Johnson) and Novartis (now GlaxoSmithKline) developed a fully liquid DTwP/HepB/Hib vaccine (Quinvaxem) containing 10 µg PRP-CRM_{197} and 10 µg HepB surface antigen.[96] A lot-consistency study demonstrated 1-month postimmunization GMTs of 7.9 µg/mL for Hib and 108 mIU/mL for HepB; 91% of subjects had HepB titers of 10 mIU/mL or more.[103] Kanra and colleagues[104] compared this combination with separately administered DTwP/Hib (QuattVaxem) and HepB (Hepavax-Gene) vaccines from the same manufacturer and found comparable adverse event and seroconversion/seroprotection rates in the two groups. Compared with separate injections, the combination elicited GMTs that were significantly higher for HepB and significantly lower (but still protective) for Hib, tetanus, and pertussis. Suarez and colleagues[91] evaluated use of the pentavalent combination versus separate DTwP and Hib vaccines to boost toddlers primed with DTwP/HepB//Hib vaccine and found no material differences in antibody responses for the two groups. A safety study involving 3000 Guatemalan infants found Quinvaxem to be associated with lower rates of healthcare visits and no increase in serious adverse events (SAEs) or hospitalizations,[105] and another study has shown Quinvaxem to be interchangeable in the primary series with a European DTwP/HepB//Hib (Tritanrix HB + Hib).[106]

GlaxoSmithKline has evaluated a DTwP/HepB/Hib vaccine containing reduced quantities of PRP-T (2.5 vs 10 µg PRP) and thimerosal (6 vs 25 µg/dose). Studies in India,[108] Latin America,[109] and the Philippines[110] compared the new vaccine with standard-formulation DTwP/HepB/PRP-T vaccine. The Philippine study showed no reduction in Hib responses after the primary series or booster dose; the other two studies showed that Hib seroprotection rates following the reduced-potency combinations were high, but mean antibody levels were about half those seen with the full-strength formulation.

Shantha Biotechnics has developed a fully liquid DTwP/HepB/Hib vaccine (Shan5), for which it reports D, T, P, HepB, and Hib seroconversion rates of 99.4%, 99.4%, 89.9%, 97.8%, and 98.3% respectively.[107] Sharma and colleagues evaluated SII's DTwP/HepB/Hib vaccine, Pentavac, and Panacea Biotec's similar Easyfive combination given to Indian infants at 6, 10, and 14 weeks.[94] Adverse reactions were similar, except that Pentavac was associated with significantly less injection-site pain and hindrance of limb movement; antibody responses did not differ after the primary series or following a dose of DTwP/Hib vaccine at 15 to 18 months.[94,95] Clinical trial data for other DTwP/HepB or DTwP/HepB/Hib vaccines from Asian manufacturers are generally not available in the indexed literature.

COMBINATIONS BASED ON ACELLULAR PERTUSSIS VACCINE

Overview

The development of numerous effective aP vaccines (see Chapter 44) and their licensure in combination with diphtheria and tetanus toxoids (DTaP) quickly stimulated efforts to combine DTaP with other routine vaccines of infancy, such as Hib, IPV, and HepB. Building on the experience with DTwP combination vaccines, efforts turned first to evaluating combinations of DTaP and conjugate Hib vaccines, in light of their similar schedules, universal use in developed countries, and lack of orally administered alternatives. However, it was soon found that combining DTaP with Hib tended to reduce, often markedly, the Hib antibody response. This discovery slowed development of combinations based on DTaP/Hib and stimulated development of alternative combinations such as DTaP/IPV, DTaP/HepB, and DTaP/IPV/HepB. It also prompted research into the clinical relevance of the reduced response, which has resulted in Hib-containing pentavalent (e.g., DTaP/IPV/Hib) and hexavalent (e.g., DTaP/IPV/HepB/Hib) combinations becoming accepted in Europe and some other jurisdictions despite reduced Hib responses. In contrast, attention in North America initially focused on DTaP/IPV/HepB and on DTaP/Hib-based combinations built on the Canadian-manufactured DTaP5 (see Chapter 44 for details of nomenclature), which does not interfere materially with Hib responses. More recently, a European DTaP/IPV/HepB/Hib vaccine (Infanrix hexa) has become widely used in Canada.

The DTaP-based combinations most widely available worldwide are produced by Sanofi Pasteur and GlaxoSmithKline. GlaxoSmithKline markets a full range of Infanrix (DTaP3)-based combinations, including the only currently available hexavalent and various pentavalent and quadrivalent combinations. Sanofi Pasteur markets products based on the French DTaP2 (e.g., Tetravac, TETRAXIM) in Europe and elsewhere and products based on the Canadian DTaP5 (e.g., Quadracel, Pentacel, Pediacel) in the Western Hemisphere, Asia, and elsewhere.

Adding IPV to DTaP, Tdap, or DTaP/Hib

Overview

The incorporation of IPV into DTaP, DTaP/Hib, or Tdap (adolescent-adult formulation tetanus, diphtheria, and acellular pertussis vaccine) has no consistent effect on antibody responses to the included components, with few comparisons achieving statistical significance. Surveillance in Sweden conducted over many years showed continued reductions in pertussis incidence among the Swedish population, concomitant with the transition from DTaP to DTaP/IPV and DTaP/IPV/Hib.[111] Multiple manufacturers are developing IPVs based on Sabin strains; early data do not suggest important differences in immunogenicity or safety between IPV and sIPV or between DTaP/IPV and DTaP/sIPV.[54,112,113]

The Studies

Although Tables 15.5 through 15.9 attempt to separate data regarding DTaP/IPV, DTaP/Hib, DTaP/HepB, DTaP/HepB/Hib, and DTaP/IPV/Hib combinations, in fact, most of the relevant studies have evaluated several of these combinations simultaneously. In the few that have looked only at DTaP/IPV, pertussis responses tend to be maintained or enhanced with the combination[114–124] and poliovirus responses varied inconsistently; few of these variations achieved statistical significance.

TABLE 15.4 Studies Comparing Combined or Simultaneous Administration of Vaccines Containing DTwP and Hepatitis B Components, With or Without Hib Components, for Primary Immunization of Infants

			Ratio of Antibody Levels With Combined Vaccine to Levels With Separate Vaccines[a]				
Place	**Ages (mo)**	**Vaccines**	**PRP**	**D**	**T**	**WBP**	**HBs**
Spain[81]	3, 5, 7	DTwP/HepB,[b] DTwP		0.79	0.70	0.79	
Thailand[82]	2, 4, 6	DTwP/HepB (5 μg), DTwP + HepB DTwP/HepB (10 μg), DTwP + HepB		1.18 1.21	0.73 0.90	0.92 0.91	0.54[c] 2.57[c]
U.S.[83]	2, 4, 6	DTwP/HepB//PRP-T,[d] DTwP/HepB[b] + PRP-T[e]		1.63[c]	1.08[c]	1.47[c]	2.02
Chile[84,85]	2, 4, 6	DTwP/HepB//PRP-T,[d] DTwP/HepB[b] + PRP-T[e]	0.70	0.95	0.98	0.86	0.94
Myanmar[86]	1.5, 3, 5	DTwP/HepB//PRP-T,[d] DTwP/HepB[b] + PRP-T[e]	1.07	0.74[c]	2.23[c]	0.95	1.00[f]
Australia[87]	2, 4, 6	DTwP/HepB/PRP-OMP,[g] DTwP/HepB + PRP-OMP[h] DTwP/HepB/PRP-OMP,[g] HepB/PRP-OMP + DTwP[i] DTwP/HepB/PRP-OMP,[g] DTwP + HepB + PRP-OMP[i]	0.53[c] 0.51[c] 1.18				0.36[c] 0.28[c] 0.07[c]
Philippines[88]	EPI	DTwP/HepB//PRP-T,[d] DTwP/HepB[k] + PRP-T[l] DTwP/HepB//PRP-T,[m] DTwP/HepB[k] + PRP-T[l]	0.78 0.60[c]	0.37[c] 0.75	1.16 1.37[c]	1.07 0.88	0.90 0.72
Myanmar[89]	EPI	DTwP/HepB/PRP-T,[d] DTwP/HepB[b] + PRP-T[n]	0.92	0.76	0.96	0.63[c]	0.67
Philippines[90]	10	DTwP/HepB//PRP-T/MenAC, DTwP/HepB[b] + PRP-T[l]	0.54[c]				
El Salvador[91]	15–24	DTwP/HepB/Hib,[o] DTwP[p] + PRP-T[q]	1.01	0.42[c]	0.53[c]	0.84[c]	
India[92]	EPI	DTwP/HepB,[r] DTwP/HepB[b]		1.07	1.11	0.85	1.78
India[93]	EPI	DTwP/HepB,[s] DTwP/HepB[b]		1.06	1.28	0.79	1.00
India[94]	EPI	DTwP/HepB/Hib,[t] DTwP/HepB/Hib[u]	1.02	1.03	0.98	1.10	1.03
India[95]	15–18	DTwP/Hib (given as booster to the two groups in the row above)	0.97	1.01	1.01	0.99	

AGG, pertussis agglutinins; D, diphtheria toxin; DTwP, diphtheria and tetanus toxoids and whole cell pertussis vaccine; EPI, Expanded Program on Immunization vaccination schedule (6, 10, 14 weeks); GSK, GlaxoSmithKline; HBs, hepatitis B surface antigen; HepB, hepatitis B vaccine; Lf, limit of flocculation; MenAC, meningococcal polysaccharide serotypes A and C conjugated to tetanus toxoid; OMPC, outer membrane protein complex of *Neisseria meningitidis*; PRP, polyribosylribitol phosphate; PRP-T, PRP-tetanus toxoid protein conjugate vaccine; PT, pertussis toxin; SII, Serum Institute of India; T, tetanus toxin; WBP, whole *Bordetella pertussis* (a mixture of serotypes 1, 2, and 3 used in a solid-phase immunoassay); wP, whole-cell pertussis.

[a]A ratio less than 1 indicates that mean antibody levels were lower with the combined vaccine than with separate injections; a ratio higher than 1, that levels were higher with combined than separate injections. A blank cell indicates that the comparison was not possible or is not available.

[b]DTwP/HepB: Tritanrix-HepB, GSK; ≥30 IU D, ≥60 IU T, 10 μg HBs.

[c]Difference significant at $P \leq .05$.

[d]DTwP/HepB/PRP-T: Tritanrix-HepB/Hiberix, GSK; ≥30 IU D, ≥60 IU T, 10 μg HBs, 10 μg PRP-T.

[e]*OmniHIB*, GSK-distributed version of *ActHIB*.

[f]Including only those subjects seronegative at birth.

[g]DTwPm/HepB/PRP-OMP: *Pentavax*, Merck & Co; 30 Lf D, 6 Lf T, 5 μg HBs, 7.5 μg PRP conjugated to 125 μg OMPC (liquid).

[h]DTwPm/HepB: *Quadrivax*, Merck & Co; 30 Lf D, 6 Lf T, 5 μg HBs. PRP-OMP: *PedvaxHIB*, Merck & Co: 7.5 μg PRP conjugated to 125 μg OMPC (liquid).

[i]HepB/PRO-OMP: *Comvax*, Merck & Co; 5 μg HBs, 7.5 μg PRP conjugated to 125 μg OMPC (liquid). DTwP: CSL Ltd: 30 Lf D, 6 Lf T.

[j]DTwP: CSL Ltd, 30 Lf D, 6 Lf T. HepB: Merck & Co, 5 μg HBs. PRP-OMP: Merck & Co, 15 μg PRP conjugated to 250 μg OMPC (lyophilized).

[k]DTwP/HepB: formulated by GSK using D and T manufactured at their facility in Hungary and wP manufactured by CSL Ltd.

[l]PRP-T: *Hiberix*, GSK; 10 μg PRP-T.

[m]DTwP/HepB/PRP-T: formulated by GSK using D and T manufactured at their facility in Hungary, wP manufactured by CSL Ltd, combined with Hiberix.

[n]A GSK formulation containing 10 μg of PRP-T.

[o]DTwP/HepB/Hib: *Quinvaxem*, Crucell; ≥30 IU DT, ≥60 IU TT, ≥4 IU inactivated *B pertussis*, 10 μg HBs, and 10 μg PRP conjugated to CRM_{197}.

[p]DTwP: SII; ≥30 IU DT, ≥60 IU TT, and ≥4 IU *B pertussis*.

[q]PRP-T: *Vaxem-Hib*, Novartis Vaccines and Diagnostics; 10 μg PRP conjugated to CRM_{197}.

[r]DTwP/HepB produced by Shantha.

[s]DTwP/HepB produced by SII.

[t]DTwP/HepB/Hib (Pentavac) produced by SII.

[u]DTwP/HepB/Hib (Easyfive) produced by Panacea Biotech.

TABLE 15.5 Antibody to PRP Among 120 Infants Given DTaP at 2 Months; Then DTaP, IPV, and Conjugate Hib Vaccine, Separately or Together, at 4 and 6 Months[114,115]

	Hib Primary Series GMC (μg/mL)	
Vaccines Given at 4 and 6 Mo	**6 mo (No.)**	**7 mo (No.)**
DTaP3, IPV, and PRP-T_2, all separate	0.19 (30)	3.94 (30)
DTaP3 and IPV mixed, PRP-T_2 separate	0.18 (28)	3.10 (30)
DTaP3 and PRP-T_2 mixed, IPV separate	0.10 (29)	0.38 (30)
DTaP3, IPV, and PRP-T_2 all mixed	0.09 (27)	0.56 (30)

DTaP3, diphtheria and tetanus toxoids and acellular pertussis vaccine (*Infanrix*, GlaxoSmithKline); GMC, geometric mean concentration of antibody; Hib, *Haemophilus influenzae* type b; IPV, inactivated poliovirus vaccine; No., number of subjects providing serum samples for assay; PRP, polyribosylribitol phosphate; PRP-T_2, PRP-tetanus toxoid protein conjugate vaccine (*Hiberix*, GlaxoSmithKline).

A number of key issues were illustrated by one of the first published studies comparing combined versus separate administration of DTaP, IPV, and Hib. Finnish infants were immunized at 2 months with DTaP3 and then at 4 and 6 months with DTaP3, IPV, and PRP-T all administered separately, all combined, only the DTaP3 and IPV combined, or only the DTaP3 and PRP-T combined.[114] As shown in Table 15.5,[114,120] PRP antibody responses were markedly reduced among infants receiving PRP-T in combination (whether or not IPV was included in the combination), but not if the PRP-T was given separately (whether or not the IPV was given separately). Pertussis responses varied little (Table 15.6); poliovirus responses were reduced with the combination. The extent to which any of these results was attributable to the nontraditional schedule, which included only two Hib doses, is unclear. In a follow-up study, available participants were boosted with DTaP3 and PRP-T at 24 months of age. Vaccines were given separately to children who had been primed with separate vaccines; children who had been primed with combination vaccines were randomized to be given a booster with separate or combined vaccines.[120] Despite the large difference in PRP antibody levels at 7 months of age, there was little difference in levels before the booster dose at 24 months of age (not unexpectedly, given the logarithmic decay in antibody). After receiving a booster dose, responses were strong but about twice as high among children primed with separate vaccines, suggesting a persistent deficit related to the initial difference in priming. The groups primed with combined vaccine had roughly equal responses to the booster dose, whether they were given combined or separate vaccines as booster doses.

Table 15.6 presents representative results from the many studies of the DTaP/IPV vaccines marketed as Tetravac, Infanrix-IPV, Quadracel, TETRAXIM, or KINRIX. In general, the combinations are noninferior with respect to seroprotection/seroconversion rates, produce mean antibody levels that do not meaningfully differ from those of the separately administered components, and can be given concomitantly with other vaccines routinely recommended for age.[116–131]

The aP component of Baxter's Certiva (a DTaP1 with a pertussis component that consists solely of pertussis toxin; see Chapter 44) has been combined with DT and IPV from Statens Seruminstitut (SSI) as DiTeKiPol.[132–134] A comparison of this DTaP1/IPV at 3, 5, and 12 months of age versus DT/IPV at 5, 6, and 15 months plus wP at 5 weeks, 9 weeks, and 10 months found that the combination achieved protective antibody levels for diphtheria, tetanus, and the polio serotypes. Adverse reactions were similar for the DTaP1/IPV and DT/IPV groups.

Although it is generally true that vaccines given simultaneously at separate injection sites do not interfere with each other, such interactions are occasionally noted. Among subjects given DTaP2//PRP-T (TriHIBit) along with IPV, sequential IPV-IPV-OPV, or OPV at 2, 4, and 6 months, Rennels and colleagues[135] found that the PRP GMTs (1.2, 1.3, and 3.1 μg/mL, respectively) and the proportions achieving PRP antibody responses of 1.0 μg/mL or greater (54%, 55%, and 79%, respectively) were reduced by coadministration of IPV. In contrast, Daum and colleagues[136] found that PRP antibody responses to the same DTaP2/PRP-T combination did not significantly differ among groups also receiving OPV or IPV at 2 and 4 months (GMTs, 4.0 vs 3.4 μg/mL; proportions ≥1.0 μg/mL, 77% vs 74%). This contradiction remains unexplained.

TdaP1/IPV (DiTekiPol Booster, SSI), Tdap3/IPV (Boostrix-IPV, GlaxoSmithKline) and Tdap5/IPV combination vaccines (Repevax, Sanofi Pasteur MSD) have been licensed for use as a booster among previously primed persons (the lower limit of licensure age varies by regulatory authority, from 3 to 10 years). Studies of Tdap3/IPV and Tdap5/IPV show that these two combinations produce antibody responses and reaction rates similar to those of separate vaccines.[128–130,137–149] When administered at age 4 to 6 years, diphtheria and pertussis antibody responses and adverse event rates are somewhat lower than with DTaP or DTaP/IPV.[130,148,149] Repeat boosting 5 or 10 years after a Tdap3 or Tdap5 administration produced good antibody responses, with unremarkable safety results.[142–144] Zimmerman and colleagues randomized adults ≥60 years of age to receive Tdap5/IPV and influenza vaccine either concomitantly or 1 month apart; immune responses did not materially differ.[145] Similarly, immune responses did not materially differ among adolescents given simultaneous or separate Tdap5/IPV and quadrivalent human papillomavirus vaccine.[146]

Theeten and colleagues[147] evaluated three doses of Tdap3, one dose of Tdap/IPV followed by two doses of Tdap3, and three doses of Td, administered on a 0–1–6-month schedule to adults age 40 years or older with no known T or d vaccination for at least 20 years. Rates of adverse reactions and immune responses to the T and d components did not differ among the groups; pertussis and polio immune responses were as expected.[147]

Adding HepB to DTaP or to DTaP/IPV

Overview

Combining HepB with DTaP or with DTaP/IPV generally produces somewhat higher DTaP and polio antibody responses than are achieved with the same components given separately on the same schedule. However, combination vaccine schedules do not provide the extended time between the second and third doses that is required for optimal HepB responses, resulting in lower HepB antibody responses.

Studies

Table 15.7 summarizes studies comparing the performance of the GlaxoSmithKline DTaP3/HepB vaccine with the separately administered components and also comparing various administration schedules.[150–155] The combination vaccine showed immune responses similar to those of separate vaccines given on the same schedule.

A comparison of combined vaccine at ages 2, 4, and 6 months versus a currently recommended schedule in

TABLE 15.6 Studies Evaluating Combined or Simultaneous Administration of DTaP or Tdap and IPV Vaccines

			PRP Antibody Levels (μg/mL)		Ratio of Antibody Levels With Combined Vaccine to Levels With Separate Vaccines[a]									
												Poliovirus Serotypes		
Place	Ages (mo)	Vaccines[b]	% >1.0	GMC	PRP	D	T	PT	FHA	PRN	FIM	1	2	3
France[116]	3, 4, 5	DTaP2/IPV, DTwP/IPV				2.47[c]	1.14[c]					1.79[c]	1.24	1.43
Chile[117,118]	3, 5, 7[d]	DTaP2/IPV+PRP-T_1, DTaP2+IPV + PRP-T_1	98, 95	19.0, 21.7	0.88			0.72	0.74[c]			1.6[c]	0.5[c]	0.4[d]
Chile[117,118]	2, 4, 6	DTaP2/IPV+PRP-T_1, DTaP2+IPV + PRP-T_1	97, 96	7.46, 14.1	0.53[c]			0.89	0.87			1.1	1.2	1.0
Korea[119]	2, 4, 6	DTaP2/IPV, DTaP2+IPV				1.00	1.10	1.01	2.97[c]			2.76[c]	2.47[c]	2.71[c]
Finland[120]	2[e], 4, 6	DTaP3/IPV//PRP-T_2, DTaP3//PRP-T_2 + IPV	48, 19[c]	0.56, 0.38	1.47	1.09	1.44	1.02	1.09	1.13		0.34[c]	0.49	0.61
Italy, Sweden[121]	48–72	DTaP3/IPV, DTaP3 + IPV				1.00	1.18	0.81	0.89	0.97		0.76[c]	0.62[c]	0.78
Australia[122]	48–72	DTaP3/IPV + MMR, DTaP3+IPV + MMR				1.06	1.24	0.88	0.84[c]	1.04		0.92	0.86	1.02
U.S.[123]	48–72	DTaP3/IPV + MMR, DTaP3+IPV + MMR				1.02	1.08	1.12	0.98	1.30		1.00	0.98	0.90
U.S.[124]	48–72	DTaP3/IPV + MMR, DTaP3+IPV + MMR				0.99	0.92	0.88	0.92	1.01		1.25[c]	1.26[c]	1.06
Canada[125]	17–19	DTaP5/IPV, DTaP5 + IPV (MRC-5 cell line)				1.28	0.98	1.23	0.96	2.05[c]	1.61	1.17	1.61[c]	0.80
Canada[125]	17–19	DTaP5/IPV, DTaP5 + IPV (Vero cell line)				0.90	1.01	1.08	0.96	1.41[c]	1.14	1.17	1.30	0.74
U.S.[126]	2, 4, 6	DTaP5/IPV + PRP-T_1, DTaP5 + IPV + PRP-T_1[f]	83, 75	3.8, 2.4	1.58[c]	1.16	1.12	1.76[c]	2.53[c]	1.33[c]	1.50[c]	1.08	1.64[c]	1.22
U.S.[126]	15	DTaP5/IPV, DTaP5 + IPV	100, 97	35.6, 32.2	1.11	0.94	0.88[c]	1.42[c]	2.14[c]	0.87[c]	1.77[c]			
U.S.[127]	4–6 y	DTaP5/IPV+MMR+V, DTaP5+IPV+MMR+V				1.20	1.17	1.96[c]	1.56[c]	1.50[c]	1.34[c]	1.28[c]	0.90	1.35[c]
UK[128]	42–60	Tdap5/IPV, Tdap5 + OPV				0.65	0.67[c]	1.02	0.76	0.71	0.83	10.0	6.7	66.9
UK[129]	13–17 y	Tdap5/IPV, Tdap5 + OPV				0.88	0.65[c]	1.27	0.73	1.82[c]	0.78			
Italy[130]	60–72	Tdap3/IPV + MMRV, DTaP2/IPV + MMRV				0.43[c]	1.13	0.75	0.91	45.5[c]		1.20	0.82	1.17

In studies incorporating Hib, the Hib administration was not different between study groups (i.e., Hib either was given separately to all subjects or was part of the combination for all subjects).
aP, acellular pertussis vaccine; D, diphtheria toxin; DTaP, diphtheria and tetanus toxoids and acellular pertussis vaccine; FHA, filamentous hemagglutinin; FIM, fimbriae; GMC, geometric mean concentration of antibody; Hib, *Haemophilus influenzae* type b; IPV, inactivated poliovirus vaccine; MMR, measles, mumps, rubella; MMRV, measles-mumps-rubella-varicella; OPV, oral polio vaccine; PRN, pertactin; PRP, polyribosylribitol phosphate; PRP-T, PRP-tetanus toxoid protein conjugate vaccine; PT, pertussis toxin; T, tetanus toxin; Tdap, tetanus, diphtheria, and pertussis; V, varicella.

[a]A ratio <1 indicates that mean antibody levels were lower with the combined vaccine than with separate injections; a ratio >1 indicates levels were higher with combined than separate injections. A blank cell indicates that the comparison was not possible or is not available.

[b]aP2 = French 2-component aP (e.g., *Triavax* or similar); aP3 = *Infanrix*; aP5 = *Tripacel* (see Chapter 44 for details of vaccines); PRP-T_1 = *ActHib* (Sanofi Pasteur); PRP-T_2 = *Hiberix* (GlaxoSmithKline); Tdap5 = *Covaxis* (*Adacel*) (Sanofi Pasteur); Tdap5/IPV = *Repevax* (Sanofi Pasteur).

[c]Difference significant at $P \leq .05$.

[d]DTaP and IPV were given at 2, 4, and 6 months; PRP-T was given at 3, 5, and 7 months. Polio antibody levels estimated from figures.

[e]Only DTaP was given at 2 months, without IPV or PRP-T.

[f]Both groups also received PCV7 (pneumococcal 7-valent conjugate vaccine) at 2, 4, and 6 months and HepB (hepatitis B) at 2 and 6 months.

TABLE 15.7 Studies Evaluating Combined or Simultaneous Administration of DTaP3 and HepB Vaccines

Place	Ages (mo)	Vaccines[b]	Comment	Mean Antibody Levels 1 Mo After Last Injection[a] HepB	D	T	PT	FHA	PRN
Turkey[150]	3, 4, 5	DTaP3/HepB	Group 1	343	2.05	4.35	56	89	129
	3, 4, 5	DTaP3 + HepB	Group 2	275	1.88	4.38	52	114	159
	3, 4, 5	DTaP3	Group 3	6	1.59	4.03	47	89	125
Lithuania[151]	3, 4.5, 6	DTaP3/HepB	Mixed in vial	667	1.40	2.21	47.9	184	170
	3, 4.5, 6	DTaP3/HepB	Mixed at time of injection	518	1.06	2.00	46.7	131	124
	3, 4.5, 6	DTaP3 + HepB	Separate administration	438	1.10	1.76	46.7	158	148
Italy[152]	2, 4, 6	DTaP3/HepB	Group 1	949	0.19		56.1	153	240
	3, 5	DTaP3/HepB	Group 2, after first 2 injections	572	0.11		31.8	86	113
	3, 5, 11	DTaP3/HepB	Group 2, after third injection	5554	1.71		65.3	232	372
U.S.[153]	2, 4, 6	DTaP3/HepB	Combined vaccine	1280[c]	1.93	3.82	72.3	459	195
	2, 4, 6	DTaP3 + HepB	HepB given at birth, 1, and 6 months	4620[c]	1.00	2.11	52.2	334	138
Not stated[154]	2, 4, 6	DTaP3/HepB		929	1.96	3.08	66.5	285	233
	2, 4, 6	DTaP3			1.88	2.22	60.0	220	170
	2, 4, 6	HepB		1895					
U.S.[155]	2, 4, 6	DTaP3/HepB	Group C	919	1.2	3.1	72.8[c]	234[c]	155
	2, 4, 6	DTaP3 + HepB	Group D	805	0.8	2.3	47.5[c]	153[c]	109

D, diphtheria; DTaP3, GlaxoSmithKline diphtheria and tetanus toxoids and acellular pertussis vaccine; EU, enzyme-linked immunosorbent assay (ELISA) units; FHA, filamentous hemagglutinin; HepB, hepatitis B, PRN, pertactin; PT, pertussis toxin; T, tetanus toxin.
[a]Units: HepB, mIU/mL; pertussis components, EU; diphtheria and tetanus, IU (international units). A blank cell indicates that data are not available.
[b]All vaccines produced by GlaxoSmithKline.
[c]Difference significant at $P \leq .05$.

the United States—HepB at birth and 1 and 6 months of age and DTaP3 at 2, 4, and 6 months of age—found significantly higher antibody responses for combined vaccine for every component except HepB, which was significantly lower.[153] However, the mean HepB antibody with combined vaccine was nevertheless high (1052 mIU/mL), and 99% of subjects had levels ≥10 mIU/mL, the level considered protective. Antibody responses to concomitantly given Hib and OPV did not differ between the two groups.

Table 15.7 also reveals a clear-cut correlation between dosing interval and HepB response of the combination. Progressively compressing the schedule from 2–4–6 to 3–4.5–6 to 3–4–5 months leads to progressively reduced HepB responses, whether given separately or in combination; in each case, results are somewhat higher with the combination. An Italian study[152] that evaluated a 3–5–11 schedule recorded higher HepB responses following the third dose, again suggesting that increasing the dosing interval is associated with an enhanced immune response.

GlaxoSmithKline's DTaP3/IPV/HepB combination vaccine (Infanrix Penta; Pediarix) has been evaluated in a variety of schedules and compared with its components given separately, with DTwP-based combinations, and as a control vaccine in studies of the GlaxoSmithKline hexavalent combination (see later text in this chapter).

In a randomized U.S. study, the response rates following DTaP3/IPV/HepB vaccine at 2, 4, and 6 months of age were shown to be noninferior (indeed, often superior) to U.S.-licensed DTaP3, HepB, OPV, and IPV vaccines administered separately on the same schedule.[155] Another randomized U.S. study comparing DTaP3/IPV/HepB plus Hib and PCV7 versus separate concomitant vaccines given at 2, 4, and 6 months found antibody responses in the combined-vaccine group to equal or exceed those in the separate-vaccine group.[156] This study also addressed the concern that PCV7 (Prevnar) vaccine might alter the immunogenicity of coadministered DTaP vaccines. A separate arm of the study administered the PCV7 2 weeks after dose of DTaP3/IPV/HepB, rather than concurrently; antibody responses in the group given DTaP3/IPV/HepB concurrently with PCV7 equalled or exceeded those in the group receiving DTaP3/IPV/HepB and PCV7 2 weeks apart, except that D titers were significantly higher in the latter group. However, point estimates for D seroprotection rates were slightly higher in the simultaneous-vaccine group, and statistical noninferiority was met.[156] The question of possible interference of PCV with DTaP3/IPV/HepB responses was further addressed by a study in which infants given DTaP3/IPV/HepB plus PRP-T received either PCV7 or PCV13 concomitantly; noninferiority criteria were met for all antigens.[157]

In a study in Moldova, 320 infants given a birth dose of HepB were randomized to receive either DTaP3/IPV/HepB + Hib or DTwP/IPV/Hib + HepB at 6, 10, and 14 weeks of age.[158] After the primary series, both groups showed equivalently high seroprotection rates and mean antibody levels. The HepB seroprotection rate in the DTaP3/IPV/HepB + Hib group was greater than 98.6% immediately before the third dose (14 weeks of age) compared with 88.7% in the DTwP/IPV/Hib + HepB group. An Estonian study administered either DTaP3/IPV/HepB or DTaP3/HepB + IPV at 3, 4.5, 6, and 15 to 27 months and found most antibody responses to be higher (some significantly so) in the combined-vaccine group

following the third and the fourth doses.[159] Adverse reactions following DTaP3/IPV/HepB are generally similar to those following the separate vaccines except for an increase in rates of fever following the first dose, which has been demonstrated in the German[160] and U.S.[161] licensing studies and in postmarketing evaluations.[162,163] The U.S. study found significantly higher rates of fever of any intensity and fever higher than 39°C after dose 1 in the combination vaccine group.[161]

Another U.S. study evaluated DTaP3/IPV/HepB versus DTaP3 or DTaP5 given separately and found fever of 38.3°C or higher to occur significantly more often with the combination than with the separate DTaP3 after all three doses (4.2% vs 2.7%, 8.8% vs 4.9%, and 5.7% vs 4.7% for doses 1–3, respectively).[162] Compared with the DTaP5, fever was significantly more common after doses 1 and 2 (4.2% vs 2.1% and 8.8% vs 6.5%) but not after dose 3 (5.7% vs 5.6%).[162]

The U.S. licensure study also collected data on unplanned visits to seek medical care following immunization; medical attention was sought for fever within 4 days following first vaccination by 1.2% of DTaP/IPV/HepB recipients versus none of the separate vaccine recipients.[161] Thompson and colleagues[163] evaluated the effect of changing from separately administered vaccines to DTaP3/IPV/HepB and found that 6- to 10-week-old infants receiving DTaP3/IPV/HepB were more likely to visit the emergency department and receive tests within 3 days of vaccination, compared with infants who received separate DTaP, IPV, and HepB/Hib. The DTaP3/IPV/HepB recipients had a sevenfold increased risk of receiving a full sepsis workup and a threefold increased risk of receiving antibiotics within 7 days of vaccination. However, a large safety study conducted following U.S. licensure of Pediarix did not identify any safety signals.[164] In particular, there was no difference in the incidence of medically attended fever events in the 4-day period after any dose of vaccine, nor in the incidence of all seizures or febrile seizures, between the DTaP3/IPV/HepB recipients and matched historical controls.

Several studies have evaluated coadministration of DTaP3/IPV/HepB with various Hib vaccines (PRP-OMP, PRP-HbOC, and two PRP-Ts [GlaxoSmithKline and Sanofi Pasteur]) and found no interference.[155,165] Five different booster options for children primed with the pentavalent vaccine have been investigated, with all regimens resulting in high rates of seroprotection or seropositivity and marked increases in GMTs or antibody concentrations.[166–168]

Safety data, based on more than 20,000 doses, show the low rates of minor local reactions typical of aP-based vaccines, with somewhat lower rates among combination recipients for most reactions.[169] Again, a rectal temperature of 38°C or greater was more common in subjects who received DTaP3/HepB/IPV and Hib concomitantly than in subjects who received DTaP3 and Hib concomitantly. The incidence of temperature greater than 39.5°C, although higher with the combination, nevertheless was low in the DTaP3/HepB/IPV + Hib vaccine group (1.4%) and the control group (0.8%). A similar pattern of adverse reactions was found in the booster phase of the same study.[170]

Adding Hib to DTaP or to DTaP/IPV

Overview

Numerous studies[108–110,117,118,126,148,155,171–243] have evaluated various DTaP/Hib and DTaP/IPV/Hib combinations and have produced remarkably consistent results (comparisons of immune responses are shown in Table 15.8 for quadrivalent combinations and Table 15.9[244–252] for pentavalent and hexavalent combinations). Adverse events following primary and booster immunization generally are similar to those seen with the separate vaccines; a modest increase in rates of low-grade fever or minor local reactions is not uncommon and is offset by the absence of local reactions at sites spared injection because a combination was used. All combinations are highly immunogenic when used to boost previously primed children. When used for primary immunization, responses to the components other than Hib have been generally similar to those obtained with separate administration. The combination vaccines (other than those containing the weaker immunogen PRP-D) also stimulate good Hib responses, with GMTs typically in the range of 2 to 5 μg/mL, but these responses almost always are substantially lower than the high responses obtained with PRP-T or HbOC when given separately.[253–255] The clinical relevance of this reduction in Hib immunogenicity, which impeded licensure of these combinations in some jurisdictions, particularly including the United States, was discussed in detail in prior editions of this textbook.[20,256–273]

Studies

DTaP1. The SSI DTaP1/IPV combination (DiTeKiPol) has been marketed with ActHIB for reconstitution as a DTaP1/IPV//PRP-T combination (DiTeKiPol-ActHIB). A study compared DiTeKiPol and ActHIB given combined or separately at 3, 5, and 12 months. Following the second and the third injections, Hib GMTs were lower with the combination, but the proportions achieving levels of 1.0 μg/mL or higher were equal (27% after the second and 92% after the third injection; see Table 15.9).[186] A large-scale safety study using Danish national datasets found that the DTaP1/IPV/HepB combination was associated with a small but significant increase in risk of febrile seizure on the day of the first or second vaccination (3 and 5 months), but no increase in the risk of subsequent epilepsy.[211]

DTaP2. TriHIBit, the combination created by using Tripedia (no longer available) to reconstitute PRP-T (ActHIB), was used in the United States as a booster in children 15 to 18 months old and produced Hib antibody levels comparable to those seen with the separate vaccines.[172] When used for primary immunization of infants, however, mean PRP antibody levels were significantly lower with the combined vaccine (4.3 μg/mL) than with the separate vaccines (7.0 μg/mL).[176,178] The magnitude of reduction in PRP antibody associated with giving a TriHIBit rather than separate Tripedia + PRP-T depended on the number of times the vaccines were administered in combination. Moreover, administration of separate vaccines for the third dose of the primary series did not overcome the reduced immune response associated with prior use of the combination, showing once again the persistent effect of the initial priming experience.[224]

DTaP2//Hib or DTaP2/IPV//Hib[209] vaccines produced by Sanofi Pasteur using their French two-component aP vaccine (Triavax; see Chapter 44) have been evaluated in evaluated in British,[223] Belgian,[177] and Turkish[177,210] infants, among others (see Table 15.8).[208] The British infants developed low levels of antibody to PRP (mean: 0.48 μg/mL) after primary immunization at 2, 3, and 4 months of age,[223] but developed higher levels (mean: 36.8 μg/mL) after a booster dose of PRP-T at 13 months of age.[223] Although the Belgian and Turkish infants (immunized at 3, 4, and 5 months of age) had markedly higher mean PRP antibody levels than the British infants after primary immunization with combined vaccine, responses nevertheless were substantially lower than with separate vaccines.[177]

Lagos and colleagues[117,118] randomized infants to five study groups: DTaP2/IPV//PRP-T; DTaP2/IPV + PRP-T; DTaP2/IPV;

TABLE 15.8 Studies Evaluating Combined or Simultaneous Administration of DTaP and Hib Vaccines

			PRP Antibody Levels (μg/mL)		Ratio of Antibody Levels With Combined Vaccine to Levels With Separate Vaccines[a]						
Place	**Ages (mo)**	**Vaccines[b]**	**% >1.0**	**GMC**	**PRP**	**D**	**T**	**PT**	**FHA**	**PRN**	**FIM**
U.S.[171]	18	DTaP2u/PRP-D, DTaP2u + PRP-D	53, 76								
U.S.[172]	15–20	DTaP2u//PRP-T_1, DTaP2u + PRP-T_1	100, 100								
U.S.[173]	15–21	DTaP4/HbOC, DTaP4 + HbOC		26.9, 32.4	0.83						
U.S.[174]	12–15	DTaP4/HbOC, DTaP4 + HbOC	98, 100	37.9, 48.4	0.78	0.71		0.55[c]	0.93	0.96	1.00
U.S.[174]	15–18	DTaP4/HbOC, DTaP4 + HbOC	98, 96	50.2, 43.3	1.16	0.94		0.79	1.32	1.04	1.00
Germany[175]	2, 3, 4	DTaP2u/PRP-D, DTaP2u + PRP-D		0.58, 0.44	1.34						
Germany[176]	2, 3, 4	DTaP2u//PRP-T_1, DTaP2u + PRP-T_1	91, 99	2.83, 4.3	0.66[c]						
Belgium[177]	3, 4, 5	DTaP2f//PRP-T_1, DTaP2f + PRP-T_1		1.78, 6.19	0.29[c]						
Turkey[177]	3, 4, 5	DTaP2f//PRP-T_1, DTaP2f + PRP-T_1		5.02, 11.7	0.43[c]						
U.S.[178]	2, 4, 6	DTaP2u//PRP-T_1, DTaP2u + PRP-T_1	85, 100[c]	4.29, 7.0	0.61[c]	1.67[c]	0.79[c]	1.00	1.29[c]		
U.S.[135]	2, 4, 6	DTaP2u//PRP-T_1 + OPV, DTaP2u + PRP-T_1 + OPV		3.17, 4.43	0.72	0.80	1.45	0.97	0.86		
Germany[179,180]	3, 4, 5	DTaP3//PRP-T_2, DTaP3 + PRP-T_2	72, 88	2.02, 7.20	0.28[c]	0.92	0.85[c]	0.89[c]	0.77[c]	0.81[c]	
Germany[179,180]	3, 4, 5	DTaP3//PRP-T_1, DTaP3 + PRP-T_1	N/A, 88	2.75, 5.44	0.51[c]						
U.S.[181]	2, 4, 6	DTaP4/HbOC, DTaP4 + HbOC	55, 94[c]	1.15, 16.4	0.07[c]						
Taiwan[182]	2, 4, 6	DTaP5//PRP-T_1, DTaP5 + PRP-T_1	95, 99	11.8, 13.0	0.91			1.25	1.00	1.31	1.30

aP, acellular pertussis vaccine; D, diphtheria toxin; DTaP, diphtheria and tetanus toxoids and acellular pertussis vaccine; FHA, filamentous hemagglutinin; FIM, fimbriae; GMC, geometric mean concentration of antibody; HbOC, PRP-diphtheria CRM_{197} protein conjugate vaccine; IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; PRN, pertactin; PRP, polyribosylribitol phosphate; PRP-D, PRP-diphtheria toxoid conjugate vaccine; PRP-T, PRP-tetanus toxoid protein conjugate vaccine; PT, pertussis toxin; T, tetanus toxin.

[a]A ratio <1 indicates that mean antibody levels were lower with the combined vaccine than with separate injections; a ratio >1 indicates levels were higher with combined than separate injections. A blank cell indicates that the comparison was not possible or is not available.

[b]aP2f = French 2-component aP (e.g., *Triavax* or similar); aP2u = *Tripedia*; aP3 = *Infanrix*; aP4 = *ACEL-IMUNE* or similar Takeda-type aP vaccine; aP5 = *Tripacel* or equivalent 5-component aP vaccine (see Chapter 44 for details of vaccines). PRP-T_1 = *ActHib* (Sanofi Pasteur); PRP-T_2 = *Hiberix* (GlaxoSmithKline).

[c]Difference significant at $P \leq .05$.

DTaP2 + IPV; or DTaP2 + OPV. These vaccines were given at 2, 4, and 6 months of age. In addition, the latter three groups received PRP-T at 3, 5, and 7 months of age. All groups had poliovirus seroconversion rates of 99% or 100%, with higher antibody levels among the groups receiving IPV in combination than in the groups receiving separate IPV or OPV (see Tables 15.6 and 15.9). Responses to pertussis toxin, filamentous hemagglutinin (FHA), and PRP were robust in all groups but were superior in infants receiving separate injections. At age 7 months, PRP antibody levels were markedly higher in the study groups given PRP-T separately at 3 and 5 months of age, even though they had only received two doses of Hib vaccine by that time whereas the other groups had received three doses. It is not known whether this result was caused by age, carrier priming, or immunologic interference. When a booster dose of DTaP2//PRP-T was given at 12 months, all groups had strong PRP antibody responses (mean levels: 48.6–95.2 μg/mL), but significantly higher antibody levels were seen in the groups primed at 3, 5, and 7 months of age.[117,118]

Philippine,[212] Indian,[213] and South African[214] studies of DTaP2/IPV//Hib (Pentaxim; Pentavac) vaccine given on the EPI schedule (6–10–14 weeks) found antibody responses to be similar to those previously reported, whether HepB vaccine was given at 0–6–14 weeks or 6–10–14 weeks. A fourth dose of DTaP2/IPV//Hib given at 18 to 19 months following primary vaccination at 6–10–14 weeks among Indian or South African children resulted in strong antibody responses to all antigens.[215,216] Antibody to PRP rose from 0.35 to 47.1 μg/mL among the South African toddlers and from 1.75 to 70.6 μg/mL among the Indian toddlers. Pentaxim was also evaluated in Thailand, as a fourth-dose booster following a primary series of a DTaP2/IPV/HepB//Hib combination (Hexavac; see later in this chapter) and as a primary series given at 2, 4, and 6 months of age.[217,218] Antibody responses again were similar to those previously reported, except that Hib antibody responses were substantially higher than those seen in the Philippine or Indian studies, probably owing to the difference in schedules. Following boosting at 18 to 19 months, antibody to PRP rose from 1.21 to 61.2 μg/mL.[219] A Chinese study evaluated Pentaxim given at 2, 3, and 4 months or at 3, 4, and 5 months versus separately administered DTaP (Wuhan Institute), IPV, and Hib (both supplied by Sanofi Pasteur) vaccines.[189] The combination vaccine was noninferior to the separate vaccines for all seroprotection and seroconversion rates; PRP GMTs were high for all groups, but significantly lower with the combination.

As noted earlier, combination vaccines—once exclusively produced by European and North American suppliers—increasingly are being developed by Asian and Latin American manufacturers. Most such efforts to date have involved

TABLE 15.9 Studies Evaluating Administration of Pentavalent or Hexavalent DTaP-Based Combinations Versus Separate Administration of One or More Contained Components

			PRP Antibody Levels (IgG, μg/mL)		Ratio of Antibody Levels With Combined Vaccine to Levels With Separate Vaccines[a]										
													Poliovirus Serotypes		
Place	**Ages (mo)**	**Vaccines[b]**	**% >1.0**	**GMC**	**PRP**	**HepB**	**D**	**T**	**PT**	**FHA**	**PRN**	**FIM**	**1**	**2**	**3**
France[183]	15–24	DTaP3/IPV//PRP-T_1, DTaP3/IPV + PRP-T_1	100, 97	60.4, 60.0	1.01		1.19	0.84	1.15	1.11	1.03		0.89	0.74	1.05
Canada[184,185]	17–19	DTaP5/IPV//PRP-T_1,[c] DTaP5/IPV + PRP-T_1	99, 100	32.5, 26.9	1.21										
Sweden[186]	3, 5	DTaP1/IPV//PRP-T_1, DTaP1/IPV + PRP-T_1	27, 26	0.4, 0.6	0.67		0.57[d]	1.86[d]	1.04				0.80	0.92	0.75
Sweden[186]	3, 5, 12	DTaP1/IPV//PRP-T_1, DTaP1/IPV + PRP-T_1	92, 92	6.9, 11.3	0.61[d]		0.77[d]	156[d]	1.11				0.94	1.00	0.89
France[187,188]	2, 3, 4	DTaP2/IPV//PRP-T_1, DTaP2/IPV + PRP-T_1	71, 88[d]	1.9, 5.2	0.36[d]		1.03	0.81	0.98	0.84			0.73	0.76	0.58
Chile[117,118]	2, 4, 6[e]	DTaP2/IPV//PRP-T_1, DTaP2+IPV + PRP-T_1	97, 98	7.5, 22	0.34[d]		0.42[d]	0.41[d]	0.73[d]	0.78			1.72[d]	1.88[d]	2.37[d]
China[189]	3, 4, 5	DTaP2/IPV//PRP-T_1, DTaP2c+IPV + PRP-T_1	99, 100	12.6, 6.3	0.51[d]		1.26[d]	0.99	2.71[d]	2.30[d]			2.30[d]	2.03[d]	2.36[d]
France[190]	2, 4, 6	DTaP2/IPV/HepB//PRP-T_1, DTaP2/IPV/PRP-T_1 + HepB		2.1, 3.7	0.56[d]	0.44[d]	0.98	1.25[d]	0.74[d]	1.19[d]			1.37	1.60[d]	1.20
South Africa[244]	EPI	DTaP2/IPV/HepB/PRP-T_1, DTwP-PRP-T_1 + OPV + HepB	80, 92	3.3, 5.2	0.64[d]	2.23[d]	1.85[d]	0.80[d]	1.74[d]	5.53[d]			2.92[d]	1.39[d]	4.28[d]
Thailand[245]	2, 4, 6	DTaP2/IPV/HepB/PRP-T_1, DTaP3/HepB/IPV//PRP-T_2	85, 71[d]	5.1, 2.4	2.10[d]	1.01	1.20	0.75	0.84	1.20			0.49[d]	0.65[d]	0.41[d]
Argentina[246]	2, 4, 6	DTaP2/IPV/HepB/PRP-T_1, DTaP2/IPV//PRP-T_1 + HepB	85, 84	4.4, 3.9	1.12	1.35	1.20	1.28[d]	0.84	1.21			1.00	1.01	0.70
Mexico[247]	2, 4, 6	DTaP2/IPV/HepB/PRP-T_1, DTaP3/HepB/IPV//PRP-T_2	93, 91	12.2, 6.7	1.83[d]	0.72	1.13	0.84	1.05	1.31[d]			0.64[d]	0.71[d]	0.51[d]
U.S.[155]	2, 4, 6	DTaP3/HepB/IPV + PRP-T_1, DTaP3/HepB + IPV + PRP-T_1	94, 98	6.2, 7.1	0.87	1.81	1.08	1.19	1.33[d]	0.51[d]	0.97		1.95[d]	1.56	4.0[d]
U.S.[155]	2, 4, 6	DTaP3/HepB/IPV + PRP-T_1, DTaP3 + HepB + OPV + PRP-T_1	94, 95	6.2, 7.8	0.79	2.06[d]	1.63[d]	1.61[d]	2.04[d]	0.78[d]	1.38		0.51[d]	0.41[d]	3.82[d]
U.S.[156]	2, 4, 6	DTaP3/HepB/IPV vs DTap3+HebP+IPV, both + PRP-HbOC	96, 91	9.6, 9.2	1.04	1.68[d]	1.27[d]	1.80[d]	1.69[d]	1.18	1.18		3.01[d]	2.54[d]	2.79[d]
Estonia[159]	3, 4.5, 6	DTaP3/HepB/IPV, DTaP3/HepB + IPV				1.72[d]	2.98[d]	1.91[d]	0.79	0.96	0.82		2.23[d]	2.21[d]	2.98[d]

Estonia[159]	15–27	DTaP3/HepB/IPV, DTaP3/HepB + IPV				2.79	1.43	1.14	1.07	1.16	1.72[d]		1.61	1.50	1.04
Germany[191]	3, 4, 5	DTaP3/IPV//PRP-T_2 + HepB, DTaP3/HepB/IPV + PRP-T_2		1.8, 5.6	0.32[d]	0.72	0.64[d]	1.00	0.78[d]	0.88	0.83		0.81	0.86	0.73
Taiwan[192]	2, 4, 6	DTaP3/IPV//PRP-T_2, DTaP3/IPV + PRP-T_2	93, 96	8.4, 20.6	0.41[d]		0.91	0.93	1.00	1.06	0.90		1.17	0.94	0.76
U.S.[193]	2, 4, 6	DTaP3/HepB//PRP-T_2, DTaP3 + HepB + PRP-T_2	71, 90[d]	1.63, 6.15	0.27[d]	0.76[d]	1.08	0.88	1.12	1.01	1.15				
U.S.[194]	2, 4, 6	DTaP3/HepB//PRP-T_2, DTaP3 + HepB + PRP-T_2	58, 88[d]	1.16, 5.45	0.21[d]	0.68	0.74[d]	0.91	0.86	0.88	0.61[d]		0.79	0.88	1.16
Germany[191]	3, 4, 5	DTaP3/HepB/IPV//PRP-T_2, DTaP3/IPV// PRP-T_2 + HepB		2.2, 1.8	1.22	1.14	1.56	1.23	1.23	1.14	1.13		1.48	1.41	1.36
Germany[191]	3, 4, 5	DTaP3/HepB/IPV//PRP-T_2, DTaP3/HepB/IPV + PRP-T_2		2.2, 5.6	0.39[d]	0.83	1.00	1.23	0.95	1.00	0.93		1.19	1.22	1.00
U.S.[195]	2, 4, 6	DTaP3/HepB/IPV//PRP-T_2, DTaP3 + HepB + OPV + PRP-T_1	84, 92	2.65, 5.53	0.48[d]	1.33	1.40	1.33	1.60	0.92	1.25		0.41[d]	0.33[d]	4.19[d]
Germany[205]	3, 4, 5	DTaP3/HepB/IPV//PRP-T_2, DTaP3/HepB/IPV + PRP-T_2	77, 89	2.62, 4.45	0.59[d]	0.75	0.94	0.78	0.91	0.98	0.95		0.82	0.97	0.75
France[197]	2, 3, 4	DTaP3/HepB/IPV//PRP-T_2, DTaP3/HepB/IPV + PRP-T_2	62, 63	1.5, 1.6	0.97	0.78	1.13	0.9	1.02	1.07	1.11		1.38	1.03	1.50
Slovakia[198]	3, 5, 11	DTaP3/HepB/IPV//PRP-T_2, DTaP3/IPV// PRP-T_2 + HepB	99, 97	19.1, 18.9	1.01	1.34	0.97	0.89	1.37	1.14	0.82		0.82	0.90	0.73
Italy, Germany[199]	3, 5, 11	DTaP3/HepB/IPV//PRP-T_2, DTaP3/HepB/IPV + PRP-T_2	99, 98	38, 32	1.19	0.78	1.03	1.06	0.92	0.87	1.13		1.14	1.33	1.18
Spain[200]	2, 4, 6	DTaP3/HepB/IPV//PRP-T_2, DTaP3/HepB/IPV + PRP-T_2	85, 94	4.7, 5.0	0.93	0.53	1.13	0.85	1/39	1.19	0.93		0.79	0.75	1.35
Canada[184,182,201]	2, 4, 6	[c]DTaP5/IPV/PRP-T_1, DTaP5/IPV + PRP-T_1	89, 89	4.86, 3.83	1.27		0.81	0.68	0.84	0.94	1.37	0.83	0.88	0.92	0.69
Philippines[202]	EPI	[c]DTaP5/IPV/PRP-T_1, DTaP5/PRP-T_1 + OPV	91, 89	5.4, 5.1	1.06										
Taiwan[203]	2, 4, 6	[c]DTaP5/IPV/PRP-T_1, DTaP5/IPV + PRP-T_1	97, 99	11.4, 21.7	0.53[d]		1.49[d]	0.88	1.02	1.13	0.83	1.03	0.89	0.79	0.84
Taiwan[204]	18–19	[c]DTaP5/IPV/PRP-T_1, DTaP5/IPV + PRP-T_1	100, 100	60, 62	0.96		1.20	0.94	1.07	1.05	0.57	0.93	0.78	0.80	0.78
Germany[205]	11–18	[c]DTaP5/IPV/PRP-T_1 + PCV7, DTaP3/IPV// PRP-T_2 + PCV7	100, 100	37.2, 30.3	1.23		1.22	1.39	1.02	0.66[d]	0.79	2.60[d]	0.77	1.18	0.75
Finland, Sweden[206]	3, 5, 12	[c]DTaP5/IPV/PRP-T_1, DTaP3/IPV//PRP-T_2	93, 97	12.2, 17.5	0.70[d]		1.83[d]	0.93	1.27[d]	0.69[d]	0.47[d]	191[d]	0.37[d]	0.46[d]	0.34[d]
Canada[184,185,201]	2, 4, 6	DTaP5/IPV//PRP-T_1, DTaP5/IPV + PRP-T_1	85, 89	5.04, 3.83	1.32		0.67[d]	0.61[d]	1.20	1.06	1.16	0.83	0.90	0.85	1.17

Continued on following page

TABLE 15.9 Studies Evaluating Administration of Pentavalent or Hexavalent DTaP-Based Combinations Versus Separate Administration of One or More Contained Components *(Continued)*

					Ratio of Antibody Levels With Combined Vaccine to Levels With Separate Vaccines[a]										
			PRP Antibody Levels (IgG, μg/mL)										Poliovirus Serotypes		
Place	Ages (mo)	Vaccines[b]	% >1.0	GMC	PRP	HepB	D	T	PT	FHA	PRN	FIM	1	2	3
U.S.[207]	2, 4, 6	DTaP5/IPV//PRP-T_1, DTaP5 + IPV + PRP-T_1	72, 71	2.31, 2.29	1.01		1.01	0.89	1.41[d]	2.52[d]	0.83[d]	1.00	0.86	1.13	1.08
U.S.[207]	15	DTaP5/IPV//PRP-T_1, DTaP5 + PRP-T_1	98, 96	17.7, 20.5	0.86		0.91	0.64[d]	1.03	1.69[d]	0.50[d]	1.08			
U.S.[126]	2, 4, 6	DTaP5/IPV//PRP-T_1, DTaP5 + IPV + PRP-T_1	75, 75	2.52, 2.38	1.06		1.00	0.78[d]	1.46[d]	2.38[d]	0.97	1.07	0.60[d]	1.03	0.81[d]
U.S.[126]	15	DTaP5/IPV//PRP-T_1, DTaP5 + PRP-T_1	98, 97	30, 32	0.93		1.02	0.69[d]	1.02	1.47[d]	0.71[d]	1.24[d]			
Canada[248]	2, 4, 6	[f]DTaP5/IPV/HepB/PRP-OMP, DTaP5/IPV//PRP-T + HepB		7.3, 4.3	1.69	1.39	1.00	1.86[d]	1.33[d]	0.80	1.03	1.23	1.26	1.05	1.33
Canada[249]	15	[f]DTaP5/IPV/HepB/PRP-OMP, DTaP5/IPV//PRP-T + HepB	95, 99	9.2, 22.6	0.41[d]		1.24	2.54	1.72[d]	1.38[d]	0.89	1.81[d]	1.19	1.62[d]	1.10
U.S.[250]	2, 4, 6	[g]DTaP5/IPV/HepB/PRP-OMP, DTaP5/IPV//PRP-T + HepB	85, 75[d,h]	4.9, 3.1	1.62[d]				1.28[d]	0.64[d]	0.83[d]	1.28[d]			
U.S.[251]	2, 4, 6	[g]DTaP5/IPV/HepB/PRP-OMP, DTaP5/IPV//PRP-T + HepB	87, 80[d]	5.6, 3.4	1.63[d]				1.20[d]	0.67[d]	1.03	1.51[d]			
Europe[i252]	2, 3, 4	[g]DTaP5/IPV/HepB/PRP-OMP, DTaP3/HepB/IPV//PRP-T_2		3.9, 0.65	6.0[d]	0.97	1.00	1.32[d]	1.55[d]	0.47[d]	0.60[d]	120[d]	0.82	0.93	0.98
Europe[i252]	12	[i]DTaP5/IPV/HepB/PRP-OMP, DTaP3/HepB/IPV//PRP-T_2	96, 98[h]	6.8, 21.4	0.32[d]	0.89	1.55[d]	2.14[d]	2.17[d]	0.62[d]	0.92	309[d]	0.82	0.84	0.87

aP, acellular pertussis vaccine; D, diphtheria toxin; DTaP, diphtheria and tetanus toxoids and acellular pertussis vaccine; EPI, Expanded Program on Immunization vaccination schedule (6, 10, 14 weeks); FHA, filamentous hemagglutinin; FIM, fimbriae; GMC, geometric mean concentration of antibody; HepB, hepatitis B; IPV, inactivated poliovirus vaccine; HbOC, PRP-diphtheria CRM_{197} protein conjugate Hib vaccine; OMP, outer membrane protein; OPV, oral poliovirus vaccine; PCVn, n-valent pneumococcal conjugate vaccine; PRN, pertactin; PRP, polyribosylribitol phosphate; PRP-T, PRP-tetanus toxoid protein conjugate vaccine; PT, pertussis toxin; T, tetanus toxin.

[a]A ratio <1 indicates that mean antibody levels were lower with the combined vaccine than with separate injections; a ratio >1 indicates that levels were higher with combined than separate injections. A blank cell indicates that the comparison was not possible or is not available.

[b]ap1 = Monocomponent aP containing PT only (*Certiva* or equivalent); aP2 = French 2-component aP (e.g., *Triavax* or similar); aP3 = *Infanrix*; aP4 = *ACEL-IMUNE* or similar Takeda-type aP vaccine; aP5 = *Tripacel* or equivalent 5-component aP vaccine, aP2c = aP2 manufactured by Wuhan Institute of Biological Products (see Chapter 44 for details of vaccines). PRP-T_1 = *ActHib* (Sanofi Pasteur); PRP-T_2 = *Hiberix* (GlaxoSmithKline).

[c]DTaP5/IPV/PRP-T combined, fully liquid, in vial.

[d]Difference significant at $P \leq .05$.

[e]DTaP and IPV were given at 2, 4, and 6 months; PRP-T was given at 3, 5, and 7 months. Polio antibody levels estimated from figures.

[f]Both groups also received PCV7.

[g]Both groups also received PCV13 and RotaTeq. Separate HepB given at birth in both groups and at 2 and 6 months in Group 2.

[h]Estimated from figure.

[i]Finland, Germany, and Belgium

[j]Both groups also received MMRV (measles-mumps-rubella-varicella).

combinations based on DTwP, but new DTaP-based combinations have begun emerging from China. Li and colleagues reported a study of 720 infants randomized 2:1 to receive a DTaP/Hib vaccine (produced by Minhai Biotechnology) or DTaP (produced by Chengdu Institute of Biological Products) + a Hib-TT (produced by Lanzhou Institute of Biological Products). Although details are not provided, it seems that both pertussis vaccines are of DTaP2 design, containing pertussis toxin (PT) and FHA. Subjects were vaccinated at 3, 4, 5, and 18 months of age. PRP antibody responses did not differ between the groups; pertussis responses were modestly higher and tetanus-diphtheria (TD) responses modestly lower in the combined-vaccine group following dose 3; there were no material differences following the booster dose.[220]

DTaP3. Schmitt[179] and Schmitt and colleagues[180] used Infanrix (GlaxoSmithKline) to reconstitute two similar PRP-T vaccines, ActHIB (Sanofi Pasteur) and Hiberix (GlaxoSmithKline). PRP antibody levels and seroconversion rates with the two Hib vaccines were reasonably comparable, but were higher with separate than combined vaccine. The combined and the separate vaccines produced high levels of antibody to the DTaP3 components; all antibody levels were slightly lower with the combined vaccine.[179,180] Available subjects were given a booster at 18 to 19 months of age with Hiberix, ActHIB, or Hiberix vaccine mixed with Infanrix. PRP antibody levels after the booster dose were high: 24 to 40 μg/mL for children boosted with the Infanrix//Hiberix combination and 85 to 137 μg/mL for children primed and boosted with the separately administered Hib vaccines.[180] Assessment of T-lymphocyte proliferative responses and cytokine production after priming and before and after the booster dose found no differences between combined and separate vaccines.[229,230]

A study comparing GlaxoSmithKline's DTaP3/IPV and PRP-T, given combined or separately at 2, 4, and 6 months of age, found antibody levels to be similar with combined vaccine or separate administration for diphtheria toxoid, the three pertussis antigens, and the three poliovirus types.[233] Anti–tetanus toxoid titer was higher with the combined vaccine, whereas the mean PRP antibody response was reduced by half with the combined product. PRP antibody levels no longer differed between the two groups before and 1 month after the booster dose given at 18 months of age. Following the fourth dose, all children in both groups had antibody levels more than 1 μg/mL; GMTs were 32.9 and 47.8 μg/mL for the combined and separate groups, respectively.

To further evaluate the question of priming by PRP-T given in combination with DTaP3, a study was conducted in Israel in which children vaccinated with DTaP3/IPV//PRP-T at 2, 4, and 6 months were boosted at 10 or 12 months with unconjugated PRP antigen.[234] The plain PRP elicited an increase in antibody titers within 7 to 10 days, achieving levels higher than the levels reported in several trials conducted in unprimed children vaccinated with the unconjugated vaccine at the same age. These findings demonstrate priming of the PRP response.

DTaP5. In addition to the previously discussed DTaP5/IPV (Quadracel), other combination vaccines based on the Canadian five-component aP vaccine, such as DTaP5//Hib (Actacel), DTaP5/IPV//Hib (Pentacel),[235] and DTaP5/IPV/Hib (Pediacel)[236] have been evaluated in various locales. In a comparative trial in Taiwan of the DTaP5//Hib combination versus the separate components, 95% of combination recipients achieved at least 1 μg/mL of antibody to PRP. Geometric mean pertussis and Hib antibody levels did not differ between combined and separate administration (see Table 15.8).[182] Another comparative trial examined vaccination at 2, 4, 6, and 18 months of age with (a) DTaP5/IPV used to reconstitute lyophilized PRP-T (Pentacel), (b) fully liquid DTaP5/IPV/PRP-T (Pediacel), or (c) DTaP5/IPV and PRP-T given separately.[184,185,201] All three groups responded well after primary and booster immunization (see Table 15.8). The liquid and lyophilized combinations performed similarly[184]; antibody responses with the liquid combination did not differ significantly from those of the separate vaccines for any antigen.[184,185,201] Halperin and colleagues[243] compared DTaP3/IPV//Hib and DTaP5/IPV//Hib as fourth doses among Canadian children and found significantly higher PRP antibody levels (29 vs 19 μg/mL) in children boosted with DTaP5/IPV//Hib. Guerra and colleagues[207] reported a comparison of Pentacel with separate Daptacel, IPOL, and ActHIB given at 2, 4, and 6 months of age; at 15 months, children received either Pentacel or Daptacel plus ActHIB. Rates of adverse reactions following Pentacel were similar or lower than following separate vaccines. Antibody responses did not materially differ between the two groups. Chatterjee and colleagues reported results of a comparison of Pentacel versus Daptacel + IPOL + ActHIB given at 2, 4, 6, and 15 months of age, along with the usual concomitant vaccines.[126] Seroprotection rates and PRP antibody levels did not materially differ between the groups. Rates of adverse reactions were somewhat lower with the combination than with the separate vaccines. Large postlicensure safety studies of Pentacel have not identified any safety signals.[274,275]

Lin and colleagues[203] compared Pediacel with Quadracel + PRP-T and found both regimens highly immunogenic. PRP antibody levels of 1.0 μg/mL or higher were achieved in 97% and 99%, respectively; GMTs were very high, at 11.4 and 21.7 μg/mL, respectively. Responses to other contained or concomitant antigens were excellent and similar between the two groups, and adverse reaction rates did not materially differ. One year later, 93% of Pediacel recipients had PRP antibody levels of 0.15 μg/mL or higher.[204] Following a booster dose of Pediacel, geometric mean antibody concentrations (GMCs) rose to 60 and 62 μg/mL in the combined and separate vaccine groups, respectively.

Grimprel and colleagues[241] reported a comparison of Pediacel with Infanrix/IPV//Hib given at 2, 3, 4, and 12 to 18 months of age along with PCV7. Most antibody levels were similar between the two groups. As would be expected from their formulations, FIM (fimbrial) responses were higher in the Pediacel group and PRN (pertactin) responses were higher in the Infanrix/IPV//Hib group. Hib responses were significantly higher in the Pediacel group following the third dose (1.38 vs 0.59 μg/mL) and the fourth dose (32.4 vs 19.3 μg/mL). Berner and colleagues randomized German toddlers ages 11 to 18 months who were previously primed with 3 doses of a hexavalent vaccine, to receive Pediacel or Infanrix hexa as boosters; both groups received concomitant PCV7.[205] There were no material differences in rates of adverse reactions nor in seroprotection or booster antibody response rates, except that FHA responses were lower, and FIM responses higher, in the Pediacel group. Both groups had strong anti-PRP antibody responses. Vesikari and colleagues compared Pediacel with Infanrix-IPV-Hib given at 3, 5, and 12 months of age.[206] Post–dose 2 seroprotection rates did not differ between the two groups. Post–dose 3, significantly higher D and lower PRP antibody responses were found in the Pediacel group. Polio GMCs were also lower in the Pediacel group, but seroprotection rates were 99% to 100%. Pertussis antibody levels differed as would be expected based on the respective formulations. Langley and colleagues randomized Canadian infants to receive Pediacel at 2 and 4 months and Infanrix at 6 months (PPI) or Infanrix at 2 months and Pediacel at 4 and 6 months (IPP). Adverse events were similar in the two groups; significantly higher responses to PT and FIM and a significantly lower response to FHA were seen in the IPP group.[242]

It has long been speculated that the less-pronounced Hib interference seen with the DTaP5-based combinations might relate to the fact that they alone use aluminum phosphate rather than aluminum hydroxide as adjuvant. Mawas and colleagues[276] found that rat Hib antibody responses were reduced with DTaP3/PRP-T compared with PRP-T alone, but were not reduced with DTaP5/IPV//PRP-T. The investigators next evaluated the administration of unadjuvanted Hib; Hib adsorbed onto aluminum hydroxide or aluminum phosphate; DTaP3; PRP-T combined with DTaP3; and PRP-T combined with the individual unadjuvanted components of DTaP3. The addition of PRP-T to DTaP did not affect DTaP responses. The combined administration of pertussis toxoid and PRP-T enhanced Hib responses, compared with their coadministration, whereas combining PRP-T with tetanus toxoid or FHA suppressed Hib responses. Finally, they found that adsorption of PRP-T onto aluminum phosphate reduced responses twofold, whereas adsorption onto aluminum hydroxide reduced responses 5- to 11-fold.[277]

Although it has been suggested that PCV7 might alter the immunogenicity of coadministered DTaP vaccines, several studies have shown no material differences in antibody responses between groups who received PCV7 concomitantly with, or 1 month separate from, each dose of Pentacel.[278–280] Reaction rates tended to be higher in the groups receiving concomitant PCV7, particularly for fever.[280]

Halperin and colleagues[243] compared Pentacel and DTaP3/IPV/Hib as fourth-dose boosters among toddlers primed with three doses of Pentacel. Local reactions were more common with Pentacel, whereas systemic reactions were generally similar for the two groups. There were no differences in seroresponse or seroprotection rates. As would be expected given their respective formulations, mean Hib and FIM titers were higher and PT, FHA, and PRN titers lower in the Pentacel group. The Canadian National Advisory Committee on Immunizations concluded that these two combination vaccines, as well as Pediacel, could be used interchangeably at the fourth (toddler) and fifth (preschool) doses to boost properly primed children.[281]

Effect of Age, Schedule, and Prematurity. Various studies suggest that immunization at an earlier age is associated with reduced antibody responses to Hib when given in combination with DTaP. In a French study in which PRP-T was administered at 2, 3, and 4 months of age, combined with or separately from DTaP2/IPV, PRP antibody levels were 1.95 μg/mL with the combined vaccine and 5.18 μg/mL with separate vaccines (see Table 15.9).[187,188] Another French study compared administration of DTaP2/IPV//PRP-T at ages 2, 3, and 4 months or 2, 4, and 6 months and found higher mean antibody levels for all antigens in the group immunized later.[188,225,226] Responses to PRP differed significantly, for the proportions exceeding 1 μg/mL (70% vs 89%) and for mean antibody levels (1.7 vs 4.7 μg/mL).[188] However, when given a booster at 15 months of age, both groups responded equally well to all antigens (PRP antibody levels were 36.8 and 31.8 μg/mL for 2–3–4 and 2–4–6 months, respectively).[188,226] Similar results have been obtained among Swedish children immunized at ages 3, 5, and 12 months or 2, 4, 6, and 13 months. Not surprisingly, antibody levels were higher after primary immunization with the 2–4–6 schedule than with the 3–5 schedule, although there was no significant difference in the proportion who achieved defined protective levels or had fourfold rises in antibody titer.[227] Following booster immunization at 12 or 13 months of age, antibody levels were virtually identical in the two groups.

Premature birth also has been shown to accentuate the reduction in antibody responses to Hib when given in combination with DTaP. A study comparing antibody responses to DTaP3//PRP-T in infants born at less than 32 weeks' gestation and full-term infants found markedly reduced Hib GMTs (0.27 vs 0.81 μg/mL) and proportions achieving 1 μg/mL (21% vs 46%) among the premature infants.[228] Both groups of children responded well to a fourth dose administered in the first year of life.[282] In contrast, another study by the same investigators found that premature infants given DTaP5/IPV/Hib (Pediacel) + meningococcal serogroup C conjugate vaccine showed substantially higher Hib GMCs than had been seen with the DTaP3//Hib combination (1.21 vs 0.27 μg/mL).[283]

Omeñaca and colleagues[284] compared responses to DTaP3/HepB/IPV/Hib (Infanrix hexa) among 94 preterm and 92 full-term infants and found that tetanus and pertussis responses were similar in the two groups. Responses to the other antigens were lower in the preterm infants, but seroprotection rates nonetheless were still 100% for polio and only moderately reduced for HepB and PRP (Hib). The vaccine was well tolerated in both groups. This study population was followed through booster vaccination in the second and fifth years of life.[285–287] At age 18 to 20 months, prior to receiving the fourth dose, antibody levels were essentially identical in the two groups except for PRP (0.3 vs 0.5 μg/mL). Following the fourth dose, strong antibody responses were seen in both groups; anti-PRP GMCs were now higher in the preterm group (70.9 vs 63.3 μg/mL). DTaP booster at 4 years of age resulted in essentially identical postboost antibody levels in the two groups.[285]

Under close monitoring, Faldella and colleagues[288] gave DTaP/HepB/IPV/Hib (Hexavac) at 7 weeks of age to 45 infants (23 with chronic disease, 22 stable and healthy) born at less than 31 weeks' gestation who were still in the neonatal intensive care unit. Immunization was not associated with variations in cardiac electrical activity or cerebral blood flow, but five (11%) of the 45 infants with chronic disease required medical support for vaccination-related apnea, bradycardia, and/or desaturation.[288] The authors concluded that vaccination of infants with chronic conditions preferably should be conducted before neonatal intensive care unit discharge.

DTaP/HepB/Hib and DTaP/IPV/HepB/Hib Combinations

A number of studies have evaluated GlaxoSmithKline's DTaP3/HepB//PRP-T combination vaccine.[193,194,289–292] As seen with most of the DTaP3//Hib and DTaP3/IPV//Hib combinations previously described, DTaP3//Hib-based combinations typically produce lower PRP antibody levels than are obtained with separate administration of the Hib component. For example, among children randomized at 2, 4, and 6 months of age to receive DTaP3/HepB//PRP-T, DTaP3/HepB + PRP-T, or DTaP3 + HepB + PRP-T, mean PRP antibody levels at 7 months of age were 1.6, 6.3, and 6.2 μg/mL, and the proportions achieving 1 μg/mL were 71%, 92%, and 90%, respectively.[193] No interference was seen in the antibody responses to the other components. Subjects with 7-month PRP antibody less than 1.0 μg/mL were administered a booster dose at 11 to 15 months of age; postboost GMTs ranged from 3 to 5 μg/mL, depending on original study group, indicating that priming was achieved with all regimens.

Pichichero and Passador[194] evaluated the DTaP/HepB//Hib combination versus separately administered vaccines and found antibody responses to be lower in the combined vaccine group for nearly all antigens and substantially lower for Hib (GMC, 1.2 vs 5.5 μg/mL; proportion achieving 1.0 μg/mL or higher, 58% vs 88% for the combined and separate groups, respectively). Children with low antibody responses to primary

immunization had excellent responses when given booster doses.[194] The same PRP results were found in a German study in which infants were immunized at 3, 4, and 5 months of age; mean PRP antibody levels were 1.2 and 5.5 μg/mL, respectively, for DTaP3/HepB//PRP-T and DTaP3/HepB + PRP-T.[289] Zepp and colleagues[290] gave unconjugated PRP to children at 12 months of age who had been immunized at 3, 4, and 5 months of age with DTaP3/HepB//PRP-T. Again, the children developed good antibody responses to PRP promptly after the booster immunization, demonstrating that the combination vaccine had successfully primed the immune system.[290] A consistency study with three lots of DTaP3/HepB mixed with three lots of PRP-T found Hib antibody levels of 0.15 μg/mL or greater in 100% and 1 μg/mL or greater in 85% of subjects; the GMT was 4.05 μg/mL.[291]

Hexavalent (DTaP/HepB/IPV/Hib) vaccines from GlaxoSmithKline and Sanofi Pasteur have been licensed in Europe and elsewhere. The GlaxoSmithKline product (Infanrix hexa) consists of GlaxoSmithKline's liquid DTaP3/HepB/IPV used to reconstitute its lyophilized PRP-T. The Sanofi Pasteur product (Hexavac, now withdrawn; see below) was a fully liquid combination of DTaP, IPV, and Hib components manufactured by Sanofi Pasteur plus HepB antigen manufactured by Merck. Both products have been extensively studied to characterize their immunogenicity, safety, and adaptability to a variety of primary and booster schedules.[166,167,189,190,191,195–198,212–220,293–307] Initial studies of both products showed good immunogenicity for their included antigens, producing seroprotection or seroconversion rates and GMTs similar to those seen with separate injections, except that Hib responses were somewhat lower than seen with separate administration of the Hib component (see Table 15.9). Rates of local and systemic adverse events following administration of the hexavalent combined vaccines are comparable to those seen with other licensed DTaP-based vaccines.

In 2005, reports appeared documenting a reduction in immunogenicity of the HepB component of Hexavac,[308–310] and the European licensing authority suspended its marketing authorization.[311,312] Although the manufacturing issue with the Merck HepB component was eventually resolved, Hexavac was not reintroduced. Subsequent booster studies of children primed with Hexavac or a comparator HepB-containing combination vaccine found little or no difference in postboost antibody responses, indicating that children in the Hexavac group were successfully primed.[313–318]

Studies in which either Hexavac or Infanrix hexa was administered alone or concomitantly with CRM_{197}-conjugated PCV7 found that antibody responses to the hexavalent antigens were not materially altered by coadministration with PCV7.[319–322] Rates of adverse reactions did not differ between study groups, except that low-grade fever (temperature 38–39°C) was more common, both in the infant series and with the toddler booster, in the groups receiving concomitant PCV7.[168,312,323]

Administration of a conjugate meningococcal C vaccine (Meningitec, Pfizer) simultaneously with Infanrix hexa at 2, 4, and 6 months or separately at 3, 5, and 7 months showed no material differences in proportions seroprotected or rates of adverse reactions.[324] A follow-up study at 18 months of age demonstrated superior antibody persistence in the separate vaccines group for diphtheria and meningococcus C.[325] Following a booster dose of DTaP/IPV//PRP-T, the group primed with simultaneous vaccines had significantly higher Hib, tetanus, polio, PT, and FHA titers and a significantly lower diphtheria titer.[325] Studies evaluating concomitant or separate administration of NeisVac-C meningococcal vaccine[326] (Baxter BioScience) or rotavirus vaccine[327] (Rotarix, GlaxoSmithKline) with Infanrix hexa found no material effects on immunogenicity or reactogenicity. A study evaluating administration of Infanrix hexa either simultaneously, one month before, or one month after GlaxoSmithKline investigational ACWY-TT conjugate meningococcal vaccine demonstrated that Infanrix hexa antibody responses did not materially differ for concomitant vs sequential administration.[328]

A number of studies have evaluated antibody persistence following use of Infanrix hexa to prime and boost according to a variety of national schedules. In general, antibody persistence was comparable to that seen with individual vaccines.[329–333] Anti-HBs antibody concentrations of 10 mIU/mL or higher were seen in 85% to 86% of children at 4 to 6 years of age, and 72% to 78% remained above that threshold at 7 to 9 years of age. Nearly all children (96–99%) had seroprotective levels following a challenge dose of vaccine. Seroprotective antibody levels persisted up to 9 years of age in ≥90% of children for diphtheria, Hib, and polio and in 65% of children for tetanus, but in only 38% for PT.[331] Among Scandinavian children vaccinated at 3, 5, and 11 to 12 months with Infanrix hexa or with DTaP/IPV//Hib and evaluated for antibody persistence at age 5 years, most had protective antibody titers for diphtheria, tetanus, Hib, and (in the hexa group) HepB; antibody to PT persisted less well, particularly in the hexa group.[323] Another study population vaccinated with Infanrix hexa or HepB plus DTaP/IPV//Hib at 3, 5, and 11 to 12 months was given HepB vaccine at age 10 to 11 years.[334] Distributions of antibody titers and responses to booster were similar in the two groups. Long-term safety surveillance studies of Infanrix hexa have not identified any safety signals.[335–337]

Sanofi Pasteur has developed a fully liquid hexavalent DTaP2/HepB/IPV/Hib combination vaccine (Hexaxim, Hexacima, or Hexyon, depending on location) that combines a novel HepB component manufactured in Argentina with the DTaP2/IPV/Hib combination Pentaxim.[338] A study in South African infants vaccinated at 6, 10, and 14 weeks of age compared this new hexavalent combination, with or without a dose of HepB vaccine at birth, with separately administered DTwP/Hib, HepB, and OPV vaccines.[244] Hib antibody responses were lower and all other antibody responses were higher in the groups receiving the combination vaccine. As expected, the group receiving a birth dose of HepB vaccine had substantially higher HepB antibody responses. There were no material differences in adverse reactions among the groups. At 15 to 18 months, study participants received the same pertussis-containing combination vaccine given before, along with concomitant vaccines; antibody responses in the hexavalent-combination group equaled or exceeded those in the DTwP/Hib group.[339] Hexaxim was compared to Infanrix hexa among Thai infants vaccinated at 2, 4, and 6 months.[245] Seroprotection rates and GMCs were similar between the two groups except for Hib (proportions with antibody to PRP ≥1.0 μg/mL, 85.2% with Hexaxim vs 71.1% with Infanrix hexa; GMCs, 5.07 vs 2.41 μg/mL), tetanus (100% of both groups achieved ≥0.1 mIU/mL but GMCs were 1.38 mIU/mL for Hexaxim vs 1.83 mIU/mL for Infanrix hexa), and polio (100% achieved seroprotection but GMCs were significantly lower in the Hexaxim group). Argentinean infants were randomized to receive Hexaxim or Pentaxim plus HepB (control group) at 2, 4, and 6 months of age. Postvaccination antibody responses did not materially differ between the two groups. HepB antibody levels were 1148 and 848 mIU/mL in the Hexaxim and control groups respectively.[246] At 18 months, antibody persistence was similar in the two groups except for antibody to HepB, which persisted better in the group originally given Pentaxim plus HepB (99.5% vs 85.5% with antibody 10 mIU/mL).[340] In a study conducted in Peru and Mexico, infants were randomized to receive Hexaxim

or DTwP/HepB//Hib plus OPV.[341] A HepB antibody level ≥10 mIU/mL was achieved by 100% of each group, but HepB GMCs were significantly higher in the group given DTwP/HepB//Hib (3376 vs 1075 mIU/mL). Another study in Mexico randomized infants to receive one of three lots of Hexaxim or Infanrix hexa; there were no material differences in antibody responses between Hexaxim lots or between Hexaxim and Infanrix hexa.[247] GMCs were significantly higher in the Hexaxim group for antibody to PRP and to FHA, and in the Infanrix hexa group for poliovirus antibodies.

COMBINATIONS BASED ON HEPATITIS VACCINE, WITHOUT DTP OR DTAP

HepB/Hib Combinations

Comvax (Merck & Co; also marketed by Sanofi Pasteur MSD in Europe as Procomvax and other trade names) combined HepB vaccine (Recombivax, 5 µg) and PRP-OMP conjugate Hib vaccine (PedvaxHIB, 7.5 µg). Merck discontinued production of this vaccine in 2014; readers interested in further information regarding Comvax are referred to the 2012 edition of this text.[20]

HepB/HepA Combinations

A combination incorporating HepA and HepB antigens (Twinrix; GlaxoSmithKline) is available in the United States, Canada, Europe, and elsewhere. The combined vaccine is available in an adult formulation that contains 720 EU of inactivated HepA antigen and 20 µg of recombinant HepB antigen (Twinrix Adult), in a pediatric formulation that contains half these amounts (Twinrix Pediatric), each given as a three-dose series, and in a pediatric formulation containing the adult dosage but given as a two-dose series (Ambirix).

A comparative trial in adults of Twinrix versus its individual components (Havrix and Engerix-B) given separately at 0, 1, and 6 months found excellent antibody responses, with 100% of combined-vaccine recipients achieving protective levels of both antibodies before the 6-month injection.[342] Another comparative trial[343] confirmed the equivalence of Twinrix and its component vaccines given separately at 0, 1, and 6 months: 99.6% of 264 Twinrix vaccinees developed antibodies against HepA (GMT: 4756 mIU/mL) versus 99.3% of 269 recipients of the monovalent vaccines (GMT: 2948 mIU/mL); for HepB, 95.1% of the Twinrix vaccinees achieved protective titers (GMT: 2099 mIU/mL) versus 92.2% (GMT: 1871 mIU/mL) of the subjects receiving monovalent vaccines. Other studies have provided comparable data.[344,345]

Northdurft and colleagues evaluated an accelerated schedule, developed for persons (e.g., travelers) in need of rapid protection against HepA and B, in which Twinrix is given at 0, 7, and 21 days, with a booster at 1 year, and compared it to HepB given on the same schedule plus HepA given at day 0 and at 1 year (the control group).[346] One week after the third dose, 100% of Twinrix recipients and 99% of controls were positive for anti–HepA antibodies (GMT, 845 mIU/mL vs 512 mIU/mL) and 82% of the Twinrix vaccinees were protected against HepB versus 84% in the control group (GMTs: 65 vs 98 mIU/mL, respectively). At 3 months after the first dose, 100% of Twinrix recipients versus 98% of controls were seroprotected against HepA and 95% of Twinrix recipients versus 91% of controls were seroprotected against HepB. In all studies, Twinrix was well tolerated, with no increased reactogenicity compared with the individual components.

Several long-term follow-up studies among children, younger adults, and older adults have found excellent antibody persistence.[347,348] Seventeen years following a three-dose Twinrix Adult series given to persons at 17 to 43 years of age, 96 to 100% remained seropositive for antibody to HepA and 88 to 92% had protective anti-HBS concentrations.[348] Those given HepB vaccine boosters because their antibody had dropped below 10 mIU/mL demonstrated strong anamnestic responses.[348]

A two-dose pediatric Twinrix schedule also has been evaluated.[349–353] Children given three doses of the pediatric formulation at 0, 1, and 6 months or two doses of the adult formulation at 0 and 6 months or at 0 and 12 months had equivalent immune responses and adverse reaction profiles. Therefore, the adult formulation of Twinrix can be administered to children and adolescents in a two-dose schedule, with the second dose following the first by 6 to 12 months (in Europe, the product packaged for use in this indication and schedule is called Ambirix).[354] Follow-up of subjects randomized at age 12 to 15 years to receive HepB/HepA vaccine on the two-dose or three-dose schedule showed very high seroprotection levels 10 years later, not differing by schedule.[355] Ten years following a two-dose Ambirix series given to children ages 1 to 11 years, 100% were anti–hepatitis A virus (HAV) positive and 82% had anti-HBs concentrations ≥ 10 mIU/mL. All those with anti-HBS concentrations <10 mIU/mL mounted a vigorous antibody response to a booster dose of HepB vaccine.[356]

HepA/Typhoid Combinations

Vaccines composed of *Salmonella typhi* capsular polysaccharide Vi (Vi vaccine) have proven safe and effective in prevention of typhoid fever. The combination of Vi with HepA offers an attractive option for travelers or others who need protection from both diseases, given that typhoid fever and HepA are among the most common vaccine-preventable diseases in travelers and share overlapping areas of endemicity. Two such vaccines are available, Hepatyrix (GlaxoSmithKline) and Viatim (Sanofi Pasteur; also marketed as Vivaxim). Hepatyrix is provided as a premixed liquid preparation in a single syringe, whereas Viatim is presented in a dual-chambered syringe that mixes the two liquid components at the moment of injection.

The proof of concept for these combinations was obtained in preliminary studies that found similar GMTs when HepA vaccine (Havrix) and typhoid vaccine (Typherix) were administered separately or combined.[357] One month postvaccination, GMTs did not significantly differ between the two groups; seroconversion rates in the two groups were 94–100% for Vi and 98–100% for HepA.[357] A lot-consistency study among 462 persons 15 to 50 years old found that greater than 95% of subjects had seroconverted for anti-Vi antibodies and greater than 86% for HepA antibody by day 14 following Hepatyrix, rising to 96% and 100%, respectively, after 1 month.[358] After a booster dose of Havrix, all vaccinees were seropositive against HepA, with a 7.5-fold increase in GMT. No increase in reactogenicity was seen with the combination vaccines.

Viatim was evaluated in a study that compared separate administration of Typhim Vi typhoid vaccine and Avaxim HepA vaccine with a single injection using the dual-chambered syringe (Typhim Vi in one chamber and Avaxim in the other chamber). Seroconversion rates at 14 days for combined and separate administration were 86.4% and 88.2%, respectively, for Vi and 95.6% and 94.2%, respectively, for HepA; at 28 days, Vi rates were essentially unchanged, but HepA rates had risen to 98.7% and 100%, respectively (because of differing definitions of seroconversion, these rates cannot be directly compared with those cited for Hepatyrix in the preceding paragraph).[359] Three years later, 99% in each group were seroprotected for HepA, and 32% of combined and 36% of separate vaccine recipients were seroprotected against typhoid;

these proportions rose to 67% and 70% following a booster dose. Another study evaluated three lots of Viatim and found that 92.1% of subjects achieved a fourfold or greater rise in Vi antibodies; all previously seronegative subjects seroconverted for HepA.[360]

A direct comparison of Viatim with Hepatyrix found statistically higher Vi and HepA seroconversion rates and GMTs at 14 and 28 days after Viatim, whereas systemic and local reactions (mainly pain at the injection site) were lower after Hepatyrix.[361]

An extemporaneous mixture of Typherix with Twinrix did not differ significantly from separate injections with respect to immunogenicity or adverse reactions, except that the likelihood of injection site pain was higher (77% vs 57%).[362]

COMBINATIONS BASED ON MMR VACCINE

After substantial effort (detailed in the fifth edition of this text), MMRV vaccines are now available from GlaxoSmithKline (Priorix Tetra)[363–367] and Merck (ProQuad).[368,369] The two MMRV vaccines incorporate different measles strains but the same rubella strain (see Chapters 37 and 53), plus each company's derivative of the Jeryl-Lynn mumps strain and the Oka varicella strain (see Chapters 40 and 62). For each MMRV vaccine, multiple clinical trials comparing the combination with separately administered MMR + V vaccines demonstrated that the MMRV combination stimulated immune responses equivalent to those achieved with separate MMR + V (for rubella, GMCs were somewhat lower with MMRV but seroprotection rates were 99–100%).[363–369] However, rates of fever and of febrile convulsions during the period from the fourth to the tenth day following initial vaccination of children 12 to 23 months old were materially higher (in the range of 30–40 per 100,000) with the quadrivalent combinations than with separately administered MMR + V; no similar increase was seen following the second dose.[370–375] Accordingly, Advisory Committee on Immunization Practices (ACIP) recommendations express a preference for use of separate MMR and V for children 12 to 47 months old first receiving these vaccines.[375] MMRV is preferred for the second dose and for persons first immunized at 4 years or older, and is an acceptable first-dose option for younger children with informed parental consent. Further information regarding these combinations can be found in Chapter 62.

COMBINATIONS INCORPORATING MENINGOCOCCAL OR PNEUMOCOCCAL CONJUGATE VACCINES

In the prevaccine era, the three most common causes of bacterial meningitis in children were Hib, meningococcus, and pneumococcus. Effective conjugate vaccines exist for all three agents, and combining these vaccines would offer obvious epidemiological synergies. Unfortunately, efforts to incorporate PCV into such combinations have been stymied by interference among the agents or overtaken by increases in the number of included serotypes in standalone PCV vaccines.

The first successful combination in this class, a combined meningococcal C conjugate (MnC)/Hib vaccine called Menitorix, was licensed by GlaxoSmithKline in Europe for use in infants and toddlers. Clinical trials[376–382] indicated that the MnC/Hib combination, given in association with DTaP3/IPV or DTaP3/IPV/HepB at 2, 3, and 4 or 2, 4, and 6 months, produced Hib antibody levels that exceeded, and meningococcus serogroup C seroprotection rates that were noninferior to, those seen in groups given MnC + Pediacel or MnC + Infanrix hexa. Administration of MnC/Hib as a booster at 12 to 18 months of age was associated with excellent antibody responses, with no interference with MMR responses. Similar results were obtained in preterm and full-term infants.[383] Infants vaccinated at 3, 5, and 11 months with MnC/Hib plus Infanrix penta had significantly higher Hib and HepB responses, and significantly lower meningococcal C responses, than control infants given Infanrix hexa plus MnC.[384] Adult laboratory workers with potential workplace exposure to Hib or meningococcus C who were given a single dose of MnC/Hib had good antibody responses to Hib, meningococcus C, and tetanus.[385] Adverse reaction profiles were unremarkable throughout. Several study populations were followed for up to 5 years; antibodies persisted at least as well in the MnC/Hib recipients as in the separate-vaccine control groups.[386–388]

GlaxoSmithKline has also developed a combined meningococcal serogroups CY (MnCY)/Hib vaccine (licensed as MenHibrix in the United States), in which each polysaccharide antigen is conjugated to tetanus toxoid. Data from clinical trials[377,389–396] indicate that the administration of this combination concomitantly with Infanrix and PCV7 at 2, 4, and 6 months resulted in antibody responses that were equivalent for most antigens to those seen with separate administration of Infanrix, PCV7, and ActHIB. Pertussis and pneumococcal antibody responses were somewhat lower with the combination, but predefined noninferiority end points were met; Hib responses were significantly higher. At ages 12 to 15 months, subjects previously given the combination received it again while subjects previously given ActHIB were randomized to receive ActHIB or the combination.[391] Hib antibody responses were significantly higher in the two groups that received a fourth dose of the same vaccine given in the primary series, as compared with subjects who had received three doses of standalone Hib vaccine followed by one dose of the MnCY/Hib combination. As expected, antibody responses to meningococcal serogroups C and Y were significantly higher with the combination than were antibody responses among a group of children age 3 to 5 years given polysaccharide meningococcal vaccine. Five years after the booster dose, antibody levels remained higher in the group given MnCY/Hib than the group given Hib alone.[397] There were no material differences in safety end points in any study.[398]

An Australian study[394] compared the same MnCY/Hib combination vaccine with Meningitec (an MnC vaccine conjugated to CRM_{197}) plus ActHIB and with ActHIB alone, in each case coadministered with Infanrix penta (Pediarix) and PCV7. At 12 to 15 months of age, participants in the first two groups received MnCY/Hib and participants in the third group received PedvaxHIB. Following the third and the fourth doses, Hib antibody levels were highest in the group given the MnCY/Hib combination and were the lowest in the group given Meningitec. Antibodies to meningococcal serogroup C were significantly higher both before and after the fourth dose in the MnCY/Hib group compared with the MnC group.

Serological follow-up was conducted for up to 5 years following administration of MnCY/Hib vaccine given as a four-dose infant–toddler series[390] or as a single-dose booster to children primed with three doses of Hib vaccine.[391] Antibody levels did not significantly differ between groups with respect to administered antigens; Hib and meningococcal antibody titers were somewhat higher in the group given 4 doses of MnCY/Hib.

COMBINATION VACCINES NO LONGER IN DEVELOPMENT

Wyeth Lederle conducted clinical trials of PCV/Hib, PCV/MnC, and PCV/MnC/Hib combinations.[399–403] In general, these studies found that the combinations produced lower

antibody responses for either the pneumococcal or the Hib components compared with separate vaccines, possibly a result of antigen overload with the carrier protein. It seems unlikely that any such PCV-containing combinations will achieve licensure.

GlaxoSmithKline conducted clinical trials[404,405] of a heptavalent vaccine, reconstituting MnC/Hib with DTaP/IPV/HepB, but development of this vaccine was stopped due to lower MnC responses with the combination than with separate vaccines.[404] A study was reported comparing DTaP/IPV used to reconstitute MnC/Hib versus DTaP/IPV//Hib plus MnC; good antibody responses were reported for the hexavalent combination but this vaccine is no longer in development.[406]

A study of liquid combination full-, half-, or one-third-strength IPV combined with DTwP/HepB, then used to reconstitute Hib so as to provide a hexavalent whole-cell combination vaccine, found postvaccination seroprotection or seropositivity rates that did not differ significantly from those of separately administered DTwP/HepB//Hib plus IPV. However, for every hexavalent formulation, there were one or more antigens for which postvaccination GMCs or GMTs were significantly lower than seen in the separate-vaccine group.[407] This hexavalent combination is no longer in development.

FUTURE COMBINATION VACCINES

Sanofi Pasteur and Merck have developed a fully liquid hexavalent DTaP/IPV/HepB/Hib vaccine composed of DTaP5 and IPV from Sanofi Pasteur plus HepB and PRP-OMP from Merck. Prelicensure studies of the vaccine (referred to during development as V419 or PR5I) have been conducted in North America and Europe. The vaccine, now listed in the EU under the trade name Vaxelis, will be marketed by a Merck-Sanofi joint venture, MCM Vaccines.[407a] Initial studies evaluated alternate formulations that differed in amount of the HepB component and the choice (PRP-OMP vs PRP-T) and amount of the Hib component.[248–252,408–413] Following an initial evaluation of two formulations as a fourth dose in toddlers,[408] Halperin and colleagues[409] evaluated four alternative formulations (12 μg PRP-T, 3 μg PRP-OMP, or 6 μg PRP-OMP, each combined with 10 μg HepB; or 6 μg PRP-OMP combined with 15 μg HepB; in each case, further combined with DTaP5/IPV) given at 2, 3, 4, and 12 to 14 months of age. Diaz-Mitoma and colleagues[410] compared Pentacel plus HepB vaccine with three alternative formulations of the liquid hexavalent combination (12 μg PRP-T, 3 μg PRP-OMP, or 6 μg PRP-OMP, each combined with DTaP5/IPV and 10 μg HepB) given at 2, 4, 6, and 12 to 14 months of age. Based on the foregoing, development of this DTaP5/IPV/HepB/Hib combination vaccine proceeded using a formulation containing 10 μg HepB, manufactured with a modified process, and 3 μg PRP-OMP. A subsequent study compared this DTaP5/IPV/HepB/Hib combination at 2, 4, 6, and 15 months, with PCV7 given concomitantly or 1 month later, to Pentacel plus PCV7 at 2, 4, 6, and 15 months with Engerix-B given concomitantly at 2, 4, and 6 months of age.[248,249] Antibodies to diphtheria were high in all groups, but significantly higher in the groups not receiving concomitant PCV7. Otherwise, the timing of PCV7 administration had no material impact on antibody responses to the hexavalent vaccine, nor did coadministration with the hexavalent vaccine impact pneumococcal antibody responses. Antibody responses to the DTaP, IPV, and HepB components of the hexavalent vaccine equaled or exceeded those seen with the standard-of-care separate vaccines. PRP antibody levels were excellent in all groups, but differed, being substantially higher in the hexavalent group after the primary series and substantially higher in the separate-vaccines group after the booster. Subsequent Phase III studies[250–252,411–413] evaluated safety, immunogenicity, lot consistency, and coadministration with other routine vaccines given to infants and toddlers. Marshall and colleagues compared the hexavalent plus PCV13 and RotaTeq at 2, 4, and 6 months to Pentacel plus PCV13, RotaTeq, and (at 2–6 months) HepB, and found the immune response to the hexavalent to be noninferior to control except for FHA GMCs (but FHA seroresponse rates were noninferior).[250] At 15 months, both groups received Daptacel plus PCV13; the hexavalent group also received PRP-OMP while the control group received PRP-T. Postbooster antibody responses in the group primed with the hexavalent were noninferior to those of the control group.[250] In another U.S. study, Block and colleagues evaluated immunogenicity and safety of three lots of the hexavalent, given with PCV13 and RotaTeq at 2, 4, and 6 months, versus a control group given Pentacel, PCV13, and RotaTeq at 2, 4, and 6 months and HepB at 2 and 6 months; both groups received Pentacel plus PCV13 at 15 months.[251] Following the infant series, all lot-consistency hypotheses were met, as were all immunogenicity noninferiority hypotheses except for GMCs for FHA and for one (6B) of the 13 PCV serotypes (but for each, noninferiority was demonstrated for seroresponse rates). Following the toddler booster, noninferiority was met for all antigens except for PRN GMCs (but noninferiority was met for PRN seroresponse rates). Adverse event rates were similar in the two groups, except that fever was more common in the hexavalent group (47.1%) than in the control group (33.2%). Vesikari and colleagues evaluated the hexavalent vaccine versus Infanrix hexa at 2, 3, 4, and 12 months; each group also received PCV13 and RotaTeq with the infant doses and MMRV with the fourth dose.[252] Immune responses in the investigational group were all noninferior to those of the control group; adverse event rates did not materially differ between the groups. Silfverdal and colleagues evaluated the hexavalent vaccine versus Infanrix hexa at 2, 4, and 11 to 12 months; each group also received PCV13 and either RotaTeq or Rotarix with the infant doses and PCV13 with the third dose.[411] Immune responses in the investigational group were all noninferior to those of the control group, and were superior for anti-PRP following the second dose. Adverse event rates were similar except for fever (6.4%) and somnolence (5.8%), which were higher in the investigational group. Martinón-Torres and colleagues investigated immune responses to a mixed schedule in which Spanish infants given HepB at birth were given the hexavalent plus MnC, PCV13, and RotaTeq at 2 months; Pediacel, MnC, PCV13, and RotaTeq at 4 months; and the hexavalent plus RotaTeq at 6 months.[412] At 7 months of age, 98.9% of subjects were seroprotected (antibody levels ≥10 mIU/mL) against HepB (GMC: 1055 mIU/mL) and 100% against Hib (≥0.15 μg/mL antibody to PRP; GMC: 8.0 μg/mL). The proportion reporting fever was lower following the hexavalent at 2 and 6 months than after the comparator vaccine at 4 months (4.9% and 4.7% vs 6.3%). Finally, Oliver and colleagues evaluated the hexavalent given at 2, 3, and 4 months concomitantly with PCV13 (at 2 and 4 months) and either MnC-TT or MnC-CRM at 3 and 4 months. All children received MnC/Hib at 12 months. In the group given MnC-TT, 100% had serum bactericidal antibody (SBA) titers of 1 : 8 or greater following the first, second, and booster doses; in the group receiving MnC-CRM, the corresponding percentages were 96.4%, 99.1%, and 97.3%. Following three doses of the hexavalent, 97.8% to 100% had ≥0.15 μg/mL of antibody to PRP and 96.3% to 96.8% had ≥10 mIU/ML of antibody to HepB; all subjects were seroprotected against D, T, and the three poliovirus types.[413]

GlaxoSmithKline is exploring the development of vaccines that provide broad-based (i.e., not serogroup-specific) protection against pneumococci, nontypeable *Haemophilus influenzae* (NTHi), or both.[414–416]

In the first study, vaccines composed of pneumococcal histidine triad protein D (PhtD) and/or detoxified pneumolysin (dPly), combined with alum or AS02$_v$ adjuvant system (an oil-in-water emulsion combined with monophosphoryl lipid A and the saponin QS21) and formulated either as free proteins or in an eight-valent PCV, were evaluated in adults 65 to 85 years of age given one dose or two doses 60 days apart.[414] The AS02$_v$ groups had higher rates of solicited local and systemic adverse reactions and better immune responses than the other groups. Another study evaluated a combination vaccine containing PhtD plus NTHi protein D (PD) and dPly (dPly), which we will refer to as HiP. Healthy young adults were randomized to receive two doses 60 days apart of HepB (as a control) or HiP formulated with or without AS03 adjuvant (a tocopherol and squalene-based adjuvant, as used, for example, in GlaxoSmithKline's European Union-licensed pandemic influenza vaccines). Local and systemic adverse events were more common in the AS03 group than the other two groups and increased more with the second dose in the two HiP groups than the HepB group. GMCs were highest and persisted best in the AS03 group.[415] A third study evaluated PhtD vaccines formulated with no adjuvant, alum, or AS02$_v$, administered to young (18–45) or elderly (≥65 years of age) adults. Humoral and cellular responses improved with the AS02$_v$ adjuvant system, such that antibody responses in the elderly AS02$_v$ group were comparable to those in the young-adult groups receiving vaccine formulated with alum or no adjuvant.[416]

Wuxi Biological is developing a MnAC/Hib vaccine to address meningococcal epidemiology in China. Hu and colleagues compared this vaccine to separate administration of Wuxi MnAC plus ActHib among children ages 6 to 23 months or 3 to 5 years.[417] Noninferiority was demonstrated for all safety and seroprotection endpoints; meningococcal GMTs did not differ between the investigational and control groups but PRP antibody levels, although very high in both groups, were significantly higher in the control groups (16.2 vs 20.0 μg/mL in the younger children and 45.2 vs 70.7 μg/mL in the older children). ZhiFei Biotech apparently has received marketing approval in China for a similar vaccine, but no study results are available in the indexed literature.[418]

There are also combination vaccines licensed or under development that incorporate multiple serotypes of a single antigen. Among these potentially important combination vaccines are human papillomavirus vaccines (see Chapter 30); trivalent or quadrivalent influenza vaccines, including those of heightened potency or novel modes of delivery (see Chapters 31 and 32); polyvalent pneumococcal vaccines (see Chapters 46 and 47) and meningococcal vaccines (see Chapter 38), especially the conjugate products that are progressively replacing the older polysaccharide vaccines; polyvalent rotavirus vaccine (see Chapter 52); and quadrivalent dengue vaccines (see Chapter 17). Other targets for combination vaccines include tickborne infections, arboviruses, and additional agents of diarrheal diseases.

PRACTICAL ISSUES IN THE USE OF COMBINATION VACCINES

Choice of Combination Versus Separate Component Vaccines

In its 2011 update of the General Recommendations on Immunization, the ACIP states that "Licensed combination vaccines can be used whenever any components of the combination are indicated and its other components are not contraindicated…. The use of a combination vaccine generally is preferred over separate injections of the equivalent component vaccines."[5] Similarly, the Red Book notes that "Parenteral combination vaccines may be administered instead of their equivalent component vaccines if licensed and indicated for the patient's age….When patients have received the recommended immunizations for some of the components in a combination vaccine, administering the extra antigen(s) in the combination vaccine is permissible if they are not contraindicated and doing so will reduce the number of injections required."[419]

When a combination consists of the same components that the practitioner would have given separately at the same visit, the combination is preferred. In other circumstances (e.g., the combination does not incorporate the same components that the practitioner would have given; the components are the same, but the practitioner would have given one or more on a different schedule; the patient does not need one or more of the components, but receipt is not contraindicated), use of the combination is acceptable but not necessarily preferred.

The risk of failing to deliver an indicated vaccination increases with the number of vaccinations indicated at a given visit.[420] Accordingly, the use of combination vaccines may help ensure timely completion of indicated vaccinations. Investigators from the New York Health Department reported that vaccination timeliness rose from 84% to 93% following introduction of a DTaP/HepB/IPV combination vaccine,[421] and investigators from the Georgia Health Department reported significant improvements in coverage rates and timeliness of vaccination for the antigens included in the combination vaccine.[422] A study in a large U.S. managed care health plan found that use of a DTaP-based combination vaccine did not alter the timeliness of DTaP immunization but significantly improved the timeliness of the other included components.[423] None of these ecological studies, however, controlled for other factors that might explain their observations. For example, in each study, the timeliness of vaccination also improved for components not included in the combination, raising the possibility that improvements in coverage were the result of improvements in administrative, provider, or patient performance unrelated to choice of vaccine. A German study that did control for temporal (e.g., birth cohort) effects evaluated the proportion of children having received one dose, the full primary series, or full immunization by 3, 5, and 15 months of age respectively.[424,425] Comparing hexavalent with separate vaccines, mean age at first immunization improved by 0.5 month for Hib, 0.4 month for polio, and 0.9 month for hepatitis B virus (HBV), and mean age at full immunization improved by 2.2 months for Hib, 3.2 months for polio, and 1.4 months for HBV. A study using data from the 2012 (U.S.) National Immunization Survey found that use of combination vaccines was associated with 60% lower likelihood of an incomplete immunization series among children age 24 months.[426] As might be expected, the use of combination vaccines has also been shown to reduce administration time and associated labor costs.[427]

Administration of Superfluous Antigens

As a variety of vaccines become available that combine different antigens, physicians will increasingly find that the simplest (and even, perhaps, the least expensive) alternative will be use of a combination vaccine that includes an antigen that the patient does not need, having already received that antigen in the recommended quantity and timing. Some antigens are known to be associated with increased adverse effects when administered too frequently (e.g., diphtheria toxoid), but these are few. Fortunately, it has been shown for many vaccine antigens that an extra dose may be given without adverse consequence. In particular, the low reactogenicity of Hib, IPV,

and HepB vaccines makes it unlikely that an extra dose of any of these antigens would cause a problem.

Interchangeability

When multiple combination vaccines are available from several different manufacturers, practitioners will question whether the vaccines can be used interchangeably. This question can be difficult to answer even for monocomponent vaccines, and there is little hope of definitive answers for multicomponent vaccines. Consider, for example, a study performed to evaluate the interchangeability of the three conjugate Hib vaccines indicated for the infant primary series.[428] Of the 27 theoretically possible permutations of three vaccines and three injections, five were evaluated. In a world of multiple, distinct DTaP, DTaP/Hib, DTaP/Hib/IPV (and so on) combination vaccines, there is little likelihood that any particular substitution will have been studied.

The ACIP has recognized certain vaccines as interchangeable within their respective series: Hib, HepB, HepA, rotavirus, and quadrivalent meningococcal conjugate.[5] For the vaccines for which there are no data on the interchangeability of licensed products from various manufacturers (e.g., DTaP[5,429,430] and the newer combination vaccines[5]), the ACIP has recommended that the same product be used throughout the primary series. However, if the identity of the previously used product is unknown or if the product is not available at the time of the child's visit, any licensed product appropriate to the child's immunization status and requirements may be used. As the Centers for Disease Control and Prevention notes, "Unavailability of the vaccine used for earlier doses is not a reason for missing the opportunity to administer a dose of acellular pertussis vaccine for which the child is eligible."[431] Similarly, Canada's National Advisory Committee on Vaccination (NACI) has stated that "Based on expert opinion and using the limited data available to date, NACI recommends that the DTaP-IPV/Hib and DTaP-IPV combination vaccine products currently approved for sale in Canada may be used interchangeably for the 18-month and 4- to 6-year booster, respectively."[281]

In this day of bulk vaccine purchase through competitive bidding by large health maintenance organizations and the government, it is unrealistic to presume that the same vaccine will always be available for each child at the time of each vaccination. The situation is further complicated by the fact that 25% of U.S. children see at least two different healthcare providers for their vaccination series and that the average time for a child to remain in a publicly funded healthcare plan is 10 months. Different practitioners are likely to stock different combination vaccines. Because the need to interchange vaccines will arise often, it is reassuring to know that interchanging one brand of vaccine for another has never been shown to result in performance that is outside the range expected for the vaccines in question.

Ad Hoc Combinations

Providers should not create their own ad hoc combinations by mixing separate vaccines in the same syringe unless there is evidence establishing the stability, safety, and immunogenicity of the resultant combination, as reflected in the package inserts.

PUBLIC HEALTH CONSIDERATIONS

Cost Issues

Whether the combination vaccine purchaser is a government, a health maintenance organization or some other intermediary, or the practitioner and patient, it must be expected that economic factors will influence decisions. It is obvious that premium pricing of the combination or reduced provider reimbursement (as a result of administration of fewer injections) may inhibit use of the combination. Less obvious, but equally important, are the economic benefits that flow from use of a combination, which include savings resulting from simplified vaccine purchase, storage, and handling; reduced costs for labor and supplies; elimination of the need for scheduling several vaccination visits to avoid multiple injections; and, of course, increased patient satisfaction and greater compliance with vaccination recommendations.

Many of the newer vaccines, such as DTaP, conjugate Hib, and complex combinations, are substantially more expensive to produce than DTwP, OPV, and other traditional combination vaccines. Certainly, cost considerations will constrain the use of some of these vaccines in certain parts of the world. DTwP, for example, is produced locally at low cost in many countries, and its replacement with DTaP must be weighed against other healthcare expenditures. However, some other relatively expensive vaccines, such as HepB or Hib, offer such clear benefits that they are being used in many developing countries, and once a country has committed to using (for example) HepB or conjugate Hib vaccine, it is likely that combinations that also provide such antigens as DTP or IPV can be purchased for less than the cost of purchasing, storing, shipping, tracking, and injecting the separate component vaccines.

Tracking of Vaccinations

The increasing variety of alternative vaccines (including those with multiple antigens) that might be used to satisfy childhood vaccination requirements makes it unlikely that a practitioner will be able to deduce the precise vaccination history of a new patient. Although U.S. legislation has mandated since March 1988 that medical records of vaccination should contain the identity of the vaccine manufacturer, the date of administration, and the lot number of the vaccine given, such practitioner-based records typically are not retrievable by others. Immunization registries (also termed immunization information systems) are confidential, population-based, computerized information systems that collect vaccination data about all children within a geographic area. Registries can elucidate uncertain vaccination histories, identify children who are due or late for vaccinations, generate reminder and recall notices to ensure that children are vaccinated appropriately, identify provider sites and geographic areas with low vaccination coverage, and provide denominator information for outbreak investigations. One of the U.S. health objectives for 2020 is to increase to 95% the proportion of children younger than 6 years whose immunization records are included in a fully operational, population-based immunization registry. As of 2009, some 77% of U.S. children younger than 6 years participated in a registry.[432] Efforts should continue to increase the percentage of children participating in registries.

Assisting these efforts are improvements that facilitate the accurate and convenient recording of vaccine information in the medical record. Increasingly, vaccine unit-dose containers are being supplied with machine-readable barcodes and/or peel-off stickers that can be placed in the medical record. Since 2011, the Centers for Disease Control and Prevention (CDC) and the FDA have been moving toward implementation of 2D barcodes, which can contain more information than linear barcodes in a smaller space.[433] Linear barcodes currently contain vaccine product identification information only, whereas 2D barcodes can contain the vaccine product identification information as well as the lot number and

expiration date, offering the potential for a marked improvement in medical record accuracy. Numerous vaccines are now distributed in the United States labeled with 2D barcodes.

In summary, combination vaccines have dramatically altered vaccination practices, particularly among the pediatric population, permitting protection against increasing numbers of diseases without equivalent increases in the number of injections required while maintaining the safety and protection associated with the individual vaccines.[434]

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16 Cytomegalovirus Vaccines

Mark R. Schleiss and Stanley A. Plotkin

Cytomegalovirus (CMV) is a ubiquitous β-herpesvirus that only replicates in human cells.[1] CMV infections are generally asymptomatic in immunocompetent individuals, but may produce a heterophile-negative mononucleosis syndrome in approximately 10% of primary infections in older children and adults.[2–4] Like other herpesviruses, it becomes latent after primary infection, but may reactivate from latency, particularly in the setting of immune suppression, leading to disease. Infection typically requires intimate contact with body fluids (blood, urine, saliva, breast milk). There is no seasonality to infection. Patient populations with an increased incidence of primary infection include breastfeeding infants,[5] toddlers and care providers in group childcare settings,[6–8] and sexually active adolescents.[9–13] Seroprevalence is higher among nonwhites and among individuals of lower socioeconomic status.[14,15]

From a public health perspective, the most important medical impact of CMV is the damage caused to a fetus when infection occurs in utero. A recent metaanalysis of published studies concluded that the overall birth prevalence of congenital CMV infection was 0.64%, although this study also noted that rates varied considerably among different study populations.[16] Current estimates suggest that there are approximately 60,000 congenital infections annually in the United States and Europe (Table 16.1). Nonwhite race, low socioeconomic status, premature birth, and neonatal intensive care unit admittance are risk factors for congenital CMV infection. Congenital infections correlate directly with maternal CMV seroprevalence in the subpopulation assessed. Recent efforts to develop newborn screening programs for congenital CMV should provide more rigorous estimates regarding the overall prevalence of this infection in future studies.[17,18] Interestingly, the prevalence of congenital CMV infection in developing countries is even higher, ranging from 0.6% to 6.1%.[19,20] The risk of intrauterine transmission is highest when primary infection occurs during pregnancy, with a significantly increased risk of adverse fetal effects if infection occurs during the first half of pregnancy.[21] CMV infection of the placenta interferes with the maintenance and differentiation of trophoblast progenitor cells, possibly resulting in decreased transport of oxygen and substrate to the fetus and leading, through indirect mechanisms, to intrauterine growth retardation.[22,23] Among congenitally infected infants, approximately 10% have signs and symptoms of disease at birth, and these symptomatic infants are reported to have a 40% to 90% risk of subsequent neurologic sequelae, including mental retardation, microcephaly, development delay, seizure disorders, and cerebral palsy.[24–26] Although the remaining 90% of infants are asymptomatic at birth, between 7% and 20% of these children are reported to subsequently develop permanent sequelae, particularly sensorineural hearing loss.[27–30] Overall, it has been estimated that in a given cohort of 1000 infants with congenital CMV, 170 to 190 will have permanent sequelae, of whom one-third are from the symptomatic group and two-thirds are from the asymptomatic group (Fig. 16.1).[24]

The public health impact of congenital CMV infection is significant and underrecognized,[31–33] and carries with it substantial costs to society. In the early 1990s, the disease burden associated with congenital CMV infection was estimated to cost the U.S. healthcare system approximately $1.9 billion annually, with an estimated cost of more than $300,000 per child.[34] Although partially effective antiviral therapy is available,[35–37] development of a CMV vaccine is the most promising strategy for addressing the problem of congenital CMV. An effective vaccine could, by preventing neurological sequelae and other disabilities, provide a lifetime of benefit for newborns. A study modeling the financial impact of routine vaccination of adolescent females demonstrated that such a strategy would be highly cost-effective.[38] A report from the Institute of Medicine of the National Academy of Sciences categorized development of a CMV vaccine for adolescents as a "Level 1" (highest level) priority.[39] The optimal timing for administration of a CMV vaccine aimed at preventing congenital infections may depend on the age-dependent seroprevalence in the population being vaccinated.[40] Some models suggest that immunization of toddlers and young children may be preferable to vaccination of adolescents, as this strategy could protect young women of child-bearing age from exposure to CMV by decreasing or eliminating infectious virus in the urine and secretions of these children.[41,42]

In addition to young women of childbearing age, patient populations that could benefit from development of a CMV vaccine include immunocompromised patients, in particular solid organ transplant (SOT) patients and hematopoietic stem cell transplant (HSCT) recipients, who are at high risk for CMV disease (manifested variably as interstitial pneumonia, hepatitis, nephritis, encephalitis, bone marrow depression, and potentiation of bacterial and fungal infections).[43–50] CMV-seropositive HSCT recipients and seronegative recipients with a seropositive donor have an enhanced and persistent risk when compared with seronegative recipients of a seronegative donor.[47] SOT recipients are also at risk for CMV disease and graft rejection, particularly seronegative recipients of organs from seropositive donors.[43,44,46,48–50] Although the advent of highly active antiretroviral therapy has dramatically decreased the risk of CMV disease in HIV-infected patients in the developed world,[51,52] HIV-CMV interactions continue to be of substantial importance in the developing world, where HIV therapies may be less available. This may have particular importance in the context of vertical transmission, since maternal HIV infection confers a high risk of congenital CMV infection, even if the infant remains HIV uninfected.[53,54] CMV vaccines may ultimately also prove to be useful in this high-risk setting. In addition, an emerging body of evidence suggests that CMV infection may play a role in the pathogenesis of atherosclerosis, autoimmune diseases, and malignancies, in particular glioblastoma multiforme.[55] CMV serostatus may also impact the clinical course of burns, trauma, and sepsis.[56,57] CMV seropositivity has also been shown to affect B-, T-, and natural killer (NK)-cell responses to influenza vaccination.[58–60] Finally, studies have noted an intriguing association between CMV infection and immunosenescence.[61–67] In light of these observations, universal vaccination against CMV might confer benefits to *all* individuals over the course of a lifetime, and not just women of reproductive age and the special populations described above.

CORRELATES OF CYTOMEGALOVIRUS IMMUNITY: IMPLICATIONS FOR VACCINE DESIGN

Congenital CMV infection can occur as the result of a primary infection, reactivation of latent infection, or reinfection with a new strain, although the relative importance of reactivation versus reinfection has not been fully elucidated. The degree of effectiveness of natural immunity in preventing congenital CMV infection and its attendant sequelae is not established with certainty. A number of studies have described fetal CMV transmission in women with preconception immunity, a result of reinfection with new strains of CMV.[68–73] Such infections can produce sequelae identical to those observed in congenitally infected infants born to women with primary CMV infection during pregnancy. These observations complicate CMV vaccine design, and suggest that (a) for full protection, a CMV vaccine may need to stimulate responses superior to those conferred by natural immunity; and (b) there may be a strong rationale for vaccinating seropositive individuals, with the goal of increasing immunity and preventing reinfections.

TABLE 16.1 Annual Impact of Estimated Congenital Cytomegalovirus Infection and Disease in Europe and the United States

Category of Infants	Total
Total number of live births per year	8,600,000
Average rate of congenital CMV infection (%)	0.7
Total number of newborns with CMV infection	60,200
Number with symptomatic infection (12.7%)	7645
Number with fatal disease (~4%)	306
Number of survivors with sequelae (40–58%)	3058–4434
Number with asymptomatic infection (87.3%)	52,555
Number with sequelae (13.5%)	7095
Total number with sequelae or death	**10,459–11,835**

CMV, cytomegalovirus.
From Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol. *2007;17:355–363, 2007.*

Although imperfect, several studies indicate that preconception immunity clearly does confer reduction in both the risk of congenital CMV transmission and the risk of sequelae if transmission occurs. Reported rates of transmission of CMV to the fetus in the setting of proven primary maternal infection are quite high, with ranges spanning 24% to 75%; an overall risk of transmission of approximately 30% was estimated in a 2007 metaanalysis.[16] Transmission rates are much lower (in the range of 1–2%) in women with preconception immunity who are reinfected during pregnancy,[68–71] compared to women with primary infection, although the relative contributions of secondary infection with novel strains and reactivation of latent strains remain incompletely defined.[72,73] At least two studies have compared transmission of CMV to the fetus from seronegative women and seropositive women, in an attempt to gauge the protective benefit of preconception immunity. Protection in seropositive women was 60% in one study and 91% in another.[74,75] In addition to decreasing the risk of infection, preconception immunity also reduces the risk of severe sequelae in those infants who acquire infection in utero. It has been reported that 25% of congenitally infected infants born to mothers with a primary CMV infection during pregnancy had at least one sequela, compared with only 8% in infants born to women with recurrent infection.[76]

A major hurdle encumbering CMV vaccine development is the lack of a clearly defined correlate of protective immunity. Considering that human CMV is a large and complex virus encoding at least 200 proteins, it is not surprising that immune control involves more than one arm of the immune system. Several lines of evidence suggest a key role for antibody in protection against congenital infection and disease. Antibodies to virally encoded envelope glycoproteins have also been shown to be protective against congenital guinea pig CMV

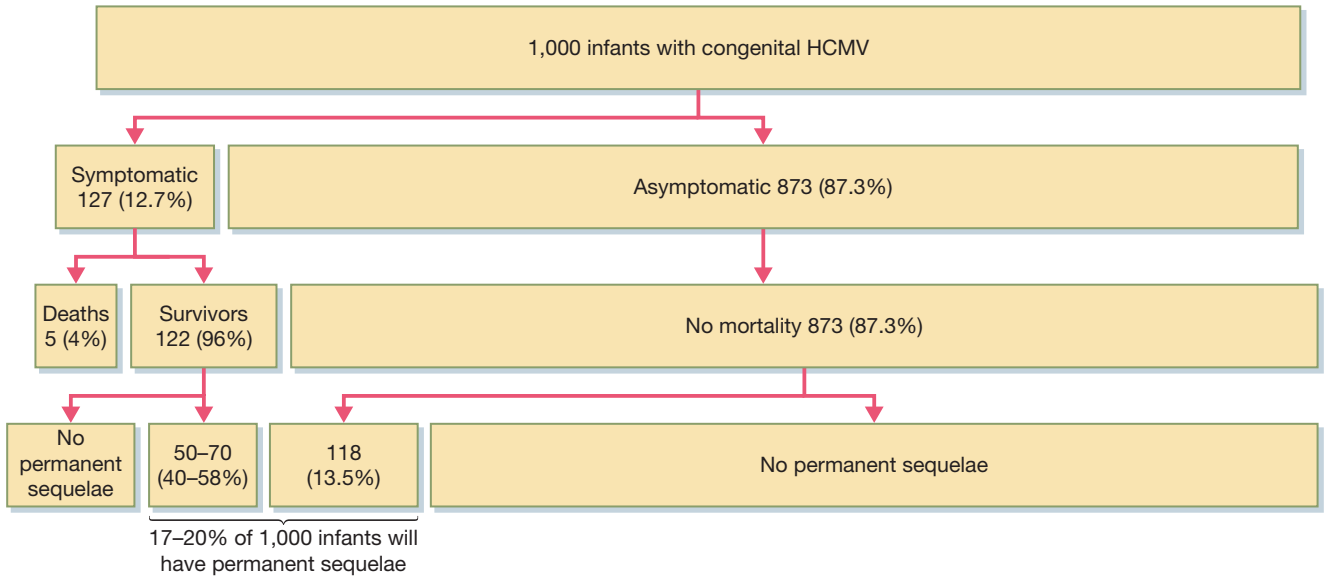


Figure 16.1. Current estimates of magnitude of long-term sequelae in infants with congenital cytomegalovirus (CMV) infection in the United States. In a given cohort of 1000 infants with congenital CMV infection, 170 to 190 will have permanent sequelae, with one-third from the symptomatic group and two-thirds from the asymptomatic group. HCMV, human cytomegalovirus. *(Data from Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection.* Rev Med Virol. *2007;17:355–363.)*

infection.[77–79] Moreover, the use of human CMV immunoglobulin has proved to be useful in SOT recipients, in whom good evidence for approximately 50% prevention of CMV disease has been demonstrated.[80–87] In addition to modifying the risk of infection in transplant recipients, the importance of antibodies in protection of premature infants against postnatally acquired CMV infection is supported by the observations of clinical efficacy of passive maternal IgG against virus transmission via blood transfusion.[88,89] There may be some evidence for protection of the fetus conferred by passive administration of antibodies to pregnant women who are at high risk for transplacental transmission of virus.[90] In an uncontrolled study conducted by Nigro and colleagues, hyperimmune CMV immunoglobulin therapy was associated with significantly lower risks of congenital CMV infection and disease.[91] However, in a Phase IIB, randomized, double-blind study conducted by Revello and colleagues (CHIP study), CMV hyperimmunoglobulin, when compared to saline placebo, did not have a statistically significant impact on the rate of CMV transmission in pregnant women with primary CMV infection.[92,93] An National Institutes of Health (NIH)-supported multicenter trial is ongoing to resolve the question of the impact of passive CMV immunoglobulin therapy on congenital CMV transmission in pregnant women with primary CMV infections.[94] Putative beneficial effects of CMV hyperimmunoglobulin treatment for the infected fetus appear to be mediated in part at the placental-fetal interface.[95–97] In the Nigro study, hyperimmunoglobulin treatment was associated with maintenance of normal placental size and structure, as measured by ultrasound, and with normal placental histology, as compared to untreated CMV-infected controls.[98,99] Finally, as discussed below, glycoprotein B (gB) vaccine protected women and solid-organ transplant recipients against CMV infection in clinical trials, providing additional support for a key role for antibody in mediating protection.

Until recently, CMV-neutralizing antibodies were measured in the laboratory based upon their ability to prevent CMV infection of human fibroblast cells. In 2005, Wang and Shenk demonstrated that the ability of CMV to infect epithelial cells was dependent on the presence of intact CMV genes capable of encoding proteins UL128-131, genes that were routinely deleted or mutated in viral strains highly passaged in fibroblasts.[100] Moreover, these proteins formed a pentameric complex (PC) with the gH and gL glycoproteins of the virus. Although the gH/gL proteins are capable of inducing neutralizing antibodies, neutralization is improved by including the UL128-131 subunits in a vaccine formulation.[101–103] Studies in many laboratories indicate that viral neutralization at epithelial cell surfaces requires antibody to the CMV PC (described below; see Table 16.2). A recent analysis of the virus-neutralizing characteristics of CMV hyperimmunoglobulin performed by serial depletion of antibody to cell-surface expressed surface proteins indicated that the major neutralizing antibody response was directed to PC, suggesting that the contribution of anti-gB antibodies to the overall CMV neutralization profile may have been overestimated in earlier studies.[104] Studies by the group in Pavia, Italy, suggested that early appearance of antibodies against the PC correlated with protection against transplacental virus transmission in women with primary CMV infection, while development of gB antibodies was not protective.[105]

Although antibody is a vital component of protection, it is evident that cellular responses are also important in the control of CMV infection. In the setting of transplantation, the correlation between recovery of host cellular immunity and freedom from CMV disease has been apparent for many years.[106–111] $CD4^+$ cells sensitized to the virus appear to be necessary to resolve the persistent excretion of CMV by congenitally infected children,[112] and delayed development of a CMV-specific $CD4^+$ T-cell response has been observed in infected mothers who transmitted virus to the fetus.[113] $CD8^+$ cytotoxic T cells are also important in immunity to CMV, particularly those that target the immunodominant CMV tegument phosphoprotein, pp65. This has been confirmed by the demonstration of the therapeutic efficacy of adoptive transfer of both pp65-specific and mixed populations of cytotoxic T lymphocyte (CTL) cells to bone marrow transplant recipients in preventing development of CMV disease in the posttransplantation setting.[106,114,115] Interestingly, CMV-specific $CD8^+$ cells are also found in congenitally infected children who have infections that do not disseminate after birth,[116,117] although T-cell responses in these infants are reduced in both frequency and functionality when compared to adult subjects.[118] The targets of $CD8^+$ T cells that appear to be of paramount importance in protection are the immediate-early antigen 1 (IE1) and, as noted, the pp65 tegument protein.[119–121] T-cell epitopes encoded by additional CMV proteins may also be required for optimal T-cell–mediated protection, and may be employed in future subunit vaccines.[122–124]

Table 16.2 lists several of the CMV proteins thought to be responsible for the induction of neutralizing and nonneutralizing antibodies as well as cellular immune responses (particularly CTL responses) in the normal seropositive host. Many are currently being evaluated in the various clinical trials of candidate CMV vaccines that are described below.

TABLE 16.2 Cytomegalovirus-Encoded Proteins That Might Be Included in a Subunit Vaccine

CMV Gene Product	Host Immune Response
ENVELOPE GLYCOPROTEINS	
gB	Major target of neutralizing antibodies; target of CTLs
gH/gL	Important target of neutralizing antibodies; target of CTLs
gH, gL, UL128–131 (PC)	PC of gH/gL/UL128/UL130/UL131 on viral envelope; target of neutralizing antibodies; antibodies neutralize CMV infection at epithelial and endothelial cell surfaces
gM/gN	Targets of neutralizing antibody responses
STRUCTURAL PROTEINS	
pp65	Major target of CTLs; target of nonneutralizing antibody responses
pp150, pp28	Targets of CTLs and nonneutralizing antibody responses
pp50	Target of CTLs
pp71, pp52	Targets of nonneutralizing antibody responses
NONSTRUCTURAL PROTEINS	
IE1	Major target of CTLs; target of nonneutralizing antibody responses

CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; IE1, immediate-early antigen 1; PC, pentameric complex.

VACCINES IN CLINICAL TRIALS

Recent years have seen a notable increase in the number of candidate CMV vaccines that are undergoing testing in human trials. The history of efforts to develop a CMV vaccine has been reviewed.[125] The various vaccine candidates are listed in

TABLE 16.3 Status of Cytomegalovirus Vaccines Evaluated in Clinical Trials

LIVE ATTENUATED AND DISABLED VIRUS VACCINES	
AD169 vaccine	• Elicited CMV-specific antibodies in seronegative vaccine recipients • Significant injection-site and systemic reactogenicity
Towne vaccine (± rhIL12)	• Elicits humoral and cellular immune responses • Favorable safety profile; no evidence for latency or viral shedding in recipients • Reduced CMV disease but not infection in renal transplant recipients • Augmented immunogenicity when administered with recombinant IL-12 in Phase I studies
Towne/Toledo chimera vaccines	• Favorable safety profile; no evidence for latency or viral shedding in CMV seropositive subjects • Attenuated compared to Toledo strain of HCMV • No efficacy data available; studies in seronegatives in progress
V160–001 replication-defective vaccine	• AD169 backbone with restoration of UL128/130/131 PC components • Rendered replication-incompetent by inclusion of ddFKBP/Shld1 • Administered with alum-based adjuvant • Phase I studies ongoing
SUBUNIT VACCINE	
Glycoprotein B (CHO cell expression) MF59 (Sanofi)/AS01 (GSK) adjuvants	• Favorable safety profile • High-titer neutralizing antibody and strong cell-mediated immune responses; augments humoral immunity in seropositives (gB/MF59) • Demonstrated efficacy in young women against primary infection and against CMV disease in solid-organ transplant patients (gB/MF59) • Safety and immunogenicity demonstrated with gB/AS01 in Phase 1 (no efficacy data)
PADRE-pp65-CMV and Tet-pp65-CMV fusion peptide vaccines ± CpG DNA adjuvant	• Lipidated fusion peptides constructed from pp65 CTL epitopes • Linked to either a synthetically derived pan-DR or Tet epitope • Phase I studies ongoing
eVLP VACCINE	
eVLP gB vaccine (HEK cells) ± alum adjuvant	• eVLPs formed by cotransfection of MMLV gag and gB constructs • Extracellular domain fused with transmembrane and cytoplasmic domains of VSV G protein • Phase I studies currently in progress
VECTORED VACCINES	
Glycoprotein B/canarypox vector	• Favorable safety profile • Suboptimal immunogenicity • "Prime-boost" effect upon combined administration with Towne
pp65 (UL83)/canarypox vector	• Favorable safety profile • Strong antibody and cell-mediated immune responses
gB/pp65/IE1 trivalent DNA vaccine; gB/pp65 bivalent DNA vaccine	• DNA adjuvanted with poloxamer adjuvant and benzalkonium chloride • Phase II study with bivalent gB/pp65 vaccine in HSC transplant recipients demonstrates impact on CMV disease • Phase III study of bivalent vaccine ongoing in transplant patients • Trivalent vaccine (gB/pp65/IE1) was evaluated with Towne in prime-boost vaccination study
gB/pp65/IE1 alphavirus replicon trivalent vaccine	• Engineered using replication-deficient alphavirus technology • Generation of virus-like replicon particles • Phase I clinical trial recently reported • Virus-neutralizing antibody and cell-mediated immune responses
gB/pp65 LCMV bivalent vectored vaccine	• Vectored using LCMV backbone • LCMV GP gene replaced by gB, pp65 • Disabled, single round of replication • No antivector immunity (allows for boosting) • Virus-neutralizing antibody, cellular ($CD4^+$, $CD8^+$) responses

CMV, cytomegalovirus; CpG, cytosine phosphate guanine; CTL, cytotoxic T lymphocyte cell; HCMV, human cytomegalovirus; IE1, immediate-early antigen 1; IL, interleukin; LCMV, lymphocytic choriomeningitis virus; PC, pentameric complex; rhIL, recombinant human interleukin.

Table 16.3 and are discussed in detail below. They can be broadly grouped into three categories: *live attenuated vaccines*; *subunit vaccines* based on recombinant proteins and peptides; and *vectored vaccines* comprising combinations of key CMV immunogens in various expression systems. These categories are individually reviewed in the following sections.

Live Virus Vaccines

The first approach to CMV vaccine development employed live attenuated strains of virus passaged in tissue culture. Elek and Stern[126] initially used the AD-169 laboratory strain to immunize normal adults, but the strain was not further developed. Plotkin and colleagues[127] isolated a strain (Towne) from a congenitally infected infant and passaged it in human embryo fibroblasts until the 125th passage, in the process performing three clonings by plaque purification; pools were then prepared at the 128th passage for vaccine trials.[128–130] Initial clinical trials were performed in healthy adult volunteers.[131,132] In these studies when the vaccine was given subcutaneously or intramuscularly, seroconversion was seen in nearly 100% of volunteers (Fig. 16.2), but intranasal administration did not stimulate immunity. During the second week after immunization, a local reaction consisting of erythema

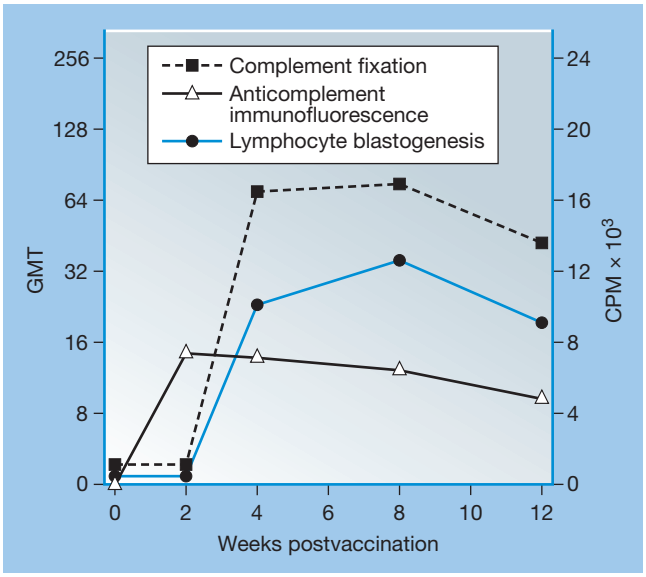


Figure 16.2. Typical immune responses to the Towne strain of live attenuated cytomegalovirus (CMV) vaccine showing the development of complement fixation antibodies, anticomplement immunofluorescence antibodies, and CMV-specific blastogenic responses. CPM, counts per minute; GMT, geometric mean antibody titer.

and induration appeared at the site of inoculation and lasted approximately 1 week, but systemic reactions were absent. Surprisingly, no virus was excreted in the pharynx or urine and virus was not recovered from the blood. Routine clinical laboratory tests also showed no abnormalities. Subsequent studies showed the persistence of antibodies for at least several years in adult female pediatric nurses who were vaccinated with the Towne strain.[133]

In the Towne vaccine studies, lymphocyte proliferation assays demonstrated sensitization to CMV antigens induced by vaccination. $CD8^+$ cell-mediated human leukocyte antigen (HLA)-restricted cytotoxicity of CMV-infected cells also was elicited in the Towne vaccinees.[134,135] Towne virus thus induced both cellular and humoral immunity to CMV.[136] However, tests of lymphocyte subsets after vaccination failed to show the increase in suppressor cells and decrease in the helper-to-suppressor cell ratio characteristic of acute CMV disease.[137] Studies of cellular immune responses in Towne vaccine recipients performed by Jacobson[138] confirmed that all vaccinees developed both $CD4^+$- and $CD8^+$-mediated responses, but over the course of 12 months those responses declined, particularly to the pp65 protein.

In related studies, sera of vaccinees were tested for antibodies to early antigens (EAs) and immediate early antigens (IEAs) of the virus.[139,140] These proteins are not present in the virus particle and are synthesized only during viral replication. Assessment of serologic, biopsy, and polymerase chain reaction (PCR) data led to the conclusion that Towne vaccine produced an abortive infection at the site of inoculation and stimulated a range of antibody and cellular responses similar to those noted after natural infection, including delayed-type hypersensitivity. However, immune responses were lower than after natural infection, perhaps because no virus excretion or systemic reaction occurred after vaccination.[141]

Vaccine Efficacy in Transplantation Patients

To evaluate efficacy of Towne vaccine, advantage was taken of the high morbidity and mortality that CMV causes in seronegative renal transplant recipients who receive a kidney from a seropositive donor.[142–144] After a pilot study to demonstrate tolerance of the vaccine, controlled, double-blind trials were conducted in prospective renal transplant recipients at academic hospitals at both the University of Pennsylvania and the University of Minnesota. The seronegative patients were randomized to receive vaccine or placebo subcutaneously prior to transplantation. Following transplantation, the patients were observed clinically, virologically, and serologically and scored for CMV-associated disease severity by individuals blind to the patients' vaccine status.[145–147] CMV disease scores were calculated based on assessment for fever; laboratory abnormalities including leukopenia and thrombocytopenia; hepatitis; pneumonia; gastrointestinal bleeding or ulceration; central nervous system changes including lethargy, stupor or coma; renal insufficiency; arthritis; secondary infection; and death.[147] Despite the induction of relatively poor antibody and cellular immune responses, the vaccine appeared to provide protection against severe CMV disease of a magnitude similar to that of prior natural infection. Two additional studies in renal transplantation patients, one of which was a multicenter study, reached the same conclusion.[148,149] In the double-blind, randomized, placebo-controlled multicenter trial, severe CMV disease was markedly reduced by vaccination, with an incidence of 17% in controls, versus 0% in vaccine recipients ($P < .03$).[148] Thus, Towne immunization rendered seronegative patients more resistant to the effects of CMV infection. Notably, no study has ever demonstrated evidence of shedding of Towne virus in any vaccine recipient; this has been confirmed by examination of viral strains in subjects in Towne vaccine studies that were later found to be shedding virus. In subjects shedding virus who received kidneys from seropositive donors, viral strains were shown by DNA restriction-endonuclease assays to be identical to strains that were latent in the donor kidneys that had been reactivated after transplantation, and not identical to the Towne strain.

TABLE 16.4 Doses of Subcutaneous Cytomegalovirus Challenge Required to Infect or Cause Disease

	Infection (PFU)	Disease (PFU)
Seronegative	≤10	≤10
Natural seropositive	~500	1000
Vacinees	100	≥100

PFU, plaque-forming units.
From Plotkin SA. Is there a formula for an effective CMV vaccine? J Clin Virol. *2002;25(Suppl 2):S13–S21.*

Challenge Studies in Immunocompetent Towne Vaccinees

After this demonstration of safety and efficacy, volunteer Catholic priests who lived in a closed community and who were CMV-seronegative were vaccinated with Towne vaccine to determine whether they could be protected against a subsequent artificially administered challenge. One year later, in company with unvaccinated seronegative and naturally seropositive priests, they were challenged subcutaneously with varying doses of an unrelated and less attenuated wild-type CMV isolate called Toledo (see Fig. 16.3 for a comparison of the respective genome structures of Towne and Toledo virus). Table 16.4 shows the results. Although subsequent molecular studies demonstrated that the Toledo strain is not a fully authentic wild-type strain, since it contains both a genomic inversion and a mutation that abrogates synthesis of an intact UL128 (PC subunit),[150] a challenge dose of 1000

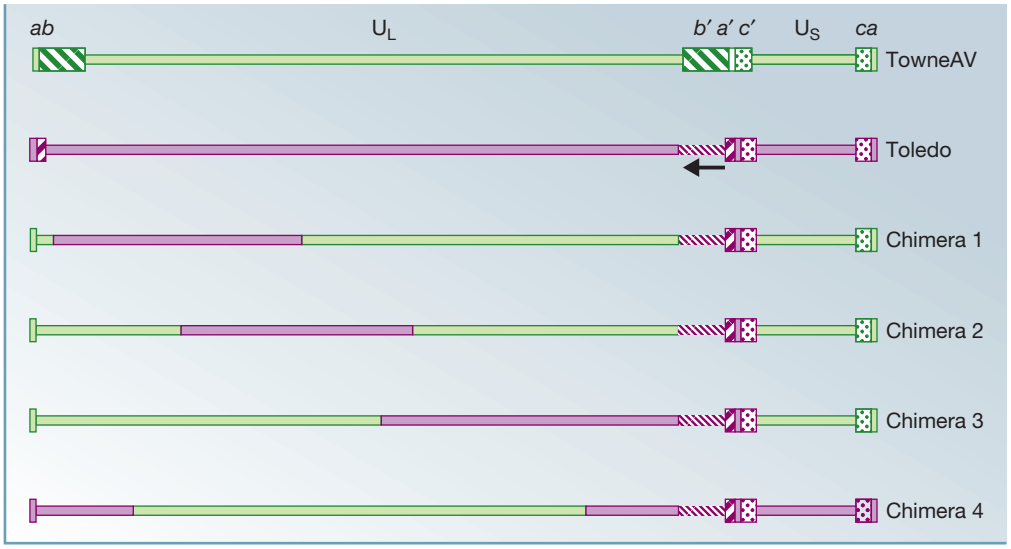


Figure 16.3. Genomic structures of cytomegalovirus Towne, Toledo, and four live attenuated "chimeric" vaccine candidates currently in clinical trials. The specific regions of each chimera's genome derived from Towne and Toledo are shown. The ULb′, unique long (U_L), unique short (U_S), and repeat (ab, b′a′c′, ca) regions of the genomes are shown. *(Modified from Heineman TC, Schleiss M, Bernstein DI, et al. A phase 1 study of 4 live, recombinant human cytomegalovirus Towne/Toledo chimeric vaccines*. J Infect Dis. *2006;193:1350–1360.)*

plaque-forming units (PFU) in this study nonetheless caused illness, even in those who were naturally seropositive; therefore, this dose was not given to other groups.[151] With a reduction to a 100-PFU dose, the challenge virus caused a mild infectious mononucleosis syndrome with virus excretion in seronegative individuals, but it was asymptomatic in naturally seropositive individuals.[152] Vaccinees also were protected against disease caused by 100 PFU, but about half transiently excreted virus asymptomatically. After injection of 10 PFU, CMV infection and symptoms were seen in the seronegative individuals, whereas both vaccinated and naturally seropositive individuals resisted the challenge. Thus, vaccination of normal individuals rendered them resistant to an artificial parenteral challenge, but with protection of lower magnitude than that conferred by natural immunity.

Despite the protection thus demonstrated against parenteral infection, in another study Towne vaccine failed to prevent infection of seronegative mothers in close contact with CMV-excreting children, although naturally seropositive women were protected.[75] This controlled trial showed no reduction in the infection rate of Towne-vaccinated mothers compared with placebo-inoculated seronegative mothers. The authors speculated that the 20-fold lower neutralizing antibody levels induced by Towne vaccine in comparison with those observed in the setting of natural seropositivity accounted for the vaccine's failure. Route of infection may have also played a role, since these young women presumably acquired CMV infection at mucosal surfaces following exposure to infectious body fluids (urine, saliva), in contrast to the parenteral route of inoculation carried out in the Toledo challenge studies.

Strategies to Improve Towne Immunogenicity

A number of strategies to improve the immunogenicity of the Towne vaccine are currently being explored. One approach is to coadminister Towne with recombinant human interleukin (IL)-12 (rhIL-12). The immunogenicity and safety of this approach was evaluated in a Phase I, dose-escalation, randomized clinical trial in CMV-seronegative healthy volunteers.[153] The adjuvant effect of rhIL-12 was associated with dose-related increases in peak anti-CMV antibody titers and improved $CD4^+$ T-cell proliferation responses. The vaccine with adjuvant rhIL-12 at doses up to 2 μg was well tolerated and did not lead to Towne persistence, confirmed by whole blood CMV DNA PCR and urine CMV culture.

In another approach aimed at improving the immunogenicity of the Towne vaccine, a CMV DNA vaccine (described in greater detail in the following section), designated as VCL-CT02, was used to prime for memory immune responses to subsequent Towne vaccination. The priming efficacy of VCL-CT02, containing the three CMV genes pp65, IE1, and gB cloned from AD169 strain, along with Towne vaccine challenge was evaluated in a series of Phase I clinical trials in healthy CMV-seronegative volunteers.[154] The median time to first pp65 T-cell response and gB antibody response after Towne vaccine was 14 days for DNA-primed subjects, and 28 days for controls who were administered Towne vaccine only, suggesting a more rapid induction of antigen-specific responses with the DNA vaccine-primed group. Vaccination with VCL-CT02 was found to safely prime for an anamnestic response following subsequent administration of Towne vaccine. Urine CMV cultures obtained after the Towne booster were negative in all subjects. Preclinical studies of DNA vaccine priming followed by boosting with Towne vaccine in mice demonstrated that priming significantly enhanced T-cell responses against gB, pp65, and IE1 as measured by interferon (IFN)-γ secretion.[155]

Towne/Toledo Chimeric Vaccine Approach

Scientists at MedImmune (formerly Aviron) discovered that some variants of the Towne vaccine strain had genetic deletions (~13.5 kb) in a particular part of its genome, designated as the ULb′ region.[156,157] This region, present in all low-passage clinical isolates, contains at least 17 genes not found in the Towne strain. Some of these gene products have emerged as important targets of host immune responses, latency, and pathogenicity.[158,159] Another difference in the genome of the Towne strain of CMV compared to clinical isolates is the discovery of a mutation in the Towne UL130 gene coding sequence that abrogates synthesis of a functional UL130 protein, a constituent of the PC[160]: this mutation may play a role in the attenuation of the Towne vaccine. In light of these observations, it was hypothesized that restoration of the

Toledo ULb′ sequences in the background of Towne virus might provide one or more genes coding for proteins that would increase the replication and immunogenicity of the Towne strain in vivo, resulting in a more efficacious vaccine. To address this possibility, four recombinant chimeric variants of Towne and the low-passage virulent Toledo strain have been made in which different segments of Towne have been replaced by the analogous sequences of Toledo (Fig. 16.3).[161] Phase I trials of safety and immunogenicity of these recombinant vaccines were initially conducted in seropositive subjects. These recombinants were well tolerated but not immunogenic in the seropositive volunteers. The lack of evidence of boosting of immune responses in seropositive subjects in this initial Phase I study may have implications for the prospects of vaccine-mediated protection against reinfection (and potential vertical transmission) in seropositive individuals. This theoretical shortcoming aside, subsequent studies of these chimeras in seronegatives focused on identifying distinctions in safety and immunogenicity among the recombinants, and correlating these findings with genetic markers that could elucidate the impact of viral gene variation on immune response as well as pathogenicity. A study of these chimeric vaccines in healthy, CMV-seronegative males conducted by Adler and colleagues at Virginia Commonwealth University has recently been published (NCT01195571, clinicaltrials.gov).[162] All these chimeras were safe, and none was shed by vaccinees, which is reassuring with respect to safety. Notably, all the chimeras were found to carry a mutation in *UL128* that presumably renders the vaccine viruses incapable of forming a functional PC. The impact of this mutation on immunogenicity and protection will require additional study.

Replication-Defective Virus Vaccine Approach

Recently, Tong-Ming Fu and colleagues at Merck have initiated a randomized, double-blind, placebo-controlled, dose escalation study of a candidate vaccine, V160, in healthy adult subjects.[162–165] This vaccine restored the UL128/UL130/UL131 proteins to the genome of the AD169 strain of CMV, in the process allowing a functional PC to be formed toward the goal of eliciting high-titer antibody capable of neutralizing virus infectivity at the epithelial-endothelial cell interface. In addition, it linked two CMV gene products, UL51 and IE1/2, to the destabilization domains of FK506 binding protein, ddFKBP, in the process rendering protein stability dependent on the presence of the small molecule Shield 1 (Shld 1). Accordingly, viral progeny can only form in cell culture when Shld 1 is provided by stable transfection, which allows production of a replication-defective candidate vaccine. Because Shld 1 is a synthetic ligand that does not exist in any natural biological system,[166] the resulting virus is safe for human use, as it can give only one cycle of replication in the human host, but as a vaccine it should be able to, in principle, confer the full breadth of immune responses to all relevant CMV antigens. In addition to eliciting high-titer antibody to the PC, preclinical animal testing indicates that this vaccine engenders cellular responses. This Phase I study is currently ongoing (NCT01986010, clinicaltrials.gov).

SUBUNIT VACCINE

Glycoprotein B Vaccines

The administration of purified protein subunit vaccine in CMV vaccine trials has chiefly focused, to date, on the glycoproteins in the envelope of the CMV virion that induce virus-neutralizing antibodies (see Table 16.2). Among the numerous glycoproteins present in the envelope, most attention has been directed toward the *gB protein* (so-called because of its analogy with the herpes simplex gB). All CMV-seropositive individuals have antibody to gB.[167,168] The glycoprotein analogous to gB has both been purified from guinea pig CMV-infected cell lysates and/or expressed via a number of recombinant methodologies, and when administered as a vaccine has been shown to protect pups born to mothers immunized before pregnancy and subsequently experimentally challenged with virus during gestation.[169–172] The gB protein initially was purified from the virus by immunoaffinity chromatography and inoculated into animals and then humans, with induction of neutralizing antibodies.[173–176] Subsequently, the gene for gB was inserted into several expression vectors for in vitro production and purification of the protein. Spaete and his colleagues at Chiron (Chiron was acquired by Novartis vaccines, which was subsequently acquired by Glaxo-Smith-Kline [GSK] vaccines) produced a truncated gB in Chinese hamster ovary cells, engineered to be secreted as a truncated, uncleaved polypeptide into cell supernatants.[177] Clinical trials have been performed using this purified protein (the gB vaccine was acquired from Chiron by Sanofi-Pasteur vaccines, prior to Chiron's acquisition by Novartis) combined with an oil-in-water emulsion adjuvant known as MF59. The initial report of the use of gB vaccine was a Phase I randomized, double-blind, placebo-controlled trial, in adults, in which gB was combined either with MF59, or alum, with vaccine administered at a 0-, 1-, and 6-month schedule.[178] Levels of gB-specific antibodies and total virus-neutralizing activity were determined 2 weeks after the third dose of vaccine, and were found to exceed those observed in CMV-seropositive control subjects. Formulation with MF59 was substantially more immunogenic than with alum. The optimal dose of vaccine appeared to be between 5 μg and 30 μg of gB. A fourth vaccine was given at 12 months to a subgroup in this study and neutralizing antibody increased from a geometric mean titer of 26 to 113, although 1 year later it fell to 57.[178] Antigen dose and immunization regimen were further evaluated in a Phase I study of 95 CMV-seronegative adult volunteers.[179] A 5-μg dose of gB/MF59 was compared with a 30-μg dose, at dose intervals of 0, 1, and 2 months; 0, 1, and 4 months; or 0, 1, and 6 months. There was no significant difference in antibody production between the two dose levels, but the vaccine induced the highest antibody titers when given as the 0-, 1-, and 6-month regimen. The immunogenicity and safety of recombinant gB were subsequently studied in toddlers.[180] A 20-μg dose was evaluated in 15 children (age range: 12–35 months) administered at 0, 1, and 6 months. After the third dose of vaccine, antibody responses of toddlers were greater than those of naturally infected adults and were notably higher than those of adults who had received three doses of the same vaccine in earlier trials. Interestingly, both mucosal immunoglobulin G and secretory immunoglobulin A antibodies were induced in many vaccinees studied in clinical trials of gB/MF59 vaccine. Presence of the former in vaccinees has been correlated with a serum neutralizing antibody titer of over 1 : 64.[181]

Because the immune responses engendered against gB/MF59 target the version of the gB sequence encoded by the "Towne" strain of CMV, there is a theoretical concern that antibodies elicited by this vaccine may fail to cross-neutralize clinical isolates of CMV, which may encode different amino acid sequences in key gB epitopes. This concern, however, does not appear warranted, as serum from vaccinees is capable of broad cross-neutralization of clinical isolates of CMV.[182] Moreover, the sequence and structural modifications introduced into the vaccine form of gB do not appear to have impaired the authenticity of the anti-gB response, as antibodies from subjects vaccinated with gB/MF59 recognize the major neutralizing domains of the protein.[183] Vaccinees exhibit high levels

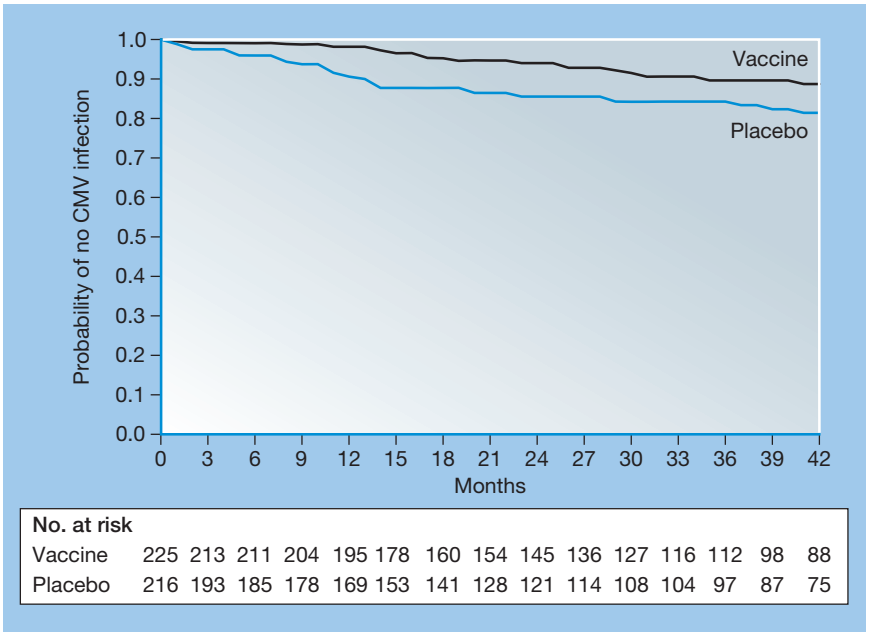


Figure 16.4. Efficacy of recombinant, adjuvanted cytomegalovirus (CMV) gB vaccine in seronegative women from a placebo-controlled study. Kaplan-Meier estimates of probability of remaining CMV-uninfected are shown. Up to 42 months after study enrollment, subjects in the vaccine group were more likely to remain free of CMV infection than were subjects in the placebo group ($P = .02$). In the vaccine group, 18 subjects were found to have CMV infection compared with 31 in the placebo group. *(Data from Pass RF, Zhang C, Evans A, et al. Vaccine prevention of maternal cytomegalovirus infection*. N Engl J Med. *2009;360:1191–1199.)*

of antibody avidity, comparable to those observed in natural infection.[184] Vaccine recipients also demonstrated an antibody response to the AD-2 epitope of gB, an important epitope in antibody-mediated neutralization, that was comparable to natural seropositives.[185] Recent insights into the postfusion conformation of gB should facilitate an improved understanding of the mechanisms of antibody-mediated neutralization.[186]

Pass and colleagues reported the results of a Phase II efficacy trial of the gB-MF59 vaccine conducted at the University of Alabama, Birmingham. This trial was undertaken in adolescent and young adult women who were at high risk for acquisition of CMV between pregnancies, with an annualized seroconversion rate of 7.8%.[74] The study was a randomized, double-blind, placebo-controlled clinical trial in seronegative women of childbearing age; 21% of the subjects had a toddler (age 13–36 months) at home. Vaccine (20 µg with MF59 adjuvant) or placebo was administered according to a 0-, 1-, and 6-month schedule.[187] The primary end point reported in this study was the time to primary CMV infection, documented by seroconversion using a gB-absorbed CMV immunoglobulin G assay.[188] Fig. 16.4 demonstrates the impact of gB vaccine on acquisition of CMV infection in this patient population. Primary CMV infection was confirmed by virus culture or immunoblotting in 18 of 225 (8%) in the vaccine group compared to 31 of 216 (14%) in the placebo group, an efficacy of 50% (95% confidence interval, 7–73%). The beneficial effect of vaccine appeared somewhat short-lived, being apparent for approximately 18 months, during the time window when vaccine-derived antibodies to gB were demonstrable. Limited information could be gleaned from this study about the impact of vaccine on congenital CMV infection. Congenital CMV infection occurred in 1 of 81 (1%) and 3 of 97 (3%) infants born to vaccine and placebo recipients, respectively. One infant in the placebo group had severe symptomatic congenital CMV infection. However, the sample size was too small to draw conclusions about efficacy against congenital infection. In another recently reported study of gB/MF59 vaccine in seronegative adolescent women, efficacy was somewhat lower than in the Pass study. In that study, adolescents completing the three-dose series (164 vaccine, 170 placebo) had 18 documented CMV infections in the placebo group and 11 in the vaccine group, with an efficacy of 38% (95% confidence interval, −31 to 72; $P = .20$) in the intent-to-treat analysis.[189]

In addition to studies in CMV seronegative individuals, gB-MF59 vaccine has also been evaluated for its potential to boost immune responses in individuals who are already CMV-seropositive. In a study of 120 seropositive women receiving a three-dose series of gB-MF59 vaccine (0-, 1-, and 6-month schedule), both CMV-specific antibody (enzyme-linked immunosorbent assay [ELISA] and neutralizing titer) and $CD4^+$ T-cell responses were significantly boosted.[190] This demonstration that antibody and T-cell responses could be augmented in an individual already infected with CMV may have implications for vaccination of seropositive women. Because approximately 75% of congenital CMV infections in the United States result from recurrent infections among pregnant women,[191] these data suggest an important potential strategy of immunizing seropositive individuals, toward the goal of preventing CMV reinfection and subsequent congenital transmission.

Further evidence of the potential usefulness of the gB-MF59 vaccine comes from a placebo-controlled Phase II study in solid-organ transplant recipients.[192] Seventy CMV seronegative and 70 CMV seropositive patients awaiting kidney or liver transplantation were randomized to receive either gB-MF59 adjuvant (20-µg dose, administered on a 0-, 1-, and 6-month schedule) or placebo. Anti-gB ELISA and neutralizing titers were stimulated in seronegative transplant recipients and were significantly boosted in seropositive subjects. Posttransplantation, the magnitude and duration of CMV viral DNA detection in blood (DNAemia), as well as the total number of days of antiviral (ganciclovir) therapy, were compared across groups. The results were striking, particularly for gB-MF59–vaccinated seronegative recipients of organs from seropositive donors, in

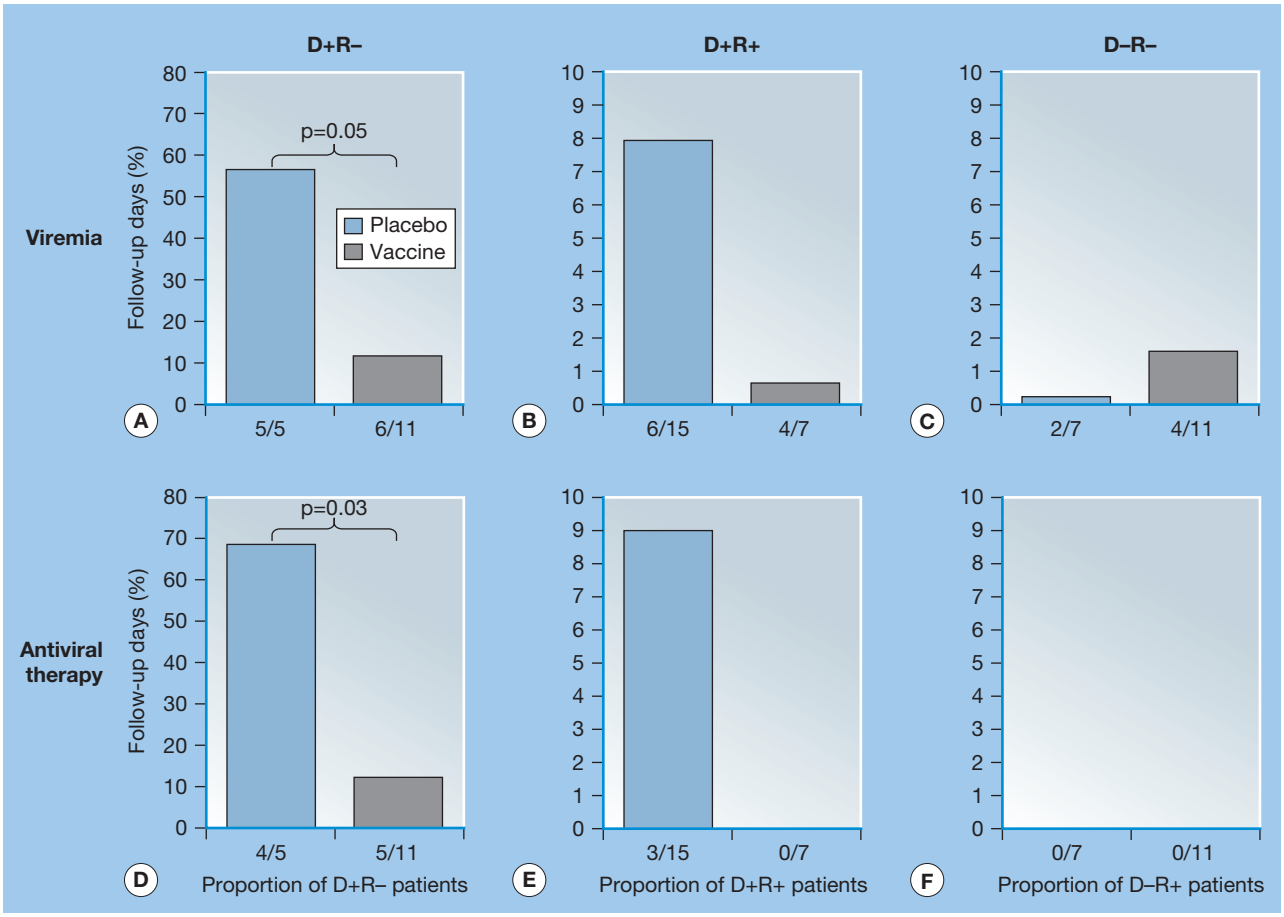


Figure 16.5. Impact of gB/MF59 vaccine in kidney or liver transplantation patients. The proportion of days that patients in the three subgroups at risk of CMV infection (D+R–, D+R+, or D–R+) experienced either viremia (panels **A** to **C**) or required antiviral therapy (panels **D** to **F**). The total number of patients with viremia or requiring antiviral therapy is indicated below each column. Recombinant gB vaccine had a statistically significant impact on both end points in the D+R– subgroup. D–, cytomegalovirus seronegative donor; D+, cytomegalovirus seropositive donor; R–, cytomegalovirus seronegative recipient; R+, cytomegalovirus seropositive recipient. *(Data from Griffiths PD, Stanton A, McCarrell E, et al. A randomised placebo controlled pharmacodynamic trial of cytomegalovirus glycoprotein B vaccine with MF59 adjuvant in transplant patients*. Lancet. *2011;377:1256–1263.)*

whom both the duration of DNAemia and the total number of days of ganciclovir administration were significantly reduced when compared to placebo recipients (Fig. 16.5). Only one patient (placebo recipient) was observed to have CMV end-organ disease, so no assessment of the efficacy of vaccination on the development of CMV disease could be made. Anti-gB antibody titer correlated directly with the virus-neutralizing capacity of serum, and there was an inverse correlation between antibody titer and the duration of DNAemia. These observations suggest that a gB subunit vaccine may ultimately prove useful in not only preventing or modifying congenital CMV infection, but also in reducing CMV disease following solid organ transplantation.

Other formulations of recombinant gB vaccine have been evaluated in early Phase I studies. A secreted, uncleaved form of CMV gB, fused to a portion of the herpes simplex virus-2 gD protein, was developed by GlaxoSmithKline vaccines, and has been evaluated with adjuvant AS01 for immunogenicity and safety (NCT00435396, clinicaltrials.gov). The gB/AS01 vaccine, designated as GSK1492903A, induced virus-neutralizing antibody responses and $CD4^+$ T-cell responses, and neutralizing responses following vaccination persisted longer than those noted by historical comparisons to gB adjuvanted with MF59.[125] Other adjuvants for recombinant gB based on synthetic Toll-like receptor (TLR) 4 agonists have also been evaluated in preclinical studies but have not yet entered human trials.[193]

Peptide Vaccines

Another type of subunit approach evaluated in clinical trials has been peptide-based vaccines, based on identification of key immunogenic sequences important for T-cell responses in the CMV proteome. A peptide vaccine to induce cytotoxic T-lymphocyte (CTL) cells has been developed by the Beckman Research Institute at the City of Hope (California) and is currently being evaluated in Phase I studies (NCT00712634, clinicaltrials.gov). Lipidated fusion peptides were constructed from multiple pp65 CTL epitopes, linked to either a synthetically derived pan-DR epitope peptide (PADRE) or one of several tetanus T(H) epitopes.[194,195] The epitopes cover the most prevalent HLA alleles in the Caucasian population, and induced robust cytotoxic cellular immune responses in HLA A*0201/K(b) transgenic mice. In preclinical testing the fusion peptides were immunogenic when administered in saline solution by either subcutaneous or intranasal routes.[196] CpG-containing single-stranded DNA (CpG7909; ss-oligodeoxynucleotide) added to the fusion peptides dramatically upregulated immune recognition by either route.[197] A Phase I trial of these PADRE-CMV fusion peptide vaccines, administered either with or

without the cytosine phosphate guanine (CpG) 7909 DNA adjuvant, was conducted in CMV seronegative and seropositive subjects ages 18 to 55 years.[197] This study demonstrated expansion of vaccine-elicited pp65-specific T cells, particularly in those subjects receiving vaccine in combination with adjuvant.[198] A recent Phase Ib study (NCT01588015, clinicaltrials.gov) demonstrated safety of the adjuvanted Tet-pp65 fusion peptide vaccine, with no evidence of an increase in adverse events related to HSCT in vaccine recipients.[199] City of Hope and Fortress Biotech have recently collaborated to form a new subsidiary company, DiaVax Biosciences, that will advance these candidate peptide vaccines into clinical trials. A Phase II study of the Tet-pp65 fusion peptide vaccine, CMVPepVax, is currently planned in HSCT patients (NCT02396134, clinicaltrials.gov). Outcome measures will include CMV-related events such as viremia, initiation and use of antivirals, and CMV end-organ disease.

VECTORED VACCINES

In general, vectored vaccines against CMV express, through a variety of techniques, the key immunogens described in Table 16.2, alone or in combination. The various expression techniques currently in clinical trials are outlined below.

Canarypox-Vectored gB and pp65 Vaccine

CMV proteins believed to be essential for an effective vaccine have been expressed using viral vectors suitable for clinical trials. In addition to inducing antibody, such vectors are particularly useful in inducing CTL responses to heterologous proteins encoded by inserted foreign genes. One such approach uses recombinant canarypox as the viral vector. Because avian poxviruses do not replicate in mammalian cells, canarypox virus vectors are safe for human use. Sanofi Pasteur developed canarypox gB and pp65 recombinants that were evaluated in a series of clinical trials.[200–202] Canarypox gB vaccine was not as immunogenic as subunit gB, but the pp65 recombinant gave excellent antibody responses. In addition, all 14 seronegative subjects given canarypox containing the gene for pp65 developed CTLs specific to CMV after two doses. The CTLs were $CD8^+$ cell–mediated and reached precursor frequencies similar to those found in naturally seropositive individuals.[201] Clinical trials have also explored the usefulness of ALVAC-gB employed in a "prime-boost" approach, in which ALVAC vaccine is administered to "prime" immune responses for subsequent "boost" with live attenuated vaccine or recombinant protein. In the first prime-boost study, ALVAC-gB was evaluated alone or in combination with Towne vaccine.[200] ALVAC-gB vaccine induced low neutralizing and ELISA antibodies in seronegative adults, but subjects primed with ALVAC-gB and then boosted with a single dose of Towne vaccine developed binding and neutralizing antibody titers comparable to naturally seropositive individuals. A subsequent study compared three immunization regimens: subunit gB/MF59; a "prime-boost" regimen in which ALVAC-gB was followed by gB/MF59; or a regimen in which both vaccines were administered concomitantly. All three approaches induced high-titer antibody and lymphoproliferative responses, but little benefit with priming was detected.[202]

Venezuelan Equine Encephalitis–Vectored gB, pp65, and IE1 Vaccine

Alphavirus replicon vector systems are being used as platforms for the development of many prophylactic and therapeutic vaccines for infectious diseases and cancer, and this vector technology has also been applied to CMV vaccines. One such vaccine developed by AlphaVax Vaccines, AVX601, is a two-component alphavirus replicon particle vaccine expressing CMV gB and an inframe fusion protein of pp65 and IE1[203,204]; thus, it contains both the immunodominant antibody and T-cell targets for CMV. This was evaluated in a randomized, double-blind, Phase I trial in healthy, CMV-seronegative adult volunteers.[205] This dose comparison study was performed in CMV-seronegative subjects; 40 subjects were randomized to either receive low-dose vaccine (1×10^7 infectious units) or high-dose vaccine (1×10^8 infectious units) by either subcutaneous or intramuscular route, or placebo, at 0, 8, and 24 weeks after enrollment. Local reactogenicity was more pronounced when vaccine was given by subcutaneous route, but immunizations were generally well tolerated. Four weeks after the third dose, 93% of the subjects in the low-dose and 100% of subjects in the high-dose groups developed neutralizing antibodies, as assessed by a microneutralization assay. T-cell responses to each of the CMV antigens measured by direct IFN-γ enzyme-linked immunospot (ELISPOT) assay were detected in 90% to 97% of vaccine recipients after the second dose, with similar rates of response in both low-dose and high-dose groups. The magnitude of the T-cell response was greatest for pp65, followed by gB and IE1. Polychromatic flow cytometry demonstrated that CMV antigen-specific $CD4^+$ and $CD8^+$ effector T cells producing multiple cytokines in response to peptide stimulation were induced in all subjects. The AlphaVax platform was acquired by Novartis Vaccines, who in turn (as noted) sold their vaccine portfolio to GSK. Therefore, the future development plan for AVX601 is unclear at this time. Similar Venezuelan equine encephalitis (VEE)-vectored vaccines have been developed targeting CMV gH and gL, and vaccine studies of the gH/gL complexes formed in vitro using the VRP platform have been performed in mice. These studies have demonstrated potent and cross-reactive neutralizing antibody responses.[206] No plans for clinical studies in humans of these vaccines have been reported to date, however.

Lymphocytic Choriomeningitis Virus-Vectored gB/pp65 Vaccine

A novel vector strategy that has recently entered phase I study utilizes an attenuated recombinant lymphocytic choriomeningitis virus (LCMV) platform. This vector employs producer cells that constitutively express the LCMV viral glycoprotein, allowing for replacement of this gene with the vaccine immunogen(s) of interest.[207] These vectors are replication defective, but elicit not only high-level antibody but also $CD4^+$ and $CD8^+$ T-cell responses, including cytotoxic responses. Hookipa Biotech AG (Vienna, Austria) has developed a replication-deficient LCMV-vectored CMV vaccine based on a truncated, membrane-anchored form of gB as well as pp65 (NCT02798692, clinicaltrials.gov). The combination of gB and pp65 in the LCMV-vectored vaccine platform has been shown in the guinea pig CMV system to confer additive protection in a model of congenital infection.[207] A phase I dose escalation study of this bivalent vaccination approach is currently ongoing with a bivalent vaccine, HB-101, which contains two vectored vaccines: one expressing pp65, and the other a truncated variant of gB. The primary study endpoint is safety, and the secondary endpoints include ELISA and virus-neutralizing antibody responses, cytokine responses, and T-cell responses.

DNA gB, pp65, and IE1 Vaccines

Vical (now Astellas) Corporation has developed two CMV DNA vaccines. VCL-CB01 (now ASP0113), a bivalent CMV DNA vaccine that contains two plasmids encoding CMV pp65 and gB, in combination with poloxamer CRL1005 and

benzalkonium chloride to increase immunogenicity,[208] was evaluated in a Phase I, open-label, dose-escalating trial in healthy CMV-seropositive and seronegative adults.[209] Through week 16 of the study, immunogenicity, defined by gB-binding ELISA antibody and/or ex vivo IFN-γ ELISPOT positive assay with pp65 or gB antigens, was documented in 45.5% of CMV-seronegative subjects and in 25% of CMV-seropositive subjects who received the full vaccine series. An additional assay, the cultured IFN-γ ELISPOT assay, was employed to evaluate whether the vaccine primed the memory T-cell response at week 32 or 16; this assay demonstrated a positive response to pp65 and/or gB in 68.2% (15/22) of CMV-seronegative subjects, including subjects who did not have detectable responses by ex vivo IFN-γ ELISPOT assay or ELISA. These results suggest that ASP0113 could prime antigen-specific T cells for the capacity to proliferate and secrete IFN-γ on restimulation with antigen.[209] A limitation of ASP0113 was the lack of gB antibody response in CMV-seropositive subjects, although the vaccine boosted existing pp65 T cell response. A Phase II, randomized, double-blind, placebo-controlled clinical trial to assess the safety and immunogenicity of ASP0113 was performed in donors and recipients undergoing allogeneic HSCT.[210,211] In immunogenicity analyses, higher frequencies of CMV-specific pp65 and gB T cells were observed at days 56 and 84 after transplantation in 33 CMV-seropositive recipients, who were immunized at 3 to 5 days prior to, and 3 to 6, 12, and 28 weeks after transplantation compared to placebo recipients. In the Phase II study of 80 HSCT recipients, ASP0113-vaccinated recipients demonstrated reductions in recurrence of CMV infection, duration of DNAemia, area under the curve (AUC) for cumulative viral load post-HSCT, and peak magnitude of DNAemia compared with recipients receiving placebo. Overall rates of CMV viremia were lower in the ASP0113 group when measured by a central laboratory, and time-to-first episode of viremia was longer in subjects receiving ASP0113.[210,211] These findings have set the stage for an ongoing placebo-controlled Phase III analysis aimed at enrolling 500 subjects who are undergoing allogeneic HSCT (NCT01877655, clinicaltrials.gov).

Another DNA vaccine, a trivalent formulation targeting gB, pp65, and IE1 (VCL-CT02), was evaluated in a Phase I trial involving a total of 40 healthy adult subjects (24 CMV-seronegative, 16 CMV-seropositive). Subjects received either a 1-mg or 5-mg dose of unadjuvanted trivalent vaccine in several multidose regimens, with modest immunogenicity; however, when participants received VCL-CT02 as a prime and the Towne vaccine as a boost, significantly enhanced pp65 T-cell and gB antibody responses were observed.[153] Efforts to improve the immunogenicity of DNA vaccines using cationic lipid-based adjuvants[212] and electroporation[213,214] are ongoing, although these approaches have not yet entered clinical trials.

Another group has approached CMV vaccine development for the transplantation setting by using messenger RNA (mRNA) to transfect dendritic cells, followed by adoptive transfer of these cells to stem cell transplant recipients.[215] Four CMV-seronegative healthy volunteers and three allogeneic HSCT recipients were immunized in this way, and CMV pp65-specific T cell responses were induced or expanded in a majority of recipients. Efforts to adapt these techniques to high-throughput good manufacturing practice (GMP) techniques are ongoing.[216,217]

NOVEL CYTOMEGALOVIRUS VACCINE APPROACHES ENTERING CLINICAL TRIALS OR IN PRECLINICAL DEVELOPMENT

Several novel vaccine approaches have been recently proposed for CMV; some have been evaluated to varying degrees in preclinical animal models.[218,219] Other new strategies are now beginning to be explored in Phase I studies. An interesting new approach to CMV vaccine development uses enveloped virus-like particles (eVLPs) that express gB in a strategy that delivers this antigen in a membrane-associated formulation that mimics that observed in viral particles. Preclinical evaluation of eVLPs expressing either full-length CMV gB (gB eVLPs) or the full extracellular domain of CMV gB fused with the transmembrane and cytoplasmic domains from vesicular stomatitis virus (VSV) G protein (gB-G eVLPs) demonstrated the superior immunogenicity of the gB-G form of the protein in mice.[220] Based on these findings, a candidate eVLP vaccine was generated by cotransfection of the Moloney murine leukemia virus (MLV) *gag* gene with the CMV gB gene. Following transcription and translation in human embryonic kidney (HEK) cells, the MLV matrix proteins and CMV gB form fully enveloped eVLPs which are then used as the candidate vaccine. This vaccine is designated as VBI-1501A, and has now entered a Phase I clinical trial. This study is being performed by VBI Vaccines, who generated the eVLP vaccine (NCT02826798, clinicaltrials.gov). The study is currently comparing the safety and immunogenicity of four dose formulations of vaccine, ranging from 0.5 to 2.0 μg of gB content with aluminum phosphate (alum) adjuvant or, in the case of one subgroup of vaccinees in the study, 1.0 μg of gB without alum. Another approach to generation of virus-like particles, originally developed using a baculovirus expression system by Redvax GmbH, a spin-off from Redbiotec AG,[221] was recently acquired by Pfizer Vaccines. RNA-based vaccines, in which "self-amplifying" RNAs are synthesized in vitro and then delivered as a vaccine, are currently being developed against CMV antigens by Novartis Vaccines (now GSK).[222] A recombinant vectored vaccine approach, expressing the murine cytomegalovirus (MCMV) gB in recombinant vesicular stomatitis virus, was effective when administered via a mucosal vaccine approach in a mouse challenge study.[223] A CMV "triplex" vaccine based on another attenuated poxvirus, modified vaccinia virus Ankara (MVA), has been developed by investigators at the City of Hope Research Institute (Duarte, California), expressing gB, pp65, and IE1.[224–226] A Phase II study is currently enrolling CMV-seropositive allogeneic HSCT transplant recipients (NCT02506933, clinicaltrials.gov), although no results have been reported at this time. The efficacy of the MVA vaccine approach has been established in challenge studies in a rhesus macaque model of CMV infection.[227,228] Recombinant adenovirus can also be engineered to express CMV proteins that elicit antibody and/or stimulate cellular targets. Like the recombinant vesicular stomatitis virus vectored vaccine, systemic and mucosal immunity can be induced by intranasal immunization using a replication-deficient adenoviral vector expressing MCMV gB or gH.[229,230] A novel replication-deficient adenoviral vectored vaccine, Ad-gBCMVpoly, has been developed by the Khanna Laboratory at the Queensland Institute of Medical Research to induce a broad repertoire of CMV-specific immune responses. Ad-gBCMVpoly encodes 46 CMV T-cell epitopes from multiple antigens such as IE1, IE2, pp50, pp65, pp150, gB, gH, and DNase, covalently linked to the extracellular domain of CMV gB antigen to allow the expression of the CMV polypeptide and gB proteins as a single fusion protein.[231–233] Recent evidence suggests that the immunogenicity of the polyepitope vaccine can be further augmented by the inclusion of TLR4 and/or TLR9 agonists.[234] An adenovirus-vectored vaccine using adenovirus serotype 4 is in early stage development by PaxVax Vaccines of Redwood City, California.[235]

Another novel approach to live, attenuated vaccine design is the targeted deletion of CMV genes modulating the host immune response through targeted mutagenesis of the viral genome maintained as a bacterial artificial chromosome

(BAC) in *Escherichia coli*. Such vaccines could, in principle, have enhanced safety profiles, as the genes responsible for immune evasion can be deleted, while retaining the broad repertoire of immune responses to multiple viral proteins intrinsic to live virus vaccines.[236] A BAC-derived MCMV recombinant vaccine lacking a total of 32 genes regulating immunoevasive functions in major histocompatibility complex (MHC)-1 presentation and NK cell response induced protective humoral and cellular immunes responses.[237] A recombinant live attenuated vaccine with a targeted deletion of a CMV protein kinase R antagonist was recently shown to be immunogenic and protective in the guinea pig model of congenital CMV infection, supporting the hypothesis that blocking viral immune evasion functions may improve the safety and immunogenicity of live virus vaccines.[238] The importance of genes promoting evasion of host $CD8^+$ T-cell responses, with subsequent reinfection of the previously immune host, was recently demonstrated convincingly in the rhesus CMV model,[239] underscoring the importance of developing vaccine strategies capable of overcoming these functions. An attenuated vaccine strategy was recently described based on a recombinant CMV that expresses the host ligand, ULBP2, for the natural killer cell receptor NKG2D. The vaccine strain was found to be susceptible to control by NK cells, but retained the ability to stimulate CMV-specific T cells.[240] Another novel, recently described vaccine strategy in a preclinical model elicits antibodies to CMV-encoded IL-10 mimics of the natural cytokine.[241]

Other new vaccine strategies are exploiting recent studies elucidating the mechanisms of attachment and entry of CMV into epithelial and endothelial cells. These studies have demonstrated that that a substantial component of the host neutralizing antibody response targets the PC protein complex, comprised of gH/gL/UL128/UL130/UL131.[100–105,242–244] The UL128, UL130, and UL131 gene products interact with the CMV gH and gL glycoproteins to form this multimeric complex and this complex has emerged as a major vaccine target (Fig. 16.6). Support for development of recombinant expression strategies for these proteins as potential vaccine candidates is strengthened by studies demonstrating that polyclonal and monoclonal antibodies targeting various components of the PC potently neutralize virus infectivity in cell culture.[245,246] Antibodies to the PC appear to be of particular importance in preventing transplacental transmission of CMV to the fetus.[103,105,247] Neither Towne vaccine nor the gB-MF59 vaccine appear to be as efficient at eliciting antibody responses that interfere with this pathway of infection.[248,249]

Another vaccine approach in preclinical development exploits the fact that CMV infection of human cells produces both infectious virus and noninfectious "dense bodies," which are particles that contain many of the proteins found in infectious virus, including both gB and pp65.[250] Since dense bodies do not contain viral DNA, they cannot replicate. However, these particles are immunogenic. Dense bodies exploited as vaccines could, in principle, elicit responses to the key CMV immunogens, without the attendant risk of inducing CMV infection. Moreover, other viral proteins (or, indeed, any protein of interest) can be incorporated into the dense bodies by genetic engineering.[251,252] In mice, neutralizing antibodies and cytotoxic T cells have been elicited with dense body vaccines without the use of adjuvant, but no human data are yet available.[253] It has been observed in mice that dense bodies derived from the Towne strain of CMV induce broad cellular immune responses and neutralizing antibodies that are capable of blocking infection of both fibroblasts and epithelial cells.[254]

In summary, the large (and increasing) number of candidate vaccines currently in preclinical development and clinical trials provides considerable hope that vaccines against both congenital and transplantation-associated CMV disease will be licensed imminently. Although the optimal age for implementation of a universal CMV vaccine remains uncertain,[40] it is most likely that once a licensed vaccine is available, the primary target population would initially include adolescent girls and boys, and women intending pregnancies. Immunization of both sexes during adolescence was assumed in the modeling of the potential impact of a CMV vaccine by the Institute of Medicine in a cost-benefit analysis of economic benefits of new vaccine development.[39] A secondary target for a licensed CMV vaccine would be HSCT and SOT patients. An ideal vaccine in this setting may need to be primarily focused on T-cell responses[255] rather than neutralizing antibody responses, although both arms of adaptive immunity are likely required for protection in all susceptible patient populations (including women of child-bearing age). Vaccine modeling has been performed based on the force of infection for CMV, which is the instantaneous per capita rate of acquisition of infection that approximates the incidence of infection

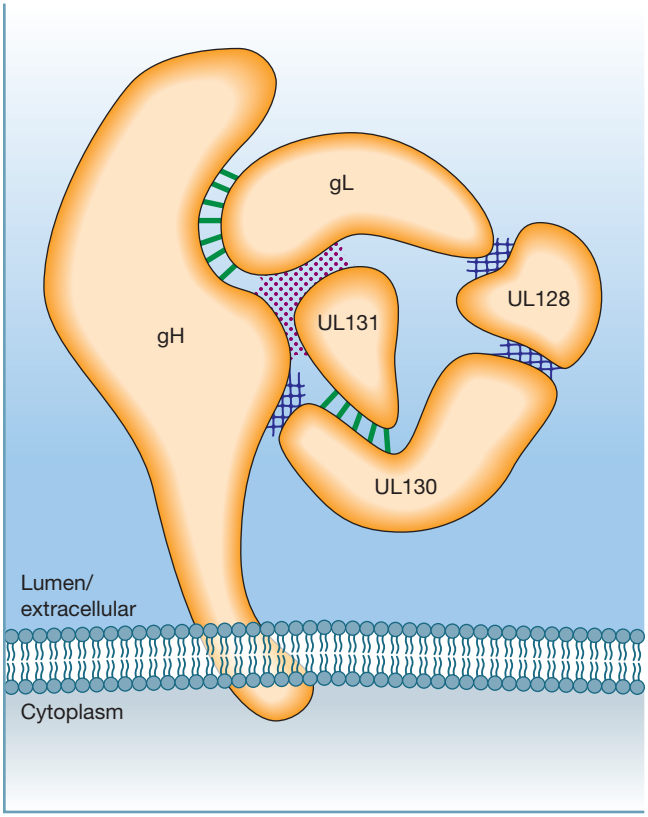


Figure 16.6. Novel cytomegalovirus (CMV) vaccine candidates aimed to elicit immune responses that block virus entry into epithelial/endothelial cells. Model demonstrating putative protein-protein interactions that make up a CMV protein complex consisting of glycoprotein H (gH)/glycoprotein L (gL)/UL128/UL130/UL131 complex. CMV gH protein is predicted to be inserted in the virion envelope and interacts with gL through disulfide linkage(s) (indicated by straight green lines). Interactions between UL130 and UL131 also involve a disulfide bond(s). UL128 interacts with both UL130 and gL through noncovalent interactions (indicated by crosshatching), and a surface created by both gH and gL is required for UL131 binding (indicated by red dots). *(From Ryckman BJ, Rainish BL, Chase MC, et al. Characterization of the human cytomegalovirus gH/gL/UL128–131 complex that mediates entry into epithelial and endothelial cells.* J Virol. *2008;82:60–70.)*

in the seronegative population. This modeling suggests that the force of CMV infection is quite low (1.6 infections per 100 susceptible persons per year), meaning that, on average, an infected person transmits CMV to just under two susceptible people annually.[256] Thus, even a modestly effective vaccine and moderate rates of vaccination could potentially have a significant impact on CMV transmission and lead to reductions in congenital CMV infection. A 2013 U.S. Food and Drug Administration workshop explored the clinical path toward licensure of a CMV vaccine, based on the assumption that prevention of congenital CMV infection would be the primary desired efficacy end point.[257] Analytic vaccinology approaches aimed at detailed dissection of the B-cell repertoire engendered in response to CMV infection may provide insights that inform and direct future subunit vaccine research.[258] Other future uses of a CMV vaccine, aside from HSCT and SOT recipients, remain speculative and dependent on further elucidation of the biology of the virus. Intriguingly, the use of the CMV virus itself as a possible vector for novel vaccines against other pathogens, including as a vectored vaccine against HIV infection, is an area of active investigation.[259] The very paradox of CMV immunity—the lack of long-term protection against reinfection observed in seropositives—could be exploited in the design of CMV-vectored vaccines for other pathogens, since preexisting immunity to CMV would not, in theory, be a barrier to effective immunization against the agent of interest. CMV-vectored vaccines for heterologous pathogens would ideally be modified to delete CMV genes involved in pathogenesis and, importantly, genes that increased the risk of transplacental transmission. Given the adverse health consequences of CMV infection that accrue over the life course for all individuals, CMV eradication effected by universal vaccination could become an eventual goal of large-scale immunization programs.[260]

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17 Dengue Vaccines

*Scott B. Halstead and Stephen J. Thomas**

Since World War II, the four dengue viruses (DENVs) have spread throughout tropical and many subtropical areas, achieving pandemic status. They are estimated to have caused 390 million infections and 96 million overt disease occurrences in 2010. Of these, perhaps 1 to 2 million persons are hospitalized with severe illnesses, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), and 0.1% to 5% die.[1–3] Dengue exacts a high socioeconomic toll on inhabitants of more than 120 tropical countries (Fig. 17.1). In addition, visitors to dengue-endemic countries are infected. In 2012, 91.7 million tourists arrived in dengue-affected areas in Asia.[4] Dengue illnesses exact a severe impact on quality of life.[5,6] In Asia and the Americas, the economic burden of dengue on the poor has been estimated at 1289 disability-adjusted life years per million population, similar to the burden imposed by the childhood and tropical disease clusters and tuberculosis.[7–10] An eight-country survey found costs for both outpatient and inpatient dengue illnesses to be substantial, ranging from 8 to 56 days of gross domestic product (GDP) per capita, costing the studied countries an estimated $587 million (in 2005 dollars) per year.[11] The global burden of dengue is both significant and underestimated.[12,13]

DENGUE VIRUSES AND DENGUE DISEASE

DENV-1 through DENV-4 are members of the *Flavivirus* group of the virus family Flaviviridae. The flavivirus genome consists of approximately RNA 11,000 base pairs (bp), which translate into three structural and seven nonstructural proteins (see "Chimeric Virus Vaccines" later). The four distinct DENVs all evolved from a common sylvatic ancestor, with separate introductions into the urban cycle of transmission—humans to *Aedes* mosquitoes to humans.[14,15] The basic virology of the four DENVs is similar to that of yellow fever (see Chapter 63) and Japanese encephalitis (see Chapter 33). The structural arrangement of DENV surface proteins controls how antibodies neutralize viruses. Dengue is an enveloped, positive-strand RNA spherical viral particle with a diameter of approximately 500 Å. RNA, when complexed with capsid proteins, forms the inside of the virus particle. It is surrounded by a bilayer lipid membrane. Anchored on the outside of the membrane are the M and E proteins. The M protein is a small protein that is hidden under the E protein but that projects to the surface in its immature state. The major protein that stimulates antibody response is the E protein. Each virus particle has 180 monomers of E that are organized into 90 tightly packed dimers that lie flat on the surface of the viral membrane. Each E protein monomer has three domains: DI, DII, and DIII.[16–19] DIII is thought to be involved in receptor binding. DII contains the fusion loop, which interacts with the endosomal membrane to facilitate fusion of the virus permitting entry into the cell.[19] The hinge that connects DI to DII is very flexible and is used to flex DII in the low pH environment of the endosome, leading to the exposure of its fusion loop.[18] The viral envelope contains two integral membrane proteins designated envelope (E) and premembrane (prM). E protein binds to cellular receptors and mediates fusion of viral and cellular membranes during viral entry into cells and is the main target of neutralizing antibodies. Individual subunits of E protein consist of three β-barrel domains designated domains I (EDI), II (EDII), and III (EDIII), with the native protein forming a head-to-tail homodimer. The hydrophobic viral fusion peptide is located at the tip of domain II and is shielded by domain III of the adjacent subunit. Domain III appears to be responsible for binding to cellular receptors as several mutations that affect receptor binding are located in this domain.[20]

Infection of humans follows the bite of an infected *Aedes* mosquito (primarily *Aedes aegypti, Aedes albopictus,* or *Aedes polynesiensis*). Infection with DENV-1 (five genotypes), DENV-2 (six genotypes), DENV-3 (five genotypes), or DENV-4 (four genotypes) may be asymptomatic, or it may result in a mild febrile illness, dengue fever (DF), or severe dengue, including DHF/DSS.[21,22] DF is a self-limited febrile illness, with an average incubation period of 5 days, characterized by acute onset of fever, and, variously, headache, myalgia/arthralgia, poor appetite, prostration, nausea, vomiting, abdominal pain, and rash. The continuum from DF to severe dengue is differentiated physiologically by the onset of the dengue vascular permeability syndrome (DVPS) which culminates around the time of defervescence, when virus-infected cells are being immunologically eliminated (Table 17.1). DVPS is the central component of DHF/DSS as described in many published observations over the past 50 years.[23–26] Hemorrhage, mild or severe, may be a component of either DF or DHF. Recent evidence shows that the nonstructural protein 1 (NS1) that is excreted from infected cells circulating at high concentrations, activates toll-receptor 4 (TLR4), directly damages endothelial cells, contributes to the damage of liver cells, activates complement, and stimulates the release of cytokines that are known to accompany the onset of the DVPS.[27,28]

Diagnosis

Dengue illnesses are documented either by detection of the etiologic agent or viral protein in blood or by documenting a specific antibody response after infection. Determination of etiology during the acute phase centers on isolating virus in any of several host systems or detecting circulating dengue RNA. An alternative method, recently commercialized, is the detection of NS1, which circulates in the blood in the acute and early convalescent phases.[29–32] Many tests, some commercialized, can be used to measure antibody responses to dengue

*Disclaimer: The opinions or assertions contained herein are the private views of the author (S.J.T.) and are not to be construed as reflecting the official views of the United States Army or the United States Department of Defense.

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to the principles stated in the *Guide for the Care and Use of Laboratory Animals,* NRC Publication, 1996 edition.

One author (S.J.T.), as an employee of the United States Army, has been assigned to work on dengue vaccine codevelopment efforts with numerous commercial entities, some mentioned in this article. The author discloses these relationships not because there is a conflict of interest, but for transparency.

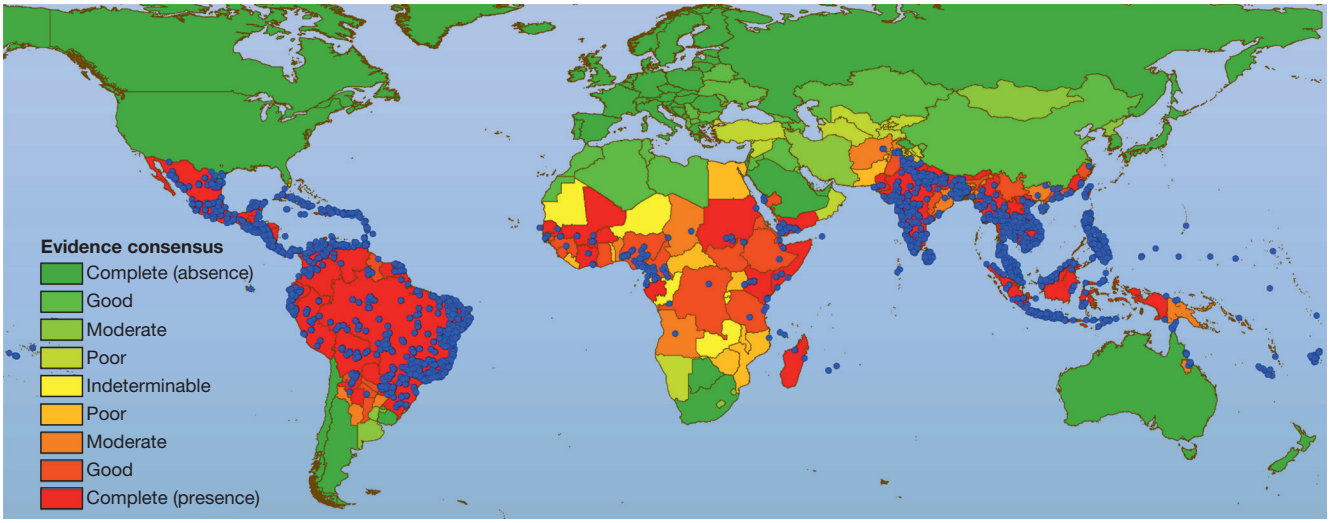


Figure 17.1. National and subnational consensus on complete absence *(green)* through complete presence *(red)* of human dengue cases. *Blue dots* are past reported cases or outbreaks. *(From Brady OJ, Gething PW, Bhatt S, et al: Refining the global spatial limits of dengue virus transmission by evidence-based consensus.* PLoS Negl Trop Dis. *2012;6:e1760.)*

TABLE 17.1 Dengue Vascular Permeability Syndrome

Dengue vascular permeability syndrome is a syndrome that occurs late in the course of an acute dengue illness (on or near defervescence) and consists of:

- Thrombocytopenia (<100,000/mm^3)
- Altered hemostasis: most commonly prolonged bleeding time, elevated activated partial thromboplastin time, and reduced fibrinogen levels
- Activated complement, by classical and alternative pathways
- Elevated liver transaminase enzyme levels usually accompanied by enlarged liver
- Vascular permeability with clinically significant loss of fluid and small macromolecules (e.g., albumin) into interstitial spaces, commonly serosal cavities

Data from Halstead SB: Dengue haemorrhagic fever-a public health problem and a field for research. Bull World Health Organ. *1980;58:1–21.*

infection. The most common is the detection of DENV immunoglobulin (Ig) M antibodies in late acute or early convalescent sera using the enzyme-linked immunosorbent assay (ELISA).[33,34] In the United States, the Food and Drug Administration (FDA) regulates the marketing of several commercial tests to detect dengue (IgM Capture ELISA). Tests may be used to distinguish primary dengue infections from infections that occur in persons immune to another dengue virus or to other flaviviruses (secondary infections). During a primary DENV infection, there should be no DENV IgG ELISA antibodies in acute-phase sera, and there should be a high ratio of IgM to IgG DENV antibodies in early convalescent sera. IgG DENV antibodies accompany secondary infection with a heterotypic DENV, accompanied in some instances by low levels of IgM DENV ELISA antibodies.

Classical DHF/DSS also accompanies first DENV infections in 5- to 11-month-old infants born to dengue-immune mothers[35]; but overall, 90+% of cases occur during second (occasionally third) heterotypic DENV infections in individuals older than 1 year.[24,36,37] These two epidemiologic settings have in common a single immune factor—IgG_1 DENV antibodies. Numerous studies show that DENV antibodies at subneutralizing concentrations enhance DENV infections in Fc-receptor–bearing cells, a process referred to as antibody-dependent enhancement (ADE).[38,39] In studies on humans, peak viremia titers during secondary DENV infections correlate with subsequent occurrence of DHF/DSS.[40] The DHF/DSS response is rare, seen in only 2% to 4% of second DENV infections.[41] Although supportive intensive care and careful fluid management can be lifesaving, specific antiviral medications are not available.[42] The single preventive measure, comprehensive mosquito control, has conspicuously failed.[43]

CORRELATES OF PROTECTION

Early studies to understand how antibodies neutralize or enhance DENV were performed using mouse monoclonal antibodies (mAbs). Many of these mice had been immunized with E proteins. Neutralizing mouse mAbs have been mapped to all three domains (ED). The strongest serotype specific neutralizing antibodies bind to domain III. Two partially overlapping epitopes on EDIII designated the lateral ridge and A strand are the main targets of mouse mAbs that neutralize DENV. The lateral ridge epitope interacts with serotype-specific strongly neutralizing antibodies. Mouse mAbs that bind to the A strand epitope cross react with more than one serotype of DENV and are designated dengue subcomplex neutralizing mAbs. It is also becoming clear that the hypothetical structure of the mature flavivirus particle generated by cryoelectron microscopy and molecular fitting does not always predict epitope exposure. Binding of some E-reactive antibodies depends on the dynamic movement of protein molecules ("breathing") in the virion particle leading to transient exposure of hidden epitopes.[44,45]

Recently, studies on dengue antibodies have shifted to those raised by DENV infections of humans. Technological developments now enable the rapid generation of human mAbs. Polyclonal human monotypic dengue-immune sera contain mostly weakly neutralizing, cross-reactive and enhancing antibodies.[46,47] DIII antibodies, although highly neutralizing, are only present in human sera at low levels. Only a small fraction of antibodies is serotype specific and highly neutralizing and these recognize a quaternary structure on the intact virion.[46,48] A similar DENV-1 mAb has been studied by cryoelectron microscopy.[49] Presumably, live attenuated tetravalent DENV vaccines should raise neutralizing antibodies directed at each of the four type-specific quaternary structures.

This has been confirmed to be the case for the attenuated DENV-1 developed by the National Institutes of Health (NIH).[50]

As discussed below, the Sanofi Pasteur Phase IIb and Phase III clinical trials have demonstrated that vaccine-induced neutralizing antibodies measured in epithelial cell monolayers are not correlates of protection against DENV infection and disease. However, when the infection experience of humans has been a single wild-type DENV, homotypic neutralizing antibodies are a protection correlate as demonstrated in challenge experiments conducted by Sabin.[51] Following an initial wild-type DENV infection in humans, the development of heterospecific neutralizing antibodies correlates with protection against developing severe disease during a heterotypic DENV infection.[52] While these predictive correlates may extend to live attenuated viruses, as discussed below, full protection may require infection by a complete dengue virus, not a chimera, as protective T-cell immunity may depend on presentation of DENV nonstructural antigens.[53,54] Of interest, administration of the monovalent or tetravalent NIH vaccine elicits type-specific and broadly reactive human T-cell responses.[55]

DEVELOPING DENGUE VACCINES

The numerous challenges that face the dengue vaccinologist have made the goal of developing a safe and efficacious dengue vaccine elusive for more than 4 decades.[56] Each DENV type is capable of causing disease and death, and many dengue-endemic areas have concomitant cocirculation of multiple types, mandating development of a vaccine that protects against all four DENV. After natural infection of human volunteers with DENV-1, a few months of cross-protection against infection with DENV-2 was observed, after which heterotypic infection often resulted in overt illness.[51] It is now well established that heterotypic protective immunity following infection with any first DENV reduces the risk that severe disease will accompany a second heterotypic DENV infection when the two infections occur within a period of 2 years. As the interval between first and second DENV infections lengthens so does the risk of severe disease.[57,58] Because classical DHF has been documented in adults with secondary DENV-2 or DENV-3 infections 20 years after infection with DENV-1, it must be assumed that immunologically primed persons are at risk for enhanced disease essentially for a lifetime.[59,60] The mechanism of early heterotypic protection is not established. Studies on DENV-immune humans and experimental studies using mouse models of severe disease suggest a role for T cells.[53,61] But, there is also evidence that antibodies contribute to protection.[62]

The suboptimal efficacy of the Sanofi Pasteur tetravalent yellow fever chimeric dengue vaccine against infection and disease caused by each of the four DENV, especially DENV-2 in large clinical trials (reviewed below) underscores the lack of understanding of the mechanisms of protection in DENV.[63]

Dengue vaccine development is still an empirical process. All attempts to combine the four live attenuated DENV into a final candidate vaccine have been plagued by an interference phenomenon. Because of the uncertainty of preclinical efficacy testing in animal models, it is prudent to demonstrate monotypic and tetravalent protective immunity by challenging vaccinated individuals using attenuated or partially attenuated DENV before proceeding to expensive and time-consuming large field efficacy studies.[63] Future DENV vaccine developers are well advised to follow the model pioneered by the NIH group, where reactogenicity and immunogenicity of each of four monotypic vaccines were separately evaluated in susceptible human volunteers before tetravalent vaccine candidates were tested. In several instances, vaccine protection was demonstrated by challenge using monovalent live DENV vaccine candidates.[64] Whatever the composition of dengue vaccines, as recommended by the World Health Organization, prolonged Phase IV clinical observation and testing will be required to monitor effectiveness and safety of dengue vaccines once deployed.[65] Progress toward developing a human DENV challenge model is described in greater detail below.

VACCINES IN DEVELOPMENT

The development of dengue vaccines began in 1929 using virus inactivated with phenol, formalin, or bile.[66,67] During World War II, live attenuated virus vaccines were developed independently by passaging DENV-1 serially in suckling mouse brain.[68,69] DENV-2 was similarly attenuated.[70] These early live virus approaches were abandoned because of concerns about the safety of inoculating mouse tissues into humans.[71] Since the 1970s, numerous new dengue vaccine constructs have entered preclinical and clinical development. Currently, six different vaccine approaches have been tested in human clinical trials, and a single candidate has now finished Phase III clinical testing. The Sanofi Pasteur chimeric vaccine was licensed in several countries in 2015. It is feasible that the NIH and Takeda vaccines will be licensed within 5 years (Table 17.2).

A large number of useful reviews on dengue vaccine development provide supplementary information.[56,72–86]

TABLE 17.2 Dengue Fever Vaccines in Clinical Testing

Type of Vaccine	Dengue Virus Genes (No.)	Stage of Development
Live attenuated virus (traditional)	10 (all)	Phase II tetravalent (Walter Reed Army Institute of Research and GlaxoSmithKline Biologicals)
Live attenuated virus (molecular)	10	Phase II tetravalent (Butantan, U.S. National Institutes of Health)
Yellow fever chimera	Chimera, 2 (PrM, E) + 8 (yellow fever virus)	Phases IIb and III tetravalent (Sanofi Pasteur); licensed in 2015
Dengue chimera	Chimera, 2 (PrM, E) + 8 (DENV-2)	Phase II tetravalent (Takeda)
Purified inactivated	3 (c, PrM, E)	Phase I tetravalent (GlaxoSmithKline, Fiocruz, Walter Reed Army Institute of Research)
Recombinant subunit	<1	Phase I tetravalent (Merck Inc.)
DNA	2+ (PrM, E)	Phase I monovalent DENV-1 (Naval Medical Research Center and Maxygen)

DENV, dengue virus.

LIVE ATTENUATED VIRUS VACCINES IN CLINICAL TESTING

Selection of Viral Mutants

Historically, flaviviral live attenuated virus vaccines have proved to be safe and durably efficacious. Examples include the 17D yellow fever vaccine[87,88] and the SA 14-2-2 Japanese encephalitis vaccine.[89,90] Live attenuated viral vaccines replicate in the recipient and in so doing present all viral antigens in a manner analogous to that of wild viruses eliciting both antibody and T-cell responses. A tetravalent vaccine approach is designed to induce primary-type immune responses to each of the four DENVs simultaneously, a phenomenon demonstrated to be protective in susceptible rhesus monkeys.[91,92] In dengue endemic countries most adults during their lifetimes have experienced three to four DENV infections sequentially, resulting in the circulation of neutralizing antibodies to all four DENV types, and are demonstrably protected against severe dengue disease.

Hawaii-Mahidol Universities/Aventis Vaccine

A major effort at live attenuated dengue vaccine development was initiated at the University of Hawaii and transferred to Mahidol University in Bangkok by investigators who used the classical method of serial passage of virus in a nonhuman host. Virus strains DENV-1 16007, DENV-2 16681, and DENV-4 1036 were passaged 15 to 50 times in primary dog kidney (PDK) cells in Hawaii and in Thailand where candidate vaccines were developed and Phase I testing completed.[93] Studies in flavivirus-susceptible Thai adult volunteers identified strains with acceptable reactogenicity and immunogenicity for DENV-1, DENV-2, and DENV-4 viruses at PDK13, PDK53, and PDK48 passage levels, respectively.[94–96] DENV-3 viruses did not replicate in PDK cells and were attenuated by 48 passages in primary African green monkey kidney cells (DENV-3 16562), and three final passages in fetal rhesus lung cells. All 10 U.S. Army soldiers inoculated with DENV-2 PDK53 developed neutralizing antibodies in the absence of dengue-like symptoms.[97] The vaccine was licensed to Aventis Pasteur for further development.

In subsequent studies, the four monovalent candidates elicited neutralizing antibody seroconversions in 3/5, 5/5, 5/5, and 5/5 American volunteers, respectively, after a single dose of 10^3 to 10^4 plaque-forming units (PFU).[98] Bivalent and trivalent formulations using DENV-1, DENV-2, and DENV-4 vaccine candidates elicited balanced seroconversions.[99] However, when all four serotypes were combined into a tetravalent vaccine, the predominant response was to the DENV-3 component.[98] The vaccine was reformulated using lower viral concentrations of DENV-3 and resulted in more balanced tetravalent seroconversion.[100,101] Vaccinated children living in Thailand were followed for intercurrent dengue infections for 8 years without occurrence of severe disease.[102] Despite these promising results, all 10 adult Australian volunteers given tetravalent vaccine had DENV-3–related systemic reactions, including fever, headache, arthralgia/myalgia, eye pain, and rash—essentially, mild DF.[103] Similar results occurred in adult Asian volunteers inoculated with a molecularly rederived DENV-3 strain, which had been selected to reduce reactogenicity but had the reverse effect.[104,105] Further testing and development of the Mahidol/Aventis live attenuated vaccine has now stopped.

Walter Reed Army Institute of Research Vaccine

A second tissue culture–passaged vaccine developed at the Walter Reed Army Institute of Research (WRAIR) was derived by serial passage of all four DENVs in PDK cells with final passages in fetal rhesus lung cells. Ten monovalent candidates were tested in 65 flavivirus-naïve volunteers between 1987 and 1996.[97–101] Vaccine candidates (specific virus strains, viral concentrations, and PDK cell passage levels) were evaluated. Candidates DENV-1, 45AZ5 PDK-20; DENV-2, 16803 PDK-50; DENV-3, CH53489 PDK-20; and DENV-4, 341750 PDK-20 with single-dose seroconversion rates of 100%, 67%, 50%, and 63%, respectively, and acceptable reactogenicity were selected for continued development.

In a follow up study, 49 flavivirus-naïve adult volunteers were administered the monovalent candidates.[102] Neutralizing antibody seroconversion rates for DENV-1, DENV-2, DENV-3, and DENV-4 were 100%, 92%, 46%, and 58%, respectively. A second dose administered 1 to 3 months later failed to improve immunogenicity—an observation reminiscent of Sabin's observed period of cross-protection after primary challenge.[40,103] There were no serious adverse reactions. The DENV-1 candidate was the most reactogenic, with a 42% rate of fever (>38°C) after a single dose, and reactogenicity was uniformly less after dose 2.

The monovalent candidates were combined into a tetravalent preparation and administered to 10 volunteers.[102] Seroconversion rates after a single dose of tetravalent vaccine by DENV type were 70%, 60%, 50%, and 30%, respectively. Dose 2 failed to improve antibody response but dose 3 improved seroconversion rates—100%, 75%, 100%, and 50%, respectively. There were no serious safety concerns.

Tetravalent vaccines made using combinations of high ($10^{5,6}$ PFU/dose) or low ($10^{3.5–4.5}$ PFU/dose) viral concentration formulations of each of the four DENVs were inoculated in 64 flavivirus naïve adult volunteers.[104] Formulations 1 to 15 were administered to 54 volunteers, three to four volunteers per formulation, on days 0 and 28. Formulation 16 was tested in 10 volunteers, five volunteers inoculated on days 0 and 30, one volunteer on days 0 and 120, and four volunteers on days 0, 30, and 120. Reactogenicity was variable across the formulations and positively correlated with immunogenicity. Similar proportions of volunteers seroconverted to DENV-1, DENV-2, and DENV-3 (69%, 78%, and 69%, respectively), but significantly fewer volunteers seroconverted to DENV-4 (38%). Seven formulations were found to be serologically superior and three of these sufficiently nonreactogenic to justify further testing.

New tetravalent dengue vaccine (TDEN) lots were produced to try to reduce the theoretical risk of transmissible spongiform encephalopathy arising from use of animal products of incompletely documented origin in the vaccine seeds or during decades of cell culture. TDEN lots were prepared from rederived vaccine strains using the same manufacturing process, except that each strain has three additional passages in fetal rhesus lung (FRhL) cells, monovalent bulks were formulated with a carbohydrate stabilizer rather than human serum albumin, and the final vaccine was lyophilized as a tetravalent product. TDEN formulations had an acceptable safety profile in primed and unprimed volunteers with antibody response rates superior in primed compared to unprimed vaccine recipients.[106] The WRAIR entered a codevelopment partnership with GlaxoSmithKline Biologicals to further develop tetravalent vaccine candidates and conducted extensive additional tests in humans.[107–110a] The future of the WRAIR/GlaxoSmithKline TDEN vaccine in its current form is in question because of manufacturing complexities and the desire by both organizations to shorten the time to protection by altering the dosage schedule or to explore other vaccine approaches.

Directed Mutagenesis

National Institutes of Health Vaccine

Workers at the National Institute of Allergy and Infectious Diseases at the NIH introduced a new era of dengue vaccine research when they constructed viable complementary DNA (cDNA) clones of DENV-4 (WRAIR 814669, Dominica, 1981).[111,112] A DENV-4 mutant virus (rDENV-4 Δ30) with a 30-nucleotide deletion in the 3′ untranslated region (10478–10507) produced lower viremia levels than parental DENV-4, but the neutralizing antibody responses were comparable to those of parental DENV-4 when inoculated in susceptible rhesus monkeys.[113] Phase I trials in flavivirus-naïve volunteers showed low-level viremia; frequent mild, largely rashes without other symptoms or signs; infrequent elevations in alanine transferase blood levels; occasional depression of absolute neutrophil count; and high levels of neutralizing antibodies at dosages of 10^1 to 10^5 PFU/0.5 mL.[74,114] *A. aegypti* mosquitoes fed on vaccinees did not become infected.[115] By screening mutants, a DENV-4 with paired amino acid substitutions at positions 200 and 201 of NS5 was obtained.[116] This virus showed reduced replication in severe combined immunodeficiency mouse, human hepatoma, and rhesus monkey models.[74,117] In flavivirus-naïve humans, inoculation of this virus abrogated hepatotoxicity and viremia but retained full immunogenicity.[118] Introduction of Δ30 into DENV-1 resulted in a successful vaccine virus.[119] Successful human vaccines for DENV-2 were obtained only by use of chimeras with rDENV-4 Δ30.[117,120,121] A DENV-3 human vaccine candidate was obtained by substituting the entire 3′ untranslated region (UTR) with that of rDENV-4 Δ30.[122]

Tetravalent formulations containing these viruses along with rDENV-1 Δ30 and rDENV-4 Δ30 have been tested in monkeys and shown to be attenuated, and they elicit balanced antibody responses.[123] In total, eight monovalent vaccine candidates were tested in 15 Phase I clinical trials to identify optimal candidates for tetravalent preparations. A subcutaneous injection of 10^3 PFU induced neutralizing antibody seroconversions to the vaccine parent virus in 80% to 100% of vaccine recipients.[64] Subsequently, it was observed that most of the candidate vaccines raised high seroconversion rates in susceptible adults even at doses as low as 10 PFU. Each of the four vaccines resulted a light maculopapular, nonitchy rash on the trunk usually unobserved by the vaccinated individual.[124]

Four different tetravalent vaccine candidates (TetraVax-DV) were tested in a randomized, placebo-controlled study in 113 flavivirus-naïve adults. A single dose of each TetraVax-DV admixture induced a trivalent or better neutralizing antibody response in 75% to 90% of vaccinees. Black volunteers had a significantly reduced incidence of rash and vaccine viremia. TV003, a vaccine comprised of 1000 PFU each of rDENV-1 Δ30, rDENV-2/4 Δ30, rDENV-3 Δ30/31, and rDENV-4 Δ30, raised a trivalent or greater antibody response in 90% of flavivirus-naïve vaccinees.[125] To improve the DENV-2 seroconversion rate and raise the neutralizing antibody titer, another formulation, TV005, containing 10,000 PFU of DENV-2 vaccine was tested in 84 susceptible volunteers while TV003 was given to another 84 volunteers.[126] TV005 outperformed TV003 with a 90% neutralizing antibody seroconversion to all four DENV at 3 months. Vaccinees failed to develop rash, viremia, or a boost in neutralizing antibodies when revaccinated at 6 months. TV003 produced solid protective immunity to DENV-2 challenge and was selected for Phase II and Phase III testing.[126a] These vaccine trials are ongoing in Brazil.

The NIH has licensed vaccine candidates to Butantan Institute, Sao Paolo, Brazil; Biological E Ltd, Hyderabad, India; Panacea Biotech, Ltd, New Delhi, India; Vabiotech, Hanoi, Vietnam; and to Merck & Co.

Chimeric Virus Vaccines

Chimera of Yellow Fever and Dengue (CYD)

Insertion of a flavivirus *preM* and *E* genes into the cDNA backbone of yellow fever (YF) 17D was pioneered at the St. Louis University Health Sciences Center.[127,128] Using this technology, DENV chimeras were developed by Acambis, Inc., and licensed for manufacture to Sanofi Pasteur.[98,129] For the purpose of manufacture, it was expected that these vaccines would benefit from the high fidelity of YF 17D polymerase.[130] Studies to date suggest that chimeric structure of dengue and West Nile viruses provides attenuation.[128,129,131–133] Contrary to the case with DENV, when wild-type *prM-E* genes of Japanese encephalitis (JE), tickborne, and St. Louis encephalitis viruses were inserted into YF 17D, they retained encephalitic properties and required additional attenuation of the *prM-E* genes to produce vaccine viruses.[128,131,134–136] Vero cells serve as the substrate for vaccine virus production.

Chimeras of dengue and YF viruses have been developed using the *PrM-E* genes from DENV-1, PUO-359; DENV-2, PUO-218; DENV-3, PaH881; and DENV-4, 1228 viruses. The results of extensive preclinical and Phase I and Phase II tests in flavivirus-susceptible and flavivirus-immune individuals were described at length in the sixth edition of this book.

Phase IIb and Phase III clinical trials have been completed in 35,146 children. In NCT00842530, 4002 Thai schoolchildren, ages 4 to 11 years, were randomly assigned (2:1) to receive three injections of dengue vaccine or control (rabies vaccine or saline) at months 0, 6, and 12.[137] On entering the trial, in a serologically sampled subset, 90% of children had circulating antibodies against DENV or JE virus. Dengue etiology of acute febrile illnesses in vaccinees and controls was investigated to month 25 after initial vaccination by detecting serotype-specific reverse-transcription polymerase chain reaction (RT-PCR) and/or NS1 in acute phase blood. Of 3673 children included in the primary analysis (2452 vaccine, 1221 controls), there were 45 cases of virologically confirmed dengue disease among vaccinees and 32 among controls, respectively. The overall vaccine efficacy at 2 years was 30.2%, with marked individual differences in protection against disease caused by the four DENV (Table 17.3).

TABLE 17.3 CYD Protection Against Serotype Specific Disease

Clinical Study	DENV-1 (CI)	DENV-2 (CI)	DENV-3 (CI)	DENV-4 (CI)
Thailand	55.6% (–21.6 to 84.0)	9.2% (–75.0 to 53.3)	75.3% (–375.0 to 99.6)	100.0% (24.8 to 100.0)
Southeast Asia	50.0% (24.6 to 66.8)	35.0% (–9.2 to 61.0)	78.4% (54.5 to 87.0)	75.3% (–190.3 to 97.2)
Americas	50.8% (29.1 to 65.2)	42.3% (14.0 to 61.1)	74.0% (61.9 to 82.4)	77.7% (60.2 to 88.0)

CI, confidence interval; DENV, dengue virus.
Data from references 137,138, and 139.

TABLE 17.4 Year 2 CYD Vaccine Protective Efficacy in an Intention to Treat (Post Dose 1) Analysis in Persons Who Were Seronegative or Seropositive for DENV or JE antibodies (Southeast Asia) or DENV Antibodies (Americas) Before Vaccination

	Seronegatives			Seropositives		
Clinical Study	Vaccine (Cases/ Person-Years)	Control (Cases/ Person-Years)	Vaccine Efficacy (%) CI	Vaccine (Cases/ Person-Years)	Control (Cases/ Person-Years)	Vaccine Efficacy (%) CI
Southeast Asia	23/424	18/216	34.9% (–18.0 to 64.1) $P = .16$	18/901	34/444	73.9% (54.3–86.3) $P < .001$
Americas	9/258	9/149	42.2% (–42.3 to 76.6) $P = .24$	8/1073	23/512	83.4% (63.2–92.5) $P < .001$
Combined	32/682	27/365	36.6% (–4.2 to 61.4) $P = .08$	26/1974	57/956	77.9% (65.1–86.0) $P < .001$

Data from Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med. *2015;373(13):1195-1206.*

TABLE 17.5 CYD Protective Efficacy Against Hospitalization for Dengue Disease

Clinical Study	Vaccinated Cases/Total	Control Cases/Total	Protective Efficacy
Thailand	32/2669	30/1333	53.3%
Southeast Asia	40/6851	61/3424	67.4%
Americas	17/26,883*	43/13,204*	80.9%

*Person-years of observation.[137–139]

TABLE 17.6 CYD Prevention of Plasma Leakage

Clinical Study	Vaccinated Cases/Total	Control Cases/Total	Protective Efficacy
Thailand	4/2669	3/1333	33.3%
Southeast Asia	29/6851	46/3424	68.6%
Americas	11/26,883*	26/13,204*	79.2%

*Person-years of observation.[137–139]

TABLE 17.7 CYD Prevention of "Severe" Dengue Disease

Clinical Study	Vaccinated Cases/Total	Control Cases/Total	Protective Efficacy (CI)
Southeast Asia (DHF)	28/6848	46/3424	68.6% (52–92.4)
Americas (severe dengue)	11/26,883*	26/13,204*	79.2% (68.8–99.9)

CI, confidence interval; DHF, dengue hemorrhagic fever.
*Person-years of observation.[138,139]

In a separate trial conducted in the Philippines, Vietnam, Thailand, Malaysia, and Indonesia (NCT01373281), 6710 children, ages 2 to 14 years, received three doses of vaccine while 3350 received placebo; 79% of a sample from both groups circulated DENV or JE antibodies.[138] In this trial, 117 dengue cases were recorded in the vaccine group and 133 in the control group, respectively, with an overall protective efficacy of 56·5% (95% confidence interval [CI], 43·8–66·4). Again, there was differential protection against disease caused by each of the four serotypes, although DENV 2 protection rates were higher than in the Thailand trial (Table 17.3).

A second Phase III trial was performed in Puerto Rico, Mexico, Honduras, Colombia, and Brazil (NCT01374516). Among vaccinated children, ages 9 to 16 years, 68% to 75% of 1334 in a random subgroup circulated DENV-1 to DENV-4 neutralizing antibodies prior to vaccination.[139] There were 176 dengue cases (11,793 person-years at risk) in the vaccine group and 221 cases (5809 person-years at risk) in the control group, for a vaccine efficacy of 60.8% (95% CI, 52.0 to 68.0). Modest differences in DENV type-specific protective efficacy were observed (Table 17.3).

Vaccine protection among Asian and American children identified as seronegative from prebleeds was poor, while among seropositives efficacy was high (Table 17.4). The poor protection of seronegatives is mirrored by a rapid decline in neutralizing antibodies reported 5 years after immunization of seronegative adults in Singapore.[139a] Many of the "seropositives" have had one lifetime DENV infection, and this is the seropositive group who are at risk for disease accompanying a dengue infection. Individuals who have two or more lifetime DENV infections are at low risk of hospitalized disease.[140] An additional observation was that chimeric yellow fever-dengue vaccine (CYD) protected against hospitalized disease better than protection against mild disease (Table 17.5). This protective efficacy was mirrored by high protection rates against the occurrence of plasma leakage (Table 17.6), DHF or "severe" disease (Table 17.7).

In July 2015, Sanofi published surveillance data from years 3 and/or 4 postvaccination for the three efficacy trials.[141,142] During this period, surveillance shifted from intense fever case finding to virological studies on hospitalized vaccine cohort members. Shown in Tables 17.8 and 17.9 are an increased frequency in hospitalization for dengue illness among vaccinated children younger than 5 years of age compared with controls over a 6-year period.

Table 17.10 provides more stark evidence that it was dengue infections with increased vascular permeability that occurred more frequently in vaccinated than in control young children. Despite the occurrence of two children with DSS among vaccinated children, the investigators expressed doubt that the excess of severe dengue in young children suggested enhancement by the vaccine because of the absence of prolonged viremia and cytokine secretion typical of enhanced disease.[141] However, the possibility that this observation does reflect enhanced disease owing to waning immunity from the vaccine in previously seronegative children is concerning and not

TABLE 17.8 Age Group Hospitalization Rates During the Third Year of Surveillance of Children Receiving CYD or Controls

Trial (Study Period)	Age Group	Vaccine Cases/Total (%)	Control Cases/Total (%)	Relative Risk
Southeast Asia CYD 14 (year 3)	2–5 yr 6–11 yr 12–14 yr	15/1636 (0.9%) 10/3589 (0.3%) 2/1544 (0.1 %)	1/813 (0.1%) 8/1806 (0.5%) 4/768 (0.6%)	7.45 (1.15–313.8) 0.63 (0.22–1.83) 0.25 (0.02–1.74)
Americas CYD 15 (year 3)	9–11 yr 12–16 yr	10/6029 (0.2%) 6/7239 (<0.1%)	9/3005 (0.3 %) 6/3625 (0.2 %)	0.55 (0/20–1.54) 0.50 (0.13–1.87)
Thailand CYD 57 (year 3)	4–5 yr	5/393 (1.4%)	1/192 (0.6%)	2.44 (0.27–115.54)
Year 3	6–11 yr	17/1738 (1.1%)	10/880 (1.2%)	0.86 (0.37–2.10)

From Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med. *2015; 373(13):1195-1206.*

TABLE 17.9 Age Group Hospitalization Rates During the Fourth to Sixth Years of Surveillance of Children Receiving CYD or Controls

Trial (Study Period)	Age Group at Vaccination	Vaccine Cases/Total (%)	Control Cases/Total (%)	Relative Risk
Southeast Asia CYD 14				
Year 4	2–5 yr 6–11 yr 12–14 yr	20/1636 (1.2%) 30/3589 (0.84%) 7/1544 (0.45 %)	7/813 (0.86%) 12/1806 (0.66%) 10/768(1.3%)	1.424 (0.60–3.33) 1.26 (0.64–2.44) 0.35 (0.13–0.92)
Year 5	2–5 yr 6–11 yr 12–14 yr	6/1636 (0.37%) 8/3589 (0.22%) 1/1544 (0.065%)	2/813 (0.25%) 5/1806 (0.28%) 2/768 (0.26 %)	1.49 (0.30–7.36) 0.8 (0.26–2.46) 0.25 (0.02–2.74)
Americas CYD 15				
Year 4	9–11 yr 12–16 yr	6/6029 (0.1%) 0/7239 (<0.1 %)	5/3005 (0.2 %) 2/3625 (0.06 %)	0.60 (0.18–1.96) 0.10 (0.0048–2.07)
Year 5	9–11 yr 12–16 yr	1/6029 (0.01%) 0	1/3005 (.03%) 0	0.498 (0.03–7.97)
Thailand CYD 57				
Year 4	4–5 yr 6–11 yr	5/393 (1.4%) 17/1738 (1.1%)	1/192 (0.6%) 10/880 (1.2%)	2.43 (0.28–20.6) 0.86 (0.37–1.87)
Year 5	4–5 yr 6–11 yr	4/393 (1.02 %) 4/1738 (0.23%)	0/192 (0) 4/880 (0.45 %)	4.36 (0.2–80.65) 0.5 (0.127–2.02)
Year 6	4–5 yr 6–11 yr	11/393 (2.8%) 26/1738 (1.4%)	4/192 (2.08%) 9/880 (1.02%)	1.33 (0.43–4.14) 1.46 (0.69–3.09)

Numerator data from Secretariat. Background Paper on Dengue Vaccines, SAGE Working Group on Dengue Vaccine. *Geneva: World Health Organization; 2016.*
Denominator data from Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med *2015; 373(13):1195-206.*

TABLE 17.10 Signs of Increased Vascular Permeability in Year 3 of Vaccinated and Control Children Younger Than 9 Years of Age in the SE Asian Clinical Trial and in Children of All Ages in the Thailand Clinical Trial

	SE Asia CYD 14		Thailand CYD 23/57	
Plasma Leakage	Vaccine	Control	Vaccine	Control
Any evidence	15/27	2/13	5/22	0/11
Clinical signs	3/27	0/13	1/22	0/11
Increased Hct	15/27	2/13	5/22	0/11
Shock	0/27	0/13	2/21	0/9

Hct, hematocrit; SE, Southeast.
Data from Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, et al: Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med. *2015;373:1195–1206.*

disproven. Hospitalized disease among placebos may be due to secondary DENV infections, while hospitalizations of vaccinated children may accompany primary DENV infections, invalidating classical efficacy calculations.[142a] In April 2016, the WHO Strategic Advisory Group of Experts on Immunization (SAGE) recommended that developing country regulatory authorities release CYD vaccine for use in populations with a dengue antibody seroprevalence of at least 70%.[142b] To date, CYD has been licensed in six countries with large-scale immunizations initiated for 9-year-olds based on mathematical models predicting that immunization of 80% of 9-year-olds over a prolonged period would result in 10% to 30% reduction of hospitalizations.[142b] If enhancement is the reason for the excess of disease several years after vaccination this may also have an implication for vaccinated older individuals not previously exposed to natural infection. However, according to the trial sponsors, among children age 9 years or older in all three trials there was evidence of modest vaccine efficacy.

To allay concerns about dengue-naïve individuals careful follow up studies both in children and in adults are needed.

Comment on the Safety and Protection by CYD Dengue Vaccine

For live attenuated DENVs two safety issues have been identified: (a) preexisting dengue or nondengue flavivirus antibodies in the vaccinated person may increase reactogenicity to vaccine virus, and (b) in the event of primary or secondary vaccine failure or waning neutralizing antibody, enhanced infection and disease may accompany breakthrough wild-type DENV infections. A safety signal, an increased relative risk of hospitalizations of vaccinated children ages 2 to 5 years, has been recognized by WHO in the Phase III clinical trials of the SanofiPasteur CYD vaccine.[142b,142c] Others have suggested dengue infections of vaccine-sensitized children resulted in these hospitalizations.[142d]

The CYD dengue vaccine was designed to raise type specific antibodies to structural antigens for each of the four dengue-like viruses and, in addition, with successive doses to raise dengue group reactive cross protective neutralizing antibodies in flavivirus-immunes. Whether CYD raises type-specific neutralizing antibody responses as defined by the deSilva group to any of the four DENV either in susceptibles or partially-immune persons (immune to a single wild-type DENV) is not known.[46] Whatever the mix of immune responses that follows administration of one or more doses of CYD, these have failed to prevent symptomatic primary DENV infections. There is an urgent need to solve this problem at a fundamental level.[142a] In addition, based upon data from children 5 years of age or younger, one or more doses of CYD vaccine has resulted in an immune response that when followed by a wild-type DENV infection resulted in hospitalization because of increased vascular permeability. It has been recommended that before testing tetravalent DENV vaccine mixtures, each of the four monotypic CYD viruses should have been inoculated into susceptible humans who should then be challenged at an interval no earlier than 6 months with a suitable homotypic vaccine virus (see "Dengue Human Infection Model" later).[63] In the Sanofi vaccine clinical trials, vaccination of seropositives provided moderate to good protection against severe DENV disease. Presumably, this reflects a booster effect that removes children who have had one single DENV infection from being hospitalized with enhanced vascular permeability disease during a second heterotypic DENV infection.

There are a number of intriguing but as yet unestablished explanations for the poorer protection observed in seronegatives compared with seropositives. First, CYD vaccines do not present DENV nonstructural proteins to the immune system. This is likely to result in suboptimal protective CD8+ T-cell responses. In humans, nonstructural DENV antigens may contribute importantly to T-cell protection against DENV-2 infections and disease.[55] The observed protection against severe disease obtained by vaccinating partially immune humans is good news, but it is troubling that CYD vaccines do not present DENV NS1 antigens to the immune system. As described above, new evidence strongly suggests that NS1 is a viral toxin and the direct cause of the DVPS.[27,28]

Some vaccinologists have published concerns that CYD, when given to seronegative children ages 2 to 5 years, has resulted in enhanced disease during the third year postvaccination, and that this same phenomenon can be expected to occur in seronegatives of all ages.[139a,142a] Testing sera for dengue antibodies to identify those who will receive optimal benefit from vaccine has been advised.[142a,142d,143,143a] The Pan American Health Organization does not recommend integration of CYD vaccine into routine immunizations at this time.[143b] In contrast, the SAGE committee of WHO has taken the position that there is no empiric evidence supporting safety concerns in the target population,[142b,142c] and that testing to eliminate seronegatives from vaccination is impractical on a large scale. SAGE further noted that in populations with a high prevalence of prior dengue infection, vaccine will prevent considerable serious disease and hospitalization, a view also expressed by Wilder-Smith and colleagues[143c] and by the manufacturer.[143d,143e,143f] See Gailhardou et al.[143g] for more extensive safety data. Since the CYD vaccine has been licensed in five countries, it is likely there will soon be evidence from Phase IV studies to determine which view is correct.

Dengue-Dengue Chimera

The Centers for Disease Control and Prevention (CDC), Vector-borne Diseases Branch developed a tetravalent chimeric dengue vaccine by introducing DENV-1, DENV-3, and DENV-4 *prM* and *E* genes into cDNA derived from the successfully attenuated DENV-2 component of the Mahidol University–Aventis Pasteur live attenuated virus vaccine (DENV-2, 16681 PDK-53) (see above).[97,144–146] Attenuating mutations of the DENV-2 vaccine are located in the nonstructural genes and reportedly are stable.[146–148] DENV-1–DENV-2 chimeras have been produced using DENV-1 PDK-13 and parental DENV-1 16007 structural genes.[147] DENV-3–DENV-2 and DENV-4–DENV-2 chimeras using parental virus structural genes are stable in cell culture, and when all constructs were inoculated in cynomolgus monkeys, monomeric and tetravalent formulations resulted in neutralizing antibody responses, low viremia titers, but some evidence of interference.[146,149]

Dengue-dengue chimeras were formulated as a DENV-1/DENV-2, DENV-2, DENV-3/DENV-2, and DENV-4/DENV-2 tetravalent vaccine candidate and licensed to Inviragen, Inc., and subsequently to Takeda Pharmaceuticals, Inc. The vaccine candidate (DENVax) has undergone preclinical testing in mice and nonhuman primates, comparing intradermal and subcutaneous deliveries.[150] The intradermal delivery of DENVax (10^5 PFU per DENV type) produced superior immune responses in cynomolgus macaques; viremia was not detected after challenge with 10^5 PFU of DENV-1 West Pacific or DENV-2 New Guinea C viruses.[151,152]

In a Phase I clinical trial, high and low doses of tetravalent vaccine and placebo were given by the subcutaneous and intradermal routes to 96 adult residents of an area of Colombia without *A. aegypti*.[153] Both formulations were well tolerated with mostly mild and transient local or systemic reactions. Both high-dose and low-dose DENVax formulations induced neutralizing antibody responses to four dengue virus serotypes in two-thirds of recipients 30 days after a second dose with the lowest response to the DENV-4 component. Immune responses following intradermal inoculation were somewhat higher than responses following subcutaneous delivery.[153] The vaccine has been shown to have acceptable thermal stability.[154]

Additional Phase I and Phase II clinical trials are in progress. Phase III clinical trials are in advanced planning.

VECTORED VACCINES

Recombinant poxviruses and adenoviruses expressing foreign proteins have been demonstrated to induce strong humoral and cellular responses in humans against various pathogens.[155–157] These viruses can infect cells and express their proteins de novo in the cell. The antigens are then naturally processed, glycosylated, and associated with the cell membrane. Major histocompatibility complex (MHC)

class I–dependent immune responses are induced as a result of intracellular translation and processing of the gene products.

Vaccinia Virus Vector

Use of recombinant *Vaccinia* viruses to express the structural proteins of DENV-2 or DENV-4 have been disappointing.[158,159] These constructs, expressing prM and a full-length E protein, failed to induce neutralizing antibodies or to protect monkeys from wild-type challenge. Recombinants expressing truncated DENV E proteins did raise neutralizing antibody in mice and in rhesus macaques, providing some protection against wild-type challenge in both models.[160,161]

Adenovirus Vector

Replication-defective adenovirus vectors have been used to construct flavivirus vaccine candidates.[162,163] Many vaccine candidates have been created by inserting the EDIII with or without prM of one or more DENV into an adenovirus vector. These include DENV-2 New Guinea C (NGC) with adeno 5,[164] other constructs tested in a prime-boost format,[165,166] bivalent vaccines,[167,168] or tetravalent mixtures of monovalent vaccines,[169] or a tetravalent single antigen.[169] Subsequently, to ensure that the prM and E antigens are expressed at similarly high levels, the genes from each DENV were placed into identical, immediate-early cytomegalovirus (CMV)–bovine growth hormone polyadenylation cassettes and inserted into either end of the adenovirus (cAdVax) vector. DENV-1 and DENV-3 were injected into one arm and DENV-2 and DENV-4 into the other.[168,170] A two-dose tetravalent vaccine was shown to partially protect monkeys against challenge viremia.[171] Subsequently, it was shown that small interfering RNA (siRNA) of the nontranslated region of the dengue genome can be presented using an adenovirus vector to achieve the blocking of DENV infection.[172]

Venezuelan Equine Encephalitis Virus Vector

Nonpropagating Venezuelan equine encephalitis (VEE) virus replicon particles (VRPs) permit translation and expression of inserted genes during a single round of replication.[173–175] Monotypic DENV-1 and DENV-2 vaccines have been constructed using VEE VRPs.[176,177] In a prime-boost regimen, after two doses of a DNA vaccine, a single dose of VEE VRP resulted in complete protection of cynomolgus macaques.[177] It has been possible to use VEE VRPs to overcome maternal-antibody interference in a mouse model[176] and raise tetravalent neutralizing antibodies in vaccinated mice.[178] Antibodies raised are type-specific.[179]

Measles Virus Vector

Genes for the dengue virus EDIII protein of DENV-1 were fused to those of the ectodomain of the measles virus attachment protein (ectoM) and inserted into the Schwarz measles vaccine (MV) strain.[180] Measles-susceptible mice inoculated intraperitoneally with two doses of this construct produced DENV-1 neutralizing antibodies. Mice were challenged with live DENV-1 6 months after the initial immunization. The presence of ectoM was critical to the immunogenicity of inserted EDIII. The adjuvant capacity of ectoM correlated with its ability to promote the maturation of dendritic cells and the secretion of proinflammatory and antiviral cytokines and chemokines involved in adaptive immunity. Subsequently, a recombinant MV vector containing genes for all four DENV EDIII fused with ectoM was prepared. After two injections in mice susceptible to MV infection, the recombinant vector induced antibodies that when passively transferred to mice failed to protect mice from death resulting from intracerebral challenge with any of the four DENV types.[181]

REPLICATION-DEFECTIVE VACCINES

Capsid-Gene Deletion

Several flavivirus vaccines have been made by deleting the capsid gene (C) and propagating the virus in cells that express high levels of C.[182] Such a virus undergoes only a single cycle of infection in vaccinated hosts. A DENV-2 RepliVAX, produced by replacing the *prM/E* genes of RepliVAX West Nile virus with the same genes of DENV-2, resulted in partial protection of AG 129 mice challenged with a mouse-adapted DENV-2.[183]

Recombinant Immune Complexes

A consensus DENV domain III gene was fused to the binding epitope of the 6D8 anti-Ebola mAb resulting in a hybrid dengue-Ebola recombinant immune complex (DERIC). Human and mouse versions of these molecules were expressed in tobacco plants using a geminivirus-based expression system. Following purification from the plant extracts by protein G affinity chromatography, three doses of DERIC were used to immunize mice inducing neutralizing antibodies against DENV-4 but not DENV-2.[184]

Inactivated or Subunit Vaccines

The simplest approach to produce viral vaccines is the use of inactivated whole virus or viral subunits. Such vaccines have potential advantages; for example, they cannot revert to a more pathogenic phenotype, and when combined they are unlikely to produce immune interference. Thus, they may be suitable for a wider age range than live vaccines, immunosuppression would not be a contraindication to immunization, an accelerated immunization schedule may be possible, and, theoretically, there could be fewer safety issues. Cell-mediated as well as humoral immune responses have been demonstrated with an inactivated flavivirus vaccine.[185] It should be noted that inactivated virions do not present nonstructural antigens to the immune system. This is likely to cripple protective T-cell immunity as described above. Furthermore, killed or subunit vaccines raise antibodies to only a portion of the structural proteins and not to normal virion-based structural conformations. Other disadvantages include the requirement for high concentrations of antigens, the need for multiple doses and the expected waning of immunity with the passage of time. Inactivated flaviviral vaccines have been licensed and are in wide use to prevent JE[186,187] and tickborne encephalitis.[188] Inactivated vaccines against flaviviral encephalitis may succeed because immune enhancement is not part of the disease process. Whether inactivated or subunit dengue vaccines can be used as standalone products is debatable. They may be useful for relatively short-term protection or in a prime-boost strategy. Augmentation of inactivated vaccines with new proprietary adjuvants may improve manufacturing and performance parameters.[189] Recent studies on respiratory syncytial and measles viruses show that formalin not only alters the structure of viral proteins, resulting in poor B-cell and T-cell antigen processing by dendritic cells.[190,191] This result suggests that other, less-stressful mechanisms of inactivating DENV should be sought. Psoralen-inactivated DENV-1 was used to immunize *Aotus* monkeys but with only equivocal protection on challenge.[192,193]

Cell Culture–Based Inactivated Vaccines

The WRAIR developed a purified inactivated virus (PIV) dengue vaccine candidate by inactivation of each of the DENV types with formalin.[194–196] A Phase I proof-of-concept trial of a monovalent DENV-1 PIV vaccine candidate adjuvanted with aluminum hydroxide (AlOH) was completed in the United States in a small number of flavivirus-naïve volunteers. Two doses administered 28 days apart had an acceptable safety profile with low to moderate immunogenicity.[197] The WRAIR granted an exclusive license to GlaxoSmithKline Vaccines and the two are codeveloping, with the Oswaldo Cruz Foundation (FioCruz), a tetravalent PIV formulation (DPIV) adjuvanted with AlOH or a GlaxoSmithKline proprietary Adjuvant System, shown to be protective in rhesus monkeys.[198] For nonflavivirus vaccines, adjuvants, particularly newer complex adjuvant systems (AS), have been shown to mediate long-lasting antibody responses.

Two Phase I trials of this vaccine are near completion, one in the United States and the other in Puerto Rico. Each is exploring the safety and immunogenicity of two doses (administered day 0 and 28) of DPIV candidates adjuvanted with AlOH, AS01E, or AS03B in DENV unprimed (U.S.) and primed (Puerto Rico) adults. Publicly available data indicate an acceptable safety profile for all vaccine formulations through study day 56 and greater than 90% tetravalent antibody seroconversions in DPIV formulations adjuvanted with higher doses of AlOH and both AS01E and AS03B. There was a trend toward higher GMTs in the AS03B adjuvanted formulation (Thomas SJ, unpublished data).

Recombinant Subunit Vaccines

T-cell and B-cell epitopes have been mapped on DENV structural and nonstructural proteins.[55,199,200] The right combination of epitopes expressed in protein subunit vaccines might be the basis for an effective vaccine produced at moderate cost.[201] Structural and nonstructural DENV proteins can be produced in large quantities in many expression systems including *Escherichia coli*,[202,203] baculovirus in *Spodoptera frugiperda* insect cells,[204–206] yeast,[207] Vaccinia virus,[159,161,208] and *Drosophila* cells.[209,210] The last approach, which resulted in 80% *E* gene expression in *Drosophila* cells, is being developed by the Hawaii Biotech Inc/Merck & Co. (see Table 17.2).

A tetravalent vaccine has been developed consisting of 80% E proteins of each of the four DENVs. Efficacy expectations are based on experiments showing that 1 μg of DENV-2 E protein with the proprietary GlaxoSmithKline adjuvant, SBAS5, protected rhesus monkeys from viremia after challenge with wild-type virus.[210] Early development included a DENV-2 vaccine candidate with 80% E + nonstructural protein 1 (NS1), but the NS1 added little to performance and this candidate has been sidelined. Various formulations have been tested in rhesus macaques producing high-titer neutralizing antibodies to all four DENVs and protecting animals from viremia after wild-type viral challenge.[210] A Phase I, single-center, double-blind, randomized study to assess the safety and tolerability of a DENV-1 to DENV-4 candidate in healthy U.S. adults was recently completed. Vaccine was tested at three escalating dosage levels and three different adjuvant formulations against in 98 subjects including 18 placebo controls. Results are pending (B.A. Coller-Griswold, personal communication, May 21, 2015).

Cuban investigators have produced candidate dengue vaccines using recombinant proteins. DENV-3 DIII protein fused with a P64k *Neisseria meningitidis* carrier protein, administered with Freund's complete adjuvant, protected nonhuman primates after challenge.[211] However, only partial protection was obtained using AlOH as the adjuvant, and this required use of the *Neisseria* serogroup A polysaccharide.[212] The authors have also expressed a domain III capsid chimeric protein of the four DENV in *E. coli* and are testing this construct as a vaccine.[213] The authors suggest priming with dengue protein and boosting with live attenuated virus.[214] This group has also tested a tetravalent vaccine formulation using AlOH as adjuvant in mice. After three doses, neutralizing antibody titers were detected against the four viral serotypes, the lowest seroconversion rate being against DENV-4. One month after the last dose, immunized animals were challenged with the four wild-type DENV with partial protection.[215]

NUCLEIC ACID–BASED VACCINES

DNA vaccines consist of a plasmid (or plasmids) containing DENV genes reproduced to high copy number in bacteria such as *E. coli*. The plasmid contains a eukaryotic promoter and termination sequence to drive transcription in the vaccine recipient. The transcribed RNA is translated to produce proteins to be processed and presented to the immune system in the context of MHC molecules. Additional genes such as intracellular trafficking and immunostimulatory sequences can be added to the plasmid. Expressed antigens lead to B- and T-cell responses. DNA vaccines afford numerous theoretical advantages over conventional vaccines, including ease of production, stability and ability to be transported at room temperature, ability to add new genes to the vaccine, ability to immunize with or without adjuvant systems against multiple pathogens with a single construct, and reduced reactogenicity.[216]

PrM-E DNA Vaccines

Workers at the Naval Medical Research Center evaluated two eukaryotic plasmid expression vectors (pkCMVint-Polyli and pVR1012; Vical, Inc., San Diego, CA) expressing the PrM protein and 92% of the E protein for DENV-1 and DENV-2 virus (see Table 17.2). These constructs induced neutralizing antibody in mice,[217] and they were subsequently improved by adding immunostimulatory CpG motifs, and the full-length *E* gene with *PrM*.[217–220] In recent studies, a DENV-1 DNA vaccine protected a portion of challenged monkeys from viremia for varying lengths of time.[221] With the recognition that dendritic cells are the percutaneous portal for dengue virus replication,[222] efforts are being made to target DNA vaccines to these cells.[223] A DENV-1 DNA vaccine was administered intramuscularly to 22 flavivirus-negative volunteers, half of whom received high and low dosages, respectively, in a three-dose series (at day 0, and at 1 and 5 months). At the completion of this series, none of the low-dosage recipients and only five of 11 high-dosage recipients developed neutralizing antibodies.[224] More recently, protection has been achieved in a rhesus monkey model by boosting tetravalent DNA vaccination with a tetravalent live attenuated virus vaccine.[215,225]

TDENs have been created by shuffling the envelope genes from the four DENVs. Selected shuffled DNA was transfected into human cells, subjected to flow cytometry, and reacted with type-specific dengue antibodies. Antibody markers permitted rapid screening of libraries and identification of novel expressed chimeric antigens. A panel of chimeric clones expressing C-terminal truncated antigens that combined envelope and prM epitopes from all four DENV types when inoculated in mice and monkeys successfully raised neutralizing antibodies. Monkeys resisted challenge with DENV-1 but not challenge with DENV-2.[226,227] Plans for testing TDENs in humans have been discussed.[223]

DENGUE HUMAN INFECTION MODEL

Because of the requirement to achieve solid protective immunity against each of the four DENV following the administration of a combination of antigens the absence of a validated dengue animal model, the absence of immune protection correlates and/or the lack of understanding protective immunity have crippled dengue vaccine development. Obviously, early tests of candidate vaccine protective immunity would be useful. To that end, the NIH group has used monovalent attenuated DENV as challenge viruses (described above). Remarkably, there has been a huge experience with experimental infections of humans using wild-type DENVs beginning in the early 1900s with no adverse outcomes.[51,66,68,69,228–235] In view of this experience, a group of investigators at the WRAIR proposed to reestablish a dengue human infection model (DHIM) and use partially attenuated DENV to test vaccine-induced protective immunity.[236,237] Human safety studies on DENV candidates are in progress at State University of New York (SUNY) Upstate School of Medicine, Syracuse, with a goal of selecting strains that induce mild dengue-like illness. Human infections with these strains should contribute immeasurably to a better understanding of clinical and immunological correlates of disease responses, to candidate drug evaluation, to studies on vector-human interactions, and to simplifying, speeding-up, and reducing the cost of dengue vaccine development. A crucial benefit of early tests of candidate vaccines by the DHIM is the early identification of vaccines that do not protect humans against dengue virus infection or disease.[238,239]

CONCLUSIONS

Dengue virus infections inflict significant morbidity and mortality on nearly 4 billion residents of tropical and subtropical countries. In addition, dengue infections are consistently among the leading causes of febrile diseases in tourists and expatriate residents of tropical countries and among military personnel assigned to combat or peacekeeping roles in tropical countries. In view of the widespread failure of national mosquito-control programs, the social and clinical impacts of disease create a significant market for dengue vaccines as evidenced by the interest shown by major vaccine manufacturers. One vaccine is currently licensed in several dengue endemic countries and is currently being employed. Several other promising dengue vaccine candidates are nearing Phase III testing. If safety concerns about Dengvaxia can be solved by the manufacturer, WHO, and regulatory agencies, market forces should continue to support the introduction of new, improved vaccines. Important deficiencies in basic understanding of protective immunity to dengue remain. These may be addressed via a newly developed human dengue infection model as well as by careful Phase III and Phase IV clinical trials. Huge requirements for dengue vaccination will require heavy financial investments in vaccine production and in strategic methods to achieve optimal reductions in virus transmission and in disease burden.

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18 Diarrhea Caused by Bacteria

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Diarrheal infections are estimated to cause 1.7 billion disease episodes and 700,000 deaths each year, mainly in children in low- and middle-income countries.[1] Travelers to endemic areas are also at high risk for being infected. However, today there are only licensed vaccines against two bacterial enteric pathogens, *Salmonella enterica* serovar Typhi (*S.* Typhi) and *Vibrio cholerae.* There are intense efforts under way to develop vaccines against additional important agents, in particular enterotoxigenic *Escherichia coli* (ETEC) and *Shigella*. This chapter focuses on vaccine development efforts for these two groups of pathogens and also briefly discusses the development of vaccines against *Campylobacter*, Shiga toxin–producing *E. coli* (STEC), and nontyphoidal *Salmonella* (NTS). While ETEC and *Shigella* have exclusively human reservoirs, *Campylobacter*, STEC, and NTS are also zoonotic pathogens with known animal reservoirs (chicken, cattle, etc). Our discussion here is limited to vaccines for human use, but it should be noted that there are also ongoing efforts to develop veterinary vaccines aimed at indirectly reducing the spread to human populations.

PATHOGENESIS AND PROTECTIVE IMMUNE MECHANISMS

Enteric pathogens differ in the way they cause infection and disease and this influences what type of immune responses they elicit and how vaccination may protect against infection and disease.[2] The different bacterial enteropathogens can cause disease by: (a) colonizing the intestinal mucosa without invasion or morphological damage but eliciting diarrhea by means of powerful secreted enterotoxins (e.g., *V. cholerae* and ETEC); (b) adhering to the mucosa and inducing enterocyte effacement by "injecting" bacterial proteins into the epithelial cells (e.g., enteropathogenic *E. coli*); (c) inducing enterocyte killing by producing powerful exotoxins that block cellular protein synthesis (e.g., enterohemorrhagic *E. coli* [EHEC/STEC]); (d) locally invading and destroying the mucosa (e.g., *Shigella*); (e) locally invading the mucosa and inducing inflammation (e.g., NTS); and (f) translocating across the mucosa, invading the bloodstream and spreading to distal organs (e.g., *S.* Typhi and *S.* Paratyphi A and B).

Secretory immunoglobulin A (SIgA) produced by plasma cells in the intestinal lamina propria provides an important first line of defense against enteric infections.[3] Immunoglobulin (Ig) A is actively transported over the epithelial barrier into the lumen, where it is released in the form of SIgA, which is resistant to degradation and can neutralize bacterial enterotoxins (and viruses) and inhibit epithelial attachment, thereby preventing colonization and cell invasion. Low levels of IgG may also be present in gastrointestinal secretions.[3] However, the small intestine (at least in individuals from industrialized country settings) is essentially nonpermeable to blood proteins if uninflamed. This is in contrast to the lower respiratory tract and the female genital tract, which are relatively permeable to transudated IgG antibodies. Consistent with this observation, the noninflammatory cholera and ETEC infections of the small intestine represent examples where vaccine protection is mediated by locally produced SIgA antibodies and probably requires vaccination via the oral route. Many young children in developing countries manifest environmental enteropathy, a syndrome that includes a chronic state of inflammation of the proximal small intestine, small bowel bacterial overgrowth and excitation of the innate immune system.[4] In such children, circulating IgG antibodies may transudate to the mucosal surface of the gut where they may play some a role in protection. However, IgG is rapidly degraded by intestinal proteases and, together with the normally low levels and lack of function of complement in intestinal secretions, limits the protective significance of IgG in the gut. Long-lasting immunological memory, mainly through the generation of long-lived memory B cells in intestinal Peyer patches and lymphoid follicles, can mediate persistent protection after the acute SIgA antibody response has vanished. The memory B cells can rapidly evolve into IgA-secreting plasma cells upon reexposure to antigen; the anamnestic SIgA antibody response may then control infection before it causes disease.[5] Mucosal memory B cells can be very long lived; for example, functional antigen-specific B-cell memory has been demonstrated for longer than 10 years after primary oral cholera vaccination.[6]

Cell-mediated protection against pathogens that enter the host via mucosal surfaces can be mediated by intraepithelial and lamina propria $CD4^+$ helper and $CD8^+$ cytotoxic T lymphocytes. These cell-mediated responses are believed to explain the durable protection that has been observed in subjects immunized with Ty21a live oral typhoid vaccine.[7] Numerous T-helper cells producing interferon-γ (Th1) and cells producing interleukin (IL)-17A (Th17) are often found in the lamina propria throughout the gastrointestinal tract.[8] Although believed to be major effector T cells against *Helicobacter pylori* and intracellular organisms such as *S.* Typhi and *S.* Paratyphi, their role in the gut mucosa is not fully understood. There are also data suggesting an important role for IL-17A in promoting intestinal IgA production and secretion.[9,10] T-follicular helper cells support antibody production and affinity maturation to protein antigens in mucosal germinal centers,[11] thereby having an important indirect role in forming protective antibody responses in the gut. However, the roles of different T-cell subsets in immune responses to oral vaccines remain to be fully elucidated.

MUCOSAL VERSUS PARENTERAL VACCINATION

Traditional injected vaccines primarily induce systemic immune responses and are generally poor in inducing mucosal immunity. In contrast, mucosal vaccines, administered via oral, nasal, or sublingual routes, generally give rise to both mucosal and systemic immune responses.[12] The mucosal response is anatomically compartmentalized reflecting the migratory properties of lymphocytes activated at different mucosal inductive sites, thus imposing distinct constraints on the choice of mucosal route for vaccine administration.[13] In general, the strongest immune response is obtained at the site

of vaccine application and next after that in anatomically adjacent or evolutionarily linked sites. An example of the latter is the intestine–mammary gland link in lactating women which ensures that the breastfeeding baby receives epidemiologically relevant breast milk SIgA antibodies from the mother, which result from the current microbial exposure in the mother's intestine.

While vaccination via the oral route has potential to prevent clinical illness by all enteric pathogens, parenteral vaccination may work against those enteric infections in which the pathogen is first translocated across the intestinal epithelium. An example is shigellosis (bacillary dysentery) in which translocated *Shigella* infect other enterocytes from the basolateral side. At this point the organisms can readily be attacked by serum-derived antibodies in concert with complement and phagocytic neutrophils. Likewise, serum antibodies can effectively attack pathogens that cause disease by inducing inflammation in the submucosal lymphoid tissues (e.g., NTS serovars and *Campylobacter jejuni*) or after entering the bloodstream (such as *S.* Typhi or *S.* Paratyphi).[14] Previous exposure to the pathogen leading to mucosal immunological priming also increases the ability of parenteral vaccination to protect against mucosal infections. A general rule seems to be that parenteral vaccines may provide some degree of boosting in already primed individuals, but they are unable to induce an effective mucosal response in individuals who have not been primed by previous mucosal exposure to the pathogen or by oral vaccination.[12] As described below, ongoing efforts to develop vaccines against ETEC are mainly focused on oral administration, while both oral and parenteral vaccination is considered for *Shigella*, *Campylobacter*, STEC, and NTS.

SPECIFIC VACCINES

Enterotoxigenic *Escherichia coli*

Epidemiology

ETEC is among the most common bacterial causes of diarrhea-associated morbidity and mortality in children younger than age 5 years in developing countries. The peak incidence occurs in children 6 to 36 months of age, where ETEC together with rotavirus and *Shigella* are the most common pathogens.[15] ETEC is also the number one cause of travelers' diarrhea,[16] reportedly accounting for 20% to 60% of all travelers' diarrhea globally with at least 20% of affected travelers being bedridden for part of their trip and 40% changing their itinerary because of diarrhea (reviewed in Reference 17). Travelers' diarrhea, primarily caused by ETEC, is also the most common medical problem for military personnel from industrialized countries deployed in less-developed areas of Asia or Africa, with an average incidence of 29% per month and up to 60% per month in hyperendemic regions.[18]

Clinical Manifestations and Mechanisms of Disease and Immunity

Disease caused by ETEC is characterized by profuse watery diarrhea lasting for several days. Infection is usually self-limited but may lead to dehydration and malnutrition in young children. ETEC causes disease after ingestion of contaminated food or water by attachment to the small bowel mucosa by so-called colonization factor (CF) antigens, mostly fimbriae, followed by bacterial multiplication and production of heat-labile toxin (LT) and/or a heat-stabile toxin (ST).[19] More than 20 types of CF antigens have been identified on human ETEC isolates, with a few types occurring most frequently, including CFA/I and "coli surface" antigens CS1 through CS6. Another important CF antigen is CS6, which is an outer membrane antigen rather than a fimbrial protein. Mucosal SIgA antibody responses against these CFs are believed to be protective by inhibiting epithelial binding of bacteria.[19] Importantly, although there are some cross-reactions between selected fimbriae, immune responses against one CF type are in general not cross-protective against several other types. Therefore, a broadly effective vaccine would require inclusion of multiple CF antigens.

Once attached to the intestinal epithelium, ETEC produces LT and/or ST enterotoxins, which induce the characteristic watery diarrhea. LT, which is highly homologous to cholera toxin (CT), is composed of a single catalytic A subunit and five identical B subunits, comprising the cell-binding domain. The A subunit mediates activation of adenylate cyclase, leading to increased production of cyclic adenosine monophosphate (cAMP), which activates ion channels and causes massive fluid secretion. Short-term protection against ETEC disease has been documented in persons immunized with a CT B subunit containing cholera vaccine because of immunologic cross-reactivity between B subunits of CT and LT.[20–22] ST is a small peptide toxin (18 to 19 amino acids) that stimulates guanylate cyclase in target cells. Efforts to use ST as a vaccine antigen have been hindered because of the low immunogenicity of this small toxin and other considerations discussed later.

Several lines of evidence support the feasibility of an efficacious ETEC vaccine. Epidemiologic as well as volunteer studies have demonstrated homologous protection against ETEC reinfection. In animal models, antibodies to LT and CF antigens have been shown to cooperate synergistically for protection.[17,23] Intestinal antibodies protected against ETEC challenge with ETEC strain H10407 that expresses CFA/I fimbriae, LT and ST in 90% of volunteers who had received passive oral milk-derived immunoglobulin from cows hyperimmunized with several fimbriated ETEC strains and LT.[24]

Enterotoxigenic *Escherichia coli* Vaccine Candidates

Although the diversity of ETEC, with more than 100 O serogroups and more than 25 recognized CF antigens, represents a major challenge for vaccine development, several ETEC vaccine candidates are under development. Most vaccines are intended for oral administration and based on toxin-derived antigens alone or combined with CFs, the latter being either purified or expressed on the bacterial surface. These vaccines are in different stages of preclinical and clinical (Table 18.1) development. The oral cholera vaccine Dukoral is licensed in several countries for prevention of ETEC travelers' diarrhea based on its demonstrated 50% to 70% short-term cross-protective effect through the B subunit component against LT-mediated ETEC diarrhea.[20–22]

Toxoid and Fusion Protein Vaccines. To develop an ETEC vaccine based solely on toxin-derived antigens may seem to be a rational strategy, as all ETEC strains produce either LT or ST or both. However, because the low-molecular ST is not immunogenic, chemical conjugates or gene fusion proteins between ST or ST-derived peptides and various carrier proteins have been studied—for example, LT (or mutated versions thereof), CT B subunit, and CFs. This approach has usually resulted in only modestly immunogenic conjugates with regard to the ST component. Immunization of animals with such preparations have typically yielded immune sera with only low-titer ST neutralizing activity even when strong adjuvants were used, and notably, the immunogenic preparations have also retained ST toxic activity.[25] A special problem for antibody-mediated protection directed against ST would

TABLE 18.1 Enterotoxigenic *Escherichia coli* Candidate Vaccines Tested in Humans

Vaccines	Development Stage	References
KILLED WHOLE-CELL ORAL VACCINES		
ETVAX		
Multivalent ETEC vaccine; recombinant *E. coli* strains overexpressing CFA/I, CS3, CS5, and CS6 + LCTBA toxoid ± dmLT	Phase I trial in Sweden	37
rCTB-CF vaccine		
ETEC strains expressing CFA/I, CS2, CS3, CS4, and CS5 + rCTB	Phase III trials in North American travelers to Mexico and Guatemala; Phase III trials in Egypt	31
LIVE ATTENUATED ORAL WHOLE-CELL VACCINES		
ACE527		
ETEC strains with deletions in *aroC*, *ompC,* and *ompF* expressing CFA/I, CS1, CS2, CS3, CS5, and CS6, and LTB ± dmLT	Phase I trials in U.S. Phase IIb trials in U.S.	41, 42, 132
SUBUNIT ORAL VACCINES		
Dukoral		
rCTB toxoid component of oral killed whole-cell cholera vaccine	Licensed Phase III trials in Bangladesh, in Finnish travelers to Morocco, and North American travelers to Mexico	20, 21, 22
dmLT		
Double mutant LT (R192G/L211A)	Phase I trial in U.S.	133
CS6		
Free protein or in microspheres	Phase I trial in U.S.	134
SUBUNIT TRANSCUTANEOUS/INTRADERMAL VACCINES		
LT patch	Phase III trial in U.S.	46
CF tip proteins		
CfaE or CfaE-sCTA2/LTB chimera + mLT (R192G)	Phase I trial in U.S.	47

CF, colonization factor; CS, coli surface antigen; dmLT, Double mutant labile toxin; ETEC, enterotoxigenic *Escherichia coli*; LCTBA, heat-labile toxin B subunit/cholera toxin B subunit hybrid protein; LT, labile toxin; rCTB, recombinant cholera toxin subunit B.

also be that the low molecular size of ST in comparison with a SIgA antibody would require a large amount of locally produced anti-ST SIgA to effectively neutralize ST. Another obstacle to vaccine development is the structural similarity between ST and human guanylin and uroguanylin, which might cause anti-ST antibodies to cross-react with endogenous ligands. Surprisingly, successful preliminary results have been reported by parenteral immunization of sows with ST-LT fusion proteins in protecting suckling piglets against challenge with a porcine ST-producing ETEC strain.[26,27] However, the capacity of these fusion proteins to induce intestinal antibodies is still unknown. Furthermore, residual ST or LT toxicity may be less of a problem in animals than in young infants and children; thus it remains to be seen if this approach, however appealing, will succeed for vaccination in humans.

Vaccines Containing Killed Whole-Cell Bacteria Plus B Subunit. A more complex, but arguably more realistic, strategy to protect against both LT-mediated and ST-mediated ETEC diarrhea is to develop a vaccine that is capable of inhibiting the intestinal attachment and colonization of ETEC and thereby diminishing the production of enterotoxins, especially by ST-producing ETEC. For additional protection against LT-induced diarrhea, the vaccine would preferably be combined with an LT "toxoid" component such as LT or CT B subunit in addition to ST. Based on this strategy, a first-generation oral ETEC vaccine consisting of both formalin-inactivated ETEC bacteria expressing various prevalent CF antigens and recombinant CT B subunit (rCTB) was developed by researchers at the University of Gothenburg and extensively tested in Phase I and Phase II trials.[17,28–30] This vaccine, given orally with a buffer in a two- or three-dose regimen, was safe and induced intestinal mucosal immune responses in 70% to 90% of Swedish, Bangladeshi, and Egyptian vaccinees of different ages. However, when tested in two Phase III trials in North American travelers to Mexico and Guatemala and one Phase III study in 6- to 18-month-old children in Egypt, the vaccine failed to significantly reduce overall (usually mild) ETEC diarrhea[17,31] (also L. Bourgeois, personal communication, 2012). Nevertheless, in both of the traveler studies in adults the vaccine reduced more severe ETEC diarrhea by almost 80%. Thus, vaccine protection against ETEC appears to be in line with observations for already licensed enteric vaccines against cholera and rotavirus disease, which have greater impact on more severe than on milder disease.[12]

A second-generation oral killed ETEC vaccine based on the same approach has recently been developed, in which several improvements have been made compared to the first-generation vaccine[32,33]: (a) vaccine strains are engineered to express four- to 15-fold increased amounts of CFA/I, CS3, and CS5 antigens, and to express high levels of the CS6 antigen in an immunogenic form (which was missing in the previous vaccine); (b) the rCTB is replaced by a hybrid rCTB/LTB*subunit* protein (LCTB*A*) eliciting a stronger anti-LT immune response; and (c) the double-mutant LT (dmLT), being a combined mucosal adjuvant and LT antigen, is optionally included in the vaccine. The dmLT molecule (R192G/L211A), which has two mutations in the enzymatic A subunit compared to the

native LT toxin, is nontoxic, and has shown potent adjuvant activity when tested in combination with various vaccines in mice and in vitro with human cells.[34–36] When the improved ETEC vaccine was given to mice, strong intestinal-mucosal IgA antibody responses were induced (as well as serum IgG and IgA responses) to each of the vaccine CFs as well as to LTB, and responses to the vaccine were further enhanced by the dmLT adjuvant.[32] Based on these data, a Phase I study was undertaken in Swedish adult volunteers (30 to 35 subjects per group) given either the vaccine alone, the vaccine together with 10 or 25 μg of dmLT adjuvant, or placebo.[37] Adverse reactions were few and mild with no differences between subjects receiving vaccine with or without dmLT or placebo. The majority of the vaccinated subjects (74% of all vaccine recipients and 83% of those receiving vaccine plus low-dose dmLT) showed significant mucosal IgA responses to all five of the primary vaccine antigens. The mucosal IgA antibodies were measured from cultured gut-derived blood lymphocytes (the antibody in lymphocyte supernatants method) and/or as specific SIgA antibodies in stool samples. Addition of low-dose dmLT to the vaccine significantly enhanced antibody in lymphocyte supernatants responses to the CS6 antigen, which was the CF present in lowest amounts in the vaccine. It is possible that the already excellent immunogenicity of the vaccine alone in this adult population explains why dmLT did not have any major effect on responses to the other vaccine antigens. Studies are in progress to examine the safety and immunogenicity of the improved oral inactivated ETEC vaccine with and without dmLT adjuvant when given to children living in areas endemic for ETEC as well as the protective efficacy in travelers.

Live Attenuated Vaccines. Several observations established the feasibility of immunoprophylaxis against ETEC using live oral vaccines. In a challenge model of ETEC diarrhea in adult volunteers, an initial clinical diarrheal infection caused by a fimbriated LT/ST ETEC strain conferred approximately 80% protection against clinical illness upon subsequent homologous rechallenge.[38] Immunization with a single 5×10^{10} colony-forming unit (CFU) dose of an ETEC strain (serotype O6:H16 expressing CS1 and CS3) that had lost the genes encoding LT and ST stimulated significant intestinal SIgA anti-CF antibody responses and conferred 75% protection against challenge with a heterologous ETEC serotype (O139:H28) expressing CS1 and CS3 and LT and ST.[39] More recently, the ACE527 live vaccine was developed containing three attenuated ETEC strains with deletions in *aroC*, *ompC*, and *ompF*, which collectively express CFA/I, CS1, CS2, CS3, CS5, and CS6, as well as LTB.[40] In a Phase I trial, two doses of ACE527 (10^{10} and 10^{11} CFUs) were well tolerated, and the higher vaccine dosage induced a high frequency of gut-derived IgA antibody in lymphocyte supernatants responses against LTB as well as CFA/I, CS6, and to a lesser extent CS3.[41] Irrespective of dose, immune responses to other antigens were low and infrequent. These observations were extended in a Phase IIb study in subjects who received two 10^{11} CFU doses and were then challenged with ETEC strain H10407 (O78, CFA/I, LT/ST).[42] In this study, 19% of vaccinees, but none of the placebo recipients, vomited upon immunization, and other gastrointestinal symptoms also tended to be more common in the vaccine group. A nonsignificant 27% reduction was seen in moderate to severe diarrhea, the primary end point, although the vaccine decreased the intestinal colonization by the challenge strain. Efforts are underway to further improve the immunogenicity of this vaccine by addition of dmLT adjuvant.

Labile Toxin Administered Transcutaneously. The safety and immunogenicity as well as protective efficacy of transcutaneously administered *E. coli* LT via skin patches have been extensively tested in human volunteers.[43,44] The patches, which did not give rise to any significant systemic side effects and only mild local skin reactions, induced significant anti-LT serum IgA and IgG, as well as IgA antibody-secreting cell responses in most vaccinees. However, two transcutaneous immunizations with LT did not protect against challenge with LT-producing ETEC.[44] In a placebo-controlled Phase II study in adult travelers to Mexico or Guatemala who were given two transcutaneous immunizations with LT there was also no significant protection against ETEC disease, although, quite surprisingly, significant protection against travelers' diarrhea of any origin was observed.[45] However, a subsequent Phase III trial did not show significant protection against travelers' diarrhea caused by either ETEC or other pathogens.[46] In a subgroup analysis by ETEC enterotoxin type, the vaccine seemed to be somewhat protective against diarrhea caused by LT-only–positive ETEC, but not against ST-positive or LT/ST-positive ETEC.[46] The manufacturer has ceased further development of LT skin patches as a human ETEC vaccine.

Fimbrial Tip Adhesion Vaccines. Another approach to develop an ETEC vaccine that provides combined anticolonization and antitoxic immunity is to produce chimeric proteins between ETEC CF antigens and LTB subunit by linking the tip protein of the CFA/I fimbriae (CfaE) to LTB. Recent Phase I clinical trials have been undertaken with a complex CfaE adhesin-toxoid chimera in which CfaE replaces the A1 domain of CT A subunit and is noncovalently assembled with LTB pentamer (CfaE-sCTA2/LTB chimera). Escalating doses of these prototype ETEC adhesin vaccines, admixed with single mutant LT (LTR192G) as adjuvant and antigen, were given by transcutaneous patch (CfaE only) and intradermal (CfaE chimera) vaccination. Although local skin reactions were common, there were no serious adverse reactions. Preliminary immunogenicity results also indicated anti-LTB as well as anti-CfaE IgG and IgA responses were seen after either route of immunization.[47] Studies to determine efficacy against ETEC challenge are in progress.

Shigella

Epidemiology

Shigella spp. are the most common cause of acute, bloody diarrhea (dysentery) and are responsible for a significant proportion of morbidity and mortality associated with diarrheal disease. Shigellosis only affects humans, and even though all age groups are susceptible, most cases occur in children younger than 5 years of age. The *Shigella* genus comprises four pathogenic species: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, and *Shigella boydii*, including 50 serotypes and subserotypes; however, *S. dysenteriae* 1, which caused severe outbreaks up to the 1990s, has now almost disappeared. *S. flexneri* is endemic in developing countries, whereas *S. sonnei* is the most common cause of shigellosis in highly industrialized countries. *Shigella* (mostly *S. flexneri*) was found to be one of the top four attributable pathogens overall in children younger than 5 years of age with moderate to severe diarrhea in four sites in sub-Saharan Africa and in three sites in South Asia, and was the most common diarrhea pathogen in the 2- to 5-year-old age group.[15]

Clinical Manifestations and Mechanisms of Disease and Immunity

Symptoms of *Shigella* infection vary from mild, watery diarrhea to severe inflammatory bacillary dysentery with fever,

severe abdominal pain, and blood and mucus in the stools. *Shigella* bacteria cause disease by invading the human lower intestine and causing intense inflammation and epithelial destruction.[48] When the orally ingested organism reaches the terminal ileum and colon, it traverses the follicle-associated epithelium, is taken up by phagocytic cells, and can then invade neighboring epithelial cells at the basolateral pole, spreading from cell to cell. Apoptosis of infected macrophages releases proinflammatory cytokines and elicits a strong inflammatory response with accumulation of large numbers of neutrophils and destruction of the intestinal epithelium. Depending on serotype, *Shigella* can also produce one or more toxins that contribute to virulence, including Shiga toxin (*S. dysenteriae* 1); *Shigella* enterotoxin 1 ("ShET1"; *S. flexneri* 2a and some *S. flexneri* 2b strains); and *Shigella* enterotoxin 2 ("ShET2"; all *Shigella* serotypes). ShET1 and ShET2 are thought to be responsible for the watery diarrhea phase of clinical shigellosis that often precedes the dysenteric phase.[48]

There are still no licensed vaccines against shigellosis. Because *Shigella* is antigenically diverse, a *Shigella* vaccine should protect against all 15 *S. flexneri* serotypes plus *S. sonnei* (which is a single serotype) and ideally also *S. dysenteriae* 1. It is believed that a vaccine comprising four to five *Shigella* strains (*S. sonnei*, *S. flexneri* 2a, 3a, and 6, and possibly *S. dysenteriae* 1) could protect not only against the serotypes contained in the vaccine but also against the other 11 *S. flexneri* serotypes because of shared antigens.[48]

Studies in healthy adult volunteers have shown that an initial episode of clinical shigellosis confers significant (approximately 75%) protection against illness upon rechallenge a few months later with the homologous virulent serotype.[49,50] A similar level of protection against homologous reinfection was observed in Chilean children.[51] Serum and/or mucosal antibodies directed against the O antigen appear to play a major role in protection.[48] Both oral and parenteral prototype *Shigella* vaccines have induced significant protection against clinical shigellosis, and the presence of gut-derived O-antigen–specific IgA antibody-secreting cells in blood 7 to 10 days after oral vaccination have correlated with vaccine efficacy. Cell-mediated immunity, including interferon-γ production, may also contribute.[48]

Shigella Vaccine Candidates

Two main strategies have been pursued to develop effective vaccines against shigellosis: (a) primarily orally administered live or killed whole-cell vaccines and (b) parenterally injected conjugate vaccines containing purified O-antigen polysaccharide (PS) coupled to a carrier protein. The main challenge for the oral live attenuated vaccine candidates has been how to achieve adequate attenuation while retaining the ability of the vaccine strain to multiply and stimulate immunity in the gut. For oral killed whole-cell or parenterally injected conjugate vaccines reactogenicity is much less of a problem and instead the main challenge has been to stimulate the right type and magnitude of immune response. These vaccines may need to be combined with an effective adjuvant. Some of the *Shigella* vaccine candidates and those that have been tested in humans are summarized below and in Table 18.2.

Live Oral *Shigella* Vaccines. In the 1960s, Mel and others evaluated in Phase I to Phase III trials a first generation of live oral attenuated *Shigella* vaccines. One type consisted of streptomycin-dependent (SmD) *Shigella* bacteria of different serotypes that had lost the ability to invade epithelial cells. A second type was a spontaneous mutant *S. flexneri* 2a strain (T32) which was noninvasive because of a large deletion in the invasiveness plasmid. Overall, these vaccines were well tolerated and protective in both adults and children in randomized, controlled field trials in Romania and China.[52,53] These early live oral *Shigella* vaccines were later superseded by better defined genetically engineered *Shigella* bacteria, and a multitude of strains have been tested in humans (see Table 18.2).

The SC602 vaccine was constructed by researchers at the Institut Pasteur by creating in a *S. flexneri 2a* strain deletion mutations in the *icsA (virG)* gene, which encodes a protein involved in intracellular motility and cell-to-cell spread of *Shigella*, and in *iuc*, which encodes the iron-scavenging protein aerobactin. A dosage of 10^6 CFUs was strongly immunogenic but unacceptably reactogenic in North American volunteers, whereas 10^4 CFUs was well tolerated and still significantly immunogenic. When vaccinated volunteers were challenged with virulent *S. flexneri* 2a, significant protection was observed 2 months later.[54] SC602 was also tested in a Phase II trial in adults and children in Bangladesh. All dose levels (10^4, 10^5, or 10^6 CFUs) were well tolerated but in this population even the highest dose was minimally shed and poorly immunogenic.[55]

The SC599 vaccine is another live *S. dysenteriae* type 1 strain attenuated by deletions of *icsA* (inhibiting invasion), *ent* and *fep* (inhibiting iron chelation), and *stxA* (blocking Shiga toxin A subunit synthesis) genes. In Phase I and Phase II trials, this vaccine was well tolerated and immunogenic at dosage of 10^5 to more than 10^8 CFUs, but induced only modest IgA antibody responses.[56,57] The clinical development of this vaccine has not been pursued further.

Investigators at Walter Reed Army Institute of Research constructed strain WRSS1 by deleting the *icsA* gene in a virulent strain of *S. sonnei*.[58] Safety and immunogenicity trials in North American volunteers,[59] and subsequently in Israeli adults,[60] indicated that the vaccine strain was well tolerated in dosages up to 10^4 CFUs. At this dosage all subjects examined had detectable IgA anti-O antigen antibody-secreting cell responses. An analogous *S. dysenteriae* 1 vaccine candidate, WRSd1, with a mutation in *icsA* and a chromosomal deletion in *stxAB*, which encodes Shiga toxin, was constructed[61] and doses of 10^3 to 10^7 CFUs were given to North American volunteers. However, approximately 20% developed diarrhea and 60% developed a detectable IgA response.[62] The adverse reactions seen with WRSs1 and WRSd1 led to efforts to introduce additional mutations to try to decrease the reactogenicity without compromising the immunogenicity. Several new vaccine strains have been developed, but none has yet been tested in humans.

Investigators at the Center for Vaccine Development (CVD) have constructed and clinically tested a number of live attenuated *Shigella* vaccine candidate strains. Their work with two early vaccine strains, CVD 1204 and CVD 1208, established the attenuating effects of deletion of the *guaBA* (leading to inhibition of the ability to biosynthesize guanine nucleotides) and of the *sen* chromosomal and plasmid genes encoding ShET1 and ShET2, respectively.[63] Based on this, the CVD 1208S *S. flexneri* 2a vaccine strain having deletion mutations in *guaBA* as well as in both *sen* genes was developed. When CVD 1208S was tested in North American adults (10^7 or 10^8 CFUs) it was well tolerated and immunogenic.[64] Analogous strains of other serotypes have now also been constructed to combine with CVD 1208S in a pentavalent live oral vaccine (CVD 1213 *S. flexneri* 3a, CVD 121 *S. flexneri* 6, CVD 1233S *S. sonnei*, and CVD 1254 *S. dysenteriae* 1) which remains to be tested in humans.

Killed Whole-Cell Vaccines. This strategy aims to develop a safe multivalent oral vaccine comprising the bacterial components needed for achieving broad protection against *Shigella*

TABLE 18.2 *Shigella* Candidate Vaccines Tested in Humans

Vaccines	Development Stage	References
LIVE ATTENUATED ORAL WHOLE-CELL VACCINES		
SmD		
Mono-serotype and multivalent *Streptomycin*-dependent *Shigella* strains with spontaneous loss of invasive ability	Several efficacy field trials and locally licensed in Romania (but no longer used)	52,135, 136, 137, 138
T32		
Shigella flexneri 2a strain with spontaneous deletion in invasive plasmid	Phase III field trials in Romania and China (but no longer used)	53, 139, 140
SC602		
S. flexneri 2a strain with specific deletions in *icsA (virG)* and *iuc*	Phase I studies in U.S. and Bangladesh; Phase IIb trial in U.S.	54, 55
SC599		
Shigella dysenteriae 1 strain with deletions in *icsA, ent, fep*, and *stxA*	Phase I and Phase II trials in U.S. and France	56, 57
WRSS1		
Shigella sonnei strain with deletion in *icsA*	Phase I and Phase II trials in U.S. and Israel	59, 60
WRSd1		
S. dysenteriae 1 strain with mutations in *icsA* and *stxA*	Phase I trial in U.S.	62
CVD1208s		
S. flexneri 2a strain with deletions in *guaBA* and *sen*	Phase I trial in U.S.	64
KILLED WHOLE-CELL ORAL VACCINES		
Historical vaccines		
Heat-killed *S. dysenteriae* 1 bacteria	Several efficacy trials	65
Formalin-killed *S. sonnei*	Phase I trial in U.S.	66
PARENTERAL CONJUGATE VACCINES		
S. sonnei O polysaccharide (PS) conjugated to rEPA carrier protein	Phase III trial in Israel	71
S. sonnei O PS-rEPA + *S. flexneri*-rEPA conjugates	Phase III trial in Israel	74
S. dysenteriae O PS-EPA biosynthetic conjugate	Phase I trial in Switzerland	75
Flexyn2a		
S. flexneri 2a O PS-EPA biosynthetic conjugate	Phase I trial in U.S.	76
INTRANASAL PROTEIN VACCINE		
Invaplex		
Invasion complex vaccine; *IpaA, IpaC, IpaD* + LPS	Phase I and Phase II trials in U.S.	77, 78

EPA, *Pseudomonas aeruginosa* exotoxin A; LPS, lipopolysaccharide; rEPA, recombinant *P. aeruginosa* exotoxin A; SmD, streptomycin dependent.

infections. Heat-killed *S. dysenteriae* 1 bacteria administered orally were used in many places in the 1920s to control outbreaks of dysentery.[65] These trials were neither blinded nor placebo-controlled but the results clearly suggest that the vaccines were effective, reducing dysentery incidence among vaccinated when compared to unvaccinated subjects by 50% to 70%. More recently, a formalin-killed *S. sonnei* vaccine was evaluated in a Phase I clinical study in North American volunteers.[66] This vaccine was well tolerated and induced an immune response to *Shigella* antigens in most individuals. A trivalent formalin-killed vaccine comprising *S. flexneri* 2a and 3a components in addition to the *S. sonnei* component has been produced and will undergo clinical testing.[67]

Parenteral Conjugate Vaccines. A strategy that has been demonstrated to confer protection in adults and older children living in endemic settings is the parenteral conjugate *Shigella* vaccine approach. Early parenterally administered killed whole-cell vaccines stimulated high levels of serum antibodies but failed to protect monkeys against challenge[68] or children from natural infection in field trials,[69] leading many to conclude that serum antibodies alone could not protect against shigellosis. This notion was contested by studies of parenteral conjugate vaccines consisting of lipopolysaccharide (LPS)-derived O PSs of relevant *Shigella* serotypes covalently linked to a carrier protein (recombinant *Pseudomonas aeruginosa* exotoxin A [rEPA]). A single dose of *S. sonnei* O PS conjugated to rEPA was well tolerated and induced high levels of serum IgG anti-PS antibodies in Israeli soldiers.[70,71] In a randomized, controlled, double-blind Phase III efficacy trial in Israeli soldiers, the vaccine conferred 74% protection against *S. sonnei* diarrhea.[71] These results were followed by Phase II trials in Israeli children, ages 4 to 7 years and 1 to 4 years,[72,73] and, subsequently, a Phase III trial in children 1 to 4 years of

age,[74] where children were given two injections of a combination of *S. sonnei* PS conjugated to rEPA and *S. flexneri* 2a PS conjugated to succinylated rEPA 6 weeks apart. While the Phase II studies showed promising immunogenicity to both serotypes in the pediatric age groups, the Phase III trial did not provide significant protection against *S. sonnei* disease, the overall efficacy being 27%; protection against *S. flexneri* could not be determined because of too few cases for analysis. Efficacy against *S. sonnei* in children ages 3 to 4 years was 71% (95% confidence interval [CI] −4.4, 92.0; $P = .04$), whereas it was 36% (95% CI −56.4, 73.4; $P = .36$) in children 2 to 3 years of age and 4% (95% CI −101.1, 46.5; $P = .91$) in children 1 to 2 years of age. This was associated with an age-related increase in serum IgG antibody levels. It is not clear if the apparent protection in the oldest children was a consequence of higher serum IgG antibody levels allowing more antibodies to transudate into the intestine or, whether the older children were immunologically primed from prior exposure to *S. sonnei* and therefore able to produce mucosal SIgA that mediated the protection.

Investigators at GlycoVaxyn have developed conjugate vaccines to various enteric pathogens using a genetic system for low-cost production of glycosylated proteins in *E. coli*. A *S. dysenteriae* 1 O PS-EPA (*P. aeruginosa* exotoxin A) conjugate was administered intramuscularly in a Phase I trial, with or without adjuvant (aluminium hydroxide), in Swiss adults. It was well tolerated and highly immunogenic irrespective of dose or formulation.[75] An analogous bioconjugate vaccine of *S. flexneri* 2a O PS-EPA (Flexyn2a) was also found to be safe and highly immunogenic when administered intramuscularly with and without aluminum hydroxide to healthy adult volunteers.[76] Plans to prepare and test a multivalent bioconjugate *Shigella* vaccine are in progress.

Additional *Shigella* Vaccine Strategies. All four species of *Shigella* produce identical type III secretion proteins (Ipa invasion proteins). Based on this concept, a *Shigella* invasion protein complex vaccine (Invaplex) containing IpaB, IpaC, and IpaD and LPS has been prepared. Administered intranasally, Invaplex was well tolerated and elicited relevant anti-*Shigella* immune responses in Phase I and Phase II trials.[77,78] Other approaches in preclinical testing include injectable or intranasal vaccines based on outer membrane vesicles being developed by Novartis in Italy,[79] vaccines based on recombinant shared *Shigella* outer membrane proteins such as PSSP-1 developed at the International Vaccine Institute in South Korea,[80] and various combined *Shigella*-ETEC vaccines based on oral live or killed *Shigella* bacteria expressing ETEC CFs and LTB developed at CVD, USA[48] or University of Gothenburg, Sweden.

Campylobacter jejuni

C. jejuni is a major cause of diarrheal disease in both industrialized and developing countries. The pathogen is the most common bacterial cause of diarrhea in the United States and the second most common cause in United Kingdom.[81] In many developing countries, almost all children are infected during early childhood, and asymptomatic reinfection and prolonged carriage are common.[82] *Campylobacter* is one of the most common causes of travelers' diarrhea[16] and also of diarrheal outbreaks during military exercises.[83] Importantly, the infection is associated with many life-threatening cases of Guillain-Barré syndrome (GBS), a form of symmetric ascending paralysis with autoimmune etiology.[81,84] *Campylobacter* species have also been associated with a range of gastrointestinal conditions, including inflammatory bowel disease (IBD), Barrett esophagus, and colorectal cancer, as well as some extragastrointestinal manifestations.[81] *Campylobacter* spp. are frequent commensals in the intestinal tracts of chickens and are commonly implicated in foodborne diarrheal illness.[85] In developing countries, *Campylobacter*, because of the heterogeneous antigenic properties of clinical isolates, may cause several infections per year per child and has been identified as an important cause of moderate to severe diarrhea in infants and young children.[15,82,86] On the other hand, *Campylobacter* infections are often asymptomatic in children older than 5 years of age and in adults in endemic areas.[87]

Campylobacter infection typically begins with a watery diarrhea phase. The infection commonly manifests with signs of inflammation, fever, abdominal pain, and bloody diarrhea.[85] Infection in developing countries is more commonly watery in nature without frank blood.[82] Untreated infection may subside after 3 to 7 days, but persistent disease is not uncommon. GBS following *Campylobacter* infection is usually self-limiting, but death may occur, especially if clinical management is not optimal.[84] The pathophysiology of *Campylobacter*-associated GBS is not fully elucidated, but antibodies to some *C. jejuni* lipooligosaccharide serotypes cross-react with human neural sheath gangliosides.[88,89]

Campylobacter Vaccine Candidates

Vaccine approaches to protect against *Campylobacter* infection are complicated by the organism's antigenic heterogeneity and by the threat of GBS. Two antigenic typing systems are in use: the Lior system and the Penner system. The Lior typing scheme includes 108 serotypes and is based on a heat-labile surface antigen that is still not characterized. The Penner system recognizes more than 60 serotypes and is based on LPS and lipooligosaccharide antigens.[90,91] Immunity to *Campylobacter* appears to be strain specific, but the antigens conferring immunity are unknown. Human challenge studies have demonstrated homologous strain-specific immunity against rechallenge with some but not all strains. The well-characterized outbreak strain, *C. jejuni* 81-176, gave complete protection against homologous rechallenge 1.5 months after the initial infection, which had decreased to 57% against rechallenge after one year.[92] *Campylobacter*-specific production of interferon-γ by peripheral blood mononuclear cells has been strongly associated with protection from illness. In contrast, there was a lack of homologous protection against *C. jejuni* CG8421 in a human challenge model.[93]

Because of the uncertainty regarding the mechanism of GBS, *Campylobacter* whole-cell vaccines, either viable or killed, must be generated and evaluated with extreme caution. The most intensively studied *Campylobacter* vaccine candidate comprises a mixture of heat-killed and formalin-killed whole bacteria derived from prototype strain 81-176, combined with *E. coli* LT as a mucosal adjuvant.[94] In mice, a single dose provided homologous protection against intestinal colonization. Initial Phase I trials indicated that the vaccine is safe and immunogenic in humans,[95] but the vaccine was unsuccessful in a Phase IIb challenge study with the same strain.[96]

Other vaccine strategies against *Campylobacter* are also being considered. A conjugate vaccine composed of capsular PS from strain 81-176 linked to CRM197 (mutated diphtheria toxin) administered subcutaneously conferred 100% protection against diarrhea in nonhuman primates after homologous challenge,[97] and Phase I trials of this vaccine are being planned.[98] The flagellin antigen is an attractive vaccine candidate because of its relatively high antigenic conservation and the expectation that this molecule will not elicit GBS. A truncated recombinant flagellin subunit vaccine administered intranasally together with single mutant LT (R192G) has provided more than 80% protection against disease symptoms

and colonization after intranasal challenge with a heterologous *C. jejuni* strain in mice.[99] Other proteins secreted through the *Campylobacter* flagellum have also been evaluated as vaccine candidates,[100] but to our knowledge none has been tested in humans.

Enterohemorrhagic *Escherichia coli*/Shiga Toxin–Producing *Escherichia coli*

EHEC was first described in 1983 after having been isolated from patients with hemorrhagic diarrhea in two diarrheal outbreaks associated with eating poorly cooked hamburgers in restaurants.[101] The *E. coli* strain isolated was O157:H7 serotype and produced the same cytotoxins (Verotoxins or Shiga toxins) as those described in cases of hemolytic uremic syndrome (HUS),[102] also caused by *E. c*oli O157:H7 bacteria. EHEC is considered as a subgroup of STEC. Approximately 450 serotypes of STEC have been isolated from humans with disease, and in most countries EHEC O157:H7 is the most common serotype causing outbreaks. EHEC is endemic in the United States with an estimated incidence of one case per 100,000 individuals. In Europe the incidence rate is lower but increasing. Many EHEC outbreaks have also been reported from low- and middle-income countries, although the importance of the pathogen in these environments is likely to be underestimated because of lack of proper diagnosis.[103]

STEC infection is acquired by ingestion of contaminated food or water or by person-to-person spread. A natural reservoir for the pathogen exists in the intestines of cattle.[104] The difficulty in eliminating the organism from bovine populations presents significant challenges to control that may be best addressed by vaccination. Whether this is best achieved by vaccination of cattle or of risk group humans or both remains an open question. Currently, two parenteral vaccines for control of EHEC in livestock have been licensed (Econiche in Canada and Epitopix in the United States). Both of these vaccines significantly decrease colonization and stool contents of O157:H7, but do not eliminate this organism completely.[105,106] It is not known whether either of these vaccines have any effect on other STEC serogroups that also cause HUS in young children and elderly people.

STEC infection is accompanied by watery diarrhea, frequently progressing to hemorrhagic colitis.[104,107] Systemic absorption of Shiga toxin (Stx), an oligomeric toxin related to the toxin produced by *S. dysenteriae* 1,[108] leads to serious sequelae, including HUS, which develops in up to 20% of pediatric patients infected with *E. coli* O157:H7. Other potentially fatal complications include intestinal perforation and cerebrovascular accident. In addition, non-O157:H7 STEC strains are increasingly being recognized as causes of HUS, bloody diarrhea, and other gastrointestinal illnesses.[109,110] Similar to CT and *E. coli* LT, Stx is composed of a single catalytic toxic—active A subunit and five cell-binding B subunits.[108] The B subunit binds to the cell by the glycolipid globotriaosylceramide (Gb3). The A subunit catalyzes the depurination of a single residue in the 28S eukaryotic ribosomal RNA, thereby inhibiting protein synthesis and leading to target cell death.

Shiga Toxin–Producing *Escherichia coli* Vaccine Candidates

The most advanced vaccine candidate appears to be a conjugate vaccine based on O157 O-specific PS conjugated to rEPA. A Phase I trial in adults indicated that a single parenteral dose was safe and stimulated strong O-antigen–specific responses.[111] All children ages 2 to 5 years participating in a Phase II study responded with anti-O157 IgG titers with high bactericidal activity, but efficacy was not reported.[112] An alternative approach is to link the O157 PS to the StxB1 subunit, to elicit both antitoxic and anticapsular antibody responses. In young mice, this formulation induced bactericidal antibodies to *E. coli* O157 as well as neutralizing antibodies to Stx.[113]

The importance of Stx in STEC disease has made it a natural target for immunization. Antibodies against the B subunit have been shown to prevent binding of the toxin to target cells[114] and immunization with the Stx2B subunit conjugated to keyhole limpet hemocyanin protected mice from a lethal dose of Stx2 holotoxin.[115] However, the A-B complex is more immunogenic than the B subunit alone, and thus both B and A-B toxoids have been examined as candidate vaccines. Vaccine approaches have been summarized[116] and include both parenteral and mucosal vaccination with inactivated holotoxin, toxoids generated by site-directed mutagenesis, genetically detoxified hybrid toxoids and fusion proteins carrying both Stx1 and Stx2 B subunits. However, the toxoid approach will likely be tested first as a vaccine for *S. dysenteriae*, which is a more important pathogen than STEC worldwide.

Several attenuated vector vaccines have been modified to express STEC antigens,[117,118] but none of these strains is being seriously considered as a vaccine candidate. Orally administered *Salmonella landau*, a *Salmonella* strain that naturally expresses the O157 antigen, is able to elicit protection of mice from colonization by *E. coli* O157.[119] These results suggest that local exposure to the O157 antigen may induce resistance when delivered by live *Salmonella*, although humans exposed to STEC do not generate highly protective O157 responses after natural infection. Proteins translocated through the type III secretion system, such as Tir, and the outer membrane protein intimin, have also been investigated as vaccine candidates, sometimes in the form of fusion proteins, also including Stx subunits.[116] There is also a continued search for novel vaccine antigens which may induce broad-based protection. Studies using comparative genomics[120] or immunoproteomics[121] have described a number of antigens expressed by STEC strains which are absent in commensal *E. coli* strains and may be potential candidates for vaccine development.

Nontyphoidal *Salmonella*

Salmonellae causing human disease are divided into thousands of NTS serotypes with a broad host range and a small number of invasive typhoidal serotypes (e.g., *S. enterica* var Typhi and *S. enterica* var Paratyphi A). NTS infection can occur by consumption of both animal and nonanimal food products, contaminated water, or by contact with animals. In healthy individuals in high-income countries, NTS predominantly causes localized and self-limited gastroenteritis, which is very distinct from the severe systemic disease caused by *S. enterica* serovars Typhi and Paratyphi, which are primarily seen in developing countries. However, invasive NTS disease can cause severe clinical disease, meningitis, and death in young infants, the elderly, and immunocompromised hosts in developed countries.[122,123] A large proportion of the episodes of invasive NTS disease, as well as of gastroenteritis in North Americans and Europeans, are caused by *Salmonella enteritidis*, *Salmonella typhimurium* or a monophasic variant of the latter that does not produce Phase II flagella. Importantly, NTS has also been identified as a major cause of invasive bacterial infections in infants and toddlers in sub-Saharan Africa.[124] These strains are often multiply antibiotic resistant and have high case fatality rates (typically 20% to 25%). In addition in this region unusual strains of *S. typhimurium* and *S. enteritidis* are circulating that cause severe, often fatal invasive disease, usually without a history of gastroenteritis.[124]

Nontyphoidal *Salmonella* Vaccine Candidates

Vaccine efforts targeting NTS include live attenuated and conjugate vaccine strategies. The live oral vaccines include one vaccine candidate, *S. typhimurium* WT05, which has deletions in the *ssaV* gene, which encodes a structural protein in the Type III secretion system, and in *aroC* gene, encoding chorismate synthase which is critical for the synthesis of aromatic amino acids and for bacterial growth. In human volunteers, oral administration of 10^7 to 10^9 CFUs of WT05 was reported to be well tolerated, but only the highest dose gave rise to a detectable immune response.[125] A number of additional *S. typhimurium* vaccine candidate strains harboring additional or alternative mutations have been generated as has also two attenuated *S. enteritidis* strains. These vaccines (reviewed in reference 126) have all shown promising safety and immunogenicity profiles in mice and the *S. typhimurium* strains have also been found to be highly protective in the mouse model. None of these vaccine candidates have yet been tested in humans.

The conjugate vaccines consist of the O PS extracted from *S. enteritidis* (group D) and *S. typhimurium* (group B) linked to carrier proteins. For *S. typhimurium* PS the carrier proteins used include, for example, tetanus toxoid[127] and bacterial porin,[128] or in a more recent conjugate using CRM_{197}.[129] These conjugate vaccines were reported to be well tolerated, highly immunogenic, and protective against challenge in mice or rabbits. A conjugate between the O PS and Phase I flagellin subunits of *S. enteritidis* stimulated high titers of opsonizing and bactericidal IgG antibodies, and the conjugates were also highly protective in the mouse model.[130] Phase I and Phase II clinical trials are being planned.

PUBLIC HEALTH PERSPECTIVES, INDICATIONS, AND CHALLENGES FOR VACCINATION

Since the first licensure of the Sabin oral polio vaccine more than 50 years ago, only a few vaccines have been licensed for enteric infections. As discussed above, there are currently intense efforts to develop vaccines against additional major diarrheal pathogens. There are strong indications to use vaccines once available for the control of ETEC diarrhea and shigellosis, which are both major causes of enteric infection and disease worldwide. Weanling infants and young children in ETEC and *Shigella* endemic countries—that is, practically all low- and middle-income countries in tropical and subtropical settings—are prime target populations for ETEC and *Shigella* vaccination. Travelers to and military personnel deployed to endemic areas are additional important target groups for such vaccines.

Likewise, *Campylobacter* vaccination is a priority in developing countries, where infants experience a high burden of life-threatening diarrhea. Travelers to developing nations may also benefit from *Campylobacter* vaccination, especially those traveling for prolonged periods such as soldiers. Although *Campylobacter* infection is less common than *E. coli* and *Shigella*, the risk of GBS increases the importance of *Campylobacter* infection. At the same time, GBS presents a particular concern in relation to vaccination, since strong safe-guards will obviously be required against triggering rather than protecting against GBS by vaccination.

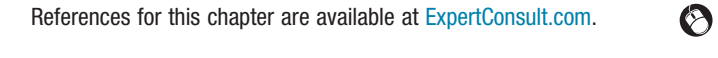
There is a growing interest in the development of safe and effective EHEC/STEC vaccines both for veterinary and human use. At the same time, there are significant policy issues regarding STEC vaccination of humans. Given the low incidence of serious infection and HUS, a vaccine would need to be highly effective and extraordinarily safe. Vaccination of cattle would not pose the same requirement for safety, however practical effectiveness of available vaccines is not fully defined. Vaccines against NTS are also under development and may become particularly important for control of invasive disease. However, the development of these vaccines are complicated by the immunocompromised nature of susceptible individuals.

Despite the strong indications for vaccination, especially against ETEC and *Shigella*, no vaccines for human use against these pathogens are available. There are several reasons for this situation, some being pathogen-specific and others being more generic. Pathogen-related challenges for vaccine development include the wide antigen diversity of different strains of the infecting organisms, problems in achieving adequate safety especially in live-attenuated vaccines while retaining protective immunogenicity and for some bacteria important animal reservoirs or risks of inducing neuro-inflammatory conditions. The more generic challenges are largely related to our still limited knowledge about the gut mucosal immune system. Mucosally administered vaccines are probably essential for inducing immune protection in subjects that have not been immunologically primed by previous natural exposure, and in those with noninvasive infections not readily reached by serum antibodies. They are also inherently safer and easier to deploy than injectable vaccines. However, development of mucosally administered vaccines have been hampered by several factors including, for example, (a) a lack of reliable animal models for predicting vaccine-induced immune protection in humans together with limitations in available adult human challenge models to predict practical vaccine efficacy in developing countries and in children; (b) a general lack of immunological correlates of mucosal immune protection; (c) a lack of safe yet effective mucosal adjuvants, limiting mucosal vaccine development to date to live-attenuated or inactivated whole virus and bacterial vaccines; (d) a limited knowledge of the factors responsible for the underperformance of (especially live) oral vaccines in children in developing countries; and (e) until recently a lacking recognition of the importance of herd protection as a means to increase the public health impact of oral enteric vaccines (for a further discussion of these factors and possible means to overcome them the reader is referred to a recent review[131]). There are reasons to believe that the development of promising safe and effective mucosal adjuvants and programmatically improved intervention strategies could enhance the efficacy of both current and next generation enteric vaccines. As emphasized throughout this chapter, this would be of special importance in low- and middle-income countries, which are often co-endemic for enteric infections, environmental enteropathy ("tropical sprue") and malnutrition.

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19 Diphtheria Toxoid

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Respiratory diphtheria is an acute communicable upper respiratory illness caused by toxigenic strains of *Corynebacterium diphtheriae*, a Gram-positive bacillus. The illness is characterized by a membranous inflammation of the upper respiratory tract, usually of the pharynx but sometimes of the posterior nasal passages, larynx, and trachea, and by widespread damage to other organs, primarily the myocardium and peripheral nerves. Extensive membrane production and organ damage are caused by local and systemic actions of a potent exotoxin produced by toxigenic strains of *C. diphtheriae*. A cutaneous form of diphtheria commonly occurs in warmer climates or tropical countries.

HISTORY OF THE DISEASE

Historical descriptions of diphtheria-like illness (throat membrane, neck swelling, frequent suffocation) first appeared in ancient Egyptian writings from the second millennium BC[1] with increasingly detailed descriptions by Greek writers, including Hippocrates (fifth century BC), Aretaeus (second century AD), and Aëtius (sixth century AD).[2–4] Only isolated reports of the disease appeared until the 17th century, when devastating outbreaks occurred in Spain.[3] Indeed, in Spanish history, 1613 is known as the Year of Diphtheria (Año de los Garrotillos).[2] Successive outbreaks occurred in southwestern Europe approximately every 12 years through the 18th century. The earliest definitive description of diphtheria in America was that of Samuel Bard in New York in 1771, although outbreaks had previously been noted in the American colonies.[2,3,5] In the early 19th century, Bretonneau clearly delineated the clinical picture of diphtheria, convincingly argued for its communicability, successfully pioneered tracheostomy as a method of treatment, and gave the disease its name, derived from the Greek word for leather or tanned skin.[2,3]

In the second half of the 19th century, increasingly severe epidemics swept many parts of Europe and the large cities of the United States, and vigorous efforts were made by researchers in the new field of bacteriology to identify the causative agent.[4] In 1883, Klebs first described the characteristic organisms in stained preparations of diphtheritic membranes, and Löffler reported the successful growth of these organisms in culture a year later.[3] Other investigators soon confirmed the pathogenicity of the organism for guinea pigs, and in 1888, Roux and Yersin demonstrated the presence of a potent exotoxin (see "Passive Immunization," later). Over the next decade, antisera produced in animals by injection of sublethal or inactivated cultures were first shown to prevent death in nonimmune animals that were challenged with virulent organisms, and then to prevent death in children with clinical diphtheria.[2,3]

The concept of active immunization began with Theobald Smith in 1907, who noted that long-lasting immunity to diphtheria could be produced in guinea pigs by the injection of mixtures of diphtheria toxin and antitoxin and suggested that these mixtures might do the same for humans. After successful immunization of children by von Behring with toxin–antitoxin mixtures, immunization programs began in selected European and American cities. However, these immunizations, although usually effective, were not free of adverse reactions. In 1913, Schick introduced a skin test for immunity that consisted of the injection of a small, measured amount of diphtheria toxin; in immune persons, circulating antibody neutralized the toxin, and no local lesion was observed.[2] The Schick skin test was widely used to distinguish immune individuals and target immunization to those susceptible. In the early 1920s, Ramon showed that diphtheria toxin, when treated with heat and formalin, lost its toxic properties but retained its ability to produce serologic protection against the disease. Thus the current immunizing preparation, diphtheria toxoid, came into being.[6]

WHY THE DISEASE IS IMPORTANT

Before the introduction of diphtheria immunization, diphtheria was a major cause of childhood mortality, and it remains endemic in many developing countries. A large outbreak of diphtheria in the newly independent states of the former Soviet Union in the 1990s highlighted the potential for this ancient scourge to reemerge in countries that fail to maintain high levels of population immunity through immunization.[7]

BACKGROUND

Clinical Description

Classic diphtheria has an insidious onset after an incubation period of 1 to 5 days (rarely longer). The gradual onset of diphtheria is in contrast to the usually sudden, almost explosive manifestations of streptococcal pharyngitis. Symptoms of diphtheria are initially nonspecific and mild; throughout the course of the disease, the patient's temperature usually does not exceed 38.5°C. Other early symptoms in children include diminished activity and some irritability. At the very onset of symptoms, the pharynx is inspected on examination but no membrane is present. About a day after onset, small patches of exudate appear in the pharynx. Within 2 or 3 days, the patches of exudate spread and become confluent and may form a membrane that covers the entire pharynx, including the tonsillar areas, soft palate, and uvula. This membrane becomes grayish, thick, and firmly adherent to the underlying mucosa. Efforts to dislodge the membrane result in bleeding. Anterior cervical lymph nodes become markedly enlarged and tender. In a proportion of patients, the lymph node swelling is associated with considerable inflammation and edema of the surrounding soft tissues, giving rise to the so-called bull-neck appearance, which is associated with a higher morbidity and mortality. Although fever is rarely high, the patient characteristically appears toxic and displays a rapid, thready pulse. In untreated patients, the membrane begins to soften about a week after onset and gradually sloughs off, usually in pieces but sometimes as a single unit. As the membrane detaches, acute systemic symptoms, such as fever, begin to disappear.

Although pharyngeal diphtheria is by far the most common form of disease seen in unimmunized populations, other skin or mucosal sites may be involved. Laryngeal diphtheria occurs in 25% of cases; in 75% of these instances, the pharynx is also involved. Isolated nasal diphtheria is uncommon (approximately 2% of cases). Cutaneous, aural, vaginal, and conjunctival diphtheria together account for only approximately 2%

of cases and are often secondary to nasopharyngeal infection. Laryngeal diphtheria may occur at any age, but is particularly likely to occur in children younger than 4 years. It is marked by an insidious onset with gradually increasing hoarseness and stridor. Fever is usually slight. The diagnosis is often missed or delayed when the pharynx is not simultaneously involved. Laryngeal diphtheria is associated with greater morbidity and mortality as a result of airway obstruction and the greater degree of toxin absorption from the extensive membrane.

Cutaneous diphtheria is an indolent skin infection that often occurs at sites of burns or other wounds and may act as a source of respiratory infection in others.[8–12] It is more common in warmer climates and in poor social conditions.[8–11] Although sufficient diphtheria toxin is absorbed from skin lesions to frequently produce immunity, systemic complications are uncommon with cutaneous diphtheria. In warmer climates, the high incidence of cutaneous diphtheria appears to have played a major role in producing immunity in the population in the absence of high rates of respiratory diphtheria.

Invasive disease caused by *C. diphtheriae* occurs rarely, most commonly as a result of nontoxigenic strains. Bacteremia, endocarditis, osteomyelitis, and arthritis have been reported.[13–17] Molecular epidemiology studies show that several of the reported clusters of invasive disease were associated with clonally related organisms.[18–20]

Complications

The impact of diphtheria is largely measured by complications attributable to the local disease and to the effect of absorbed toxin on other organs. The major threat from laryngeal diphtheria is respiratory obstruction. Life-endangering obstruction is generally managed by tracheostomy. Even with a tracheostomy tube in place, fatal acute respiratory obstruction occasionally occurs when a portion of a laryngeal membrane is dislodged and aspirated. The membrane may extend down into the tracheobronchial tree, resulting in pneumonia and expiratory respiratory obstruction. Because of edema of the upper respiratory tract, pharyngeal and nasal diphtheria are frequently associated with secondary otitis media and sinusitis.

The majority of deaths from diphtheria result from the effects of absorbed diphtheria toxin on various organs; severe complications from toxin absorption include acute systemic toxicity, myocarditis, and neurologic complications, primarily peripheral neuritis. The risk for complications is directly proportional to the extent of local disease, presumably because of increased production and absorption of the toxin in larger membranes. In addition, the frequency of these various complications appears to vary considerably between epidemics, for which no clear explanation is available. In the past, it was erroneously believed that the severity of the disease could be related to strains of the organism that were morphologically different on culture, being designated gravis, intermedius, and mitis.[21,22] A possible explanation for variation in reported frequency of complications is variability in the timeliness of therapy with diphtheria antitoxin, as well as differences in susceptibility among affected populations.

Severe acute systemic toxicity with myocardial involvement usually occurs between the third and seventh day of the illness; many investigators classify this complication as early myocarditis.[23] Others, however, believe that the effects on the myocardium are only part of diffuse systemic toxicity, including fever, purpura, peripheral circulatory collapse, restlessness, somnolence, and disturbances of carbohydrate metabolism.[24,25] This so-called early myocarditis is usually fatal. Late myocarditis usually appears in the second or third week of illness, when the local symptoms of diphtheria in the respiratory tract are resolving and the patient is otherwise improving.

In either early or late myocarditis, a wide variety of clinical and electrocardiographic findings may be noted.[24] Tachycardia, distant heart sounds, and a weak pulse may be observed. Electrocardiography most often shows conduction changes and alterations in T waves. Supraventricular and ventricular ectopic rhythms are common in severe diphtheria, even in the absence of evidence of heart failure.[26] The earlier electrocardiographic changes appear, the worse is the prognosis. Complete heart block frequently occurs and is usually fatal; ventricular pacing may not improve survival.[27–30] Echocardiograms show decreased contractility and ventricular dilation proportional to the severity of the clinical carditis; a left ventricular ejection fraction of less than 35% is associated with an increased risk for death.[31,32] Although electrocardiograms and echocardiograms return to normal in most survivors, residual changes are seen in some survivors of severe carditis for up to several years after illness.[26,31,33] Left bundle-branch block at hospital discharge has a poor prognosis.[34]

Neurologic complications of diphtheria are primarily toxic peripheral neuropathies, and they occur in approximately 15% to 20% of cases.[35,36] The manifestations are more motor than sensory and usually begin 2 to 8 weeks after onset of the illness. In severe cases, palatal paralysis with consequent nasal voice and nasal regurgitation of ingested fluids may occur during the acute membranous phase, particularly with extensive pharyngeal disease, and are believed to be attributable to local effects of the toxin. With milder disease, palatal paralysis is common as late as the third week. Symmetric peripheral neuritis of the lower extremities is a frequent neurologic complication, usually occurring 3 to 10 weeks after onset of the infection. Diaphragmatic paralysis occasionally occurs, usually a month or more after onset, and may require mechanical respiratory support. Ocular paralysis, involving either the extraocular muscles or those of accommodation, sometimes appears, usually 5 or 6 weeks after onset. Fortunately, functional recovery from these neuropathies is the rule, even in severe disease.[37] An association between diphtheria and delayed-onset hearing loss has also been reported.[38]

BACTERIOLOGY

Corynebacterium diphtheriae is a slender, Gram-positive bacillus, usually with one end being wider, thus giving the often-described club-shaped appearance. On culture, particularly under suboptimal conditions, characteristic bands or granules appear. On smear, the organisms often have a "pick-up sticks" relationship, assuming parallel (palisade-like), or V- or L-type patterns. The organisms are resistant to environmental changes, such as freezing and drying. There are four biotypes of *C. diphtheriae* (gravis, mitis, belfanti, and intermedius), which historically were identified by colonial morphology and biochemical differences; however, in practice, only the intermedius biotype can be distinguished reliably by colonial morphology.[39] No consistent differences are found in severity of disease caused by different biotypes. Biotype differentiation is not supported by phylogenetic analyses.[40]

PATHOGENESIS AS IT RELATES TO PREVENTION

The exotoxin produced by *C. diphtheriae* is by far the most important pathogenic factor associated with the organism. The extensive study of the biology of diphtheria toxin has pioneered many biomedical developments over the past century. The basic biology of diphtheria toxin, including its production and actions, has become reasonably well understood, although some gaps remain.[41,42]

The ability of strains *C. diphtheriae* to produce toxin results from a nonlytic infection by one of a series of related bacteriophages that contain a genetic sequence encoding the toxin. The phage integrates into specific sites present in *C. diphtheriae* and other *Corynebacterium* species. The presence of the phage is thought to confer a survival advantage to the bacterium by increasing the probability of transmission in a susceptible population; transmission may be facilitated by local tissue damage resulting from the toxin.[43,44] The sequence of diphtheria toxin has been demonstrated to be highly conserved in *C. diphtheriae* strains, suggesting that immunologically important differences among the toxins produced by different strains are unlikely to occur.[45] Once integrated, the *tox* gene is part of a multiple bacterial gene operon; other bacterial gene products in this operon are involved in the liberation and uptake of host iron.[46] The entire operon is under the control of a repressor gene, *dtxR*, which in the presence of iron binds to and inhibits the *tox* gene; toxin is produced only under low-iron conditions.[47]

Diphtheria toxin is a polypeptide with a molecular weight of approximately 58,000 Da. The toxin is secreted as a proenzyme, requiring enzymatic cleavage into two fragments (fragments A and B) to become active. Fragment B is responsible for attachment to and penetration of the host cell. Although nontoxic by itself, fragment B appears to be the antigen responsible for clinical immunity. The receptor domain of fragment B binds to a cell surface receptor, heparin-binding epidermal growth factor precursor,[48] with CD9 as a coreceptor.[49] After receptor-mediated endocytosis and penetration of the cell, fragments A and B are detached.[50] The released fragment A is the toxic moiety, and it acts by inhibiting protein synthesis, resulting in cell death.[44] Unless cell penetration occurs, fragment A is inactive. Differences in the tissue distribution of the receptor and coreceptors may account for the differential effects of diphtheria toxin on different organs.[51,52]

The ability of *tox* gene–containing bacteriophages to infect nontoxigenic strains of *C. diphtheriae* provides a potential explanation for the fact that, during outbreaks of diphtheria, both toxin-producing and non–toxin-producing strains of the organism may be isolated on culture surveys. Some evidence suggests that the introduction of a toxin-producing strain of *C. diphtheriae* into a community may occasionally initiate an outbreak by transfer of phage to nontoxigenic strains of the organism carried in the respiratory tracts of community inhabitants, rather than a new strain being the responsible agent.[53]

On mucous membranes, the toxin causes local cellular destruction, and the accumulated debris and fibrin result in the characteristic membrane. More important, absorbed toxin is responsible for remote manifestations affecting various organs, including, for example, the myocardium, nervous system, and kidneys. Because the lethality of diphtheria is almost entirely determined by the organism's toxin, clinical immunity depends primarily on the presence of antibodies to the toxin. In the presence of small amounts of formaldehyde, diphtheria toxin loses its attachment and enzymatic activities while retaining its immunogenicity, thus becoming a toxoid. This process is the basis of active immunization against diphtheria.

Certain cell wall antigens of *C. diphtheriae* are also thought to contribute to the pathogenesis of the disease in humans. The cell wall contains a heat-stable O antigen, which is found in all corynebacteria. The cell wall also contains K antigens, which are heat-labile proteins that differ among strains of *C. diphtheriae* and therefore permit categorization of the organism into a number of types.[54] The K antigens play two roles in relation to humans: first, they appear to be important in the establishment of infection, and second, they produce local type-specific immunity. Lack of immunity to K antigens appears to be responsible for the fact that local upper respiratory tract diphtheria can occur with non–toxin-producing organisms, and toxin-producing organisms may infect persons with ample serum antitoxin levels, but neither of these instances is associated with systemic manifestations, even though a faucial membrane is produced.[55] Another factor responsible for the local invasiveness of the organism is the so-called cord factor, which is a toxic glycolipid. This glycolipid has been shown to disrupt mitochondria, depress cell respiration, and interfere with oxidative phosphorylation.[56] The term *cord factor* is derived from a similar substance found in *Mycobacterium tuberculosis* that results in the growth of the organism in serpentine coils. Undoubtedly, there are other factors as well that help *C. diphtheriae* establish residence and provide nutritional substrates.

Some strains of two other closely related *Corynebacterium* species, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*, have been demonstrated to produce diphtheria toxin,[57] and nontoxigenic strains can be converted to toxigenic strains by infection with β-corynebacteriophage.[58] Sequencing studies demonstrate that both the base-pair sequence of the *tox* genes and the amino acid sequence of the diphtheria toxins from *C. diphtheriae* and *C. ulcerans* differ by approximately 5%.[59] Disease indistinguishable from that caused by toxigenic strains of *C. diphtheriae* is associated with *C. ulcerans* infection.[60–63]

DIAGNOSIS

Diphtheria is rare in the United States. However, physicians need to be aware of the signs and symptoms that suggest diphtheria. Some developing countries continue to have high levels of circulation of toxigenic strains of *C. diphtheriae*. Some circulation persists in a few countries of the former Soviet Union after a large epidemic in the 1990s,[64,65] and until recently limited persisting foci were reported in some highly developed countries.[66,67]

In countries where the disease is endemic, a patient with confluent pharyngeal exudate should be suspected of having diphtheria until proven otherwise. The onset is usually gradual over the course of 1 to 2 days and is associated with low-grade fever. The hallmark of respiratory diphtheria is the presence of pseudomembrane in the pharynx. Although certain clinical characteristics of membranous pharyngitis caused by diphtheria, such as the color, adherence, and odor of the membrane, can be recognized as being different from other forms of exudative pharyngitis by experienced clinicians, very few physicians in industrialized countries currently have the experience required to base a differential diagnosis on the clinical appearance of the lesion.

Because laryngeal diphtheria usually occurs concomitantly with pharyngeal involvement, membranous pharyngitis with stridor should be considered to be diphtheria until proved otherwise. However, about a quarter of all cases of laryngeal diphtheria do not display a pharyngeal lesion and therefore may often be misdiagnosed. The differential diagnosis includes epiglottitis caused by *Haemophilus influenzae* type b (Hib), although uncommon in immunized populations, spasmodic croup, the presence of a foreign body, or viral laryngotracheobronchitis. There should be little confusion regarding the first three because the onset and clinical characteristics of each are well known and are different from diphtheritic croup, which is ordinarily associated with gradual onset and steady progression through hoarseness to stridor during a period of 2 or 3 days. Viral laryngotracheobronchitis may be more difficult to differentiate, and, if diphtheria is suspected for epidemiologic or other reasons, laryngoscopy is indicated.

Nasal diphtheria may be difficult to distinguish from many other causes of nasal discharge and accordingly is most likely to be suspected if the patient has been exposed to diphtheria, such as during an outbreak. Suspicion should be heightened if a serosanguineous discharge is present and if the upper lip is ulcerated. However, the latter also occurs with streptococcal infections. Any cutaneous or mucous membrane lesions at other sites should be considered suspicious if a membrane is noted.

The risk of complications and mortality from diphtheria are inversely related to the promptness of diagnosis and treatment. Thus, it is critical that the diagnosis be considered, appropriate clinical specimens be obtained, and a decision made regarding administration of antitoxin as early as possible in the course of illness. When diphtheria is suspected, treatment for the disease should be initiated immediately after bacteriologic specimens are obtained, without waiting for results. Delay, even for a few hours, may increase the risk of complications and death.

Swabs for culture should be obtained under direct visualization, preferably from the edge or beneath the edge of the membrane. Directly stained smears are usually grossly misleading even in experienced hands and should not be used. Swabs should be inoculated promptly onto tellurite-containing media and onto blood agar.[68] Cultures should be incubated promptly and interpreted by an experienced microbiologist. Because not all *C. diphtheriae* recovered on culture are toxigenic, testing for toxin production must be performed. The modified Elek immunoprecipitation test for detection of toxin is the standard assay, but it generally requires 24 to 48 hours, and more rapid approaches to toxin detection have been described.[69] Many laboratories now use a polymerase chain reaction (PCR) assay for the detection of the *tox* gene.[70–73] Although this assay can provide a rapid indication that an isolate may be toxigenic, in some isolates the *tox* gene is detected but is nonfunctional.[74] For this reason, toxin production in PCR-positive isolates should be confirmed by an immunoprecipitation test. PCR can also be performed directly on clinical specimens.[75] Real-time PCR assays that detect toxin genes associated with both *C. diphtheriae* and *C. ulcerans* have been developed.[76,77]

Several approaches to typing of strains of *C. diphtheriae* as an adjunct to epidemiologic investigations have been developed. During the 1960s, Saragea and Maximescu[78] developed a system of phage typing and demonstrated considerable diversity of circulating strains in different countries. Subsequently, the usefulness of molecular typing methods was demonstrated in analysis of outbreak-related strains in Sweden[79] and the United States.[80] Since then, ribotyping,[81] pulsed-field gel electrophoresis,[81] and multilocus enzyme electrophoresis[82] have been used for molecular subtyping, as has PCR–single-strand conformation polymorphism (SSCP) analysis.[83] A rapid ribotyping method using PCR-SSCP has been described.[84] Ribotyping has been used most extensively and standard nomenclature has been developed.[85] Multilocus sequence typing also has been used and appears useful for discriminating reliably among strains of *C. diphtheriae*.[86] Molecular methods have been used to characterize the dominant strains of *C. diphtheriae* associated with the outbreak in the former Soviet Union,[81] and to document ongoing endemic circulation in certain communities in the United States and Canada.[66,87,88] However, these tests are only available in research laboratories.

TREATMENT AND PREVENTION WITH ANTIMICROBIALS

Although diphtheria antitoxin is the mainstay of diphtheria therapy, penicillin or, alternatively, erythromycin should be given to hasten clearance of the organism, prevent transmission, and cease further production of diphtheria toxin.[89] Before the availability of antibiotic therapy, convalescent carriage of toxigenic organisms was a major problem. Up to 50% and 25% of patients continued to harbor the organism 2 and 4 weeks after onset, respectively. As late as 2 months after onset, reported carriage rates varied between 1% and 8%.[3] Long-term convalescent carriers were often subjected to tonsillectomy, probably with some effect.[90]

Treatment with penicillin or erythromycin should be continued for 2 weeks. On completion of treatment, patients should be cultured twice at least 24 hours apart to confirm elimination *C. diphtheriae*. Patients who continue to harbor the organism after treatment with either penicillin or erythromycin should receive an additional 10-day course of oral erythromycin, and specimens for follow-up cultures should be obtained on completion of the course.[89] Although treatment with penicillin or erythromycin has no apparent effect on the clinical course of the disease, in most instances the organism can no longer be recovered on culture within a week of therapy and subsequent convalescent carriage is thus uncommon.

EPIDEMIOLOGY

Incidence and Prevalence Data

Active immunization of children with diphtheria toxoid has markedly altered the epidemiology of diphtheria, reducing diphtheria to extremely low levels in both developed countries and developing countries that have sustainable and well-implemented vaccination programs. However, diphtheria continues to produce substantial childhood morbidity and mortality in developing countries with incompletely implemented childhood immunization programs.[91,92]

From 1980 to 2014, only 57 cases of respiratory diphtheria were reported in the United States.[93–101] The last case in the United States was reported in 2014 in a 17-year-old white female resident of Ohio.[101] However, the isolated organism *C. diphtheriae* was nontoxigenic. The patient was fully immunized. No other family member or close contact was ill.

The decline of reported cases in the United States was abrupt partially because cutaneous diphtheria ceased to be nationally notifiable in 1980 (Fig. 19.1). However, improved childhood immunization in Mexico and other developing countries as part of the World Health Organization (WHO) Expanded Programme on Immunization (EPI) beginning in the late 1970s is likely to have contributed to improved diphtheria control in the United States by reducing importations of toxigenic strains. In the mid-1990s, with so little disease reported in the United States, it seemed likely that toxigenic strains of *C. diphtheriae* were no longer circulating in this country.[93] However, in 1996, surveillance revealed widespread circulation of the organism in one American Indian community in the Northern Plains.[102] Similarly, although recognized cases of diphtheria remain rare, endemic transmission of *C. diphtheriae* has been documented in some native communities in Canada.[103–105] Strains from the United States and Canada were assayed by ribotyping and multilocus enzyme electrophoresis and found to be closely related to strains that circulated in the same areas during the 1970s and 1980s, suggesting ongoing endemic circulation.[66,88] Circulation has also been reported among the aboriginal population in central Australia.[106] The common denominator in these communities is likely to be poverty and crowding.

In temperate climates, respiratory diphtheria occurs year-round but most often during colder months, probably because of the close contact of children indoors. In tropical climates,

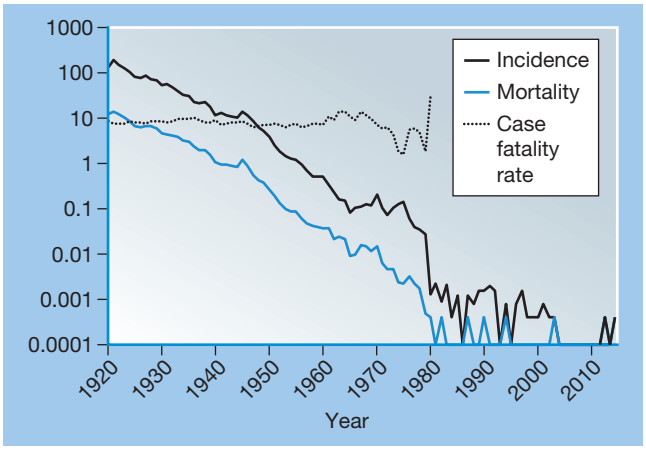


Figure 19.1. Diphtheria incidence in the United States, 1920–2014, and mortality rates, 1920–1980. Years in which no case was reported are plotted with an incidence rate of 0.0001 per 100,000 population. Because of the small number of diphtheria deaths since 1980, case fatality rates (CFRs) are unstable and are not shown on this graph. CFRs have varied widely since 1980 because of the small number of cases, but the overall CFR from 1980 to 2010 was 16%. *(Data from the Centers for Disease Control and Prevention, Atlanta, GA.)*

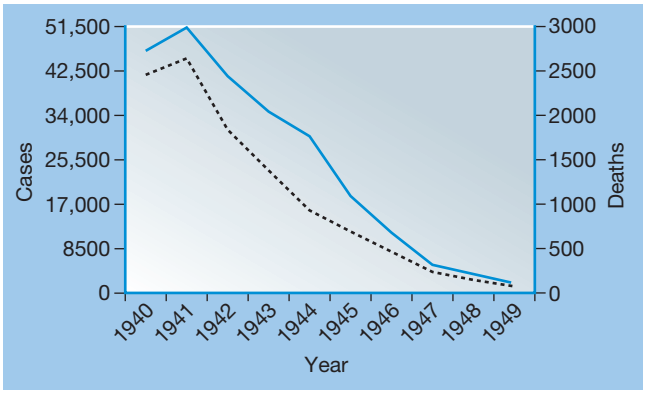


Figure 19.2. Diphtheria cases (solid line) and deaths (dashed line) in England and Wales, 1940–1949. *(From Mortimer PP. The diphtheria vaccine debacle of 1940 that ushered in comprehensive childhood immunization in the United Kingdom.* Epidemiol Infect. *2011;139: 487–493.)*

cutaneous diphtheria is more common and is unrelated to season.

Risk Groups

Unvaccinated or inadequately vaccinated preschool and school-age children are most often affected by respiratory diphtheria. Diphtheria is rare in infants younger than 6 months of age, presumably because of the presence of maternal antibody, and rare among adults, especially those living in urban areas, as a result of acquired immunity. Although no differences in diphtheria incidence were noted by sex in the prevaccine era, an increased risk of diphtheria among women was reported in several outbreaks among adults in the 1940s and subsequently; an increase in risk of diphtheria among women was observed in Russia and some of the other countries of the former Soviet Union during the outbreaks that occurred there in the 1990s.[64,107–111]

Reservoirs of Infection and Modes of Transmission

Humans are the only natural host for *C. diphtheriae*. Transmission is from person to person, most likely by intimate respiratory and physical contact. The organism is reasonably hardy and has been isolated from the environment of persons infected with *C. diphtheriae*.[112–115] Nonetheless, the occurrence of indirect transmission by airborne droplet nuclei, dust, or fomites has not been established. Evidence of outbreaks caused by contaminated milk and milk products has been reported.[23,115,116] Cutaneous lesions appear to be important in transmission under poor social conditions.[9,10,117]

The precise microbial events responsible for the transmission of diphtheria remain unclear. However, the molecular epidemiologic data showing clonal identity of bacteria in large numbers of infected people in the outbreaks in Seattle,[80] Sweden,[79] and Russia[87] strongly suggest that direct spread of toxigenic bacteria from one individual to another is a major factor in large epidemics.

Significance as a Public Health Problem

In the past, in the absence of immunization, most people acquired immunity to diphtheria as measured by the Schick test without experiencing clinical diphtheria. Transplacental antibody to diphtheria toxin is present at birth in most infants but declines to nonprotective levels during the second 6 months of life. Thereafter, the proportion of immune children (Schick-negative) in unimmunized populations gradually increases to 75% or more, presumably as a result of repeated subclinical infection with the organism.[118]

In the 21st century, it is difficult to comprehend what a major cause of morbidity and mortality diphtheria was in the past. Before 1900, the best data for the United States were from Massachusetts. From 1860 to 1897, death rates from diphtheria ranged between 46 and 196 per 100,000 population annually, with a median of 78, with the proportion of total deaths attributable to diphtheria ranging from 3% and 10% annually.[119] By 1900, a considerable fall in the death rate had occurred and continued to decline from 40 to 15 per 100,000 over the next 20 years, presumably because of the therapeutic use of diphtheria antitoxin and, perhaps, other measures such as intubation. However, even in 1900, more than half as many deaths from diphtheria were recorded in the United States as from cancer.[119] Several interesting and readable histories of diphtheria in the late 19th and early 20th centuries have been published.[120–122]

Excellent data on morbidity, mortality, and case-fatality rates for diphtheria were available for the Province of Ontario for 1880 to 1940 and for several Canadian cities for some of those years.[123] Mortality from diphtheria exceeded 50 per 100,000 population in most years before the advent of diphtheria antitoxin. Mortality subsequently declined to approximately 15 per 100,000 by World War I, although morbidity rates did not decline. With the widespread use of diphtheria toxoid vaccine beginning in the late 1920s in Canada, the disease nearly disappeared.[124]

At the beginning of the 20th century, diphtheria was a major cause of death of children in the United Kingdom. In 1934, the diphtheria mortality rate among children was 38.5 per 100,000. Widespread diphtheria immunization programs began there in 1940 and by 1944, it was reduced to 9.2 per 100,000, and by 1949 both morbidity and mortality were reduced more than 10-fold compared with the period from 1940 to 1941 (Fig. 19.2).[125]

Since the introduction of vaccination with diphtheria toxoid, a number of diphtheria outbreaks have occurred in

industrial countries. During World War II, a major outbreak spread throughout western Europe with well over 1 million cases reported.[126,127] The outbreaks spread from Europe to North America. A major outbreak affecting nearly 1% of the population of Halifax, Nova Scotia, during the winter of 1940–1941 was linked to disease imported by Norwegian sailors.[128] Another outbreak occurred in Alabama in 1943 among German prisoners of war.[129]

By the late 1950s, the incidence of diphtheria was markedly reduced in the United States, but disease continued to occur in some other areas. From 1959 to 1970, 5048 cases of diphtheria were reported in the United States, with the highest incidence rates reported in the southeast, south central, and northern plains states. Incidence rates were 20-fold higher for Native Americans and sevenfold higher for blacks than for whites.[130]

Diphtheria incidence continued to decline (see Fig. 19.1), and from 1971 to 1981, 853 noncutaneous and 435 cutaneous cases were reported in the United States.[131] Incidence rates exceeded 1 per million population in South Dakota, New Mexico, Alaska, Washington, Arizona, and Montana, and incidence rates were 100-fold greater for Native Americans than for whites and blacks.[131] During this period, there were seven outbreaks with 15 or more cases in the United States.[131] From 1972 to 1982, a large outbreak of predominantly cutaneous diphtheria occurred among residents of Skid Road in Seattle, Washington.[10,80] The Seattle outbreak and most of the other outbreaks from 1969 to 1980 were caused by toxigenic strains of the intermedius biotype, which had previously been uncommon; clonality of these strains was suggested by molecular epidemiologic studies of strains from outbreaks in Seattle[80] and the southwestern states.[132]

Although diphtheria has become a rare disease in most developed countries, a major epidemic of it began in the Russian Federation in 1990 and subsequently spread throughout the countries of the former Soviet Union (Fig. 19.3), with more than 157,000 cases and 5000 deaths reported between 1990 and 1998.[111] A compendium summarizing the knowledge gained from this outbreak has been published.[133] Although the cause of the epidemic was uncertain, it was multifactorial. Contributing factors included decline in childhood vaccination acceptance and coverage, exaggerated physicians fears about adverse events, the use of three doses of Td (diphtheria and tetanus toxoids–adult) instead of DTP (diphtheria, tetanus, pertussis) in the childhood immunization schedule, and the absence of an adult Td immunization program resulted in inadequate population immunity among children and adults. In addition, delayed recognition of cases and public health response, and changing social conditions facilitated spread once the outbreak began.[111] In the Russian Federation, the emergence of an epidemic clone of *C. diphtheriae* biotype gravis was demonstrated.[82] The epidemic clone was present in Russia as early as 1985, and strains of this clonal group have been retrospectively identified in different geographic areas of Russia from 1985 to 1987.[134] Many cases were also caused by biotype mitis strains as well, especially in the newly independent states of Central Asia, suggesting that microbial factors alone did not account for the epidemic.[135] The Russian epidemic peaked between 1994 and 1995 and subsequently was brought under control by increasing immunization coverage with diphtheria toxoid among both children and adults (see later).[64,111]



Figure 19.3. Reported cases of diphtheria, by year, in the Soviet Union (1965–1990) and in the newly independent states of the former Soviet Union (1991–2013).

Diphtheria toxoid has been included in the WHO's EPI since its inception in 1974. In developing countries, the implementation of the EPI has led to dramatic falls in the global number of reported cases of diphtheria since 1980; however, marked disparities remain in reported rates between countries. Some countries have achieved control of diphtheria comparable to that seen in highly developed countries. In others, disease rates have fallen dramatically but sporadic outbreaks still occur, for example, Thailand,[136] India,[137–139] Brazil,[140] Indonesia,[141] and Laos,[142] which have recent evidence of widespread circulation of toxigenic strains. Low vaccination coverage rates and population immunity were reported in the affected areas in these countries.

Before the WHO EPI began, it was estimated that close to a million cases of diphtheria occurred annually in the Third World, with 50,000 to 60,000 deaths.[143] From 1980 to 2013, reported cases of diphtheria globally decreased from 97,774 to 4680 in 2013 with control of the outbreak in the former Soviet Union. Approximately 87% of cases worldwide in 2013 were reported from the WHO Southeast Asian region (Fig. 19.4); 77% of cases in this region were reported from India.[144]

Under the EPI, the goal was to achieve 90% or higher immunization rates in 1-year-old children by the year 2000. By this time, it was estimated that the proportion who had received three doses of DTP vaccine (DTP3) had risen from negligible levels in the early 1970s to 81% worldwide, with Africa being the lowest at approximately 55%.[145] By 2013, global coverage improved to 84% while 79% of the countries of the African region reported DTP3 coverage of 80% or more by 1 year of age.[144] Some of the regional differences in these data no doubt reflect differences in either the capacity or quality of surveillance in member countries.

PASSIVE IMMUNIZATION

The history of the development of diphtheria antitoxin was reviewed in detail by Andrewes and colleagues.[3] In brief, in 1888 Roux and Yersin reported their observation that bacteria-free filtrates of broth cultures of *C. diphtheriae*, when injected into animals, produced all the manifestations of diphtheria except for the membranous local lesions.[146] In rapid succession, other advances followed. von Behring showed that inactivated cultures of the organism injected into animals subsequently rendered them protected against living cultures.[147] Ultimately, von Behring demonstrated the transfer of protection from an immunized animal to another unimmunized one by serum, which he named antitoxin. Diphtheria antitoxin was first given to a child in 1891, and antitoxin was

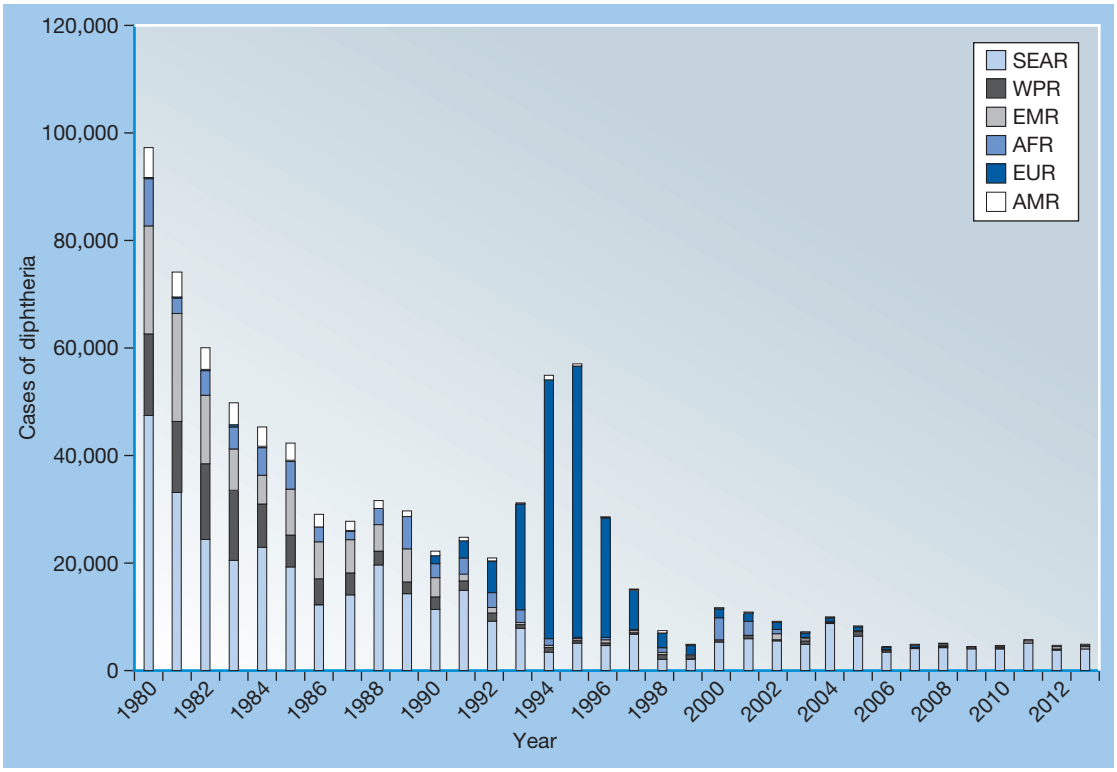


Figure 19.4. Reported cases of diphtheria, by year and region, 1980–2013. AFR, African region; AMR, American region; EMR, European region; EUR, European region; SEAR, Southeast Asia region; WPR, Western Pacific region. *(From World Health Organization, Department of Vaccines and Biologicals, Vaccine-Preventable Diseases: Monitoring System 2014 Global Summary. http://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencediphtheria.html.)*

commercially produced in Germany in 1892. The use of horses for the production of antitoxin began in 1894 and was widespread within a few years. The lack of regulated standards for the production of equine diphtheria antitoxin resulted in the release of contaminated or counterfeit antisera and contributed to the development of the predecessors of the present Center for Biologics Evaluation and Research of the U.S. Food and Drug Administration (FDA).[148–150]

Equine diphtheria antitoxin continues to be used to date. The product is prepared by hyperimmunizing horses with diphtheria toxoid and toxin.[149] To diminish reactivity from horse serum, current preparations are semipurified by techniques that concentrate immunoglobulin G and remove as much extraneous protein as possible. There must be at least 500 units of antitoxin per milliliter, and sterility is attained by microfiltration. A cresol derivative is added as a preservative.

Diphtheria antitoxin is used for the treatment of diphtheria and occasionally for prevention in exposed persons. Its therapeutic efficacy is well established, although it is in no way a substitute for prior active immunization with diphtheria toxoid. No antiserum or hyperimmunoglobulin of human origin is currently available.

Globally, the production and supply of equine antitoxin for human therapeutic use has become increasingly problematic. Almost all industrialized countries that traditionally manufactured and supplied antitoxin have ceased production. Unavailability of an antitoxin supply increases the likelihood of mortality, as highlighted by shortages during the epidemic in the newly independent states of the former Soviet Union. With low demand for the product, many manufacturers have left the market, so only a few manufacturers supply diphtheria antitoxin to other countries, many of which have reported difficulty in identifying a supplier and maintaining a supply for domestic use.[151,152] As of January 6, 1997, licensed diphtheria antitoxin with a valid expiration date was no longer available in the United States, and there was no manufacturer proposed to produce it. However, for treatment of the disease in the United States, the Centers for Disease Control and Prevention has a supply of antitoxin that can be distributed for treatment under an investigational new drug protocol.[153] This antitoxin is comparable to the prior U.S. product and may be requested by calling 770-488-7100.

Novel approaches to passive immunity could include the commercial development of human monoclonal antibodies to diphtheria toxin or the development of recombinant modified diphtheria toxin receptor molecules to bind diphtheria toxin.[154,155] A neutralizing monoclonal antibody of human origin has been developed which completely protected guinea pigs from lethal challenge in an in vivo assay. The monoclonal antibody binds to the receptor-binding domain of diphtheria toxin and blocks the toxin from binding the receptor.[156]

Postexposure Use of Antitoxin and Toxoid

The value of antitoxin in postexposure prophylaxis is dubious. Theoretically, it should be useful in preventing the establishment of infection in exposed, susceptible people because the toxin plays a role in local invasiveness. However, there is no acceptable clinical evidence of prophylactic efficacy; all that exist are anecdotes and small, uncontrolled series of experiments.[3] Even if it were effective, antitoxin would be of little use in controlling community outbreaks, because asymptomatic carriers, rather than persons with overt disease, are usually the major source of transmission.[157] For these reasons,

antitoxin is not recommended for exposed, susceptible persons, particularly in view of the high rates of subsequent serum sickness and occasional anaphylaxis. The preferred treatment for exposed, unimmunized, asymptomatic persons is to obtain a throat culture, begin immunization with a preparation containing diphtheria toxoid that is appropriate for age, and institute prophylaxis with erythromycin or penicillin for 7 days, during which time the patient must be kept under surveillance for development of symptoms.[89]

USE OF ANTITOXIN FOR TREATMENT OF DIPHTHERIA

Many studies have demonstrated that therapy with antitoxin is efficacious in reducing mortality from diphtheria primarily by preventing cardiovascular toxicity.[4,158] However, only a single controlled therapeutic trial is discussed in the literature.[159,160] This nonblinded trial consisted of treating all patients admitted on alternate days with antitoxin and comparing their outcomes with those of patients admitted on nontreatment days. Eight (3.3%) of 242 patients treated with antitoxin died, compared with 30 (12.2%) of 245 control subjects.

In addition, many observations of the direct relationship between mortality and the day of disease when antitoxin was administered provide ample evidence of its efficacy. For example, among 3558 patients observed by Ker,[158] 320 cases of paralysis occurred. There was a strong direct relationship between the frequency of postdiphtheritic paralysis and the number of days between onset of illness and administration of antitoxin. Only 4.8% of 1168 patients developed paralysis when antitoxin was administered no later than the second day of illness, in contrast to 12.1% of 1375 patients who received antitoxin on the fourth day of the disease or later.

Antitoxin is given intramuscularly or intravenously; many authorities prefer the intravenous route for at least part of the dose because a therapeutic blood level can be reached more rapidly.[161] The entire therapeutic dose should be administered at one time, and the amount of antitoxin recommended varies between 20,000 and 100,000 units. Larger amounts are recommended for persons with extensive local lesions, because the amount of toxin produced depends on the size of the membrane. Furthermore, the longer the interval since onset, the higher should be the dose of antitoxin. Toxin that has already entered host cells is unaffected by antitoxin.

ACTIVE IMMUNIZATION

History of Vaccine Development

After the discovery of diphtheria toxin and the development of antitoxin in the 19th century, the first successful approach to active immunization was the use of balanced mixtures of toxin and antitoxin, which successfully immunized both animals and humans after injection.[162,163] The combination preparation, toxin-antitoxin, was rapidly accepted as an active immunizing agent. It was widely used in the United States beginning in 1914, and was found to protect approximately 85% of recipients.[164] There is little question that the toxin-antitoxin preparation developed by von Behring created active immunity against diphtheria, on the basis of the results of Schick testing and clinical observation, despite the absence of well-controlled studies.[3]

In the early 1920s, Ramon treated diphtheria toxin with small amounts of formalin and found that the product retained most of its immunizing capacity while losing its toxic properties.[6] Ramon dubbed this preparation anatoxine; this name has since been replaced in English usage with the term toxoid. For primary immunization, the toxin-antitoxin preparation was gradually replaced by toxoid in the United States and Canada during the next 15 years, and elsewhere thereafter. In 1926, Glenny and coworkers[165] found that alum-precipitated toxoid was more immunogenic, and by the mid-1940s, diphtheria toxoid was combined with tetanus toxoid and pertussis vaccine as diphtheria and tetanus toxoids and whole-cell pertussis vaccine. Adsorption of all three onto an aluminum salt followed shortly thereafter. It is clear that the immunogenicity of diphtheria toxoid, as well as that of tetanus toxoid, is enhanced by the adjuvant effects of both pertussis vaccine and the aluminum salt.[166–168] In recent years, diphtheria and tetanus toxoids with acellular pertussis components (DTaP) have been licensed, and various other combinations of DTaP with Hib vaccine, inactivated poliovirus vaccine, and hepatitis B vaccine have been developed.[169] Combination vaccines have been shown to be as comparably immunogenic as the monovalent preparations.

Vaccine Constituents

A preservative to prevent antimicrobial growth is required when diphtheria toxoid is dispensed in multidose vials. Thimerosal, a preservative containing ethyl mercury, has been used in vaccines and biologics for this purpose since the 1930s.[170] Some diphtheria toxoid–containing vaccines containing only trace (<1 μg of mercury) or no thimerosal are available in single-dose preparations (single-dose vials or prefilled syringes). Diphtheria toxoid is adsorbed onto an adjuvant (most commonly aluminum hydroxide or aluminum phosphate) to improve the immunogenicity of the vaccine. Only adjuvanted diphtheria toxoids are available in the United States.

Manufacture of Vaccine

Diphtheria toxoid is produced worldwide in a standard fashion; in the United States, production and testing procedures are specified in the Code of Federal Regulations. Specifically, a strain of *C. diphtheriae* that is known to produce large amounts of toxin (such as the Park Williams 8 strain) is grown in a liquid medium conducive to toxin production. After appropriate incubation, sterilization is achieved by centrifugation and filtration. After ascertainment of potency, the filtrate is incubated with formalin for conversion to toxoid. The product is then further purified and concentrated to achieve the necessary dosage. It is adsorbed onto an aluminum salt, usually aluminum hydroxide or aluminum phosphate. After each step, appropriate tests for potency and sterility are conducted. Toxoid concentration is ascertained by determining the content of flocculating units (Lf) in established fashion; 1 Lf is the amount of toxoid that flocculates 1 unit of a standard reference diphtheria antitoxin. The purity of diphtheria toxoids in vaccines currently licensed in the United States is usually at least 1500 Lf/mg nondialyzable nitrogen; WHO recommends a similar standard. Toxoid potency is determined by assays, with different tests currently required by WHO, the European Pharmacopoeia, and the FDA.[148,171,172] Although there are ongoing efforts to harmonize potency testing requirements globally, no single approach has yet been universally adopted.

Producers

Diphtheria toxoid is produced both by large multinational vaccine companies and by developing country manufacturers, many of which produce vaccine only for domestic use. A 1995

assessment of global DTP manufacturing capacity reported DTP production by 63 manufacturers in 46 countries. At that time, it was estimated that approximately two-thirds of children received DTP that was manufactured in their own country, and that more than one-half of the global DTP supply came from developing country manufacturers.[173] In 2006, it was estimated that more than 500 million doses of DTP were produced globally, with many manufacturers producing vaccine for domestic use only. More than 100 million doses of DTaP are produced as well, with major production by Sanofi Pasteur and GlaxoSmithKline.

Preparations Available, Including Combinations

Currently in the United States, diphtheria toxoid is available in combination with tetanus toxoid (DT [diphtheria and tetanus toxoids–pediatric], Td) and in combination with tetanus toxoid and acellular pertussis vaccine (DTaP), as well as in other combination vaccines including DTaP; additional combination vaccines are available in Europe, Canada, and Australia that are not licensed for use in the United States. Globally, diphtheria toxoid continues to be used in combination with tetanus toxoid and whole-cell pertussis vaccine (DTP) as well as in other combinations including DTP. The product is available only in adsorbed form in the United States.

Since September 2011, DTaP vaccines from two manufacturers are marketed for use in infants in the United States: Infanrix (manufactured by GlaxoSmithKline Biologicals and distributed by GlaxoSmithKline) and Daptacel (manufactured by Aventis Pasteur Ltd., and distributed by Sanofi Pasteur). Two pentavalent combination vaccines—a combined DTaP (Infanrix), hepatitis B vaccine, and inactivated polio vaccine (GlaxoSmithKline) and a combined DTaP, Hib conjugate vaccine, and inactivated polio vaccine (Pentacel, Sanofi Pasteur, Ltd.)—are available for use at 2, 4, and 6 months of age; Pentacel is also licensed for use for the fourth dose of the series. A DTaP-inactivated polio vaccine combination, KINRIX, is licensed and marketed by GlaxoSmithKline Biologicals for use as the fifth dose of the DTaP series at 4 to 6 years of age. Another DTaP-inactivated polio vaccine combination, Quadracel, was licensed and marketed by Sanofi Pasteur Limited for active immunization against DTP and poliomyelitis in the United States. A single dose of Quadracel is licensed for use in children 4 through 6 years of age as a fifth dose in the DTaP vaccination series, and as a fourth or fifth dose in the inactivated poliovirus vaccination (IPV) series, in children who have received 4 doses of Pentacel and/or Daptacel vaccine.

The amounts of diphtheria toxoid in the DTaP vaccines currently licensed in the United States range from 6.7 to 25 Lf/0.5-mL dose. They provide levels of serum antitoxin that are considerably lower than those seen after receipt of whole-cell DTP, probably reflecting the adjuvant effect of the whole-cell pertussis component.[174,175] However, the lower antitoxin levels induced by vaccination with DTaP are probably of no clinical consequence, being manyfold higher than protective levels.[169] For routine immunization of children, five doses are recommended (at 2, 4, 6, and 15 to 18 months of age and at school entry before the seventh birthday).[176] The fourth dose should be administered at least 6 months after the third dose.[177]

Combined adult formulation tetanus and diphtheria toxoids (Td) are licensed in the United States for use in persons 7 years of age and older. These vaccines are licensed for primary vaccination as a three-dose series in previously unvaccinated persons 7 years of age and older, and as a decennial booster for use in adolescents and adults. These products contain reduced amounts of diphtheria toxoid (<2 Lf). Since 2005, formulations of tetanus and diphtheria toxoids with acellular pertussis vaccine (Tdap) have been licensed in the United States for use in adolescents and adults. Boostrix (GlaxoSmithKline) is currently licensed for use in persons 10 years of age and older, and Adacel (Sanofi Pasteur) is licensed for use in persons 11 to 64 years of age. These vaccines are licensed for use as a single booster vaccination in persons who have previously received a primary series of DTP or DTaP. A single dose of Tdap is recommended by the ACIP in the United States for persons aged 11 years and older, with the preferred timing at 11 to 12 years of age.[178] A dose of Tdap is recommended for women during each pregnancy to provide maternal antibodies to their newborn for added protection against pertussis until they are eligible to receive their first dose of DTaP.[179]

Dosage and Route

All products currently in use in the United States are administered as a 0.5-mL dose. Preparations containing diphtheria toxoid should always be injected intramuscularly, not subcutaneously.

Vaccine Stability

Expiration dates for diphtheria toxoid are established through the licensing process for each vaccine by national authorities. Preparations containing diphtheria toxoid should be stored at refrigerator temperatures (2°C to 8°C) but not frozen. If vaccine has been frozen, it should be discarded.

Immunogenicity of Vaccine

Several laboratory assays for diphtheria antitoxin are available. Vero cell neutralization assays are highly accurate but technically cumbersome and are available in only a few research laboratories.[180] Standard enzyme immunoassays are widely available and technically less demanding; these assays generally correlate with results from neutralization assays at concentrations greater than 0.01 IU/mL.[181] Double-antigen enzyme-linked immunosorbent assay (ELISA), double-antigen delayed time-resolved fluorescence immunoassays, and toxin-binding inhibition tests, which are more accurate than standard enzyme immunoassays but technically less demanding than Vero cell neutralization, have been developed.[182,183]

Maternal antitoxin levels do affect the immune response of infants. Diphtheria antitoxin levels greater than 0.1 IU/mL inhibit the response to active immunization, but no effect is seen at levels of less than 0.02 IU/mL.[184,185] This is more likely to be of significance in areas where *C. diphtheriae* continues to circulate, resulting in high levels of antibody in mothers and their infants. However, it appears that high maternal antibody titers suppress, but do not prevent, adequate responses of infants to two doses of vaccine, and after the third dose the suppressive effect is gone.[186,187] The half-life of diphtheria antitoxin has been estimated to be 30 days.[188]

After three doses of diphtheria toxoid, nearly all infants develop diphtheria titers greater than 0.01 IU/mL.[189] Geometric mean titers vary among vaccine preparations, with some DTaP products producing significantly lower geometric mean titers than those observed after vaccination with DTP[174]; however, these differences are unlikely to be clinically significant. When the toxoid is used for primary immunization of adults, data suggest that nearly all adults develop diphtheria antitoxin titers greater than 0.01 IU/mL after administration of three doses of diphtheria toxoid, and that most develop titers greater than 0.1 IU/mL.[190]

Vaccination with protein conjugate vaccines containing diphtheria toxoid may result in a booster response to the

carrier protein in persons who have previously received diphtheria toxoid.[191] One of the tetravalent meningococcal polysaccharide diphtheria toxoid conjugate vaccines currently licensed in the United States (Menactra, Sanofi Pasteur) contains approximately six times as much diphtheria toxoid as is contained in the adult formulations of Td vaccine. Simultaneous administration of Td and Menactra resulted in much higher geometric mean titers of diphtheria antitoxin than did Td (120.0 IU/mL compared with 8.4 IU/mL); consistent with the increase in diphtheria toxoid content, Menactra alone was also substantially more immunogenic than Td alone (46.5 IU/mL compared with 8.4 IU/mL).[192] There is also cross-reactivity between diphtheria toxoid and CRM_{197} in protein conjugate vaccines; the other tetravalent meningococcal polysaccharide conjugate vaccine licensed in the United States, Menveo (Novartis Vaccines and Diagnostics), uses CRM_{197} as the protein carrier. Children receiving meningococcal serogroup C-CRM_{197} vaccines develop higher diphtheria antitoxin levels than are seen in children who did not receive the vaccine.[193,194] Lack of baseline immunity to diphtheria may result in poor antibody responses to vaccines conjugated to CRM_{197}.[195] Although immunologic interference is a potential concern with simultaneous administration of diphtheria toxoid with diphtheria toxoid or CRM_{197}-containing protein conjugate vaccines,[196–198] experience to date does not suggest that the immunogenicity of diphtheria toxoid is adversely affected by simultaneous administration with these vaccines.

Correlates of Protection

Several lines of evidence suggest that persons with diphtheria antitoxin levels of less than 0.01 IU/mL should be considered susceptible. Ipsen reported results of studies in which rabbits were administered antitoxin and then challenged with intravenously administered diphtheria toxin; rabbits with a serum level of 0.01 IU/mL were almost completely protected from death with the standard lethal dose.[199] However, higher doses of toxin required higher serum antitoxin levels for equivalent protection. On the basis of studies of diphtheria antitoxin levels early in the course of disease, persons with diphtheria antitoxin levels of less than 0.01 IU/mL appear to be highly susceptible to disease, and higher levels are generally associated with progressively less-severe symptoms.[199–202] Probably no level of circulating antitoxin confers absolute protection; Ipsen reported two cases of fatal diphtheria in patients with antitoxin levels above 30 IU/mL the day after onset of symptoms.[199] Historically, clinical diphtheria was rare among persons with a negative Schick test; the minimal serum antitoxin level associated with a negative Schick test was approximately 0.005 IU/mL.[203] Overall, the data allow some general conclusions regarding protective levels in most circumstances. An antitoxin level of 0.01 IU/mL is the lowest level providing some degree of protection, and 0.1 IU/mL is considered a protective level of circulating antitoxin. Levels of 1.0 IU/mL and greater are associated with long-term protection.[204]

Efficacy and Effectiveness of Vaccine

No controlled clinical trial of the efficacy of the toxoid in preventing diphtheria has ever been conducted. There is, however, strong evidence from observational studies to support the effectiveness of vaccination. Some evidence of the protective efficacy of diphtheria toxoid is provided by observations during the Halifax epidemic.[128] During the course of this outbreak, an intense effort was made to administer diphtheria toxoid to previously unimmunized individuals, and the subsequent incidence of diphtheria in these children was compared with the incidence in the unimmunized population during the next few months. Among those immunized, the monthly incidence of diphtheria fell to 24.5 per 100,000 population, about one-seventh of the rate in the unimmunized children during that same period (168.9 per 100,000). In Britain in 1943, the rate of clinical diphtheria among the unimmunized was 3.5 times that among the immunized, and mortality was 25-fold greater.[127] In an outbreak in Elgin, Texas, in 1970, only two of 205 fully immunized, exposed elementary schoolchildren acquired the disease.[205] In contrast, among 97 children who had received inadequate or no immunization, a 13% attack rate occurred.

In a household study during a diphtheria outbreak in San Antonio, Texas, in 1970, vaccine efficacy was estimated at only 54%.[206] However, because index cases were included and denominators of exposed individuals were unknown, the data are difficult to interpret. Furthermore, any differences in attack rates between immunized and nonimmunized persons might have been blunted by the institution of antibiotic therapy in all members of the household on recognition of a case. Thus, the apparent efficacy of 54% is probably low. In an outbreak in Yemen, the protective efficacy of diphtheria toxoid was determined to be 87% by the case-control method.[116]

The effectiveness of Russian-manufactured diphtheria toxoid was evaluated in several case-control studies during the epidemic in the former Soviet Union. Three or more doses of diphtheria toxoid were demonstrated to be highly effective in prevention of diphtheria among children younger than 15 years in a preliminary study in Ukraine in 1992 and a subsequent study performed in Moscow in 1993. In Ukraine, the effectiveness of three or more doses was 98.2% (95% confidence interval [CI], 90.3% to 99.9%).[207] In Moscow, the effectiveness of three or more doses was 96.9% (95% CI, 94.3% to 98.4%), increasing to 99.0% for five or more doses (95% CI, 97.7% to 99.6%).[208] In addition, administration of a booster dose of diphtheria toxoid within 2 years was shown to decrease risk of diphtheria among children 6 to 8 years of age compared with those who had received the last dose 3 to 4, or 5 to 7 years previously.[209] Among adults in the Russian Federation, the effectiveness of three or more doses compared with no doses was 70% (95% CI, 10% to 90%).[210] Similarly, recent vaccination also was found to be highly effective among adults in Ukraine.[211]

Thus, it appears that the effectiveness of diphtheria toxoid is high but not 100%. However, most reports indicate that the disease in previously immunized individuals is milder and less likely to be fatal.[130,131,205,212–214] In Britain in 1943, case-fatality rates in unimmunized children were more than sevenfold greater than rates in those who had been immunized (6.4% versus 0.9%).[127] The failure to protect 100% of individuals on exposure indicates the importance of herd immunity in the disappearance of diphtheria from developed countries.[215]

Duration of Immunity and Protection

Both the diphtheria toxoid formulation and the schedule of administration affect the level of diphtheria antitoxin achieved and the duration of protection. Globally, various schedules for primary immunization of infants are used, but after three doses of diphtheria toxoid, most children achieve antitoxin titers greater than the minimally protective level.[216] However, in the absence of ongoing exposure, immunity wanes over time, requiring booster doses of diphtheria toxoid to maintain protective antitoxin levels. Longitudinal studies indicate that after a period of rapid decline of antitoxin levels, there is a prolonged, slower decline, presumably reflecting the initially activated immune system and half-life of immunoglobulin, followed by a sustained period of less active production of

immunoglobulin.[217,218] Both the four-dose schedule used in the United States, with 15 Lf doses at 2, 4, 6, and 15 months of age, and the three-dose schedule used in Sweden, Denmark, and Norway, administering 25 Lf doses at 3, 5, and 12 months of age, resulted in geometric mean levels well in excess of the minimally protective level at 48 months of age.[219] Similar geometric mean levels were found 23 months after dose three was administered as DT (pediatric diphtheria and tetanus toxoids), DTP, or DTaP, with varying diphtheria toxoid contents in a two-, four-, and six-dose schedule.[218] In 1990, the United Kingdom moved to an accelerated schedule, administering DTP at 2, 3, and 4 months instead of at 3, 5, and 9 months of age. Although postvaccination geometric mean concentrations of diphtheria antitoxin were lower among children vaccinated on the accelerated schedule, geometric mean antitoxin levels did not differ at age 4 years among children who completed the series at 8 to 13 months, 6 to 7 months, or before 6 months of age, suggesting that adequate protection would be maintained until administration of the preschool booster dose.[220–222]

Although various schedules for primary immunization appear to provide adequate protection from diphtheria in the early years of life, in the absence of a booster dose at 4 to 6 years, protection may not be maintained throughout the school-age years. In Sweden, the first booster dose after the primary series is not administered until age 10 years, resulting in lower levels of antitoxin among children 5 to 9 years of age than is observed in countries administering a preschool-age booster.[223] In one study, 12% of 10-year-old children had diphtheria antitoxin levels below 0.01 IU/mL before receipt of a booster dose.[224] In the Soviet Union, the immunization schedule was changed in 1986, delaying the age 6 (years) booster dose to age 9 years. During the diphtheria outbreak in Russia in the 1990s, receipt of the booster dose at 6 to 8 years of age was found to decrease the risk of diphtheria in this age group.[209] In countries with longstanding childhood immunization programs, adults who have neither been exposed to diphtheria nor received booster doses of diphtheria toxoid may become susceptible to diphtheria as a result of waning immunity.[216] During the outbreak in the former Soviet Union, waning of immunity was thought to contribute to the high incidence rate observed among adults. A large proportion of the population of adults, although seronegative, were previously primed by prior immunization or infection with toxigenic *C. diphtheriae*, as evidenced by development of protective titers after a single booster dose of toxoid.[225–228] Although the immunization histories of the adult patients were difficult to ascertain, the overall population data suggested that many probably had been immune but lost immunity over time. With implementation of booster vaccination for all age groups, the outbreak came under control.

The experience of this massive epidemic strongly suggests that sustaining high immunization coverage with a primary series of diphtheria toxoid among infants and administering booster doses at school entry and subsequently throughout life are important for maintenance of population immunity.[114] WHO now recommends that people living in low-endemic or nonendemic areas should receive booster doses of diphtheria and tetanus toxoids approximately 10 years after completing the primary series, and subsequently every 10 years throughout life.[229]

Postexposure Prophylaxis and Therapeutic Vaccination

The immunization status of close contacts of diphtheria cases should be assessed, and any contacts found to not be fully up to date with diphtheria toxoid immunization should be vaccinated.[90] Diphtheria patients should be immunized in the convalescent phase of illness because clinical infection does not always induce adequate levels of diphtheria antitoxin. However, vaccination after onset of disease has no role in treatment of diphtheria; diphtheria patients should be promptly treated with diphtheria antitoxin (see "Treatment and prevention with Antimicrobials" earlier).

Safety

Common Adverse Reactions. Extensive data on adverse reactions after administration of currently available preparations of diphtheria toxoid, adsorbed, are not available because the toxoid is usually administered in combination with tetanus toxoid and, in children, with pertussis vaccine as well. When it is given in combination with pertussis vaccine, local reactions often are ascribed to the pertussis-containing component. In several large clinical trials, the reactogenicity of DT was compared with that of DTaP for primary vaccination of infants. In general, the frequencies of reported common systemic symptoms (temperature of 38°C or higher, crying for 1 hour or longer, irritability, drowsiness, loss of appetite, vomiting) and local reactions (redness, swelling, tenderness) after vaccination with DT or DTaP were comparable.[230–232] In clinical trials in Sweden and Italy, DT vaccines containing 15 or 25 Lf of diphtheria toxoid and 3.75 or 10 Lf of tetanus toxoid, respectively, were given to more than 7000 infants. The frequency of temperature of 38°C or higher after any vaccine dose was 35% in the Swedish trial and 9% in the Italian study. Other common systemic symptoms occurred with similar frequencies in the two studies: crying for 1 hour or longer in 5% and 6% of infants, irritability in 67% and 55% of infants, drowsiness in 54% and 43% of infants, loss of appetite in 22% and 26% of infants, and vomiting in 15% and 9% of infants. Redness and tenderness after any vaccine dose were reported in 42% and 22% of infants in the Swedish trial, respectively, and in 19% and 9% of infants in the Italian study, respectively. The frequencies of marked redness or swelling were substantially lower in both studies, with redness and swelling of 2 cm or more reported in only 4% and 6% of infants in the Swedish trial, respectively.[230,231,233] The frequency of adverse reactions after DT increased with increasing dose number.[232,234]

Available data suggest that both diphtheria and tetanus toxoids contribute to the reactogenicity of Td and DT. Among Swedish medical personnel with a history of receipt of previous primary immunization in childhood, adverse events (local tenderness and swelling >5 cm, or general discomfort) were reported by 11% of those who received 2.5 Lf of diphtheria toxoid, compared with 20% of those who received 2.5 Lf of diphtheria toxoid combined with 0.75 Lf of tetanus toxoid, documenting the additive effects of the two toxoids.[235] Data from several controlled studies suggest that fever and local reactions are more common after administration of Td than after tetanus toxoid alone.[236,237]

In some populations, large numbers of previously primed persons develop local reactions and fever in response to diphtheria toxoid, even at low dosages. In a small study of Israeli military recruits who had been previously vaccinated in childhood, mild to moderate pain at the injection site was reported by 38% and severe pain by 20% after receipt of a booster dose of 2 Lf diphtheria toxoid without tetanus toxoid; limitation of abduction was reported by 8%. Systemic symptoms of mild to moderate or severe weakness were reported by 24% and 9%, respectively, and a temperature of 38°C or higher was reported by a single subject (<1%).[238] Similarly, a booster dose of 1.5 Lf was administered to 215 university students with prevaccination diphtheria antitoxin levels of less than 0.1 IU/mL. Eight percent reported tenderness at the injection site, and 13% reported pain with abduction, which

was marked in 2% of subjects; none had erythema or swelling noted on examination.[239]

With current formulations of diphtheria toxoid, the frequency of reported adverse events varies by prior vaccination history, prevaccination diphtheria antitoxin level, and dosage of diphtheria toxoid administered. Among 123 persons 30 to 70 years of age with diphtheria antitoxin levels of 0.05 IU/mL or less, adverse events after vaccination were more severe among those who received 12 Lf of diphtheria toxoid without tetanus toxoid as a booster than among those who received dosages of 5 Lf or 2 Lf of diphtheria toxoid without tetanus toxoid, supporting the recommendation to administer reduced dosages of diphtheria toxoid to adults.[240] A second study in military recruits 18 to 25 years of age, most of whom had documentation of receipt of a complete primary vaccination series in childhood, also showed no differences in adverse events between dosages of 5 Lf and 2 Lf of diphtheria toxoid when combined with tetanus toxoid.[241] A newer study in 180 adults evaluated the reactogenicity of DTaP (9 Lf) with Td (2 IU) and monovalent diphtheria toxoid (2 IU). Although the proportion of vaccinees with local reactions (e.g., erythema, induration, warmth, tenderness) was generally lower among recipients of the monovalent diphtheria toxoid than was observed in the other two groups, there was no consistent pattern of increased reactogenicity among recipients of DTaP compared with Td.[242]

Lower diphtheria toxoid content generally results in decreased reactogenicity of booster doses among children. Among children who had previously received three doses of DTaP containing 25 Lf of diphtheria toxoid at 3, 4, and 5 months of age, booster doses containing varying amounts of antigen and adjuvant were administered as a fourth dose at 15 to 27 months of age. Among 117 children who received vaccine containing a reduced amount of diphtheria toxoid (7.5 Lf) and tetanus toxoid (7.5 Lf compared with 10 Lf), there were significant reductions in the proportion of children with redness (27% vs 50%), swelling (18% vs 39%), or pain (17% vs 30%) compared with the frequency in 859 children who received the 25 Lf-containing preparation. A temperature higher than 38°C was also significantly more common in the group receiving vaccine with higher diphtheria toxoid content (27% compared with 18%). A third group of 117 children received vaccine containing 1.5 Lf of diphtheria toxoid, 10 Lf of tetanus toxoid, and reduced aluminum adjuvant (0.3 mg, compared with 0.5 mg); the proportion of children experiencing local reactions was lower than those seen with the 25 Lf vaccine and similar to that seen with 7.5 Lf (28% redness, 25% swelling, 14% pain), but there was no decrease in the proportion with temperature greater than 38°C (28%).[243] Diphtheria toxoid content of one-fifth the dosage at 4 to 6 years of age was evaluated in a study of 593 children in Canada, in which the licensed DTaP-IPV combination (Quadracel, Sanofi Pasteur) (15 Lf) was compared with Tdap (Adacel, Sanofi Pasteur) (2 Lf). The proportions of children developing erythema, swelling, pain, and fever were significantly reduced in the group receiving Tdap with 2 Lf of diphtheria toxoid.[244] In the United Kingdom, among children who had previously received meningococcal conjugate vaccine containing CRM_{197}, there were similar rates of local reactions and fever among children receiving 30 Lf- and 2 Lf-containing vaccines at ages 3.5 to 5 years.[245]

Use of adsorbed vaccine for primary immunization has been reported to result in higher rates of local adverse reactions after subsequent booster vaccination. A higher incidence of local reactions after booster vaccination with DT was observed among Swedish schoolchildren who received adsorbed DT for primary immunization in infancy compared with those who had received nonadsorbed fluid DTP. Seventy-three percent of children who had been primed with DT reported redness, 56% reported swelling, and 47% reported itching, compared with 23%, 15%, and 21%, respectively, among those who had received DTP.[246] In contrast, local reactions did not differ among children who had received adsorbed DT for the primary series when they were boosted with either adsorbed or nonadsorbed DT.[247,248]

Although local injection site reactions are common, only a small proportion of these reactions are clinically significant. A study in the Vaccine Safety Datalink found a rate of medically attended local reactions after Td of 3.6 per 10,000 among persons 9 to 25 years of age. Although infection is an unlikely cause of injection site swelling after vaccination, persons with medically attended local reactions were frequently prescribed antibiotics for cellulitis.[249] A similar rate of medically attended local reactions has been reported after Tdap.[250] Because administration of the U.S.-licensed tetravalent meningococcal conjugate vaccine Menactra (Sanofi Pasteur) contains a relatively large amount of diphtheria toxoid and may produce a high level of diphtheria antitoxin in persons who have previously received childhood immunization with diphtheria toxoid–containing vaccines, there are at least theoretical concerns about administration of Td or Tdap after Menactra.[192] Prelicensure data were not available on adverse events after Td or Tdap administered nonsimultaneously after Menactra; postlicensure data are limited but do not suggest that significant adverse events result from sequential administration of diphtheria toxoid–containing vaccines, including Menactra.[250] Similarly, a second U.S.-licensed tetravalent meningococcal vaccine, Menveo (Novartis Vaccines and Diagnostics) contains CRM_{197} as the protein carrier; prelicensure evaluation of this vaccine found a similarly high proportion of vaccinees reporting injection site pain associated with administration of Tdap, regardless of whether it was given before, simultaneously, or after Menveo.[251]

Rare Adverse Events

The potential for anaphylaxis exists with any protein antigen, but has not been attributed to diphtheria toxoid. A recent case series from a food allergy clinic identified eight cases of anaphylaxis after DTaP or Tdap among children with severe milk allergy; the authors hypothesized that the reaction could have been caused by residual casein.[252] This observation has not been replicated in other patient populations. The British National Childhood Encephalopathy Study, designed to examine the incidence of brain damage after the administration of pertussis vaccine, showed a slight but statistically insignificant excess of acute encephalopathy in the first 7 days after a dose of DT.[253] It is likely, however, that this excess is attributable to the induction of inevitable manifestations of preexisting central nervous system disorders by the systemic effects of DT, as was observed with infantile spasms.[254] Newer studies suggest that many cases of encephalopathy after DTP are among children with severe myoclonic epilepsy of infants (Dravet syndrome), a disorder associated with mutations of the gene encoding the sodium channel.[255,256]

In 1994, the Institute of Medicine (IOM) reviewed the possible association between tetanus and diphtheria toxoids and Guillain-Barré syndrome (GBS) or polyneuritis. Of 29 reports identified in the medical literature, most cases were in adults who had received tetanus toxoids alone (21 cases) or tetanus toxoid and tetanus antitoxin (four cases). Few cases of GBS or polyneuritis after diphtheria toxoid–containing vaccines have been reported. In 1994, the IOM concluded that the evidence favored a causal relationship between tetanus toxoid and GBS,[257] but a more recent report concluded that the evidence was inadequate to either accept or reject a causal relationship

between diphtheria toxoid, tetanus toxoid, or acellular pertussis–containing vaccines and GBS.[258] Although there is little evidence to support an independent association between receipt of diphtheria toxoid and GBS, cases of GBS have been reported in the United States among recipients of a tetravalent meningococcal conjugate vaccine, in which the meningococcal polysaccharides are conjugated to diphtheria toxoid (Menactra, Sanofi Pasteur).[259] A causal relationship between receipt of Menactra and GBS has not been established.

Indications for Vaccine. DTaP is ordinarily not given to infants who are younger than 6 weeks of age because responses to pertussis vaccine in the young infant are suboptimal; responses to tetanus and diphtheria toxoids, however, are satisfactory in such young infants regardless of the presence of maternally derived serum antibody and without induction of immunologic tolerance.[260] The optimal age for immunization of premature infants cannot be stated with confidence, although available data suggest that satisfactory responses are achieved by initiating the usual DTP series according to the routine immunization schedule regardless of pregnancy duration.[261–264] Follow-up of a small group of children born at less than 29 weeks' gestation suggests lower diphtheria antibody levels at age 7 years, after a five-dose series, than in children born at term.[265] There is evidence that high titers of transplacental antibody to diphtheria toxin inhibit serologic responses to the first two doses of diphtheria toxoid in infants, but after the third dose (administered in the Swedish schedule at 12 months of age), the effect is no longer evident.[186]

Diphtheria and tetanus toxoids, adsorbed, for pediatric use (DT) is recommended for the primary immunization of children younger than 7 years in whom pertussis vaccine is contraindicated. DT contains 10 to 12 Lf of diphtheria toxoid; infants who begin the series before 1 year of age should receive DT at 2, 4, 6, and 15 to 18 months of age. Satisfactory responses are obtained even in the absence of the adjuvant effect of pertussis vaccine.[266,267] For unimmunized children 1 to 7 years of age, two doses 2 months apart and a third dose 6 to 12 months later constitute primary immunization.[268]

Tetanus and diphtheria toxoids, adsorbed, for adult use (Td) contains approximately the same amount of tetanus toxoid as do DTP and DT, but the amount of diphtheria toxoid is reduced to no more than 2 Lf per dose. This reduction minimizes reactivity in persons who may have been sensitized previously to diphtheria toxoid, and it is sufficient to provoke satisfactory anamnestic responses in previously immunized persons.[216] In addition, in previously unimmunized older children and adults, Td is satisfactory for primary immunization[190,269] when administered as a three-dose series, with the second dose given 4 to 8 weeks after the first dose, and the third dose 6 to 12 months after the second dose.[262] Td should be administered approximately every 10 years after the completion of childhood immunization, with the first booster dose given as Tdap. Although Td is slightly more reactive than tetanus toxoid alone,[235] it is preferable to monovalent tetanus toxoid for prophylaxis of tetanus after wounds to maintain satisfactory population immunity against diphtheria. In the United States, DT and Td are distributed by Sanofi Pasteur, Inc. Monovalent diphtheria toxoid is no longer available in the United States.

Tdap contains a reduced dose of acellular pertussis vaccine and adult formulation diphtheria and tetanus toxoids. Two Tdap vaccines are licensed for use in the United States, with different age indications (see "Preparations Available, Including Combinations" earlier). Both Tdap products are currently licensed for use as a single booster dose. After 10 years of follow-up from receiving Tdap, 97% to 100% of participants had protective levels of tetanus antibodies (>0.01 IU/mL) and 73.5% to 85.9% had protective antibody levels for diphtheria antibodies (>0.01 IU/mL) even when the booster dose was combined or given coadministered with other vaccines such as IPV or hepatitis B (HepB).[270–272] For added protection against pertussis, a dose of Tdap is recommended to replace one decennial dose Td in persons who have not received a prior dose of Tdap previously. In some industrialized countries like the United States, United Kingdom, Canada, New Zealand, and Belgium, Tdap is recommended during every pregnancy to protect the newborn against pertussis.[273–277] In Latin America, Tdap is recommended for use during pregnancy situations of high risk such as outbreaks.[278]

Limited data regarding simultaneous administration of the first three doses of DTaP with other childhood vaccines indicate no interference with response to the diphtheria toxoid component. Data are available regarding administration of DTaP with other vaccines recommended at the same time as the fourth and fifth doses of the diphtheria, tetanus, and pertussis series (i.e., Hib conjugate vaccine, measles-mumps-rubella vaccine, and varicella vaccine) and regarding administration of whole-cell DTP (all doses in the series) with these vaccines.[177,279] DTaP may be administered simultaneously with other routinely recommended childhood vaccines, including HepB vaccine, Hib vaccine, inactivated poliovirus vaccine, pneumococcal conjugate vaccine, and rotavirus vaccine, to infants at ages 2, 4, or 6 months.

As with tetanus toxoid and pertussis vaccine, prolonging the interval between doses does not require restarting the series; indeed, immune responses achieved after longer intervals between doses than those recommended are often higher than after the regular schedule, although the subject may be left unprotected in the interim.

In other countries, DTP is administered according to alternative schedules (see Chapters 72 and 74). According to the recommended schedule for the WHO's EPI, DTP is administered at 6, 10, and 14 weeks. In a number of European countries, two doses of DTP or DTaP are administered early in the first year of life, followed by a third dose late in the first year or early in the second year of life. Recommendations regarding subsequent boosters vary by country. In response to the epidemic of diphtheria in the former Soviet Union, booster doses have been recommended or reinstated in a number of countries outside the former Soviet Union. WHO now recommends that the primary vaccination series of three doses starting as early as 6 weeks of age and with a minimum interval of 4 weeks between doses. Where resources are available, additional doses can be given after completion of the primary series. In some countries, additional booster doses are given in the second year of life and a second booster is given at 4 to 7 years of age. WHO recommends that people living in low-endemic or nonendemic areas should receive a booster dose of diphtheria toxoid approximately 10 years after the primary series and subsequently every 10 years throughout life to sustain immunity, and that particular attention should be given to revaccination of healthcare workers.[229]

In some countries, booster doses are administered as adult formulations of Tdap to some age groups.

Contraindications and Precautions

There are few contraindications to the use of diphtheria toxoid. Severe hypersensitivity reactions after a previous dose are considered contraindications to further doses.[268] Even though causation by pertussis vaccine is not established, children who experienced encephalopathy within seven days after administration of a previous dose of DTaP or Tdap vaccine and not attributable to another cause should not receive additional

doses of a combination vaccine that contains pertussis antigens. Local side effects alone do not preclude continued use. Vaccination of persons with severe, febrile illness generally should be deferred until recovery, but mild illnesses with or without fever should not preclude vaccination.

Some diphtheria toxoid products are packaged in containers (vials or syringes) containing latex rubber. If a person reports a severe anaphylactic allergy to latex, vaccines supplied in vials or syringes that contain natural rubber should not be administered, unless the benefit of vaccination outweighs the risk of an allergic reaction to the vaccine. For latex allergies other than anaphylactic allergies (e.g., a history of contact allergy to latex gloves), vaccines supplied in vials or syringes that contain natural rubber or natural rubber latex can be administered.[280]

Persons with a bleeding disorder or receiving anticoagulant therapy may receive indicated vaccinations by the intramuscular route if, in the opinion of a physician familiar with the patient's bleeding risk, the vaccine can be administered with reasonable safety by this route. A fine needle (23 gauge or smaller) should be used for the injection and firm pressure applied to the site, without rubbing, for 2 or more minutes. The patient or family should be instructed concerning the risk for hematoma from the injection.[281]

PUBLIC HEALTH CONSIDERATIONS

Epidemiologic Effects of Vaccination

Despite the relatively low levels of immunity among adults in many countries, diphtheria has remained well controlled in most countries with effective childhood immunization programs. Historically, it has been thought that 70% or more of a childhood population must be immune to diphtheria to prevent major community outbreaks[281]; the herd immunity threshold has been estimated to be 80% to 85%, based on the average age of infection in the prevaccine era.[282] Whether an epidemic of a given infectious disease occurs is influenced by a number of factors other than the proportion of susceptible persons in the population, including the age distribution of immune and susceptible persons, the extent of mixing of individuals and subgroups in the community, and the infectivity and routes of transmission of the organism.[215] In countries with high rates of childhood immunization against diphtheria, it may well be that epidemics do not occur among adults, up to half of whom may be susceptible, because the reservoir of disease in the childhood population has been eliminated and because the strains of *C. diphtheriae* circulating in the community are less likely to be toxigenic.

Serologic studies in Europe and the United States demonstrate that many adults in these countries remain susceptible to diphtheria.[223,283–289] Differences in seroprevalence among countries reflect the varied immunization schedules and vaccination coverage among countries, the effect of immunization during military service, and the unknown effects of natural exposure to toxigenic *C. diphtheriae*.[141,142,223] Although there is some variability among countries, studies of diphtheria seroprevalence frequently have demonstrated low levels of immunity among older adults. In 1996, a serologic study in England and Wales showed that only 29% of adults age 60 years and older had diphtheria antitoxin titers of 0.01 IU/mL or greater.[290] A similar pattern of susceptibility is seen among elderly persons in other countries of Western Europe.[223] Some studies also demonstrated less susceptibility among males; in some countries, this may reflect immunization during military service.[223]

These concerns have been heightened by the epidemic of diphtheria in the former Soviet Union. A striking feature of this epidemic was the proportion of cases occurring among adults, varying from 38% in Azerbaijan to 82% in Latvia and Lithuania in 1994.[290] Before 1986, the last dose of diphtheria toxoid was routinely administered at 14 to 16 years of age in the Soviet Union; in response to an increase in reported cases of diphtheria in the early 1980s, targeted vaccination of certain occupational groups was initiated, but routine use of booster vaccinations among adults was not recommended. Immunogenicity studies in Russia, Ukraine, the Baltic States, and Georgia demonstrated that some adults failed to develop a booster response to a single dose of diphtheria toxoid, suggesting that they may never have received an effective primary series in childhood.[225–228,291] Childhood immunization coverage was low in some regions in the late 1980s and early 1990s, in part because of an extensive list of contraindications to vaccination,[292] which undoubtedly contributed to the epidemic.[293] For control of the epidemic, the WHO recommended identification, isolation, and appropriate treatment of all cases; prevention of secondary cases by optimum management of close contacts of cases; and rapidly increasing population immunity by sustaining high coverage among children with four doses of DTP in all districts and administering a single dose of an age-appropriate formulation of diphtheria toxoid to the entire population.[290] By 1997, all countries had made significant progress in immunization of children and adults; the declines in disease incidence were most dramatic in countries that had achieved high coverage.[64] Diphtheria incidence has continued to decrease. During the period 2000–2009, 7032 cases of diphtheria were reported in the WHO European Region, with more than 60% of the cases reported from the Russian Federation. During the decade, diphtheria incidence decreased by more than 95% in the region. By 2009, only Latvia reported an incidence of greater than 1 case per million population.[64] During the period 2001–2013, a total of 65,226 cases of diphtheria were reported from the WHO South East Asia Region; approximately 84% were from India.[65,140]

Disease Control Strategies: United States and Others

The primary control strategy for prevention of diphtheria globally is routine childhood vaccination with diphtheria toxoid as DTP or DTaP. In countries rendered nonepidemic through high immunization coverage, WHO recommends that the primary vaccination series of three doses be extended by at least one booster dose, and acknowledges that revaccination of adults against diphtheria every 10 years may be necessary to sustain immunity.

In the United States, DTaP, is administered as a five-dose series in childhood followed by decennial booster doses of Td beginning at age 11 to 12 years. For added protection against pertussis, Tdap is now recommended for the booster dose at age 11 to 12 years. Td, rather than monovalent tetanus toxoid, should be used in emergency departments, physicians' offices, and other situations in which tetanus-prone wounds are treated.[294–296] There are essentially no indications for monovalent tetanus toxoid at present in the United States or elsewhere.

Epidemiological surveillance to ensure the early detection of cases should be in place in all countries, and all countries should have access to laboratory facilities that can provide reliable identification of toxigenic *C. diphtheriae*. Rapid public health response to a suspected case of diphtheria, with case investigation to identify the source of infection and other persons who may have been exposed, can help limit spread of diphtheria.

Cost-to-Benefit Information

The costs and benefits of diphtheria toxoid as a component of DTP or DTaP, administered as part of the routine immunization series have been evaluated on several occasions. In 1997, it was estimated that diphtheria vaccination prevented almost all the 276,750 diphtheria cases and 27,675 diphtheria deaths that were estimated to occur in the absence of vaccination among a simulated birth cohort of 4.1 million children during the first 15 years of life; both were cost-saving from the societal and healthcare system perspectives.[297] A similar analysis evaluated DTaP as part of the 2001 recommended childhood immunization schedule, which also included Hib vaccine, IPV, measles-mumps-rubella vaccine, HepB vaccine, and varicella vaccine. Vaccination of a hypothetical U.S. birth cohort was estimated to result in savings of more than $2 billion in direct costs and $24 billion in total costs for cases of diphtheria prevented and was cost saving both in terms of direct costs (benefit-to-cost ratio: 5.3) and from the societal perspective (benefit-to-cost ratio: 16.5).[298]

ERADICATION

Although no animal reservoir exists for *C. diphtheriae*, *C. ulcerans* may carry the β-corynebacteriophage that encodes diphtheria toxin,[299] and an animal reservoir does exist for this organism[300,301] and thus for the bacteriophage. Given the worldwide ubiquity of carriage of *C. diphtheriae* and the bacteriophages implicated in toxin production, prospects for eradication of diphtheria currently seem remote. Continuing active immunization with diphtheria toxoid is the key to the control of diphtheria.

FUTURE VACCINES

New combination vaccines for use in young children built on both the DTaP and DTP backbone are under development.[302] Alternative approaches to diphtheria immunization in the future may include vaccination by the oral[303,304] or nasal[305] routes or use of a highly purified, less-reactive antigen[306–308] or carrier systems[309,310] for diphtheria toxoid that would require fewer injections. Such products would be particularly useful in the developing world, where severe limitations in healthcare personnel and financial resources are major barriers.

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20 Ebola Vaccines

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Named for the site of the first outbreak in the Ebola River valley in 1977, Ebola virus and the closely related filovirus, Marburg virus, represent a family of pathogens that cause hemorrhagic fever with a high associated mortality. The potential significance of these viruses was first appreciated with the occurrence of a series of Ebola virus outbreaks in the 1970s. After a hiatus in the 1980s, the disease reemerged with greater frequency in the mid-1990s, likely as a consequence of zoonotic transmission from nonhuman primates (NHPs) to humans in equatorial Africa. In the past decade, Ebola virus outbreaks have occurred at regular, almost yearly intervals, typically during the latter part of the year and localized to the same regions of Central Africa; however, in 2014 an unprecedented outbreak in terms of scale and mortality began in Guinea (West Africa), an area where Ebola virus previously had little impact. From Guinea, the outbreak spread to several countries, requiring an international response to bring disease spread under control.[1,2] This most recent outbreak highlights the serious public health threat that natural transmission of Ebola virus represents, above and beyond its potential use as a bioterror agent. Because of its unusual pathogenesis, and the concern over its dissemination into the population through natural or deliberate means, an understanding of Ebola virus infectivity, molecular pathogenesis, and transmission, as well as the development of vaccines and therapies, have become high priorities.

BACKGROUND

Clinical Description

The initial clinical manifestations of Ebola virus begin 2 to 21 days after infection (average: 8 to 10 days) with "flu-like" symptoms including fever, fatigue, and myalgia. Within days, abnormalities related to liver function and clotting appear, with nausea, abdominal pain, rash, petechiae, diarrhea, and vomiting. As the disease progresses, in addition to the occasional development of respiratory disorders, the gastrointestinal tract becomes involved, leading to signs of bleeding from mucosal sites, including melena and hematochezia. In the terminal phases, typically within 1 to 2 weeks after infection, there is progression to disseminated infection, with abnormal clotting parameters, hepatic parenchymal damage, disseminated bleeding through various sites including but not limited to venipuncture sites and the conjunctiva, and cardiac distress resulting in terminal hypovolemic shock or septic shock in the absence of bacterial superinfection.[3–7] The mortality rate ranges from 36% to 90% in those with recognizable infection.

Complications

Clinical complications of Ebola virus infection stem from the nature of the cells targeted during the viral life cycle. In early infection, the virus replicates in reticuloendothelial cells, monocytes, macrophages, and dendritic-like cells.[8] Damage to these cell types results in the release of cytokines that likely contribute to early symptoms of infection, including fever. Subsequent damage to the liver and vasculature as the disease progresses results in impaired blood vessel integrity and gives rise to a bleeding diathesis accompanied by hypotension.[3] Ebola virus infection of the adrenal gland (at later stages of disease) may also contribute to hypotension.[9] The inability to maintain blood pressure in the setting of fever, blood loss, profuse vomiting and diarrhea, and shock leads to a high mortality rate, particularly in the absence of effective supportive care. Some case reports from the 2014 outbreak suggest that a rapid clinic visit and carefully monitored resuscitation improved patient outcomes.[3,10,11]

Virology

Ebola virus and the closely related Marburg virus (MARV) are members of the *Filoviridae* family, named for the filamentous morphology of virions that appear by electron microscopy to be a consistent diameter of approximately 80 nm, but nonuniform in length.[12] There are five species in the genus *Ebolavirus: Zaire ebolavirus* (Ebola virus/EBOV), *Sudan ebolavirus* (Sudan virus/SUDV), *Taï Forest ebolavirus* previously named Côte d'Ivoire (Taï Forest virus/TAFV), *Reston ebolavirus* (Reston virus/RESTV), and the most recent *Bundibugyo ebolavirus* (Bundibugyo virus/BDBV) discovered in 2007.[13] Of these species, RESTV is the only member that has not caused any reported human fatalities.[14]

The 19-kb nonsegmented negative-strand RNA genome of Ebola virus contains a linear array of seven genes, six of which each encode a single protein product. The seventh gene contains two open reading frames: the major transcript, secreted glycoprotein (sGP), is a secreted dimer, while trimeric glycoprotein (GP) is synthesized only after addition of a nontemplated adenosine by the viral polymerase, yielding the only virion surface protein.[15] Several cellular surface proteins, such as phosphatidylserine binding proteins, C-type lectins, DC-SIGN, and TIM-1, facilitate Ebola virus particle attachment. The virions are then taken up by macropinocytosis into intracellular compartments.[16–22] Cathepsin cleavage of GP within these acidified internal compartments exposes an occluded receptor binding site, allowing binding of GP to the endosomal/lysosomal cholesterol transporter Niemann-Pick C1 (NPC1), the cellular receptor for filoviruses.[23–27] A 3.4 Å x-ray structure of GP trimer lacking several regions was solved after cocrystallization with neutralizing antibody KZ52, isolated from an Ebola virus survivor.[28] The determination of several subsequent structures and the mapping of the cathepsin cleavage site, in combination with previous work on enzymatic processing during entry and identification of the cellular receptor, have contributed to a greater molecular understanding of GP function.[25,29]

Among the other viral proteins (VPs), VP40/VP24 form the inner matrix while the nucleoprotein (NP), VP30, VP35, and the RNA-dependent RNA polymerase, L, comprise the ribonucleoprotein complex (Fig. 20.1). Nucleocapsid-like filamentous structures can be formed by overexpression of NP, VP24, and VP35 in the absence of other VPs.[30,31] A T7-polymerase-based minireplicon system demonstrated that the minimal replication machinery for Ebola virus requires the cooperative activity of NP, VP30, VP35, and L,[32] whereas VP40 and GP are sufficient to form virus-like particles.[33] The structure of the

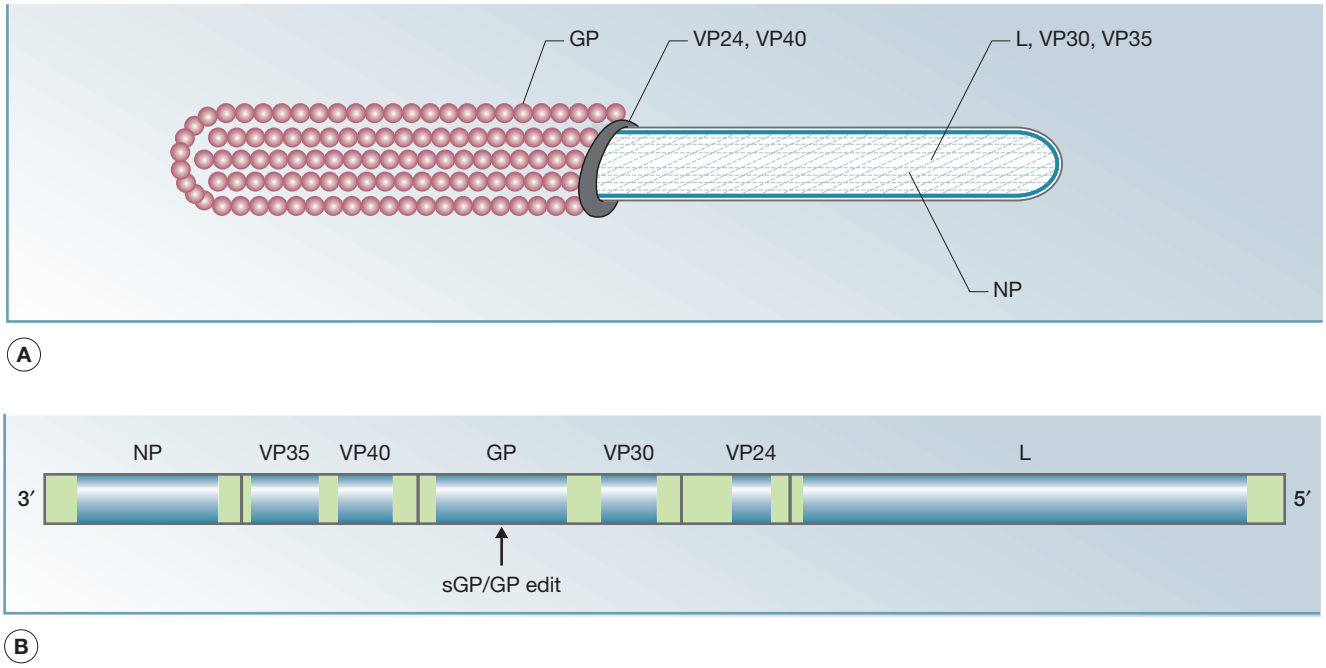


Figure 20.1. Schematic of Ebola virus components and its genomic organization. A, Model structure of Ebola virus. Among the virion proteins (VPs), VP40/VP24 and the glycoprotein (GP) are present in the membrane matrix and outer envelope, respectively, while the nucleoprotein (NP), VP30, VP35, and the RNA-dependent RNA polymerase, L, comprise the ribonucleoprotein complex. **B,** Ebola virus genomic organization. The 19-kb nonsegmented negative strand RNA genome contains a linear array of seven genes. Open reading frames (ORFs) are shown in *blue* and noncoding bases at gene termini, in the genome leader (3′) and trailer (5′), regions are *gray*. The presence of additional sequence, also known as intergenic regions, between the stop and start signals of sequential ORFs is also indicated *(dark blue lines)*. Each gene encodes a single protein except for the GP gene that contains two ORFs. The major transcript is secreted (sGP) and the minor transcript, GP, is synthesized only after addition of a nontemplated adenosine by the viral polymerase.

carboxyterminal domain of Ebola virus VP35, which possesses both its double-stranded ribonucleic acid (dsRNA) binding and interferon inhibitory activity, was determined to atomic resolution by x-ray crystallography.[34] In addition to its role in viral RNA replication and in virion assembly, VP35 plays a crucial role in Ebola virus infectivity and inhibition of host innate immune responses including suppression of type I interferon responses,[35] inhibition of RNA interference (RNAi),[36,37] and impairment of dendritic cell functions.[27,38] Another member of the replication machinery, VP30, also suppresses host cell silencing of viral genes through interaction with the RNAi pathway.[36] Advances in our molecular understanding of Ebola virus proteins strengthen the basis for future structure-function-guided identification of susceptible targets for therapeutic intervention.

Pathogenesis as It Relates to Prevention

Ebola virus enters the body through abrasions in the skin or exposure to mucosal surfaces, either by contact with bodily fluids or blood from an infected individual (Fig. 20.2).[39] It is thought that the virus traffics rapidly from these sites, possibly by transport on dendritic or other cells bearing DC-SIGN or related lectins, which bind the virus through their recognition of glycans on GP.[40] Cells of the reticuloendothelial system in the liver, lung, and spleen are the initial targets of infection, which then spreads to the endothelium.[9] In effective immune-mediated prevention, viral recognition would most likely have to occur early after infection by cells of the innate and adaptive immune system, as both $CD8^+$ T-cell and humoral responses are detected in Ebola survivors.[41–43] The mechanism of viral clearance is not fully understood but seems to require cellular immune responses.[27,185]

Diagnosis and Prevention

A diagnosis of Ebola virus disease (EVD) is suggested when a patient with high-risk epidemiological factors presents with symptoms of fever, flu-like illness, petechial rash, diarrhea, vomiting, abdominal pain, and unexplained hemorrhage.[44] Definitive diagnosis is best made using a reverse transcription polymerase chain reaction (RT-PCR) detection assay and can be confirmed by detection of viral replication in Vero cells. Additional diagnostic support can be made through the measurement of immunoglobulin (Ig) M antibodies to VPs and various more specialized research assays. During the recent outbreak in West Africa, mobile laboratories were organized to analyze suspected case samples more expeditiously. In addition, rapid Ebola virus in vitro diagnostics were approved for emergency use by the World Health Organization (WHO) and the U.S. Food and Drug Administration (FDA) as a first line of screening.[45,46,46a] The majority of those rapid assays are based on RT-PCR using whole blood (venipuncture or fingerstick), plasma, serum, or urine from patients starting to develop signs of disease. Most methods were developed for the hospital environment and not for mass population screening. Genome amplification targets different genes with NP, VP40, L, and GP used alone or in combination depending upon the kit used. EBOV is the principal target, but in some assays, detection of SUDV, BDBV, and MARV is also possible using degenerate primers. However, those assays lack species distinction if the result shows a positive signal, reinforcing the need for a follow-up species-specific test. In addition to genomic detection, a few diagnostic methods have focused on antigenic protein detection. For example, a rapid test kit reveals the presence of VP40 from any variant of EBOV in blood and plasma within 15 to 25 minutes, although results

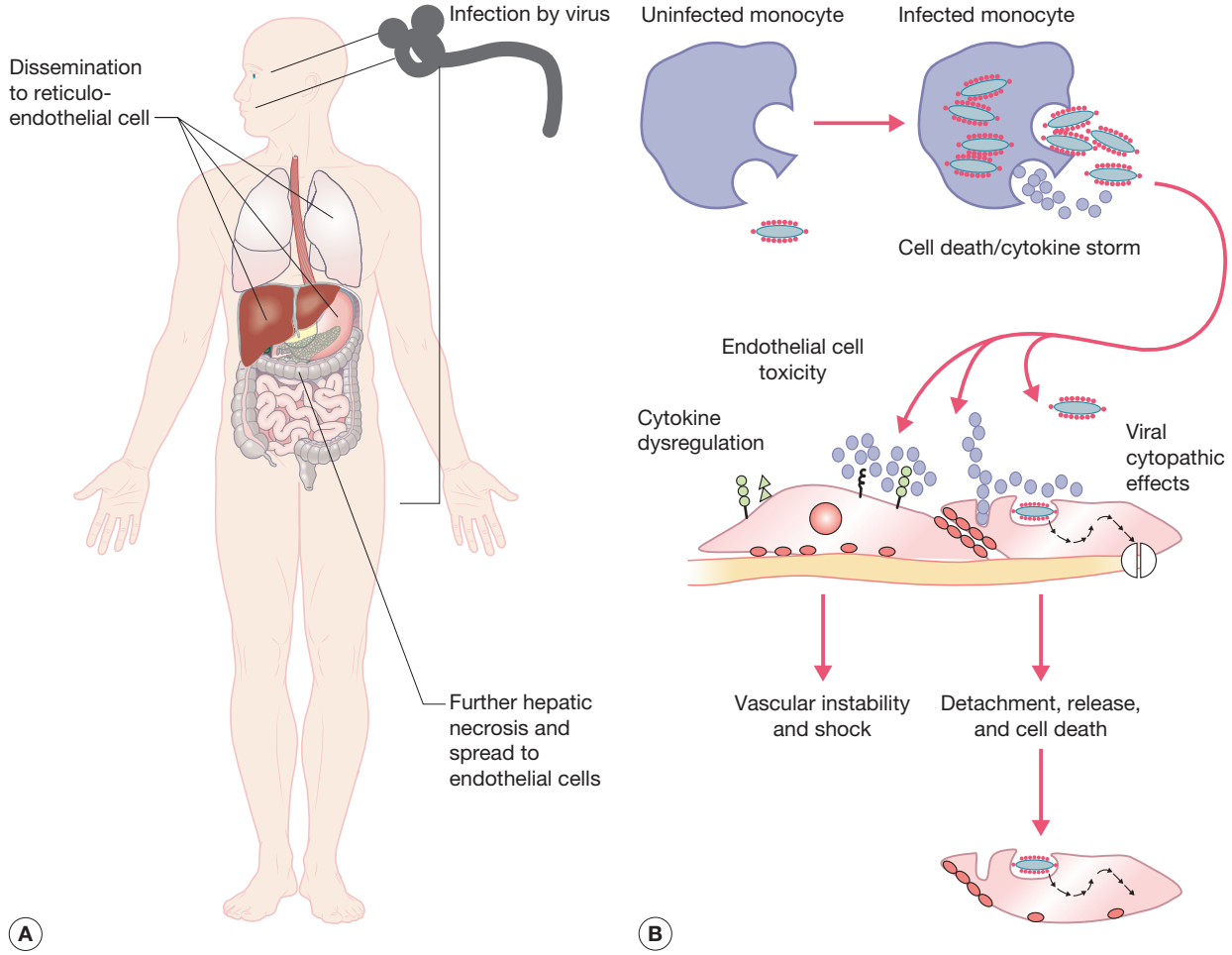


Figure 20.2. Pathophysiology of Ebola virus infection. A, After initial infection through mucosal sites or through cuts or abrasions in the skin, Ebola virus is thought to disseminate first to reticuloendothelial cells of the liver, lung, and spleen. **B,** Further replication at these sites is postulated to cause damage to these cells that result in the release of cytokines that may precipitate fever and alter vascular permeability/tone. Additional replication in the liver and endothelial cells further compromises thrombosis and hemostasis, as well as vascular integrity, leading to hemorrhage and the ultimate circulatory collapse responsible for its high mortality.

should be followed-up with the more rigorous RT-PCR detection assay.[45,46] With the emergence of new species of *Ebolavirus* like BDBV[47] increasing the number of possibilities that have to be considered with any new outbreak and the sequence diversity between *Ebolavirus* species, more than one diagnostic assay should be used to prevent false negatives. This testing redundancy is particularly critical in the early stages of an outbreak where the circulating variant has not yet been definitively identified.

In an outbreak situation, contact tracing is essential to halt spread of the disease through the population.[48,49] When an index case is identified, strict public health measures should be implemented, including use of adequate personal protective equipment (PPE), cleaning and decontamination of all equipment, isolation and quarantine to reduce transmission, and identification and testing of all contacts of the index case followed by immediate quarantine of any contacts that also develop signs of illness. Collaboration between healthcare workers and local community representatives is crucial to improve communication with the population and ensure dissemination and understanding of public health directives. To provide efficient basic healthcare during an outbreak, the number of treatment centers needs to be in accordance with the size of the susceptible population.[11] The Centers for Disease Control and Prevention (CDC) and WHO established guideline declares a country to be considered free of Ebola virus if during a 42-day window (twice the length of the maximum incubation period) no patients are diagnosed Ebola positive.[50]

EPIDEMIOLOGY

Incidence and Prevalence

Historically, outbreaks of Ebola virus infection have occurred sporadically, primarily in the countries of sub-Saharan Africa, including the Democratic Republic of Congo (DRC), the Republic of Congo (Brazzaville), Côte d'Ivoire, Sudan, and Uganda.[51] Depending on the proximity to larger population centers and the virulence of the *Ebolavirus* variant involved, outbreaks have ranged in size from dozens to hundreds of fatalities, resulting in cumulative mortality numbers of less than 1200 over the past 2 decades. Outbreaks have mainly centered in the rain forest regions of northern Congo and the DRC, particularly, but not exclusively, at the beginning of the rainy season in October or November. Fortunately, all these outbreaks remained localized and were contained quickly.

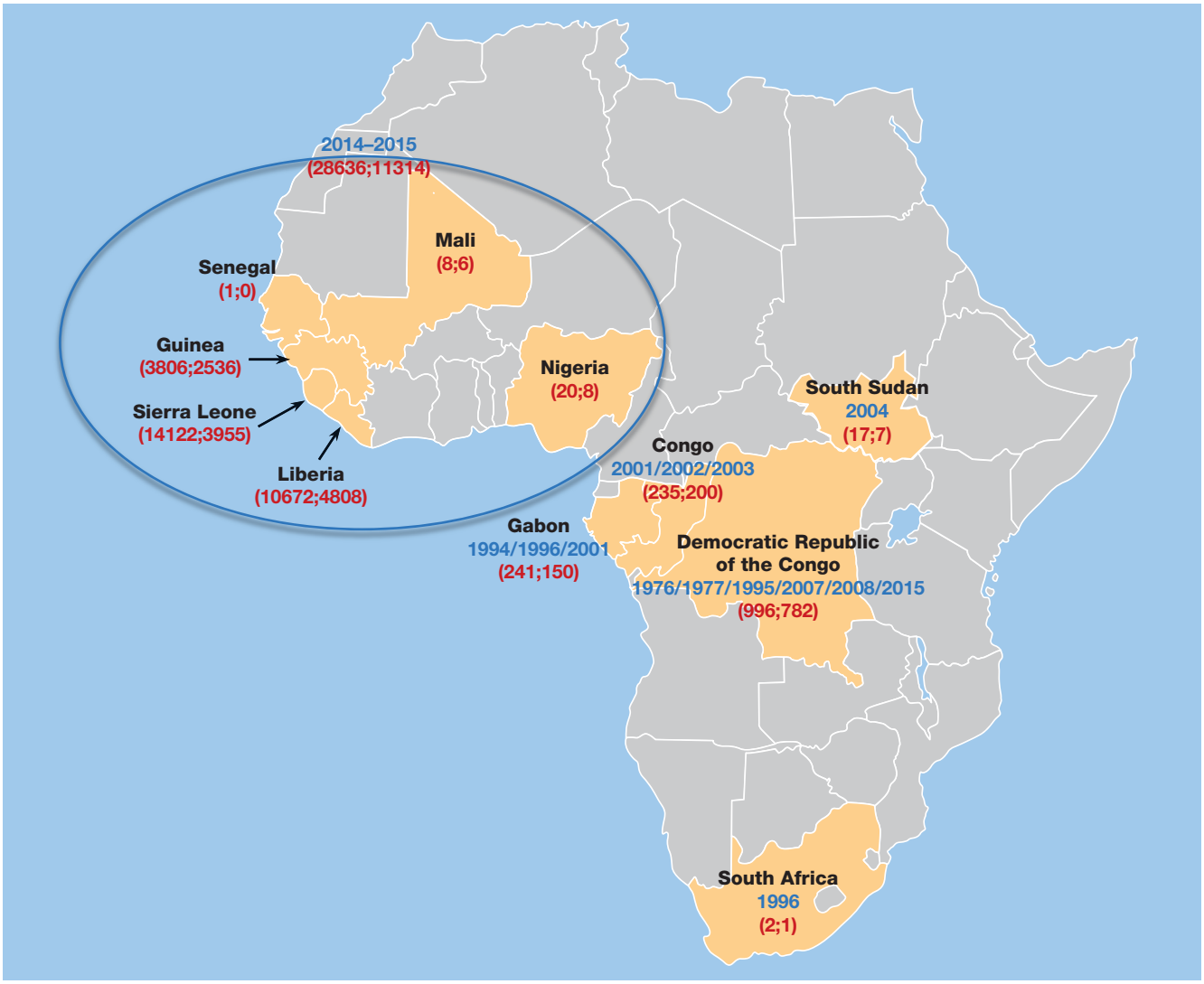


Figure 20.3. Ebola virus disease case reports in Africa between 1976 and 2015. Geographic schematic view of Africa representing in orange color countries affected by an Ebola virus disease outbreak (*Zaire ebolavirus*). Under the name of each country, the number of cases (suspected/probable/confirmed; death) is indicated in *red* and the date for each outbreak is reported in *blue*. The *blue circle* surrounding West Africa represents the spacial localization of the 2014–2015 outbreak. The case counts shown are accurate as of November 7, 2015. *(Data from Centers for Disease Control and Prevention. Outbreaks chronology: Ebola virus disease. 2015. Available at www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html.)*

The 2014 outbreak stands apart from previous outbreaks, as the outbreak region encompassed several countries (including Guinea, Liberia, and Sierra Leone) over a period of almost 2 years, resulting from rapid spread of the virus across borders and compounded by the difficulty of contact tracing and quarantine maintenance. As a result of international travel from the area of outbreak, Ebola virus–positive patients were identified for the first time in more distant countries, such as Nigeria, Mali, and the United States, and the total number of cases from the 2014 outbreak exceeds that of all previous outbreaks combined (Fig. 20.3).[52] Sequencing of clinical viral isolates from the outbreak revealed that the circulating variant, now named Makona, formed a separate clade from previously identified EBOV variants.[1]

Risk Groups

In several Central African outbreaks, ape carcasses testing positive for EBOV have been discovered in proximity to areas experiencing human EBOV infection.[53–55] Although no definitive chain of transmission has been established between infected apes and humans, it is thought that the reservoir of infection may reside in close proximity to great apes, and therefore bushmeat hunters and their family and village contacts represent a potentially high-risk population. The reservoir has been postulated to be the fruit bat,[56] creating risk groups among those close to their habitats, including miners who can be exposed to bat nests and tourists who explore caves. Any other causative factors that may play a role in initiating outbreaks remain unknown.

Local and international healthcare workers acting as first responders in outbreak settings represent a significant high-risk group for infection.[57,58] The identification of RESTV in pig farms in the Philippines in 2008 has suggested swine as another potential reservoir or intermediate host for Ebola virus, making pig farmers an additional potential risk group for the disease.[59] In addition to natural transmission, laboratory workers and handlers of NHPs are at increased risk for infection. Indeed, the very first cases of filovirus hemorrhagic fever were recognized in a primate facility in Marburg,

Germany, where the prototype filovirus, MARV, was identified. Finally, because the virus has been the object of development for bioterrorism and is classified as a Category A agent, there is concern about its potential use against military and civilian targets as a weapon of widespread human destruction.

Modes of Transmission and Reservoirs of Infection

During outbreaks, Ebola virus is transmitted by direct contact with the bodily fluids (blood, urine, saliva, feces, vomit, breast milk) or tissues of an infected subject, such as can occur during burial rites.[60] Ebola virus antigens and viral particles have been detected in the skin of infected individuals, with high antigen load around the sweat glands.[61] Previous filovirus outbreaks have suggested that virus may persist in immune privileged sites, such as the testes or the eye, even after peripheral viremia and clinical illness have normalized.[62,63] Observations after the 2014 outbreak showed that the semen of survivors can remain positive for virus for periods as long as 3 months or even longer, reemphasizing the risk of viral transmission through unprotected sexual contact.[64–66] Ebola virus has also been detected in the aqueous humor of the eyes of a survivor weeks after his remission, although no virus was detected in the conjunctivae or tears of the patient.[67] Although some evidence indicates that Ebola virus can also be transmitted via the aerosol route[68,69] and NHPs have been experimentally infected with Ebola virus via the aerosol route, the contribution of this mode of transmission in an outbreak setting is thought to be low.[8] Transmission from animals to humans is believed to occur via direct contact with infected animal blood or carcasses. Although the natural reservoir of infection is not known, the virus is presumed to be zoonotic because human index cases have been linked to direct contact with gorillas, chimpanzees, duikers,[55] swine,[59] or to unknown contacts within or around mineral mines[70] that harbor multiple bat species. Declines in great ape populations are reported to be coincident with EVD outbreaks in humans; however, NHPs are not thought to be the primary reservoir since the virus is highly pathogenic in these hosts, and long-term virus circulation in its reservoir presumes nonlethal infection. Epidemiological reporting from primary cases has led to speculation that bats may be the natural transmitting host for Ebola virus while other mammals may be amplifying hosts. Evaluation of field-caught bats revealed that partial viral sequences could be recovered from some animals, while others were seropositive against antigens from Ebola virus or the closely related MARV as measured by enzyme-linked immunosorbent assay (ELISA).[56,71] Even though Ebola virus has not yet been directly isolated from seropositive bats, rescue of replicating MARV from bats strengthens the hypothesis that they could also be a natural reservoir of Ebola virus.[72,73] Swine have also been proposed as another potential reservoir of Ebola, as some studies suggest that infection of pigs with either RESTV or EBOV is sublethal and generates high-titer virus in the lungs of infected animals.[74,75]

Significance as a Public Health Problem/Health Burden

For all *Ebolavirus* species from the first recognized outbreak in 1976 until 2013, the cumulative number of fatalities was approximately 1500 people. Most outbreaks were in isolated Central African locations and small in number, with sizes ranging from a handful of fatal cases to several hundred deaths in some of the larger outbreaks.[51,52] A similar pattern of outbreaks has been observed for the related filovirus MARV.[76] Despite the high mortality rate of EVD, the limited nature of these outbreaks meant that filoviruses were not a major focus of global public health initiatives. In March 2014, a new outbreak began in West Africa, a location atypical for *Ebolavirus* outbreaks, which continued unabated for nearly 2 years and grew to an unprecedented size and geographical coverage. In August 2014, the Ebola outbreak was designated an international public health emergency by the WHO[77]; however, as a result of challenges associated with population density, cultural beliefs, and contact tracing, the outbreak still continued through the following year. The West African outbreak changed public perception of the significance of ebolaviruses, as this outbreak alone had almost an order of magnitude more fatalities (11,315 people as of December 30, 2015) than the cumulative fatality total observed in all previous outbreaks combined.[14,52] The 2014 outbreak demonstrated that an outbreak of this size can have severe worldwide economic, social, medical, and public health consequences, affecting international travel and potentially limiting resources for treatment of other significant diseases such as malaria that have increased prevalence in the countries affected by ebolavirus epidemics. Although ebolaviruses are not the most significant pathogen in terms of absolute disease burden in Africa, the West African outbreak highlights the need for these viruses to remain a significant public health priority.

POSTEXPOSURE ANTIVIRAL TREATMENTS

Antisense and Small Interfering RNA Therapy

Experimental treatments including anticoagulant[78] and recombinant human protein C[79] therapy have shown limited protection against Ebola virus infection in NHPs. A viral RNA polymerase inhibitor, Favipiravir, efficient against influenza virus, also showed no benefit against infection in NHPs, but it was nonetheless given to human EVD patients under "compassionate use" in the latest outbreak.[80,81] Advanced antisense technology against EBOV VP35 and VP24 or MARV NP and VP24 administered within 1 hour of exposure showed partial protection against EBOV challenge and complete protection against MARV challenge in rhesus macaques, suggesting that complete protection against MARV does not imply a similar result for other filoviruses.[82] Similarly, repeated administration of small interfering RNA (siRNA) targeting VP24, VP35, and L protein was fully protective against EBOV challenge in NHPs when administered within 1 hour of infectious challenge.[83] More recently, EBOV-targeting siRNAs encapsulated in lipid nanoparticles protected 100% of NHPs against Ebola virus 3 days after viral exposure.[84] A Phase II clinical trial of this treatment began in Sierra Leone in early 2015, but was terminated because of a lack of therapeutic benefit. Although both antisense and siRNA treatment demonstrated significant protection in NHPs, naturally occurring mutations in the target sequence and a short window for treatment postinfection, possibly because of inhibition of RNAi by Ebola virus proteins,[36] may limit their use.

Passive Immunization

Enveloped viruses such as Ebola virus represent a potential target for passive immunization because high-titer Ig against the viral GP can be generated by either vaccination or natural infection of an appropriate host. However, the broad virus tropism for nearly all tissues, the large filamentous virion and the high quantity of surface GP trimers necessitates a high concentration of antibody for in vivo virus inactivation. The primary mode of virus entry is via macropinocytosis, with exposure of the GP receptor binding domain occurring only after the virus enters intracellular acidic compartments, thus

reducing accessibility of critical epitopes to passively transferred antibodies.

Early studies showed some success with passive immunotherapy in mouse[85,86] and guinea pig models[87,88] where the disease is less virulent, as compared to that seen in primate models; however, NHPs are the preferred animal model for studying EBOV because pathogenicity and immune responses in NHPs recapitulate human disease better than the responses seen in rodent models.[89] Infection of rodents occurs only after serial passage of human EBOV in the rodent host, and inherent differences between wild-type and adapted viruses and/or the animal model become evident when vaccine or treatment successes in rodents fail to translate into NHP protection.[42,88] One of the first successful passive transfer EBOV NHP studies involved administration of hyperimmune equine Ig to *Papio hamadryas* baboons prior to infectious challenge. Injection of the hyperimmune equine serum (1:8192) prevented viral replication in 100% of treated animals when injected 2 hours before challenge.[90] In contrast, 30 minutes after Ebola challenge, injection of a higher serum titer of 1:65,536 failed to protect all the animals. In another study, the administration of equine IgG to cynomolgus macaques just after EBOV infection only delayed fatal outcome by 1 to 2 days.[91]

Surprisingly a human survivor monoclonal antibody, KZ52, which showed potent in vitro neutralizing activity, was administered using a prechallenge schedule similar to that used for the equine serum but failed to protect macaques, suggesting that a polyclonal formulation may be required.[42,92] Therefore, mixtures of mouse-derived chimeric monoclonal antibodies (mAbs) given as cocktails of three antibodies were developed and showed protection from death when administered up to 5 days after EBOV challenge.[93–99] The FDA allowed the use of these mAb cocktails as an emergency treatment under a "compassionate use" clause, and clinical trials in Liberia and Sierra Leone will evaluate their efficacy as postexposure treatment. More recently, a simplified cocktail of two human mAbs—mAb100 and mAb114, both isolated from a survivor of the 1995 Kikwit EBOV outbreak—was tested in NHPs with the first administration given 1 day after infectious challenge. This mAb therapy prevented death in all animals and, unlike previous cocktails, protected against all clinical signs or symptoms of infection. mAb114 was further evaluated as monotherapy and showed complete protection from death in NHPs, with transient viremia but no clinical symptoms.[100] These more recent results indicate that mAb therapy can be optimized and suggest the potential for simplified and efficacious mAb-based therapeutics in humans.

Studies in pursuit of other possible immunotherapies in humans evaluated infusion of blood from convalescent patients into infected subjects. The treated patients survived EBOV infection, but administration of treatment was late in the course of infection so natural immune responses and/or clinical care could have played a role in recovery, and furthermore no control group was included in the study.[101] Currently, several Phase II/III trials are ongoing in West Africa to study the efficacy of convalescent plasma as a possible future treatment.[102]

ACTIVE IMMUNIZATION

Initial Approaches That Were Abandoned

Despite many years of experimental vaccine failures in NHPs, studies analyzing human samples showed that it is possible to develop adaptive immunity against Ebola virus, and suggested that an effective vaccine should induce cellular immune responses. However, because of the limited number and availability of human clinical samples over the course of historical Ebola outbreaks, the determination of a definitive correlate of survival was not possible. Therefore, studies of protective immunity against Ebola virus as well as vaccine efficacy have largely focused on animal models of infection. NHPs (rhesus or cynomolgus macaques) undergo a disease course that closely resembles clinical infection in humans, whereas guinea pigs and mice can be infected with an adapted virus, but undergo pathogenic changes that differ from those seen in human cases.[103,104] One of the earliest approaches for an Ebola virus vaccine tested the ability of a Vero cell-produced, formalin-inactivated virus to protect against infection in the guinea pig model.[105] Antigen-specific immune responses were not reported, and although protection was observed in the vaccine group, control animals receiving cell culture fluid lacking vaccine exhibited less than 30% mortality, suggesting that protection may have been nonspecific. Contradictory results regarding this vaccine have been observed in NHPs, with one study showing 50% protection and one study showing a failure to protect.[106,107] The lack of translation from the small animal model to primates was likely explained by either non-specificity of the guinea pig protection, greater sensitivity of the NHP model, or lack of relevant cellular immune responses necessary to clear virus-infected cells. In support of the latter interpretation, a recombinant GP protein subunit vaccine generated specific neutralizing antibody titers but yielded only partial protection in guinea pigs, likely because of the generally inefficient induction of cellular immunity by subunit protein vaccines.[108]

Early approaches to the development of an Ebola virus vaccine made use of a vaccine modality (virus inactivation) classically considered to elicit primarily humoral responses.[109] With the goal of establishing effective immune protection in primates, and ultimately in humans, the conflicting results of the early EBOV studies implied that a vaccine platform designed to elicit both humoral and cellular immunity might be more successful. The most common approach to elicit such a response is through the use of live attenuated vaccines, but the lethality of Ebola virus made this option untenable because of the potential risks associated with incomplete attenuation.

The limitations that impeded early Ebola virus vaccine development were finally overcome by the introduction of gene-based vaccination approaches that elicited both humoral and cellular immunity. The effectiveness of this approach was borne out with the first demonstration of immune protection against Ebola virus infection in the guinea pig infectious challenge model,[110] where immunization with plasmid DNA vectors encoding GP or NP conferred complete protection against lethal Ebola virus challenge, while uniform mortality was observed in unvaccinated controls. Analysis of vaccine-induced immune responses showed robust $CD8^+$ cytotoxic T lymphocyte (CTL) responses in both mice and guinea pigs to GP but not NP, and both antigens generated potent IgG titers that correlated with survival. Because passive transfer of hyperimmune sera from these animals did not protect naïve recipients, it is likely that IgG titers of sufficient quality or magnitude were not achieved or that protection required effector functions not present in serum. Indeed, subsequent studies in mice and guinea pigs illustrated that Ebola virus genes delivered by either plasmid DNA or viral replicons likely elicited protection through a cell-mediated mechanism since passive immunization with vaccine-induced immune serum did not prevent mortality.[111,112] Increased survival was observed with immunization regimens that produced both humoral and $CD8^+$ CTL responses,[112,113] and depletion of $CD4^+$ or $CD8^+$ T cells in immune animals eliminated protective immunity against Ebola virus challenge in mice.[114,115]

Description of Current Vaccines and History of Development

Vaccine efficacy trials cannot be ethically conducted in humans for lethal pathogens such as Ebola virus, so the FDA in 2002 instituted a new rule, 21 Code of Federal Regulations (CFR) Parts 314 and 601, that allows approval of biological products based on "evidence of effectiveness derived from appropriate studies in animals." In place of human field efficacy trials, marketing approval for a new product can be based upon efficacy demonstrated in an animal species that has been shown to predict relevant responses in humans.[116] Ebola virus infection of cynomolgus macaques represents a model that satisfies the requirements for vaccine licensure because the disease time course, pathophysiology and clinical symptoms closely mimic those observed in human EVD,[9] making this NHP infectious challenge model the most appropriate for advancing potential vaccine candidates into humans. The strategy for Ebola virus vaccine development has been to identify the most potent vaccines in rodents that showed promise for testing in primates. This goal proved elusive for several years because successful immunization in the rodent model was not recapitulated in primate models.[117] Although recombinant vaccinia viruses or Venezuelan equine encephalitis virus replicons encoding EBOV GP showed some protection in guinea pigs (rodents) against Ebola virus challenge, protection was not observed in NHPs.[118,119] Lack of protection in monkeys was not a result of a species defect in the ability of primates to mount an effective immune response because animals that survive infection develop immune memory that protects against a subsequent infectious challenge.[120,121] This suggested that protection of NHPs required the generation of broader and/or higher levels of immunity. To develop a more potent vaccine for primates, investigators augmented gene-based immunization that had been shown to protect rodents[110] by employing a heterologous prime-boost strategy in which immune responses primed by inoculation with plasmid DNA vectors expressing Ebola virus GP and NP were boosted by a subsequent injection of replication-defective recombinant adenovirus (rAd) vectors encoding the same gene products. rAd boosting of DNA-primed cynomolgus macaques generated Ebola virus-specific cellular and humoral immunity, and vaccinated animals were completely protected from challenge with a lethal dose of Ebola virus, providing evidence for the first time that vaccination could elicit protective immunity against EVD in primates.[122] Since that observation, Ebola virus immunization strategies have yielded increasingly promising approaches to prevention of infection in primates.[123] A single inoculation with rAd encoding GP and NP from Ebola virus elicited protective immunity against homologous challenge within 4 weeks of vaccination,[124] raising the possibility of accelerated immunization during outbreaks.[125] This single-shot approach has been extended to other vaccine platforms including replication-competent vesicular stomatitis virus (VSV) vectors,[126] and with a two-dose human parainfluenza virus vectored vaccine.[127] Generation of Ebola virus-like particles (VLPs) is based on the self-associating properties of some EBOV structural proteins.[30,128] 293T-cell–derived VLPs containing EBOV GP, NP, and VP40 administered intramuscularly with RIBI adjuvant three times at 42-day intervals protected NHPs against challenge with approximately 1000 PFU (plaque-forming units) of EBOV. The vaccinated animals had no detectable viremia following challenge, although minor hematologic and liver-enzyme effects, and increases in antibody titers and T-cell repertoires were observed postchallenge, indicating a failure to induce sterile immunity.[129]

Each of the protective vaccine platforms has potential advantages and limitations for their development as a human vaccine. VLPs require a more complicated (multishot) regimen, may face challenges for bulk production,[8] and are less flexible than gene-based strategies for rapid adaptation to target newly emerging variants or multiple *Ebolavirus* species. Viral-vectored vaccines may present challenges for safety or manufacturing if they are replication competent, and all viral vectors may exhibit reduced potency in the context of natural or vaccine-induced immunity against the vaccine vector.[130–132] This latter issue has been mitigated for rAd vectors through the development of nonhuman-derived rAds exhibiting low seroprevalence in humans.[133,134] These vectors have shown success for immunization against various diseases: chimpanzee (Ch) Ad3 for Ebola virus[135] and cancer,[136] ChAd63 in combination with modified vaccinia Ankara (MVA) for malaria,[137] and ChAd32 in combination with rAd6 for hepatitis C virus.[138] Although alternative rAd vectors are insensitive to preexisting immunity in humans[133,136,139] genetic differences between adenovirus vectors result in vector-specific T-cell immunity qualities that associate with inferior protection in NHPs. A single injection of rAd26, rAd35 or ChAd63 was inferior for protection against challenge in NHPs.[134,135,140] Prime/boost combinations with less potent rAd vectors protected animals from challenge for a short period postvaccination but long-term protection was still found to be compromised, likely because of suboptimal priming of antibody and T-cell immunity.[125,134,135]

Despite previous failures of Vero cell–produced, formalin- or γ radiation–inactivated Ebola virus to protect against challenge in the guinea pig and NHP models,[105,117,141] inactivated, whole Ebola virus was reassessed for its capacity to protect NHPs. A single intramuscular injection of hydrogen peroxide–inactivated, replication-defective (VP30-deleted) whole Ebola virus completely protected cynomolgus macaques against challenge 4 weeks after vaccination.[142] However the potential risk associated with manufacturer error during the inactivation process or recombination events with replication-defective virus in an environment with circulating wild-type virus decreases the safety of this approach as a vaccination strategy.

An alternative vaccine platform in development is based on human parainfluenza virus type 3 (HPIV3). A two-dose regimen of a combined intranasal and intratracheal inoculation of NHPs with HPIV3 encoding EBOV GP showed complete protection against EBOV challenge 39 days after the second immunization.[127] A newer study found that a single aerosol dose of HPIV3-EBOV-GP conferred complete protection against EBOV challenge 27 days postvaccination in NHPs, although low-level viremia was detected in some animals.[143] Because HPIV3 is a common infection, preexisting immunity in the human population could affect the platform's effectiveness in people, as was seen during early development of the rAd5 platform.[144–147]

Because an outbreak situation would demand fast-acting vaccines, following the strategy used to eradicate smallpox virus in 1988,[148] rapid protection such as that observed with VSV and rAd Ebola vaccines[123,124,126,135,149–151] would be needed for effective ring vaccination during an outbreak. Inducing long-term protection would also be crucial for assuring that the vaccinated individual, especially healthcare workers, would be protected during the entire outbreak period. Durable immunity was maintained with a ChAd3/MVA prime-boost combination in NHPs, where protection was still observed following infectious challenge approximately 1 year after vaccination.[135]

Translational development of experimental vaccines for human use through clinical trial testing began in 2003 with a Phase I trial of the first lead candidate, rAd5-GP demonstrating safety and immunogenicity.[124] This vaccine strategy was not pursued because of the high prevalence of preexisting immunity against Ad5 in humans, potentially impacting the

TABLE 20.1 Filovirus Vaccine Clinical Phase I Trial (2003 to 2014)

Study Start Date (Year)	Institution (Country)	Vaccine Platform	Constituents	Dose	No. of Subjects	Trial Number	Reference
2003	NIH (U.S.)	DNA	(EBOV + SUDV) ΔTM GP	2 mg or 4 mg or 8 mg	21	NCT00072605	152
2006	NIH (U.S.)	Ad5 vectors	(EBOV + SUDV) mutant GP	2×10^9 or 2×10^{10} PFU	36	NCT00374309	157
2008	NIH (U.S.)	DNA	(EBOV + SUDV) WT GP or MARV WT GP	3 × 4 mg	20	NCT00605514	161
2009	NIH (Uganda)	DNA	(EBOV + SUDV) WT GP or MARV WT GP	3 × 4 mg	108	NCT00997607	159
2014	NIH (U.S.)	ChAd3 vectors	(EBOV + SUDV) WT GP	2×10^{10} or 2×10^{11} PFU	20	NCT02231866	158

This table represents the different filovirus vaccine platforms evaluated in humans for their safety and immunogenicity.
Ad, Adenovirus; ChAd, Chimpanzee adenovirus; ΔTM, transmembrane deletion; EBOV, Ebola virus; GP, glycoprotein; MARV, Marburg virus; mg, milligrams; NIH, National Institutes of Health; PFU, plaque-forming unit; SUDV, Sudan virus; WT, wild-type.

immunogenicity of rAd5-based vaccines.[144–146] This trial was followed by a strategy of iterative and parallel candidate vaccine testing in NHPs and humans over the course of the next decade (Table 20.1).[116,123,140] The extensive database of safety and immunogenicity of these early clinical trials enabled accelerated review of the lead candidates, ChAd3-GP and VSV-GP during the 2014 outbreak. The vaccines were tested at international clinical trial sites, and the transition to Phase I and Phase II trials was streamlined by enhanced collaboration across research institutions, regulatory agencies, and manufacturing sponsors, and culminated in the first human efficacy trial based on ring vaccination using VSV-GP (Table 20.2).[152]

Constituents

Of five possible *Ebolavirus* species that might be targets for immunization, EBOV and SUDV have been targeted for inclusion in a candidate vaccine because of their high incidence and pathogenic potential relative to the other species: BDBV,[47] TAFV, and RESTV. RESTV has not yet been shown to cause disease in humans, and only one case of TAFV has ever been reported. BDBV is a relatively recently identified ebolavirus species with a cumulative case total of 185 people. However, cross-protection against BDBV following EBOV/SUDV-GP-encoding DNA/rAd5 vaccination has been demonstrated, possibly the result of sufficient genetic similarity between the BDBV and EBOV/SUDV GP genes, and thus demonstrating the potential of vaccine strategies that target EBOV and SUDV species to be sufficient for protection against BDBV.[153]

Tests of a prototype vaccine in cynomolgus macaques showed that addition of the GP gene from the SUDV Gulu variant to the existing Ebola virus GP vaccine did not reduce efficacy against subsequent challenge with Ebola virus. The efficacy of the vaccine was not altered if the antigens were expressed by multiple vectors or coincidentally in a single vector.[135,154] Furthermore, elimination of the NP gene from the vaccine did not diminish protection, thereby simplifying the vaccine for future development in human trials.[125] The transmembrane form of GP was necessary to assure complete protection against Ebola virus but introduction of a point mutation to decrease the cytopathic effect of GP overexpression did not alter the vaccine's protective potency.[125] The emergence of the BDBV species in 2007 highlights the need for vaccines to be cross-protective against existing and future ebolavirus species. Encouraging signs of such broad cross-protection were observed using a DNA/rAd5 vaccine regimen containing EBOV and SUDV GP that demonstrated potent induction of T-cell responses and uniform protection against BDBV challenge in a NHP model.[153] The West African Ebola variant (Makona) showed 97% identity to the Mayinga variant (DRC outbreak), suggesting that a vaccine protecting against the Ebola virus DRC variant will also protect against the Makona variant.[1] Variant cross-protection has been demonstrated in recent studies using the VSV vaccine platform, using an infectious Makona challenge in NHPs vaccinated with GP from a Kikwit variant.[155,156]

Manufacturers and Producers

Ebola vaccines are developed through close collaboration between government public health agencies and pharmaceutical companies. The ChAd3 vaccine is supported by the United States National Institute of Allergy and Infectious Diseases (NIAID) and GlaxoSmithKline. The Public Health Agency of Canada and NewLink Genetics in association with Merck Vaccines USA are developing the VSV vaccine. Janssen Pharmaceutical Companies of Johnson & Johnson and Crucell Holland Bavarian Nordic are codeveloping a vaccine comprised of the rAd26 vaccine and MVA-BN Filo in a prime-boost combination.

Dosage and Route

The majority of NHP studies deliver Ebola vaccines by the intramuscular route. In the original heterologous prime-boost vaccine in primates, DNA vectors were administered at a dose of 8 mg per inoculation using a needle-free injection system, and delivery of rAd vectors was performed using a conventional needle and syringe at a dose of 10^{12} particle units (PU). A subsequent pilot dose-ranging experiment in cynomolgus macaques demonstrated that the single-dose rAd Ebola virus vaccine still achieved 100% protective efficacy with a 100-fold dose reduction to 10^{10} PU.[125] A later study similarly found that a single dose of ChAd3 10^{10} PU was sufficient to uniformly protect NHPs against EBOV infection after high-dose challenge.[135] This dose is within a range that is well tolerated in humans for other rAd systems expressing a variety of recombinant genes. The safety of intramuscular delivery of a single injection of rAd5 encoding GP from EBOV and SUDV at either 2×10^9 or 2×10^{10} was demonstrated in a Phase I clinical trial.[157] Moreover, formulation of the rAd vectors at a unit dose up to 10^{11} PU permits scalable and affordable manufacture of pharmaceutical grade vaccine, and corresponds to the dose of ChAd3-GP vaccine used in Phase I and Phase II clinical trials.[158] When MVA vector was administered to extend the duration of protective immunity to 1 year after a ChAd3 prime, an MVA dose of 1×10^8 PFU was used in both the monkey infectious challenge studies and human clinical trials.[135,152]

DNA vectors are stable and easily manufactured at the doses needed for the induction of immune responses in

TABLE 20.2 Filovirus Vaccine Clinical Trials (2014 to 2015)

Phase[a]	Vaccination Strategy	Vaccine Type	Insert	No. of Trials	Countries	No. of Subjects	Trial Numbers	References
I	Single injection or prime boost	ChAd3 MVA	EBOV GP or (EBOV + SUDV) GP or (EBOV + SUDV + MARV) GP	10	U.S./UK/Switzerland/ Mali/Uganda/ Senegal	877	NCT02231866 NCT02408913 NCT02240875 NCT02451891 NCT02267109 NCT02368119 NCT02289027 NCT02354404 NCT02485912	158, 172, 173
I	Single injection	VSVΔG	EBOV GP	7	U.S./Canada/ Germany/Kenya	782	NCT02269423 NCT02280408 NCT02283099 NCT02287480 NCT02374385 NCT02296983 NCT02314923	48, 167, 202
I	Single injection or prime boost	Ad5	EBOV GP or EBOV Makona variant GP	3	China	301	NCT02326194 NCT02533791 NCT02401373	192
I	Prime boost injection	Ad26 ChAd3 MVA	EBOV GP or (EBOV + SUDV + MARV) GP	6	U.S./UK/Tanzania/ Uganda/Kenya/ Ghana	929	NCT02495246 NCT02313077 NCT02376400 NCT02325050 NCT02376426 NCT02543567	152
I	Prime boost injection	Nanoparticles	EBOV GP	1	Australia	230	EBOV-H-101 NCT02370589	152
II	Single injection	ChAd3	EBOV GP	1	Senegal	2796	NCT02485301	152
II/III	Single injection	ChAd3 or VSVΔG	EBOV GP	1	U.S./Liberia[b]	28,170	NCT02344407	152
II/III	Prime boost injection	Ad26 MVA	EBOV GP (EBOV + SUDV + MARV) GP	2	Europe/Sierra Leone	1052	NCT02509494 NCT02416453	152
II/III	Single injection	VSVΔG	EBOV GP	2	Guinea[c]/Sierra Leone[d]	9200	NCT02378753	152, 195

Ad, Adenovirus; ChAd, Chimpanzee adenovirus; EBOV, Ebola virus; GP, glycoprotein; MARV, Marburg virus; MVA, modified vaccinia Ankara; SUDV, Sudan virus; VSVΔG, vesicular stomatitis virus deleted from G glycoprotein.
[a]Phase I, safety; Phase II, immunogenicity; Phase III, efficacy.
[b]Partnership for Research on Ebola Vaccines in Liberia (PREVAIL).
[c]"Ebola ça suffit!"
[d]Sierra Leone Trial to Introduce a Vaccine Against Ebola (STRIVE).

humans as shown by Phase I human studies that delivered the vaccine using a pressure injection device. A dose of 4 mg given three times at monthly intervals was shown to be safe and well tolerated.[159–161]

The VSV vaccine required 1 to 2 × 10^7 PFU of replicating vector for protection against challenge in NHPs,[162,163] and a similar dose was used during subsequent Phase I, Phase II, and Phase III clinical trials. In addition to the intramuscular injection route, some studies have evaluated the potency of Ad vectors delivered thought the respiratory tract or by sublingual administration but NHPs were only partially protected from an Ebola virus challenge by these alternative routes, thus favoring the use of intramuscular injection in vaccination.[164,165]

IMMUNOGENICITY OF VACCINES

Antibodies

To assess immunogenicity of Ebola virus vaccines, assays to evaluate humoral and cellular immune responses have been developed that allow bridging between animal efficacy studies and human clinical trials. Antigen-specific antibody responses are assessed using ELISAs to measure serum levels of IgG or IgM directed against Ebola virus GP. As measured by ELISA both the DNA and rAd components of the Ebola virus vaccine used alone or in prime-boost combination generated robust GP-specific Ig titers that correlated well with vaccine protective efficacy in the mouse, guinea pig and monkey models of infectious challenge.[110,122,124,125,140] In NHPs, antigen-specific IgG is detected by ELISA even when samples are diluted 1000-fold (10^3 titer) within 1 month after the last vaccination with three DNA inoculations, and the titers are boosted to 10^5 after subsequent inoculation with rAd. Accelerated immunization using a single rAd injection generates antibody titers that are generally observed to be somewhat lower than with the prime-boost regimen, in the range of 10^4, but peak titers are achieved earlier than with DNA immunization.[124] Three weeks after a single injection with ChAd3, IgG titers are on the order of 10^3 and increased after an MVA boost to peak at a titer of 10^5 4 weeks after the second injection.[135] Replicating VSV vectors generate antibody titers ranging from 10^2 to 10^4 2 to 3 weeks after a single immunization in NHPs.[149,163,166]

In a Phase I randomized human clinical trial, 20 of 20 vaccinees immunized with three doses of an Ebola virus DNA vaccine candidate generated positive antibody titers ranging from 1:30 to 1:4000 against at least one of the three vaccine-encoded antigens (Zaire NP, Zaire GP, and Sudan GP) as measured by ELISA irrespective of vaccine dose, which ranged from 2 to 8 mg of DNA.[160] A similar Phase I clinical trial testing a single 2×10^{10} VP dose of EBOV/SUDV-GP encoding rAd5 ($n = 23$) in humans detected antibodies, with titers peaking at 4 weeks postvaccination, against SUDV GP and EBOV GP in 100% and 55%, respectively, of vaccinated subjects.[157] More recently, a human study using a high dose of ChAd3 vector yielded titers on the order of 10^3 against Ebola virus GP, and showed cross-reactivity against SUDV GP and the Ebola virus Makona variant.[158] VSV vectors at a dose of 2×10^7 PFU also induce 10^3 titers against Ebola virus GP.[167] Except for the DNA vaccine human trial, which showed development of lower than expected antibody response 4 weeks after the last immunization ($\sim 10^2$ titer),[159,161] the range of antibody titers in the human trials was comparable to those observed in NHPs vaccinated with similar regimens, indicating that responses in the monkey model are predictive of human immune responses against ebolavirus antigens.

Cellular Responses

A role for cellular immunity in survival is supported by the observation of CTL activation approximately 10 days after onset of symptoms, in the recovery phase of infection.[41] Furthermore, early after symptom onset following SUDV infection, a greater number of activated $CD8^+$ T cells were detected in patients with a nonfatal outcome as compared to fatal cases, a result probably associated with increased clearance of infected cells.[43] More recently, the cellular responses in four EBOV-infected patients from the West African outbreak were analyzed and characterized.[168] While these patients were concurrently receiving several therapeutic treatments that could have affected progression of the natural immune response[168,169] and even though all four patients developed lymphopenia, activated B cells, $CD4^+$ and $CD8^+$ T cells were all observed from 10 days to as long as 2 months following onset of symptoms. $CD8^+$ T-cell responses were mostly specific for NP antigen, with more moderate responses against VP40 and very low-level responses against GP. Taken together, these data suggest that the immunosuppressive effects of EBOV previously reported, may be overcome in survivors.[170,171]

In an early study showing protection in macaques, proliferative $CD4^+$ T-cell responses were observed in DNA/Ad5 vaccinated NHPs.[122] In follow-up studies of a single-shot rAd vaccine, antigen-stimulated cytokine secretion was induced within 3 weeks of immunization in both $CD4^+$ and $CD8^+$ T lymphocytes.[124] Similar results were obtained in Phase I human trials testing Ebola virus GP vaccines delivered with rAd5 or DNA vectors; both $CD4^+$ and $CD8^+$ antigen-specific responses were detected in vaccinated study volunteers.[157,160] More recently, the quality of the $CD8^+$ T cells induced by Ad vectors was observed to correlate with survival in NHPs,[135,140] and this T-cell quality was also found to be conserved following vaccination in humans.[158,172,173]

Correlates and Mechanism of Protection

Vaccine development is facilitated by the identification of host immune responses that correlate with attenuated symptoms in exposed individuals. As a result of relatively small case numbers in historical Central African outbreaks and challenging conditions for clinical research, limited information was previously available concerning disease outcome and host responses in surviving cases. The much larger number of infected subjects in the latest West African outbreak is yielding new pathogenesis information and will hopefully provide novel observations regarding correlates of survival.[7,11,174–177] Data on individual immune responses are not yet available for the 2014 outbreak; however, it has been shown that serum from outbreak fatalities exhibited elevated proinflammatory cytokine levels, including interferon (IFN)-γ, interleukin (IL)-2, IL-10, tumor necrosis factor (TNF)-α, and IFN-α, as compared to levels in survivor or uninfected control sera, indicating that exaggerated and uncontrolled immune activation might be deleterious.[1,41,178–181] A study of convalescent patients showed a correlation between elevation in IgM or IgG levels and the disappearance of viral antigen,[63,182] suggesting that adaptive immune responses may have the potential to control viral loads during EVD. In studies of human cases from the 1996 Gabon outbreak, IgG against NP was detected in all survivors and against VP35 and VP40 in the majority of survivors after the onset of symptoms.[41,179] Interestingly, a study of the 2000 SUDV outbreak found that major histocompatibility complex class I genes (MHC-I) were a predictor of case outcome with a specific subclass of alleles of MHC-I, the human leukocyte antigen (HLA)-B locus, associated with higher virus load and fatal outcome.[183]

Although gene-based vaccines generate both humoral and cellular immunity, $CD8^+$ cellular immune responses appear to be crucial for protection against Ebola virus infection in experimental animal models. The potential importance of T lymphocytes was initially demonstrated by studies in rodent models using an adapted Ebola virus variant. Unfractionated T-lymphocyte transfer achieved protection from infection with an efficacy in recipient mice that was comparable to that in immunized donors.[112] In later studies, it was observed that knockout mice lacking specific T-cell effector functions were more susceptible to lethal infection than their wild-type counterparts,[114,184] and that adoptive transfer of Ebola virus antigen-specific CTL to naïve mice protected against subsequent infectious challenge.[112] In addition, early studies with DNA and rAd vaccines indicated a trend toward greater survival in animals exhibiting higher $CD8^+$ T-cell responses.[125] More recent studies employing immunodepletion techniques have identified $CD8^+$ T cells as the critical cellular component responsible for virus clearance in vaccinated NHPs and studies using passive transfer of antibodies failed to protect the animals from lethal challenge.[185] The depletion antibody used in this study (anti-CD8α) may have also depleted natural killer (NK) cells. Because NK cells are known to have an important role in innate immune responses and antibody-dependent cell-mediated cytotoxicity, the possibility of NK cell involvement in the mechanism of protection cannot be excluded. Furthermore, cross-protection against BDBV infection in NHPs vaccinated with an EBOV-specific DNA/rAd vaccine was achieved in the absence of BDBV-directed antibodies, reinforcing the requirement for T cells in vaccine-induced protection.[153]

However, the magnitude of T-cell responses is not the sole determinant of protective immunity because some vectors elicit robust antibody and T-cell responses but fail to afford uniform protection. In these cases, a specific quality of $CD8^+$ T cells capable of simultaneous secretion of IFN-γ and TNF-α upon antigen stimulation and performance of effector functions associates with protection.[186,187] Durable protective immunity requires this population in combination with a sufficient proportion of cells comprising a memory phenotype that secrete three cytokines simultaneously, IFN-γ, TNF-α, and IL-2.[140,188] This population of polyfunctional $CD8^+$ T cells has also been observed after ChAd3-GP injection in NHPs and in human vaccinees.[135,140,158,172,173] The role of cellular responses

in VSV EBOV vaccine protection is less clear. In some studies there appears to be a role for cellular immunity, as some animals survived in the absence of measureable antigen-specific IgG.[162] In contrast, animals depleted of $CD8^+$ T cells survived EBOV challenge; however, $CD8^+$ T-cell depletion was at the time of vaccination.[189] Because vaccination was several weeks before challenge, it is possible that antigen-specific cellular responses from residual nondepleted or rebounding T cells may have contributed to protection. This is supported by simian immunodeficiency virus (SIV) vaccine studies where rebound of $CD8^+$ T cells was observed within a week following infusion of anti-CD8 antibody.[190]

While $CD8^+$ cellular immunity is central to the mechanism of protection, rAd-based Ebola virus vaccines have demonstrated that GP-specific IgG ELISA titers provide a strong immune correlate of protection and provide a statistically significant marker of the overall protective immune response induced by vaccination. More specifically, studies in NHPs with rAd5-based vaccines demonstrated that an antibody titer of 1:3700 was predictive of 100% protection.[116] The strength of the ELISA correlate likely reflects the robust specificity and sensitivity of assays for IgG, and the cumulative nature of the antibody response. Concerning the VSV-based Ebola virus vaccines, it has been inferred that antibodies play a role in protection; however, depletion of circulating $CD20^+$ lymphocytes did not abrogate protection, and passive transfer which would show a definitive role of vaccine-induced antibodies, was not performed. Depletion of circulating $CD4^+$ lymphocytes did abrogate protection, but lack of protection cannot be definitively ascribed to deficits observed in the humoral immune response because $CD4^+$ T cells play an important role in both humoral and cellular immune responses.[189] A quantitative correlate of protection in terms of antibody titer has not been reported for the VSV-EBOV vaccine.

The current body of data suggests that Ebola vaccine antibody and T-cell responses are tightly linked, and the magnitude and quality of both the humoral and cellular immune responses determine protection against Ebola virus in the NHP model. The level of IgG response is an easy parameter to measure and to correlate with survival, but because T-cell quantity and quality affect survival, further evaluation of T-cell responses are also necessary.[135,140]

EFFICACY OF VACCINES

By the beginning of 2015, the number of infected people had been dramatically reduced giving hope for the end of the outbreak; but on the other hand, it has become more difficult to assess the efficacy of the different vaccines in the Phase I and Phase II clinical trials initiated during the outbreak.[48,158,159,161,167,172,191,192]

Although the window has closed for efficacy trials in the absence of high case numbers, clinical trials originally established to evaluate efficacy of ChAd3 and VSV Ebola vaccines in West Africa will continue as Phase II and survivor follow-up studies (see Table 20.2). Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) was a Phase II/III, randomized controlled trial (RCT), with three arms: ChAd3, VSV, and a placebo conducted in a randomized and double-blind fashion.[193] The second trial, Sierra Leone Trial to Introduce a Vaccine against Ebola (STRIVE), was a Phase III study of the VSV vaccine. This clinical trial was a nonblinded, randomized trial that was changed from a stepped-wedge cluster trial (SWCT) into an RCT because of concerns that the original study design was complicated by regional variation in EVD incidence within the population, and therefore lacked the statistical power to determine true efficacy.[194] Finally, a Phase III ring vaccination trial conducted in Guinea for the VSV vaccine, "Ebola ça suffit" ("Ebola it's enough") used a study design that allowed all participants to receive the vaccine, by randomly assigning clusters to either immediate or delayed vaccination. An interim analysis reported that in clusters of contacts vaccinated immediately, there were no cases of EBOV with onset more than 10 days after entry into the study, whereas in the clusters vaccinated 21 days after randomization there were 16 cases, for an early point estimate of 100% efficacy.[195] The authors' reported a *P* value of .0036 for the estimated vaccine efficacy. However, this value did not fall below the predetermined threshold of success of .0027.[196,197] The absence of a placebo control group in this trial means that at least 190 clusters (95 clusters per arm) of approximately 50 subjects each would be required to evaluate true efficacy, numbers that will be difficult to attain with the decline of the West African outbreak.[195,198]

An initial efficacy signal is suggested by preliminary Phase III results but, in the absence of placebo-controlled studies, it is probable that vaccine efficacy determinations will have to continue in NHP models to support licensure using the Animal Rule or Accelerated Approval regulatory pathways.[116,199,200] In these pathways, immune responses will be bridged between NHP protection studies and the level of immune responses observed in human clinical trials.

Once a licensed vaccine becomes available, immunization of primary responder health professionals who are at risk of infection may permit further assessment of vaccine efficacy and evaluation of the durability of protection should another outbreak occur in the future.

DURATION OF IMMUNITY

The durability of Ebola virus vaccine-induced protective immune responses is best addressed in prospective long-term follow-up of immunized subjects, but requires empirical determination of an immune correlate in NHPs who are challenged following a long delay from the time of vaccination. In human volunteers immunized with DNA vectors only, both humoral and cellular Ebola virus GP-specific immune responses were detected through 52 weeks of follow-up, with antibody titers waning over time, with a half-life of approximately 6 months.[160] The duration of the antibody response may be enhanced by subsequent boosting of DNA vaccine recipients with rAd as has been shown in monkeys immunized with SIV antigens.[201] In these studies, antigen-specific antibody titers were maintained at constant levels for greater than 2 years following the rAd boost, and cellular responses, although decreasing somewhat relative to immediate postboost levels, were sustained at a magnitude greater than post-DNA prime responses. Similarly for the Ebola virus vaccine, rAd inoculation of DNA-primed cynomolgus macaques yielded antibody titers that were boosted by greater than 100-fold over those obtained with DNA or rAd alone.[122,125] Furthermore, DNA primed immunologic memory was durable for at least 1 year.[153] A single injection of ChAd3 provided 50% protection after 10 months in NHPs; a boost with MVA, 2 months after the ChAd3 injection increased the durability to 100% protection during 10 months, with a sustained high T-cell quality comprising both effector and memory T-cell responses.[135] In addition, ChAd3 vaccination of human subjects induces specific IgG responses up to 6 months after a single immunization or up to 5 months after a ChAd3/MVA prime boost combination.[172,191] The antibody kinetics will need to be followed yearly and the level of antibody responses will also need to be correlated with survival probability. Persistent immunity for the VSV-EBOV vaccine remains to be determined since data have not been reported regarding long-term

protection in NHPs or durability of antibody titers in human vaccinees.

SAFETY

In Phase I human trials, DNA and Ad5 vector Ebola virus vaccines proved safe and well-tolerated.[157,160] Minor injection site symptoms included pain or tenderness following injection using a needle-free injection system, and systemic symptoms reported by vaccinees were mild to moderate headache, myalgia, malaise, and fever; however, these symptoms were also reported by placebo controls. In a more recent clinical trial with study sites in the United States and Uganda, in which subjects received three doses of DNA vaccine, some participants reported mild to moderate pain at the injection site, headache, muscle ache, fatigue, or aching joints but these symptoms resolved rapidly.[159,161] In the ChAd3 trial, the majority of symptoms reported at all study sites (United States, Europe [United Kingdom and Switzerland], and Africa) were mild to moderate fever (resolved in 1 day), mild pain at the injection site, chills, headache, and fatigue, resolving within a day.[158,172,173,191]

In general, the EBOV VSV vaccine tested in the United States, Europe, and Africa showed mild to moderate injection site pain, fever, headache, myalgia, and fatigue.[167] However, at the Geneva, Switzerland study site, some subjects developed oligoarthritis, in association, in some cases, with vesicular dermatitis, skin vesicles containing VSV virus, and, in rare cases, some cutaneous vasculitis, all appearing during the first 2 weeks after vaccination[48,202]

PUBLIC HEALTH CONSIDERATIONS

An Ebola virus vaccine is indicated primarily for populations that can be identified as being at high risk for exposure to the virus, such as bushmeat hunters and others who are exposed to the natural virus reservoir in the forest, or international or local healthcare workers who are first responders during outbreaks. Although the mode of virus transmission to hospital personnel has not been definitively proven, exposure of healthcare professionals likely occurs through mucosal surfaces. Strict compliance with barrier and universal precaution practices can reduce exposure, but cases continue to occur because first responders are placed into direct contact with viremic patients under challenging conditions, often in temporary field hospitals and/or prior to diagnosis.[58] The discovery of Ebola virus in swine has raised considerable concerns since the proximity of humans and pigs in farms may favor adaptation to a more lethal form, and suggests that pig farmers may be potentially at risk. Laboratory workers who conduct research with infectious Ebola virus also constitute another risk group. Although research personnel perform experiments wearing protective suits under Biosafety Level 4 biocontainment, exposures have occurred in this population, usually involving accidental needle sticks. Finally, in the event of an Ebola virus-based bioweapon or bioterrorist attack, the ability to have immunized military personnel or first responders would be beneficial.

Until 2012, outbreaks of EVD have remained geographically localized and relatively contained, but since 2014, the high transmissibility and mortality of ebolaviruses, with their demonstrated ability to spread from human to human, has placed it among the most serious of emerging infectious diseases from the perspective of public health. In Africa, the endemic area of infection in the equatorial rain forest lies within reasonable proximity of two highly populated cities, Kinshasa and Brazzaville. Freetown and Conakry in Sierra Leone and Guinea respectively, all affected by the 2014 outbreak, are also important centers of West African economy and commerce. A formerly unrecognized public health concern has arisen with the discovery of virus remaining in "cured" subjects for weeks or months after resolution of EVD.[64,65,67,203] Therefore, close contacts of survivors may comprise a new risk group that should be considered in any vaccination strategy.[66,204]

The modern ease of international travel can facilitate spread of Ebola virus, as illustrated by imported cases on two occasions of MARV first from a Dutch traveler and later from an American traveler, both returning from Uganda. In the most recent outbreak, again a traveler was subsequently diagnosed as infected following his return to Texas from Liberia.[174,205,206] Exposure of infected individuals to a high-density population could result in a catastrophic outbreak. Fortunately, in the recent outbreak, new cases of EVD from imported patients in the United States were efficiently curtailed through rapid patient identification, isolation, and aggressive follow-up, rapidly limiting the potential for disease spread. More positively, the 2014 outbreak created a worldwide movement fostering international collaboration between laboratories and pharmaceutical companies, resulting in the rapid construction of research laboratories and hospitals and the development of coordinated treatment and vaccine strategies. Despite these advances, more effort is still needed to develop a healthcare emergency response system (PPE and treatment) within endemic areas, and to optimize protocols for rapid contact tracing and efficient mobilization of fully trained medical teams in the case of future outbreaks.

Finally, in addition to the public health concerns raised by ebolaviruses, previous reports of weaponization of the virus in the former Soviet Union raise concerns about the possibility of its use as a military threat or a bioterrorist agent.[207–210] In the case of either natural or deliberate introduction of these viruses into any population, it is important to recognize not only the potential loss of human life but also that fear and chaos caused by this highly aggressive, visible infection ultimately lead to economic disruption and a breakdown of social order if not swiftly and effectively countered.[211,212]

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References for this chapter are available at ExpertConsult.com.

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21 Enterovirus 71

Pele Choi-Sing Chong and Michel Klein

Enteroviruses (EVs), which have been classified into four species (A, B, C, and D), are positive-sense, single-stranded RNA viruses within the *Picornaviridae* family. They cause various clinical manifestations, including severe neurological diseases in young children.[1–4] More than 100 EV serotypes have been identified including polioviruses, coxsackieviruses A (CV-A), coxsackieviruses B (CV-B), echoviruses, and numerous EV serotypes. Since 1990, EV-A infections have become the primary cause of hand, foot, and mouth diseases (HFMDs). Since the almost complete eradication of poliovirus, EV-A71 has emerged as a major neurotropic virus responsible for severe neurological complications and has become a serious public health threat to infants and young children across the Asia-Pacific region.

HISTORY OF DISEASE

EV-A71 strain, BrCr,* was isolated in California in 1969 from the brain of a patient with encephalitis, although the emergence of the virus can be traced back to 1941.[5,6] Sporadic epidemics have since occurred worldwide, in particular, in Asia.[1–4,7–9] Major HFMD epidemics in Malaysia (1997), Taiwan (1998), Singapore (2008), and China (2008) were associated with severe neurological complications and fatal outcomes and prompted several countries to establish national surveillance programs for HFMD and/or EVs.[1–4] More than 10 million cases of HFMD with more than 3000 fatalities were reported in China between 2008 and 2012.[10,11] As a consequence of the considerable socioeconomic impact of EV-A71 infections on healthcare systems and the absence of an effective treatment, the urgent development and clinical evaluation of EV-A71 vaccines have been a national priority in some Asian countries such as China, Taiwan, Singapore and Malaysia.[4,12]

CLINICAL DESCRIPTION

EV-A71 preferentially infects infants and children younger than 5 years of age. However, approximately 70% of infections remain asymptomatic.[1–4] EV-A71 together with coxsackievirus 16 (CV-A16) is a major cause of herpangina and HFMD. HFMD is typically a self-limiting illness characterized by a papulovesicular or maculopapular rash, blisters of the hands, soles, and buttocks associated with painful ulcerative lesions of the mouth. It is highly contagious and efficiently propagated through household contacts by oropharyngeal secretions or fecal–oral transmission depending on the level of hygiene, water quality, and the extent of crowding.[13] EV-A71 infections may cause severe neurological disorders, including brainstem encephalitis, that can lead to fulminant neurogenic pulmonary edema associated with high mortality.[14] The average yearly fatality rate of patients with severe disease in Taiwan was reported to be 13%.[15] Children who recover from brainstem encephalitis are left with significant neurologic sequelae, including long-term impairment of neurological development and cognitive functions.[1–3,8,14–19] Symptomatic EV-A71 infections progress through four stages: HFMD/herpangina (Stage 1), central nervous system (CNS) involvement (Stage 2), cardiopulmonary failure (Stage 3), and convalescence (Stage 4).[20,21] EV-A71 elicits both humoral and cellular responses, but there is no correlation between neutralizing antibody levels and disease severity. This indicates that altered cellular responses, such as an imbalance in T-helper (Th)1/Th2 and Th17/regulatory T cell (Treg) ratios, play a significant role in disease outcome and are of potential prognostic value.[22–25] The presence of neutralizing antibodies did not reduce the incidence of infections caused by EV serotypes other than EV-A71.[26]

VIROLOGY

EV-A71 has been divided into three genetic lineages: A, B, and C; however, four new genotypes have been recently identified as D, E, F, and G.[4,12,27] After infection, viral RNA is translated into a polyprotein containing three regions: P1, P2, and P3 (Fig. 21.1). P1 encodes four viral structural proteins—VP4, VP2, VP3, and VP1—that are released by proteolytic cleavage (Fig. 21.1). The VP1, VP2, and VP3 proteins are exposed on the virion surface in its crystal structure[4,28] and are responsible for immune responses and host-receptor binding. VP1 contains the major neutralization epitopes and is used in viral identification and evolutionary analyses.

Pathogenesis as It Relates to Prevention

Sites of Infections

EV-A71 can infect a wide spectrum of cells. Virus tropism and pathogenicity depends on host-cell surface receptors and cellular *trans*-acting factors.[29–31] The identification of these receptors and the availability of transgenic mouse models have facilitated the understanding of EV-A71 pathogenesis.[32–37] Human skin and mucosal lesions likely result from direct infection of squamous cells. The tonsillar crypt epithelium supports viral replication and may be the source of replication at other sites, as well as of viremia, leading to neuroinvasion, possibly via a retrograde peripheral motor nerve route.[38–41] EV-A71 infection triggers apoptosis of brain-derived neural progenitor cells responsible for neurological sequelae.[42]

Host Receptor Factors

EV-A71 enters host cells via receptor-dependent mechanisms. Infected host cells produce both infectious and defective particles as shown in Fig. 21.1.[43–45] Cell-to-cell propagation contributes to pathogenesis. EV-A71 uses the ubiquitous human scavenger receptor class B, member 2 (hSCARB2) and the P-selectin glycoprotein ligand 1 (PSGL-1) to infect host cells. hSCARB2 is capable of viral binding, uncoating, and internalization, resulting in high EV-A71 infectivity. In contrast, the low infection efficiency of PSGL-1 expressing cells is a result of the inability of this receptor to induce viral uncoating.[35,37] Because of its ubiquity, hSCARB2 likely mediates systemic and neural cell infections. Indeed, hSCARB2 transgenic mice develop encephalitis after EV-A71 challenge.[36,37] PSGL-1, a sialomucin

*The nomenclature of EV-A71 strains is defined as patient's initials/year of isolation. For more information, see http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

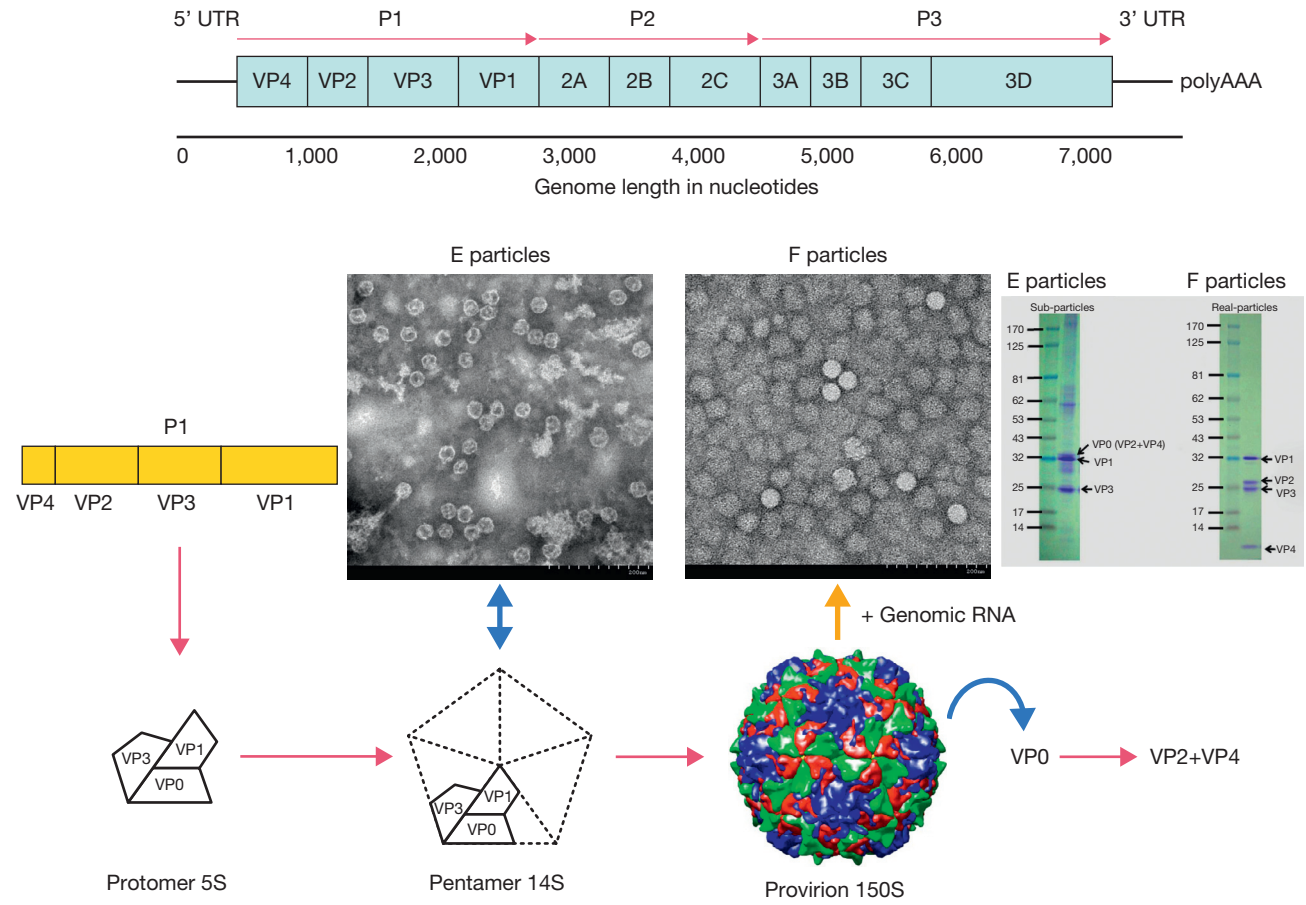


Figure 21.1. Schematic flowchart of EV-71 assembly. The single RNA transcript is translated into a polypeptide containing the P1, P2, and P3 domains. As shown in the figure, P1 is proteolyzed into the structural proteins VP0, VP1, and VP3. These proteins assemble into protomers (5S), five of which self-assemble to form a pentamer. Pentamers (14S) assemble into the provirion by procapsid self-assembly followed by progeny genome packaging. The final step of maturation involves the cleavage of VP0 to generate VP2 and VP4 and produce F-particles. A few purified E-particles (1/1000) contain viral RNA and VP0 (Liu CC et al., unpublished results). *(Modified from Cifuente JO, Lee H, Yoder JD, et al. Structures of the procapsid and mature virion of enterovirus 71 strain 1095.* J Virol. *87:7637–7645, 2013.)*

membrane-bound protein expressed on leukocytes, plays an important role in the early stage of inflammation. Replication of EV-A71 in endothelial cells, leukocytes, and dendritic cells result in the production of proinflammatory mediators. Extensive inflammatory responses with abnormal interleukin (IL)-6, IL-10, IL-13, and interferon (IFN)-γ cytokine levels as well as an increase in the levels of interferon-inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), monokine induced by interferon-γ (MIG), and IL-8 chemokines in serum and cerebrospinal fluid (CSF) appear to be involved in the pathogenesis of EV-A71 brainstem encephalitis, autonomic nervous system (ANS) dysregulation, and pulmonary edema (PE).[46–49] Annexin-2, DC-SIGN, sialylated glycans, and heparin sulfate are other receptors that also play a role in EV-A71 pathogenesis.[50–52] The administration of intravenous immunoglobulins (IVIg) and milrinone has been shown to modulate inflammation and reduce ANS and PE.[46] The use of monoclonal antibodies against hSCARB2 and DC-SIGN, polycationic poly-D-lysine and/or an anti–heparin sulfate peptide could potentially inhibit viral entry and limit EV-A71 infection.[51]

EV-A71 Genotypes and Disease Severity

The relationship between EV-A71 genotypes and disease severity remains controversial. There is some evidence that specific mutations in the 5′ UTR (untranslated region), as well as in the VP1 and protease 2A coding regions,[53–57] are critical determinants of neurovirulence. Subgenotype C1 is rarely the cause of CNS complications,[58,59] whereas C2 was strongly linked to severe neurological disease.[3,59] Although subgenotype B4 was found to be less pathogenic than other subgenotypes in Malaysia,[60] other studies report that genotype B viruses are more likely responsible for neurological complications than genotype C viruses.[5] In Taiwan, EV-A71 genotypes B and C have been isolated from both mild and fatal HFMD cases.[61–63] Therefore, more careful and systematic analyses will be necessary to determine whether there is a correlation between EV-A71 genotypes and disease severity.

Prevention and Control Measures

Prevention and control measures of EV-A71 infections are primarily nonpharmaceutical, and meant to interrupt the chain of virus transmission using strict hygiene practices and avoiding contact between infectees and exposed individuals. Based on the World Health Organization (WHO) report, HFMD is a notified infectious disease in Japan (1993), Taiwan (1998), Malaysia (1998), Singapore (2000), and China (2009).[64] Early detection of outbreaks through physician-based sentinel clinics and automated alert and response systems to recognize the rare but severe forms of the disease are among the key

principles applied to minimize morbidity and mortality. WHO has proposed a risk-assessment framework[64] that defines criteria for appropriate interventions. However, an efficacious prophylactic vaccine is the best option to prevent EV-A71 infection and disease.

Diagnosis

Viral diagnosis is critical to confirm the EV-A71 nature of HFMD. Sample collection includes swabs from throat/nasopharynx, ulcers, and rectum, stools specimens, and samples from sterile sites such as vesicle fluid, CSF, serum, and urine. Virus isolation and identification are generally laborious and time-consuming to perform. Some clinical specimens require appropriate pretreatment before cell inoculation. Following virus isolation, the EV-A71 serotype can be identified by a conventional neutralization assay using "in house" reagents. However, reverse-transcription polymerase chain reaction (RT-PCR) amplification is the method of choice for the molecular identification of the virus.[43,65–67] It is a rapid, simple, and inexpensive method with 80% or greater sensitivity for blood specimens.[68,69] EV-A71–specifc primers should be adapted to the sequences of newly emerging EV-A71 genotypes and variants.[43,66] The indirect immunofluorescence assay (IFA) can also provide rapid identification,[70] but is relatively expensive.

An EV-A71 reference virus bank and reference reagents (broad neutralizing antibodies) are critical to the establishment of quality assurance system and test harmonization between laboratories to ensure the validity of serotyping and/or genotyping results in the future. The development of high-throughput multiplex polymerase chain reaction (PCR) assays for the simultaneous identification of multiple clinical EV isolates should greatly facilitate molecular epidemiology investigations in the future.[71,72]

Antiviral Drug Development

Currently, there is no approved antiviral drug against EV-A71. Pleconaril, a viral capsid inhibitor active against a number of EVs is inefficacious against EV-A71.[73]

EPIDEMIOLOGY

Incidence and Prevalence Data

Two patterns of epidemics have been reported since EV-A71 isolation in 1969: small-scale sporadic outbreaks with limited severe complications and large-scale epidemics with frequent CNS complications and deaths.[1,3,5,6,8,74–76] Fig. 21.2 summarizes the global incidence of large-scale epidemics with fatal outcomes observed in Europe, Malaysia, Taiwan, Singapore, Japan, Korea, China, and Vietnam. Japan was the first country in Asia to establish a comprehensive EV surveillance program with symptom-reporting and virus identification using molecular typing (http://idsc.nih.go.jp/iasr/index.html). Two

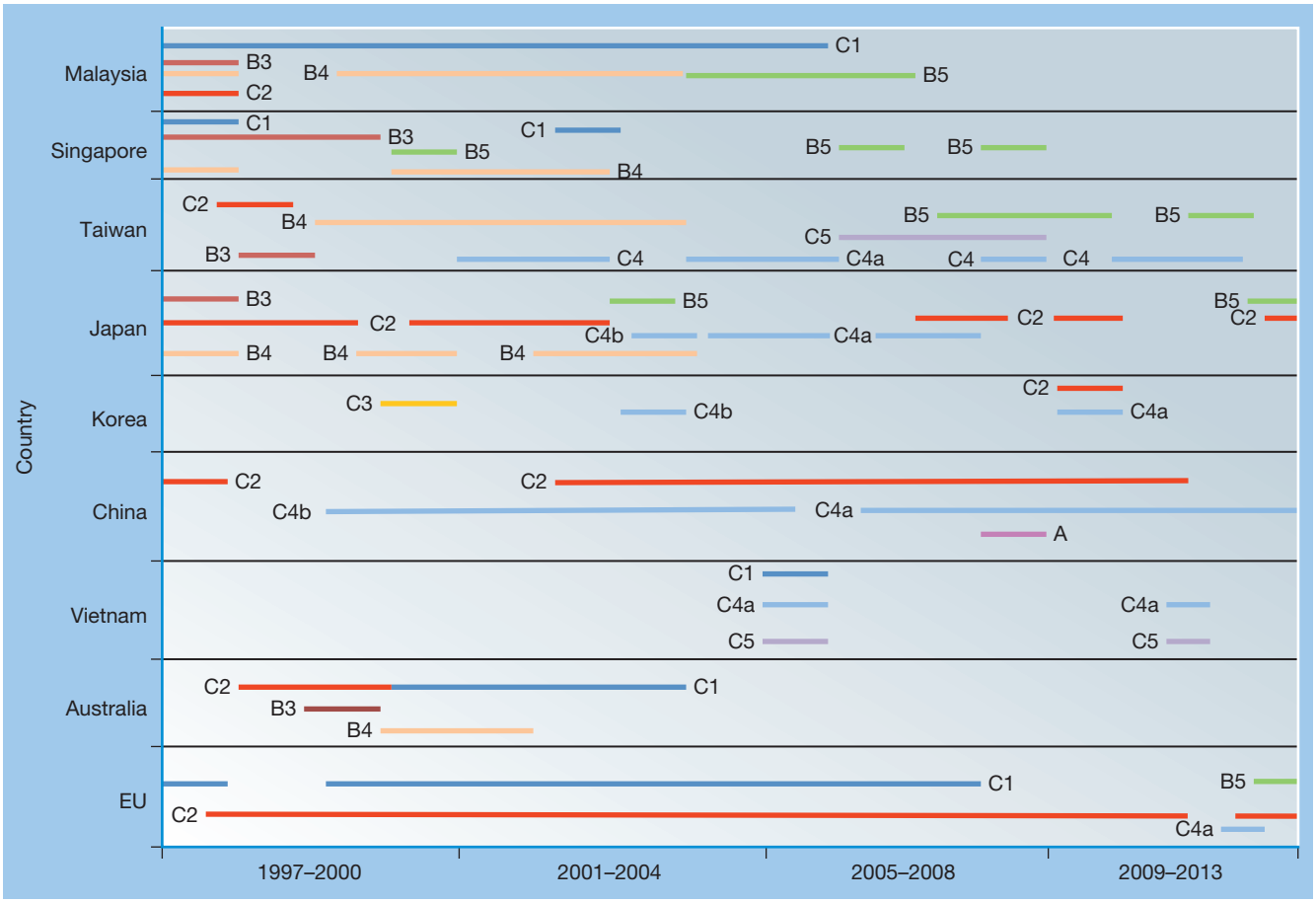


Figure 21.2. Geographic distribution of prevalent circulating EV-A71 genotypes and subgenotypes from 1997 to 2012. The annual distribution of EV-A71 genotypes and subgenotypes reported in this figure is a compilation of references 1, 3, 4, 12, and 126. EU includes the Netherlands, United Kingdom, Germany, France, Denmark, and Austria.

large-scale outbreaks occurred in 2000 and 2003.[75] While EV-A71 epidemics occur every 2 to 3 years, CV-A16 is more frequently the prevalent serotype.[77] Since 2000, Taiwan has a surveillance system for disease-reporting with systematic sampling of clinical specimens for virus isolation.[74,75] EV-A71 was among the top five EV serotypes isolated each year during the period 2000 to 2009 and was responsible for the most severe cases of HFMD. In Malaysia, EV-A71 epidemics occurred every 3 years while CV-A16 was detected during both epidemic and interepidemic periods.[75–78] In Singapore, large HFMD outbreaks were detected in 2002 and in 2005–2007, and nearly 30,000 cases were reported during the largest epidemic in 2008 with CV-A6 and EV-A71 being the predominant strains, followed by CV-A10.[79,80] In China, EV-A71 was the predominant serotype in the first HFMD epidemic in 2007 responsible for more than 80,000 cases, including 17 deaths (http://www.chinacdc.cn/). From 2009 to 2012, the surveillance registry recorded more than 10 million cases of HFMD of which more than 3000 were fatal.[9–12]

Clinical and Molecular Epidemiology

There is only one EV-A71 serotype, but EV-A71 has now been classified into seven genotypes: A to G.[4,27,81] Genotype A contains only the prototypic strain (BrCr). Genotypes B and C have six and five subgenotypes, B0 to B5 and C1 to C5, respectively. No association could be established between genotype and disease severity.[3] Since the 1990s, recurrent HFMD epidemics with neurological complications and fatalities have been reported in Europe and the United States.[1–6] The most severe EV-A71 epidemics spread through the Asia-Pacific region, including Australia, China, Hong Kong, Japan, Malaysia, Singapore, Taiwan, and Vietnam (see Fig. 21.2).[1–6] The first major EV-A71 outbreak occurred in 1997 in Malaysia where cocirculating neurovirulent B3, B4, C1, and C2 genotypes were responsible for 41 deaths among young children.[82] In Singapore, 30% of HFMD cases that affected thousands of children over a period of 7 years were caused by EV-A71, and subgenotypes B5 and C2 caused the largest epidemic in 2008.[80] Outbreaks of HFMD with severe neurological complications and herpangina that occurred in Japan between the late 1990s and 2013 were attributed to four cocirculating genotypes B4, B5, C2, and C4.[83] Between 2000 and 2011, Taiwan's CDC (Center for Disease Control) reported that between 93,000 and 140,000 cases of HFMD and herpangina occurred annually. EV-A71 was associated with 82% of severe cases. A major epidemic of EV-A71 subgenotypes C2 and B4 that caused 1.5 million infections and 78 deaths in 1998 was followed by smaller outbreaks of switching genotypes (Fig. 21.2).[84] More than 7 million HFMD cases caused by subgenotype C4 infections were reported in China between 2008 and 2012 of which more than 2450 were fatal.[10] Recently, subgenotype C5 was observed in severe pandemics associated with high mortality rates and spread through Vietnam in 2011 and Cambodia in 2012.[3,12]

Switching Genotypes and Genetic Recombination

EV-A71 epidemics occur cyclically every 2 to 4 years as a result of infections with single or multiple genotype(s)/subgenotype(s) and the unpredictable switching of genotypes/subgenotypes (see Fig. 21.2).[3,84,85] The cocirculation of several EV-A71 genotypes and CV-A16 during epidemics has been responsible for the intertypic and intratypic genetic recombinations responsible for the emergence of new variants.[3,83,85] Nearly all major HFMD outbreaks correlate with genetic variations caused by EV-A71 switches.[3,83–86] Thus a continuous monitoring of antigenic variation and genetic evolution is critical for epidemic control and vaccine design.

High-Risk Groups and Risk Factors

EV-A71 infections occur annually or semiannually depending on the country.[1–9] Their incidence is influenced by warmer temperatures and elevated humidity. The cyclic occurrence of epidemics in Malaysia coincides with new, naïve birth cohorts.[77–80,82] Human leukocyte antigen (HLA)-A33 and HLA-A2 have been linked to disease susceptibility and cardiopulmonary failure, respectively.[87] In general, children older than 2 years of age who are attending kindergarten or child-care centers, as well as coming from a family with more than 2 children and living in a rural area, were found to be risk factors for EV-A71 infections.[13,88,89] Only an early diagnosis can significantly reduce the risk of severe HFMD.[89–93]

SIGNIFICANCE AS A PUBLIC HEALTH BURDEN

Newer studies demonstrate that EV-A71 infection could cause long-term neurological sequelae.[90] In a prospective hospital-based case-finding study, 38 (21%) of 183 EV-A71 infections in children younger than 18 months of age developed neurological complications such as meningitis and encephalitis.[21] Based on national EV surveillance and two cross-section serological surveys, Lu and colleagues estimated that 130,617 Taiwanese children younger than 3 years of age were infected with EV-A71 in 1998 and that 273 (0.21%) developed neurological complications.[93] EV-A71 mortality rates heavily depend on healthcare accessibility and standards.

PASSIVE IMMUNIZATION

The indication for passive administration of IVIg is based on the assumption that immune sera have not only potential virus-neutralizing activity[64,68,69,94,95] but also nonspecific anti-inflammatory properties that could be beneficial in case of ANS dysregulation and PE associated with the production of proinflammatory cytokines (IL-6, IL-10, and IL-13), chemokines (IL-8, IP-10, MCP-1), and possibly MIG.[47–49,64,94–96] Their therapeutic effect may be IgG subclass, IgG concentration, and genotype dependent.[95] Although the early administration of IVIg had beneficial immunomodulatory effects in children with ANS dysregulation and brainstem encephalitis by reducing proinflammatory cytokine levels, and viremia,[13,90,94–97] its therapeutic activity remains controversial. The production of hyperimmune IVIg from Chinese donors with high titers of EV-A71 neutralizing antibodies could be a promising development; an early treatment with IVIg from selected donors in the mouse model showed a dose-dependent reduction in mortality.[94] However, the use of IVIg in significant infusion volume is not without risk and is very costly. Thus, IVIg efficacy needs to be unambiguously demonstrated before their use is recommended for passive immunization.

ACTIVE IMMUNIZATION

History of Vaccine Development

First EV-A71 Vaccine Candidate

During the epidemic in 1975 in Bulgaria, an inactivated EV-A71 whole-virus vaccine candidate was produced in Moscow. The vaccine was well tolerated and immunogenic in children 1 to 4 years of age,[98] but in the absence of subsequent outbreak, the vaccine was not evaluated for clinical efficacy.

Recent EV-A71 Vaccine Development

To avoid the risk of potential reversion to virulence of attenuated vaccines, chemically inactivated virion, synthetic peptides, recombinant subunits, virus-like particles, genetic and vector-based vaccines have been the preferred approaches to EV-A71 vaccine development.[4,9–12,75] In the absence of standardized antigens and immunological assays to assess the potency of vaccine candidates, it has been difficult to compare the efficacy of immune responses induced by prototypic EV-A71 vaccines. We used "in-house" standardized viral antigens and microneutralization assays to compare the immunogenicity of vaccine candidates produced by different platform technologies.[99] Vaccines were ranked in order of potency and efficacy: formalin-inactivated virion > recombinant virus-like particles > recombinant VP1 > synthetic neutralization epitope (VP1–43).

Synthetic Peptide-Based EV-A71 Vaccine

Synthetic peptides containing neutralization epitopes are safe. They also are well-defined and cost-effective immunogens that could be produced in developing countries. However, formulation in complete and incomplete Freund's adjuvants (CFA/IFA) that is not acceptable for human use is necessary to elicit only low neutralizing antibody titers, thus rendering peptide vaccines unattractive.[99]

Recombinant Subunit-Based EV-A71 Vaccines

It is well accepted that recombinant subunit vaccines are safe and cost-effective, but require strong adjuvants. Purified recombinant VP1 (rVP1) subunits produced in different expression systems could elicit good virus neutralizing antibody responses and protect newborn mice against lethal EV-A71 challenge.[1–4] rVP1, rVP2, and rVP3 elicited subunit-specific antibodies,[99] but only rVP1 formulated with CFA/IFA was capable of eliciting a 1/128 virus neutralization titer.

Virus-Like Particle–Based EV-A71 Vaccine Candidates

Recombinant virus-like particles (VLPs) produced from baculovirus or yeast expression systems mimic the conformation of authentic native viruses, but are safe because they are devoid of viral genome. VLPs elicit virus-neutralizing antibody responses in adult mice and protect newborn mice against lethal EV-A71 challenges by passive immunization. VLP-induced neutralizing responses are stronger than those elicited by synthetic peptides or rVP1.[99–101] Their safety, immunogenicity, and high-yield production make them ideal candidates for future combination vaccines.

Chemically Inactivated EV-A71 Virion Vaccines

Formalin-inactivated EV-A71 virions (FI-EV-A71) based on a B4 subgenotype formulated with alum elicited antibody responses that cross-neutralized different EV-A71 genotypes but failed to neutralize CV-A16.[99] Immunization with FI-EV-A71 C4 vaccines yielded similar results. Inactivated virions were shown to be significantly more immunogenic than the other EV-A71 vaccine prototypes, and to elicit potent cross-genotype neutralizing antibody responses in mice and non-primate models.[99,101–103] These results suggest that common neutralization epitopes (most likely conformational) are shared by different EV-A71 genotypes/subgenotypes, and that amino acid sequence substitutions between EV-A71 and CV-A16 can partially explain the failure of EV-A71 vaccines to cross-neutralize CV-A16. Reciprocally, inactivated CV-A16 virions could not elicit cross-neutralizing antibodies against a panel of EV-A71 genotypes.[67,104] Based on their promising protective ability and the success of inactivated polio vaccines (IPVs), inactivated EV-A71 virions were selected for the clinical development of stable and cost-effective HFMD vaccines.

Manufacturing Process of EV-A71 Inactivated Vaccines

The manufacturing process of EV-A71 vaccines includes several stages: selection and characterization of a genetically stable EV-A71 virus vaccine strain; qualification of cell substrates and virus seeds; evaluation and selection of serum-free culture medium for production; pilot scale upstream and downstream process development; optimization of chemical inactivation; and preclinical animal immunogenicity studies.

The EV-A71 E59 vaccine strain of the B4 subgenotype was isolated in 2000 and selected among more than 100 EV-A71 clinical isolates deposited at Taiwan's CDC as potential vaccine strains.[105] Full documentation and clear patient's medical history were provided as part of the regulatory requirements. As shown in Table 21.1, Sinovac Biotech Co Ltd, Vigoo Biologicals Co, and Chinese Academy of Medical Sciences (CAMS) in China developed the C4 H07, FY7VP5, and FY23 seed strains, respectively. Inviragen used a B2 subgenotype as vaccine strain.[4,12] Good manufacturing practices (GMP)-compliant Vero cell banks were readily available from American Type Culture Collection (ATCC), and used for EV-A71 production by National Health Research Institutes (Taiwan) (NHRI), Sinovac, Vigoo, and Inviragen, whereas CAMS used the KMB-17 cell-line. NHRI Vero-cell Master Cell Bank and Master Virus Seed of EV-A71 E59 strain were characterized according to U.S. Food and Drug Administration (FDA) guidelines,[2] tested by BioReliance (UK), and found to be free of retroviruses, bovine, and porcine viral contaminants and other adventitious agents.[105]

VP-SFM (GIBCO) serum-free medium was selected for the manufacturing process. Optimal virus yields were obtained at a 10^{-5} multiplicity of infection (MOI) at 37°C. Titers of infectious virus particles in production bulks were monitored using the $TCID_{50}$ (tissue culture infective dose) assay and measuring VP2 epitope content by quantitative enzyme-linked immunosorbent assay (Q-ELISA).[105–107] The overall recovery of virus purified by liquid chromatography after concentration ranged from 30% to 57%. Purified EV-A71 bulks prepared by NHRI and the Chinese manufacturers were inactivated with formalin (0.025%) whereas Inviragen used binary ethyleneimine.[108] The quality of inactivated EV-A71 virions was biochemically analyzed and used for immunogenicity studies. No contamination was observed in sterility tests at 18 months. The vaccine was stable after 18 months of storage at 4°C as confirmed by the absence of decline in virus neutralizing antibody titers over time in mouse immunogenicity studies. Microgram doses of vaccine formulated in alum elicited high neutralizing antibody responses against the E59 strain in mice, rats, rabbits, and macaques, as well as strong cross-neutralization titers against other EV-A71 B and C subgenotypes. These results provided the information required by regulatory authorities to move the vaccine to human Phase I clinical trials.

Chemically Inactivated EV-A71 Vaccines and Efficacy Trials

For regulatory, economic and market acceptability reasons, five chemically inactivated EV-A71 vaccines have been rapidly developed in the past few years.[4,12] The Vaccine R&D Center of the NHRI produced a B4-based FI-EV-A71 vaccine (EV-A71vac) and launched the first human Phase I clinical trial in adults in 2010. A single vaccine dose of 5 μg or 10 μg was safe and

TABLE 21.1 Summary of EV-A71 Candidate Vaccines[a] Tested in Human Clinical Trials

	Manufacturing Process		Clinical Trials				
Organization	Cell Lines and EV-A71 Vaccine Strain	Bioprocesses	Dosage (μg of Antigen)	Population Target	Sample Size	Status	References (ClinicalTrials.gov Identifiers)
NHRI (Taiwan)	Vero cell and EV-A71 B4 subgenotype (GMP certified)	Roller-bottle (SFM) Gel-filtration chromatography Formalin inactivation	5 and 10	Adults 20–43 yr	60	Phase I completed	109, 110 (NCT01268787)
Sinovac Biotech Co. Ltd. (China)	Vero cell and EV-A71 C4a subgenotype H07 strain	Cell factory/ bioreactor (SFM) Gel-filtration chromatography Formalin inactivation	0.25, 0.5, and 1.0[b] 1 1	Adults, children (>5 yr) Children (18–60 mo) Children (6–35 mo)	10, 245	Phase I completed Phase II completed Phase III completed	113 (NCT01273246, NCT01273233, NCT01507857)
Beijing Vigoo Biological Co. Ltd (China)	Vero cell and EV-A71 C4a subgenotype FY7VP5 strain	Cell factory (SFM) Gel-filtration chromatography Formalin inactivation	0.4, 0.8, and 1.6 0.8 0.8	Adults, children (>5 yr) Children (18–60 mo) Children (6–35 mo)	10,077	Phase I completed Phase II completed Phase III completed	112 (NCT01313715, NCT01399853, NCT01508247)
CAMS (China)	Human diploid cell KMB-17 and EV-A71 C4a subgenotype FY23 strain	Cell factory (medium + serum) Gel-filtration chromatography Formalin inactivation	0.25	Adults, children (>5 yr) Children (18–60 mo) Children (6–71 mo)	12,000	Phase I completed Phase II completed Phase III completed	114 (NCT01391494, NCT01512706)
Takeda Pharmaceuticals Co. (Inviragen, Singapore)	Vero cell and EV-A71 B2 subgenotype	Cell factory (SFM) Gel-filtration/ ion-exchange chromatography Binary ethyleneimine	0.6 and 3.0	Adults	36	Phase I completed	4, 12, 108 (NCT01376479)

CAMS, Chinese Academy of Medical Sciences, Kunming, China; NHRI, National Health Research Institutes, Taiwan; SFM: serum-free medium.
[a]The Chinese Academy of Medical Sciences (CAMS) and Sinovac Biotech vaccines have been approved by China Food and Drug Administration (CFDA).[127]
[b]The antigen dosage is calculated based on the report by Liang, et al.[122] that the specific activity of the EV-A71 antigen reference standard established in China is 421.1 U/μg.

highly immunogenic.[109] It elicited 100% seroconversion in naïve volunteers and strong virus neutralizing antibody (VNA) responses (geometric mean antibody titer [GMT] = 210) against the vaccine strain as well as against B1, B5, and C4a isolates in 85% of the vaccinees.[110] In contrast, neutralizing responses against C4b and CV-A16 were weak in 20% of the subjects and 90% of the vaccinees did not develop any VNA against an atypical C2 strain. Inviragen (Takeda Pharmaceuticals Co. Ltd) reported the results of a Phase I trial in adults with an inactivated EV-A71 B2 vaccine. All subjects who received 0.6 μg or 3 μg of vaccine at days 0 and 28 seroconverted and developed VNA GMTs of 323 and 452, respectively.[12]

Inactivated EV-A71 vaccines based on different C4 isolates were independently developed and evaluated by three different Chinese companies (see Table 21.1.)[4,12,111] The clinical efficacy of these vaccines formulated in alum was assessed in large Phase III trials involving more than 30,000 healthy infants and young children (6 to 35 months of age) who received two doses of vaccine 28 days apart or a placebo control. All three vaccines were found to be safe and well tolerated.[112–114] The most common side effects observed were induration, erythema, and pain at the injection site that resolved within 24 to 72 hours and a grade 3 fever. The rate of seldom-reported serious adverse events (SAEs) in vaccinees was not different from that observed in the control groups and were not causally related to vaccination. The Vigoo's vaccine[112] was greater than 90% efficacious against EV-A71–related HFMD and greater than 80% protective against EV-A71–associated serious diseases, including herpangina. In the Sinovac's trial, the incidence rate of EV-A71–associated disease was 0.3% versus 2.1% in the control group, corresponding to an 89.3% efficacy.[113] In the CAMS's study,[114] the seroconversion rate was 100% after two vaccinations, with a VNA GMT of 170.6. The vaccine was 97.4% efficacious against EV-A71–related diseases. All C4-based vaccines prevented herpangina and EV-A71–associated hospitalizations. Immune sera from subjects immunized with the Vigoo's and Sinovac's vaccines cross-neutralized the circulating EV-A71 genotypes and subgenotypes (B4, B5, C2, C5) associated with epidemics in recent years.[115] Furthermore, pre-existing antibodies due to stealth infections of young children did not interfere with vaccine efficacy against different EV-A71 genotypes.[115]

Immunological Correlates of Protection

Interestingly, the VNA titers decreased by half after 6 months but this waning did not affect vaccine efficacy.[111] Most importantly, Phase III results suggest that a VNA titer of 1/16 can serve as a correlate of protection against EV-A71–related HFMD.[12,111–114] A VNA titer of 14.7 U/mL was found to be

associated with a 50% protection against EV-A71 disease.[128] Despite differences in seed strains and manufacturing processes, C4-based vaccines have shown batch consistency and efficacy.[116]

Challenges for EV-A71 Vaccine Registration

Cross-Protective Ability Against Other EV-A

Both C4- and B4-based vaccines cross-neutralized the current circulating EV-A71 isolates,[110–115] but the B4 vaccine poorly neutralized an atypical C2 strain.[110] However, the degree of cross-protective ability of these vaccines against viruses from genotypes D, E, F, and G remains to be evaluated. In addition, they failed to protect against CV-A16 infections that are predominantly responsible for annual HFMD outbreaks.[110–115] It is likely that FI-EV-A71 vaccinations may not significantly reduce the number of clinical cases of HFMD during outbreaks. In this regard, bivalent FI-EV-A71/FI-CV-A16 vaccines that are being developed have elicited balanced protective responses against both viruses.[67,104] Furthermore, because of the possible emergence of new variants with increased virulence, only results from multinational efficacy trials will reveal if a monovalent vaccine can elicit broad protection against divergent epidemic viruses. To this end, a global surveillance network for EV outbreaks is urgently needed to monitor immune responses to EV-A71 vaccines in the future.

Duration of Immunity and Protection

A significant waning of antibody titer was noticed during the first 6 months after 2 vaccinations.[117] To ensure long-term antipoliovirus antibody persistence, the WHO recommended that a primary series of immunizations be followed by booster administrations.[118] Volunteers primed with one dose of EV-A71 vaccine at least in a Phase II clinical trial were enrolled to receive a booster dose.[116,117,119] A 10-fold increase at least in neutralizing antibody responses was induced by the booster injection, compared to placebo ($P < .0001$). The frequencies of adverse reactions were similar between vaccine and placebo groups. Therefore, a booster dose of EV-A71 vaccine 1 year after priming showed excellent immunogenicity and good safety profile. Although the Sinovac vaccine elicited robust protectino over a 2-year period,[127] only Phase IV studies will help define the optimal immunization schedule and determine whether a third immunization at 24 months is recommended for long-lasting protection. The development of mucosal vaccines that are attractive may not be necessary since parenteral immunization confers protection. Prospective studies should be conducted during EV-A71 and coxsackievirus epidemics to assess the role of cellular immunity in long-term cross-protection and viral pathogenesis. In addition, longitudinal studies are necessary to evaluate the efficacy of EV-A71 vaccines at controlling antigenic shift, virus fitness, and the emergence of new viruses.

Standardized Animal Models for Vaccine Potency Test

Macaques develop antibody responses to EV-A71 vaccines similar to those observed in human, but their use is limited by ethical and economic considerations.[37] Neonatal suckling mice and immunodeficient animals have widely been used in evaluating the protective efficacy of EV-A71 vaccine candidates, but they do not mimic human infection.[120] NHRI[36] and Fujii and colleagues[37] have successfully developed transgenic mice carrying the human receptor hSCARB2 that develop clinical symptoms similar to those observed in humans. The hSCARB2 transgenic mouse model once standardized will be useful to assess the cross-protective ability of vaccines against EVs in the future.

Vaccine Manufacturing Considerations

An ideal EV-A71 vaccine should be inexpensive, safe, compatible with large-scale production, easy to administer, and acceptable to parents. To this end, cooperation between research and development institutes and both Asian and global companies are urgently needed to improve and scale-up the current manufacturing processes for broad approval by regulatory authorities. As a result of intellectual property rights and proprietary technologies, information on the influence of culture medium and production systems on vaccine yields is lacking. However, both the roller-bottle and cell factory technologies used to produce current clinical lots are easy to operate, although labor intensive.

Clinical trials have revealed that administration of a 400-unit (1 μg) dose of EV-A71 vaccine twice achieved efficacy.[111–113] A 2012 publication[99] reports that a 40-L pilot-scale production batch could yield fifty thousand 1 μg doses of FI-EV-A71 at a cost of 0.4 U.S. dollar/dose. This translates into 200,000 doses of the lowest C4-vaccine protective dose (0.25 μg) as determined in a Phase III trial[114] at 0.1 U.S. dollar/dose. Although Chinese manufacturers could produce 40 million doses annually,[127] larger-scale production of EV-A71 vaccines will require an improvement of the current manufacturing processes. The use of bioreactors, microcarriers, and perfusion technology could increase cell growth and virus yield by one order of magnitude. To lower the production cost, a simple and efficient downstream chromatographic purification step could be optimized to copurify immunogenic defective and infectious virus particles.[107] Lee and colleagues[121] forecasted that routine immunization with a 70% efficacious EV-A71 vaccine sold at 25 U.S. dollars/dose would be of great economic value. With this profit margin and the new emerging vaccine markets in the Asia-Pacific region, the global vaccine companies should become interested in manufacturing EV-A71 and future multivalent HFMD vaccines.

Public Health Perspectives

Based on the current Phase III results, national EV-A71 immunization programs should be implemented in China to build strong herd immunity. A two-dose regimen vaccination should be administered to 6- to 7-month-old children and possibly a third dose at 18 to 24 months. Multinational efficacy trials will be critical to evaluate the degree of cross-immunity against circulating genotypes and subgenotypes. A global HFMD surveillance network is mandatory for continuous epidemiological monitoring and the detection of emerging EV-A71 variants. Harmonization and standardization of vaccine strains, quality control (QC) reagents and assays and animal models at the international level are urgently needed to evaluate the potency of vaccine candidates and determine which manufacturing process yields the most potent and most affordable products. In this regard, China has established national standards for vaccine evaluation.[122] Although the coadministration of FI-EV-A71 with Pediacel (Sanofi Pasteur) did not affect antibody responses against the individual components of the pediatric pentavalent vaccine, clinical trials should be conducted to determine whether EV-A71 vaccines could ultimately be combined with Expand Program of Immunization (EPI) vaccines which would greatly simplify immunization schedules.[123] EV-A71 vaccines also represent the first step toward the development of future multivalent HFMD vaccines.[124,125]

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22 Epstein-Barr Virus Vaccines

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Epstein-Barr virus (EBV) is a ubiquitous human pathogen that infects at least 90% of the world's population.[1] EBV causes infectious mononucleosis, which can result in significant loss of time from school or work in developed countries.[2] EBV is responsible for a number of cancers including endemic Burkitt lymphoma,[3] nasopharyngeal carcinoma (NPC),[4] a subset of Hodgkin lymphomas,[5] lymphomas in HIV-infected individuals,[6] aggressive natural killer (NK) cell leukemia/lymphoma,[7] X-linked[8] and posttransplantation[9] lymphoproliferative disorders, and gastric carcinoma.[10] Finally, EBV infection, especially if it manifests as infectious mononucleosis, is considered to be a major risk factor for the development of multiple sclerosis.[11] Additional evidence for the EBV–multiple sclerosis connection is mentioned in the "Suggestions for Future Development of Epstein-Barr Virus Vaccines" section below.

Despite the enormous disease burden attributed to EBV, progress on EBV vaccines has been painfully slow partly because of the lack of a suitable animal model other than nonhuman primates, selection of the appropriate antigen and adjuvant, and debate over what such a vaccine could actually achieve.[12] An ideal prophylactic vaccine might be one that provides sterilizing immunity, meaning that after vaccination, the host can never be infected by the same pathogen. In reality, both live attenuated and subunit viral vaccines do not elicit sterilizing immunity. Rather, they reduce the severity of disease caused by subsequent natural infection. This is likely true for all vaccines, including EBV. There actually could be an advantage to this in that subclinical or mild "reinfection" would likely boost the host's vaccine-induced immunity and potentially extend its duration of protection.

CONSIDERATIONS FOR DEVELOPING PROPHYLACTIC EPSTEIN-BARR VIRUS VACCINES

A live prophylactic EBV vaccine would be unacceptable because EBV is oncogenic. Consideration must therefore focus on choosing a viral antigen or antigens most likely to prevent infection. A prototype candidate subunit prophylactic vaccine is gp350, the major envelope glycoprotein of EBV, first proposed by Epstein in 1976.[13] The reason is that EBV has exquisite specificity for human B cells via attachment of gp350 to CD21, also known as the CR2 receptor, the C3d receptor, or the EBV receptor.[14,15] Anti-gp350 antibody would be expected to block attachment of EBV to the B cell and hence prevent endocytosis of the virions into B cells where they can establish infection and latency. In support of this concept, gp350 antibody and EBV neutralizing antibody are closely associated.[16]

A number of studies in cottontop tamarins have confirmed that vaccine containing EBV gp350 can protect against a subsequent EBV challenge infection and associated malignancy.[17–19] However, the method of preparation of the vaccine appears to be crucial, because a major EBV membrane antigen (EBV-MA) vaccine purified by monoclonal antibody immunoaffinity chromatography rather than sucrose density gradient centrifugation elicited neutralizing antibodies in cottontop tamarins, but did not protect them from lymphoma after challenge with B95-8 strain EBV.[20] The most likely reason for this was that the monoclonal antibody was too specific and selected the wrong viral antigen.

Rhesus lymphocryptovirus (RLCV) infection resembles human EBV infection in that Rhesus macaques can be infected orally, shed RLCV from their throat, develop lymphocytosis and lymphadenopathy, and become latently infected.[21] A recent investigation by Sashihara and colleagues showed that RLCV gp350 vaccine protected against challenge infection and reduced viremia.[22] Three different vaccines were compared in this study: soluble RLCV, a virus-like replicon particle expressing RLCV gp350, and virus-like replicon particles expressing RLCV gp350, EBV nuclear antigen (EBNA)-3A and EBNA-3B. Animals given soluble gp350 had the highest gp350 antibody titers, the lowest rate of infection after challenge, and the lowest level of viral DNA in the blood 2 years after challenge. These results suggest that a prophylactic vaccine containing gp350 alone might be more effective than one targeting both gp350 and EBNA-3 proteins in reducing the incidence of infection and viral load in persons who are exposed to wild virus following vaccination.

PROPHYLACTIC EPSTEIN-BARR VIRUS VACCINES TESTED IN CLINICAL TRIALS

Two very different prophylactic vaccine constructs have been tested in humans: those designed to elicit gp350 antibodies and a vaccine to induce production of EBV-specific $CD8^+$ T-cells (Fig. 22.1).

Subunit gp350 Vaccines

Two subunit prophylactic gp350 EBV vaccines have been evaluated in human subjects. The first vaccine, developed by Gu and colleagues,[23] was created by transfecting human embryonic lung cells with a *Vaccinia* virus construct from the smallpox vaccine strain *Tien Tan*, which contained the major EBV-MA. The majority of this material, we now know, was gp350. Cells demonstrating cytopathic effect were harvested and the supernatant from these lysed cells was found to contain high titers of infectious recombinant virus.

After demonstrating that the candidate vaccine was immunogenic in rabbits, the investigators conducted a series of three clinical trials. The first study assessed safety by inoculating 11 healthy adults who had previously received the *Tien Tan* vaccine. Three vaccine recipients had febrile reactions and local vaccinia lesions with antibody responses to *Vaccinia*. The other eight subjects had mild inflammatory reactions at the scarification sites but no antibody responses to *Vaccinia*. None of the 11 volunteers had a rise in antibody titers to EBV. In the second trial, six school children ages 8 and 9 years who were latently infected with EBV and had previously received *Tien Tan* vaccine were administered the EBV vaccine. The vaccine was safe and 1 month after vaccination, eight of the nine developed antibodies to EBV-MA and to *Vaccinia* virus. None had a rise in antibodies against EBV viral capsid antigen (VCA), which meant that the response to EBV-MA was not a result of reinfection by EBV circulating in the community.

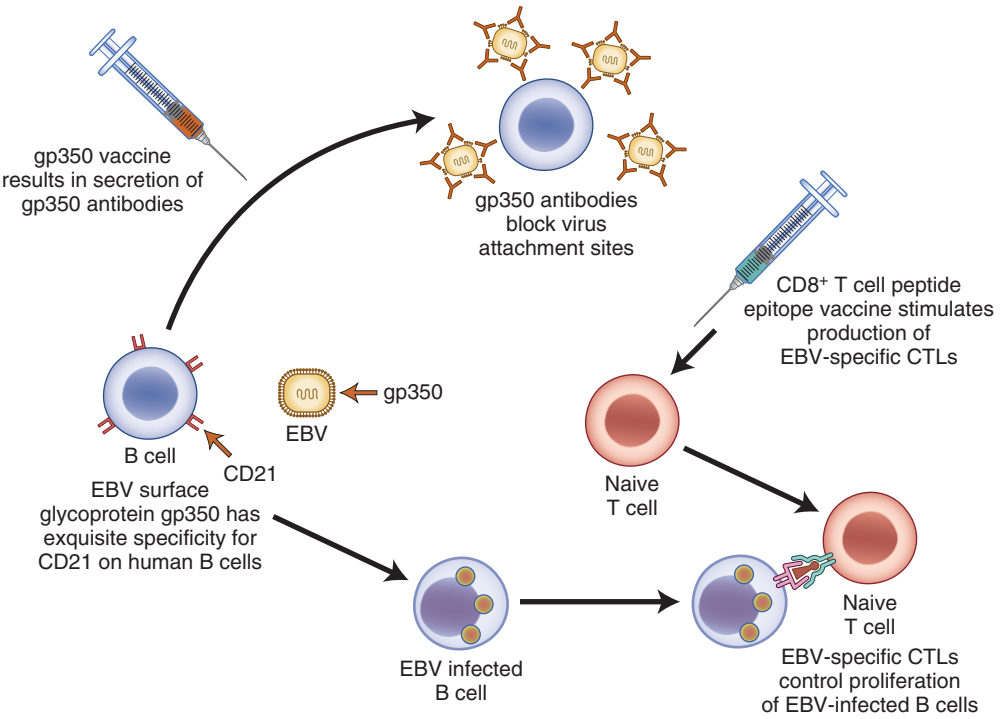


Figure 22.1. Prophylactic Epstein-Barr virus (EBV) vaccine strategies. gp350 and CD8+T cell peptide epitope vaccines. CTLs, cytotoxic lymphocytes.

The final trial enrolled 19 EBV-naïve children who were 1.7 to 2.8 years old and who had not received *Tien Tan* vaccine previously. Nine children were vaccinated while 10 served as unvaccinated controls. The vaccine was safe and all nine vaccinees developed antibodies to EBV-MA and *Vaccinia*. During 16 months of follow-up, three of nine vaccinees and 10 of 10 in the control group became infected with EBV as evidenced by development of antibodies against EBV VCA ($P = .003$, Fisher exact test, two-sided). This supports the authors' conclusion that "it has been shown for the first time that protection against and/or delay of EBV infection by the natural route is possible in humans." The authors state that "live *Vaccinia* vectors can be used and are efficacious." However, a live *Vaccinia* vector in a prophylactic vaccine is unlikely to be acceptable today given the severe adverse events associated with using smallpox vaccine to enhance bioterrorism preparedness.[24]

The second subunit prophylactic gp350 EBV vaccine that has been tested in human subjects was developed by Jackman and colleagues.[25] They reported the successful production of a recombinant gp350 in Chinese hamster ovary cells and showed by antibody and CD21 binding studies that it closely resembled native gp350. This construct administered with Freund adjuvant or alum elicited gp350 and neutralizing antibodies in rabbits. An EBV vaccine produced by this technology has been subsequently evaluated in four clinical trials.

Two sequential double-blind, randomized controlled trials were reported by Moutschen and colleagues.[26] The first was a Phase I study evaluating safety and immunogenicity of gp350 vaccine in 67 volunteers (mean age: 21.4 years; range: 18–25 years). The subjects were stratified by EBV antibody status and then randomized to receive 50 µg of gp350/0.5 mL adjuvanted with either 3-*O*-desacyl-4′-monophosphoryl lipid A and aluminum salt (AS04) or aluminum salt alone. Vaccine was administered intramuscularly according to a 0-, 1-, and 6-month schedule.

One severe adverse event was noted 10 days after receiving a second dose of gp350 adjuvanted with AS04. This previously EBV antibody-positive subject developed severe flu-like symptoms with headache and meningismus followed by arthritis of the knees, ankles, and lower back. The subject fully recovered within 2 months. The authors wrote that "a causal relationship with the vaccine could not be excluded."

Fifty-one of the subjects participating in the Phase I study were analyzed for immunogenicity. All 25 antibody-negative subjects had a gp350 antibody response when tested 1 month after the third dose of vaccine. The geometric mean titer (GMT) was significantly higher among the subjects given AS04-adjuvanted vaccine when compared with those who received alum as the adjuvant (727 vs 498). All 26 antibody-positive subjects experienced at least a fourfold rise in gp350 antibody titers, with the GMT slightly higher among those subjects given AS04-adjuvanted vaccine (1188 vs 1076). Neutralizing antibody responses were seen in 100% of antibody-negative subjects who received AS04-adjuvanted vaccine as compared with 44% among the antibody-negative subjects given gp350 with alum. Among the antibody-positive subjects, fourfold boosts in neutralizing antibody titers occurred in 71% of those given AS04-adjuvanted vaccine as compared with 56% given alum as the adjuvant. Thus, in the Phase I trial, gp350 adjuvanted with AS04 was consistently more immunogenic than gp350 adjuvanted with alum.

In the Phase I/II study, 81 EBV-naïve subjects (mean age: 21.8 years; range: 18–37 years) were randomized 1:1:1 to receive unadjuvanted vaccine, vaccine adjuvanted with AS04, or vaccine adjuvanted with alum. Like the Phase I study, vaccine was administered as a 50 µg of gp350/0.5 mL dose intramuscularly according to a 0-, 1-, and 6-month schedule.

The vaccine was considered to be safe, but one notable reaction was reported. A subject with a history of migraine experienced a severe headache associated with vomiting 27 days after receiving the second dose of vaccine. Because of the

vaccinee's past history of migraine, the relationship of migraine with the vaccination was classified as unlikely.

All 71 evaluable subjects had gp350 antibodies when tested 1 month after the third vaccination. The GMTs were as follows: unadjuvanted vaccine, 258; alum-adjuvanted, 1053; AS04 adjuvanted, 1251. The GMT of the group that received unadjuvanted vaccine was statistically significantly lower than the GMTs of the other two groups. A similar trend was noted for neutralizing antibodies assayed either by inhibition of EBV-infected B-cell proliferation[25] or by a competition immunoassay that measured the ability of vaccine-induced antibody to block binding of a murine anti-gp350 monoclonal antibody (72A1) to gp350.[27]

In summary, the Phase I/II trial of the gp350 vaccine confirmed the immunogenicity results of the Phase I study showing that AS04 was a better adjuvant than alum. The Phase I/II study also indicated that unadjuvanted gp350 was inferior to the adjuvanted preparations.

The third trial was a Phase II, placebo-controlled, double-blind study evaluating safety, immunogenicity, and efficacy of recombinant gp350 vaccine in EBV-naïve young adults ages 16 to 25 years.[28] The vaccine contained 50 μg of gp350 and 50 μg of AS04 in a 0.5 mL volume that was given intramuscularly according to a 0-, 1-, and 5-month schedule. The primary end point was the incidence of infectious mononucleosis disease in vaccine and placebo groups 18 months after administration of the second dose of clinical trial material as documented by clinical criteria along with EBV antibody responses to nonvaccine antigens. EBV infection was defined as development of EBV antibody responses to non-vaccine antigens in the absence of clinical infectious mononucleosis. The study enrolled 181 subjects (mean age: 20.6 years; 52% male; 97% white) who were assigned to vaccine or placebo in a 1 : 1 ratio. No serious adverse events were reported. Almost all (76/77; 98.7%) of the vaccine recipients who were not subsequently infected by wild-type EBV developed gp350 antibodies. The 72A1 competition immunoassay was employed to detect neutralizing antibody.[27] Neutralizing antibody levels peaked 1 month after the third dose "with 69.86% (95% confidence interval [CI], 58.00–80.06%) showing seroconversion at that point in time." Presumably, this pertained only to the vaccine recipients.

During the 19-month follow-up period, EBV vaccine did not prevent infection: 13 (14%) of 90 vaccine recipients became infected versus 18 (20%) of 91 placebo subjects. However, vaccine had a significant effect on the development of clinical disease. In the intent-to-treat population, infectious mononucleosis developed in only two (2%) of 90 vaccinees as compared with nine (10%) of 91 placebo recipients (P = .03; Fisher exact test; one-sided).

This study is noteworthy in that it was the first to demonstrate protection against clinical infectious mononucleosis by an EBV subunit vaccine.[29] However, it had limitations, including EBV viral loads were not measured, the neutralizing antibody results were difficult to interpret, and there was no assessment of cell-mediated immune responses.

The fourth study was a Phase I trial of recombinant gp350 vaccine with an aluminum hydroxide adjuvant in 16 pediatric renal transplantation candidates.[30] Subcutaneous dosing regimens of 12.5 μg or 25 μg of gp350 given three or four times (weekly for the first three doses with a fourth dose at weeks 30 to 32 if not yet transplanted) over a total of 32 weeks were well tolerated. Three subjects were excluded because they did not complete the two immunogenicity evaluations. All 13 evaluable subjects had an anti-gp350 antibody response but only four subjects made neutralizing antibody. Because there was no control group, vaccine efficacy could not be evaluated. This small Phase I trial showed that immunization of children awaiting transplantation for chronic renal disease is feasible, but the low amounts of adjuvanted gp350 given were probably insufficient to elicit an optimal immune response.

CD8$^+$ T-Cell Peptide Epitope Vaccine

A different vaccine approach is to control expansion of EBV-infected B cells by generating CD8$^+$ T-cell immunity to EBNAs. The potential role of these viral proteins in B-cell transformation precludes their use in whole-protein–based vaccines, and therefore a peptide vaccine was generated and tested in adult volunteers.[27] This trial used an EBNA-3A peptide epitope restricted by human leukocyte antigen (HLA)-B8 with tetanus toxoid formulated in a water-in-oil adjuvant as a source of T-cell help.[31] EBV-naïve adults between the ages of 18 and 50 years were given two vaccinations 2 months apart. Of the 14 enrolled subjects, eight individuals were immunized with a 5-μg dose of peptide with adjuvant, two were immunized with a 50-μg dose of peptide with adjuvant and, four received placebo. This strategy was effective at generating a peptide-specific CD8+ T-cell response in most individuals as measured by ex vivo peptide-specific interferon-γ production. During the 2- to 12-year follow-up, five of 10 vaccinees acquired wild-type EBV infection. One subject, most likely infected shortly after the first vaccination, developed infectious mononucleosis. Two of four placebo subjects were infected, one of whom developed infectious mononucleosis. Although all four subjects infected after two vaccinations were asymptomatic, suggesting that the vaccine may have prevented symptomatic EBV infection, the sample size was too small to definitively address this issue.

The practicality of epitope vaccines is limited by the fact that they only target specific HLA types and thus cannot be used in the general population. Nevertheless, epitope vaccines might be effective for prophylaxis for patients with posttransplant lymphoproliferative disorder (PTLD) whose HLA type is known. This trial was a proof of concept that an EBV vaccine that generated CD8+ T-cell immunity was safe and did not predispose subjects to disease after primary infection by wild-type EBV.

PROPHYLACTIC EPSTEIN-BARR VIRUS VACCINE CANDIDATES WITH IMMUNOGENICITY IN BALB/c MICE

Several novel EBV vaccines are promising in that they have demonstrable immunogenicity in mice. Ruiss and colleagues described the production of virus-like particles (VLPs) in which the EBV proteins EBNA-2, latent membrane protein 1 (LMP1), EBNA-3A, EBNA-3B, EBNA-3C, and BZLF1 were deleted or functionally inactivated.[32] Because the viral genome also lacked the terminal repeat signal sequences required for packaging viral DNA, the VLPs did not contain detectable EBV DNA. This vaccine was shown to have human B-cell tropism in vitro, and elicited EBV-specific neutralizing antibodies and cellular immune responses in BALB/c mice.

Cui and colleagues designed a tetramer containing the first 470 amino acids of gp350 and showed that this construct elicited approximately 19- to 20-fold higher gp350 antibody titers in vaccinated BALB/c mice as compared with its monomeric counterpart.[33] This concept could prove valuable if adjuvanted monomeric gp350 vaccines are ultimately shown to have unsatisfactory immunogenicity.

Ogembo and colleagues developed a subunit vaccine based on a novel Newcastle disease virus (NDV)-VLP platform consisting of EBVgp350/220 fused to NDV-fusion (F) protein.[34] The chimeric protein EBVgp350/220-F was incorporated into

the membrane of a VLP composed of the NDV matrix and nucleoprotein. The particles produced resembled native EBV in diameter and shape and were found to bind both CD21 and CD35. Vaccination of BALB/c mice with these EBVgp350/220-F VLPs elicited antibodies to gp350 that were predominantly of the immunoglobulin G_1 subclass, and neutralizing antibodies, which prevented infection of Raji cells by a recombinant EBV construct containing enhanced green fluorescent protein to enable quantitation.

TREATMENT OF EPSTEIN-BARR VIRUS–SPURRED DISEASES BY TRANSFERRING OR STIMULATING CELL-MEDIATED IMMUNITY

Therapeutic vaccines could be effective for a number of EBV-spurred diseases, especially NPC and EBV-positive lymphomas. The goal of therapeutic vaccines is to eliminate tumor cells by stimulating T-cell responses against the EBV antigens they express as listed in Table 22.1. Several reports suggest that providing EBV-specific cellular immunity can be beneficial at least in the short term.

In children at risk for PTLD after hematopoietic cell transplantation, intravenous infusions of EBV-specific T cells (mainly CD8+) from HLA-matched unrelated donors or mismatched family donors reduced EBV viremia and possibly caused regression of EBV disease in three of 10 patients.[35] Autologous cytotoxic T lymphocytes (CTLs) targeting LMP1 alone or LMP1 and LMP2 were given to 50 patients with histologically proven EBV lymphomas (Hodgkin lymphoma, 25; NK cell leukemia/lymphoma, 11; diffuse B-cell lymphoma, 7; miscellaneous diagnoses, 7). Twenty-nine high-risk or multiple-relapse patients treated in remission had a 2-year event-free survival rate of 82%, which the authors considered to be a therapeutic response.[36]

In another study, 10 patients with advanced, relapsed NPC were given infusions of autologous EBV-specific CTLs.[37] The CTL infusions were associated with reduced levels of EBV DNAemia and also appeared to have antitumor activity. Four of six patients with disease that was resistant to or had relapsed after intensive chemotherapy and radiation had complete or partial remissions. All four patients who were in remission at the time of CTL infusions remained disease free after 19 to 27 months.

Smith and colleagues treated NPC patients who had regional recurrent or metastatic disease with EBV-specific autologous T cells, which were generated by exposure to an adenoviral vector–based vaccine that encoded EBNA-1 fused to multiple CD8+ T-cell epitopes from LMP1 and LMP2.[38] EBV-specific autologous T cells were successfully expanded in 16 (73%) of 22 patients. The median overall survival of the 14 patients given CTLs was 523 days as compared with 220 days for six subjects who did not receive CTLs. The authors did not say whether or not this was statistically significant. They commented that "these preliminary observations will require confirmation in a formal Phase II randomized clinical trial."

Finally, in the realm of adoptive immunotherapy, 35 patients with metastatic and/or locally recurrent NPC were treated with chemotherapy followed by EBV-specific CTLs, which resulted in better response and survival rates than chemotherapy alone.[39] The authors concluded, "Our study achieved one of the best survival outcomes in patients with advanced NPC, setting the stage for a future randomized study of chemotherapy with and without EBV-CTL."

In the realm of therapeutic vaccines for NPC, Lin and colleagues used autologous dendritic cells incubated with LMP2 peptides to vaccinate patients with NPC.[40] After four injections into inguinal lymph nodes, $CD8^+$ T-cell responses to LMP2 were induced by the peptides in nine of 16 patients, two of whom also experienced tumor reduction.

Taylor and colleagues created a candidate poxvirus vector EBV vaccine expressing a fusion protein containing the carboxyl terminus of EBNA-1 (to stimulate $CD4^+$ T cells) fused to LMP2 (to stimulate EBV-specific $CD8^+$ T cells).[41] They showed that dendritic cells infected by this vaccine reactivated LMP2-specific $CD8^+$ T cells and EBNA-1–specific memory T cells in peripheral blood mononuclear cells from EBV antibody-positive donors.

A Phase I dose-escalation study using this same poxvirus vector EBV vaccine (called modified vaccinia virus Ankara with EBNA-1 and LMP2 [MVA-EL]) was conducted in 18 subjects with NPC.[42] Subjects received one of five doses ranging from 5×10^7 to 5×10^8 plaque-forming units (PFU) intradermally at 3-week intervals for a total of three inoculations. The vaccine was well tolerated with no evidence of dose-limiting toxicity. Overall, 15 of 18 patients developed T-cell responses to one or both vaccine antigens. There was a dose–response relationship with the highest dose producing the strongest response. A parallel dose-escalation study of EBV MVA-EL vaccine was performed on 16 subjects in the United Kingdom, with the recommendation being that the highest dose (5×10^8 PFU intradermally) be used for subsequent Phase I and Phase II trials because it produced the broadest and strongest immune response.[43]

TABLE 22.1 Expression of Latent Epstein-Barr Virus (EBV) Genes on EBV-Spurred Tumor Cells Are Targets for Therapeutic Vaccines or Adoptive Immunotherapy

Condition	Latent EBV Genes Expressed by Tumor Cells
Posttransplant lymphoproliferative disorder	EBNA-1, -2, -3A, -3B, -3C LMP1, LMP2
X-linked lymphoproliferative disease	EBNA-1, -2, -3A, -3B, -3C LMP1, LMP2
Hodgkin lymphoma	EBNA-1 LMP1, LMP 2
Nasopharyngeal carcinoma	EBNA-1 LMP 1, LMP 2
Burkitt lymphoma	EBNA-1

EBNA, Epstein-Barr virus nuclear antigen; LMP, latent membrane protein.

SUGGESTIONS FOR FUTURE DEVELOPMENT OF EPSTEIN-BARR VIRUS VACCINES

The global burden of EBV-associated diseases is enormous. EBV vaccines could potentially reduce this burden significantly. For prophylactic vaccines, ideal diseases for efficacy trials in order of probable success are infectious mononucleosis, PTLD, and endemic Burkitt lymphoma. A vaccine containing gp350 antigen is a logical first choice to test because gp350 vaccine has been shown to protect against infectious mononucleosis in a Phase II clinical trial.[28] Prevention or reduction in severity of infectious mononucleosis should be the end point of the next Phase II or Phase III efficacy trial because a susceptible population with a high incidence of this illness—college freshmen—is at hand. In prospective studies of EBV-naïve University of Minnesota undergraduates, the incidence of primary EBV infection among freshmen was 26%, and 75% of those infected had typical infectious mononucleosis.[44,45] Such a proposed efficacy trial of the vaccine would include

periodic viral load measurements on all subjects after vaccination, because there is a statistically significant correlation between the quantity of virus in the blood and disease severity.[44,46] If the vaccine is shown to reduce blood viral load among subjects who are infected with wild virus after vaccination, then its effectiveness in preventing malignancies appears promising. Increased blood viral loads have been associated with the development of PTLD,[47] and increased plasma EBV DNA correlates with a worse outcome in Hodgkin lymphoma.[48] To gain insight into the duration of protection, it would also be worthwhile to evaluate EBV gp350-specific memory T-cell responses in vaccinees and to determine whether T cells in vaccinated individuals undergo EBV-induced changes in interleukin-15 receptor expression.

PTLD would be the next logical disease to study because EBV-naïve hematopoietic stem cell or solid-organ transplantation candidates who are at the highest risk could be identified and vaccinated pretransplantation. This trial also could also assess the effect of vaccination on the quantity and duration of EBV DNAemia, which could be responsible for posttransplantation morbidity in the absence of full-blown PTLD. Endemic Burkitt lymphoma and NPC are serious malignancies and hence worthy targets for preventive trials. Burkitt lymphoma may be the better candidate for a field trial because its "incubation period" is shorter and there are known high-incidence geographic foci where investigations could be conducted.[49] EBV vaccine might also reduce the incidence of Hodgkin lymphoma. The gp350 vaccine has been shown to protect against infectious mononucleosis and a history of infectious mononucleosis increases the risk of EBV-positive Hodgkin lymphoma by more than threefold.[50]

An EBV vaccine could conceivably reduce the incidence and/or severity of multiple sclerosis (MS), which is a common neuroinflammatory disease with more than 2,000,000 cases worldwide. EBV is a prominent, if not the number one, environmental risk factor for MS. The evidence for this is that nearly 100% of MS patients have EBV antibody[51]; their antibody levels are markedly elevated especially against EBNA-1[52]; a history of infectious mononucleosis increases the risk of developing MS[53]; EBV-specific $CD8^+$ T-cell responses are elevated during active MS[54]; and therapies with monoclonal antibodies that deplete the B-cell reservoir of latent EBV[55] or infusions of autologous virus-specific T cells[56] have a beneficial effect in MS.

In terms of therapeutic vaccines, the MVA-EL vaccine that contains part of the carboxyl terminus of EBNA-1 to stimulate CD4+ T cells fused to LMP2 to stimulate EBV-specific CD8+ T cells is the leading candidate.[41–43] In addition to NPC, this vaccine could have activity against EBV-positive lymphomas because these tumor cells also express EBNA-1 and LMP2.

BARRIERS TO EPSTEIN-BARR VIRUS VACCINE DEVELOPMENT

The promising prospects for EBV vaccines cannot be fulfilled until some knowledge gaps and practical issues are overcome. A major problem in EBV vaccine development is to find an industry sponsor or a consortium of investors willing to support it. Sponsors, if found, face challenges in implementing successful field trials in humans. For example, the optimum vaccine formulation—including both the antigen construct and the adjuvant—needs to be determined. Would the best vaccine be adjuvanted gp350 alone or would gp350 combined with EBV gH, gL, and gB (to induce protection from infection of oral epithelial cells) be more effective?[57,58] Could adjuvants more potent than those used in the published trials be incorporated?

If the first Phase III trial is to prevent infectious mononucleosis, the major beneficiaries would be EBV antibody-negative individuals. Because EBV antibody prevalence is tightly related to age, and if the goal is to choose a vaccine cohort in which 50% of individuals are EBV-naïve, what age should be chosen? Two studies have found that the age-specific EBV antibody prevalence is different among whites and nonwhites.[59,60] Whites do not reach 50% antibody prevalence until their teenage years, whereas nonwhites reach it between the

TABLE 22.2 Status of Epstein-Barr Virus (EBV) Vaccines

Disease	Clinical Trials Reported	Comments
Infectious mononucleosis	Prophylactic vaccines: Adjuvanted gp350 vaccine 4 Phase I[23,26,30]; 1 Phase II[28] CD8+ T-cell epitope vaccine 1 Phase I[31]	The logical target for further development of a prophylactic vaccine
Nasopharyngeal carcinoma	Adoptive immunotherapy[37–39] Therapeutic vaccines: dendritic cells incubated with LMP2 peptides 1 Phase I[40] Modified vaccinia Ankara with EBNA-1 and LMP2 (MVA-EL) 2 Phase I[42,43]	Best data are for MVA-EL vaccine
Posttransplant lymphoproliferative disorder	Adoptive immunotherapy[35]	The second choice as target for further development of a prophylactic vaccine
EBV-positive lymphomas	Adoptive immunotherapy[36]	Clinical trial possible but would be lengthy and require a large number of subjects
Endemic Burkitt lymphoma		Clinical trial feasible in focal areas of East Africa where disease is endemic
Miscellaneous		
Chronic active EBV Gastric carcinoma X-linked lymphoproliferative syndrome Multiple sclerosis		Severity of these entities makes them worthy of prophylactic and/or therapeutic vaccine trials

ages of 4 and 6 years. This creates a dilemma for establishing a uniform vaccination policy.

A daunting challenge for therapeutic EBV vaccine trials is that subjects for these, most likely patients with NPC or lymphomas, have complex management strategies that can confound interpretation of the results. Nevertheless, based on some encouraging results discussed above, continuation of therapeutic vaccine trials is warranted.

CONCLUSIONS

EBV is a common worldwide human pathogen that causes acute and chronic infections, cancers, and autoimmune disease. Prophylactic and therapeutic vaccines could prevent or reduce the severity of some, if not all, of these conditions, but they are not yet available for general use (Table 22.2). Our charge is to find the resources from government, industry and/or philanthropy to conduct the studies necessary to make effective EBV vaccines available to all those who will benefit from them.

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23 *Haemophilus influenzae* Type b Vaccines

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Prior to use of vaccination, *Haemophilus influenzae* type b (Hib) was responsible for significant morbidity and mortality worldwide in children, especially in those younger than 2 years of age. In 2000 alone, an era when Hib vaccine was in routine use in nearly all high-income and most middle-income countries, and had virtually eliminated Hib pneumonia, meningitis, and other related illnesses, Hib was estimated to still cause 8.13 million cases and 371,000 deaths,[1] a burden of disease that ranked as a leading cause of death among children younger than 5 years of age globally. In addition to mortality, more than 30% of children who develop Hib meningitis suffer from neurologic sequelae.

In November 2006, the World Health Organization (WHO) universally recommended the implementation of Hib vaccination in all infant immunization programs worldwide, regardless of the availability of local or national surveillance data.[2] In 2008, the WHO estimated that more than 200,000 children died from Hib. While introduction of Hib conjugate vaccine was slower in developing countries than in industrialized countries, all countries except for China and Thailand have included Hib vaccine in the Universal Immunization Programs (UIPs), although China has achieved more than 50% uptake through the private market. India, which accounts for more than 20% of global child mortality, has introduced Hib into the UIP; however, the vaccine has been implemented in the UIP in only 16 of the 36 States and Union Territories as of August 2015. As of May 2015, a report from the International Vaccine Access Center (IVAC) at Johns Hopkins University noted that more than 35% of the world's children still do not have access to Hib vaccine because of lack of introduction or underimmunization.[3]

Routine use of Hib conjugate vaccines has resulted in a remarkable decline in Hib disease in developed and developing countries. Because most serious *H. influenzae* disease is caused by serotype b, efforts in vaccine development largely focused on Hib. Therefore, this chapter focuses primarily on the vaccines for Hib disease. However, other strains of *H. influenzae* are also important causes of illness, and more recent efforts in vaccine development for the other strains are also briefly reviewed.

HISTORY

H. influenzae was first identified as a pathogen in 1883 by Koch, who described small gram-negative rods in pus obtained from patients with conjunctivitis.[4] During the 1889 influenza pandemic, Pfeiffer isolated *H. influenzae* (which he called "Pfeiffer's bacillus") from postmortem cultures and described it as the causative organism of "influenza."[5] During the 1918 influenza pandemic, *H. influenzae* was recognized as a separate entity from the organism that causes influenza.[6]

In 1931, Pittman categorized *H. influenzae* into two major groupings: encapsulated and nonencapsulated.[7] Encapsulated strains produce a polysaccharide capsule, which is a major virulence factor. These capsules were further characterized into six antigenically distinct serotypes (a through f), which differ in chemical and antigenic composition.[7]

In 1933, Fothergill and Wright demonstrated that the great majority of Hib meningitis cases occurred in children younger than 5 years of age,[8] and that older children and adults had naturally acquired antibodies that were protective against serious Hib disease. In subsequent years, rabbit hyperimmune serum was used as an immunotherapy for Hib infections, and antibodies against the Hib polysaccharide capsule were demonstrated as the curative component.[9]

Despite the discovery of effective antimicrobial agents in the mid-1940s, Hib continued to cause significant morbidity and mortality. The case fatality ranged from 40% to 90%, and 30% to 40% of survivors had neurologic sequelae. Thus scientific advancements and recognized public health impact prompted efforts to develop effective Hib vaccines.

The first-generation Hib vaccine contained the pure polysaccharide of the Hib capsule. The polysaccharide vaccine was licensed for routine use in the United States in the early 1980s but was poorly immunogenic in children younger than age 18 months, and did not protect the age group with the highest Hib disease burden.[10] Several of the Hib conjugate vaccines that were evaluated in the mid 1980s were immunogenic in children younger than 2 years of age. Initially, the Hib conjugate vaccines were licensed for use in the United States in children between 12 and 18 months of age.[11] Subsequently, in 1990, two vaccines (PRP-OMP[polyribosylribitol phosphate–outer membrane protein] and PRP-CRM [polyribosylribitol phosphate–CRM_{197}]) were licensed for routine use among infants younger than 6 months age based on two efficacy trials.[12] The incidence of Hib disease dropped dramatically soon after introduction. Other developed countries adopted the vaccine soon after the United States with equally dramatic results. These vaccines have virtually eliminated Hib disease in areas in which they are routinely used.[13]

Uptake of the Hib vaccine was slow in low- and middle-income countries. In 2000, no Asian countries and only one country in the sub-Saharan African, South Africa, had adopted the Hib vaccine in the UIP. A few additional countries began to adopt Hib vaccines between 2000 and 2005, but uptake by low- and middle-income countries remained low. In 2005, the Hib Initiative which consisted of a consortium of four institutions (Johns Hopkins University, London School of Hygiene and Tropical Medicine, CDC [Centers for Disease Control and Prevention], and WHO) was established through funding by GAVI, the vaccine alliance.[14] The Hib Initiative helped stakeholders and Ministries of Health establish disease burden and strongly encouraged all GAVI-eligible countries to adopt Hib-containing vaccines. Currently, all the GAVI countries have introduced Hib-containing vaccines.

BACKGROUND (BOX 23.1)

Microbiology

H. influenzae is a non–spore-forming gram-negative coccobacillus. It requires two accessory (X and V) factors for growth in vitro, both naturally present in erythrocytes. The X factor is a porphyrin, such as hemin, and the V factor is nicotinamide adenine dinucleotide.[15] The requirement of both of these factors for multiplication differentiates *H. influenzae* from other *Haemophilus* species.

BOX 23.1 Key Points: Background

- Serotype b of *Haemophilus influenzae* (Hib) is the most important in human pathogenesis.
- Hib is a non–spore-forming, gram-negative coccobacillus.
- Hib was a leading cause of serious disease, particularly in childhood.
- Antibodies to the Hib polysaccharide capsule (polyribosylribitol phosphate [PRP]) provide protection against Hib disease.
- Compared with the pure polysaccharide vaccine, Hib conjugate vaccines are more immunogenic in younger children and induce immunologic memory.

A polysaccharide capsule is a major virulence factor for several bacteria, including *H. influenzae*. The capsule is thought to facilitate evasion of the mucosal immune response by resisting the bactericidal and opsonic activities of complement and protecting the bacterium from phagocytosis.[16,17] The greater pathogenic potential of type b encapsulated *H. influenzae* strains as compared with the other encapsulated *H. influenzae* serotypes has been the subject of intense investigation.[18–21] Hib possesses a capsule composed of polyribosylribitol phosphate (PRP), the structure of which was initially described in 1975.[22] The Hib polysaccharide capsule is thought to be effective for evading complement-mediated killing and to avoid splenic clearance.[16,23]

In addition to the chemical composition of PRP, certain aspects of the genetic basis of PRP capsule production could also explain the particular virulence of the type b capsule. All encapsulated strains have a *cap* locus that contains the genes necessary for capsule production. For type b strains, this region of genes is designated as *cap b*. Experiments have shown that the *cap b* region of particularly invasive type b strains contains a DNA duplication.[24–26] Several mechanisms have been proposed to explain how this *cap b* duplication/multiplication could result in increased virulence. First, strains with this duplication make more of the hydrophilic capsular polysaccharide, which may make them more resistant to dehydration during transmission or mucosal invasion.[18,27] Second, the presence of the *cap b* duplication has been shown to result in decreased susceptibility to complement-mediated lysis and opsonization.[28] Several isolates from the blood and cerebrospinal fluid (CSF) of invasive Hib disease cases have been shown to be heavily encapsulated strains with five or more copies of the *cap* locus.[24,25] (Importantly, some isolates of invasive strains of non–type b *H. influenzae* organisms have also shown amplification in their *cap* loci; the significance and prevalence of this mutation in non–type b strains is the subject of ongoing investigation.[29,30])

There are also noncapsular factors that contribute to the virulence of Hib and other *H. influenzae* strains. An important component of the *H. influenzae* cell envelope is the lipooligosaccharide (LOS), which contains endotoxin. It has been suggested that genes encoding LOS of type b strains result in the expression of certain surface-exposed epitopes that are toxic to the epithelial cells of the upper respiratory tract of animals.[31,32] The LOS of certain Hib strains may also aid in protecting the organism from host immune defenses by affecting the ability of the organism to survive and replicate in macrophages.[26,33] Another noncapsular virulence factor is the expression of pili. Several strains of *H. influenzae* are capable of expressing pili, which are considered to have a role in mediating adherence to host cells.[34] Piliated variants of Hib have shown increased binding to human oropharyngeal cells in vitro compared with nonpiliated organisms.[35] In addition, the ability to alternate between piliated and nonpiliated forms may also increase the virulence of the organism.[36] In a nasopharyngeal tissue culture model, Hib was shown to bind to the mucus above the epithelial surface, with piliated organisms having a growth advantage at this stage. At 12 hours into infection, Hib organisms were seen intracellularly, with nonpiliated bacteria having an advantage at this invasion stage.[37] This variable expression of pili enables the organism to adapt successfully at the different stages of colonization and infection, thus acting as an important virulence factor.[38]

Immunology

Anticapsular Antibodies and Titers

Pure polysaccharide antigens are recognized by B-cell receptors, but because they cannot be presented to T cells in conjunction with major histocompatibility complex class II molecules, they are T-cell independent.[20] With T-cell independent immune responses, the development of memory B cells does not occur, and immunologic memory is not induced. Thus, with the pure polysaccharide vaccines, there is no booster antibody response to revaccination.[39] In addition, polysaccharide antigens induce relatively low titers of serum antibodies in children younger than 2 years of age.[39] The reason for the poor antibody response in young children remains unclear, but on the basis of animal models has been attributed to immunologic immaturity, increased suppressor T-cell activity, immature antigen processing by macrophages, and absence of a late-appearing class of T-cell independent B cells that play a role in the production of antipolysaccharide antigen immunoglobulin (Ig) G antibody.[20,40]

Protein Conjugation

In the early 1930s, Avery and Goebel showed that the immunogenicity of small oligosaccharide or polysaccharide molecules could be increased by covalently bonding them to protein carriers.[36] Conjugating a protein carrier to Hib polysaccharide is an effective method for creating a vaccine capable of recruiting T cells into the immune response.[20] The resulting T-cell–dependent conjugate antigens differ from T-cell–independent polysaccharide antigens in two important ways. First, the protein-polysaccharide conjugate antigens are immunogenic in infants younger than 6 months of age. Second, protein-polysaccharide conjugate antigens are capable of eliciting a booster response.[20,41]

Pathogenesis

Hib is an obligate human pathogen that spreads from person to person via respiratory droplets and direct contact with respiratory secretions. It enters the body via the upper respiratory tract and colonizes the pharyngeal mucosa. Hib disease is caused by direct spread via the respiratory tract or by invasion of the pharyngeal mucosal surface and subsequent hematogenous spread. Disease episodes in which Hib is isolated from blood, CSF, or other normally sterile fluids such as pleural fluid are termed "invasive."

Individuals may remain colonized with Hib for up to 6 months without clinical symptoms or contracting disease.[42] In the prevaccine era, 3% to 5% of healthy preschool-age children in developed countries were asymptomatic Hib carriers.[43–45] Prevalence of pharyngeal carriage varies with age,[43,44,46] geographic location,[44,46] crowding,[43,44] and vaccine coverage[47,48] in the population. Before routine vaccine use in

the United States, carriage prevalence among white infants was low (0.7%) in the first 6 months of life and increased to approximately 2% in the 6- to 11-month age group.[44] The highest prevalence (up to 5%) was seen in the preschool-age group, and the prevalence gradually declined in adulthood.[43] However, among Navajo and Apache infants before the use of the Hib conjugate vaccines, the prevalence of carriage was 3% in children as young as 3 months of age,[46] which may have contributed to the high incidence rates of invasive Hib disease seen in young children in these populations.[49,50] Studies in India and Thailand from the prevaccine years showed Hib carriage prevalence of 6% to 8%.[51,52] Household crowding and attendance at a daycare center where there is an infected child are associated with an increased risk of carriage.[44,53,54] A study in Alaskan Native children showed that a ratio of more than two persons per room in a household was associated with increased Hib carriage risk (odds ratio [OR] 1.2; 95% confidence interval [CI], 1.1–1.5).[55] Only a small fraction of people colonized with Hib become ill. It has been hypothesized that disruption of the respiratory epithelium, such as with viral infection, cigarette smoke, or indoor air pollution, facilitates binding to and/or penetration of the upper respiratory tract by various bacteria.[56–58]

Recent viral infections are a risk factor for developing invasive Hib disease.[59] Viral infections could increase the risk for developing invasive Hib disease by facilitating attachment of Hib to the pharyngeal epithelium or invasion of Hib into the bloodstream.[60] One study showed that rats infected intranasally with influenza A virus had increased frequency and magnitude of bacteremia following subsequent inoculation with Hib.[61]

Once Hib enters the bloodstream, the organism causes disease at distant sites if not cleared by the host immune system. The occurrence of different disease syndromes is thought to depend, at least partially, on the density of organisms circulating in the host bloodstream.[62] For Hib meningitis, a density of 10^3 colony-forming units (CFU) per milliliter was needed in the bloodstream for at least 6 hours in infant primate models.[63] Studies in children with Hib meningitis demonstrate that the majority had more than 10^3 CFU/mL of Hib circulating in the blood at admission implying that serious consequences of infections with Hib such as meningitis are more likely to occur when there is a higher concentration of Hib in the blood.[64,65]

Laboratory Diagnosis

Hib is difficult to isolate in blood cultures. Culturing Hib from blood or other infected body fluids requires specific transport and growth conditions. Ideally, processing should occur within a few hours of specimen collection. Bacterial growth requires chocolate agar or another medium that contains the accessory factors hemin and nicotinamide adenine dinucleotide, a 3% to 5% carbon dioxide environment, and a constant temperature between 35°C and 37°C. Of note, antibiotic use before specimen collection reduces the sensitivity of culture significantly.[66]

Newer, immunologic, nonculture diagnostic methods are more sensitive and rapid, especially in settings where conditions for Hib isolation from traditional culture methods are not ideal because of the use of antibiotics prior to obtaining laboratory specimen or poor laboratory facilities for performing bacterial cultures. Studies demonstrate that analysis of CSF by polymerase chain reaction (PCR) has 100% sensitivity and 75% to 96% specificity compared with culture.[67,68] In addition, PCR is able to identify Hib in CSF even in the presence of prior antibiotic use.[68,69] Detection of the Hib polysaccharide antigen in the CSF is diagnostic of Hib meningitis. Available CSF antigen-detection methods include counterimmunoelectrophoresis (CIE), coagglutination, latex agglutination (LA), and latex particle agglutination (LPA). The CIE method uses an applied electrical field across a diffusion medium to evaluate the binding of an antibody to an antigen. Coagglutination involves mixing an agglutinin into a sample to observe the aggregation of the antigens. The LPA method involves mixing a sample with latex beads coated with an antibody to observe whether clumping occurs by an antigen binding with the antibodies. One study compared all three detection methods with CSF culture and found that LPA had a sensitivity of 78% and specificity of 100%; CIE, a sensitivity and specificity of 67%; and coagglutination, a sensitivity of 78% and specificity of 97%.[70] As with PCR, antigen detection can be more useful than culture in the diagnosis of Hib meningitis cases that have been pretreated with antibiotics.[71] These newer diagnostic techniques were essential to demonstrate the burden of disease, particularly in Asia where blood culture rates for Hib in CSF were low because of antibiotic use prior to diagnosis or poor/limited diagnostic capabilities.

The Hib polysaccharide antigen can also be detected in the urine. There has been interest in the use of urine antigen detection to aid in the diagnosis of Hib disease, particularly Hib pneumonia. In studies of patients with bacteremic pneumonia and meningitis, detection of antigenuria using LPA had a sensitivity of 90% to 100%.[72–74] In contrast, CIE had a very low sensitivity of 44%.[74] The specificity of the Hib antigenuria approach is limited by four factors: first, Hib carriage can cause Hib antigenuria even in the absence of clinical disease[75]; second, nonspecific agglutination can occur[76,77]; third, there is cross-reactivity with certain bacteria such as *Escherichia coli* K1 and K100 and *Streptococcus pneumoniae* type 6[74,76,78]; and fourth, vaccination with conjugate Hib vaccine has been shown to induce transient Hib antigenuria.[79] Studies in Navajo infants in the United States and in The Gambia have shown a prevalence of antigenuria in children of more than 80% in the first week following Hib conjugate vaccine, and antigen excretion persisted for several weeks after vaccination in some children.[80,81] In contrast, a study in Bangladesh showed transient antigenuria in only 8% of 90 vaccine recipients that lasted only 1 to 4 days after vaccination.[73] Urine antigen testing was explored as a field based/easier methodology; however, it was limited by poor specificity as it also was positive in carriage states. Given the widespread use of vaccine and decrease in disease burden, there is less interest now to explore such diagnostic methods.

Clinical Entities Caused by *Haemophilus influenzae* Type b

Hib can cause a variety of clinical syndromes such as meningitis, pneumonia, orbital cellulitis empyema, osteomyelitis, septic arthritis, cellulitis, bacteremia without focus, and infections of other organ systems. Other diseases that have been associated with Hib include epididymitis, endocarditis, peritonitis, and tracheitis. These diseases have virtually disappeared in areas that have adopted the conjugate vaccine. Nonetheless, it is important to educate healthcare providers in countries about the devastating consequences of Hib disease in countries where Hib vaccines are not used. Fig. 23.1 shows the distribution of bacterial culture–positive invasive Hib diseases seen globally in the prevaccine era. Unfortunately, even in developing countries that have adopted the vaccine, the poorest children who are at highest risk for disease and serious consequences of the disease may not have access to the vaccine. Hib can also cause diseases in the respiratory tract disease,

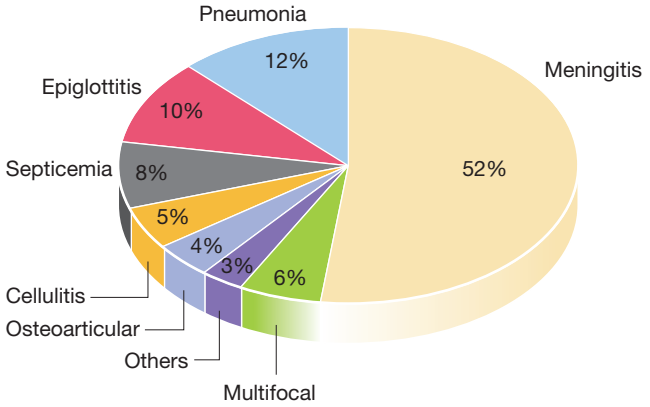


Figure 23.1. Distribution of clinical syndromes associated with invasive *Haemophilus influenzae* type b (Hib) disease worldwide. Worldwide spectrum of all classic (nonbacteremic pneumonia excluded) Hib diseases, taken from data for 3931 patients in 21 studies from various parts of the world. *(From Peltola H. Worldwide Haemophilus influenzae type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates.* Clin Microbiol Rev. *2000;13:304.)*

including otitis media, sinusitis, and bronchitis. However, the majority of upper respiratory *H. influenzae* disease is caused by nonencapsulated organisms and not Hib.

Haemophilus influenzae Type b Clinical Features

Otitis Media/Sinusitis

Otitis media results when Hib reaches the middle ear by way of the eustachian tube from the site of colonization in the nasopharynx. Although nonencapsulated *H. influenzae* causes more than 90% of *H. influenzae* otitis media, one study from the preconjugate vaccine era showed that approximately 6% of *H. influenzae* otitis media confirmed by middle-ear fluid culture was caused by Hib.[82] In addition, Hib otitis media was often a precursor to more serious invasive disease. Otitis media caused by Hib is a risk factor and in some studies the primary focus of infection for invasive disease.[83,84] As with otitis media, nontypeable *H. influenzae* strains are the most common causes of acute and chronic sinusitis.[85,86] However, in the prevaccine era, Hib was also a documented cause of bacterial sinusitis.[87,88]

Pneumonia

Hib pneumonia cannot be clinically distinguished from pneumonia caused by many other bacteria or viruses. Furthermore, there are no laboratory methods that are noninvasive, sensitive, and specific for identifying Hib as the cause of pneumonia, as blood cultures are often negative.[89] Culturing of fluid collected transthoracically from peripheral consolidation or pleural effusion can identify the causative organism in some cases of pneumonia.[90] However, the procedure cannot be used to identify the etiology of nonconsolidative pneumonia without effusion, and transthoracic aspirates are too invasive for routine use. Blood cultures are highly specific for Hib pneumonia but have low sensitivity (10–20%).[91] Reliance on blood cultures alone can result in underestimations of the role of Hib in causing pneumonia. Bronchoscopy has sensitivity and specificity of more than 85% for the identification of the etiologic agent of pneumonia; however, the procedure is invasive and requires highly trained personnel with specialized equipment.[92] Sputum culture is of little value because young children usually cannot produce adequate sputum specimens, and there are high rates of false-positive results because many persons are asymptomatic carriers of Hib and it is difficult to differentiate carriage from infection. Also, oropharyngeal or nasopharyngeal carriage of Hib does not correlate with the occurrence of Hib pneumonia.

Because there is no sensitive, specific, noninvasive test to identify Hib pneumonia cases, the incidence of Hib pneumonia is difficult to estimate. An alternative approach to estimating the burden of pneumonia caused by Hib is the "vaccine probe" method.[93–95] A vaccine probe study is a randomized clinical trial of a vaccine of known efficacy. By measuring the difference in disease incidence between vaccinated and unvaccinated groups, a vaccine probe trial can estimate the vaccine-preventable disease burden. It should be noted that the burden of Hib disease estimated in this way is an underestimate of the true Hib disease burden because most vaccines are not 100% effective and most studies do not have 100% case ascertainment. In Indonesia and The Gambia, Hib vaccine was shown to prevent approximately 5% of cases of clinical pneumonia as defined by the presence of lower chest wall retractions.[93,95] In The Gambia and Chile, Hib vaccine prevented approximately 20% of pneumonia cases with alveolar consolidation.[94,95] In contrast, a significant reduction in radiologically confirmed pneumonia was not seen with Hib vaccine in Indonesia.[93] A case-control study in Bangladesh showed that three doses of Hib conjugate vaccine reduced radiologically confirmed pneumonia by 17% to 44%, with the measured effect varying depending on the radiologist and control group used.[96] Two case-control studies from South America also found that Hib conjugate vaccine prevented a substantial portion of radiologically confirmed pneumonia—31% in Brazil and 56% in Colombia.[97,98] These results from studies of Hib vaccine effectiveness against pneumonia vary owing to different methods and different case definitions, but they all show that Hib was an important cause of pneumonia in young children (Table 23.1). A review and metaanalysis of vaccine probe studies in children younger than age 5 years estimated that Hib is responsible for 5% (uncertainty range: 1–9%) of clinical pneumonia and 21% (uncertainty range: 3–36%) of pneumonia with radiographic consolidation worldwide in children younger than age 5 years.[1]

Meningitis

Infectious meningitis occurs when an organism invades the meninges, resulting in inflammation and edema. Several studies in animal models have shown that Hib enters the CSF via the choroid plexus, where inflamed capillaries allow the organism to enter the ventricular fluid.[99] The organism then multiplies and stimulates the release of cytokines and other inflammatory mediators. The classic symptoms of meningitis are fever, bulging fontanelle, stiff neck, and altered mental status; however, in young infants, these classic signs may not be present and meningitis may instead manifest as altered consciousness, poor feeding, hypothermia or hyperthermia, and/or irritability. Without treatment, case fatality ratios from Hib meningitis are as high as 90%. Even with appropriate treatment, case fatality ratios for Hib meningitis range from 3% to 5% to 60% depending on the type of healthcare available to the child and complications such as mental retardation, cerebral palsy, hearing loss, and seizure disorders are common.[100–102] Reported rates of neurologic sequelae following Hib meningitis in survivors range from 15% to 30% in

TABLE 23.1 Calculated Vaccine Efficacy for Pneumonia Using Different Case Definitions in Various Vaccine Probe Trials

Reference	Location	Case Definition	Vaccine Efficacy (95% Confidence Interval [CI])
CLINICAL TRIALS			
Gessner et al.[98]	Indonesia	Radiologically confirmed[a] Clinically defined[b]	–3.9% (not specified) 4.0% (0.7% to 7.1%)
Mulholland et al.[96]	The Gambia	Radiologically confirmed[a] Clinically defined[b]	21.1% (4.6% to 34.9%) 4.4% (–5.0% to 12.9%)
VACCINE EVALUATION			
Levine et al.[97]	Chile	Radiologically confirmed[a] Likely bacterial[c]	22% (–7% to 43%) 26% (7% to 44%)
CASE-CONTROL TRIALS			
de Andrade et al.[100]	Brazil	Radiologically confirmed[a]	31% (-9% to 57%)
de la Hoz et al.[101]	Colombia	Radiologically confirmed[a]	47% (2% to 72%)
Baqui et al.[99]	Bangladesh	Radiologically confirmed[a]	34% (6% to 53%) in community control subjects; 44% (20% to 61%) in hospitalized control subjects

[a]Exact case definition varies, but this corresponds to consolidation or pleural effusion on chest radiograph as described by the World Health Organization (WHO).
[b]As defined by the WHO; includes tachypnea and retractions.
[c]Case definition included any of the following: alveolar consolidation, pleural effusion, erythrocyte sedimentation rate ≥40 mm/h, or bronchial breath sounds.

developed and developing countries.[103–105] A metaanalysis showed a median risk of 9.5% (95% CI, 7.1–15.3%) of at least one major sequela from Hib meningitis, with highest rates of long-term disabling sequelae occurring in low-income countries.[106] In developed countries, Hib meningitis had a case fatality ratio of at least 3%,[107] and case fatality ratios of 20% to 60% have been reported in developing countries, probably a result of poorer access to care and appropriate antibiotics.

Diagnosis of meningitis requires analysis of a CSF sample obtained by lumbar puncture. With appropriate transport and processing of specimens, analysis of CSF is a highly sensitive method for the diagnosis of Hib meningitis. However, in areas where appropriate laboratory support is not readily available, or antibiotics are given pre-CSF specimen collection, diagnosis of Hib meningitis can be difficult. A review and metaanalysis estimated a global Hib meningitis incidence rate of 31 per 100,000 (uncertainty range: 16–39 per 100,000) in children younger than age 5 years in 2000.[1] A study in Indonesia showed a vaccine-preventable, laboratory-confirmed Hib meningitis incidence rate of 16 (95% CI, 1.4–31) per 100,000 children younger than 2 years, but the rate of meningitis with CSF findings consistent with a bacterial cause (not necessarily culture-confirmed) prevented by Hib vaccine was 67 (95% CI, 22–112) per 100,000 children younger than 2 years of age.[93] The results of this study suggests that bacterial cultures identify less than 25% of probable meningitis cases in areas of the world where antibiotics are extensively used prior to obtaining CSF specimen.

Epiglottitis

Acute epiglottitis is the swelling and inflammation of the epiglottis and surrounding structures. Classic symptoms include sore throat, dysphagia, stridor, and high fever. If appropriate management, including airway establishment and antibiotic therapy, is not instituted, the disease can progress rapidly to airway obstruction and death. In the prevaccine era, Hib was responsible for 75% to 90% of epiglottitis cases in U.S. children.[108] Epiglottitis also was a common manifestation of Hib disease in other developed countries.[109,110] Rates in Scandinavia were extremely high; annual rates in Sweden ranged from 4.5 to 32 per 100,000, with the highest rates in children 3 to 4 years old.[109] However, epiglottitis was rare among indigenous populations in developed countries, including Australian Aboriginals and Alaskan/Canadian Inuit,[111,112] and in most developing countries.[113] The reason for this geographic difference is not clear but may relate to age at exposure. In populations with low epiglottitis incidence, the majority of serious Hib disease cases occurred in children younger than 1 year of age, suggesting that while early exposure to Hib led to other clinical presentations, later exposure predisposed to epiglottitis.

Septic Arthritis and Osteomyelitis

Septic arthritis is a result of an organism invading the synovial fluid and most commonly occurs in the lower extremities. Common clinical manifestations include joint pain, local swelling and erythema, limited range of motion, and fever. Sequelae are fairly common, including limitation of motion, limping gait, limb-length discrepancy, and abnormal bone growth.[114,115] In the prevaccine era, Hib was the most common cause of septic arthritis in children younger than age 2 years in the United States.[116] According to one study in Canada conducted before the availability of Hib vaccines, Hib was identified in 41% of cases of culture-positive septic arthritis.[117] Hib was a less-common cause of osteomyelitis in the prevaccine era. An estimated 5% of culture-positive osteomyelitis was caused by Hib.[117]

Facial and Orbital Cellulitis

Before conjugate vaccine introduction, Hib was a common cause of orbital, periorbital, and facial cellulitis.[118] Periorbital cellulitis caused by Hib was associated with bacteremia in up to 80% of cases, which was often associated with meningeal seeding and subsequent intracranial infection.[118–120] Hib was also a cause of facial cellulitis, including a syndrome of buccal cellulitis accompanied with bacteremia. In one series of facial cellulitis cases in the United States before the use of Hib vaccines, 82% were attributed to Hib.[121]

Non–Type b Encapsulated *Haemophilus influenzae* Disease

While non–type b encapsulated *H. influenzae* can cause meningitis and sepsis, the overall invasive disease burden resulting from non–type b encapsulated *H. influenzae* is low in relation to that caused by the type b strains. In the United States between 1998 and 2000, the rate of non–type b *H. influenzae* invasive disease was 0.8 cases per 100,000 children younger than 5 years of age.[122] In Canada, the incidence of non–type b disease was 0.11 per 100,000 per year in 2011.[123] During the years 1992 to 2006, surveillance data from 14 European and other countries identified a 3.5% (CI 2.1–5.2) annual increase in invasive non–type b *H. influenzae* disease.[124]

Nontypeable *Haemophilus influenzae* Disease

Nontypeable *H. influenzae* strains are ubiquitous colonizers of the respiratory tracts of children and adults.[125,126] Studies document pharyngeal carriage rates of approximately 50% in children younger than 2 years of age.[127] Nontypeable *H. influenzae* is a well-known cause of otitis media, sinusitis, and pneumonia.[128,129] Rarely, the organisms can cause invasive disease as well, particularly in children younger than 4 years of age and in people with underlying serious medical conditions, especially in those who are immunocompromised.[126,130,131]

Treatment and Antibiotic Resistance

Treatment of Hib disease requires early assessment and identification of the illness, administration of appropriate antibiotic therapy, and supportive management of sequelae. Therapy should be based on patterns of antibiotic resistance in the community. In the United States, the American Academy of Pediatrics (AAP) recommends that initial empiric therapy for suspected Hib meningitis should be cefotaxime or ceftriaxone. Meropenem or the combination of ampicillin and chloramphenicol are alternative regimens.[132] The WHO recommends initial therapy with chloramphenicol in combination with ampicillin or benzylpenicillin for suspected Hib meningitis.[133] If the cause is unknown, some experts recommend the addition of vancomycin to the empiric regimen owing to widespread resistant pneumococci.[132] Subsequent therapy should be guided by the antibiotic susceptibility profile of the organism and clinical/national guidelines.

Chemoprophylaxis of household contacts is recommended in certain cases because of the occurrence of secondary infections. In the prevaccine era, several documented cases of Hib occurred in close contacts of index cases, such as co-daycare attendees and household contacts.[54,134,135] Rates of secondary disease were documented to be approximately 0.6% in one study, with contacts younger than 2 years of age at highest risk.[136,137] One national study showed that for Hib meningitis in the 30 days following initial illness, the risk for household contacts was 585 times the risk in the age-adjusted general U.S. population.[138] Currently, chemoprophylaxis with rifampin is recommended for all household contacts of a case of Hib if the household has a child younger than 12 months of age who has not received the primary series, a child younger than age 4 years who is incompletely immunized, or an immunocompromised child. Daycare contacts should receive prophylaxis only if two or more cases of invasive Hib disease have occurred within 60 days.[132] Antibiotics that are usually used for the treatment of meningitis often do not eliminate Hib from the upper airway; therefore, the index case may require a different antibiotic to eliminate carriage.[139]

The relevance of the recommendation for rifampin prophylaxis should be reevaluated in countries where there is widespread use of Hib vaccines. If the index case has been in contact with unimmunized children, it is appropriate to consider rifampin prophylaxis. Between 1972 and 1974, the first antibiotic-resistant Hib isolates were reported in Europe and the United States. Since then, the proportion of resistant strains has increased across the world.[140] Studies show that 20% to 60% of isolates produce β-lactamase and are resistant to ampicillin.[141,142] Other antibiotics to which resistance has been shown include chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole (TMP-SMX). Emerging resistance to the cephalosporins has been shown; for example, in Mali, of 207 Hib isolates from blood, 0.5% were resistant to ceftriaxone.[143,144] A study in Korea carried out between 1992 and 1997 showed that of 55 Hib strains, 15% had only intermediate susceptibility to cefprozil and cefaclor.[143] Multidrug-resistant Hib has also been demonstrated. In Bangladesh in a study carried out from 1999 to 2003, 31% of Hib isolates were multidrug-resistant.[145] In Spain, 11% of isolates in one study from year 2000 were resistant to multiple antibiotics, with 3% of strains resistant to ampicillin, tetracycline, and chloramphenicol.[146] In a study from Kenya looking at Hib susceptibility to amoxicillin, chloramphenicol, and TMP-SMX, of a total of 236 blood or CSF isolates from children admitted with meningitis or sepsis, 40% were resistant to at least two antibiotics and 28% were resistant to all three antibiotics. In addition, resistance to these three antibiotics increased significantly during the 9 years of the study spanning from 1994 to 2002.[147] Surveillance studies in Latin America from before the year 2000 demonstrated that 21% of invasive *H. influenzae* (type b and non–type b) isolates were β-lactamase producers, 21% to 32% were ampicillin-resistant, and 26% to 49% were TMP-SMX–resistant.[148,149] Significant levels of resistance to ampicillin, chloramphenicol, and TMP-SMX in addition to an increasing trend of β-lactamase–producing strains have also been seen in other Asian and African countries.[145,150,151] The problem of antibiotic resistance is particularly challenging in the developing world because Hib is often resistant to the antibiotics commonly used for empiric therapy and antibiotics that remain effective against Hib are expensive or unavailable. Although judicious use of antibiotics and continued development of new antimicrobial drugs should remain priorities, vaccination remains the most powerful tool for reducing the burden of antibiotic-resistant disease.

EPIDEMIOLOGY IN THE PREVACCINE ERA (BOX 23.2)

Before the routine use of Hib conjugate vaccines, more than 95% of all invasive *H. influenzae* disease was caused by serotype b.[152] The annual incidence of invasive disease caused by Hib in the general U.S. population was estimated at 20 to 88 per 100,000 children younger than 5 years of age,[153–155] with approximately 20,000 recognized cases each year, more than 50% of which were cases of meningitis.[156] Reported rates of invasive disease in Europe varied. Studies in Spain and France showed invasive disease rates of 12 and 21 cases per 100,000, respectively.[157,158] Reported rates in Scandinavia were higher; the incidences reported from Finland and Sweden were 41 and 54 cases per 100,000 children younger than age 5 years, respectively.[158,159] It is not clear whether these differences in reported incidence rates reflect differences in surveillance methods or true differences in disease incidence.[1]

Certain U.S. populations were at higher risk of Hib disease. For example, the incidence of invasive Hib disease in Navajo and Apache children was found to be 152 and 250 per 100,000 children younger than 5 years of age, respectively.[49,160] Alaskan Eskimos were documented to have rates of invasive Hib disease of 491 per 100,000 children younger than 5 years of

BOX 23.2 Key Points: Epidemiology in the Prevaccine Era

- Before routine use of *Haemophilus influenzae* type b (Hib) conjugate vaccines, more than 95% of invasive *H. influenzae* disease was caused by serotype b.
- The annual incidence of Hib invasive disease in the general U.S. population was estimated at 20 to 88 cases per 100,000 children younger than age 5 years before Hib conjugate vaccine introduction.
- Young age (<2 years) is the predominant risk factor for invasive Hib disease.
- Other risk factors for invasive Hib disease include belonging to certain racial or ethnic groups, lower socioeconomic status, living in crowded conditions, and having concomitant serious medical conditions, particularly HIV infection.
- The WHO estimates that in 2000 (before widespread vaccine use), Hib was associated with more than 8 million serious illnesses and 371,000 deaths per year. The majority of these occurred in resource-limited settings where the Hib conjugate vaccines were not in routine use.

age.[161] Australia reported similar population differences in rates of Hib invasive disease. Nonindigenous children had an incidence rate for Hib invasive disease ranging from 33 to 60 cases per 100,000 children younger than 5 years of age, while indigenous children were found to have rates as high as 500 per 100,000 children younger than 5 years of age.[162]

Studies in Africa have consistently found high rates of Hib disease. For example, in Uganda and The Gambia, the incidence rates of Hib meningitis in children younger than 5 years of age were 88 and 60 per 100,000 before vaccine introduction, respectively.[162] Significant rates of Hib disease have also been documented in the Middle East and the Pacific island countries.[163–165]

In Asia, data on Hib disease burden have emerged more recently.[166–168] A review of studies from Asia published between 1998 and 2009 showed that Hib was identified in 60% of studies that reported the etiology of bacterial meningitis and that the incidence rate of Hib meningitis ranged from 0.98 to 28 per 100,000 children younger than 5 years of age.[168] A surveillance study of children younger than age 5 years in Sri Lanka showed Hib was responsible for 50% of the 108 meningitis cases in which an etiology was identified.[169] A prospective population-based surveillance study in India showed a Hib meningitis incidence of 7.1 per 100,000 (95% CI, 3.1–14.0) children younger than 5 years.[170] An expert panel that reviewed the literature on Hib disease in Asia concluded that many studies underestimated the true incidence of Hib because of antibiotic use before diagnostic testing, delayed contact with health providers, low rates of lumbar puncture, and inadequate specimen processing.[166] Another possible explanation for the low observed incidence of Hib meningitis in some countries is that widespread antibiotic use may actually change the epidemiology of Hib disease by altering transmission patterns or the progression of disease. In an effort to address these issues, vaccine probe studies were carried out in Indonesia and Bangladesh.[93,96] In Indonesia, Hib conjugate vaccine prevented 67 (95% CI, 22–112) cases of probable bacterial meningitis and 158 (95% CI, 42–273) cases of clinically diagnosed meningitis or seizures per 100,000 child years in children younger than 2 years of age.[93] Of note, the incidence of laboratory-confirmed Hib meningitis was only 16 per 100,000 child years in children younger than 2 years of age, suggesting that even under clinical trial conditions, laboratory confirmation of Hib meningitis is difficult. As noted, the burden of Hib pneumonia is difficult to measure, but several studies have shown that Hib conjugate vaccine prevents a significant proportion of clinical pneumonias and pneumonias with radiographic consolidation in children.[1,93,96]

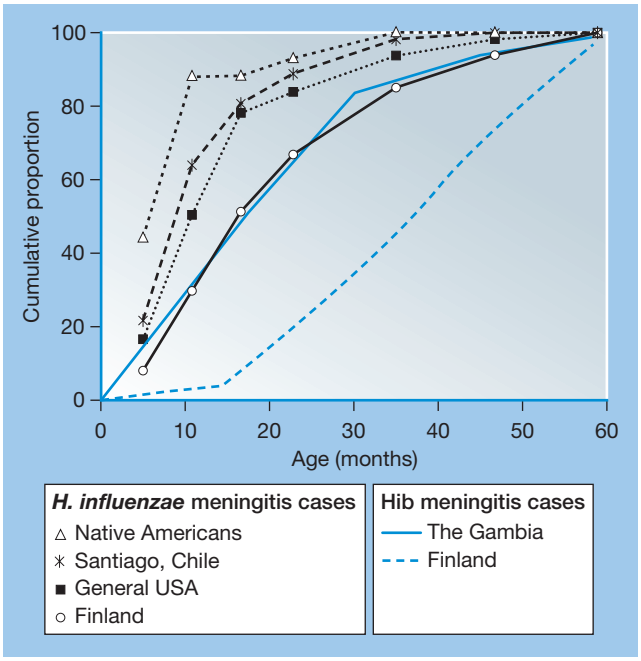


Figure 23.2. Cumulative proportion of *Haemophilus influenzae* type b (Hib) meningitis cases by age and geographic location. Figure shows cumulative proportion of cases of *H. influenzae* meningitis at successive ages <60 months for children in Santiago, Chile and cumulative distribution of Hib meningitis cases by age in The Gambia and Finland. *(Data from Ferreccio C, Ortiz E, Astroza L, et al. A population-based retrospective assessment of the disease burden resulting from invasive Haemophilus influenzae in infants and young children in Santiago, Chile.* Pediatr Infect Dis J. *1990;9: 491 and Watt JP, Levine OS, Santosham M. Global reduction of Hib disease: what are the next steps? Proceedings of the meeting Scottsdale, Arizona, September 22–25, 2002*. J Pediatr. *2003;143[6 Suppl]: S165.)*

Risk Factors

Age

Age is a major risk factor for invasive Hib disease, with children younger than 2 years of age at highest risk. Of note, the incidence of invasive Hib disease in children younger than 2 months of age is low, presumably owing to protection from transplacentally derived maternal antibodies. The age at which peak attack rates occur varies by geographic location. Fig. 23.2 shows the distribution of Hib meningitis cases by age in different geographic locations. Data from 1978 to 1998 from Western Europe showed that the majority of cases occurred in the second year of life, and fewer than 20% of cases had occurred by 6 months of age,[171] while in indigenous and developing country populations, the majority of cases occurred in the first year of life, with 30% to 50% of cases having occurred by 6 months of age.[49,112,151,172]

Race/Ethnicity

The risk of invasive Hib disease has been reported to be elevated in several ethnic groups. In the prevaccine era, African American children in the United States had rates of Hib meningitis that were up to fourfold higher than white children.[173–175]

As mentioned in "Epidemiology in the Prevaccine Era" above, Native American populations in Alaska and the continental United States had the highest incidence of invasive Hib disease in the world.[49,50,112,160] A similar higher incidence of invasive Hib disease was seen in Australian Aboriginal populations when compared with the non-Aboriginal population.[111] Whether differences in rates between various ethnic groups are a result of biological differences in the host or other factors, including genetic or environmental factors, remains unclear.[176] One study found that after controlling for various sociodemographic factors and for sickle cell disease, African Americans did not have a higher rate of Hib disease than other races.[176] Some studies have looked for genetic markers that may be associated with increased disease risk in certain communities.[177,178] Genes that increase *and* decrease susceptibility to Hib disease have been identified. For example, in Alaskan Eskimos, children with a γ-marker (GM) allotype were found to have increased susceptibility to invasive disease if associated with the human leukocyte antigen (HLA)-DR8 allele but a decreased susceptibility if associated with the HLA-DR5 allele.[179]

Sociodemographic Factors

Several factors that are often used as surrogate markers for low socioeconomic status have been associated with increased risk of Hib disease, including low household income, low parental education, and large household size.[173,180–182] The independent effect of each of these and other related risk factors has been difficult to isolate. In a multivariate analysis, Cochi and colleagues showed that household crowding was associated with a 2.7-times increased odds of Hib disease (95% CI, 1.3–5.6).[176] Other investigators also found that household crowding resulted in increased risk of Hib disease.[183–185] An increased number of siblings, particularly of preschool and elementary school age, and the presence of extended family groups were associated with an increased risk of Hib disease.[175,181,182,184] Daycare attendance also had a strong association with increased risk of invasive Hib disease, particularly in children younger than 2 years of age.[84,137,176,186] Hib can survive for up to 48 hours on toys that were placed in the mouths of children who were asymptomatic Hib carriers; therefore, communal toys may serve as fomites for the transmission of Hib.[187] Given that these sociodemographic factors would also impact vaccine access and coverage, their influence on disease still remains relevant and difficult to quantify.

Concomitant Medical Conditions

Serious medical conditions, particularly conditions resulting in immunosuppression, are also recognized risk factors for Hib disease. Hemoglobinopathies such as sickle cell disease, complement deficiency, antibody deficiency, and asplenia, are associated with increased risk of Hib disease.[188–191] The incidence and severity of Hib disease is also higher in adults and children with HIV infection.[192,193] Antecedent viral respiratory infections are associated with increased Hib carriage and increased susceptibility to Hib meningitis.[46,59] This could be from the enhanced transmission of virus through the increased production of respiratory secretions or damage to the respiratory epithelium by the virus, resulting in increased attachment or invasion by Hib into the bloodstream.[194]

Breastfeeding

Several studies show that in children younger than 6 months of age, breastfeeding is protective against invasive Hib disease.[84,176,181,186] Although the mechanism for protection is unknown, it is thought to be from immunologic or nutritional cofactors that are transmitted from the mother. Studies have shown that human milk contains secretory antibody to the Hib PRP capsule.[195]

PASSIVE IMMUNIZATION

Bacterial Polysaccharide Immunoglobulin

Passive immunization with bacterial polysaccharide immunoglobulin (BPIG) hyperimmunoglobulin was tested in high-risk children in the late 1980s. BPIG was prepared from the plasma of adult volunteers immunized with Hib polysaccharide vaccine, a four-valent meningococcal vaccine, and pneumococcal polysaccharide (14- or 23-valent) vaccines. Compared with conventional immunoglobulin, BPIG contained 10 to 60 times higher concentrations of antibody to Hib polysaccharide.[196] Infants were randomized to receive BPIG or saline placebo at 2, 6, and 10 months of age in a double-blind trial among White Mountain Apache infants in Arizona. The point estimate of efficacy for 4 months following BPIGG administration was 86% (95% CI, −11% to 100%).[197] However, because of the necessity of frequent large-volume injections, the relatively high cost and the subsequent availability of effective conjugate vaccines for this age group, BPIG was not licensed for use in the United States.

ACTIVE IMMUNIZATION (BOX 23.3)

The first definitive demonstration that invasive Hib infections were vaccine preventable was a Finnish trial during an epidemic of meningococcal disease during which a Group A meningococcal vaccine efficacy study was conducted with an investigational Hib capsular polysaccharide (PRP) vaccine as control.[198,199] Statistically significant efficacy of the Hib vaccine was demonstrated in children older than 24 months of age. There were too few children in the 18 to 24 months age group to evaluate efficacy, while children younger than 18 months were not protected.[200] The age-related differences in efficacy correlated well with age-related differences in antibody responses to vaccination. Analysis of infection rates in children who received the vaccine suggested that a level of 1.0 μg/mL of anti-PRP antibody, obtained 1 month after vaccination, was predictive of protection against infection for a period of at least 3 years—that is, the presence of an antibody level of at least 0.15 μg/mL. Analysis of infection rates in children who received the meningococcal vaccine showed that children having a naturally acquired level of 0.15 μg/mL of anti-PRP antibody were resistant to infection.[201] The antibody assay used for this study was a Farr-type radioimmunoassay for total antigen binding protein, which, while useful and well standardized, provided no information regarding the nature of the immune response. For the purposes of vaccine development, the Finnish study provided two useful benchmarks: (a) 1.0 μg/mL of anti-PRP antibody 1 month after vaccination was predictive of protection for longer than 3 years (i.e., presence of

BOX 23.3 Key Points: Active Immunization

- The pure polysaccharide (polyribosylribitol phosphate [PRP]) vaccine is effective in children older than 24 months of age but not in children younger than 18 months of age.
- The *Haemophilus influenzae* type b (Hib) conjugate vaccines are safe and effective in children as young as 6 weeks of age.
- Invasive disease rates have decreased dramatically with routine use of Hib conjugate vaccine in a variety of settings.

antibody levels of at least 0.15 μg/mL), and (b) naturally occurring antibody levels above 0.15 μg/mL were associated with short-term protection. Vaccine development was principally guided by the goals of achieving a 1.0 μg/mL or higher response to vaccination, considered predictive of protection, and an acceptable duration of immunity assessed in long-term follow-up studies as the maintenance of a minimum 0.15 μg/mL circulating antibody.

POLYSACCHARIDE VACCINE

Immunogenicity and Correlates of Protection

As mentioned above the first Hib vaccine to be field-tested was the pure capsular polysaccharide (PRP) vaccine. The level of antibody response to PRP vaccine is age-dependent. After one dose, 8% to 20% of children younger than 12 months of age had an antibody concentration of more than 0.15 μg/mL, and only 2% had a concentration of more than 1.0 μg/mL.[159,202] By contrast, up to 45% of children ages 12 to 17 months had an antibody concentration of more than 1.0 μg/mL, and 50% to 75% of children ages 18 to 23 months mounted such an antibody response.[39,159,201] More than 90% of 4- to 5-year-olds responded with a strong antibody response.[203] While a range of doses elicited a significant immune response in older children, no difference in response was seen in children younger than 12 months of age who were given doses ranging from 0.2 to 50 μg of PRP.[9,204]

The PRP capsular polysaccharide of Hib bacteria is characterized as a T-cell–independent immunogen. While inducing a strong immune response in adults,[205,206] it induces a more modest but still protective level of immunity in children older than 24 months of age.[198] However, the unconjugated PRP vaccine did not induce protective antibody levels in infants younger than 1 year of age.[201,207,208] Characteristic features of the T-cell–independent antibody response to polysaccharide immunogens include the absence of booster responses after administration of repeated doses of the vaccine and the absence of anamnestic responses on reexposure to the antigen.

Persistence of antibody response following immunization is also age-dependent. In children younger than 2 years of age, antibody concentrations rapidly declined to levels similar to unimmunized children in the first few months after vaccination.[39,209] In a follow-up study of children vaccinated with one dose of PRP at ages 18 to 35 months, children who were 1.5 years postvaccination had geometric mean antibody titers of more than 1.0 μg/mL, but children who were 3.5 years postvaccination had geometric mean titers of only around 0.5 μg/mL.[39]

Efficacy

As discussed previously, in a trial designed to evaluate the efficacy of a meningococcal vaccine during an epidemic, the Hib polysaccharide vaccine was used as the control. Finnish children ages 3 months to 5 years were randomized to receive meningococcal vaccine or the Hib polysaccharide vaccine. Children ages 18 to 71 months who received Hib polysaccharide vaccine had a statistically significant reduction in bacteremic Hib disease incidence, and vaccine efficacy was estimated to be 90% (95% CI, 55–98%). Of note, in children younger than 18 months of age, there was no statistically significant difference in rates of bacteremic Hib disease between the two groups.[159,200] Based on these results, the vaccine was licensed in the United States in 1985 for use in children older than 23 months of age and for children 18 to 23 months of age who are considered at high risk for invasive Hib disease.[210] Subsequent case-control studies in the United States demonstrated a range of efficacy of 55% to 92% in children 24 to 72 months old.[211,212]

In general, PRP vaccines were found to be extremely safe in field trials and postmarketing studies, with no association between PRP vaccine and serious reactions found in a 1-year postmarketing surveillance study.[213] There was some concern about an increased risk for Hib infection in the 1 week following vaccination with PRP.[214–217] This corresponded to the observation that the anti-PRP antibody concentrations fell in the first few days after vaccination.[218] Black and colleagues found a 6.4-fold increased risk in the week after vaccination (95% CI, 2.1–19.2) versus a 1.8-fold increase (95% CI, 0.3–10.2) shown by a CDC study conducted across the United States.[214,216] The affected children in both studies were thought to be high risk; they were African American, attended daycare, and had underlying medical conditions.[217] Owing to the subsequent availability of Hib conjugate vaccines, interest in further defining the safety and efficacy of PRP vaccines waned.

CONJUGATE VACCINES

History and Development

While the potential for prevention of Hib disease by vaccination was clear from the Finnish efficacy trial using the pure PRP vaccine, it wasn't until the 1980s that innovative vaccine development accelerated. In addition to the pure PRP polysaccharide vaccine, investigational approaches included partially purified capsular polysaccharide complexes with bacterial membrane fragments,[219] adjuvanting PRP by coformulation with whole-cell pertussis vaccine,[220–222] and PRP conjugated to either diphtheria toxoid, PRP-D,[223] CRM_{197} mutant diphtheria toxoid (HbOC),[224] group B meningococcal outer membrane protein (OMP)[225] or tetanus toxoid PRP-T.[226]

Vaccine development in the 1980s was also stimulated by The National Childhood Vaccine Injury Act of 1986, which included the creation of National Vaccine Program. Another major contributor to the very productive decade of Hib vaccine development was the creation, starting in 1980, of a program for the accelerated development of vaccines within the U.S. Department of Health and Human Services.[227] Through a consultative process managed by the National Academy of Sciences, priorities for the development of new vaccines addressing diseases of importance in the United States and globally were evaluated. Critically, a very high priority was established for the development of vaccines for the prevention of Hib. This allowed the National Institute of Allergy and Infectious Diseases (NIAID) to focus its resources on approaches to accelerating development of new vaccines. Core to this initiative was the NIAID's announcement that it would sponsor a Phase III pivotal efficacy study of a candidate vaccine. NIAID issued two requests for proposals, one to clinical investigators for the design and conduct of the Phase III study and a second parallel request for proposals to vaccine developers for the selection of the best available vaccine for accelerated development in the sponsored efficacy study. In addition to requesting written proposals, the NIAID formed an expert panel to advise on the selection of both the study design/site and of a vaccine to be evaluated in the NIAID study. Investigators were invited to periodic meetings of the expert panel to present and defend their proposals; however, it was a precondition of eligibility that, while confidential to the public, all presentations had to be made in the presence of all contenders. This process assured not only the benefits of the expertise of the independent panel members, but also the deep subject-matter expertise of the principal developers of each of the vaccines and study plans. The NIAID sponsored efficacy study[112,220] was only one of several ways in which the

NIAID program has assisted in the accelerated development of Hib and other vaccines.

Hib vaccines used in the United States include PRP 1985-1988, PRP-D 1987-2000, PRP-CRM_{197} 1990-1997. Hib vaccines currently distributed in the U.S. include PRP-OMP, first licensed in 1989, and PRP-T vaccines, first licensed in 1993. As with all vaccines there has been continued product development well past initial licensure. The first two vaccines to be licensed for immunization of infants below 6 months of age were the PRP-OMP and the HbOC. Both were licensed in 1990. The HbOC vaccine was licensed as a three-dose primary series with a booster dose at 15 months of age or as soon as possible thereafter, and the PRP-OMP was licensed as a two-dose primary series with a booster dose at 12 months of age.[12] While all the PRP conjugate vaccines produced good immune responses after the primary series, only the PRP-OMP vaccine induced a "protective" antibody response after a single dose at 2 months of age. In the efficacy trial conducted on the Navajo reservation, there were eight cases of Hib in the placebo group and zero cases in the vaccine group between the first and second doses ($P < .005$).[12,228] Subsequently an efficacy trial was conducted in The Gambia, which was the first trial to demonstrate efficacy in a developing country against meningitis and blood culture–positive pneumonia. In addition, in a post hoc analysis, The Gambia trial demonstrated a 21% reduction in X-ray–documented pneumonia.[95]

Molecular Basis for Conjugate Vaccines

The objective of developing PRP polysaccharide–protein conjugate vaccines was to convert PRP from a T-cell–independent immunogen to a T-cell–dependent immunogen. The underlying principal was originally described by Landsteiner[229] and first applied to bacterial polysaccharides by Avery and Gobel.[36] Essentially the approach is to combine the T-cell–independent PRP polysaccharide and a T-cell–dependent protein into a single molecule by covalent conjugation. The resulting hybrid molecule has the ability to stimulate a T-cell–dependent antibody response conferred by the carrier protein and the antigenic epitopes of both the carrier protein and the PRP capsular polysaccharide. The carrier protein stimulates activation and proliferation of antigen-reactive T cells, which influence how antigen-reactive B cells respond to the antigenic epitopes on the conjugate. In a T-cell–dependent response, antigen-reactive B cells can either proliferate resulting in an expanded population of antigen reactive B cells or may differentiate into antibody-secreting plasma cells. The proliferation of antigen-specific T and B cells, referred to as clonal expansion, is largely responsible for immunologic memory, the ability to mount an anamnestic or accelerated antibody response on subsequent exposure to the antigen. B-cell differentiation into antibody secreting plasma cells is, however, an end-stage process. The design of a conjugate vaccine consequently involves balancing the stimulation of high levels of circulating antibodies versus high levels of memory cells, enabling anamnestic response to infection. Although the balance will differ between vaccines, Hib conjugate vaccines stimulate both protective levels of circulating antibody and the ability to mount anamnestic responses on challenge.[230–232]

Formulations

Four conjugate Hib vaccines have been developed. Table 23.2 lists the manufacturers and formulations available for use in the United States. Table 23.3 is a similar list of WHO prequalified vaccines for purchase by United Nations agencies for global use. The protein carrier, PRP component size, and chemical linkages are different for all four conjugate vaccines, and there are some differences in the elicited immune response. The vaccines should all be stored between 2°C and 8°C.

PRP-D was produced by Connaught Laboratories. It contained medium-sized lengths of the polysaccharide linked to the diphtheria toxoid carrier. There were no added adjuvants. Thimerosal was added as a preservative. PRP-D was the first Hib conjugate vaccine to be licensed in the United States. It was licensed in the United States for children 15 to 59 months of age in 1987. This product is no longer available. Licensure was based on the demonstration of efficacy in a large-scale field trial conducted in Finnish infants.[233]

The HbOC vaccine contains oligosaccharides derived from purified PRP from the Hib Eagan strain. These are coupled by reductive amination to purified CRM_{197}, a nontoxic variant of diphtheria toxin isolated from *Corynebacterium diphtheriae* C7 (β197). The HbOC vaccine was licensed in the United States in October 1990 for immunization of infants younger than 6 months of age based on a trial conducted at Kaiser Permanante in Southern California by Black and colleagues.[234] This product is no longer available.

The PRP-OMP vaccine is based on a purified PRP from the Hib Ross strain. This is covalently bonded to an outer membrane protein complex of the B_{11} strain of *Neisseria meningitidis* type B. The mixture is formulated with aluminum hydroxyphosphate sulfate and 0.9% sodium chloride. The vaccine was licensed for use in all infants in the United States in December

TABLE 23.2 *Haemophilus Influenzae* Type b Conjugate Vaccines Available in the United States

Product	Trade Name (Manufacturer)	Hib Protein Carrier	Amount of *Haemophilus* b PRP Per Dose (μg)
PRP-OMP	PedvaxHIB (Merck & Co.)	Outer membrane protein complex of *Neisseria meningitidis* strain B_{11}	7.5
PRP-T	ActHIB (Sanofi Pasteur)	Tetanus toxoid	10
PRP-T	Hiberix (GlaxoSmithKline) (booster dose only)	Tetanus toxoid	10
PRP-T/MenCY	MenHibRix (GlaxoSmithKline)	Tetanus toxoid	2.5
PRP-OMP/hepatitis B	Comvax (Merck & Co.) (primary series and booster dose)	Outer membrane protein complex of *N. meningitidis* strain B_{11}	7.5
PRP-T/DTaP-IPV	Pentacel (Sanofi Pasteur)	Tetanus toxoid	10

DTaP, diphtheria, tetanus toxoids, and acellular pertussis; Hib, *Haemophilus influenzae* type b; IPV, inactivated polio vaccine; MenCY, meningococcal C- and Y-specific memory B cells; PRP, polyribosylribitol phosphate; PRP-OMP, PRP–outer membrane protein; PRP-T, PRP conjugated to tetanus toxoid.

TABLE 23.3 *Haemophilus influenzae* Type b Conjugate Vaccines Prequalified by the World Health Organization for Purchase by United Nations Agencies Globally

Product	Trade Name (Manufacturer)	Hib Protein Carrier	Amount of *Haemophilus* b PRP Per Dose (μg)
PRP-T/DTaP-HepB-IPV	Hexaxim (Sanofi Pasteur)	Outer membrane protein complex of *Neisseria meningitidis* strain B_{11}	12
HbOC/DTwP	QuattVaxem (Novartis)	Diphtheria toxin CRM_{197}	10
PRP-T/DTwP	TETRAct-HIB (Sanofi Pasteur)	Tetanus toxoid	10
PRP-T/DTwP	Diphtheria-Tetanus-Pertussis (whole cell)–*Haemophilus influenzae* type b (Serum Institute of India)	Tetanus toxoid	10
HbOC/DTaP-HepB	Quinvaxem (Berna Biotech Korea Corporation, a Crucell Company)	Diphtheria toxin CRM_{197}	10
PRP-T/DTwP	Pentabio (Biofarma, Indonesia)	Tetanus toxoid	10
PRP-T/DTwP	Diphtheria-Tetanus-Pertussis (whole cell)–Hepatitis B–*Haemophilus influenzae* type b (Biological E, India)	Tetanus toxoid	11
PRP-T/DTwP	Tritanrix-Hib (GlaxoSmithKline)	Tetanus toxoid	10
PRP-T/DTwP	Euforvac-Hib (LG Lifesciences, Republic of Korea)		
PRP-T/DTwP	Easyfive-TT (Panacea Biotech, India)	Tetanus toxoid	10
PRP-T/DTwP	Shan-5 (Shantha Biotechnics Private Ltd., India)	Tetanus toxoid	10
PRP-T	Quimi-Hib (Center for Genetic Engineering and Biotechnology, Cuba)	Tetanus toxoid	10
PRP-OMP	Pedvax (Merck & Co.)	Outer membrane protein complex of *N. meningitidis* strain B_{11}	7.5
HbOC	Vaxem Hib (Novartis Vaccines and Diagnostics s.r.l.)	Diphtheria toxin CRM_{197}	10
PRP-T	ActHIB (Sanofi Pasteur)	Tetanus toxoid	10
PRP-T	*Haemophilus influenzae* type b (Serum Institute of India)	Tetanus toxoid	10

DTaP, diphtheria, tetanus toxoids, and acellular pertussis: DTwP, diphtheria and tetanus toxoids combined with whole-cell pertussis; HbOC, Hib oligosaccharide conjugate; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; IPV, inactivated polio vaccine; PRP, polyribosylribitol phosphate; PRP-OMP, PRP–outer membrane protein; PRP-T, PRP conjugated to tetanus toxoid.

1990 based on an efficacy trial conducted on the Navajo Indian reservation.[228]

PRP-T is made by covalently binding PRP to tetanus toxoid derived in some instances from *Clostridium tetani* Harvard strain. The PRP-T currently available in the United States is produced as a lyophilized powder that is reconstituted at the time of use with 0.4% sodium chloride and contains no preservative. This vaccine was licensed in the United States in March 1993. The licensure of PRP-T was based on immunologic correlates of protection. None of the vaccine manufacturers report the presence of antibiotics in any of the monovalent or combination Hib vaccines.

Immunogenicity

PRP-D elicited a geometric mean antibody response of more than 1.0 μg/mL in a group of 141 children 15 months or older after just one dose.[235] In children 9 to 15 months of age, all subjects achieved antibody titers of more than 1.0 μg/mL after two doses; although titers waned in the following year, a booster dose induced a strong antibody response.[236] However, in children younger than 6 months, the immune response was limited.[221] In addition, the antibody response was suboptimal in some high-risk children, including children with cancer.[237]

The three other conjugate vaccines are highly immunogenic when given to children 18 to 24 months or older.[238,239] The response in younger infants varies among vaccines (Fig. 23.3). HbOC, while relatively poorly immunogenic after one or two doses at 2 and 4 months of age, elicits antibody titers greater than 1.0 μg/mL in the majority of recipients after the third dose at 6 months of age.[240,241] The antibody levels induced after the third dose at 6 months of age were slightly higher than with PRP-OMP and were similar to those from PRP-T.[242] The induced antibody is bactericidal and has a statistically significantly greater avidity than the antibody induced by PRP-OMP and PRP-T.[243] The clinical significance of this increased antibody avidity is unknown.

PRP-T has an immunogenicity pattern similar to that of HbOC.[240,242] Some studies have shown that this vaccine is the most immunogenic of the three available Hib conjugate vaccines after completion of a three-dose primary series[240,241]; whether that translates to improved efficacy is unclear. The elicited antibodies are bactericidal, with a similar isotype distribution as HbOC.[244]

Unlike the other Hib conjugate vaccines, PRP-OMP elicits antibody titers of more than 1.0 μg/mL in 70% to 80% of children after just one dose given at 2 months of age.[225,245–248] After the second dose at 4 months of age, some studies showed that more than 90% of children achieved antibody

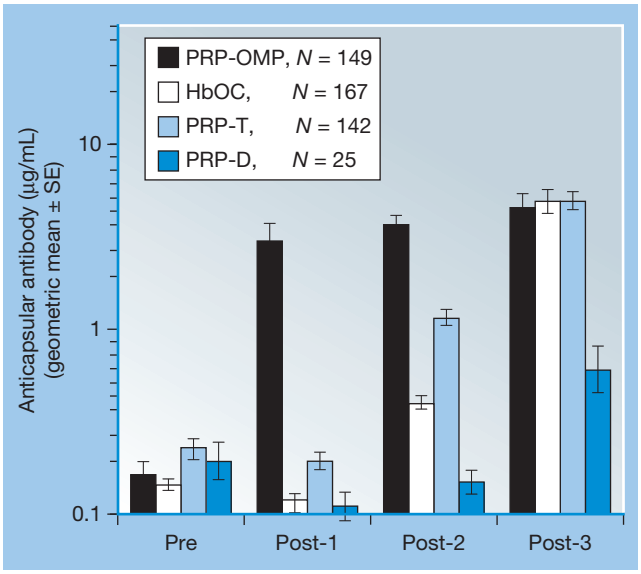


Figure 23.3. Immunogenicity of the *Haemophilus influenzae* type b conjugate vaccines in infants vaccinated at 2, 4, and 6 months of age. HbOC, *H. influenzae* type b oligosaccharide conjugate; PRP, polyribosylribitol phosphate; PRP-D, PRP-diphtheria; PRP-OMP, PRP–outer membrane protein; PRP-T, PRP conjugated to tetanus toxoid; SE, standard error. *(Modified from Granoff DM, Anderson EL, Osterholm MT, et al. Differences in immunogenicity of three Haemophilus influenzae type b conjugate vaccines in infants*. J Pediatr. *1992;121:87–194.)*

titers more than 1.0 µg/mL.[249,250] A study in high-risk Native American children showed that 1 month following the second dose at 4 months of age, 67% of Apaches and 75% of Navajos had antibody titers more than 1.0 µg/mL.[248] A third dose did not result in significantly increased antibody concentrations in most studies; therefore, the primary series for this vaccine consists of only two doses.[240,241,247] In children given a booster dose of PRP-OMP at 12 to 15 months of age, a clear booster response is seen.[251] However, the peak antibody titer achieved after the booster dose is lower than with the other two vaccines. It has been shown that as against Hib conjugated to CRM_{197} and tetanus toxoid, Hib conjugated to the outer membrane protein (OMP) of *N. meningitidis* engaged human Toll-like receptor 2 (TLR2) expressed in human embryonic kidney (HEK) cells. In mice where the TLR2 was knocked out, the antibody response to Hib-OMP was not as robust as in wild-type mice with the TLR2 intact.[252] This immune-stimulating capacity of the OMP has been attributed to the meningococcal PorB protein, which was shown elsewhere to activate B cells and other antigen-presenting cells through upregulation of CD86 with the involvement of TLR2.[253] This additional immune stimulating activity when compared to the other carrier proteins might be a reason for the relatively higher immunogenicity of the Hib-OMP vaccine after the first dose.

Because of the ability to induce antibody responses after the first dose in young infants, PRP-OMP was evaluated in newborns as a possible means of providing earlier protection against invasive Hib disease; unfortunately, antibody levels were substantially diminished in children through the first year of life, suggesting immune tolerance if the vaccine is administered soon after birth.[254] In contrast, a study in Finland found that children receiving PRP-T at 2 days of age had a geometric mean antibody concentration of 0.12 µg/mL at 4 months of age, which was significantly higher than in unimmunized children. Subsequently vaccinating the children again at 4 months of age elicited a strong antibody response, suggesting that children vaccinated as neonates are protected against invasive Hib disease.[255] The antibody induced by PRP-OMP has lower avidity and bactericidal activity compared with the antibody elicited by the other conjugate vaccines.[243] The differences in antibody titers and avidity induced after the primary series and booster doses of the different conjugate vaccines have not been shown to have any clinical significance. PRP-OMP may have a theoretical advantage in populations in which high rates of Hib disease are seen in children younger than 6 months of age or in which incomplete immunization is more likely. In populations such as the Navajo and Apache in the United States where high rates of Hib disease were observed in children younger than 6 months of age, routine use of PRP-OMP has virtually eliminated invasive Hib disease.[256]

Some studies showed differences in immunogenic response to the Hib polysaccharide vaccine in certain ethnic groups; for example, Siber and colleagues showed that Apache children had 10-fold lower geometric mean Hib antibody concentration after immunization than white children (0.34 vs 3.6 µg/mL; $P < .01$) at the age of 24 months, approximately 5 to 6 months after immunization. Similarly, PRP-D had very low immunogenicity in Alaskan Native infants compared with nonnative children.[257,258] However, ethnic differences in immune response have not been shown with the other Hib conjugate vaccines when tested in numerous developed and developing country settings.

Interchanging HbOC and PRP-OMP, as well as HbOC and PRP-T, for the primary series or the booster dose is equally or more immunogenic than using a single vaccine for the entire series.[259,260] Use of HbOC for the booster dose following a two-dose primary series with PRP-OMP has been shown to induce a higher antibody response compared with giving PRP-OMP as the booster dose (geometric mean antibody titer 7.46 vs 29.5 µg/mL; $P < .05$).[261] Use of a single vaccine has been highly effective in many different settings; whether certain combinations of vaccines in the Hib series can improve effectiveness has not been established.[244]

As previously discussed, serum anti Hib capsular polysaccharide antibody levels greater than 1 µg/mL are associated with long term protection and 0.15 µg/mL with short-term protection. An interesting finding with significance for decrease in carriage of Hib at the population level was that among vaccinated infants, the serum anti Hib capsular polysaccharide antibody level required for protection against carriage was greater than 5 µg/mL as measured a month after the third dose. The levels needed for protection against colonization are much higher than those needed to protect against invasive disease.

Combination Vaccines. Combination vaccines containing Hib conjugate vaccine have also been shown to be highly immunogenic in a variety of settings.[262–264] The initial combination vaccines contained the Hib conjugate vaccine in combination with diphtheria, tetanus, and pertussis (DTP) containing the whole-cell pertussis. In fact, this is the combination that is currently used in most developing countries. However, the hepatitis B surface antigen has also been added to the current Hib-containing combination vaccines that are used in developing countries. In the United States and other developing countries, DTP component of the Hib containing combination was replaced with diphtheria, tetanus, and acellular pertussis (DTaP) because of the reduced reactogenicity of the latter vaccine. Initial studies demonstrated reduced

immunogenicity in Hib containing combination vaccines that were combined with DTaP; however, this reduced immunogenicity has never been shown to be of clinical significance. Several other combination vaccines containing different antigens are now available in the market. All these new combination vaccines are safe and induce adequate Hib antibody levels. For example, the DTaP-HBV (hepatitis B virus)-IPV (inactivated polio vaccine)-PRP-T hexavalent vaccine when studied in Western Europe and the Philippines showed that 93% to 100% of children had an anti-PRP titer of 0.15 μg/mL 1 month after the primary vaccination series.[264]

Carrier Priming

Administration of a vaccine's carrier protein at a time before the administration of a conjugate vaccine that contains that same protein is termed *carrier priming*. Carrier priming may result in enhanced immune responses to Hib conjugate vaccine, but the immunologic consequences of carrier priming are unclear.[265–267] Because most children have early exposure to vaccines containing diphtheria and tetanus toxoids, it has been suggested that use of diphtheria or tetanus toxoid as the carrier for Hib conjugate vaccine may confer an immunologic advantage.[268] Animal studies have shown immunologic enhancement with carrier priming.[269,270] Several clinical trials have demonstrated an enhanced antibody response following PRP-T administration in children who had previously received DTaP vaccine and suggested that the effect may be at least partially from carrier priming.[271–273] In a clinical trial in Denmark, infants were given PRP-T at 5 to 6 months of age and randomized to receive tetanus toxoid as part of the diphtheria-tetanus–inactivated polio (DT/IPV) vaccine before, during, or after this dose. The primed group that received prior priming with DT/IPV had threefold higher antibody levels than the unprimed group. The group that received only concomitant priming had no difference in antibody levels compared with the unprimed group.[274] The clinical significance in terms of vaccine efficacy of carrier priming for the Hib conjugate vaccines remains unclear.

Efficacy

Clinical trials of the protective efficacy of PRP-D were conducted in Finland and Alaska. Between 1985 and 1987, an open-label non–placebo-controlled trial was conducted in Finland in which all children born on the odd days of the month received 25-μg doses of PRP-D at ages 3, 4, 6, and 14 to 18 months. The protective efficacy of PRP-D against invasive Hib disease after three doses was 90% (95% CI, 79–96%). Following the booster dose, the protective efficacy was 100%.[233] The Alaskan trial recruited Native Alaskan infants between 1984 and 1988. Infants were randomized to receive PRP-D or placebo at 2, 4, and 6 months of age. The protective efficacy after three doses was 35% (95% CI, −57% to 73%).[257] Because of the availability of other conjugate vaccines with superior immunogenicity and efficacy, particularly in young and high-risk children, PRP-D was not used in most countries and is no longer manufactured.

Two large prospective trials showed the clinical efficacy of HbOC. In the late 1980s, an unblinded, nonrandomized study was conducted in California, in which all children between 6 weeks and 6 months of age were given a three-dose series with HbOC vaccine; findings were compared with those for unvaccinated children between 7 and 18 months of age. Vaccine efficacy after two doses was 100% (95% CI, 47–100%) and after three doses was 100% (95% CI, 68–100%); however, efficacy after just the first dose was lower (26%; 95% CI, −166% to 80%), but the data are limited to the small number of cases occurring after a single dose.[234] A second trial in Finland compared the efficacy of HbOC with PRP-D when given at 4, 6, and 14 to 18 months of age. After two doses, the vaccine efficacy was 87% (95% CI, 69–96%) for PRP-D and 95% (95% CI, 76–99%) for HbOC. There were no episodes of invasive Hib disease following the booster dose in either group.[275]

PRP-OMP was evaluated in a randomized, double-blind, placebo-controlled trial on the Navajo Indian reservation, a population previously established to be at high risk for invasive Hib disease. Infants were randomized to receive the vaccine or placebo at 2 and 4 months of age. Overall efficacy was 95% (95% CI, 72–99%). Of note, protection began after the first dose. Between the first and second doses of vaccine or placebo, there were eight cases of Hib disease in the placebo group and none in the vaccine group (vaccine efficacy, 100%; 95% CI, 15–100%).[228] The PRP-OMP vaccine was subsequently found to be effective in postlicensure trials in several populations and countries, including Los Angeles County, California, Navajo and Apache Indians, Australia, and Israel.[276–279] Dramatic reductions in Hib disease incidence have been demonstrated in all populations in which the vaccine has been used.

Two large PRP-T trials were started in the United States but were discontinued in October 1990 when the recommendations for use of HbOC in infants were instituted. In the mid-1990s, a large vaccine trial was conducted in The Gambia, comparing PRP-T mixed with DTP with DTP alone. This trial showed a protective efficacy of 95% (95% CI, 67–100%) against all invasive Hib disease after three doses. Efficacy after a single dose for all invasive Hib disease was 44% (95% CI, −85% to 85%).[95] PRP-T reduced clinical pneumonia and radiologically confirmed pneumonia in several clinical trials. The efficacy for the prevention of clinical pneumonia as defined by the WHO was 4.4% (95% CI, −5% to 12.9%), and for radiologically defined pneumonia, efficacy was 21.1% (95% CI, 4.6–34.9%). The efficacy for the prevention of blood culture–positive pneumonia after three doses of vaccine was 100% (95% CI, 55–100%).[95] PRP-T was also highly efficacious against invasive Hib disease in trials in Britain, Chile, and Finland.[280–282] There have been no comparative efficacy trials of the three commonly used Hib conjugate vaccines. All three seem highly and equally efficacious.

In many of the developing countries, a large number of children do not receive all the recommended doses of multidose vaccines like Hib. A metaanalysis of eight prospective controlled trials of different conjugate Hib vaccines found that pooled vaccine efficacies against invasive disease after one, two, and three doses were 59%, 92%, and 93%, respectively.[283] This analysis did not address the long-term protection or the requirement for booster dose of conjugate vaccines as the trials followed up participants for 24 months only. This is discussed later in the chapter.

Effectiveness/Impact

Hib vaccine has been highly effective in developed and developing countries. In the United States, the introduction of the Hib conjugate vaccine resulted in a decrease in the incidence of Hib disease of more than 98%.[122,154] There were also substantial indirect effects ("herd immunity") induced by the vaccine.[278] Similar success has been documented in Canada and several countries in Western Europe.[284–289] Several developing countries have also noted dramatic declines in Hib disease with use of the Hib vaccines.[162,290] In Chile, a 90% decline in the rate of invasive Hib disease was demonstrated.[282] After Hib conjugate vaccine introduction, the annual incidence rates of Hib meningitis in The Gambia decreased from

more than 200 per 100,000 children younger than 1 year of age in the early 1990s to none per 100,000 in 2002 and from 60 to no cases per 100,000 in children younger than 5 years of age.[47] In Uganda, vaccine effectiveness, with two or more doses, was 99% (95% CI, 92–100%) against Hib meningitis using neighborhood control subjects and 96% (95% CI, 80–100%) using hospitalized control subjects.[291] In Senegal, the annual rate of Hib meningitis in children younger than 1 year of age went from 33 to 1.4 per 100,000; similar success was documented in Mali.[292,293] Some studies have shown decreases in rates of clinical pneumonia, as well as pneumonia with radiographic consolidation and clinical pneumonia, following Hib vaccine introduction.[97,98,294] Following routine introduction in Chile, Hib vaccine was shown to have 80% effectiveness ($P = .039$) against bacteremic pneumonia and empyema.[295] In the Nha Trang province of Vietnam, within 2 years of introduction of the Hib vaccine, the annual incidence of radiographically confirmed pneumonia decreased by 39% among children younger than 5 years of age.[294]

The importance of sustained vaccination against Hib was highlighted during a Hib vaccine shortage in the United States between November 2007 and March 2008 because of a vaccine recall that left only one manufacturer of Hib vaccine. Small outbreaks were reported in Minnesota and Pennsylvania during this time; the five cases reported to the Minnesota Department of Health in 2008 represented the highest number since 1992.[296] Even though the CDC had emphasized that all children should complete the primary immunization series during this period, vaccine coverage for the Hib primary series was 7.8% to 10.3% lower than that for DTaP and pneumococcal vaccine coverage at eight sentinel sites around the country.[297] This finding highlights the need for all children to complete the primary Hib immunization series and the need to ensure a consistent vaccine supply.

Substantial declines in levels of pharyngeal Hib carriage have been documented following Hib vaccination.[298] In Navajo and Apache children in the United States ages 3 months to 4 years, inadequate vaccination increased the odds of carriage by 2.66 (95% CI, 1.00–7.05).[46] Following routine immunization in this population, oropharyngeal carriage rates decreased to 0.3% (95% CI, 0–1.3%).[299] The carriage rates in The Gambia in unvaccinated children fell from 7.7% to 3.8% following routine vaccine introduction.[47,172]

There are conflicting data on differences in effectiveness among the available Hib conjugate vaccines. Delayed immunization and continued circulation of Hib may have caused an increase in Hib disease among Alaskan Native children following a shift from PRP-OMP to HbOC.[300] The data from Alaska suggest that under certain unusual epidemiologic circumstances, PRP-OMP may be more effective than HbOC. As a consequence, PRP-OMP is the primary vaccine used for certain Alaskan Native and Native American populations. In contrast, a review of surveillance data from 1993, when the Hib vaccine was introduced, to 2013, found that there was no increase in incidence of Hib disease in indigenous Australian children living in high incidence regions in the first 4 years following a change from the PRP-OMP vaccine to PRP-T vaccine.[301] However, all three available Hib conjugate vaccines have been successfully used to achieve very low levels of Hib disease. For example, PRP-T has been highly effective in The Gambia despite delayed immunization in many children, suggesting that indirect effects may contribute to protection of infants who have received only a single dose of PRP-T.[47] It is possible that in most situations, the differences in immunogenicity of the Hib conjugate vaccines may not translate into differences in effectiveness because of reduced circulation of Hib seen in most mature immunization programs.[302]

Haemophilus influenzae Type b Vaccine in Special Populations

Premature Infants

In the United States, the AAP recommends that premature infants be immunized according to their chronological age.[132] Data on the immunogenicity of Hib conjugate vaccines in premature infants are mixed. Two studies showed that following two doses of PRP-OMP at 2 and 4 months of age, just over 50% of premature infants achieved a PRP antibody titer of more than 1 μg/mL compared with more than 90% of term infants.[303,304] Similarly, one study showed that a significantly higher percentage of preterm infants had antibody titers less than 1.0 μg/mL compared with term infants (77% vs 57%) following three doses of PRP-T.[305] However, other studies comparing three doses of HbOC or PRP-T in preterm with term infants showed relatively little difference in the immune response.[306,307] In a study looking at persistence of the antibody response, Heath and colleagues[305] showed that 5 to 6 years following three doses of PRP-T, preterm infants had a significantly lower geometric mean antibody titer than did term infants ($P < .05$). The optimal dosing schedule for premature infants is unclear, and most experts continue to recommend that premature infants receive the Hib conjugate vaccine in accordance with their chronological age to provide protection against Hib disease in the first 2 years of life.[132]

Immunosuppressed Populations

HIV-Positive Persons. HIV-positive children and adults have been documented to have higher rates of invasive Hib disease with increased morbidity and mortality compared with HIV-negative persons.[308–310] A recent metaanalysis of three studies from South Africa estimated that HIV-infected children have a 7.4-fold increased risk of invasive Hib disease (uncertainty range: 2.9- to 22-fold).[1] Therefore, there has been great interest in the immunogenicity and efficacy of Hib conjugate vaccines in this population.

Madhi and colleagues studied 66 HIV-positive children who were not receiving antiretroviral therapy in South Africa.[311] The children were approximately half as likely (relative risk: 0.54; 95% CI, 0.43–0.69) to have antibody concentrations of 1.0 μg/mL or more following vaccination with PRP-T than were children without HIV infection. The estimated vaccine failure rate based on geometric mean antibody titers in HIV-positive children was 35.1-fold higher (95% CI, 14.6–84.6) than in their HIV-negative counterparts.[311] Similarly, poor antibody responses in HIV-positive persons have been documented in other studies, particularly in persons with high HIV viral loads.[312,313] Children with more severe or symptomatic HIV disease also demonstrate less antibody response.[314] In contrast, HIV-positive persons receiving antiretroviral treatment have an improved antibody response to the vaccines compared with before treatment.[315] Overall efficacy of Hib conjugate vaccine in untreated HIV-positive persons was shown to be 43.9% (95% CI, −76.1% to 82.1%); although this is lower than in HIV-negative persons, vaccination for Hib is still considered an important preventive measure for children infected with HIV.[316] There has been some suggestion that a booster dose might be warranted in HIV-positive children in countries where the routine immunization program uses only the primary series; however, because it is unclear whether antibody levels are an appropriate marker of protection in HIV-positive persons, this issue warrants further study.[314]

Other Immunosuppressive Conditions. Persons with deficient immune systems are at increased risk for invasive Hib

disease. Hib vaccines have been evaluated in persons with a number of immunodeficiency conditions, including organ transplantation and sickle cell disease. Hib vaccines are safe and effective in renal transplant recipients, although close monitoring must continue for invasive Hib disease because children undergoing dialysis and transplantation for chronic renal failure have occasionally been documented to experience Hib conjugate vaccine failures.[317,318] Studies suggest that immunizing a bone marrow transplant recipient before transplantation will result in improved antibody responses to Hib vaccines in the recipient compared with other techniques such as increasing the number of lymphocytes in the graft.[319] Some studies suggest that recipients immunized after transplantation may require an added booster dose at 24 months of age.[320] Persons with hemoglobinopathies may benefit from vaccination before splenectomy.[321] An open-label study in children younger than age 5 years with sickle cell disease demonstrated a geometric mean antibody titer of more than 3 μg/mL following one dose of PRP-OMP, and more than 18 μg/mL following a booster dose of PRP-OMP.[322]

Duration of Protection and a Booster Dose

Currently, most developed countries are using a two- or three-dose primary vaccination series with a booster dose in the second year of life, although the timing of the primary series is different in different countries. An immunogenicity study in Finland showed that the geometric mean antibody titer in children immunized with the primary series between 3 and 7 months of age fell to approximately 0.3 μg/mL at 18 months of age. Titers rose sharply following a booster dose and declined to approximately 5 μg/mL during the next 6 months. However, children between 2 and 8 years from their last vaccine dose did not exhibit significant further decline in antibody level; the mean antibody concentration for this group was 3.4 μg/mL. In addition, children 9 to 10 years of age who had received the primary series plus booster schedule in infancy had antibody titers 3.6-fold higher than children who had never received the vaccine.[323] A study in Sweden also showed long-term antibody persistence. Nine years after receiving three doses of PRP-T at 3, 5, and 12 months of age, the geometric mean antibody titer in the 56 study children was 4.16 μg/mL.[324] A follow-up study was conducted in Canada of children initially having been randomized to receive four doses of PRP-T (at 2, 4, 6, and 18 months of age) or PRP-D at 19 months of age.[325] At 4 to 5 years of age, the anti-PRP geometric mean antibody concentration in the PRP-T group was 2.2 μg/mL vs 0.7 μg/mL in the PRP-D group ($P < .001$). Four weeks following a booster dose of PRP-T, the geometric mean antibody titer rose to 98.4 μg/mL in the PRP-D group and 102 μg/mL in the PRP-T group (difference not significant). These results were consistent with persistent immunologic memory resulting in a booster immune response several years after immunologic priming.

In the United Kingdom, routine vaccination with the Hib conjugate vaccine was begun in 1992 with a three-dose primary series only at ages 2, 3, and 4 months without a booster dose.[110] A catch-up dose of Hib conjugate vaccine was given to older children. There was a rapid and dramatic decline in the incidence of Hib disease; however, starting in the late 1990s, a small increase in Hib cases in preschool-age children was noted (134 cases in 0- to 4-year-olds in 2002 vs 31 cases in 1996 in the same age group).[308,326] An anti-PRP antibody concentration study in 3- to 5-year-olds in the United Kingdom showed that antibody concentrations were higher in children who had been immunized in 1991 than children in the late 1990s ($P < .02$); this difference may have been a result of differing immunogenicities of vaccine formulations (including a change from whole-cell to acellular pertussis) or varying rates of Hib carriage in the community over time (see below).[327] A similar experience was noted in Ireland, with one to four cases seen from 1996 to 2003, compared with 10 cases in 2005.[328] As a result, in the United Kingdom in 2003, a second catch-up campaign was launched, offering all children ages 6 months to 4 years another dose of Hib vaccine.[329] Subsequently, the incidence of invasive Hib disease declined rapidly.[110]

The United Kingdom and Ireland now recommend a three-dose primary series at ages 2, 3, and 4 months and a booster dose at 12 months of age. In contrast, The Gambia uses a three-dose primary vaccination series without a booster, and Hib disease in preschool-age children has not been seen.[47] It has also been suggested that the experience in the United Kingdom may have been unique because of the vaccination catch-up campaign. High coverage achieved by the campaign may have reduced natural boosting opportunities due to reduced circulation of Hib.[330,331] A study in the United Kingdom comparing geometric mean antibody titers following an episode of invasive Hib disease between vaccinated and unvaccinated children found that children who had been vaccinated with the primary immunization series had higher geometric mean antibody titers than unvaccinated children ($P < .001$).[332] This study suggests that children who received the primary series responded to natural infection with a boosted antibody response, but that for some children, the primary series alone did not protect against invasive Hib disease.

Interestingly, the increase in rates of invasive Hib disease in the United Kingdom coincided with the distribution of Hib vaccine in combination with acellular pertussis vaccine as compared with whole-cell pertussis vaccine. Unlike combined vaccines with whole-cell pertussis, DTaP-Hib combinations have shown reduced immunogenicity to the Hib component (geometric mean antibody concentration, 0.38 μg/mL; 95% CI, 0.19–0.78) than with Hib administered separately (geometric mean antibody concentration, 3.10 μg/mL; 95% CI, 1.78–5.39), and, therefore, the concern was raised that acellular pertussis–containing Hib vaccines resulted in defective immunologic priming.[333] It has been suggested that the avidity of the anti-PRP antibody to Hib antigen may be a better measure of immunologic priming rather than antibody concentration.[334] One study showed a lower prebooster ($P = .0189$) and postbooster ($P < .001$) anti-PRP antibody avidity to Hib antigen in persons vaccinated with DTaP-Hib compared with persons vaccinated with DTaP and Hib separately.[335] In comparing geometric mean antibody titers after administration of DTaP-Hib, Goldblatt and colleagues showed an anti-PRP IgG geometric mean concentration of 1.23 μg/mL (95% CI, 0.98–1.58) just after initial vaccine administration, but then a titer of 0.25 μg/mL (95% CI, 0.21–0.30) before the booster dose, and a titer of 7.86 μg/mL (95% CI, 5.3–11.7) following the booster dose. This led them to suggest that despite the relatively lower immunogenicity of DTaP-Hib, adequate priming does occur following the primary series, but that the waning antibody concentration before the time of the booster dose may warrant the introduction of a booster.[336] A similar phenomenon with DTaP-HBV-IPV/Hib combination primary series yielding lower antibody levels than when Hib conjugate vaccines were administered separately is seen. However, it was also shown that the avidity and opsonic activity of the antibodies are the same. Maturation of antibody response as indicated by an increase in avidity over the postprimary to prebooster period was similar with separately injected Hib conjugate vaccine and the combination vaccine.[337] A later analysis of data from clinical trials concluded that there was no difference between the functional

activity of the DTaP-IPV-Hib combinations and DTwP-Hib combinations with the authors positing that the IPV component might have additional immunostimulating activity. Furthermore, all the different combination vaccines elicited the same opsonophagocytic and bactericidal activity as well as the same ability to protect in a passive infant rat protection assay as diphtheria and tetanus toxoids combined with whole-cell pertussis (DTwP) containing Hib vaccines after a booster dose.[338]

The importance of a booster dose and ongoing exposure to Hib for long-term protection remains controversial. Most developing countries that have introduced the Hib vaccine are using a three-dose primary schedule, mainly using combination vaccines with a whole-cell pertussis component, without a booster dose. A multicountry evaluation of Hib meningitis and nasopharyngeal carriage in four South American countries among children younger than 5 years of age suggested that high coverage of the vaccine was very important regardless of whether a booster dose was used.[339] In The Gambia, while in the Western region Hib disease has been under control for the 15 years since the vaccine was launched, in the Eastern region there has been a resurgence in Hib cases leading a few in the country to suggest the need for a booster dose.[340,341] Long-term surveillance for invasive Hib disease in developing country settings will be needed to determine whether a booster dose will be needed.

Safety

The three conjugate Hib vaccines have been given to millions of children during the past 20 years with few reports of serious adverse events.[342] Rare reports of transverse myelitis, thrombocytopenia, anaphylaxis, and Guillain-Barré syndrome have been temporally associated with Hib vaccine administration.[343,344] A review by the Institute of Medicine of the polysaccharide and conjugate Hib vaccines did not find adequate evidence to establish or reject a causal relationship between the vaccines and these disease entities.[344] A review of adverse events reported to the Vaccine Adverse Event Reporting System (VAERS) for the years 1990 to 2013 did not identify any new or unexpected safety concerns for Hib vaccines.[345] HbOC is extremely safe.[346] PRP-OMP has a similar safety profile; local and febrile reactions were seen in early trials but decreased after the addition of the aluminum phosphate adjuvant that presumably slowed the release of the reactogenic components.[228,245] PRP-T is associated with higher rates of local reactions such as erythema or induration than the PRP vaccine; however, higher rates of systemic or severe adverse events were not seen in one comparison study.[324] The higher rate of local reactions is thought to be a consequence of preexisting high levels of tetanus immunity in older children.[281,324]

Immunization Schedules and Recommendations. The AAP, the American Academy of Family Physicians, and the Advisory Committee for Immunization Practices have issued indications and recommendations for Hib vaccine in the United States.[132,347] These groups have recommended that for the HbOC and PRP-T vaccines, children should receive three doses at 2-month intervals and a fourth dose at 12 to 15 months of age. For PRP-OMP, two doses 2 months apart comprise the primary series, with a booster at 12 to 15 months of age. Immunization can start as early as 6 weeks of age, but because of possible immune tolerance, Hib conjugate vaccine should not be given earlier. For all three vaccines, any conjugate vaccine may be interchanged for the primary series or the booster dose. For premature infants, immunization should be given at the infant's chronological age in accordance with the schedule for all infants. Children who have completed the series but who are undergoing a splenectomy should be revaccinated 7 to 10 days before the procedure. Although there is some evidence that immunosuppressed patients (as a result of HIV, immunodeficiency, or malignancy) may benefit from additional booster doses, there is no absolute recommendation at this time.

The WHO recommends that HbOC, PRP-T, or PRP-OMP be given as a complete primary series with the three doses given at the same time as the DTP vaccine doses. It is suggested that the first dose be given as early as 6 weeks of age and the second and third dose given at 4- to 8-week intervals. A typical schedule for the primary series is doses at 6, 10, and 14 weeks of age of the child.[348] Most of the developing countries that have introduced combination vaccines use the DTwP-containing combinations. There is no absolute recommendation for giving a booster dose, although some experts suggest that a booster dose may be needed if there is evidence of disease resurgence in the future.[302,348,349]

In the future, some developing countries are likely to switch to Hib-containing combination vaccines that contain DTaP. It is unclear if making such a shift would result in resurgence of disease. This issue needs to be carefully monitored in order to evaluate the long-term impact of such changes.

Contraindications

The only contraindication to the Hib conjugate vaccines is previous hypersensitivity to the vaccine components.

Maternal Immunization

Vaccination of women before or during pregnancy is a potential approach to preventing early neonatal disease. A study in Arizona compared Hib antibody levels in infants born to women who were randomized to receive HbOC, PRP-OMP, or the 23-valent pneumococcal vaccine (Pneumovax 23, Merck Research Laboratories) before pregnancy. At birth, infants born to women vaccinated with PRP-OMP or HbOC had a higher geometric mean anti-PRP titer than infants born to women vaccinated with Pneumovax ($P < .01$). Furthermore, significantly fewer infants born to women vaccinated with PRP-OMP or HbOC had antibody titers less than 0.15 μg/mL at birth and 2 months of age compared with infants born to pneumococcal-vaccinated women ($P < .01$).[350] Studies in Texas and The Gambia in which women were vaccinated during the third trimester of pregnancy also showed significantly higher levels of anti-PRP antibody in infants born to vaccinated women.[351,352] Maternal immunization has not been pursued as a strategy for prevention of Hib disease owing to the relatively low incidence of disease in newborns and the reduction in disease in incompletely vaccinated infants because of indirect protection associated with widespread Hib conjugate vaccine use.

Vaccine Failures

Although the Hib conjugate vaccines are highly efficacious, occasional cases of invasive Hib disease occur in fully immunized persons. Considerable research has been done to identify risk factors for vaccine failures. Early or accelerated dosing may be associated with vaccine failure.[331] Underlying serious medical illness and immunodeficiencies have also been implicated in vaccine failures. In a study in the United Kingdom and Ireland, Heath and colleagues found that of the children who were vaccinated before 12 months of age, 20% of vaccine failures had a clinical risk factor (defined as prematurity, malignancy, developmental delay/dysmorphism, Down

syndrome, or neutropenia).[353] Of children vaccinated after 12 months of age, 67% had a clinical risk factor, immunodeficiency, or both.[353] A long-term follow up of 175 vaccine failure cases showed that approximately 4 years after a breakthrough illness, 57% had anti-PRP antibody concentrations less than 1.0 μg/mL and 16% had concentrations less than 0.15 μg/mL, suggesting a possible need for additional vaccine doses.[354] Another study on the same cohort showed that these children with Hib vaccine failure were also more likely to have lower serotype specific pneumococcal antibody concentrations after administration of the pneumococcal conjugate vaccine as compared to age-matched controls. Younger vaccine failure cases were more likely to have nonprotective antibody levels against more pneumococcal serotypes than their age-matched controls, leading to the suggestion that children with Hib vaccine failure might be able to develop antibody concentrations against encapsulated organisms but only when they are older.[355] An underlying genetic susceptibility in the immune system has been suggested as another host factor that could be responsible for vaccine failures. In the above discussed cohort of vaccine failure cases in the United Kingdom and Ireland, two recessive single nucleotide polymorphisms (SNPs) in *TIRAP* and *IL10* genes showed evidence of association with specific clinical manifestations of invasive Hib disease.[356]

Characteristics of the Hib strain may also be associated with vaccine failure. Cerquetti and colleagues showed that strains from persons experiencing vaccine failure were more likely to have multiple copies of the *cap* locus (≥3) than strains causing invasive disease in unimmunized persons.[357] The actual role of the *cap* amplification to the virulence of the organism and any associated clinical significance warrants further study.

Alternative Formulations

Combination Vaccines. The need for combination vaccines to simplify and improve compliance with an increasingly complex childhood vaccination schedule is well recognized. Several DTP-Hib-hepatitis B combinations have been developed and are widely used.[349] Concurrent administration of Hib conjugate vaccine in the same syringe as DTaP vaccine has been extensively evaluated. In several studies, significantly lower titers of anti-PRP antibody have been demonstrated in children who receive the DTaP-Hib combination than in children who receive Hib conjugate vaccine in a separate syringe.[336,358,359] However, the clinical relevance of this decreased immune response is unclear. The majority of children still exhibited titers of more than 0.15 μg/mL.[359] This decreased response is not seen when the whole-cell DTP vaccine is combined with the Hib conjugate vaccine.[360] Infants who are given a DTaP-Hib primary series exhibit a strong booster response when given the Hib conjugate vaccine in the second year of life, showing the establishment of immunologic memory.[331,336,359] In addition, the DTaP-Hib combination vaccines have demonstrated clinical effectiveness.[333,361] A clinical vaccine effectiveness study against invasive Hib disease in Germany showed 97.5% effectiveness (95% CI, 96.3–98.4%) after receipt of at least one dose of DTaP-Hib or DTaP-IPV-Hib and an effectiveness of 98.8% (95% CI, 98.2–99.3%) after three doses of DTaP-Hib or DTaP-IPV-Hib vaccine.[362]

Numerous combinations have been evaluated, including DTaP-IPV-Hib,[362–364] Hib-Hep B,[365] DTaP-Hep B-Hib,[366,367] and DTaP-Hep B-IPV-Hib.[331,368,369] The combination vaccines containing Hib that are licensed and in common use in the United States and many developed countries are shown in Table 23.2 (see also Chapter 15).

POSTVACCINE ERA: PUBLIC HEALTH CONSIDERATIONS

Herd Protection

Immunization with the Hib conjugate vaccine prevents establishment of new pharyngeal carriage in immunized persons.[46] If the proportion of children who have been immunized in a population is sufficient, there is a reduction in transmission of Hib, and unimmunized persons are protected because they have reduced exposure to the organism; reduction of disease in an unimmunized segment of a population in which a large proportion has been immunized is commonly referred to as "indirect effect" or "herd protection." Several studies have shown evidence of herd immunity with the introduction of Hib vaccine.[45,370,371] On the Navajo Indian reservation, with coverage rates ranging from 20% to 60%, invasive disease incidence decreased by 56% to 73%.[278] A study looking at data from 1989 to 2007 from Ontario, Canada, showed that infant Hib vaccination, introduced in 1992, led to a decrease in disease caused by Hib in all age groups.[372] This has important implications, particularly in developing countries where lack of infrastructure may prevent complete vaccination of the population. For example, in The Gambia, Hib vaccine was introduced in the mid 1990s, but vaccine supply has been interrupted at times and coverage with three doses of vaccine has ranged from 62% to 75%.[47] Nevertheless, invasive Hib disease has virtually disappeared there.[47]

Serotype Shift

There has been investigation into whether widespread use of the Hib conjugate vaccine can result in a change in the serotypes of *H. influenzae* most likely to cause invasive disease. Theoretically, the decrease in Hib carriage rates could open up ecological niches in the human pharynx, resulting in increased rates of carriage and disease due to other *H. influenzae* serotypes. Whether there has been a change in the rates of disease caused by non–type b encapsulated or nontypeable *H. influenzae* since the introduction of the conjugate Hib vaccines remains unclear. In the United States, population-based surveillance by the CDC from 1998 to 2000 did not show an increase in rates of non–type b *H. influenzae* invasive disease, nor has a shift been seen in Europe.[122,373] A population-based study of Navajo and White Mountain Apache children younger than 5 years of age did not find evidence of an increase in non–type b invasive disease rates after introduction of the Hib conjugate vaccine.[299] However, some studies have shown increases in non–type b *H. influenzae* serotypes in the postvaccine era, particularly with serotype a. A study of Alaskan residents showed an increase from 0.5 per 100,000 persons to 1.1 per 100,000 persons per year in invasive infections with non–serotype b strains of *H. influenzae.*[371] Specifically, whereas there was no *H. influenzae* a disease identified prior to 2002, from 2002 to 2011, the incidence of *H. influenzae* a disease was 5.4 per 100,000 among children younger than 5 years of age. Almost all but two cases out of 29 occurred in Alaskan Native children.[374] Similarly small increases have been noted in Australian Aborigine children and in a surveillance study of five metropolitan regions in England.[375,376] In the Navajo and White Mountain Apache population, while no increase in non–type b disease was noted in the postvaccine era, the rate of *H. influenzae* type a disease continued to be significantly higher than the general U.S. population.[256] In Ontario, Canada, a nearly threefold increase in incidence rates of nontypeable and serotype f *H. influenzae* infections was noted.[372] Of note, all reported increases in invasive non–type b *H. influenzae* disease rates since the introduction of the Hib vaccine

have been very small compared with the decreases in Hib disease incidence. Following vaccine introduction, estimated annual incidences of invasive non–type b encapsulated and nontypeable *H. influenzae* disease have been approximately 30-fold lower than that of Hib disease in the prevaccine era in studies in the United States and the United Kingdom.[131,376–378] The phenomenon of serotype shift has been seen with *S. pneumoniae* since the introduction of the seven-valent pneumococcal conjugate vaccine. Increases in pharyngeal carriage, otitis media, and possibly invasive disease caused by nonvaccine serotypes have been documented.[379–381] The reasons why serotype shifts might be more likely to be associated with pneumococcal vaccination compared with Hib vaccination are unclear. One hypothesis is that pneumococcal carriage is more common and multiple colonizing events result in increased competition between strains; in contrast, encapsulated *H. influenzae* organisms have lower rates of carriage, fewer colonizing events, and, therefore, less competition.[382]

FUTURE VACCINES

As the pediatric immunization schedule becomes more and more complex, developing more combination vaccines is an area of active interest, as are vaccine candidates for nontypeable *H. influenzae* disease and *H. influenzae* type a. Several candidate vaccines have been shown to reduce carriage in the ear and nasal mucosa of animal models.[383–385] An oral vaccine against nontypeable *H. influenzae* has been shown to reduce the incidence and severity of exacerbations of chronic bronchitis in small clinical trials.[386] A lipopolysaccharide-based conjugate vaccine showed good safety and immunogenicity in a Phase I study in adults and may soon be evaluated in children.[387] Future emphasis will likely be on elucidating correlates of protection against nontypeable *H. influenzae* disease that will facilitate the development and testing of more vaccine candidates.[388]

BOX 23.4 Conclusions

- Hib is the most important cause of serious *Haemophilus influenzae* disease.
- Several safe and highly efficacious vaccines are available against Hib disease.
- The effective use of Hib conjugate vaccines has virtually eliminated disease in developed countries and in the developing countries where it has been introduced.
- Use of Hib conjugate vaccine has increased globally, but many children still do not have access to this effective vaccine.

CONCLUSION (BOX 23.4)

The evaluation and implementation of Hib vaccines around the world has been one of the major public health accomplishments in the past decade. GAVI estimates that by 2020, the routine use of Hib vaccines would have prevented more than 7 million deaths.

References for this chapter are available at ExpertConsult.com.

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24 Hepatitis A Vaccines

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Contagious jaundice was recognized by the Greeks at the time of Hippocrates and by the ancient Chinese[1]; however, the earliest recorded outbreaks of jaundice that appear epidemiologically to have been hepatitis A occurred in Europe in the 17th and 18th centuries.[2] Early in the 20th century, Cockayne[3] concluded that sporadic and epidemic forms of jaundice were probably manifestations of the same disease, and McDonald[4] postulated that a virus might be involved. Epidemic hepatitis has been a military problem for centuries, and major outbreaks occurred among British, French, German, and Romanian troops in World War I and among French, American, Commonwealth, and Axis troops in World War II.

The first scientific evidence that hepatitis A was an enterically transmitted viral infection was obtained during World War II from studies among experimentally infected volunteers. In a classical series of experiments, Havens[5] and Neefe and colleagues[6] were able to show that volunteers in whom infectious hepatitis had developed were protected from subsequent challenge with the same virus and with infectious material obtained from a separate outbreak. Havens and colleagues [5,7] also demonstrated that intramuscular injection of pooled normal human immunoglobulin could prevent or attenuate the disease, and the practice was rapidly adopted. During an epidemic among American soldiers near the end of World War II in the Mediterranean theatre, more than 2700 American soldiers were administered immunoglobulin, which resulted in an 86% reduction in the incidence of disease among immunized troops.[8]

By the end of World War II, volunteer studies had clearly established that infectious hepatitis was enterically transmitted and was caused by a filterable agent—presumably a virus—that was relatively heat-stable but could be inactivated by chlorine.[6] The disease seemed to be caused by a single agent, seemed to be associated with lifelong immunity, and was preventable by administration of normal immunoglobulin. During the 1950s, it became apparent that infectious hepatitis was clearly distinct from serum hepatitis in mode of transmission and etiology. [9–11] MacCallum earlier had proposed that the epidemiologically distinct diseases be known as hepatitis A and B, replacing the terms infectious hepatitis (hepatitis A) and homologous serum hepatitis (hepatitis B).[12] This suggestion was adopted in 1952 by the World Health Organization's First Expert Committee on Viral Hepatitus.[1] Fecal samples collected from studies during the 1950s were critical in the subsequent identification of the etiologic agent of the disease by electron microscopy in 1973 (Fig. 24.1).[13]

WHY THE DISEASE IS IMPORTANT

Annually, an estimated 100 million-plus hepatitis A virus (HAV) infections result in significant morbidity and 15,000 to 30,000 deaths globally; most of this burden occurs in lower- and middle-income countries where 80% or more of the population can be infected by late adolescence in the poorest countries.[14–16] In high-income countries, with more advanced standards of hygiene and sanitation, the greatest risk of infection is low and when it occurs, it is often from travel to endemic countries and foodborne outbreaks.[17,18]

BACKGROUND

Clinical Description

Hepatitis A is an acute infection of the liver. The incubation period for hepatitis A ranges from 15 to 50 days after exposure with the mean incubation period being approximately 28 days.[6,19,20] The average incubation period has been reported to be shorter among patients who acquired HAV infection by parenteral transmission from contaminated blood products and among nonhuman primates infected parenterally compared with those infected orally, and the incubation period also depends on the infectious dose.[21–23]

Infection with HAV, as evidenced by the detection of HAV-specific immunoglobulin (Ig) M antibody in serum, may produce a wide spectrum of outcomes ranging from inapparent (asymptomatic, without elevation of serum aminotransferase levels), to subclinical (asymptomatic, with elevation of serum aminotransferase levels), to clinically evident (with symptoms). Clinical manifestations typical of acute hepatitis include jaundice and dark urine, but symptomatic hepatitis A without jaundice also occurs.

The clinical symptoms of acute hepatitis A in an individual patient are indistinguishable from those caused by other forms of viral hepatitis. The onset of the prodromal period, particularly in older children and adults, can be abrupt and is characterized by increasing fatigue, malaise, anorexia, fever, myalgias, dull abdominal pain, nausea, and vomiting. Pediatric patients may have diarrhea or, less commonly, upper respiratory symptoms including cough and coryza.[24,25] Typical signs and symptoms of hepatitis will appear several days to a week following the onset of the prodromal period and include darkening of the urine (bilirubinuria) which is followed by jaundice (yellow discoloration of the sclera, skin and mucus membranes) and pale-colored stools. Additional physical findings may include hepatomegaly and tenderness. Pruritus, caused by cholestasis, occurs in less than 50% of patients, but may be severe.

The frequency of symptoms with HAV infection is strongly influenced by age. Children are less likely to have symptomatic infection compared with adults; 50% to 90% of infections acquired before the age of 5 years are asymptomatic, whereas 70% to 95% of infected adults will have symptoms.[26–28] Jaundice is rare among young children, occurs more frequently among adolescents, and is experienced by the majority of adults with hepatitis A.[28,29]

The duration of illness varies, but most patients feel better within several weeks. In a large 1989 Shanghai outbreak, 90% of a subset of 8647 hospitalized patients observed carefully had recovered completely in 4 months, and all had recovered in 1 year.[19] In a study of 59 patients in the United States, about two-thirds recovered within 2 months, 85% within 3 months, and nearly all by 6 months.[30] Relapse, consisting of renewed symptoms, elevated liver function test results, and detection of virus in stools, has been found in up to 10% of cases, but recovery from relapse is universal.[30–32] Infection with HAV has not been found to cause chronic infection, and immunity after infection is thought to be lifelong.

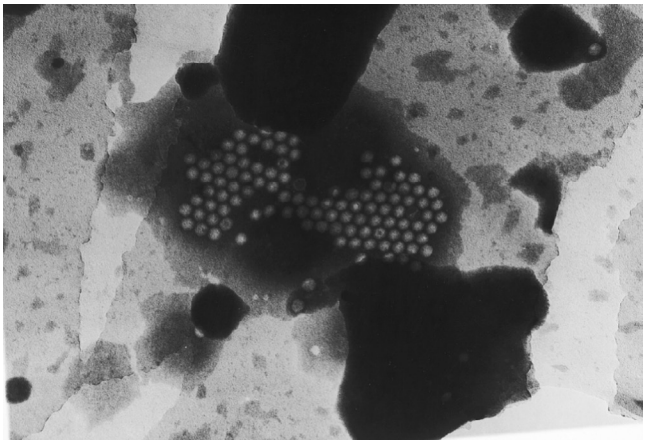


Figure 24.1. Electron micrograph of purified hepatitis A virus particles. Magnification ×125,000.

TABLE 24.1 Atypical Clinical Manifestations and Complications of Hepatitis A Virus Infection

Relapsing hepatitis A
Fulminant hepatitis A
Extrahepatic manifestations
Transient rash or arthralgias
Papular acrodermatitis of childhood
Cutaneous vasculitis
Cryoglobulinemia
Guillain-Barré syndrome
Other neurologic syndromes (e.g., myeloradiculopathy, mononeuritis, vertigo, meningoencephalitis)
Renal syndromes (acute renal failure, nephrotic syndrome, acute glomerulonephritis)
Pancreatitis
Hematologic (including aplastic anemia and thrombocytopenia)
Cholestatic hepatitis A
Hepatitis A triggering autoimmune hepatitis[8]

Data from references 30 and 35–76.

Complications

A cholestatic form of hepatitis A has been reported in which patients experience persistent jaundice, usually accompanied by pruritus.[30,33,34] Other atypical clinical manifestations and complications, including immunologic, neurologic, hematologic, pancreatic, and renal extrahepatic manifestations, are rare (Table 24.1).[30,35–76] Complications, including premature rupture of membranes or placental separation, have been reported among pregnant women with acute hepatitis A.[77–81]

The overall case-fatality rate varies according to the population studied. Among hospitalized patients with hepatitis A in Australia, the mortality rate was estimated to be 0.14%.[82] In the large 1988 Shanghai epidemic that involved primarily adolescents and young adults, there were 47 deaths (0.015%) recorded among the 310,746 diagnosed cases.[83] Case-fatality rates during communitywide outbreaks in the United States have ranged from 0.1% to 0.7%.[84,85] The case-fatality rate in the United States ranges from near 0% in healthy children to less than 2% in older adults.[86–88] The most severe form of the disease, fulminant hepatitis A, is rare, but mortality is as high as 60%.[89,90] Host factors reported to be associated with an increased risk of fulminant hepatitis include older age[69,90–92] and underlying chronic liver disease,[92–101] although some reports have identified chronic hepatitis C as a risk factor for severe disease whereas others have not.[99–102]

Morbidity and mortality associated with hepatitis A in developing countries has been documented among children; however data are limited.[103,104]

Treatment

No specific therapy is available for hepatitis A, and management is supportive and in most patients, admission to the hospital is not necessary. Activity, including exercise, should not be restricted among healthy patients who can tolerate it, as there does not appear to be an adverse impact on the course of illness. Abstinence from alcohol is recommended, although there are few objective data that demonstrate a benefit. Medications, particularly those metabolized by the liver or that are potentially hepatotoxic, should be used with caution because their half-life may be prolonged and/or promote additional insult to the liver. Hospitalization may be necessary for patients who become dehydrated from vomiting and is necessary for those who develop fulminant hepatitis.

Hepatitis A is occasionally complicated by cholestasis which can result in pruritus (itching); approximately 7% of cases were affected in one series of 59 hospitalized patients.[30] A brief course of corticosteroids has been reported to shorten the course and reduce symptoms, primarily itching, but recovery is universal, even without treatment.[34] In the rare event of fulminant hepatitis with hepatic failure, hospitalization with intensive care is indicated and most patients survive.[105,106] Liver transplantation may be indicated in some cases of fulminant hepatitis, but because survival without transplantation is relatively high, up to 80%, and no single factor is predictive of poor outcome, it has been difficult to establish criteria for choosing transplantation candidates.[35,69,70,106,107] Persistent HAV infection has been demonstrated in some transplant recipients, but whether this affects survival is unknown.[71]

Virology

HAV is a member of the Picornaviridae family, which includes the enteroviruses and rhinoviruses of humans. While HAV resembles enteroviruses in most morphologic and physical aspects, owing to several unique features, HAV has been placed into its own genus, *Hepatavirus*.[108,109] The HAV genome is composed of single-stranded linear RNA molecule of 7478 nucleotides (strain HM175) (Fig. 24.2).[110] Like other picornaviruses, the genomic RNA has positive polarity, a relatively long 5′ untranslated region (UTR) of 735 nucleotides typical of picornaviruses, followed by a single, long open reading frame of approximately 6,681 nucleotides coding for a polyprotein of 2227 amino acids, in turn followed by a short 3′ UTR ending with a virus coded poly(A) tail. Sequence analysis of the HAV genome reveals its gene order is also characteristic of picornaviruses with the structural genes coded by the 5′ third of the open reading frame and the nonstructural proteins coded by the remainder. Although HAV is similar to the other picornaviruses in physical and molecular structure, it has little similarity to other picornaviruses at the nucleotide or amino acid sequence level.[110]

As with other picornaviruses, the 5′ end of the genome does not have a cap structure but instead has a small, covalently bound, virus-coded protein termed VPg (3B protein).[111] The 5′ UTR has a predicted secondary structure similar to other picornavirus UTRs and includes an internal ribosomal entry site for cap-independent translation.[112,113] Translation begins at one of two in frame AUG codons at nucleotide position 735 or 741.[114] Following a translation terminator sequence, the genome ends with a 3′ noncoding region of 63 nucleotides that is followed by a poly(A) tail of varying lengths typical of picornavirus genomes. The polyprotein of picornaviruses has been divided

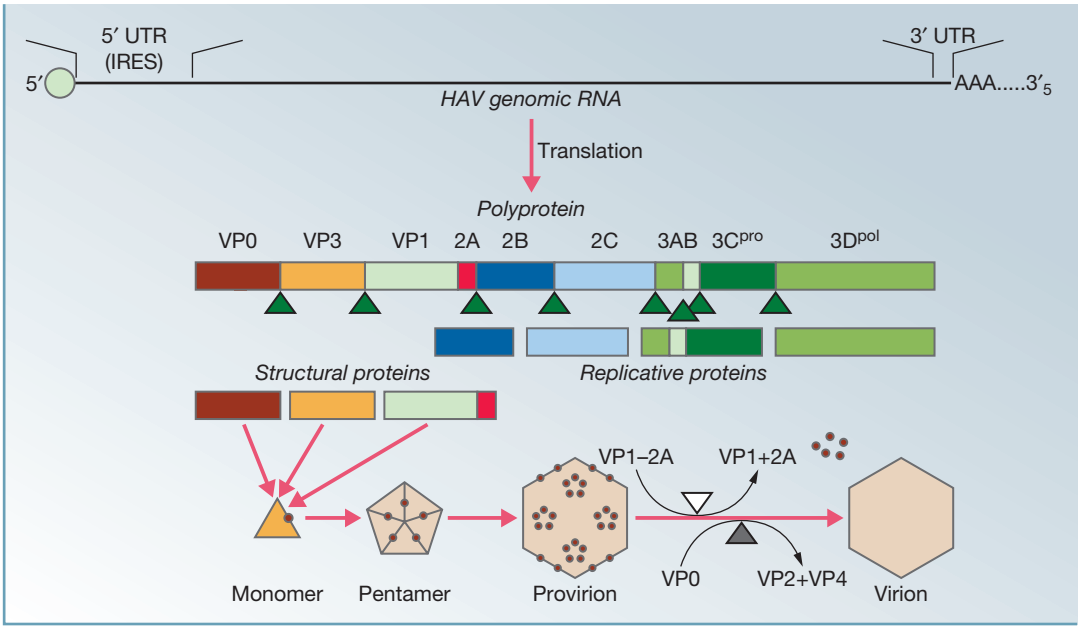


Figure 24.2. Organization of the RNA genome of hepatitis A virus (HAV), polyprotein cleavage, and viral assembly. The 7.5-kb positive-strand RNA is covalently attached to VPg (5′ end) and has a poly(A) tail. The 5′ untranslated region (UTR) of 734 nucleotides functions as an internal ribosome entry site (IRES) to initiate translation (*vertical arrow*) of the precursor polyprotein of 2227 amino acids. The single viral protease, $3C^{pro}$, cleaves itself from the polyprotein and subsequently cleaves elsewhere in the polyprotein to yield the structural protein precursors VP0, VP3, and VP1–2A (PX) and replicative proteins 2B, 2 C, 3A, 3B (VPg), and 3D (RNA-dependent RNA polymerase). VP0, VP3, and VP1–2A probably remain associated as a monomer and then form pentamers that are a stable precursor in capsid formation. Assembly of 12 pentamers together with RNA forms the provirion, after which 2A is susceptible to cleavage by host cell protease(s) *(open arrowhead)*. The final maturation cleavage of VP0 to VP2 and VP4 *(solid arrowhead)* is dependent on the encapsidated viral RNA. *(From Wasley A, Feinstone SM, Bell BP. Hepatitis A virus. In: Mandell GL, Bennett JE, Dolin R, eds.* Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, *7th ed. Philadelphia: Elsevier; 2010:2367–2388.)*

into three parts, termed P1, P2, and P3. The four capsid proteins, VP1, VP2, VP3, and VP4, are coded by the first 2373 nucleotides (P1) and the nonstructural proteins by the remainder (P2 and P3). The gene order and protein function of HAV are similar to the other picornaviruses. However, there are differences in the details of the protein cleavages, the size of the capsid proteins, myristoylation at the N-terminus, and the inclusion of the small VP4 in the virion particle.[115–121]

The 2A protein of poliovirus has proteinase activity and makes the VP1/2A cleavage. However, the 2A of HAV has no known function and is not required for replication, but at least the N-terminus is needed for particle assembly.[122,123] The 2B and C proteins are believed to be involved in replication. Mutations leading to cell culture adaptation and attenuation in animals have been mapped to these regions.[124] Protein 3C is the virion protease that is responsible for all proteolytic cleavages of the HAV polyprotein except the VP0 cleavage.[115–117] Protein 3D is the viral polymerase responsible for the replication of the genomic RNA.

Because HAV can survive in the environment, can contaminate food and water, and can be concentrated by shellfish, methods to inactivate this virus have been studied. HAV is more resistant to heat than other picornaviruses.[125] While HAV may be incompletely inactivated (depending on the conditions) by exposure to 60°C for 10 to 12 hours, complete inactivation has been reported after heating to 70°C for 4 minutes, 5 seconds at 80°C, and virtually instantly at 85°C.[126] HAV may survive extended periods in shellfish, water, soil, and marine sediment.[127] Outbreaks of hepatitis A have been reported following ingestion of partially cooked shellfish, suggesting that the usual steaming conditions used to cook shellfish may be inadequate to destroy the virus. HAV can be reliably inactivated by autoclaving (121°C for 30 minutes).[128] The virus is resistant to most organic solvents and detergents and a pH as low as 3.[129,130] HAV can be inactivated by many common disinfecting chemicals, including hypochlorite (bleach), and quaternary ammonium formulations containing 23% HCl found in many toilet bowl cleaners.[130] Photoinactivation of HAV in solution by the addition of porphyrins has been demonstrated.[131] Currently, licensed vaccines are inactivated by 1:4000 formalin at room temperature for at least 15 days to exceed complete inactivation by at least threefold.

There seems to be only one HAV serotype throughout the world.[132–134] People who were infected by HAV in one part of the world are not susceptible to reinfection in another part of the world, and immunoglobulin prepared in a variety of developed countries or vaccines developed from single strains of HAV protect travelers throughout the world. One vaccine approved in the United States was developed from a genotype I virus originally isolated from an Australian patient (HM175); the other vaccine was derived from a genotype III virus originally isolated from a Costa Rican patient (PA21). Although the two vaccine strains differ by 16.8% in the nucleotide sequence of the structural protein coding region, no significant antigenic differences were observed in a cross-cross neutralization assay, and both viruses reacted nearly identically with a panel of 18 monoclonal antibodies.[133,135]

The antigenic composition of the HAV capsid has been extensively analyzed through binding studies of neutralizing monoclonal antibodies and neutralization escape mutants.[136] Neutralization epitopes of HAV are contained primarily within predicted loop regions on the structural proteins VP1 and VP3. However, neutralizing monoclonal antibodies

do not recognize either oligopeptides predicted to contain neutralization epitopes or denatured individual viral capsid proteins, and antibodies raised against these synthetic oligopeptides do not neutralize, suggesting that these neutralization epitopes are conformational and not linear. Binding competition assays of neutralizing monoclonal antibodies have indicated that the neutralization epitopes are contained within a closely related antigenic site.[136,137]

Details of HAV replication have been incompletely elucidated owing to its relatively inefficient growth in vitro. Like other picornaviruses, HAV is believed to bind to the cell through a specific cellular receptor inserted into the plasma membrane. An HAV cellular receptor molecule from monkey kidney cells termed havcr1 is a mucin-like glycoprotein stalk topped by an immunoglobulin-like structure. Binding of HAV to cells that express havcr1 is blocked by a specific monoclonal antibody to the receptor, and the complementary DNA (cDNA) coding for havcr1 can be transfected into nonpermissive cells, rendering these cells permissive for virus binding, entry, and translation. However, nonpermissive cells transfected with havcr1 cDNA do not generally support the replication of complete infectious virus.[138] The cys-rich immunoglobulin-like region of HAVCR1 is sufficient for binding of HAV. Soluble forms of HAVCR1 containing the cys-rich region plus the mucin-like region neutralize and uncoat HAV, whereas forms containing only the cys-rich region bind but do not uncoat HAV, suggesting that the mucin-like region provides the correct scaffolding for the correct presentation of the cys-rich region or interacts with the viral particle.[139] The HAV receptor havcr1 is identical to the T-cell immunoglobulin and mucin domain (TIM1). TIM1 is known to regulate T-cell activation and has been implicated in atopic disease.[140] Some studies have identified an inverse association between HAV infection and atopy in humans, and there has been the suggestion that this could be related to the effect of HAV infection on TIM1-induced T-cell differentiation.[140]

Unlike many viruses that activate the CD4+ CD25+ regulatory T-cells (Tregs), HAV temporary impairs Treg functions during acute infection.[141] Direct binding of HAV to HAVCR1 on Treg inhibits the cell functions.[142] The transitory block of Treg may lead to removal of pathogenic T cells, providing protection against development of autoimmune and allergic states.[143] Following receptor binding and entry, HAV follows the general replication strategy of other picornaviruses in uncoating, translation, RNA replication, and assembly. Like other picornaviruses, replication of HAV is intimately related to the cytoplasmic membranes, and proteins 2C and 2BC have been shown to interact with and induce rearrangement in these membranes.[144] HAV uses a unique "membrane hijacking" mechanism. At least a fraction of HAV circulating in blood of infected humans and chimpanzees is enveloped into the host cell membrane, which prevents recognition of the form of HAV by antibody in enzyme immunoassays. Enveloping of HAV with the host membrane may affect neutralization with antibody and potentially assists in spreading HAV infection in the liver.[145]

The features of HAV replication in cell culture also distinguish it from many other picornaviruses, and these characteristics are important for vaccine production; for example, HAV is cultured in human lung fibroblast cells, MRC-5, using medium-perfused bioreactors for production of inactivated hepatitis A vaccine VAQTA. The virus generally grows slowly and to low titers. Most HAV strains do not significantly interfere with host transcription or translation, and the virus does not cause a cytopathic effect or kill the cells in which it is growing. Instead the virus readily establishes persistently infected cells, and the virus remains largely cell-associated. A few cell culture–adapted viruses that grow rapidly exhibit a cytopathic effect and cause cell death through apoptosis.[146–148]

Many HAV strains have been isolated in cell culture directly from clinical material, although the procedure may take several weeks or even months of adaptation to in vitro growth.[148] A cell line derived from human hepatoma cells, Huh-7 cells, seems to have the capacity to grow field isolates of HAV.[149] In addition, cells of nonprimate animal origin have now been shown to support at least limited growth of HAV.[150–152]

Cell culture of HAV has been used to alter the phenotype of the virus primarily for growth characteristics and attenuation of virulence. Attenuated strains of HAV have been selected by multiple tissue culture passages, and cold adaptation has been achieved by passage at reduced temperature.[153–155] Some mutations responsible for these altered phenotypes have been determined by molecular cloning and sequencing of the mutant and comparing its sequence with the parental strain. Mutations within the 5′ UTR and mutations within the 2B and 2C coding regions of HAV RNA are associated with enhanced virus replication in vitro.[124,156]

HAV is known to infect humans, at least chimpanzees among the great apes, and some species of monkeys. The most extensively characterized models are the chimpanzee and two New World monkeys, tamarins and *Aotus* (owl) monkeys.[134,157,158] The presence of antibodies in some primate species at the time of capture might indicate that there is a reservoir of infection in nature or cross-reactive antibodies with monkey viruses. In some regions of Africa and Asia, where HAV is endemic, species of macaques and *Cercopithecus* monkeys are often infected with simian or possibly human strains of HAV. Transmission of HAV to primate handlers is well documented. However it has not been determined if these monkey isolates are true simian HAVs or human viruses that have infected monkey colonies where they have persisted and adapted.[159] Limited HAV replication has been demonstrated in guinea pigs.[160] Therefore, while HAV may be transferred to nonhuman primates and even lower-order animals, it is not clear if any of these species serve as a reservoir for HAV in the environment, which could then be a source of human infections.[160]

Pathogenesis as It Relates to Prevention

HAV is generally transmitted by the fecal–oral route, and this acid-resistant virus can survive passage through the stomach. It was found in tamarins and chimpanzees that approximately $10^{4.5}$ intravenous doses were required to infect the same species by the oral route.[23] The primary site of HAV replication is in the liver, although there are also experimental data in chimpanzees that HAV may replicate in the oropharynx.[161,162] The virus has been identified by immunofluorescence in the epithelial cells of the intestinal crypts of the jejunum and ileum of experimentally infected monkeys.[161,163] The period of viremia has been measured by a sensitive reverse transcriptase–polymerase chain reaction in 13 naturally acquired human cases and five experimentally infected chimpanzees. HAV RNA was detected an average of 17 days before the peak alanine aminotransferase (ALT) level and persisted an average of 79 days after the peak ALT. The average total duration of viremia was 95 days, but the range was between 36 and 391 days.[164] Although other organs might be seeded by the viremia, HAV, like many other picornaviruses, seems to be highly organ-specific, and the primary pathologic process in hepatitis A is restricted to the liver. Virus is shed from infected liver cells into the hepatic sinusoids and the bile canaliculi, passes into the intestine, and is excreted in the feces, where it may be found in high titers early in the infection.[165–167]

Because HAV is generally not cytopathic in cell culture, the pathologic findings in experimental animals and humans show little hepatocyte damage at the peak of viral replication. Immune mechanisms, in particular cell-mediated immune responses, have been postulated to explain the hepatic injury.[168,169] In contrast, circulating antibodies are probably more important in limiting spread of virus to uninfected liver cells, which, in combination with specific T cells, are responsible for termination of the infection.[169–171] As HAV has a positive strand RNA genome virus that goes through a double-stranded intermediary during its replication cycle, infection by HAV should be a strong inducer of type I interferons. HAV uses several molecular mechanisms to disrupt early innate immune responses controlling induction of interferon synthesis,[172,173] which results in a limited type I interferon response in the liver infected with HAV. Experimental animal models showed that HAV does not noticeably induce expression of interferon-stimulated genes (ISGs) despite the approximately 100-fold greater abundance of HAV RNA in the liver than during acute infections with other hepatitis viruses.[174, 175] The cellular helicases, RIGI and MDA5, and Toll-like receptor 3 (TLR3) are pattern recognition receptors that are activated by double-stranded RNA (dsRNA) and initiate the interferon signaling cascade.[176] Many viruses have evolved mechanisms to evade the interferon system. For example, the hepatitis C virus (HCV) protease cleaves RIGI, thus disrupting downstream signaling. It was recently shown that HAV has a mechanism that is similar to HCV. The 3ABC precursor of the HAV 3C protease is targeted by the transmembrane region of protein 3A to the mitochondrial membrane, where it then cleaves the mitochondrial antiviral signaling protein, MAVS. The cleavage of MAVS in turn disrupts the downstream interferon pathway and inhibits the production of interferons and interferon-induced antiviral proteins.[173]

In addition to the 3ABC intermediate, other products of incomplete processing of the HAV polyprotein play an important role in mitigating interferon response. For example, the 3CD intermediate disrupts TLR3 signaling and, consequently, interferon production.[172]

Although liver damage (as measured by ALT elevations) begins at about the same time that circulating antibodies become detectable, there are no data suggesting that the pathology is antibody-mediated, but is more likely from cytotoxic T lymphocytes. Although passive immunization with immunoglobulin can provide complete protection against infection, no data exist suggesting that already infected patients can be treated by infusion of antibodies.[177] The effectiveness of immunoglobulin also suggests that mucosal immunity is not required for prevention of HAV infections.[178,179]

Diagnosis

Hepatitis A is indistinguishable from other forms of acute viral hepatitis on clinical grounds, although the diagnosis may be suspected in a patient with typical symptoms during an outbreak. The diagnosis requires serologic detection of specific antibody responses to HAV and is confirmed by detection of HAV-specific IgM in a single acute-phase serum sample (Table 24.2).[180–182] The HAV-specific IgM antibody appears early in the illness, is usually present at the time the patient seeks medical attention, and declines to undetectable levels within 6 to 12 months in most cases. The presence of HAV-specific IgM in the serum is regarded as evidence of current or recent infection (Fig. 24.3).[183] Serologic assays can be initially negative in up to 10% of patients who are tested soon after onset of symptoms and testing should be repeated if hepatitis A is suspected clinically, particularly in the setting of an ongoing outbreak.[184,185] In addition, cases of positive test results for IgM

TABLE 24.2 Available Tests for Hepatitis A Virus and Antibody to Hepatitis A Virus

Tests	Uses and Comments
Anti–hepatitis A virus (HAV)/total antibody (radioimmunoassay [RIA]/enzyme-linked immunosorbent assay [ELISA])[a]	Determine immunity/susceptibility before vaccination epidemiologic studies
Anti-HAV/IgM-specific (RIA/ELISA)[a]	Primary test for diagnosis of current or recent infection
Anti-HAV/oral fluid (ELISA)	Research application in epidemiology
Neutralizing antibody (radioimmunofocus inhibition [RIFIT]; HAV antigen reduction assay [HAVARNA])	Research use, particularly for characterizing types of antibody induced by vaccination Very labor intensive; not widely available or well standardized
Viral culture (cell culture)	Research use only because virus grows slowly on initial isolation
HAV antigen (RIA/ELISA)	Research use for detecting virus in various specimens (i.e., cell culture)
HAV RNA (nucleic acid testing/polymerase chain reaction)	Research applications Epidemiologic studies and outbreak investigations Environmental studies
Liver biopsy (light or fluorescent microscopy)	Research with animal inoculations Rare diagnostic dilemma or unusual clinical manifestations Not indicated for most hepatitis A cases

[a]Only ELISAs are currently commercially available.

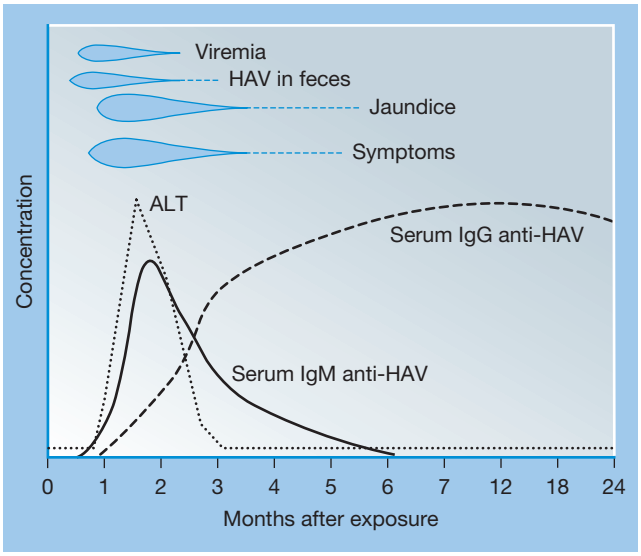


Figure 24.3. Clinical, virologic, and serologic events after hepatitis A virus (HAV) infection. ALT, alanine aminotransferase; Ig, immunoglobulin.

anti-HAV more than 1 year after infection have been reported. Furthermore, false-positive results among persons who have no other evidence of recent infection have been observed, reflecting a low positive predictive value of the test when used to test asymptomatic persons inappropriately.[186,187] Biochemical evidence of hepatitis consists of elevated levels of serum bilirubin and serum hepatic enzymes, including ALT, aspartate aminotransferase (AST), alkaline phosphatase, and γ-glutamyltranspeptidase. Elevations in AST and ALT levels occur most consistently and may precede the appearance of symptoms by a week or more (Fig. 24.3). Except for patients with relapsing or cholestatic hepatitis A, serum bilirubin and aminotransferase levels usually return to normal by 2 to 3 months after illness onset.[31]

A desire to monitor vaccine-induced protective immune responses prompted development of several in vitro assays for detection of HAV-neutralizing antibodies[188–190] and serologic assays that differentiate HAV immunity acquired after infection from immunity acquired after vaccination with inactivated hepatitis A vaccine.[191–193] None of these assays has found a broad application in the field or is commercially available. Several diagnostic assays for the detection of HAV antibody in oral fluid have been developed[194–197] to support population-based seroprevalence studies.[198–202] Although ease of oral fluid collection might simplify the logistics of anti-HAV testing, application of oral fluid tests to clinical management and practical epidemiology has been hampered by the lack of commercial assays.

Other tests are rarely used for the diagnosis of HAV infection (see Table 24.2). Viral detection assays generally have not been useful because wild-type HAV is extremely difficult to isolate in cell culture, usually requiring weeks or months. Because virus shedding peaks before the onset of clinical illness, antigen detection systems usually are insufficiently sensitive to detect HAV in stool samples. Polymerase chain reaction (PCR) techniques have been useful in certain clinical, epidemiologic, and environmental studies.[195–205] Although highly sensitive nucleic acid tests based on PCR could potentially be used in the diagnosis of acute HAV infections, no approved commercial assays are available. Commercially available serologic tests that measure total anti-HAV are used most often in epidemiologic investigations or in determining susceptibility to HAV infection as both natural infection and vaccine-acquired immunity will result in the presence of total anti-HAV (as IgG antibody).

Molecular Epidemiology

HAV is genetically heterogeneous.[135] Originally, HAV was classified into seven genotypes using a 168-nucleotide fragment of the HAV genome containing the VP1/P2A junction,[132] but it was recently reclassified into six genotypes, I to VI, based on sequences from the VP1 region.[206] Clear differences in clinical outcomes among the genotypes are not established. Despite multiple genotypes of HAV, there is one recognized serotype. Genotypes I, II, and III circulate among humans and genotypes IV, V, and VI infect simians.[132,207] Genotypes are divided into subtypes A and B. Geographic distribution of HAV genotypes and subtypes is not uniform. Genotype I has a worldwide distribution, with subtypes IA and IB frequently cocirculating. Subtype IA is prevalent in South and North America, Europe, Asia, and Africa, while subtype IB is predominant in the Middle East and South Africa. Recent evaluation, however, suggests that subtype IB is more abundant in South America than previously thought.[208] Genotype II seems to have a West African origin but it is not frequently detected.[209] Genotype III circulates globally; however, its prevalence varies among geographic regions.[207,208] Recently, an increase in subtype IIIA infections was reported in Korea, Russia, and Estonia.[210–212] Circulation of more than one subtype and genotype in certain geographic regions provides an opportunity for coinfection and recombination. Recombination between cocirculating HAV subtypes has been reported.[213–215]

HAV genetic heterogeneity is used for the identification of HAV strains and for outbreak investigations. The detection and sequencing of HAV RNA from water, food, and blood or saliva of infected individuals provide important information for tracing HAV strains and identifying the source of waterborne and foodborne hepatitis A outbreaks.[216,217] Molecular investigations are mainly conducted using short HAV genomic regions, usually VP1/P2A junction.[208] However, analysis of whole-genome sequences showed that the VP1/P2A sequences may be shared by different HAV strains and that more than one closely related HAV strain may be involved in a single foodborne outbreak.[218] Exposure to several HAV strains has been reported during outbreaks associated with person-to-person transmission in endemic regions.[219–221]

Genetic characterization of HAV strains collected during outbreak investigations has revealed important trends in HAV dissemination in different epidemiological settings. For example, identification of a few, large monophyletic clusters of the subtype IA HAV strains and sharing of these strains during outbreaks among men who have sex with men (MSM) from several countries in Europe indicates infrequent introductions of HAV strains into broad MSM networks capable of sustaining the introduced HAV variants for a prolonged period; the presence of many unique HAV variants and small monophyletic clusters of subtypes IB and IIIA among travelers returning from endemic countries emphasizes introduction of numerous HAV strains that do not spread broadly in Europe.[222,223] Detection of subtype IIIA among injection drug users was interpreted as a frequent introduction of HAV to these communities in developed countries through contaminated drugs originating in Central and South Asia where this subtype is prevalent.[222,224–227]

EPIDEMIOLOGY

Worldwide Disease Patterns

Hepatitis A occurs worldwide, but major geographic differences exist in endemicity and resulting epidemiologic features (Fig. 24.4).[16] The degree of endemicity is closely related to hygienic and sanitary conditions and other indicators of the level of development. Under conditions of overcrowding, especially when there is limited access to clean water and inadequate disposal of human feces (e.g., high endemicity; Fig. 24.5), HAV infects most people early in life, when infection is rarely clinically apparent. Where high standards of hygiene and sanitation exist, most children reach adult life without encountering the virus. Distinct patterns of HAV infection can be described, each with characteristic age-specific profiles of anti-HAV prevalence and hepatitis A incidence, attributable to prevailing environmental (hygienic and sanitary) and socioeconomic conditions (Fig. 24.5).[16,227] However, it is becoming increasingly challenging to generalize about endemicity in developing countries as prevalence patterns of HAV infection vary among regions within a country and among social strata; in addition, data are often missing or obsolete.[227]

In areas of high endemicity, represented by the least-developed countries (i.e., parts of Africa, Asia, and Central and South America), poor socioeconomic conditions allow HAV to spread readily (see Fig. 24.4).[16] The lifetime risk of infection exceeds 90%, in such countries and occurs primarily in early childhood[228] when asymptomatic infection predominates. Nearly the entire population is infected before reaching

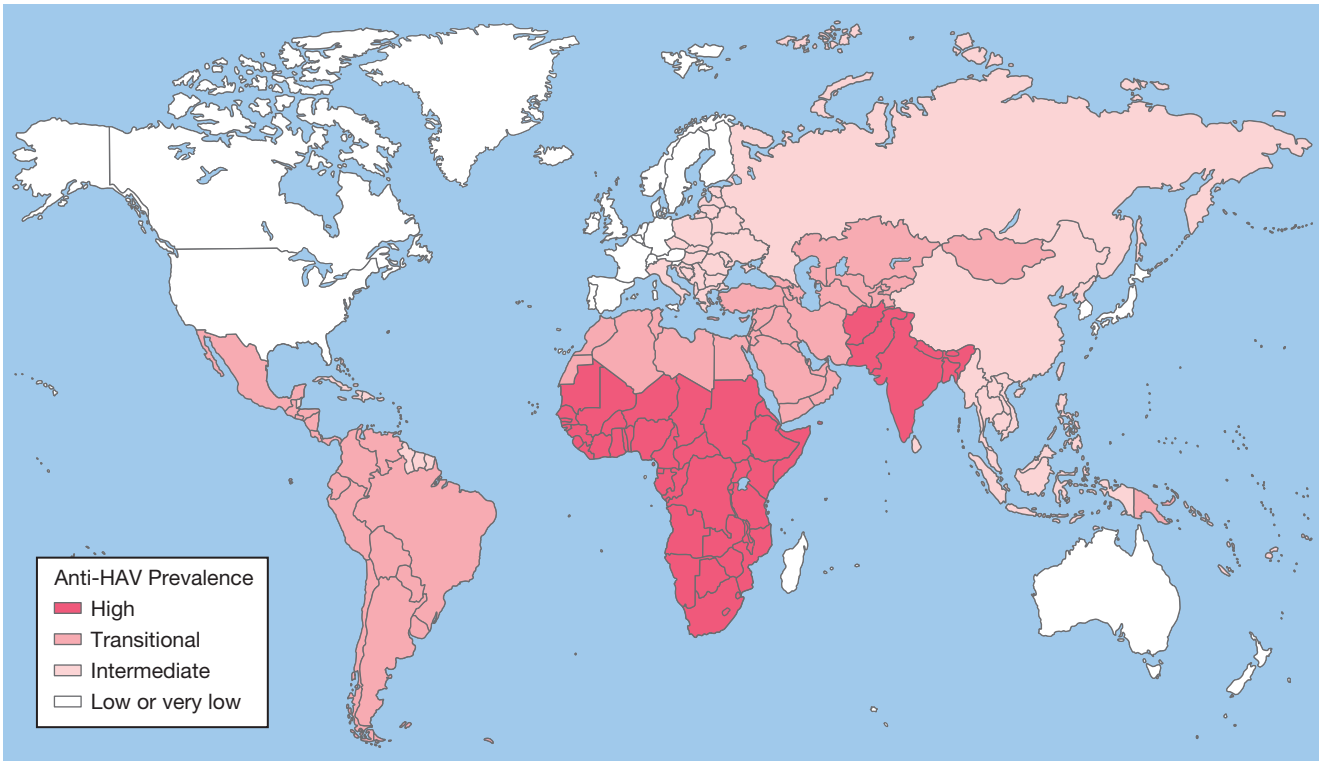


Figure 24.4. World map indicating patterns of endemicity of hepatitis A virus (HAV) infection (generalized from available data). Antibody to HAV prevalence patterns of high (red), intermediate (dark pink), low (light pink), and very low (white) endemicity are shown. *(From Jacobsen KH, Wiersma, ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005.* Vaccine. *2010;28:6653–6657.)*

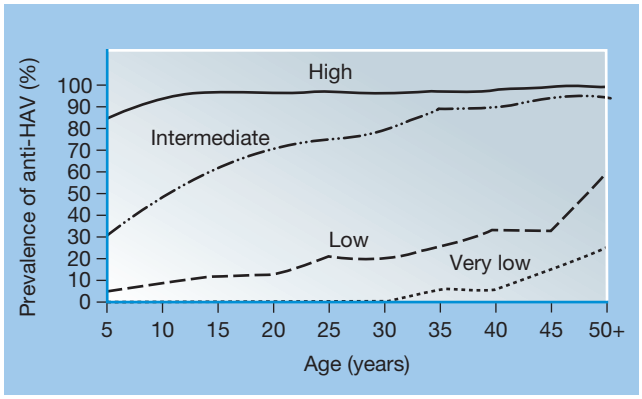


Figure 24.5. Patterns of hepatitis A virus (HAV) infection worldwide. Anti-HAV, antibody to HAV.

adolescence, as demonstrated by the age-specific prevalence of anti-HAV (see Fig. 24.5).[229–231] Susceptible adults in these areas are at high risk of infection and disease, but reported disease rates are generally low and outbreaks rarely reported, owing not only to the high prevalence of immunity in the population, but also to the lack of adequate surveillance. High endemicity patterns also may be seen in some ethnic or geographic groups within highly developed countries, such as the aboriginal children in northern Australia.[232] Travelers to high endemic areas from the developed world also are at high risk.[233]

In areas of moderate endemicity, HAV is not transmitted as readily because of better sanitary and living conditions, and the predominant age of infection is older than in areas of high endemicity (see Figs. 24.4 and 24.5).[16,234] Paradoxically, the overall incidence and average age of reported cases are often higher than in highly endemic areas because high levels of virus circulate in a population that includes many susceptible older children, adolescents, and young adults, who are likely to have symptoms with HAV infection.[235] Large common-source food- and water-associated outbreaks can occur because of the relatively high rate of virus transmission and large number of susceptible persons, especially in the higher socioeconomic level. An extreme example of an outbreak in intermediate endemicity populations occurred in Shanghai in 1988, with more than 300,000 cases associated with consumption of clams harvested from water contaminated with human sewage.[19] Nevertheless, person-to-person transmission in communitywide epidemics continues to account for much of the disease in these countries. Unimmunized travelers from the developed world to these areas also are at risk.[24,233]

In the United States, Canada, much of Western Europe, and other developed countries, the endemicity of HAV infection was described as low, but is now trending toward very low (see Figs. 24.4 and 24.5);[16] in low endemic countries relatively few children are infected. This level of endemicity is characterized by a low incidence of infection and disease, with disease often occurring in the context of communitywide outbreaks. In the United States, communitywide outbreaks have become increasingly rare and most recently transmission is characterized by common-source outbreaks, international travel, and contact with an infected person, a pattern of transmission more consistent with very low endemicity.[236] Low-endemicity countries experience a cyclic pattern of disease incidence with peaks every 5 to 10 years, as has been noted previously in the United States and in other developed countries with temperate climates. Population-based seroprevalence surveys show a gradual increase in the prevalence of anti-HAV with increasing age, primarily reflecting declining incidence, changing endemicity, and resultant lower childhood infection rates over time

(see Fig. 24.5).[237] In countries that have very low endemicity (e.g., Scandinavian countries, and now the United States and Canada), most cases occur in defined risk groups such as travelers returning from areas of high or intermediate endemicity and risk populations, such as users of injection drugs.[238] The changing epidemiology in the United States has also been influenced by universal vaccination recommendations for children.

Changes in the epidemiologic pattern of HAV have occurred in countries undergoing rapid development accompanied by improvement in sanitation standards and clean water supplies.[15] Shifts in age-specific prevalence patterns that reflect a transition from high to intermediate endemicity (see Fig. 24.5) show declines in childhood and adolescent HAV seroprevalence as the numbers of susceptible people at older ages at which there is greater associated morbidity and mortality, and greater risk for large communitywide outbreaks of hepatitis A, grow.[15,239–251] Considerable hepatitis A–related morbidity and associated costs occur with this shift in epidemiology, even in developing countries.[89,252] In the United States, as a result of significant decreases in incidence, owing in large part to universal childhood vaccination, there have been decreases in morbidity and mortality among children and adolescents, resulting in the average age of hepatitis A–related hospitalizations and deaths increasing.[253,254] Although overall hepatitis A morbidity and mortality have decreased dramatically in the United States, the proportion of persons hospitalized for hepatitis A in recent years are more likely to have liver diseases and other comorbid medical conditions.[254]

Epidemiology in the United States

Although large national outbreaks were recognized during the 1950s, data collection for hepatitis A in the United States started in 1966 (Fig. 24.6). The highest number of hepatitis A cases recorded in the United States was in 1971, approximately 60,000 cases (incidence: approximately 29 cases per 100,000 population). In 1995, when hepatitis A vaccine was licensed in the United States, more than 31,000 cases of hepatitis A had been reported (incidence: 12 cases per 100,000 persons) making hepatitis A one of the most frequently reported vaccine-preventable diseases (Fig. 24.6).[255] In the period 1996 to 1999, the Advisory Committee on Immunization Practices (ACIP) recommended targeted vaccination for children at 2 years of age in states with high hepatitis A rates.[256,257] Incidence rates declined sharply following implementation of these recommendations (Fig. 24.6).[236] In 2006, universal vaccination was recommended in all states for all U.S. children starting at 1 year of age.[86] The number of reported cases in the United States reached an all-time low in 2011 of 1398 cases, representing a 95.5% decline in reported cases since the first vaccine recommendation in 1996. The first increase in cases since 1995 occurred in 2012 (1562 total cases) and again in 2013 (1781 total cases); these increases have been coincident with a 2013 multistate outbreak of hepatitis A, and may be partly a result of increased reporting associated with heightened awareness around the outbreaks as there was substantial media reporting of the events.[17] In 2014, a total of 1,239 cases of hepatitis A were reported from 50 states to the CDC, a 30.4% decrease from 2013.[236] Most importantly, hepatitis A rates have declined since vaccine introduction—from 11.7 cases per 100,000 population in 1996 to between 0.4 and 0.6 cases per 100,000 population in 2011–14—a greater than 20-fold decline [236,258]

Young children typically have asymptomatic or unrecognized infections, and have historically, prior to vaccine introduction, played an important role in the epidemiology of hepatitis A in the United States, serving as a major reservoir for HAV transmission.[259] Prior to vaccine introduction in the United States, the highest hepatitis A rates were reported among children 5 to 14 years old, with approximately one-third of cases occurring among children younger than 15 years of age,[257] while the majority of infections were among asymptomatic children younger than 5 years of age.[260] Hepatitis A rates among children have declined more sharply than among adults since vaccine introduction and, since 2009 rates among both adults and children have been less than 1.0 case per 100,000 population despite small fluctuations in incidence (Fig. 24.7).[255,261] In 2014, persons aged 20 to 29 years had the highest rate (0.55 cases per 100,000 population) and persons aged 0 to 9 years had the lowest rate (0.10 cases per 100,000 population).

In the United States rates of hepatitis A among Hispanics have historically been higher than those of other racial/ethnic populations. Rates among Hispanics declined 94% from 1997 to 2007 (1.4 cases per 100,000 population); in 2009, the rate

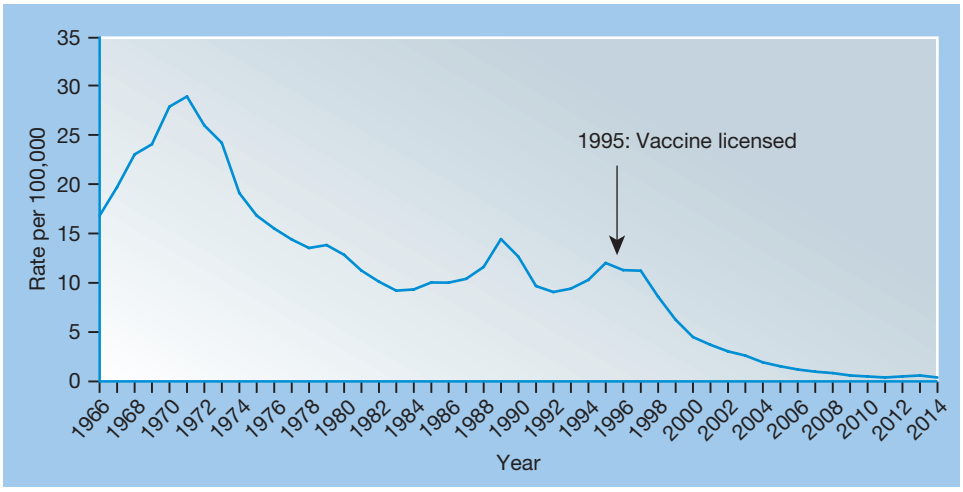


Figure 24.6. Hepatitis A incidence in the United States: 1966–2014. *(Data from the Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance System, Atlanta, GA.)*

was the lowest ever recorded (0.8 per 100,000).[255,261] Rates among all racial and ethnic groups have declined dramatically and comparably in the United States, including Alaska Natives/Native Americans who have traditionally experienced among the highest rates (Fig. 24.8).[236] Mortality associated with hepatitis A in the United States is uncommon; of the 80 deaths reported in 2013 most were among older adults, and none were reported in 2014.[236]

In the United States, before hepatitis A vaccine was widely available, hepatitis A occurred in large nationwide epidemics approximately every decade.[262] The highest rates and the majority of cases occurred in the western and southwestern states.[86,257,263] With the advent of routine vaccination of children, large communitywide outbreaks and cyclic peaks have ceased. The geographic variations that characterized hepatitis A incidence in the past[263] have disappeared.

In the United States, prior to the introduction of vaccine when community outbreaks and outbreaks in childcare centers were common, studies of outbreaks where serologic testing of household contacts was performed found that 25% to 40% of contacts younger than 6 years of age had serologic evidence of acute HAV infection,[264,265] resulting in some immunity among children as they entered adolescence and adulthood.[28,266] A nationally representative serosurvey (for the years 2007–2010) focusing on ages 6 to 19 years in the United States, reported a 37.6% prevalence of immunity to hepatitis A measured by anti-HAV levels—a 13.1% increase compared to the prevaccine era (the years 1988–1994)—reflecting the increased immunity among children as a consequence of vaccination. However, results from National Health and Nutrition Examination surveys conducted for the periods 1988–1994, 1999–2002, and 2003–2006 indicate an overall decline in the age-adjusted seroprevalence of anti-HAV in the U.S. population from 32.5% in 1988–1994 to 26.7% in 2003–2006, presumably as herd immunity has prevented infection among adults.[267] Substantial declines in HAV infections among children have resulted in decreased age-specific seroprevalence rates among U.S. adults and therefore an increase in susceptibility to HAV among older adults.[267,268] The 2013 multistate outbreak of hepatitis A occurred largely in adults with few cases among vaccinated and unvaccinated children.[17] In summary, the epidemiology of hepatitis A in the United States is fundamentally changed as endemic transmission, outbreaks in childcare centers, and large community outbreaks have been virtually eliminated by the childhood vaccination program. We are likely to continue to see a greater proportion of cases among adults because of travel and outbreaks associated with imported food contaminated with HAV, although the overall risk of hepatitis A to the United States population is greatly diminished.

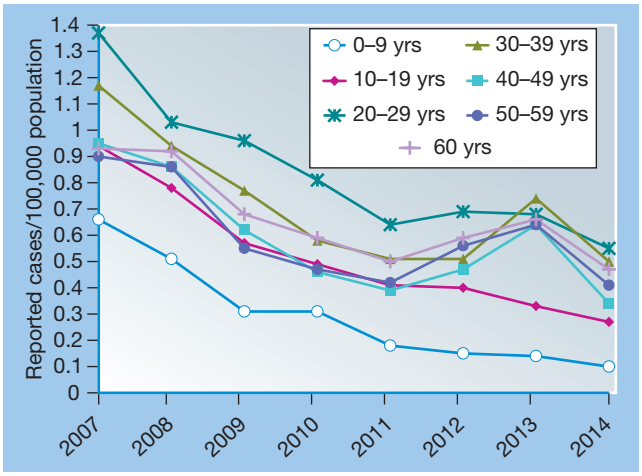


Figure 24.7. Incidence of acute hepatitis A by age group in the United States: 2007–2014. *(Data from the Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance System, Atlanta, GA.)*

Potential Sources of Infection

In 2014, only 7% of hepatitis A cases reported in the United States had a risk factor identified; the other 93% either had no risk factor identified or data were missing.[236] Of the 7% with a risk factor identified, international travel was the most commonly identified risk.[236] Other potential sources of infection identified in the United States in 2014 include injection-drug use, MSM, association with daycare (child or employee), association with a recognized food/waterborne outbreak, and other contact with a hepatitis A patient. Common source food exposures are likely to continue to occur with sporadic

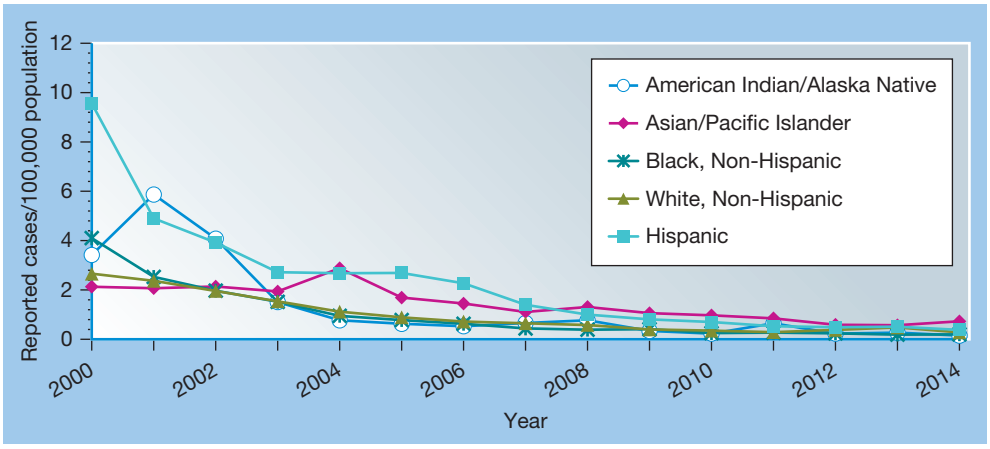


Figure 24.8. Incidence of acute hepatitis A by race/ethnicity in the United States: 2000–2014. *(Data from the Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance System, Atlanta, GA.)*

outbreaks being reported in Europe and North America, as well as other countries with low levels of endemic transmission of hepatitis A.[17,269,270]

Specific Groups and Settings

International Travel. Hepatitis A is a risk for unvaccinated persons from developed countries who travel to regions with high, transitional, or intermediate endemicity (see Fig. 24.4).[271–273] A study published in 2006 of Swiss travelers estimated the risk to be 6 to 30 cases per 100,000 months of stay in developing countries among persons who did not receive immunoglobulin or vaccine before departure.[274] The risk may be higher among travelers staying in areas with poor hygienic conditions[275] and varies according to the region and the length of stay. Declines in the number of hepatitis A cases attributed to improved infrastructure in endemic countries are now reported for travelers from some European countries.[276,277] In the United States, international travel is a commonly reported risk factor.[236,278] In the United States and Europe, hepatitis A in travelers, especially children, traveling to endemic countries to visit relatives and friends accounts for an increasing proportion of reported cases. Compared with persons traveling for work or recreation, these persons generally have trips of longer duration and, by staying in family settings rather than in hotels, may have greater exposure to HAV circulating in the community. These persons also are less likely to seek or to adhere to pretravel advice.[279–286] Travelers who acquire hepatitis A infection during their trip may also transmit the virus to others on their return.[287] Vaccination of international travelers to endemic regions remains an important consideration for pretravel preparations.[288]

Contacts of International Adoptees. International adoptees with asymptomatic hepatitis A may transmit the virus to family members and close contacts. Most adoptee-associated cases of hepatitis A are among unvaccinated nontraveling contacts of the adoptee exposed during the first 60 days after arrival in the United States; secondary and tertiary transmission have been described.[289–292] One study of 270 adoptees who received serologic testing within 4 months of arrival in the United States found that 1% had evidence of acute hepatitis A, and rates varied by country of origin; furthermore, all acutely infected adoptees were asymptomatic.[292] A retrospective review from Minnesota from 2007 to 2009 identified 21 hepatitis A cases among international adoptees younger than 60 months of age.[293] Another study found the prevalence of acute HAV infection to be 4.6% among international adoptees based on screening of adoptees from 2006 to 2010; the study made note of the substantial interruption of care and social support associated with secondary caretaker infection.[294] Screening of international adoptees from endemic areas has been suggested as a preventive measure; however, there is a need for a better understanding of the impact and public health importance of international adoptees introducing hepatitis A into the community, including consideration of the effort and expense of screening as compared to the benefits.

Users of Illicit Drugs and Homeless Populations. In recent decades, outbreaks of hepatitis A have been reported among illicit drug users in North America, Australia, and Europe.[84,87,226,295–302] In the United States, these outbreaks involved users of injected and noninjected methamphetamine, who accounted for up to 30% of reported cases in communities during outbreaks in some settings.[90,298,303,304] Cross-sectional serologic surveys have demonstrated that injection drug users have a higher prevalence of anti-HAV than the general U.S. population.[305–310] A randomized intervention trial of 469 injection (70%) and noninjection (30%) drug users in methadone maintenance, found 56% were anti-HAV–positive.[311] Transmission among injection drug users probably occurs through percutaneous and fecal–oral routes.[298] Similarly, homeless populations in the United States and Europe may have increased seroprevalence of hepatitis A and are recognized in occasional outbreaks.[312] The risk of hepatitis A may be related to sexual and illicit drug use practices, years as a homeless person, and country of origin.[299,312–314]

Men Who Have Sex With Men. Hepatitis A outbreaks among MSM have been reported in urban areas in the United States, Canada, Europe, and Australia, and may occur in the context of an outbreak in the larger community.[302,303,315–320] Outbreaks among MSM have been reported in Europe during 2008–2009 in Ireland, Poland, and Italy[321–323]; however, seroprevalence surveys have not consistently demonstrated an elevated prevalence of anti-HAV among MSM compared with a general population of similar age.[305,324] Some studies conducted during outbreaks and seroprevalence surveys have identified specific sex practices associated with illness, whereas others have not demonstrated such associations.[306,315,318] A recent large cross-sectional prevalence study among MSM conducted from 1994 to 2000 in the United States found a similar prevalence of anti-HAV as in the general population of similar age.[325] The study also found that factors associated with HAV infection among MSM in nonoutbreak settings are likely similar to those among non-MSM, although the risk of HAV infection from community outbreaks among MSM exists and could be prevented with vaccination.

Transfusion and Healthcare-Related Transmission. Transfusion-related hepatitis A is rare (see "Bloodborne" below).[326] The risk of infection in patients with hemophilia is unknown, but results of one serologic survey of persons with hemophilia before the licensure of hepatitis A vaccine suggested they might have been at increased risk.[327] Outbreaks have been reported in Europe and the United States among patients who received factor VIII and factor IX concentrates.[328,329] However, no HAV infections attributed to blood products were identified in an analysis of serosurveillance data collected from 140 hemophilia treatment centers in the United States between 1998 and 2002, suggesting that improved viral inactivation procedures, donor screening, and increased hepatitis A vaccination coverage among clotting factor recipients has reduced the risk of HAV transmission.[330]

Outbreaks of hepatitis A have been reported in neonatal intensive care units following transmission to hospital staff from a neonate with asymptomatic HAV infection acquired from a blood transfusion.[331–334] One episode of transmission was reported in association with an experimental treatment with lymphocytes incubated in serum from a donor with HAV infection.[335]

Nosocomial transmission of hepatitis A from adult patients to healthcare workers also is rare because most patients with hepatitis A are hospitalized after the onset of jaundice, when infectivity is low, but it has been reported in association with fecal incontinence of patients.[336–338] Healthcare workers were not found to have an increased prevalence of anti-HAV compared with control populations in serologic surveys conducted in the United States from 1977 to 1982.[339]

Foodborne and Waterborne. Foodborne hepatitis A outbreaks are of increasing concern globally, particularly in Europe and the United States. Historically, in the United States they were most commonly associated with contamination of food during preparation by a food handler with HAV infection.[217,340–345] Food contaminated before retail distribution,

such as lettuce or fruits contaminated at the growing or processing stage, can be the source of hepatitis A outbreaks, some of which can be large or spread over a wide geographic area.[205,346–351] Since 2000, common source food exposures, such as the 2003 outbreak associated with contaminated green onions[350] and more recently contaminated berries imported from countries with endemic transmission of hepatitis A, have been increasingly recognized in hepatitis A low and very low endemic regions.[269,270,352–354] According to the Rapid Alert System for Food and Feed database in Europe, 35 notifications of HAV in food were reported between 1999 and 2013 in eight European Union countries from multiple food sources. In such outbreaks, trace back to the contaminated product and isolation of HAV has proven to be challenging.[270,352,353]

Mixed frozen berries were implicated in a hepatitis A genotype 1A outbreak in Italy in 2013.[270,352,355] An outbreak likely associated with berry mix buttermilk cake imported frozen from Germany[357] occurred in 2013–2014. Other recent outbreaks of HAV genotype IB illness associated with food from the Middle East were also reported. A Nordic outbreak (Denmark, Finland, Sweden, and Norway) occurred in 2012–2013 from contaminated strawberries, suspected to have originated from Egypt and Morocco.[358,359] Frozen pomegranate arils from Egypt were implicated in an outbreak in Canada in 2012, and imported pomegranate arils from Turkey were associated with a multistate outbreak in the United States in 2013.[17] Semidried tomatoes from Turkey were associated with cases in Europe and Australia in 2009–2011.[269,360,361]

In low and very low endemic countries, outbreaks related to contaminated shellfish have become less common but are still occasionally identified.[362–366] Waterborne hepatitis A outbreaks are rare and generally are related to sewage contamination or inadequate treatment of water.[367–369]

Although results of some serologic surveys conducted among sewage workers in Europe indicated a possible elevated risk of HAV infection, findings have not been consistent.[370–372] In published reports of three serologic surveys conducted among U.S. sewage workers and appropriate comparison populations, no substantial or consistent increase in prevalence of anti-HAV was found among sewage workers.[373–375] No work-related instances of HAV transmission have been reported among sewage workers in the United States.

Institutions for Persons With Developmental Disabilities

Historically, HAV infection was endemic in institutions for developmentally disabled people, but with smaller facilities, improved conditions, and vaccination, the incidence and prevalence of infection have decreased and outbreaks rarely are reported in the United States.[86,255,376] However, unvaccinated disabled adults cared for in group homes may be at risk from living in close quarters, and often being incontinent and nonverbal.[377] Unvaccinated staff working in group homes may also be at risk during such outbreaks. During April to July 2013, an outbreak in Michigan occurred in which eight unvaccinated residents of five group homes for adults with disabilities were diagnosed with hepatitis A, and one died.[377]

MODES OF TRANSMISSION

HAV is primarily transmitted by the fecal–oral route. The virus replicates in the liver, is excreted in bile, and is found in highest concentrations in stool.[378] Because of the high concentration of HAV in the stool of infected persons, fecal excretion of HAV is the primary source of virus. Virus can be demonstrated in stool for 14 to 21 days before to 8 days after the appearance of jaundice. The highest concentrations are during the 2-week period before jaundice develops or liver enzyme levels become elevated, followed by a rapid decline after the appearance of jaundice (see Fig. 24.3).[13,337,378] Epidemiologic studies demonstrated that peak infectivity and the greatest risk of transmission occur during the 2 weeks before onset of symptoms.[10] Shedding of HAV may continue for longer periods in infected infants and children than in adults. With the use of PCR techniques, HAV RNA has been detected in stools of infected newborns for up to 6 months after infection.[331] Excretion in older children and adults has been demonstrated 1 to 3 months or longer after clinical illness.[164,331,379–381] Although chronic shedding of HAV does not occur, the virus has been detected in stool during relapsing illness.[30,32,382]

The infectious dose of HAV in humans is not known. During the period of viremia, which begins during the prodrome and extends through the period of liver enzyme elevation (see Fig. 24.3), HAV concentrations in blood are several orders of magnitude lower than in stool.[162,164,383,384] However, in experiments conducted in nonhuman primates, HAV was several orders of magnitude more infectious when administered by the intravenous compared with the oral route, and animals were successfully infected with low concentrations of HAV administered by the intravenous route.[23] Although HAV occasionally may be detected in saliva in experimentally infected animals,[162] transmission by saliva has not been demonstrated.

Enzyme immunoassays and PCR may detect defective (noninfectious) and infectious viral particles. Thus, the detection of HAV antigen in the stool by enzyme immunoassays or HAV RNA in the serum or stool by PCR does not indicate whether the person is infectious. It is likely that the period of infectivity is shorter than the period during which HAV RNA is detectable in stool. For practical purposes, children and adults with hepatitis A can be assumed to be noninfectious 1 week after jaundice appears.

Person to Person

Person-to-person transmission by the fecal–oral route is the predominant means of HAV transmission globally.[303,385] Most transmission occurs among close contacts, particularly in households and extended family settings.[264,265,386] In areas of endemic transmission, young children have the highest rates of infection and are often the source of infection for others, primarily because infections in this age group are usually asymptomatic and standards of hygiene are generally lower among young children than among adults.[260,264,265,386]

Foodborne and Waterborne

HAV can remain infectious in the environment,[387] allowing for common-source outbreaks and sporadic cases to occur from exposure to fecally contaminated food or water. Many uncooked foods have been implicated as the source of outbreaks. Cooked foods also can transmit HAV if the cooking is inadequate to kill the virus or if the food is contaminated after cooking, as commonly occurs in outbreaks associated with infected food handlers.[217,340,341,344,388,389] Hepatitis A inactivation has been demonstrated when exposed to 90°C temperature for 180 seconds.[390] Contaminated [390] shellfish were responsible for a large outbreak in Shanghai in 1988.[19,391] Although outbreaks continue to be reported,[362–365] shellfish-associated HAV outbreaks in the United States and other developed countries are increasingly uncommon.[366,392] Several large outbreaks traced to produce contaminated before distribution have been reported in recent years from the United

States, Europe, and Australia.[17,205,269,346,350,351,361,393] Waterborne outbreaks of hepatitis A are uncommon in developed countries, but have been reported from countries with poor water sanitation and infrastructure.[394]

Bloodborne

Transfusion-related hepatitis A is rare because HAV does not result in chronic infection. In the developed world, blood donors are routinely screened for elevated aminotransferase levels, further decreasing the risk of transmission during the viremic prejaundice phase of infection. Transmission by transfusion of blood or blood derivatives collected from donors during the viremic phase of infection has been reported, including outbreaks in Europe, Korea, and the United States among patients who received factor VIII and factor IX concentrates prepared using solvent-detergent treatment to inactivate lipid-containing viruses.[326,328,329,384,395–398] HAV is resistant to solvent-detergent treatment, and contamination presumably occurred from plasma donors with hepatitis A who donated during the incubation period. Since 2002, nucleic acid amplification tests such as PCR have been applied to the screening of source plasma used for the manufacture of plasma-derived products in the United States. These assays have sufficient sensitivity to remove most units of blood or plasma that contain HAV.[326,330,399] In settings where such procedures are not followed, infection from HAV remains as a remote possibility.

Vertical

Rare published case reports describe intrauterine transmission of HAV during the first trimester, resulting in fetal meconium peritonitis.[400,401] After delivery, two infants were found to have a perforated ileum. The risk of transmission from pregnant women in whom hepatitis A develops in the third trimester of pregnancy to newborns seems to be low.[78,402] However, newborns who acquire infection in this manner are usually asymptomatic, and an outbreak among hospital staff related to exposure to an infected asymptomatic infant has been reported.[403]

SIGNIFICANCE AS A PUBLIC HEALTH PROBLEM

The World Health Organization (WHO) estimates that more than 100 million cases occur annually, resulting in an estimated 15,000 to 30,000 deaths per year.[14,16] The burden of disease is closely linked to the economic development of a country (see "Worldwide Disease Patterns," above, and Fig. 24.5). In the United States, Canada, Western Europe, and other developed countries, endemic transmission is low to very low with small numbers of cases being reported, and a substantial proportion associated with a common-source outbreak or international travel. A recent analysis of data from the United States revealed not only cases but also hospitalizations and death have decreased substantially.[254] There have been major declines in incidence since introduction of hepatitis A vaccine in the United States in the 1990s; less than 1500 cases were reported in 2014. However, 45% of acute hepatitis A cases with available data were hospitalized, and none died.[88,236,261] The decrease in disease, coupled with the successful vaccination program in the United States, has resulted in the prevalence of immunity acquired from infection reduced among young adults, leaving a substantial proportion of this population without natural or vaccine-induced immunity.[267]

PASSIVE IMMUNIZATION

Until the licensure of hepatitis A vaccines, immunoglobulin (previously known as γ-globulin or immune serum globulin) was the mainstay of prevention for HAV after exposure through administration of passive antibodies. This product has also proven useful for prevention of hepatitis A in travelers, Peace Corps volunteers, military personnel, and persons recently in close contact with a person with hepatitis A, as well as to control outbreaks in childcare centers.[404–406] In the United States, GamaSTAN (Grifols Therapeutics Inc., Research Triangle Park, NC) is currently the only available hepatitis A immunoglobulin (IgG anti-HAV) product.[407] Immunoglobulin is prepared by a serial cold ethanol precipitation from pools of plasma, collected from tens of thousands of donors. Donors are screened for hepatitis B virus (HBV), HIV, and HCV by serologic and other assays.[408,409] Since 1995, immunoglobulin prepared in the United States has been required to be negative for HCV RNA by PCR amplification or to be produced using a method that ensures additional virus inactivation.[409] The manufacturing process removes contaminate enveloped and nonenveloped viruses, including HIV, HBV, HCV, as well as low levels of Creutzfeldt-Jakob disease (CJD)/variant CJD (vCJD) agent infectivity.[407]

Since licensure of inactivated hepatitis A vaccines, vaccination is preferred for preexposure and postexposure prophylaxis, and the use of immunoglobulin for preexposure and postexposure prophylaxis is limited. Immunoglobulin is indicated in persons for whom the vaccine is contraindicated, and in certain situations for international travel and postexposure prophylaxis (see "Preexposure and Postexposure Prophylaxis" below) (Table 24.3).[410]

Seroprotective levels (see section on correlates of protection below) of anti-HAV (ie, 10–20 mIU/mL) appear rapidly after preexposure intramuscular injection of Ig.[177] Peak levels of immunoglobulin are reached in approximately 2 days after injection.[411] Immunoglobulin is 80% to 90% effective in preventing hepatitis A when administered before exposure or within 2 weeks after exposure.[177,412–415] Whether immunoglobulin completely prevents infection or leads to asymptomatic infection and the development of persistent anti-HAV (passive–active immunity) is related to the amount of time between exposure and immunoglobulin administration.[24,414,416] Immunoglobulin has not been successful in controlling communitywide hepatitis A outbreaks, owing in part to the transient nature of protection, coverage rates, perhaps the lack of herd immunity, and the frequency of unrecognized infection.[417]

Although the prevalence of antibody to HAV in the population has been declining, and in the United States there is no standard for anti-HAV levels in immunoglobulin preparations, no clinical or epidemiologic evidence of decreased protection from immunoglobulin has been observed.[86,418]

Serious adverse events from immunoglobulin are rare. Because anaphylaxis has been reported after repeated administration to persons with IgA deficiency, these persons should not receive immunoglobulin.[419] Immunoglobulin is also contraindicated for persons who have severe thrombocytopenia or any coagulation disorder.[419] Pregnancy and lactation are not contraindications to immunoglobulin administration.

Immunoglobulin should be administered intramuscularly only, preferably in the deltoid muscle of the upper arm or the anterolateral aspects of the upper thigh in children younger than age 24 months.[410]

Immunoglobulin can be administered at the same time as most inactivated vaccines. It does not interfere with the immune response to oral poliovirus vaccine or yellow fever vaccine; immunoglobulin, however, can interfere with the response to live attenuated virus vaccines (e.g., measles,

TABLE 24.3 Recommended Preexposure Prophylaxis for International Travel and Postexposure Immunoprophylaxis

Age/Health Status	Immunoglobulin (Ig) Dosage[a]	Hepatitis A Vaccine
PREEXPOSURE PROPHYLAXIS FOR INTERNATIONAL TRAVEL		
Infants <12 mo,[b] persons allergic to vaccine or who elect not to be vaccinated	*For length of stay:* <3 mo: Ig 0.02 mL/kg 3–5 mo: Ig 0.06 mL/kg >5 mo: Ig 0.06 mL/kg (repeat every 5 mo for extended travel)	None; vaccinate infants at age 12 mo
12 mo–40 y	None	One dose of hepatitis A vaccine as soon as possible before departure[c]
>40 y and persons with special conditions[d]	*For length of stay:* <3 mo: IgG 0.02 mL/kg 3–5 mo: Ig 0.06 mL/kg >5 mo: Ig 0.06 mL/kg (repeat every 5 months for extended stay)	Ig *and* one dose of hepatitis A vaccine as soon as possible before departure[c]
POSTEXPOSURE PROPHYLAXIS FOR PREVIOUSLY UNVACCINATED PERSONS[e]		
Infants <12 mo,[b] persons with special conditions[d]	Ig 0.02 mL/kg within ≤2 wk of exposure; efficacy of Ig administered >2 wk after exposure not established	None; vaccinate infants at age 12 mo
Healthy persons 12 mo–40 y	None	Hepatitis A vaccination as soon as possible
Persons >40 y	Ig 0.02 mL/kg within 2 wk of exposure preferred	Hepatitis A vaccine can be used if Ig is not available

[a]For preexposure prophylaxis, administer Ig ≤2 weeks before departure. Intramuscular preparations of Ig should never be given intravenously, and the intravenous preparations of Ig are not intended for hepatitis A prevention and are formulated at a lower globulin concentration. Hepatitis A vaccine should be administered as soon as possible before departure. Immunoglobulin and hepatitis A vaccine should be administered at separate sites if administered at the same visit. For postexposure prophylaxis, Ig should be administered as soon as possible within 2 weeks after exposure.

[b]Children younger than 1 year (for whom vaccine is not licensed) and persons with a contraindication to vaccination should receive Ig 0.06 mL/kg, repeated every 5 months during exposure.

[c]The hepatitis A vaccine series should be completed with a second dose 6 months after the first dose.

[d]Special conditions: immunocompromised persons, persons with diagnosed chronic liver disease, and persons for whom vaccine is contraindicated.

[e]Persons who previously received a dose of hepatitis A vaccine should complete the series. Persons who previously received a complete series of hepatitis A vaccine need no further treatment.

From Novack R, Williams I, Bell B. Update: prevention of hepatitis A after exposure to hepatitis A virus and in international travelers: updated recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep. *2007;56:1080–1084.*

mumps, and rubella [MMR] vaccine and varicella virus vaccine) when administered either as individual or combination vaccines.[86,419,420]

ACTIVE IMMUNIZATION

History of Vaccine Development

Hepatitis A vaccine for active immunization was developed in a manner similar to that used for poliovirus vaccines. As for poliovirus vaccines, the initial breakthrough came with the in vitro cultivation of HAV in cell lines suitable for vaccine production.[153] HAV was successfully propagated in cell culture obtained from marmoset liver cells in 1979.[16] Since then, formalin-inactivated, cell culture–produced, whole-virus vaccines have been approved in much of the world.[421,422]

The cell culture–adapted HAV strains CR326F and HM175 were found to be highly attenuated in humans and have been tested as live attenuated vaccine candidates in primates and to a limited extent in humans.[423–426] For both strains, inoculums of greater than 10^6 tissue culture infective doses (TCIDs) were required to induce an antibody response in volunteers. That the vaccines infected volunteers was never proved because the only evidence was seroconversion, which could have been induced by the antigenic mass contained in the inoculums rather than new antigen produced by replication.

The entire nucleotide sequences of the wild-type and the vaccine variant of HM175 have been determined.[114,156] A full-length, infectious cDNA clone of the cell culture–adapted virus was made,[427,428] and the mutations responsible for cell culture adaptation and attenuation were determined by the molecular construction of chimeric viruses.[124,156,428] Substitutions and deletions in the 5′ noncoding region and substitutions in the 2B/2C coding regions were found to be important for cell culture adaptation and attenuation of virulence. However, mutations throughout the genome contributed to improved in vitro replication.[429]

Description of Current Vaccines and History of Development

Inactivated and live attenuated hepatitis A vaccines have been developed worldwide. The WHO recommends vaccination in countries with intermediate to low endemicity.[16]

In 1995, the first inactivated U.S. Food and Drug Administration (FDA)-approved HAV vaccine Havrix (GlaxoSmithKline Biologicals, Rixensart, Belgium) became available in the United States; a second inactivated HAV vaccine VAQTA (Merck & Co, Inc., West Point, PA) was introduced in the United States in1996.[86] In 2001, a combination hepatitis A and hepatitis B vaccine (Twinrix, GlaxoSmithKline, Research Triangle Park, NC) was approved in the United States for persons age 18 years or older. These vaccines have been approved for use throughout much of the world (Table 24.4).[421,422]

Similar inactivated vaccines have been licensed or registered in Europe, Canada, China, and other countries.[430–432] VAQTA is based on strain CR326F of HAV isolated in Costa Rica and was the first strain to be successfully cultivated in vitro.[153] CR326F initially was isolated in a fetal rhesus kidney

TABLE 24.4 Major Constituents and Recommended Schedules for Selected Inactivated Hepatitis A Vaccines[a]

Manufacturer	Trade Name	HAV Strain	Adjuvant	Dosage	Age	Volume (mL)	Schedule (mo)[b]
Merck & Co., Inc.	VAQTA	CR326F	Aluminum hydroxyphosphate sulfate	25 U 50 U	12 mo–18 y ≥19 y	0.5 1.0	0, 6–18 0, 6–18
GlaxoSmithKline Biologicals	Havrix	HM175	Aluminum hydroxide	720 EL.U. 1,440 EL.U.	12 mo–18 y ≥19 y	0.5 1.0	0, 6–12 0, 6–12
Sanofi Pasteur, Inc.[377]	Avaxim	GBM	Aluminum hydroxide	80 U 160 U	12 mo–15 y ≥16 y	0.5 0.5	0, 6–18 0, 6–18
Crucell Vaccines Inc.[385]	Epaxal	RG-SB	Virosomes composed of 10 μg purified influenza virus hemagglutinin and 100 μg of phospholipids (immunopotentiating reconstituted influenza virosome)	25 IU	≥12 mo	0.5	0, 6–12
Sinovac Biotech Ltd.	Healive	TZ84	Aluminum hydroxide	250 U 500 U	12 mo–15 y ≥16 y	0.5 1.0	0, 6 0, 6

EL.U., enzyme-linked immunosorbent assay units; HAV, hepatitis A virus; IU, international units; U, units of HAV antigen.
[a]Licensed ages, dosages, and schedules vary among countries. All vaccines are administered by the intramuscular route in a two-dose series. Some vaccines contain other constituents. Refer to the product insert for more information.
[b]Zero months represents the timing of the initial dose; subsequent numbers represent months after the initial dose.

cell line, FRhK6. After 15 passages, it was transferred to human embryonic lung diploid fibroblast (MRC-5) cells for an additional 28 passages.[422] Havrix is based on strain HM175 of HAV, isolated from stool of a patient in a family outbreak in Australia.[433] It was originally adapted to cell culture by a series of 30 passages in primary green monkey kidney cells followed by adaptation to MRC-5 cells.[421] Twinrix contains inactivated HAV and noninfectious recombinant hepatitis B surface antigen. The purified HBsAg is obtained by culturing genetically engineered *Saccharomyces cerevisiae* yeast cells. Each antigen is separately adsorbed onto aluminum salts and pooled during Twinrix formulation.[434]

Avaxim (Sanofi Pasteur, Lyon, France), based on the GBM strain of HAV, is licensed in Europe, Canada, and other areas.[435] This strain was isolated and propagated on primary human kidney cell culture for 10 passages, followed by adaptation to human diploid fibroblast cells during 20 passages.[436–438] Inoculation of chimpanzees showed that the strain had been attenuated by passage.[439] This vaccine also is produced in the MRC-5 human diploid fibroblast cell strain and is processed in a way similar to the U.S.-licensed vaccines.[430]

A vaccine developed at the Swiss Serum Institute, called Epaxal, is licensed by Crucell Vaccines Inc. (Leiden, The Netherlands) in most countries in Europe and Canada, in many countries in South America, and other parts of the world.[431,440–443] The RG-SB strain of HAV used in this vaccine is harvested from disrupted MRC-5 cells and inactivated by formalin. The liposome adjuvant, immunopotentiating reconstituted influenza virosomes (IRIV) is composed of phosphatidylcholine, phosphatidylethanolamine, and hemagglutinin from A/Singapore/6/86-like H1N1 strain of influenza virus. It is hypothesized that IRIVs may stimulate humoral and cellular immunity by binding to macrophages and other cells primed by influenza virus.[440]

More than one inactivated vaccine is available in China. Healive (Sinovac Biotech Ltd., Beijing) is based on formalin-inactivated HAV strain TZ84 cultivated in 2BS human fetal lung diploid fibroblast cells. Pediatric and adult formulations are available; 0.5 mL/250 U for persons ages 1 to 15 years; 1.0 mL/500 U for persons age 16 years or older.[432,444]

A freeze-dried, live attenuated HAV vaccine has also been used in China (Zhepu, Zhejiang Pukang Biotechology Co. Ltd., Hangzhou)[155,445–448] and is immunogenic in trials conducted in India.[449,450] The vaccine uses the H2 strain, a derivative of the cell culture–adapted, attenuated strain of HM175.[424,428,429] A randomized trial demonstrated detectable antibody for 8 years after the vaccine was administered either as a single subcutaneous dose or a single dose plus a booster dose at 12 months, suggesting that a booster dose is not needed in immunocompetent persons.[447] Because the virus is highly attenuated, large inoculums (≈10^7 $TCID_{50}$ [median TCID]) are needed to produce an immune response in humans.

Constituents

All available inactivated vaccines include HAV antigen, but the units by which the antigen content is expressed are different for each vaccine (see "Preparations" below). Owing to the different assays used and the lack of an accepted standard, it is not possible to compare the antigen content of the various vaccines (see Table 24.4). Havrix, VAQTA, Avaxim, and Healive contain aluminum hydroxide or aluminum hydroxyphosphate as an adjuvant. Epaxal uses liposomes as the adjuvant. Avaxim is formulated using 2-phenoxyethanol as a preservative; the other vaccines are formulated without preservatives. No hepatitis A vaccine contains antibiotics; however, residual antibiotic from the manufacturing process might be present in trace amounts in Havrix (neomycin), Avaxim (neomycin), and Epaxal (polymyxin B). Hepatitis A viral particles are concentrated and purified during the manufacture of all the vaccines. Differences in the content of nonvirion proteins exist between the vaccines, but these differences have not been found to be clinically relevant.

Manufacture

Inactivated hepatitis A vaccines are produced in similar ways. The vaccine virus is grown in cell culture, harvested by cell lysis, and inactivated with formalin. Differences relate to the viral purification processes.

Preparations

Both inactivated monovalent hepatitis A vaccines licensed in the United States are available in pediatric and adult formulations. All monovalent inactivated hepatitis A vaccines have

formulations available for persons at least 1 year old; all are available in two-dose schedules and are administered intramuscularly (see Table 24.4).[442,451,452]

Combination inactivated hepatitis A and recombinant hepatitis B vaccine (Twinrix; GlaxoSmithKline, Rixensart, Belgium) is available in the United States for persons age 18 years or older in a three-dose schedule (0, 1, 6 months), or in a four-dose "accelerated" schedule (days 0, 7, 21–30, and 12 months) for adults.[453–458] The vaccine's performance seems to be equivalent to that of each of the single-antigen vaccines administered separately.[459] Pediatric formulations of Twinrix are available in Europe, Canada, and other parts of the world.[453,454,460]

Ambirix (GlaxoSmithKline) is a combination hepatitis A and hepatitis B vaccine formulation containing the same components and content as the adult formulation of Twinrix (1.0 mL), 720 EL.U. (enzyme-linked immunosorbent assay units) of the hepatitis A component and 20 µg of the purified recombinant HBV surface antigen. Ambirix is licensed in Europe for children 2 to 15 years old in a two-dose series (0, 6–12 months) given intramuscularly.[454]

A combination inactivated hepatitis A and recombinant hepatitis B vaccine has been introduced in China for adults and children (Bilive, Sinovac Biotech Ltd.).

Combination vaccines for the prevention of hepatitis A and typhoid fever among adolescents and adults are available in some countries. ViATIM (also known as Vivaxim, Sanofi Pasteur) contains 160 antigen units of inactivated HAV (similar to Avaxim) and 25 µg of *Salmonella* Typhi Vi capsular polysaccharide antigen packaged in a dual-chamber bypass syringe. Mixing of the two antigens occurs at the time the syringe plunger is depressed to start the intramuscular injection. Also present in the final 1-mL dose are 2.5 mg of 2-phenoxyethanol as a preservative and 0.3 mg aluminum hydroxide as adjuvant. To produce long-term protection against hepatitis A, a dose of hepatitis A vaccine is recommended 6 to 12 months later; maintaining immunity to *Salmonella* Typhi requires a dose of a single-antigen typhoid fever vaccine 3 years after the dose of ViATIM (the booster schedule for the U.S.-licensed capsular polysaccharide typhoid vaccine [which does not include hepatitis A] is every 2 years).[461,462] Hepatyrix (GlaxoSmithKline) contains 25 µg of *Salmonella* Typhi Vi capsular polysaccharide antigen and 1440 EL.U. of hepatitis A antigen combined in 1 mL of saline and aluminum hydroxide as adjuvant. The second dose is given 6 to 12 months after the first. Hepatitis A–typhoid fever combination vaccine performance seems to be similar to that of the single-antigen vaccines administered separately.[458,463]

Dosage and Route

Dosages and schedules vary according to country, and country-specific recommendations should be sought for current licensure information and recommendations (Table 24.5). Only Havrix, VAQTA, and Twinrix are licensed in the United States (see Table 24.4). Only Havrix is a WHO prequalified vaccine.[464]

Vaccine Stability

The vaccines should be stored at 2°C to 8°C, and Havrix and VAQTA have been shown to retain potency when kept for at least 2 years under those conditions.[421,465,466] Freezing destroys the vaccine, causing aggregation of the adjuvant particles. Any vaccine that was frozen should be discarded. The reactogenicity and immunogenicity of Havrix after storage at 37°C for 1 week and the stability profile of VAQTA when stored at this temperature for more than 12 months did not differ from those of vaccines stored at the recommended temperature.[467,468]

TABLE 24.5 Countries With Hepatitis A Childhood Vaccination Policies Reported by Country to WHO/UNICEF, 2014

Country	Year of Introduction	Number of Doses	Age at Administration of First Dose (Months)	Schedule Interval Between Doses (Months)
Argentina	2005	1	12	0
Bahrain	2004	2	18	0, 6
Brazil	2013	1	12	0
Chile	2014	1	18	0
China	2007	1	18	0
Colombia	2013	1	12	0
Greece	2007	2	12	0, 6
Israel	2002	2	18	0, 6
Kazakhstan	2009	1	24	0
Mexico	2012	1	12	0
Mongolia	2013	2	14	0, 10
Panama	2006	1	12	0, 6
Qatar	2010	2	12	0, 6
Saudi Arabia	2008	2	18	0, 6
Turkey	2013	2	18	0, 6
United States of America	2002	2	12–23	0, 6–18
Uruguay	2008	2	15	0, 6

From WHO Reports of immunization schedules by vaccine (2014) and year of introduction of selected vaccines database (2014). http://www.who.int/immunization/monitoring_surveillance/data/en.

Immunogenicity of Vaccine

Antibodies

Anti-HAV concentrations are measured in comparison with a WHO reference immunoglobulin reagent and are expressed in milli-international units per milliliter (mIU/mL). Clinical studies with Havrix have generally measured antibody using an enzyme immunoassay and, with VAQTA, a modified radioimmunoassay. In extensive clinical trials in children and adults, the inactivated hepatitis A vaccines available in the United States have been found to be highly immunogenic. Both vaccines begin to be effective approximately 2 weeks after a single intramuscular dose. Although the concentration of antibody after vaccination varies with the dose and schedule of the vaccine, after a single dose of vaccine, antibody concentrations are higher than those produced by doses of immunoglobulin known to be protective.

A second dose 6 to 18 months later results in a boosted antibody concentration, but the final concentration is generally lower than concentrations measured after natural infection (Fig. 24.9).[469–472] By 4 weeks after one dose of vaccine, 90% to 100% of children age 1 year or older and adults respond with concentrations of antibody considered protective. The boost in antibody concentration following the second dose is likely important for long-term protection.[430,466,473–479] HAV-specific IgM antibody occasionally can be detected by standard assays, primarily if measured soon (ie, up to 4 weeks) after vaccination.[430,469]

Results of a number of studies indicate that the response of adults administered hepatitis A vaccine according to a schedule that mixed the two currently U.S.-licensed single-antigen vaccines was equivalent to that of adults vaccinated according to the licensed schedules with the single vaccine.[480,481] Mixed schedules with Epaxal and Havrix, and Avaxim and Havrix or VAQTA, have also been shown to be equivalent to schedules that used a single vaccine brand.[482–486] Based on limited data, the response to a second dose delayed for 20 to 31 months (Havrix), 24 to 66 months (VAQTA), or 30 to 54 months (Epaxal) was similar to that of the licensed schedules.[487–489] Schedules with shorter intervals between doses have not been studied using the currently licensed single-antigen formulations. Healive and Havrix have been shown to be comparable in healthy Chinese children.[490]

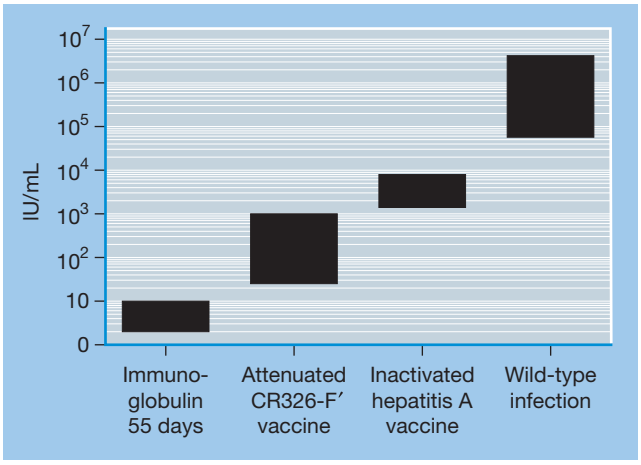


Figure 24.9. Comparative levels of antibody to hepatitis A virus (HAV) after administration of immunoglobulin, one dose of an attenuated hepatitis A vaccine, two doses of an inactivated hepatitis A vaccine, and natural infection. The detection limits of the hepatitis A virus antibody (HAVAB) assay are approximately 100 mIU/mL. *(From Lemon SM. Hepatitis A virus: current concepts of the molecular virology, immunology and approaches to vaccine development.* Rev Med Virol. *1992;2:73–87.)*

Hepatitis A vaccines are approved for children age 12 months or older (see Table 24.4). Studies in children as young as 2 to 6 months old suggest that the vaccine is safe for infants and that it is immunogenic for infants who do not have passively transferred antibody from previous maternal HAV infection.[474,491–497] Among infants who received hepatitis A vaccine according to a number of different schedules, infants with passively transferred maternal antibody at the time of vaccination responded, but final antibody concentrations were approximately one-third to one-tenth those of infants who did not have passively transferred antibody and were vaccinated according to the same schedule.[474,491–497] The clinical significance, if any, of these lower antibody concentrations is unknown. The majority of infants born to anti-HAV–positive mothers have lost detectable antibody by 12 to 15 months of age.[474,493,498–501] Most, but not all, infants vaccinated in the presence of passively transferred maternal antibody at ages 2, 4, and 6 months respond to a booster dose 6 to 12 months or later with an anamnestic response, suggesting that they had been primed by the primary series.[491,497,498,502] One long-term follow-up study found that seropositivity persists for at least 10 years in children vaccinated at younger than 2 years of age, regardless of presence of maternal anti-HAV.[503] Another study found anti-HAV persistence for at least 17 years in adults vaccinated as children.[504] A recent study evaluating cellular immunity in poor vaccine responders found that one dose of inactivated hepatitis A vaccine induced HAV-specific cellular responses; an increase in the frequencies of classical memory B cells, TCD8 cells, and central memory TCD4 and TCD8 cells, and an increase in cytokine production were observed. The HAV-specific T-cell immunity persisted independently of anti-HAV antibody levels.[505]

Correlates of Protection

The absolute lower limit of antibody needed to prevent HAV infection has not been determined. The concentrations of antibody achieved after passive transfer by immunoglobulin or active induction by vaccination are 10- to 100-fold lower than those produced in response to natural infection (see Fig. 24.9). The minimum level of neutralizing antibody to HAV necessary to prevent infection is not known, although it is likely quite low because immunoglobulin provides approximately 90% efficacy in preventing hepatitis A and very low levels of serum neutralizing antibody are detected in immunoglobulin recipients.[177,506] Anti-HAV concentrations of 10 to 20 mIU/mL 1 to 2 months after administration of immunoglobulin protect against hepatitis A.[177] Results of in vitro studies using cell culture–derived HAV indicate that even antibody concentrations less than 20 mIU/mL can be neutralizing.[507] Antibody levels ranging from 10 to 33 IU/mL using different assays have been proposed as the threshold for protection from HAV infection in humans.[508] Because no absolute protective level has been defined, the lower limit of detection of the particular assay being used has generally been considered the protective level; 10 mIU/mL has also been considered a protective level in some postvaccination studies,[509] although it has been proposed that any detectable concentration of IgG anti-HAV may even suggest protection.[178] Clinical studies of Havrix have used levels greater than 20 or 33 mIU/mL, as measured using enzyme immunoassays to define "protective levels"; studies of VAQTA have used levels greater than 10 mIU/mL as measured with a modified radioimmunoassay (hepatitis A virus antibody [HAVAB]).[474,491,510]

Some studies indicate that concentrations of antibody above the defined protective concentration can be measured as early as 2 weeks after one dose,[440,470,511] but distinct differences between the antibody induced by vaccine and the antibody detected in people who received immunoglobulin (which should be similar to antibody induced by infection) can be identified when results using different assays are compared (see Fig. 24.9).[512,513] In one study with similar radioimmunoassay (HAVAB) titers, adult immunoglobulin recipients had higher neutralization titers as measured by a radioimmunofocus inhibition test (RIFIT) and an HAV antigen reduction assay (HAVARNA), but negligible radioimmunoprecipitation titers compared with a group of vaccinated children when measured 4 weeks after receiving one dose of vaccine.[512] In another study using the RIFIT assay, the neutralizing geometric mean antibody titers of persons who received immunoglobulin with hepatitis A vaccine were five times higher compared with persons who received hepatitis A vaccine alone when measured 4 weeks after administration.[513] However, it has also been shown that immunoglobulin prepared from the serum of vaccinees could protect a chimpanzee from HAV infection when the titer of antibody achieved in the chimpanzee was similar to that found in humans receiving immunoglobulin prophylaxis.[514]

How soon neutralizing antibody appears after vaccination is not clear from published reports, owing to differences among assays, dosages of vaccine, and when antibody measurements were obtained. In one study, depending on the assay, 42% to 100% of children vaccinated with the currently licensed formulation had neutralizing antibody when measured 1 month after a single vaccine dose.[512] In another study, approximately two-thirds of adults vaccinated with a lower dosage than the currently licensed formulation were positive for neutralizing antibody 4 weeks after one dose, and virtually all were positive 2 weeks after a second dose, given 1 month after the first.[512] In a third study, 84% of adults vaccinated with one dose of the liposomal vaccine had neutralizing antibody 2 weeks later.[440]

Special Groups

Conditions that may result in reduced immunogenicity include HIV infection, chronic liver disease, and older age. Among adults with HIV infection, 50% to 95% had protective antibody concentrations after completing the vaccination series, and final antibody concentrations were considerably lower than those among HIV-negative persons.[515–521] Higher CD4+ T-lymphocyte count at baseline were associated with response to vaccination[516–519,522]; increases in CD4 cell count with highly active antiretroviral therapy and lower HIV plasma RNA levels improved vaccine response rates.[523,524] It is not clear if sex is associated with vaccine response; one study found that males had greater vaccine response than females (Armstrong)[519] and another study found that vaccine responders were more likely to be female (Weissman).[522] A retrospective observational study found that most HIV-infected adult initial responders to two doses of HAV vaccine, maintain seropositivity for 6 to 10 years.[525] From 84% to 100% of HIV-infected children with normal CD4+ cell levels may develop protective antibody concentrations after two or three doses of hepatitis A vaccine.[526–530] Higher counts or restoration of CD4+ T-lymphocyte counts and younger age at vaccination are associated with improved responses to vaccination.[529,531] The absence of detectable HIV RNA and higher CD4+ T-cell percentages at the time of vaccination are associated with higher anti-HAV responses.[530,532,533] The seroconversion rate to three and two doses of hepatitis A vaccine was similar in HIV-infected MSM, although lower than that in HIV-uninfected MSM who received two doses. Twinrix recipients might be more sensitive to HIV RNA level and higher CD4+ T-cell percentages and less likely to seroconvert than those administered Havrix. This is possibly a result of noncompletion of the vaccine series among Twinrix recipients.[532]

Among persons with chronic liver disease, seroprotection rates were similar to those observed among healthy adults, but the final antibody concentrations were substantially lower.[534–538] Seroprotection rates and seroprotective titers of anti-HAV IgG at 8 years after the last vaccine dose was comparable in children with chronic hepatitis C and controls.[539] Limited data from small immunogenicity studies among liver or kidney transplant recipients have yielded seroprotection rate estimates that vary from 0% to 97%,[540–542] and one study indicated that antibody levels declined more rapidly among successfully vaccinated transplant recipients compared with other patients.[543] The final antibody concentrations achieved among persons older than age 40 years may be somewhat lower than among younger persons, but response rates were similar.[477,509,543–545] Other factors, such as smoking and obesity, have not been evaluated for the currently licensed formulations.

Up to 2% of "normal" adults may be low responders or nonresponders to primary and booster doses of inactivated hepatitis A vaccine. In one study, low response and nonresponse was associated with lack of a hepatitis A cellular receptor (havcr1) TIM1 on CD4+ T lymphocytes. No information was available to determine if these persons might also have a different susceptibility to HAV infection.[546]

Efficacy and Effectiveness of Vaccine

Hepatitis A vaccines are highly effective in preventing clinically apparent disease. In one study, 1037 healthy seronegative children 2 to 16 years old in a community with high hepatitis A rates and periodic outbreaks received a single dose of formalin-inactivated vaccine derived from strain CR326F (used to prepare VAQTA) or placebo. No cases of hepatitis A occurred in the vaccinated group beginning 17 days after vaccination. In the placebo group, 34 cases of hepatitis A were observed, yielding an estimated 100% vaccine efficacy, with a lower bound of the 95% confidence interval of 87%.[547] In a large field trial of approximately 40,000 Thai children 1 to 16 years old, the efficacy of inactivated vaccine (HM175 strain, used to prepare Havrix) was 94% (95% confidence interval, 79%–99%) after two doses (360 EL.U. per dose) administered 1 month apart.[380] The efficacy of the virosome-formulated hepatitis A vaccine derived from the RG-SB strain (used to prepare Epaxal) was assessed in a randomized, placebo-controlled, double-blind study among Nicaraguan children ages 1.5 to 6 years. Protective efficacy beginning 6 weeks after vaccination was 100% (95% confidence interval, 79.8%-100%).[548]

A number of studies and demonstration projects have evaluated the effectiveness of hepatitis A vaccines in controlling and preventing hepatitis A in communities. In areas with the highest hepatitis A rates, such as in Native American and Alaska Native communities,[549–551] vaccination of the majority of children, and in some cases adolescents and young adults, resulted in a rapid decline in disease incidence. With ongoing routine vaccination of children, these reductions in disease incidence have been sustained.[255,261,552–557] In larger, more heterogeneous communities with lower but consistently elevated hepatitis A rates, accumulating evidence from a number of countries indicates that sustained routine vaccination of children can reduce hepatitis A incidence markedly over time (see "Public Health Considerations" below).[18,217,218,223,255,261,263,489,490,558–560]

In 2012, the WHO Strategic Advisory Group of Experts (SAGE) on Immunization reviewed evidence of efficacy after

a single dose of inactivated hepatitis A vaccine in a randomized controlled trial in Nicaragua and from national use of a single-dose schedule of hepatitis A vaccine in Argentina.[561] The SAGE concluded that national programs may consider a single-dose schedule, although additional evidence of long-term immunogenicity are needed and for certain high-risk and immunocompromised individuals, a two-dose schedule is recommended.[16,562] Argentina includes a single-dose inactivated hepatitis A vaccine in their national immunization schedule at 12 months[561]; humoral immunity has been shown to persist for at least 5 years in Argentinean children.[563]

A 5-year follow up study in India showed that the single dose of live attenuated vaccine (Biovac-A) is well tolerated and provides long-term immunogenicity in healthy Indian children.[564] Another follow-up study demonstrated 10 year protection after administration of Biovac-A.[565]

Duration of Immunity

The duration of protection after vaccination is unknown; however, anti-HAV has been shown to persist in vaccine recipients for at least 17 years in adults administered inactivated vaccine as children with a three-dose schedule.[504,566–572] Vaccine recipients vaccinated as children with the previously recommended three-dose hepatitis A schedule were found to have similar protection for longer than 14 years after vaccination compared to those vaccinated with the two-dose schedule.[573] Another study demonstrates at least 20-year anti-HAV persistence among adults vaccinated with a two-dose schedule as adults. Detectable antibodies are estimated to persist for 40 years or longer based on mathematical modeling and anti-HAV kinetic studies.[574–576] Protection following natural infection is lifelong and may also be lifelong following vaccination.

Because the average incubation period for hepatitis A is 4 weeks and the anamnestic responses observed after the second vaccine dose are rapid and robust, it has been suggested that vaccine recipients who seroconverted will be protected even if their antibody levels have fallen below protective levels.[577] Cellular immunity might contribute to this protection. HAV-specific T cells are detectable after the primary vaccine series and undergo rapid expansion after a booster dose given 28 weeks after completion of a primary series.[546,578,579] A review of the single-dose use of inactivated hepatitis A vaccine found that protective anti-HAV antibody levels can persist for almost 11 years and increase or reappear after booster vaccination. These results suggest there is no indication for a booster dose of hepatitis A vaccine within 6 years after vaccination.[580] Another study evaluating single-dose vaccine suggested proliferation of classical memory B cells, although no substantial activation of HAV-specific memory B cells after the first dose.[505]

Safety (Adverse Events)

Common

Local injection site reactions (pain, tenderness, or erythema) that are mild and transient have been reported in as many as 21% of children and 56% of adults vaccinated. Systemic reactions, including fatigue, fever, diarrhea, and vomiting, occur in fewer than 5% of vaccinees. Headache has been associated with vaccination in up to 16% of adults and 2% to 9% of children.[380,430,435,440,443,457,581–584]

Rare

Between 1995 and 2005, more than 50 million doses of hepatitis A vaccine were sold in the United States, and more than 188 million doses were sold worldwide.[86] During that period, the Vaccine Adverse Events Reporting System (VAERS; maintained by the FDA and the Centers for Disease Control and Prevention [CDC]) received approximately 6000 reports of adverse events occurring after injection with hepatitis A vaccine. The most common events were minor and of brief duration, such as fever, injection-site reactions, rash, and headache. The 871 serious adverse events included Guillain-Barré syndrome, transaminitis, idiopathic thrombocytopenic purpura, and seizures among children.[86,583] Rare adverse events reported postmarketing include syncope, jaundice, erythema multiforme, anaphylaxis, brachial plexus neuropathy, transverse myelitis, encephalopathy, and others.[465,466] No serious adverse events among children or adults have been identified that could be definitively ascribed to hepatitis A vaccine.[582,583,585] For events for which incidence rates are available, such as Guillain-Barré syndrome, reported rates were not higher than reported background rates.[86]

In Immunosuppressed Persons

Most hepatitis A vaccines are inactivated and based on limited data, administration to immunosuppressed persons does not seem to be associated with a greater risk of adverse events compared with administration to persons with normal immune systems.[516,528,540,586] No data are available regarding the safety of live attenuated hepatitis A vaccines in immunosuppressed persons.

Spread to Contacts

The inactivated vaccines do not pose a risk of spread to contacts. They do not contain viable HAV. One study of a live attenuated vaccine did not find any seroconversion among unvaccinated classmates of vaccinated children.[587]

Prevaccination and Postvaccination Testing

Prevaccination serologic testing may be considered to reduce costs by not vaccinating persons with prior immunity, but it should take into account the cost of testing, vaccine cost, and the likelihood that the person will return for vaccination if susceptible.[588] Populations with a high prevalence of infection can include children, adolescents, and adults born in areas of high hepatitis A endemicity; older MSM[86,315]; and persons using illegal drugs.[305,308] Prevaccination serologic testing can be considered for older travelers and younger travelers who were born in a country that has high or intermediate hepatitis A endemicity. In the United States, national seroprevalence data show that among all age groups, seroprevalence of anti-HAV is less than 50%; consequently, prevaccination testing is not indicated. Vaccination of immune people is not harmful. Postvaccination testing is not indicated because of the high rate of vaccine response. Also, some commercially available testing methods cannot detect the low anti-HAV antibody concentrations generated by immunization.

Indications

Israel was the first nation-state to introduce universal childhood hepatitis A vaccination, followed by Bahrain in 2004, Argentina in 2005, and Panama and the United States in 2006 (see Table 24.5). By 2016, 17 countries have introduced universal childhood hepatitis A vaccination.[589] Recommendations for use of hepatitis A vaccine in the United States are discussed below (Table 24.6). The recommended immunization schedules can be found in Table 24.4. The U.S. recommendations were first issued by the ACIP of the U.S. Public Health Service,

TABLE 24.6 ACIP Recommendations for Routine Preexposure Use of Hepatitis A Vaccine

Group	Comments
All children at age 12–23 mo[a]	Integrate into routine childhood vaccination schedule; children not vaccinated by age 2 years can be vaccinated at subsequent visits
Children age 2–18 y	Maintain existing programs[b]; can be considered in areas without existing programs
International travelers	Except persons traveling to Canada, Western Europe, Japan, Australia, or New Zealand who are at no greater risk than in the United States (see Table 24.3)
Persons who anticipate close contact with an international adoptee	Includes unvaccinated household or regular babysitting contacts age ≥12 mo[a] of international adoptees from a country of high or intermediate hepatitis A endemicity during the first 60 days following arrival of the adoptee in the United States
Men who have sex with men	Includes adolescents
Illicit drug users	Includes adolescents
Persons with chronic liver disease	Increased risk of fulminant hepatitis A with HAV infection
Persons receiving clotting factor concentrates	
Persons who work with HAV in research settings	
Anyone who wants to obtain immunity	

ACIP, Advisory Committee on Immunization Practices; HAV, hepatitis A virus.
[a]Hepatitis A vaccine is not licensed for children younger than 12 months.
[b]States covered by 1999 ACIP recommendations (AL, AK, AZ, CA, CO, ID, MN, MO, NV, NM, OK, OR, SD, TX, UT, WA, WY, and selected areas in other states).[220]
Data from Advisory Committee on Immunization Practices (ACIP), Fiore AE, Wasley A, Bell BP. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep. *2006;55(RR-7):1–23. Advisory Committee on Immunization Practices (ACIP) Centers for Disease Control and Prevention (CDC). Update: Prevention of hepatitis A after exposure to hepatitis A virus and in international travelers: updated recommendations of the Advisory Committee on Immunization Practices (ACIP).* MMWR Morb Mortal Wkly Rep. *2007;56:1080–1084. Centers for Disease Control and Prevention (CDC); Advisory Committee on Immunization Practices. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoptees.* MMWR Morb Mortal Wkly Rep. *2009;58(36):1006–1007.*

the American Academy of Pediatrics, and other groups in 1996, and were updated in 1999, 2006, 2007, and 2009 (Table 24.6).[86,256,257,289,410,590] In addition, inactivated hepatitis A vaccine is indicated for any person who wants to obtain protection from hepatitis A.

Children

Vaccination should be completed according to licensed schedules and integrated into the routine vaccination schedule specific to each country (see http://www.who.int/immunization/policy/immunization_tables/en/ and http://www.cdc.gov/vaccines/schedules/). In the United States, it is recommended that all children receive hepatitis A vaccine at 1 year of age (i.e., 12–23 months of age) to avoid interference by passive maternal anti-HAV that may be present during the first year of life. In the United States, children who are not vaccinated by 2 years of age can be vaccinated at subsequent visits; vaccination can be considered for children 2 to 18 years old and for anyone who wants protection against hepatitis A infection.[86] Booster doses are not recommended at this time.[86]

Persons at Increased Risk of Hepatitis A or Severe Consequences as a Result of Infection

Susceptible persons who travel or work in countries with high or intermediate hepatitis A endemicity should be vaccinated or receive immunoglobulin before departure; vaccination is preferred (see Table 24.3; see "Preexposure Prophylaxis" below).[410] Persons who anticipate having close contact with an international adoptee from a country with high or intermediate hepatitis A endemicity,[289] persons working with HAV-infected nonhuman primates or with HAV in a research laboratory setting, persons using illicit injecting and noninjecting drugs, and adolescent and adult MSM should receive hepatitis A vaccination.[86]

The risk of hepatitis A from blood transfusion or from plasma derivatives is extremely low, but both have been reported.[326,328,329,384] Although manufacturers continue to develop methods to improve elimination of infectious HAV from their products, vaccination remains prudent. Persons with chronic liver disease are not at increased risk for hepatitis A; however, acute hepatitis A can have serious or fatal consequences.[94] All persons with such disease, regardless of the etiology of their liver disease, should be vaccinated against hepatitis A.

Outbreaks

There has been considerable interest in using hepatitis A vaccine to control ongoing communitywide epidemics. In areas that have implemented routine vaccination of children, outbreaks are rare. In general, vaccination efforts are better directed toward sustained routine vaccination of children to maintain high levels of immunity. Achieving high levels of coverage through mass vaccination sufficient to interrupt transmission and shorten the course of community outbreaks has not been demonstrated; however, susceptible persons in a community experiencing an outbreak can benefit from vaccination to prevent infection and disease.

Hepatitis A outbreaks in childcare centers are rare when routine vaccination programs of young children are implemented. When outbreaks are recognized, aggressive use of immunoglobulin is effective in limiting transmission,[28] and vaccination is effective in interrupting outbreaks.[591–594] Unvaccinated healthy persons 12 months to 40 years can be vaccinated for postexposure prophylaxis; immunoglobulin should be used for children younger than 12 months of age,

immunocompromised children, persons who have had chronic liver disease diagnosed, and those for whom vaccine is contraindicated. For persons older than age 40 years, immunoglobulin is preferred; vaccine can be used if immunoglobulin cannot be obtained.[410]

The frequency of outbreaks in hospitals, institutions, and schools is not high enough to warrant routine vaccination of persons in these settings, and there are limited data with respect to using vaccine to control outbreaks in these settings.[377] Although persons who work as food handlers are not at increased risk of hepatitis A because of their occupation, they may transmit HAV to others when they contract hepatitis A.[217,342] To reduce the frequency of evaluations of food handlers with hepatitis A and the need for postexposure prophylaxis of patrons, public health officials in some jurisdictions have instituted measures to promote hepatitis A vaccination of food handlers. However, because transmission from infected food handlers accounts for a very small proportion of cases nationwide, vaccination of food handlers is not likely to affect overall disease incidence and has not been found to be cost-effective.[594]

Contraindications and Precautions

The inactivated hepatitis A vaccines should not be used in persons with a history of a severe reaction to a prior dose of hepatitis A vaccine or allergy or hypersensitivity to the vaccine or any of its components. The safety of the inactivated hepatitis A vaccines in pregnancy has not been determined. Because the vaccine is produced from inactivated HAV, the theoretical risk to the fetus is low. A review published in 2014 of VAERS reports from January 1, 1996 to April 5, 2013 did not identify any concerning pattern of adverse events in pregnant women or their infants following maternal hepatitis A (Havrix, VAQTA) or hepatitis AB (Twinrix) immunizations during pregnancy.[595]

Preexposure and Postexposure Prophylaxis

After decades of effective use of immunoglobulin for postexposure prophylaxis, data now indicate the equivalency of hepatitis A vaccine in preventing symptomatic hepatitis A among healthy persons age 40 years or younger.[596–599] Hepatitis A vaccine is recommended in most circumstances for preexposure and postexposure prophylaxis because of the public health advantages of vaccination compared with immunoglobulin, which include induction of active immunity and longer protection, greater ease of administration, and greater acceptability and availability (see Table 24.3).[410]

Preexposure Prophylaxis

Hepatitis A vaccination is preferred to immunoglobulin for preexposure prophylaxis for persons who plan to travel or will live in countries that have high or intermediate hepatitis A endemicity. The first dose of hepatitis A vaccine should be administered as soon as travel is considered. For most healthy persons 1 to 40 years old, one dose of a single-antigen hepatitis A vaccine given any time before departure can provide adequate protection. Prevaccination serologic testing may be considered for persons who may have preexisting immunity, such as adults and older adolescent born in areas of high hepatitis A endemicity (see Fig. 24.5). Adults older than age 40 years, immunocompromised persons, and persons with chronic liver disease or other chronic medical conditions planning to depart to an endemic area in 2 weeks or less should receive the initial dose of the vaccine and simultaneously receive immunoglobulin (0.02 mL/kg) at a different anatomic injection site (see Table 24.3). Completion of the vaccine series according to the licensed schedule is needed for long-term protection. Immunoglobulin should be used for preexposure prophylaxis for infants younger than 1 year of age and for travelers who are allergic to vaccine or components.[289,410]

The use of hepatitis A vaccine with other vaccines that might be used for travelers has been studied among adults. There was no effect on the immunogenicity or reactogenicity of hepatitis A vaccine administered concurrently with diphtheria, polio (oral and inactivated), tetanus, hepatitis B, yellow fever, typhoid (oral and intramuscular), cholera, Japanese encephalitis, or rabies vaccines.[474,600–604] In studies among infants, simultaneous administration of hepatitis A vaccine did not affect the immunogenicity or reactogenicity of diphtheria-tetanus-acellular pertussis (DTaP); inactivated polio; MMR; hepatitis B; and *Haemophilus influenzae* type b vaccines.[474,605,606]

The usual dose of immunoglobulin for preexposure prophylaxis is a single intramuscular injection of 0.02 or 0.06 mL/kg (see Table 24.3). The lower dose is adequate to provide protection for up to 3 months, and the higher dose is effective for up to 5 months.[607] Even though readministration every 5 months is necessary for extended trips, hepatitis A vaccine, if not contraindicated, is preferred for such travelers. Intramuscular preparations of immunoglobulin should never be given intravenously, and the intravenous preparations of immunoglobulin are not intended for hepatitis A prevention and are formulated at a lower globulin concentration.

Several studies have evaluated administration of inactivated hepatitis A vaccine concomitantly with immunoglobulin to produce immediate and long-term immunity.[608–611] Although the proportion of persons who respond to vaccination was not reduced by coadministration of immunoglobulin, the antibody concentrations elicited were lower as compared to persons who received vaccine alone. For example, in one study, 1 month after completion of the vaccine series, the geometric mean antibody concentration among persons who received a dose of immunoglobulin (0.06 mL/kg) with the first dose of vaccine was 4872.3 mIU/mL (range: 3716.2–6388.2 mIU/mL) compared with 6497.8 mIU/mL (range: 5110.9–8 261.0 mIU/mL) among persons who received vaccine alone.[611] Because the concentrations induced by vaccination far exceed that needed for protection, these reductions are not considered clinically significant.

Immunoglobulin does not interfere with the immune responses to oral poliovirus or yellow fever vaccines or, in general, with inactivated vaccines. Immunoglobulin can interfere with the immune response to some live attenuated vaccines (e.g., MMR, varicella). Administration of MMR or varicella vaccine should be delayed for at least 3 months after receipt of immunoglobulin for hepatitis A prophylaxis. Immunoglobulin should not be given for at least 2 weeks after administration of MMR or at least 3 weeks after administration of varicella vaccine unless the benefits of the immunoglobulin are greater than the benefits of vaccination.[420]

Immunoglobulin should be used to protect children younger than 12 months of age and persons for whom the vaccine is contraindicated (see Table 24.3).[411] Hepatitis A vaccine is not licensed for children younger than 12 months of age; prevention of hepatitis A among infants will prevent the rare severe cases that occur and transmission to others after returning from abroad.[410]

Postexposure Prophylaxis

Unvaccinated persons who were recently exposed to HAV should receive postexposure prophylaxis as soon as possible. Such persons include close personal contacts of patients with

hepatitis A and other food handlers at establishments when a food handler is identified with hepatitis A.[217] Postexposure prophylaxis can be considered under certain conditions when outbreaks occur in childcare centers.[404,612] Hepatitis A vaccination is recommended for postexposure prophylaxis of healthy persons ages 12 months to 40 years and can be used for older persons if immunoglobulin is not available (see Table 24.3). When using hepatitis A vaccine for postexposure prophylaxis, single-antigen vaccine should be used; no data exist regarding the performance of a combination vaccine for prophylaxis after exposure to HAV. Immunoglobulin is recommended within 2 weeks of exposure for infants younger than 12 months, persons with immunocompromise or diagnosed chronic liver disease, and persons for whom vaccine is contraindicated, and is preferred for adults older than age 40 years. The efficacy of immunoglobulin administered 2 weeks or more after exposure has not been established. Once cases are identified that are associated with a food service establishment, it generally is too late to administer immunoglobulin, but patrons can be vaccinated. The usual dose of immunoglobulin for postexposure prophylaxis is a single intramuscular injection of 0.02 mL/kg (see Table 24.3).

The ACIP recommends hepatitis A vaccine for postexposure prophylaxis of healthy persons ages 12 months to 40 years based on the results of a study comparing the efficacy of hepatitis A vaccine and immunoglobulin in persons 2 to 40 years of age.[410,596] Hepatitis A can be more severe in older adults; vaccine has the advantage of providing long-term protection, but the vaccine response might be less robust in this population. A small number of studies report the immune responses to hepatitis A vaccine among adults older than age 40 years at 2 weeks after the first dose, suggesting that vaccination may be adequate as prophylaxis for older adults; the sample sizes, however, were small and/or the age distributions not discrete.[509,544,613] In one study the age-related seroconversion proportions 15 days after one 1440 EL.U Havrix dose were: 74% ($n = 125$) for ages 40 to 49 years; 54% ($n = 37$) for ages 50 to 59 years; 30% ($n = 10$) for ages 60 years and older.[613] Another study found 77% of adults ($n = 68$) ages 40 to 62 (mean: 48) years seroconverted.[544] A hepatitis A multistate outbreak response in 2013 highlighted the need for more evidence on vaccine for postexposure prophylaxis in older adults.[509,544,613–615]

VACCINE COVERAGE

Two-dose vaccine hepatitis A vaccine coverage is low in the United States compared to other recommended childhood vaccinations; in 2014, hepatitis A vaccine coverage for children 19 to 35 months of age was 85.1% and 57.5% for one or more and two or more doses, respectively.[616] In 2009, coverage for children ages 13 to 17 years was 42% and 29.5% for one- and two-dose coverage, respectively.[617] Among adults age 19 to 49 years, a group for which vaccine is only recommended for those at high-risk of HAV infection, in 2013 the total two-or-more-dose coverage was 12.3%, while coverage was 18.8% for travelers outside of the United States (to countries other than Europe, Japan, Australia, New Zealand, or Canada since 1995) and 14.5% for adults with chronic liver conditions.[618] When considering other adults for whom vaccination is indicated, a study evaluating adherence to vaccination guidelines reviewed the records of 705 patients with chronic liver disease found that hepatologists recommended vaccination for HAV in only 63% of eligible patients in an academic hospital-based hepatology clinic with variation among providers.[619] This study suggests the need for better education of providers and counseling of patients about the importance of vaccination. However, the benefit of hepatitis A vaccination targeting HCV-infected persons specifically has been questioned because of the low risk of mortality to HAV superinfection in low HAV incidence areas and the cost associated with vaccination.[620] A random sample of medical records of 1329 HIV-infected MSM in eight HIV clinics in six U.S. cities from 2004 to 2007 were reviewed—only 17% of HIV-infected MSM were vaccinated with hepatitis A vaccine and 47% screened.[621] This study also emphasized the importance of increasing screening and vaccination in this at-risk population.

Decreasing hepatitis A incidence in the United States results in reduced exposure to HAV in the population. National serologic data suggest an increasing proportion of susceptible adults in the United States.[17,618] This may place high-risk adult populations and older adults at greater risk for infection than in the past when most adult Americans reached adulthood with natural acquired immunity. The era of large community outbreaks in the United States appears to have passed, owing largely to universal childhood vaccination. However, there is a need to be vigilant to the impact of potentially greater susceptibility of older adults and persons at elevated risk for complications of HAV infection, although the overall public health impact is likely to be minor.[254,622]

PUBLIC HEALTH CONSIDERATIONS

Epidemiologic Effects of Vaccination

Effectiveness in Populations

Hepatitis A transmission patterns are influenced by many factors, including the age-specific prevalence of immunity, prevailing hygienic conditions, and the degree of pervasiveness of HAV in the environment. Many European countries without childhood vaccination programs have very low disease incidence. In the United States vaccination of successive cohorts of children has contributed to a sustained reduction in disease incidence nationwide. A number of studies and demonstration projects have evaluated the effectiveness of hepatitis A vaccine in controlling and preventing hepatitis A in populations with transitional and intermediate endemicity and high hepatitis A rates. Relatively small, homogeneous, usually rural communities include indigenous communities in North America and Australia and selected communities in Central and Eastern Europe and Israel.[257,552–557,623] In the absence of universal vaccination, hepatitis A epidemics typically occur every 5 to 10 years in these communities with transitional or intermediate endemicity.[555] Few cases occur among persons older than age 15 years; seroprevalence data indicate that 30% to 40% of children acquire infection by 5 years of age, and almost all persons have been infected by young adulthood.[549,550,624,625] Demonstration projects conducted soon after hepatitis A vaccines became available showed that routine vaccination of children living in these communities was feasible and that vaccination of the majority of children and, in some cases, adolescents and young adults, resulted in a rapid decline and sustained reduction in disease incidence.[556,557] For example, a 1992–1993 communitywide epidemic among Alaska Natives in one rural area was ended within 4 to 8 weeks of vaccinating approximately 80% of children and young adults.[556] A communitywide demonstration project in an endemic region of California vaccinated children aged 2 to 17 years of age during the period 1995 to 2000, resulting in major decreases in hepatitis A incidence among all age groups.[558] Universal vaccination of children can have the greatest impact on disease in countries and populations with intermediate or transitional hepatitis A endemicity. In the United States, the initial recommendations for vaccination were published in 1996 and called for routine

vaccination of children living in communities with the highest hepatitis A rates.[256] Following implementation of the recommendations, large decreases in incidence among these populations, including Native Americans and Alaska Natives, were observed.[554] The implementation of these recommendations, however, had little impact in the overall national incidence of disease. In 1999, the recommendations were expanded to include vaccination of children living in states, counties and communities with consistently elevated rates of hepatitis A.[257] In 2006, hepatitis A vaccine licensure was expanded to include vaccinating children beginning at age 12 months (previously vaccination could not begin earlier than age 2 years) thus facilitating program implementation by being more in line with the routine childhood vaccination schedule. Subsequently, universal vaccination of children was recommended in 2006, to include all children ages 12 to 23 months nationwide.[86] In the United States, this strategy has resulted in historic lows of reported hepatitis A incidence (see Fig. 24.6). Rates have declined most dramatically among children 2 to 18 years of age and are now similar across all regions of the country.[236] Mortality from hepatitis A has also fallen in parallel with declines in incidence.[254] Similar observations have been made in a growing number of countries and regions with diverse and heterogeneous epidemiology where the implementation of routine vaccination of children in one or more age cohorts in all or part of the country was followed by dramatic overall declines in hepatitis A incidence.[363,559,560,626–635] Review of coverage and surveillance data indicate that incidence declines have occurred with relatively modest levels of vaccination coverage achieved and incidence declines have been observed among unvaccinated age groups, suggesting a herd immunity effect.[263,559,636–638] One study that modeled the relationship between incidence and vaccination coverage in the United States estimated that herd immunity accounted for one-third of the cases prevented.[636]

Economic Considerations

Studies conducted in the early 2000s found that hepatitis A vaccination of young children or adolescents in high endemic areas of the United States could be cost saving from the societal perspective and have comparable effectiveness to other recommended vaccinations.[639,640] Another study estimated that cost-effectiveness of routine vaccination at age 1 year in the United States is greatly enhanced when the effect of herd immunity was included in the model, although the benefits declined over time.[268,641] An analysis from Argentina found favorable economics of routine hepatitis A vaccination of children in that country using a number of outcomes; this study assumed that the country was transitioning from high to intermediate endemicity, a situation where greater morbidity is expected.[642] However, results of economic analyses from China and Canada of routine vaccination of children were mixed.[643,644] The underlying assumptions, particularly baseline endemicity greatly influence estimates of cost-effectiveness.

Disease Control Strategies

The strategy of widespread routine vaccination of children at age 12 months, recommended by the ACIP in 2006,[86] has contributed toward a substantial reduction in the overall incidence of hepatitis A in the United States by preventing infection among children and younger persons that previously accounted for at least one-third of cases and served as a major source of infection for others. Sustaining routine vaccination of children will make elimination of indigenous HAV transmission in the United States an attainable goal. In the interim, a susceptible cohort of American adults remain.

As of 2014, 17 countries, including the United States, had recommendations for routine vaccination of children with hepatitis A vaccine (see Table 24.5).[589] Other developed countries with very low incidence, such as those in Western Europe, might potentially benefit from routine vaccination of children in addition to vaccination of persons in certain high-risk settings. Because most older children and adults remain susceptible to HAV in low-endemicity areas, there is a risk for disease outbreaks after introduction of the virus from an external source, such as contaminated food or returning international travelers as has been described in some Northern European countries.[286,645–647] Vaccination programs that focus on children in immigrant communities has been proposed and would likely reduce the risk to immigrant children and their contacts, although one analysis did not find such a strategy to be cost saving.[646] High rates of hepatitis A among indigenous children in north Queensland Australia led to a recommendation for routine vaccination of children in indigenous communities, with subsequent large decreases in incidence among indigenous and nonindigenous members of the community.[623] Similar decreases in hepatitis A have been documented throughout the world in countries implementing vaccination strategies that target select children.[363,629,632–635] Implementing and sustaining such vaccination programs is important to prevent the return of community outbreaks. The childhood vaccination program in the United States has contributed to the elimination of large communitywide outbreaks, although small outbreaks can occur among unvaccinated communities.

At present, routine hepatitis A vaccination is generally not indicated in developing countries, particularly those with highest endemicity where infection in early childhood is nearly universal and disease is believed to be uncommon (see Fig. 24.5). However, recent seroprevalence and morbidity data suggest that some countries or parts of countries are undergoing a transition to intermediate endemicity as economic development improves.[648] Surveillance data from India suggests an enormous burden of disease from hepatitis A, including numerous outbreaks annually[394]; most developing countries do not have surveillance and thus the impact of the disease remains unknown or unreported. Countries should be alert to the possibility that this fairly rapid transition to an epidemiologic pattern more typical of countries with moderate endemicity, with a substantial proportion of the population remaining susceptible into later childhood or adulthood, is likely to result in increases in hepatitis A–related morbidity and mortality. At that point, implementation of vaccination programs might become more feasible and cost-effective, particularly with a one-dose schedule.[16] Surveillance systems that can monitor trends in viral hepatitis, such as in India and Pakistan, are needed to inform vaccination policy.[394,649] In addition, analyses examining the relative cost-effectiveness of hepatitis A vaccination compared with other major public health priorities in developing countries are needed to support development of appropriate vaccination policy.[650–653] It is likely the global disease burden associated with hepatitis A will increase in the coming years because of continuing improvements in standards of living and sanitary and hygienic conditions.[16] Without use of hepatitis A vaccine, a larger proportion of the population will remain susceptible to HAV infection into adolescence and adulthood, and when infected have severe disease, contributing to an increase in the global burden; an estimated 15,000 to 30,000 persons die every year from hepatitis A.[14–16] If vaccine were available at a low cost and vaccination was shown to be cost-effective, some countries in which a considerable susceptible adolescent and adult population has developed might find it useful to include hepatitis A vaccination among their routine childhood immunizations.[654]

ERADICATION OR ELIMINATION

In the United States, vaccination of successive cohorts of children should eventually result in a sustained reduction in disease incidence nationwide, providing the opportunity to eliminate indigenous HAV transmission. Additional information on vaccine safety and duration of immunity will continue to be important. HAV has been considered as a target for eradication, but international bodies have not made this recommendation, primarily because of considerations of cost and feasibility.[655] At present, the disease can best be controlled by improving living conditions in the developing world and evidence based application of vaccination.

FUTURE VACCINES

Full-length infectious complementary RNA (cRNA) transcribed from the cDNA has been produced, which, on transfection into permissive cells, results in complete HAV replication.[427] Many of the mutations responsible for cell culture adaptation and attenuation in primates have been identified, although a virus with the ideal characteristics of in vitro growth combined with attenuation and immunogenicity in humans has not been produced.[124,156,428] There has always been an interest in the development of a live attenuated oral vaccine for hepatitis A analogous to the Sabin polio vaccine. Oral polio vaccine (OPV) was developed by the selection of strains of poliovirus that continued to replicate well in the gut but lost their ability to replicate in the central nervous system. Although gut replication of HAV seems to exist at a low level, it does not seem sufficient to induce a vigorous immune response. The selection of a virus that grows well enough in the target organ, the liver, to produce a good immune response but not clinical disease has been difficult to achieve.[423–425] In addition, the inactivated hepatitis A vaccines have been so effective that the need for a live vaccine is not a primary focus. In China, live attenuated hepatitis A vaccines have been licensed since 2008[16]; however, only inactivated hepatitis A vaccine formulations are currently WHO prequalified.[464] Development of recombinant hepatitis A vaccines has also been explored,[657] but it is not a straightforward problem. The important neutralizing epitopes of HAV seem to be conformational, and immunization with synthetic peptides or expressed single-capsid proteins has not induced an effective neutralizing antibody response.[658] Stapleton and colleagues[659] and others[660,661] took the approach of expressing the entire open reading frame of the HAV genome in recombinant vaccinia virus or baculovirus expression systems. Complete or partial capsid assembly seems to occur in cells infected with this recombinant vaccinia, and antibodies raised to these purified HAV synthetic capsids are neutralizing in vitro and protective in animals. Although the immunizing effect of such a vaccine might not be greater than that of inactivated vaccines, the potential reduced production costs could make it an attractive alternative.

At present, there is a licensed combination hepatitis A and hepatitis B vaccine. Additional hepatitis A combination vaccines in development include antigens for protection against Japanese encephalitis virus and hepatitis E, but to date none is currently available.[662,663] With the present recommendation for universal childhood immunization at 1 year of age in the United States, manufacturers may consider other potential combinations to fit in the childhood immunization schedule.

There is interest in a vaccine that would provide long-lasting immunity after a single dose, and a single-dose schedule is used in some countries, although the long-term efficacy is unknown but is under assessment.[16,561] Improved adjuvants or possibly larger doses of the antigen may result in long lasting protection with a single dose. Both U.S.-licensed vaccines use alum as the adjuvant. Killed HAV packaged in influenza virosomes is licensed in Europe.[441] Although this vaccine has not been proven superior to alum-adjuvanted vaccines, it seems to induce an adequate protective response. Other adjuvants might improve the responses to the point that a single dose would be sufficient for long-lasting protection. Such a vaccine should reduce the cost of immunization and solve delivery problems that may exist in some areas where hepatitis A vaccination would be desirable.

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25 Hepatitis B Vaccines

Pierre Van Damme, John W. Ward, Daniel Shouval, and Alessandro Zanetti

Hepatitis has been recognized as a clinical entity since antiquity, but the diversity of viruses causing the infectious form of the disease has only recently been recognized. The first evidence that a form of hepatitis was transmitted by direct inoculation of blood or blood products was discovered when an outbreak of hepatitis occurred after a smallpox immunization campaign in Germany in 1883.[1] Jaundice developed in 15% of workers after they had been vaccinated with material prepared from human lymph; several hundred unvaccinated workers did not become ill. Further evidence of a parenterally transmitted form of hepatitis was documented early in the 20th century, when numerous outbreaks of jaundice were associated with reuse of syringes and needles without sterilization.[2–8] Blood products were confirmed as a source of hepatitis transmission through investigations of outbreaks of jaundice after yellow fever vaccination,[9,10] administration of measles and mumps convalescent plasma,[11–13] and transfusion of whole blood.[14]

The viral etiology of hepatitis was first established during the 1940s through experimental transmission to human volunteers.[15–20] The studies provided evidence of two distinct diseases: *infectious* hepatitis, transmitted by the fecal–oral route and primarily a disease of younger children, and *parenteral* or *serum* hepatitis, transmitted by percutaneous exposure to blood products and seen more often in adults. The respective terms *hepatitis A* and *hepatitis B*, first introduced in 1947,[21] were later universally adopted.[22] In the 1960s and 1970s a series of transmission and cross-challenge studies in humans confirmed the existence of these two distinct forms of hepatitis[23–25] and also that each virus generated homologous immunity after infection.

The largest outbreak of what is now recognized as hepatitis B was recorded in 1942, when 28,585 American soldiers inoculated with yellow fever vaccine developed jaundice and 62 died.[9] This outbreak was traced to a specific lot of vaccine that contained human serum; a follow-up study in the 1980s confirmed that hepatitis B virus was the cause.[26]

Of the five viruses known to cause hepatitis in humans, hepatitis B virus (HBV) is responsible for most of the global chronic hepatitis burden caused by viral hepatitis. Enormous progress has been made in understanding the etiology, natural history, epidemiology, and public health control of hepatitis B. The discovery of the etiologic agent of hepatitis B remains a remarkable scientific achievement. In 1965, Blumberg and colleagues described an isoprecipitin, which they termed *Australia antigen*, present in the serum of Australian aborigines.[27] Its distribution varied widely in healthy populations across the world, with a low prevalence (<1%) in North America and Europe, and a high prevalence (6% to 25%) in the tropics and Southeast Asia and in patients who had received multiple transfusions and blood products. The Australia antigen was shown by Prince to be related to HBV infection[28] and to be the surface protein of the virus.

HBV was visualized by electron microscopy as 42-nm viral particles that reacted with antisera to the hepatitis B surface antigen (HBsAg).[29] With the development of immunoassays for HBV antigens and antibodies,[27,29,30] the natural history of HBV infection was defined, and preventive strategies were designed, including the screening of blood for HBsAg to prevent transfusion-associated hepatitis B. In 1970, studies on the natural history of HBV infection revealed that boiling serum containing the virus destroyed its infectivity. The heat-inactivated material, however, retained its immunogenicity and partially protected subjects who were challenged with HBV.[31] The realization that HBsAg could serve as an immunogen to generate antibody to HBsAg (anti-HBs), and that this antibody protected against HBV infection, led to the development of prototype hepatitis B vaccines.[32,33] From the early 1980s, several plasma-derived hepatitis B vaccines were manufactured and licensed before recombinant DNA technology allowed the expression of large quantities of HBsAg in yeast and mammalian cells, enabling the production and licensing of recombinant hepatitis B vaccines.[34,35]

Epidemiologic studies in the 1970s and 1980s linked chronic HBV infection with chronic liver disease, including cirrhosis and hepatocellular carcinoma (HCC).[36,37] HBV-associated chronic liver disease was identified as a leading cause of death in adults worldwide, particularly in countries with a high prevalence of chronic HBV infection.[38–40] The recognition of hepatitis B as a serious global cause of morbidity and mortality led to major prevention and control efforts. Affordable vaccines are now available and effective prevention strategies are being deployed; 184 countries have introduced hepatitis B vaccine in their national infant immunization programs by the end of 2014, a major increase compared with 31 countries in 1992, the year that the World Health Assembly passed a resolution to recommend global vaccination against hepatitis B[41]; in addition, global initiatives exist to allow the world's poorest countries to obtain the vaccine. With continued expansion of routine newborn and infant hepatitis B vaccination, chronic HBV infection in children should be markedly reduced in the near future, resulting in declines in deaths from liver cancer and cirrhosis related to HBV.

BACKGROUND

Clinical Description

Infection with HBV causes a broad spectrum of liver disease, including subclinical infection, acute, clinically overt self-limited hepatitis, and fulminant hepatitis.[42,43] Persons infected with HBV can also develop persistent infection, which can lead to chronic liver disease and death from cirrhosis or HCC. The age of acquisition of HBV infection is the main determining factor in the clinical expression of acute disease and the development of chronic infection. Genetic characteristics of HBV might also contribute to outcome of infection.[44] Fewer than 10% of children younger than 5 years of age who become infected have initial clinical signs or symptoms of disease (i.e., acute hepatitis B), compared with 30% to 50% of older children and adults.[45] The risk for developing chronic HBV infection varies inversely with age: approximately 90% of infants infected during the first year of life develop chronic infection, compared with 30% of children infected between ages 1 and 4 years and less than 5% of persons infected as adults (Fig. 25.1).[46] Persons with chronic diseases such as renal failure, HIV infection, and diabetes are at increased risk for developing chronic HBV infection, presumably as a result of enhanced

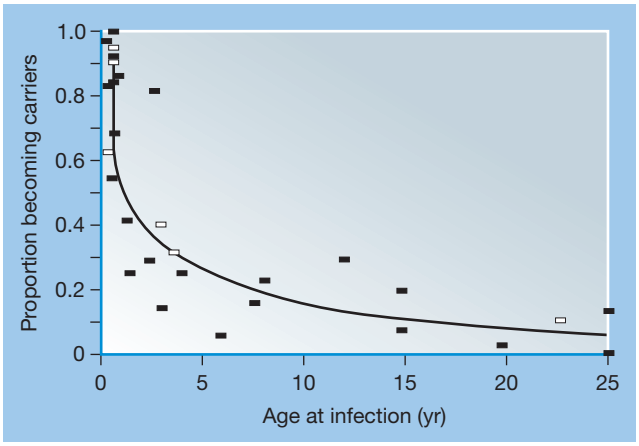


Figure 25.1. Studies evaluating the risk of chronic hepatitis B virus infection by age of infection. Filled squares represent data from developing countries; open squares represent data from developed countries. *(From Edmunds WJ, Medley GF, Nokes DJ, et al. The influence of age on the development of the hepatitis B carrier state.* Proc R Soc Lond B Biol Sci. *1993;253:197–201.)*

viral replication and ineffective immune clearance. It is estimated that 10% of the 40 million people infected with HIV worldwide are coinfected with HBV. Although HBV infection appears to have a minimal effect on the progression of HIV, the presence of HIV markedly increases the risk of developing HBV-associated liver cirrhosis and HCC.[47–50]

Acute Hepatitis B

The clinical manifestations of acute hepatitis B are indistinguishable from other causes of viral hepatitis; a definitive diagnosis requires serologic testing. The average incubation period is 90 days (range: 60 to 150 days) from exposure to onset of jaundice, and 60 days (range: 40 to 90 days) from exposure to onset of abnormal alanine aminotransferase (ALT) levels.[51,52] The prodromal phase of disease is variable and is characterized by insidious onset of constitutional symptoms that may include malaise, anorexia, nausea, occasional vomiting, low-grade fever, myalgias, and easy fatigability. In 5% to 10% of patients, a serum sickness–like syndrome develops during the prodromal phase that is characterized by arthralgias or arthritis, rash, and angioedema. Other extrahepatic manifestations that have in rare instances been reported in association with acute HBV infection include polyarteritis nodosa, membranous glomerulonephritis,[53] Gianotti-Crosti syndrome,[54,55] and aplastic anemia.[56] In patients with icteric hepatitis, jaundice usually develops within 1 to 2 weeks after onset of illness; dark urine and clay-colored stools might appear 1 to 5 days before onset of clinical jaundice. During the icteric phase, constitutional prodromal symptoms usually diminish, and right-upper-quadrant pain might develop as the liver becomes enlarged and tender. In 10% to 30% of patients with acute hepatitis B, myalgias and arthralgias have been described without jaundice or other clinical signs of hepatitis; in one-third of these patients, a maculopapular rash appears with joint symptoms.[57] Clinical signs and symptoms of acute hepatitis B usually resolve within 1 to 3 months.

Approximately 40% of persons in the United States with reported cases of acute hepatitis B are hospitalized.[58] Higher rates of hospitalization in other countries might be attributed to established patterns of medical care rather than disease severity. Fulminant liver failure occurs in approximately 0.5% to 1.0% of adults with reported acute hepatitis B cases, but rarely in infected infants and children.[59,60] Higher rates of fulminant hepatitis can occur in association with HBV–hepatitis delta virus coinfection.[61] Patients with fulminant hepatitis usually present with features of hepatic encephalopathy, including disturbances in sleep patterns, asterixis, mental confusion, disorientation, somnolence, and coma. The case-fatality rate among patients who develop fulminant hepatitis is approximately 20% to 33%, unless liver transplantation can be performed.[62,63] With liver transplantation, survival rates exceeding 80% have been achieved.[64]

The impact of treatment with the new antiviral agents on the case-fatality rate of severe or fulminant hepatitis B is unknown. Limited evidence suggests that antiviral agents such as lamivudine or other nucleoside or nucleotide analogs such as entecavir or tenofovir may alter the course and improve survival.[65–68]

Chronic Hepatitis B Virus Infection

Most of the disease burden associated with HBV infection occurs among persons with chronic infection.[69] Persons who have persistence of HBsAg in serum for at least 6 months are classified as having chronic infection. HBV replication persists throughout the course of chronic HBV infection, and the natural history of chronic HBV infection is determined by the interaction between virus replication and host immune response. Additional factors that can contribute to progression include gender, alcohol consumption, and coinfection with other hepatotropic viruses.

Chronic HBV infection is a dynamic process that may span several decades. It is characterized by up to four phases (Fig. 25.2),[70] including a period of active viral replication without active liver disease (immune tolerance phase),[71] a period of active viral replication and active liver disease (immune clearance phase), and a phase with low or absent viral replication and remission of active liver disease (inactive chronic HBV infection).[72] In addition, some patients with inactive chronic HBV infection experience reactivation of HBV replication. All four phases of chronic infection can occur in persons who acquire infection perinatally, whereas the latter three phases occur in persons who acquire infection as older children and adults.

The immune tolerance phase of perinatally acquired infections is characterized by high levels of virus replication, which correlates with hepatitis B e antigen (HBeAg) positivity and high levels of HBV DNA in serum.[73,74] However, these patients typically have no evidence of active liver disease, with normal ALT levels, no signs or symptoms of hepatitis, and minimal necroinflammatory activity on liver biopsy.[71,75] The absence of liver disease despite high levels of viral replication is believed to be attributable to immune tolerance. The initial phase of infection usually lasts 10 to 30 years. During this time, rates of spontaneous clearance of HBeAg from serum are less than 1% per year.[71,75,76] This low rate of HBeAg clearance accounts for the high prevalence of HBeAg among women of childbearing age in areas with high rates of perinatal HBV transmission.

The replicative phase in active liver disease is characterized by high levels of virus replication (see Fig. 25.2). These patients are usually HBeAg-positive, with high or fluctuating levels of HBV DNA in their serum. Evidence of active liver disease includes persistently or intermittently elevated ALT levels and necroinflammatory activity on liver biopsy. Development of fibrosis of the liver can lead to cirrhosis, which usually leads to an irreversible form of liver injury. Transition from this phase of chronic infection to the next phase occurs with reduction in viral load and sometimes clearance of HBV DNA with HBeAg seroconversion. The rate of spontaneous clearance of

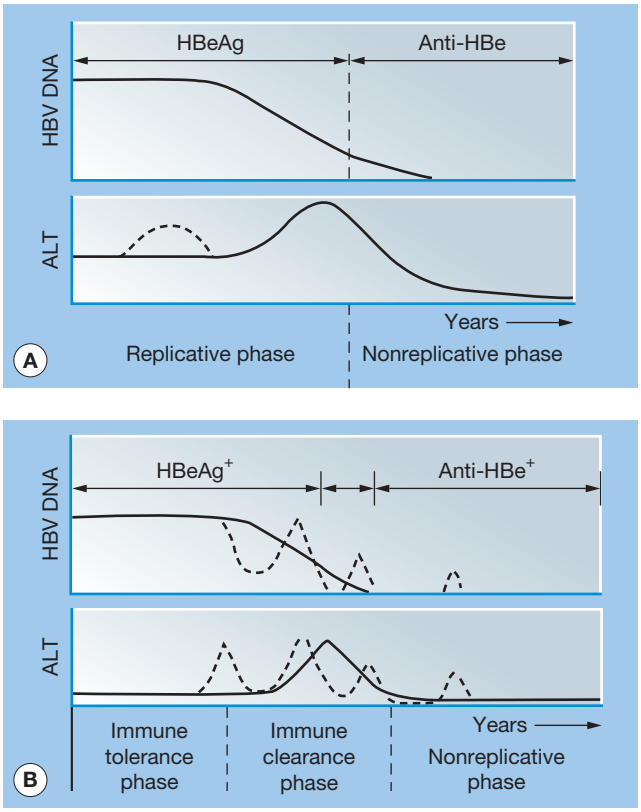


Figure 25.2. Natural history of chronic hepatitis B virus infection. A, Adult-acquired infection. **B,** Perinatally acquired infection. *Solid lines* represent typical patterns of alanine aminotransferase (ALT) levels and hepatitis B virus DNA (HBV DNA) concentrations in patients with chronic HBV infection over time. *Dashed lines* represent fluctuating ALT levels or HBV DNA titers present in some patients. Anti-HBe, antibody to HBeAg; HBeAg, hepatitis B e antigen. *(From Chan HL, Ghany MG, Lok ASF. Hepatitis B. In: Schiff ER, Sorrell MF, Maddrey WC, eds.* Schiff's Diseases of the Liver, vol. 1, *ed 8. Philadelphia, PA: Lippincott Williams & Wilkins; 1999:768.)*

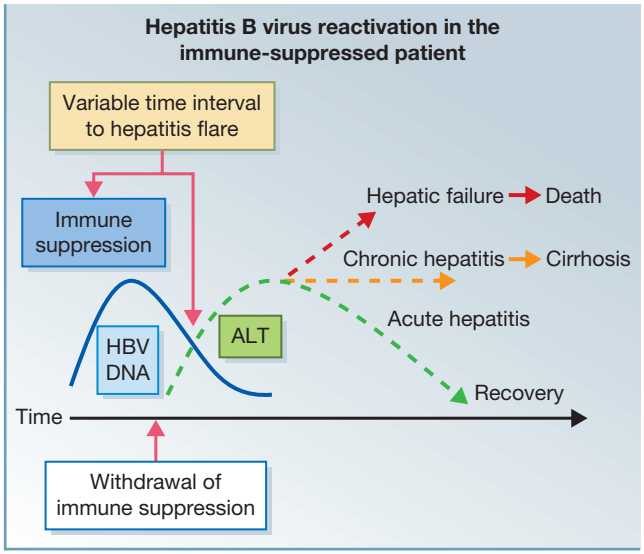


Figure 25.3. Dynamics of viral load and alanine aminotransferase (ALT) during the course of hepatitis B virus (HBV) reactivation after chemotherapy-induced suppression. *(Modified from Lalazar G, Rund D, Shouval D. Screening, prevention and treatment of viral hepatitis B reactivation in patients with haematological malignancies.* Br J Haematol. *2007;136:699–712.)*

HBeAg is approximately 10% to 20% per year, both in patients with perinatally acquired infections and in those who acquire infection at an older age.[76–79] Most patients are asymptomatic during this phase, but conversion to HBeAg negativity can be accompanied by symptomatic exacerbations, with increased ALT levels.[78–80] Hepatic decompensation might occur in approximately 2% of patients during these exacerbations, which can, in rare instances, lead to death from hepatic failure.[81] In some patients with suboptimal immune response and abortive immune clearance, repeated exacerbations can occur, with intermittent clearance of HBV DNA from serum and transient loss of HBeAg.[82,83] Repeated episodes of necroinflammation might increase the risk for developing severe chronic liver disease. Ultimately, the outcome of chronic infection depends primarily on the severity of liver disease at the time that HBeAg seroconversion occurs and HBV replication is diminished.

The phase of inactive chronic HBV infection is characterized by low or absent levels of virus replication and remission of active liver disease (see Fig. 25.2). Patients are typically HBeAg-negative, have antibody to HBeAg (anti-HBe), and have low or undetectable levels of HBV DNA in serum. Most patients who clear HBeAg have normal ALT levels and resolution of necroinflammation on liver biopsy, indicating resolution of active liver disease.[72,79,80,84,85] However, a small percentage of these patients continue to have moderate levels of HBV replication, elevated ALT levels, and chronic inflammation on liver biopsy despite HBeAg seroconversion.[84,86] These patients may also have residual liver disease and can be misdiagnosed as having chronic liver disease of unknown etiology if they are HBsAg-negative.[87,88] Patients who clear HBeAg typically remain HBsAg positive for long periods, even in the absence of detectable HBV DNA. The annual rate of HBsAg clearance among patients with chronic HBV infection is approximately 0.5% to 2%.[77,85,89,90] However, HBV DNA can be detected in up to 50% of these patients by polymerase chain reaction even after clearance of HBsAg.[91]

The phase of reactivation of HBV replication or HBeAg-negative chronic hepatitis B can occur in persons with inactive chronic HBV infection either spontaneously or as a result of immunosuppression (Fig. 25.3).[79,92,93] In addition, some patients progress directly from HBeAg-positive chronic hepatitis to HBeAg-negative chronic hepatitis.[94] In one study of Taiwanese patients followed for a median of 8.6 years after spontaneous HBeAg seroconversion, 67% had sustained remission, 4% had HBeAg reversion, and 24% had HBeAg-negative chronic hepatitis B.[94]

Chronic HBV infection is an important cause of morbidity and mortality worldwide.[95] For example, in cohort studies conducted in Taiwan, approximately 25% of persons who became chronically infected during childhood and 15% of those who became chronically infected at older ages died of HCC or cirrhosis.[36,96] In one population-based study, the incidence of decompensated cirrhosis among persons with chronic HBV infection was 0.5 per 1000 person-years.[85] In studies of persons with chronic infection referred to clinical centers, the incidence of cirrhosis is as high as 2% to 3% per year.[97,98] Risk factors for the development of cirrhosis in persons with chronic infection include older age, HBeAg positivity, heavy use of alcohol (>40 g/day), and increased ALT levels.[97–99]

Persons with chronic HBV infection are also at high risk for developing HCC.[38,40] The annual risk of HCC is estimated to be less than 1% for chronically infected persons without cirrhosis and 2% to 3% for persons with cirrhosis.[100] In a

Taiwanese study, rates of HCC were approximately 100 times higher among men who were HBsAg-positive (495 per 100,000 per year) than among those who were HBsAg-negative (5 per 100,000 per year).[37,101] Most HCC cases occur in men, with an average male-to-female ratio of cases of 3.7 : 1. Other risk factors for HCC in persons with chronic HBV infection include high levels of virus replication, older age, family history of HCC, presence of cirrhosis, HBV genotype (C > B) (see "Virology" below), coinfection with hepatitis C virus (HCV), and increased alcohol intake.[37,85,100,102–105] Although HCC is more common in persons with cirrhosis, approximately 30% to 50% of HBV-associated HCC cases occur in persons who do not have cirrhosis.[85] Clearance of HBsAg might decrease the risk for HCC[88]; however, HCC can occur in persons who develop chronic HBV infection and then clear HBsAg.[85,89,106]

Virology

Hepatitis B virus, previously called the Dane particle, is a 42-nm DNA virus that belongs to the Hepadnaviridae family comprising two genera: *Avihepadnavirus* infecting birds and *Orthohepadnavirus* infecting mammals. The avian member's include the duck HBV, heron HBV, Ross goose HBV, stork HBV, and parrot HBV. The detection of endogenous avian hepadnavirus DNA integrated into the genomes of zebra finches has revealed a deep evolutionary origin of hepadnaviruses that was not previously recognized, dating back over 40 million years.[107,108] *Orthohepadnavirus* has been identified in several mammals, including the woodchuck *(Marmota monax)*, the ground squirrel *(Spermophilus beecheyi)*, the pig *(Sus scrofa)*, and Old World primate genera including gorillas, chimpanzees, and gibbons. Orthohepadnaviruses, which show approximately 70% sequence homology to HBV, are not able to infect humans, but HBV is infectious for the great apes.

The outer lipoprotein envelope of HBV contains HBsAg, which is produced in excess amounts and also circulates in the blood as 22-nm spherical and tubular particles (Fig. 25.4). The inner nucleocapsid is a 28-nm icosahedral structure consisting of 180 copies of the HBV core protein, or hepatitis B core antigen (HBcAg), and it surrounds a single molecule of partially double-stranded DNA and a DNA-dependent DNA polymerase (Fig. 25.5).[109]

The HBV genome is a small, circular, partially double-stranded DNA molecule (complete minus strand and partial plus strand) that is approximately 3200 nucleotides in length (Fig. 25.6).[110] The virus efficiently uses its genetic information to encode four groups of proteins and their regulatory elements by shifting the reading frames over the same genetic material.[111] The pre-S/S gene has three separate open reading frames (ORFs) that encode three forms of HBsAg: the large (pre-S1), middle (pre-S2), and small (S) structural proteins of the virus envelope. The C gene has two ORFs (C and pre-C). The C ORF encodes the structural protein of the viral nucleocapsid (HBcAg), and the pre-C/C ORF encodes the nonstructural e protein, which is processed to produce soluble HBeAg. The X gene encodes the X protein (HBx), a small transcriptional transactivator that influences the transcription of HBV genes by regulating the activity of transcriptional promoters.[112] The P gene encodes a large polymerase protein that functions as both a reverse transcriptase for synthesis of the negative DNA strand from genomic RNA and an endogenous DNA polymerase for synthesis of the positive DNA strand using the negative strand as a template. Interestingly, P overlaps with all other coding regions, and mutations in the polymerase gene may also affect the overlapping S gene, with implications on viral infectivity, pathogenesis of liver disease, and resistance to treatments.

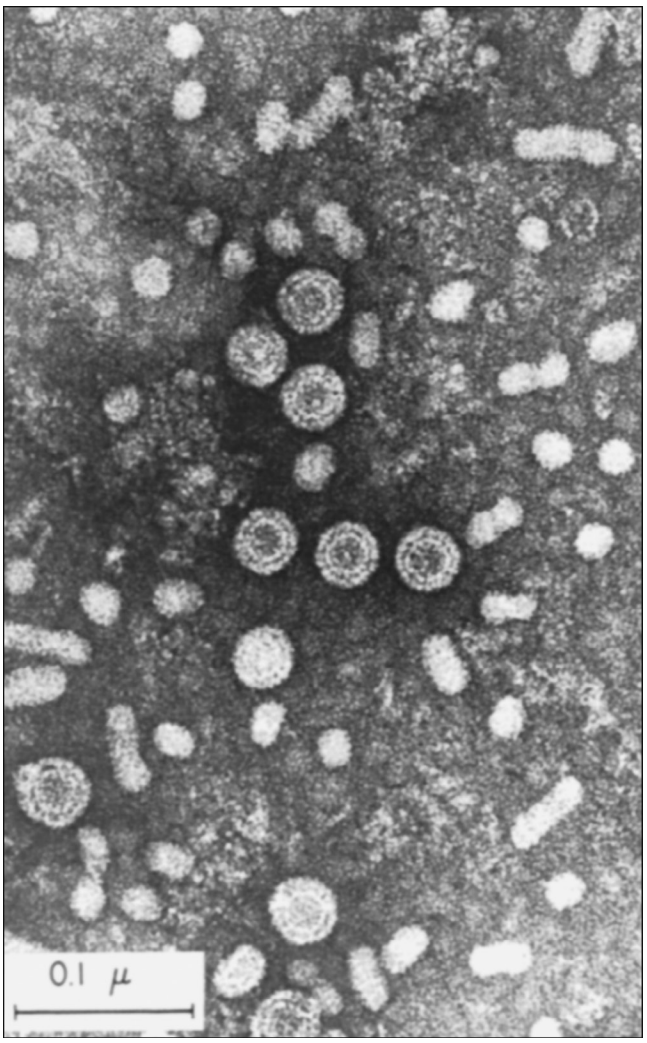


Figure 25.4. Electron micrograph of hepatitis B virus. Note Dane particles (43 nm) as well as spherical and tubular hepatitis B surface antigen particles (22 nm in diameter).

The HBV envelope contains a mixture of S, pre-S1, and pre-S2 HBsAg proteins in both glycosylated and nonglycosylated forms. These three proteins share the same carboxyterminus part but have different aminoterminal extensions. In particular, the S protein, which consists of 226 amino acids, is expressed in the largest quantities and is coded for by the S genomic region alone. The pre-S2, containing an extra N-terminal extension of 55 amino acids, is coded for by pre-S2 and S regions, and the pre-S1 protein, containing a further N-terminal of 108 or 119 amino acids—depending on the genotype—is coded for by the entire pre-S/S gene. Pre-S1 and pre-S2 proteins are also expressed individually in small quantities; these proteins contain a number of T- and B-cell epitopes and the pre-S1 protein appears to play an important role in the attachment of HBV to hepatocytes,[113–117] essentially through the sodium taurocholate cotransporting polypeptide (NTCP) receptor, recently identified as an entry receptor of HBV and HDV (hepatitis Delta virus).[118–120] Notably, the S protein contains the major site for binding of neutralizing antibody, designated the *a* determinant. Two other major determinants of the S protein also have been described; one has either *d* or *y* specificity, and the other has *w* or *r* specificity, determined by mutually exclusive amino acid substitutions in

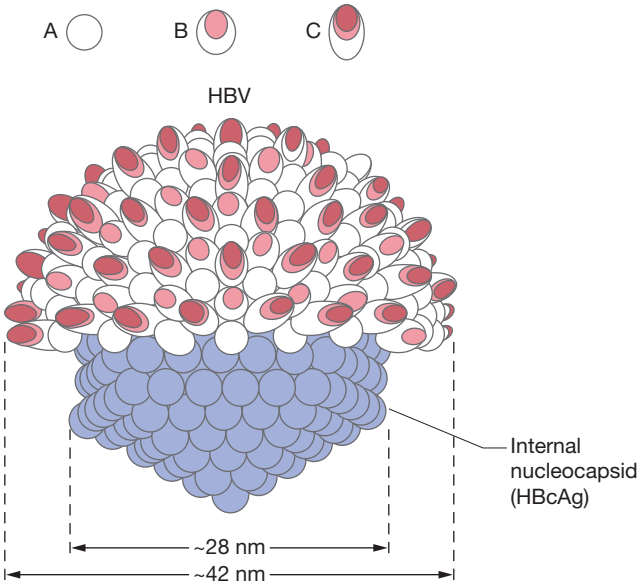


Figure 25.5. Schematic diagram of hepadnavirus particles. Individual subunits containing S protein only **(A),** S protein plus pre-S1 **(B),** and S protein plus pre-S1 and pre-S2 **(C)** are shown at the top of the figure. S proteins correspond to the white areas, pre-S2 to the light pink areas, and pre-S1 to the dark pink areas. The virus particles contain an internal nucleocapsid shown in the bottom split-open section. HBcAg, hepatitis B core antigen; HBV, hepatitis B virus. *(From Neurath AR, Thanavala Y. Hepadnaviruses. In: Von Regenmortel MHV, Neurath AR, eds.* Immunochemistry of Virus, II: The Basis for Serodiagnosis and Vaccines. *New York: Elsevier Science;1990:403–458.)*

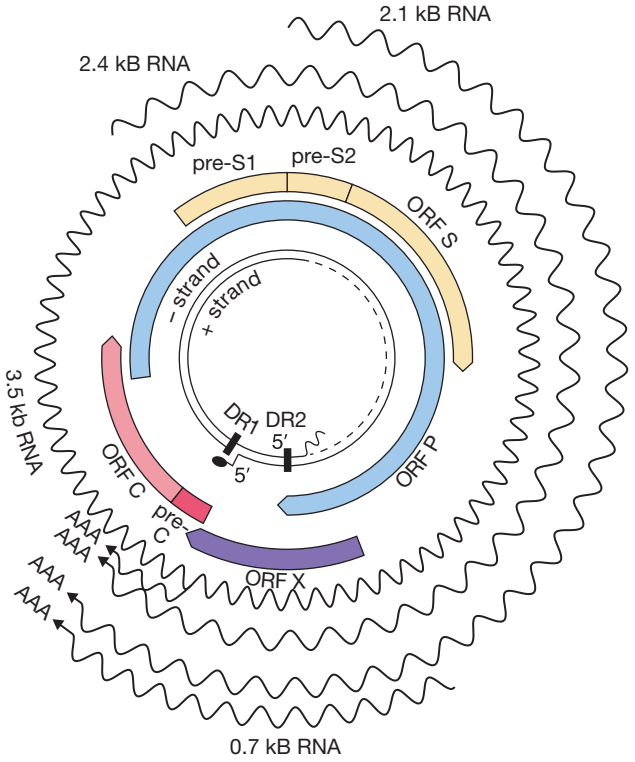


Figure 25.6. Hepatitis B virus coding organization. Inner circle represents virion DNA, with dashes signifying the single-stranded genomic region. Boxes denote viral coding regions, with arrows indicating direction of translation. Outermost wavy lines depict the viral RNAs identified in infected cells, with arrows indicating direction of transcription. DR, direct repeat; ORF, open reading frame. *(From Ganem D. Hepadnaviridae and their replication. In: Fields BN, Knipe DM, Howley PM, et al, eds.* Fields Virology, vol. 2, *ed 3. Philadelphia: Lippincott-Raven; 1996:2706.)*

positions 122 and 160 of the S region of HBV DNA. All combinations of these determinants have been found, resulting in four major subtypes—*adw, adr, ayw,* and *ayr*—and nine minor subtypes. Antibodies to the *a* determinant confer protection to all these serotypes, whereas antibodies to the subtype determinants do not.[121] No apparent differences in infectivity or virulence of HBV have been attributed to HBsAg subtypes. However, these subtypes have a distinct geographic distribution worldwide and have been used in epidemiologic studies to identify patterns of virus transmission.[122] The most common subtype among persons with chronic HBV infection in the United States is *adw*.[123] Moreover, based on a minimal divergence of 8% of the complete genome sequences derived from several HBV worldwide isolates, at least 10 genotypes labeled alphabetically from A to J and a number of subgenotypes—with a minimal genetic distance of 4%—have currently been identified, showing different geographic and ethnic distributions.[124–128] For example, genotypes A and D are prevalent in Europe, the United States, and Africa, whereas genotypes B and C are prevalent in Asia. Genotype D predominates in the Mediterranean Basin and in the near Middle East up to India. Genotype E is by far the dominant genotype in West Africa, whereas genotypes F and H have mostly been isolated in Amerindian populations in different countries in the Americas. Finally, genotype G has no specific endemic areas in the world and is usually detected in coinfection with other HBV genotypes, mostly genotype A. More recently two additional genotypes, named I and J, were isolated in Laotian and Japanese patients,[129,130] respectively. Emerging evidence suggests that HBV genotypes and subgenotypes may play a role in determining the severity of the liver disease and treatment outcomes of HBV infection.[131–133]

Replication of the viral DNA genome occurs via reverse transcription mediated by the pregenomic RNA (pgRNA), for which polymerase plays a crucial role.

Both the nucleocapsid protein (HBcAg) and the e protein are translated from the C gene. HBcAg is essential for viral packaging and is an integral part of the nucleocapsid. It is not detectable in serum by conventional techniques, but it can be detected in liver tissue in patients with acute or chronic HBV infection. The e protein is processed by the endoplasmic reticulum, where it is cleaved and HBeAg is secreted. HBeAg is a soluble protein that is not part of the virus particle, but it can be detected in the serum of patients with acute hepatitis B and in persons with chronic HBV infection who have a high viral load. HBeAg is also expressed on the surface of hepatocytes[134] and might be an important target for the immune defense mechanisms that lead to the destruction of hepatocytes.[135]

HBV replication begins with attachment of the virus to the hepatocyte and penetration (Fig. 25.7).[117,120,136,137] After uncoating in the cytoplasm, the nucleocapsid is transported to the nucleus. However, it is unclear whether the nucleocapsid enters the nucleus or disassembles in the cytoplasm and delivers the viral DNA to the nucleus at the nuclear pore. Once in the nucleus, the single-stranded gap in the viral genome is repaired, and covalently closed circular DNA (cccDNA)—which is completely double stranded—is formed. Episomal HBV cccDNA persists in the hepatocyte as a stable mini chromosome organized by histone and nonhistone proteins. In

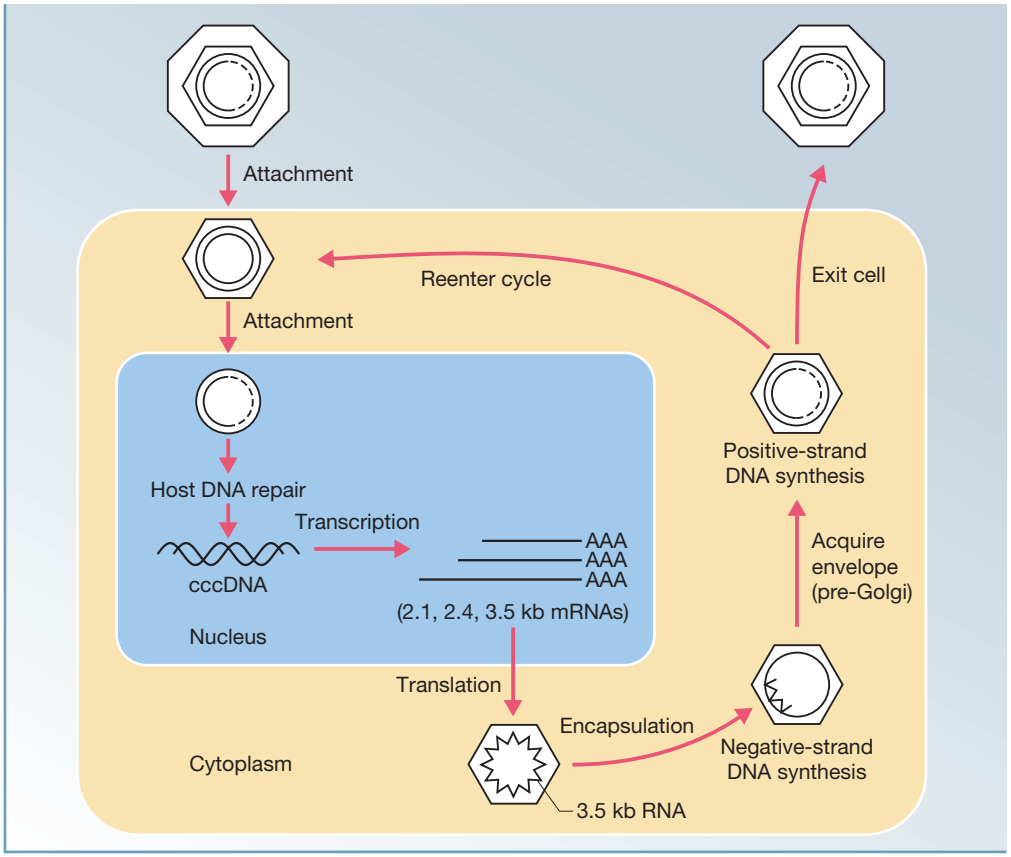


Figure 25.7. Hepatitis B virus replication cycle (see text for details). cccDNA, covalently closed circular DNA. *(From Butel JS, Lee TH, Slagle BL. Is the DNA repair system involved in hepatitis B virus-mediated hepatocellular carcinogenesis?* Trends Microbiol. *1996;4:119.)*

contrast to classic retroviruses such as HIV, integration of HBV DNA into the host genome is not necessary for viral messenger RNA (mRNA) synthesis or replication. However, integration of HBV DNA does occur during chronic infection, which might be an important factor for the development of HCC. The cccDNA acts as the template for production of four mRNAs, each of which is expressed from its own promoter.[136] A 0.7-kb mRNA transcript encodes for the X protein; a 2.1-kb mRNA transcript encodes for pre-S2 and S proteins; a 2.4-kb mRNA transcript encodes for pre-S1, pre-S2, and S proteins; and a 3.5-kb mRNA encodes for HBcAg, HBeAg, and polymerase. These mRNA transcripts are transported into the cytoplasm, where translation yields the viral envelope, core, precore, and X proteins and the viral DNA polymerase. The 3.5-kb mRNA is also a pgRNA that serves as a template for HBV DNA synthesis. Viral packaging occurs in the cytoplasm, where the pgRNA is encapsulated by newly synthesized HBcAg, and the polymerase synthesizes negative-strand DNA by reverse transcription. The pgRNA is then degraded by ribonuclease (RNase), an enzyme that resides on the polymerase.[138] After the negative strand is synthesized, the polymerase starts to synthesize the positive DNA strand.[139] However, the process is not completed, resulting in replicative intermediates consisting of full-length minus-strand DNA plus variable-length (20% to 80%) positive-strand DNA. Nucleocapsid particles containing these replicative intermediates bud from the pre-Golgi membranes, where they acquire HBsAg/pre-S protein–containing envelopes. Virus particles can then either exit the cell or be reimported into the nucleus and initiate another round of replication in the same cell.

Because of this strategy of replication, HBV shows a greater mutability (at least 100 times) than other DNA viruses. Mutations and variations that occur naturally or following antiviral therapy play important roles in viral infectivity, pathogenesis of liver disease, vaccine efficacy, and resistance to antiviral therapies.[140]

Hepatitis B Virus Mutants

HBV has a high frequency of mutations because the virus replicates via an RNA intermediate, using a reverse transcriptase (RT) that appears to lack a proofreading function leading to quasispecies. Mutations in the HBV genome can evolve from spontaneous errors of the viral polymerase, or as the consequence of pressure by the host immune system or by exogenous factors, including passive or active immunization as well as treatment with antiviral agents. As a result of overlapping ORF mutations within the HBV P can cause concomitant changes in the HBV surface gene (S) and vice versa. HBV quasispecies diversity is associated with response to antiviral therapy, disease severity, tumorigenesis, and long-term clinical outcomes. Mutations have been identified in all four HBV genes but have been most fully characterized in the pre-C/C gene, the polymerase gene, and the pre-S/S gene.[141–149]

Basal Core Promoter, Pre-C/C Gene Mutants

Two major groups of mutations that result in reduced or blocked HBeAg expression have been identified. The most common mutation in the ORF pre-C/C region is a guanine-to-adenine substitution at nucleotide (nt) position 1896 (G1896A) that results in a translational stop codon (from TGG to TAG) that abolishes the synthesis of HBeAg.[150,151] However, HBV DNA synthesis persists and may cause liver

damage with progression to cirrhosis and liver cancer. The G1896A mutation strongly depends on the viral genotype, being highly frequent among genotypes B to E and rare in A, F, and H strains; all G isolates harbor this mutation. Loss of HBeAg expression can also occur with mutations in the basal core promoter (BCP) region that regulates the expression of both HBeAg and core protein.[152] These mutations have been associated with fulminant hepatitis and severe chronic liver disease.[153,154] However, fulminant hepatitis can occur in the absence of such mutations.[155–157] Additional studies are needed to determine the pathogenesis and clinical sequelae arising from the selection of these mutants.

X Gene Mutations

Because the X ORF overlaps the BCP completely, promoter mutations can affect the amino acid sequence of the X protein. The most common BCP double mutations, occurring at nt 1762 (A1762T) and at nt 1764 (G1764A), can cause changes in the X protein that may affect its ability to transactivate the BCP. In addition, insertions or deletions in the BCP often shift the X gene frame, resulting in truncated forms of the X protein. These shortened X proteins lack the domain in the C terminus that is required for the transactivation activity of HBx antigen.[144,145,158]

Several mutations in the X gene are reported to be associated with progression of chronic hepatitis B and hepatocarcinogenesis.[159]

Polymerase Gene Mutants

Mutations of the polymerase gene have been associated with resistance to treatment with nucleoside or nucleotide analogs (NAs) and with viral persistence.[125,143–145,148,160–163] The most common of these mutations occur at codon 180 (the template binding site of the polymerase) and at codon 204 of the reverse transcriptase domain in the P gene.

Polymerase mutations have been demonstrated to emerge in up to 80% of patients after treatment with nucleoside or NAs. These mutations significantly decrease the efficacy of treatment.[165] The development of drug resistance due to mutations in the P gene is usually followed by a virological breakthrough (rise in HBV DNA levels of at least 1 log compared to nadir value), a biochemical breakthrough (ALT elevation), and worsening of liver disease.[166] Mutations of the Pol gene located within the catalytic domain of the RT region are particularly common after treatment with lamivudine (up to 80% after 48 months of treatment) but lower following treatment with other NAs.[167] To minimize the likelihood of resistance development and to increase the chance of achieving treatment goals, the currently available EASL (European Association for the Study of the Liver) guidelines recommend the choice of drugs with high potency and lowest rates of resistance as first-line treatment options.[168]

Because of the genome organization of HBV into overlapping reading frames, the emergence of polymerase mutants—favoring resistance—during long-term antiviral therapy can select viruses with crucial changes in the overlapping S gene, potentially able to alter the S protein immunoreactivity.[169–173] In this context, the widespread use of these drugs may cause the emergence of mutants potentially able to escape neutralization by vaccine-induced anti-HBs antibody and to infect successfully vaccinated people.[174,175]

Pre-S/S Gene Mutants

Isolates with pre-S deletions are often found. There is evidence that a set of mutations (deletion in the pre-S region as well as precore and BCP mutations) are significantly associated with progressive liver disease and HCC.[176,177]

Mutations in the S gene can lead to conformational changes in the common *a* determinant (shared by all HBV genotypes and serotypes), which spans amino acids 124 to 147 within the major hydrophilic region (MHR) of HBsAg and is in a form of two major loops and one minor loop with cysteine-disulphide bonds, protruding from the outer surface of the virus; the second hydrophilic loop (amino acids 139 to 147 or 149) is the major target for neutralizing anti-HBs induced by natural infection or vaccination (Fig. 25.8).[178] Neutralizing (protective) antibodies produced by vaccination are targeted largely toward the conformational epitope of the *a* determinant, providing broad protection against all HBV genotypes and subtypes. Thus, alteration of residues within this region of the viral surface antigen can determine conformational changes that can allow replication of HBV in vaccinated people.

The prototype of such mutants, the so-called G145R, which shows a point mutation from guanosine to adenosine at nt position 587, resulting in an amino acid substitution from glycine (G) to arginine (R) at position 145 in the *a* determinant of the surface antigen, was first observed in Italy some 25 years ago in infants born to HBsAg carrier mothers who developed breakthrough infections despite having received hepatitis B immunoglobulin (HBIG) and vaccine at birth.[179,180] Because the G145R substitution alters the projecting second loop of the *a* determinant, the neutralizing antibodies produced by vaccination are no longer able to recognize the mutated epitope. This mutant was infectious in experimentally infected chimpanzees.[181] Besides the G145R, other *S* gene mutations (alone or in combination) across the entire *a*-determinant region that are potentially able to evade neutralizing anti-HBs have been found worldwide.[142,182–184] Concerns have been expressed that these mutated viruses might allow replication of HBV in the presence of protective levels of anti-HBs (immunization escape mutants), negatively affecting the efficacy of vaccination.[183] In addition, these mutants might not be detected by some commercially available HBsAg assays based on antibodies to the wild-type virus, posing a potential threat to the safety of blood supply (diagnostic escape mutants).[185–188]

HBV infection with *S* gene mutant viruses has been reported to occur in anti-HBs antibody-positive infants born to HBV-infected mothers who received prophylaxis with HBIG plus hepatitis B vaccine,[179–181,189–193] in children who responded to vaccination,[194] and in liver transplant recipients who received for prophylaxis of relapse of HBV infection.[195–197] However, in population-based studies of infants born to HBsAg-positive mothers, *S* gene mutant viruses have not been found to be associated with a failure to prevent perinatal HBV transmission. In addition, preexposure vaccination of chimpanzees with currently licensed vaccines (not containing pre-S epitopes) conferred protection after intravenous challenge with the G145R HBV.[198] At present, no evidence exists that *S* gene mutants have spread in immunized populations or that these mutants pose a public health threat to hepatitis B immunization programs.[173–175,200] Further studies and enhanced surveillance to detect the emergence of these mutants, as well as those resulting from the onset of resistance to antiviral therapy, are a high priority in monitoring the effectiveness of current immunization strategies.

Pathogenesis

HBV is primarily hepatotropic. However, although HBsAg has been detected in organs other than the liver, there is little evidence to indicate primary replication in sites other than

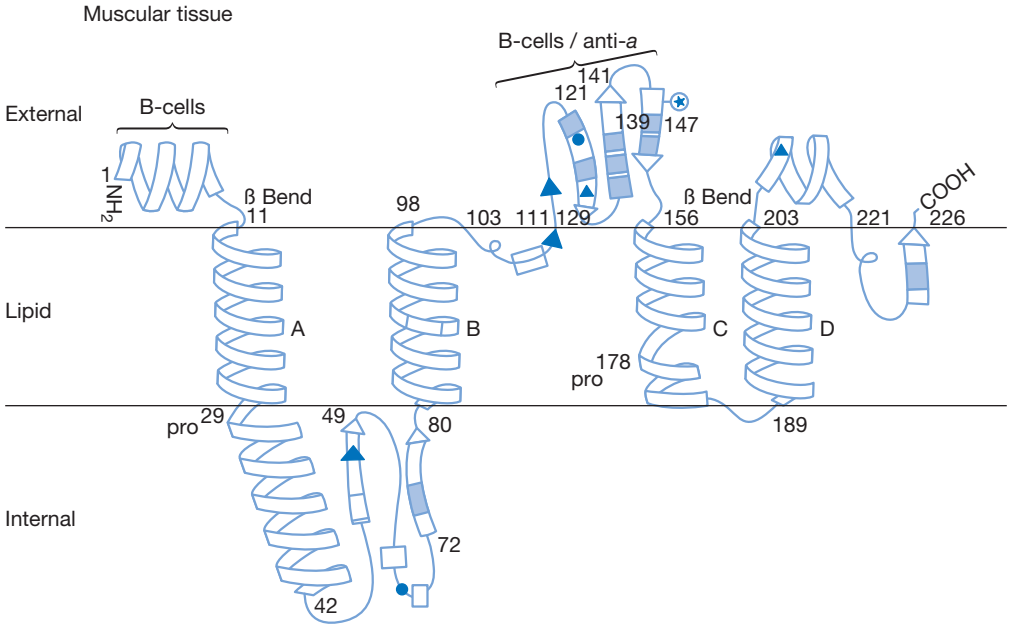


Figure 25.8. Secondary structure of hepatitis B virus surface (s) antigen in the lipid envelope as predicted by computer modeling. The shaded areas indicate the locations of sequence variations for w/r (s) and d/y (l) subtypes. *(From Howard C, Smith Stinh HJ, Brown SE, et al. Toward the development of a synthetic hepatitis B vaccine. In: Zuckerman AJ, ed.* Viral Hepatitis and Liver Disease: Proceedings of the International Symposium on Viral Hepatitis and Liver Disease, held at the Barbican Centre, London, May 26–28, 1987. *New York: Alan R. Liss; 1988.)*

hepatocytes. Most available experimental evidence suggests that HBV is not directly cytopathic, and that liver damage is produced by the cellular immune response to viral proteins in infected hepatocytes.[201] Extrahepatic manifestations that can appear during the prodromal phase of acute hepatitis B (e.g., arthritis, urticaria) and in patients with chronic infection (e.g., vasculitis, glomerulonephritis) appear to be mediated by immune complex formation.[57] Development of HCC in persons with chronic infection is apparently induced either directly by activating cellular oncogenes or inactivating tumor suppressor genes, or indirectly through chronic liver injury, inflammation, and the promotional effect of hepatocyte regeneration.[202]

Neonatal immune tolerance to viral antigens appears to play an important role in viral persistence in infants infected at birth, but the basis for poor T-cell response and the establishment of chronic infection in adults is not well understood.[73,74] Patients with acute hepatitis B whose infection has resolved have a vigorous T-cell response against multiple viral antigens, including the viral core, surface, and polymerase proteins. In contrast, persons who develop chronic infection have a weak or undetectable cellular immune response, suggesting that a less-efficient T-cell response might permit persistence of HBV.

Diagnosis

Antigens and antibodies associated with HBV infection include HBsAg, anti-HBs, total (i.e., immunoglobulin [Ig] G and IgM) antibody to HBcAg (anti-HBc), IgM anti-HBc, HBeAg, and anti-HBe. At least one serologic marker is present during the different phases of HBV infection (Table 25.1). Serologic assays are commercially available for all circulating markers except HBcAg, because no free HBcAg is secreted into the blood. However, HBcAg can be identified in liver tissue through immunohistologic assays.

The presence of a confirmed HBsAg result indicates current (or active) HBV infection. All HBsAg-positive persons should be considered infectious, yet infectivity is proportional to the magnitude of the viral load. In newly infected persons, HBsAg is the only serologic marker detected during the first 3 to 5 weeks after infection (Fig. 25.9). The average time from exposure to detection of HBsAg is 30 days (range: 6 to 60 days).[51,72] Highly sensitive single-sample nucleic acid tests can detect HBV DNA in the serum of an infected person 10 to 20 days before detection of HBsAg.[203] Transient HBsAg positivity has been reported for up to 18 days after vaccination with standard hepatitis B vaccine dosages,[204,205] and for up to 28 days after vaccination with the increased dosages used for hemodialysis patients.[206] This HBsAg positivity is clinically insignificant.

Anti-HBc appears at the onset of symptoms or liver test abnormalities in acute HBV infection and persists for life (see Fig. 25.9).[125] Acute or recently acquired infection can be distinguished by the presence of the IgM class of anti-HBc, which is detected at the onset of acute hepatitis B and persists for up to 6 months if the disease resolves. In patients who develop chronic hepatitis B, IgM anti-HBc can persist at low levels during viral replication, but they are usually not detectable by assays used in the United States. However, persons with exacerbations of chronic infection can test positive for IgM anti-HBc.[207] Because the positive predictive value of testing for IgM anti-HBc is low in asymptomatic persons, testing for diagnosis of acute hepatitis B should be limited to persons with clinical evidence of acute hepatitis or an epidemiologic link to a case.

In persons who recover from HBV infection, HBsAg is cleared from the blood, usually within 3 to 4 months, and anti-HBs develops during convalescence (see Fig. 25.9). The presence of anti-HBs typically indicates immunity from HBV infection. In addition, anti-HBs can be detected for several months after HBIG administration.[208] Persons who recover from natural infection are typically positive for both anti-HBs and anti-HBc, whereas persons who respond to hepatitis B vaccine develop only anti-HBs. In persons who become chronically infected, HBsAg and anti-HBc persist, typically for life (Fig. 25.10). HBsAg becomes undetectable in approximately

TABLE 25.1 Interpretation of Serologic Test Results for Hepatitis B Virus Infection

Serologic Marker				
HBsAg	**Total Anti-HBc**	**IgM anti-HBc**	**Anti-HBs**	**Interpretation**
–	–	–	–	Never infected
+[a]	–	–	–	Early acute infection; transient (up to 18 days) after vaccination
+	+	+	–	Acute infection
–	+	+	+ or –	Acute resolving infection
–	+	–	+	Recovered from past infection and immune
+	+	–	–	Chronic infection
–	+	–	–	False positive (i.e., susceptible); past infection; low-level chronic infection[b]; or passive transfer of anti-HBc to infant born to HBsAg-positive mother
–	–	–	+	Passive transfer after hepatitis B immunoglobulin administration or immune if concentration is ≥10 mIU/mL

Anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to hepatitis B surface antigen (HBsAg); IgM, immunoglobulin M; –, negative test result; +, positive test result.
[a]To ensure that an HBsAg-positive test result is not a false positive, samples with repeatedly reactive HBsAg results should be tested with a licensed (and, if appropriate, neutralizing confirmatory) test.
[b]Persons positive for only anti-HBc are unlikely to be infectious except under unusual circumstances in which they are the source for direct percutaneous exposure of susceptible recipients to large quantities of virus (e.g., blood transfusion or organ transplantation).

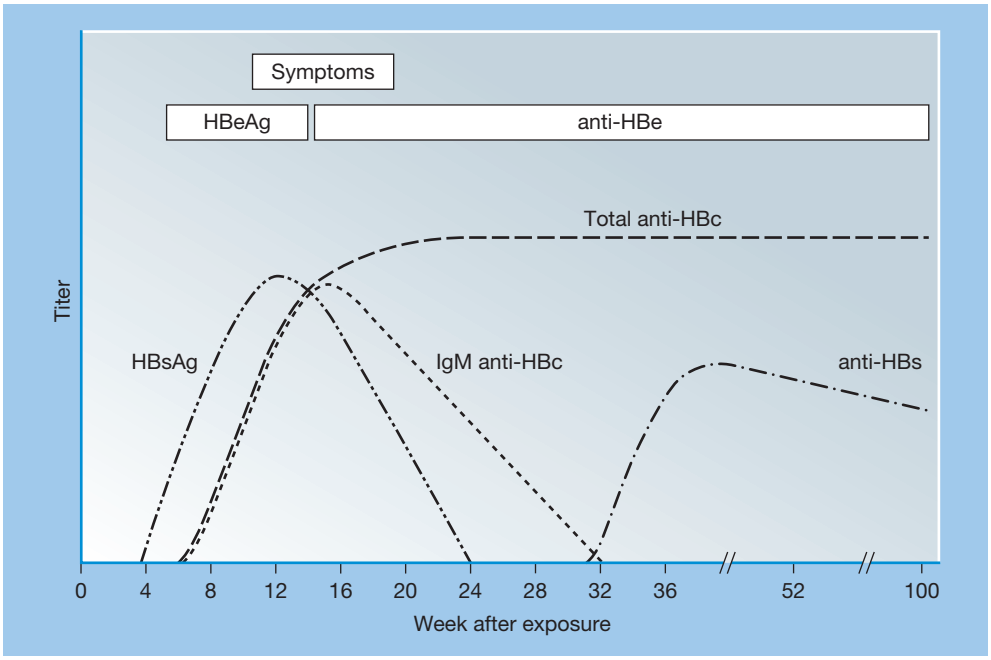


Figure 25.9. Concentrations of hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), IgM anti-HBc, and antibody to HBsAg (anti-HBs) in patients with acute hepatitis B with recovery. Anti-HBe, antibody to HBeAg; HBeAg, hepatitis B e antigen; IgM, immunoglobulin M.

0.5% to 2% of chronically infected persons yearly, and anti-HBs develops in the majority of these persons.[77,89,90]

In certain persons, the only HBV serologic marker detected in serum is isolated anti-HBc. Isolated anti-HBc can occur after HBV infection in persons who have recovered but whose anti-HBs levels have waned, or in chronically infected persons in whom anti-HBs failed to occur and in whom circulating HBsAg is not detectable by commercial serology. HBV DNA has been detected in the blood of fewer than 5% of persons with isolated anti-HBc in the absence of detectable HBsAg.[209,210] In such patients, occult HBV infection is manifested by the presence of low levels of HBV DNA in serum and intranuclear HBV cccDNA in hepatocytes.[211,212] Such persons with occult HBV are unlikely to be infectious except when they are treated with immunosuppressive agents or are the source for direct percutaneous exposure of susceptible recipients to substantial quantities of virus (e.g., through blood transfusion or after liver transplantation).[93,213] Typically, the frequency of isolated anti-HBc relates directly to the prevalence of HBV infection in the population. In populations with a high prevalence of HBV infection, isolated anti-HBc most likely indicates previous infection, with loss of anti-HBs. In these individuals, hidden immunity to HBV may be unmasked through a single HBV vaccine dose that leads to an anamnestic anti-HBs response.

For persons in populations with a low prevalence of HBV infection, isolated anti-HBc is found in 10% to 20% of persons

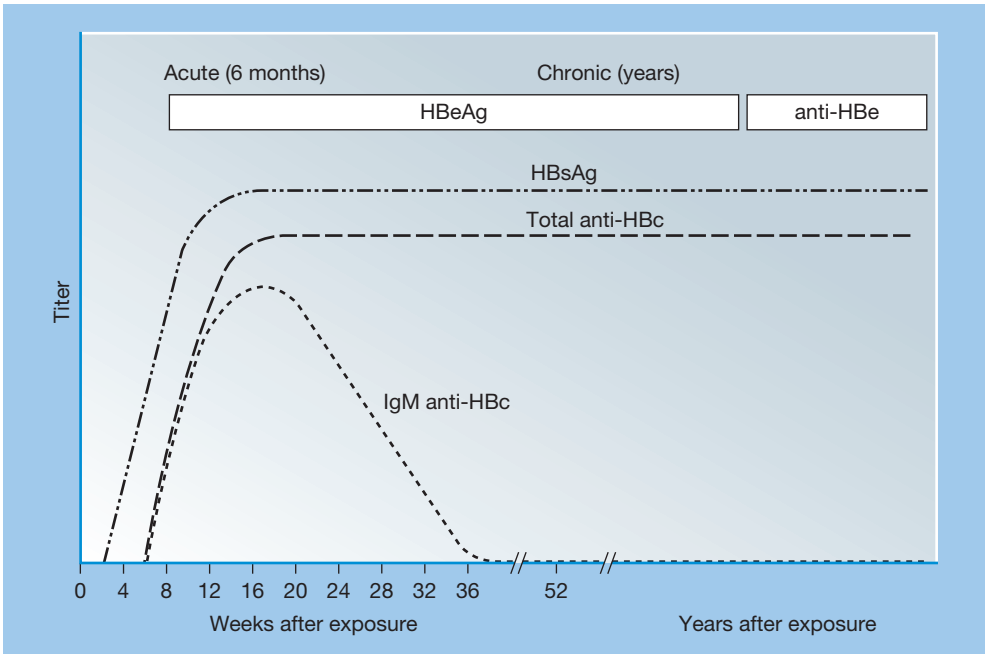


Figure 25.10. Concentrations of HBsAg, anti-HBc, and IgM anti-HBc during progression to chronic hepatitis B virus infection. Anti-HBc, antibody to hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; IgM, immunoglobulin M.

with serologic markers of HBV infection,[210] and it often represents a false-positive reaction; the majority of these persons have a primary anti-HBs response after a three-dose series of hepatitis B vaccine.[214,215] Infants who are born to HBsAg-positive mothers and who do not become infected might have detectable anti-HBc for up to 24 months after birth from passively transferred maternal antibody.

HBeAg can be detected in the serum of persons with acute or chronic HBV infection. The presence of HBeAg correlates with high levels of viral replication (i.e., HBV DNA levels of 10^7 to 10^9 IU/mL, indicating high infectivity).[216,217] Loss of HBeAg correlates with low levels (i.e., HBV DNA levels of $<10^5$ IU/mL) of replicating virus, although some HBeAg-negative persons have HBV DNA levels up to 10^8 to 10^9 IU/mL.[100] A mutation in the precore region of the HBV genome has been found in HBeAg-negative persons with high HBV DNA levels.[150,218]

Treatment

No specific antiviral treatment is recommended for persons with acute hepatitis B, as approximately 95% of infected immunocompetent adults recover spontaneously with anti-HBs seroconversion.[219–221] Consequently, supportive care is the mainstay of therapy. Antiviral treatment may, however, be considered in patients with severe acute or fulminant hepatitis B.[219,222] In contrast, in patients with chronic hepatitis B (CHB), long-term antiviral treatment has been demonstrated to reduce the risk for disease progression and HCC by approximately 50%.[223] The U.S. Food and Drug Administration has approved six antiviral drugs for treatment of CHB: interferon-α (standard[224] and pegylated[225,226]) and five oral antiviral agents (lamivudine,[227] adefovir dipivoxil,[225,228] telbivudine,[229] entecavir,[230,231] and tenofovir).[232] To date, combination therapy has not been shown to be more effective than treatment with a single drug.[226,233] Guidelines for antiviral treatment in CHB were updated by the American Association for the Study of Liver Diseases (2009) and by the EASL (2012).[219,221]

Antiviral treatment is generally recommended for chronically infected patients on the basis of the presence of active disease (i.e., ALT levels more than twice the upper limit of normal), clinical or histologic evidence of progressive disease and fibrosis, or both.[234–236] The primary goal of treatment is to minimize progression of liver injury and fibrosis by suppressing viral replication.[224,237–239] The end points used to assess response include normalization of ALT levels (biochemical response); sustained clearance of markers of active HBV replication, including HBeAg seroconversion for HBeAg-positive patients and undetectable HBV DNA by polymerase chain reaction for HBeAg-negative patients (virologic response); histologic improvement on liver biopsy (histologic response); and sustained clearance of HBsAg (complete response).

Interferon-α and pegylated interferon-α are usually administered for 4 to 6 months, with durable virologic response rates of approximately 25% among patients with HBeAg-positive CHB, and 15% to 20% among patients with HBeAg-negative CHB.[226,233] For the five oral antiviral agents, virologic response rates as determined by undetectable HBV DNA in serum in controlled trials after 48 weeks of treatment were 76% with tenofovir, 67% with entecavir, 60% with telbivudine, 40% to 44% with lamivudine, and 21% with adefovir in patients with HBeAg-positive CHB. In patients with HBeAg-negative CHB, undetectable HBV DNA was reported in 93% of patients treated with tenofovir, 90% with entecavir, 88% with telbivudine, 60% to 73% with lamivudine, and 51% with adefovir.[225,226,228,230,231,233]

The optimal end point and duration of oral antiviral treatment for CHB has not been determined, and prolonged or even lifelong treatment must be considered in individual cases. In patients with HBeAg-positive CHB, durable virologic response rates of 16% to 24% have been achieved after 48 weeks of treatment with oral antiviral agents, even if treatment is stopped. However, in the majority of treated patients, particularly those with HBeAg-negative disease, HBV is suppressed but not eliminated, and relapse occurs when treatment is stopped.[240,241] Relapse can be associated with severe

exacerbation of disease, which might cause hepatic decompensation and death.[224] Thus, once started, treatment with oral antiviral agents is difficult to stop. Although these drugs have an excellent safety profile, the safety of long-term administration has not been demonstrated. The efficacy of long-term administration has also not been determined. A major issue related to long-term effectiveness is antiviral resistance. Resistance to lamivudine develops at a rate of 15% to 25% per year, reaching 70% to 80% after 4 years.[242] Resistance to adefovir is rare before completion of 2 years of therapy but reaches 15 to 20% by 4 years.[242] Although data are limited, the development of antiviral resistance with long-term use of entecavir has been described only in patients with preexisting lamivudine resistance[243] and so far has not been observed with tenofovir.[244]

BURDEN AND EPIDEMIOLOGY

Burden

HBV infection is a highly prevalent infection around the globe, the frequency and burden of which varies by region and subpopulation. Approximately 30% of the world's population (i.e., about 2 billion persons) have serologic evidence of HBV infection,[245] and of these, more than 240 million persons are living with chronic infection.[95,125,245,246] Based on a systematic literature review and an analysis of pooled data, an estimated 3.61% of the world's population or an estimated 248 million persons are HBsAg positive. By World Health Organization (WHO) region, the largest number of HBsAg-positive persons live in the Western Pacific and African regions (~95 million persons and 75 million persons, respectively), where most countries with high endemicity (≥8% prevalence) are located. However, countries of high endemicity can also be found in the Americas (e.g., Haiti) and European (e.g., Kyrgyzstan) regions. Countries with the highest population of HBsAg-positive persons include China (74 million), India (17 million), and Nigeria (15 million).[247]

HBV causes significant morbidity and mortality worldwide.[248–251] In 2013, approximately 686,000 HBV-infected persons died from causes related to acute infection (69,000 deaths), cirrhosis (317,000 deaths), and HBV-associated liver cancer (300,000 deaths), the sixth most common cancer globally and the second leading cause of cancer-related death worldwide. Of all cases of primary liver cancer, 70% to 90% are caused by HCC, for which HBV is a major cause.[252] The proportion of HCC attributed to HBV infection varies by the prevalence of HBV infection in the population. Accordingly, in Asia, HBV causes more than two-thirds of HCC in China, the Republic of Korea, and Vietnam, and 50% of the HCC in India, Thailand, and Malaysia. In countries of sub-Saharan Africa, 19% to 64% of HCC can be attributed to HBV infection. Data from the Global Burden of Disease Project in 2013 revealed that one-third of liver cancer–related deaths are among persons with HBV infection. Despite improvements in infant vaccination coverage, rates of HBV-related liver cancer remain high in some countries, reflecting HBV infection in persons acquiring the virus during childhood (before the implementation of routine hepatitis B vaccination) and the decades-long latency period before development of HCC.[253]

The lifetime risk for HCC in a chronically infected person is approximately 10% to 25%, which is 15 to 20 times greater than that for persons without HBV infection.[38,40] In a study conducted in Taiwan, rates of HCC among men who were HBsAg-positive were approximately 100 times higher than rates among those who were HBsAg-negative (495 vs 5 per 100,000 persons per year, respectively).[37,101]

Certain populations are disproportionately affected by HBV-related HCC. For example, whereas the incidence of HCC in women chronically infected with HBV ranges from 120 to 178 cases per 100,000 persons, among men, incidence ranges from 340 to 804.[248] The reasons for gender-related differences in HCC incidence remain unknown.

Other factors place certain persons with CHB at increased risk for developing HCC, including increasing age; HBeAg serostatus; increasing serum levels of HBV DNA and ALT; HBV genotype; concurrent infection with HCV, HBV genotype C infection, HDV, or HIV[97–99,253–257]; and cirrhosis, which is found in the majority of those who have HBV-related HCC (but not all patients with HCC will have had cirrhosis).[100,258–260] Behavioral factors that increase risk for HCC among persons infected with HBV include heavy alcohol consumption and aflatoxin ingestion.[261,262] Persons who achieve clearance of HBsAg (i.e., who have minimal or no viral replication) might be at decreased risk for HCC,[88] although HCC can occur in persons who clear the virus.[85,89,106,263,264]

Rates of new cases of HBV have declined substantially in the United States over the last several decades since implementation of routine hepatitis B immunization of infants recommended by the Centers for Disease Control and Prevention (CDC) in 1991.[265–267] In the two decades prior to publication of these guidelines, approximately 200,000 to 300,000 persons acquired HBV infection annually.[267,268] In contrast, only 19,000 new or acute hepatitis B cases were reported during 2012, reflecting an 86% decline in HBV incidence.[269,270] In 2013, the number of acute HBV infections increased 5%, reflecting cases associated with healthcare-related outbreaks and an increase in transmission of bloodborne infections among persons who inject drugs (PWID).[271–273]

In the United States, surveys suggest stable or decreasing rates of chronic HBV infection attributed to routine childhood vaccination. Data from the National Health and Nutrition Examination Survey (NHANES)[258] demonstrate that from 1988 through 2012, prevalence of past or current HBV infection decreased from 5.1% to 3.9%, representing approximately 10.8 million persons.[266] In 2011–2012, an estimated 0.3% of the U.S. population, or 847,000 persons were HBsAg-positive. HBsAg prevalence was lowest among persons 6 to 19 years of age (0.03%), a population with high rates of hepatitis B vaccination coverage, and highest among Asian-Americans (3.1%), 93% of whom were born in countries other than the United States. Actual rates of chronic infection among foreign-born persons are likely higher than those based on national surveys, as these persons are underrepresented in national surveys. Given that an estimated 54,000 persons migrate to the United States annually and considering country-specific HBsAg prevalence data, up to 2.2 million foreign-born persons could be living with HBV in the United States.

In 2010, a total of 1875 HBV-associated deaths (0.54 deaths per 100,000 population) were recorded in the United States.[274] Certain populations, such as Asians and Pacific Islanders, are at increased risk for HBV-related death. HCC incidence in the United States is highest among Asians and Pacific Islanders (7.8 per 100,000 persons),[275] and studies suggest that HBV infections account for approximately 80% of liver cancer–related deaths in the Asian community.[276] In 2013, 1873 HBV-related deaths were recorded in the United States.[270]

Substantial healthcare costs are associated with HBV infection, including those incurred from HBV antiviral therapy. From the late 1990s through the early 2000s, outpatient visits and hospitalizations for hepatitis B–related diagnoses increased more than fourfold in the United States, with a concomitant increase in healthcare costs, from $357 million in 1990 to $1.5 billion in 2003. Fortunately, this number has plateaued, dropping to $1.3 billion in subsequent years.[278]

Despite these figures, cost-to-benefit analysis shows that each $1 spent on primary prevention, including immunization, saves a net $1 in medical and work-loss costs.[279]

Routes of Transmission

Hepatitis B virus is transmitted by percutaneous (i.e., puncture through the skin) or mucosal (i.e., direct contact with mucous membranes) exposure to infectious blood or body fluids. All HBsAg-positive persons are potentially infectious, but those who are also HBeAg positive are *more* infectious because their blood contains high concentrations of HBV (typically 10^7 to 10^9 virions/mL). Although HBsAg has been detected in multiple body fluids, only serum, saliva, semen, and vaginal fluid have been demonstrated to be infectious.[280] HBV remains infectious for at least 7 days outside the body and can be found in very low concentrations on fomites, even in the absence of visible blood.[281–283] Primary sources of HBV infection are perinatal exposure from infected mothers, nonsexual person-to-person contact, sexual contact, and percutaneous exposure to blood or infectious body fluids. HBV is not transmitted by air, food, or water.

Perinatal Transmission

Infants born to mothers with chronic HBV infection can acquire perinatal HBV infection, which usually occurs at the time of birth; *in utero* transmission of HBV is relatively rare (accounting for <2% of infections transmitted from mother to infant),[284–287] and the virus is not transmitted through breastfeeding.[288] The likelihood of transmission is associated with time of maternal infection—pregnant women who acquire hepatitis B in the first and second trimester rarely transmit HBV to the fetus or neonate,[289,290] whereas approximately 60% of infants born to women infected during the third trimester become infected.[290] Risk for perinatal transmission is higher from mothers with a high viral load, which generally correlates with the presence of HBeAg in serum, whereas less than 10% of infants born to HBeAg-negative mothers develop chronic HBV infection.[291–294] Of infants born to HBeAg-positive mothers, 70% to 80% develop chronic HBV infection by age 6 months in the absence of postexposure immunoprophylaxis.

Perinatal transmission is a major source of HBV infection in many countries.[284–287,291,293–296] A mathematical model estimates that globally, an estimated 21% of future HBV-related deaths among persons who were not vaccinated at birth will be attributable to perinatal HBV infection.[95] In the United States, the race-adjusted prevalence of HBsAg among pregnant women is approximately 0.6%; the prevalence of HBeAg among HBsAg-positive pregnant women is approximately 35% among women of Asian descent and approximately 20% among other races.[297,298] When applied to 2003 U.S. natality data, race- and ethnicity-adjusted HBsAg prevalence estimates revealed that approximately 25,600 infants were born to HBV-infected pregnant women during 2008; without immunoprophylaxis, approximately 9600 of these infants would develop chronic HBV infection during their lifetime.[299]

Nonsexual, Person-to-Person Contact

HBV transmission can occur horizontally via nonsexual, person-to-person contact with HBsAg-positive persons. The risk of transmission increases with duration of exposure. Person-to-person transmission probably occurs from inadvertent percutaneous or mucosal contact with blood or infectious body fluids during certain activities, such as sharing toothbrushes or razors, contact with exudates from dermatologic lesions, contact with saliva through bites or other breaks in the skin, premastication of food, sharing of gum and other food items, and contact with HBsAg-contaminated surfaces.[280,300–305]

Households are typical settings for such transmission, particularly in highly endemic areas[306]; as a result, household contacts of HBsAg-positive persons are at high risk for HBV infection. Seroprevalence of HBV infection among household contacts of persons with chronic infection varies by study, ranging from 14% to 60%.[36,78,80,90,91,95,96,307–313] According to one study involving 302 hepatitis B–infected children referred for tertiary care in Ankara, Turkey, 38% of mothers, 23% of fathers, and 11% of other siblings were also HBsAg-positive. Other settings where person-to-person transmission typically occurs include childcare centers and schools.[314–320] Of all HBV-related deaths in the 2000 birth cohort worldwide, 48% will result from infection acquired in the early childhood period, according to the mathematical model of Goldstein and colleagues[95] (Table 25.2).

Routine hepatitis B immunization prevents person-to-person transmission; integrating hepatitis B vaccination into the routine infant immunization schedule beginning at birth would prevent or eliminate person-to-person transmission among young children. Although it is often recommended that household and other close contacts of persons positive for HBsAg receive hepatitis B vaccination, levels of hepatitis B vaccination coverage tend to be low among household contacts and other persons in risk populations.[321,322]

Sexual Transmission

HBV is efficiently transmitted during heterosexual and male-to-male sexual contact.[320,323–327]

Sexual transmission is a leading cause of HBV infection among adults in areas of low HBV prevalence, including the United States and Europe,[234,328] and it is a contributing cause of HBV transmission in moderately endemic (e.g., India, Bulgaria)[329,330] and highly endemic (e.g., China)[320,331] countries. The most common risk factors for sexual transmission include number of years of sexual activity, number of lifetime sexual partners, unprotected sex with an infected partner, unprotected sex with more than one partner, and history of another sexually transmitted infection. Among persons seeking

TABLE 25.2 Hepatitis B Disease Burden Model: Proportion of Future Hepatitis B–Related Deaths Without Vaccination, as Related to Age at Acquisition of Infection

	Life Stage When HBV Infection Was Acquired		
Region	Perinatal (%)	Early Childhood (%)	Late (%)
Africa	18	52	30
Americas	23	37	40
Eastern Mediterranean	13	47	40
Europe	16	40	40
Southeast Asia	17	48	35
Western Pacific	26	47	26
Global	21	48	31

HBV, hepatitis B virus.

From Goldstein ST, Zhou F, Hadler SC, et al. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. Int J Epidemiol. *2005;34:1329–1339.*

care for sexually transmitted infections, the prevalence of HBV infection is typically several times greater for men who have sex with men (MSM) than for heterosexual patients.[332–337] Unprotected anal sex increases HBV transmission risk for both MSM and heterosexual women.[338–340]

Percutaneous Exposure

In many developing countries, unsafe injections and other unsafe percutaneous or permucosal procedures conducted in medical and other care settings (i.e., acupuncture) contribute substantially to transmission of bloodborne pathogens (e.g., HBV, HCV, and HIV).[341–344] In the United States and Europe, HBV outbreaks continue to occur in dialysis centers, medical wards, surgery services, nursing homes, and residential care facilities.[269,345,346] The risk of infection after needlestick exposures to HBV is high—approximately 30% to 60% after inoculation with HBeAg-positive blood and 10% to 30% after percutaneous or permucosal exposure to HBeAg-negative blood.[347–349] By comparison, the risks of HCV and HIV transmission from percutaneous exposures are approximately 2% and 0.2%, respectively.[350,351]

As recently as 2000, unsafe injection practices have been estimated to cause up to 20 million HBV infections worldwide. However, exposures to unsafe injections decreased over the following decade, resulting in an estimated 91% reduction in injection-related HBV infection, or about 1.68 million new infections per year.[352]

Blood transfusions were once a frequent cause of HBV transmission. However, with (a) the advent of donor exclusion guidelines, (b) recommendations to invite voluntary, nonremunerated blood donors from low-risk populations, and (c) testing of donated blood for HBsAg, anti-HBc, and HBV DNA, transfusion-associated hepatitis B is now rare in the United States and many other industrialized countries.[353] Currently, the risk of receiving potentially HBV-infectious blood after donor screening and blood bank testing is approximately 1 in 500,000 per unit exposure.[354] This risk remains higher in countries where these measures have not been as successfully implemented. A 2000 study conducted in sub-Saharan Africa revealed that approximately 30% of blood donations were not screened for HBV.[355]

However, donor recruitment and screening of blood donations for bloodborne pathogens have improved in many sub-Saharan countries. From 2000–2004 to 2010–2011, the median annual number of units donated in each of 36 sub-Saharan countries evaluated increased, as did the number of countries screening at least 95% of blood donations for HBV; over this period, the median national prevalence of donation with markers of HBV infection decreased from 7% to 4%.[356]

Clotting factor concentrates from donors not tested for HBV, along with concentrates that were not mechanically processed to ensure viral inactivation, can transmit HBV and other bloodborne infections to patients with hemophilia and other clotting disorders.[357] Since 1987, clotting factor concentrates licensed in the United States and other industrialized countries have been manufactured by processes known to inactivate HBV.[358]

Injection of illicit drugs is a major nonmedical source of percutaneous exposures to HBV. Globally, injection-drug use is a common mode of HBV transmission; incidence rates of 10% to 31% per year have been reported among persons with illicit drugs (PWID).[359–363] Risk for HBV transmission increases with the number of years of drug use, with frequency of injection, and with sharing of drug-preparation equipment (e.g., cottons, cookers, and rinse water), independent of syringe sharing.[359,361,364] Sexual risk factors (such as selling sex for money or drugs) contribute to HBV transmission among drug users.[365]

In populations of PWID, global prevalence of HBV infection (anti-HBc positivity) ranges from 42% to 85%; prevalence of HBsAg also varies widely, at 2.8% in Brazil, 11.8% in the United States, and 21.3% in Estonia.[366] In 2010, an estimated 1.2 million (range: 0.3–2.7) PWID were HBsAg-positive worldwide, with an 8.4% prevalence of HBsAg among all among PWID.

Geographic Patterns of Transmission

The frequency and patterns of HBV transmission vary markedly in different parts of the world (Fig. 25.11). HBV prevalence is greatest in areas of moderate (≥2%) to high (≥8%) HBsAg endemicity, including most of Asia (except Japan and India), most of the Middle East, the Amazon basin, most Pacific Island groups, and Africa, particularly the western portion of the continent; however, certain populations in areas of low endemicity (e.g., Australian Aborigines, New Zealand Maoris, and Alaskan Native Americans) also have elevated prevalence of HBV infection. In the absence of vaccination, the lifetime risk for HBV infection is greater than 60% in areas of high endemicity; rates of chronic liver disease and liver cancer also are very high in these areas.[95]

In highly endemic countries, most infections are acquired during the perinatal period and early childhood, when the risk for developing chronic infection is greatest; acute hepatitis B is rarely detected because most infections in early childhood are asymptomatic. In recent years, many of these countries have successfully introduced hepatitis B vaccine into their routine childhood immunization schedule, resulting in lower HBV and HCC prevalences.[367–371]

For example, in 2002, China began routinely providing infants with a dose of vaccine at birth, followed by the complete hepatitis B vaccine series. As a result, from 1992 through 2006, HBsAg prevalence among children younger than 5 years of age declined from 9.7% to 1%[367]; however, despite these declines, prevalence remains high among older unvaccinated populations. In Alaska, after routine vaccination of infants and newborns in 1984, the incidence of HCC in children declined from three cases per 100,000 population (1984 to 1988) to zero cases, where it has remained stable since 1999.

In areas of intermediate endemicity, the lifetime risk for HBV infection is 20% to 60%, and infections occur in all age groups. Acute hepatitis B is common in these areas because many infections occur in adolescents and adults. However, high rates of chronic HBV infection are maintained primarily because of infections occurring in infants and children.

Most HBV infections in areas of low endemicity occur in adults in relatively well-defined risk groups,[95] but a high proportion of chronic infections might occur as a consequence of perinatal and early childhood exposures. In the United States, more than one-third of chronic HBV infections were acquired perinatally or in early childhood before implementation of perinatal hepatitis B prevention programs and routine infant hepatitis B vaccination.[265]

The contribution of perinatal HBV transmission to the overall hepatitis B disease burden varies substantially in different geographic regions. In eastern and southeastern Asian countries and the Pacific Islands, 35% to 50% of HBsAg-positive women are also HBeAg-positive.[300,372–375] In these countries, approximately 3% to 5% of all infants develop chronic HBV infection after HBV infection during birth, and as many as 30% to 50% of all chronic infections among children result from perinatal transmission. In areas of high endemicity where the prevalence of HBeAg among pregnant

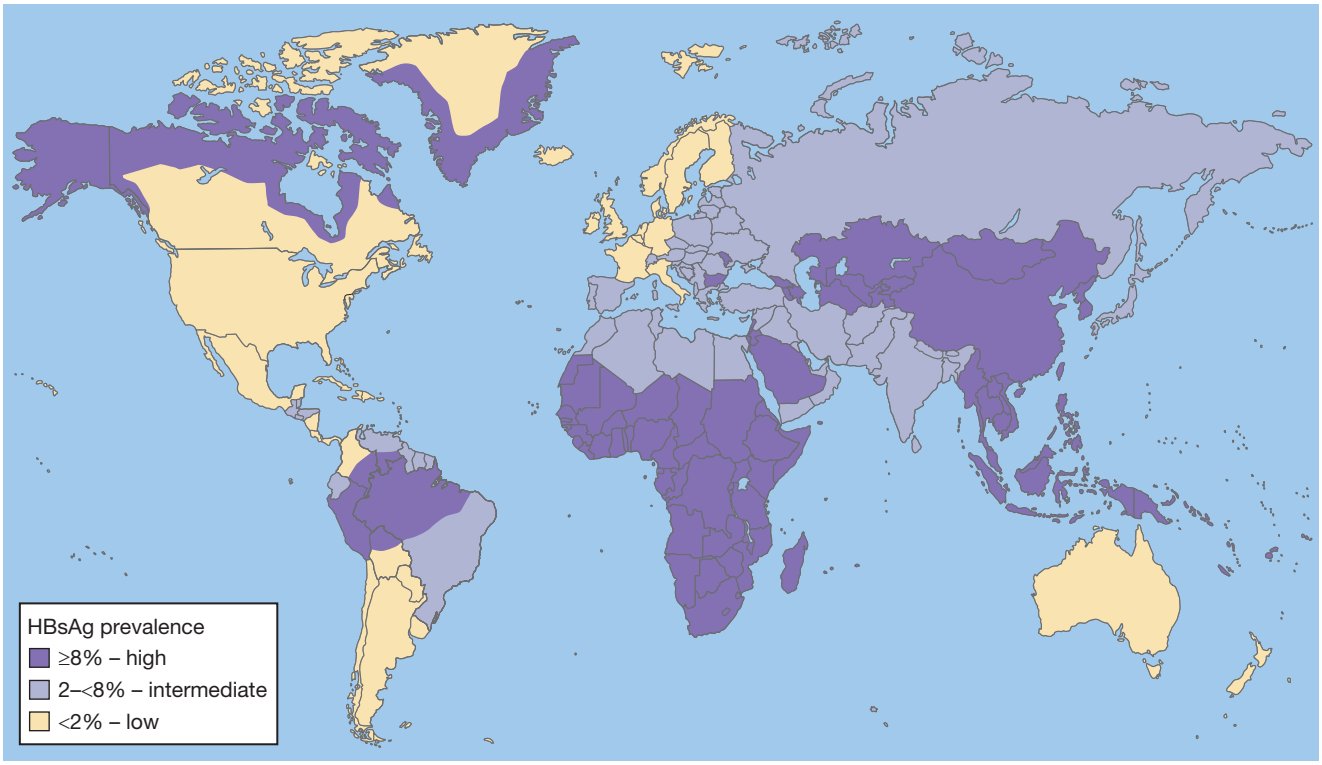


Figure 25.11. Geographic distribution of chronic hepatitis B virus infection. HBsAg, hepatitis B surface antigen.

women is low (i.e., Africa, South America, and the Middle East), perinatal HBV transmission contributes less to the pool of children with chronic infection than does postnatal early childhood transmission.[376–380] In general, fewer than 10% to 20% of all chronic infections among children in these areas result from perinatal exposures.

Risk Populations, Based on U.S. Data

In the United States the most common routes of HBV transmission are sexual contact and injection-drug use. From 2011 to 2013, injection-drug use surpassed sexual contact to become the most commonly reported exposure among cases of acute HBV infection reported to CDC accompanied by behavioral risk information. Of 606 such cases reported in 2013, a total of 55% were among persons reporting injection-drug use; 37% reported risks from heterosexual contact, and 7% were MSM. In some states, HBV transmission attributed to injection-drug use is reversing the decades-long trend of declining HBV incidence.[271]

Persons at Risk for HBV Infection Through Sexual Exposure

HBV is readily transmitted from an infected person to a susceptible partner during sexual contact. Serologic evidence of HBV infection (i.e., positive for anti-HBc) can range from 10% to 40% among adults seeking treatment in sexually transmitted disease clinics[325,337,381] and from 10% to 40% among MSM younger than age 30 years. Additional studies have identified HBV infection in 20% to 42% of susceptible heterosexual partners of persons with acute hepatitis B.[382–384] Among susceptible heterosexual spouses of persons with chronic HBV infection, the seroprevalence of HBV infection ranged from 25% to 59%.[307,308,385] Population-based serosurveys also have demonstrated an association between HBV infection and sexual health and behaviors. Whereas approximately 4.6% of the general population has serologic evidence of HBV infection,[386] NHANES data from 1999 to 2008 revealed a 9.1% and 15.5% prevalence of past or current HBV infection among persons who were positive for antibody to herpes simplex virus type 2 and among persons reporting having 50 or more sexual partners over their lifetime, respectively.[386]

Contacts of Persons With Chronic Hepatitis B Virus Infection

Studies demonstrate varying rates of HBV transmission, ranging from 15% to 60%, among susceptible household contacts of chronically infected persons[307–313]; 3% to 20% of these contacts are chronically infected.[387] Of household contacts, risk for infection is highest among sexual partners and children.[311–313] Before hepatitis B vaccine was integrated into the U.S. childhood immunization schedule, an estimated 16,000 children younger than 10 years of age were infected with HBV annually from nonsexual person-to-person contact occurring beyond the postnatal period[388]; at that time, an estimated 18% of persons with chronic HBV infection acquired their infections postnatally during early childhood.[279] Since the adoption of routine infant immunization in 1992, HBV transmission among children has declined dramatically.[389]

Hemodialysis Patients

Because hemodialysis presents frequent opportunities for blood exposures, hemodialysis patients are at increased risk for hepatitis B infection. However, in the 1970s, dialysis centers adopted infection-control procedures (i.e., routine HBV screening, hepatitis B vaccination of susceptible dialysis patients, and management of HBsAg-positive patients) that

dramatically reduced rates of transmission. In 2013, only one person with acute HBV infection reported hemodialysis as a risk.[390] According to recent surveys of U.S. dialysis centers, prevalence of HBV among dialysis patients ranges from 1.0% to 2.4%[391,392]; annual incidence of new HBV infections is low in these settings, at 0.12% (2002).[392] Despite this progress, hemodialysis patients remain at risk for infection, as evidenced by outbreaks of HBV infection among unvaccinated patients.[345,393]

Incarcerated Persons

The seroprevalence of HBV infection among incarcerated adults in the United States ranges from 14% to 47% for both prison and jail inmates; 1% to 11.4% of inmates have evidence of chronic infection,[394–399] and prevalence is highest among female prisoners.[400] The high prevalence of infection observed among incarcerated adults primarily reflects infections acquired before incarceration. However, HBV is known to be transmitted within correctional facilities; incidence of transmission in this setting has ranged from 0.82% to 3.8%.[401–403] Inmates can remain at risk for hepatitis B after release into the community. A study of recidivist women reported an HBV seroconversion rate of 12.2 per 100 person-years between incarcerations,[400–404] compared with an estimated incidence of 0.03 per 100 person-years for the general U.S. population.[400] Increased rates are likely attributed to a return to high-risk behaviors after incarceration.[405] In a nationally representative sample of adult men 15 to 44 years of age, those who were recently incarcerated were much more likely to report engaging in high-risk sexual behaviors.[406]

Injection-Drug Users

In 2013, injection-drug use accounted for more than half of new HBV infections in the United States.[269–271] In a seroprevalence study of HBV infection among injection-drug users (IDUs) admitted to drug treatment in six U.S. cities, 64% (range: 50% to 81%) were seropositive for anti-HBc, with seroprevalence increasing with age.[407] Other studies of IDU populations have revealed 20% to 70% of IDUs ever infected with HBV and a 3% to 6% prevalence of chronic infection.[405–416] IDUs are at increased risk for HDV infection, which can be associated with HIV among HBsAg-positive PWID. From 1999–2013, of 2175 HBsAg-positive U.S. military veterans who were tested for HDV, 73 (3.4%) tested positive; a history of injection-drug use was a major risk factor for HDV infection.[417] From 2005 through 2006, a total of 50% of HBV-infected IDUs in Baltimore had serologic evidence of exposures to HDV.[418] Of 115 HIV-infected persons enrolled in international clinical trials and who were also HBsAg-positive, anti-HDV was detected in 15 (13.3%) of the HIV/HBV coinfected study participants.[419]

The incidence of HBV infection among unvaccinated IDUs is high, ranging from 10 to 31 per 100 person-years.[361,363,420] Risk for HBV transmission increases with number of years of drug use, frequency of injection, and sharing of drug-preparation equipment (see Percutaneous Exposure).[359,361,363,420] The adoption of safe-infection strategies has led to decreased rates of HBV infection in this population, as have enhanced efforts to vaccinate IDUs against hepatitis B. Hepatitis B vaccination campaigns targeting IDUs through syringe exchange programs are cost saving[421]; however, rates of vaccination remain low in most cohorts of IDUs.[422–424] Recent increases in injection-drug use behaviors in the United States are contributing to increases in viral hepatitis incidence in some states.[272,425] For hepatitis B infection, most are among persons older than 30 years of age, an age group not reached by childhood and adolescent vaccination earlier in life.[271]

Persons at Risk for Occupational Exposure

In the United States, from 1998 to 2008 a total of 18 outbreaks were reported, resulting in 173 persons with incident HBV infection. Transmission was associated in most cases with unsafe injection practices occurring in inpatient, outpatient, and long-term care facilities, including nursing homes and assisted-living facilities.[426] During 2008–2014, another 23 outbreaks of HBV transmission were reported to CDC involving 175 cases; 17 of these outbreaks occurred in long-term care facilities.

Based on reported cases of hepatitis B in the United States, persons with diabetes mellitus have about a twofold greater risk of acute HBV infection.[427] Data for the period 1999–2010 from the NHANES, indicated a 60% higher seroprevalence of antibody to HBcAg (indicative of past or present HBV infection) overall among persons 18 years of age and older with diagnosed diabetes compared with those without diabetes. Lack of adherence to standard infection-control precautions and failure to implement longstanding recommendations against sharing fingerstick devices place diabetes patients at risk for acquiring infections from bloodborne pathogens such as HBV. Percutaneous exposures to HBV occur as a result of assisted monitoring of blood glucose and other procedures involving instruments or parenteral treatments shared between persons. Lapses in infection control during assisted blood glucose monitoring that have led to HBV transmission include multipatient use of fingerstick devices designed for single-patient use and inadequate disinfection and cleaning of blood glucose monitors between patients.

Studies conducted in the United States during the 1970s and 1980s demonstrated a 10% to 30% prevalence of HBV infection among healthcare workers.[428–433] At that time, infection was associated with frequency of blood contact, needlestick exposures, direct patient contact, and years of employment, and occurred mainly among certain occupational groups, including physicians, nurses, laboratory technicians, and emergency medical technicians. However, with the widespread vaccination of healthcare workers and implementation of standard precautions in healthcare settings, the incidence of new infections has declined substantially in the United States and is now less than that of the general population.[434] In many other countries, reaching a high HBV immunization coverage in healthcare workers remains a challenge.

Theoretically, public safety workers whose job duties present opportunities for exposures to blood are at increased risk for HBV infection.[435–437] However, although occupational exposures can occur, the prevalence of HBV infection in these workers (e.g., police officers, firefighters, and corrections officers) typically does not differ from that of the general population when adjusted for race and age.[422,435] Instead, infection among public safety workers is most often associated with nonoccupational risk factors.[435] No increased risk for HBV infection resulting from occupational exposures has been documented in persons infrequently exposed to blood or body fluids, such as ward clerks, dietary workers, maintenance workers, housekeeping personnel, lifeguards, teachers, and persons employed in child daycare settings.[432]

Developmentally Disabled Persons in Long-Term Care Facilities

High rates of HBV infection were documented among residents in institutions for developmentally disabled persons before hepatitis B vaccine was used in these facilities and integrated into the routine U.S. childhood immunization schedule.[438,439] In the United States, the risk for HBV infection in these institutions has declined substantially over the past three decades. However, because HBsAg-positive persons can

reside in these institutions, unvaccinated residents and workers remain at risk for HBV infection.

Travelers to Regions With Moderate or High Hepatitis B Virus Endemicity

Many travelers (24% to 49%) report activities or exposures that might place them at risk for hepatitis B transmission while visiting countries of moderate or high HBV endemicity, such as sexual contact with a new (local) partner, receipt of medical or dental care, and other direct exposures to blood.[440–442] At particular risk are persons traveling to endemic areas for the purpose of obtaining planned healthcare services (e.g., renal transplantation), which can expose these patients to HBV and lead to healthcare-associated HBV transmission to patients and their close contacts.[441,443] Although hepatitis B vaccination can greatly reduce risk for HBV infection among travelers, few (19% to 29%) travelers from low to more highly endemic areas for HBV recall having received hepatitis B vaccination before departing from their home countries.[440–442,444,445]

Studies of HBV incidence indicate that short-term travelers to areas where HBV infection is of intermediate or high endemicity are generally not at risk for HBV infection.[446–449] However, because length of time in an endemic country increases the likelihood of exposure to HBV, risk of HBV transmission is greatest for persons working or traveling in endemic countries over extended periods. One prospective study of 7887 travelers found that of two documented travel-associated HBV infections, both occurred among the 97 persons who worked abroad[448]; none of the 7317 persons traveling for vacation acquired HBV.[444,445] Another study of long-term missionaries in developing countries demonstrated that 5.5% became positive for anti-HBc, for an HBV incidence rate of 0.8 per 100 person-years.[450] In Amsterdam, a study of persons with acute hepatitis B reported over a 12-year period estimated an incidence rate of 4.5 per 100,000 travelers to HBV-endemic countries; 89% of cases were among long-term travelers or immigrants from highly endemic areas returning from visits with friends or relatives in their countries of origin.[447]

PREVENTION OF HEPATITIS B VIRUS

Passive Immunization

The discovery that passively acquired anti-HBs can provide protection against acute hepatitis B and chronic HBV infection when administered soon after exposure led to the development and therapeutic use of HBIG, a specific immunoglobulin containing high concentrations of anti-HBs. Currently, HBIG is recommended only for postexposure prophylaxis (often in combination with hepatitis B vaccine) in specific settings.[451]

HBIG is prepared by fractionation from serum containing high concentrations of anti-HBs and that has been screened for HBsAg and antibodies to HIV and HCV. The process inactivates the potential presence of HIV in the final product.[452,453] Since 1999, all HBIG products available in the United States and many other countries have been manufactured by methods that inactivate HCV and other viruses.

The efficacy of HBIG in preventing clinical hepatitis B or the development of chronic infection is approximately 75% in adults,[349,454,455] but the protection lasts only several months. HBIG is expensive and usually not affordable in the developing world.

A major use of HBIG is as an adjunct to hepatitis B vaccine in preventing perinatal HBV transmission. Untreated, 70% to 90% of infants born to HBeAg-positive mothers become infected at birth and develop chronic HBV infection.[296,456] Of these chronic infections, 75% can be prevented by treatment with HBIG started within 48 hours of birth and with subsequent doses at ages 3 and 6 months[295,457]; however, children living in a household with an infected mother often become infected after the first year of life.[300] Immunoprophylaxis with both HBIG and hepatitis B vaccine increases the efficacy of preventing perinatal HBV transmission from 85% to 95% and provides long-term protection.[296,297,458]

HBIG is also indicated for postexposure prophylaxis after needlestick or other percutaneous injuries, following the demonstration of the efficacy of HBIG alone in preventing acute clinical hepatitis B in needlestick recipients in two large multicenter trials.[348,349] With the availability of hepatitis B vaccine, postexposure prophylaxis protocols that take into account the vaccination and serologic status of the exposed person recommend administration of HBIG to unvaccinated persons and to vaccine nonresponders.

The efficacy of HBIG in preventing clinical hepatitis B or chronic HBV infection after sexual exposure to an acutely infected partner is also approximately 75% when administered within 7 days of exposure.[383] Current recommendations for postexposure prophylaxis after sexual exposure to an infected person include administration of HBIG and hepatitis B vaccine.

HBIG alone or in combination with an oral antiviral agent is also used for the prevention of HBV reinfection of liver grafts in patients undergoing liver transplantation because of fulminant or CHB.[459]

The standard dose of HBIG is 0.5 mL for postexposure prophylaxis of infants born to HBsAg-positive mothers and 0.06 mL/kg for all other indications. HBIG should be administered intramuscularly and may be administered simultaneously with hepatitis B vaccine but in a different injection site. For neonates (<1 month old) and infants (1 to 12 months old), HBIG should be administered intramuscularly in the anterolateral thigh using an appropriate needle length (usually ⅝ inch [or 1.5 cm] for neonates, and ⅞ to 1 inch [or 2.2 to 2.5 cm] for infants). For older children, adolescents, and adults, HBIG should be delivered in an appropriate muscle mass (e.g., deltoid or gluteal) using a needle length appropriate for the person's age and size.[460]

Various protocols exist that use high-dose HBIG intravenously or low-dose HBIG administered via the intramuscular or subcutaneous routes for HBV patients undergoing liver transplantation.[461,462]

In such patients who need long-term protection, HBIG was used previously as monotherapy, and it is used more recently in combination with oral nucleoside or NAs.[463]

Active Immunization

Development of Vaccines

Safe and effective hepatitis B vaccines have been commercially available since 1982. The American microbiologist Maurice Hilleman at Merck used three treatments (pepsin, urea, and formaldehyde) of blood serum together with rigorous filtration to yield a product that could be used as a safe vaccine. Hilleman hypothesized that he could make an HBV vaccine by injecting patients with hepatitis B surface protein. In theory, this would be very safe, as these excess surface proteins lacked infectious viral DNA. The first available vaccines were indeed produced by harvesting HBsAg (the 22-nm particle) from the plasma of persons with chronic HBV infection. Concerns about transmission of bloodborne pathogens, including HIV, from plasma-derived vaccines have proved to be unfounded.[464]

Subsequently, the development of recombinant DNA technology to express HBsAg in other organisms offered the potential to produce unlimited supplies of vaccine,[465–467] and recombinant DNA vaccines have replaced the plasma-derived vaccines (Table 25.3). Hepatitis B vaccines are formulated to

TABLE 25.3 Hepatitis B Vaccines[a] Prequalified[b] by the World Health Organization

Manufacturer	Country	Vaccine Type	Brand Name	Prequalification Date	Presentation	Administration	Shelf Life at 2°–8°C[c]
Sanofi Pasteur	France	DTPa-HepB-Hib	Hexacim	12/19/2014	1-dose vial	IM	36 mo
Bio Farma	Indonesia	HepB	Hepatitis B	05/13/2004	1-dose Uniject	IM	24 mo
Bio Farma	Indonesia	DTPw-hepB	DTP–Hep B	10/07/2004	5- & 10-dose vial	IM or SC	24 mo
Bio Farma	Indonesia	DTPw-hepB-Hib	DTP–Hep B-Hib	12/19/2014	5-dose vial	IM	24 mo
Center for Genetic Engineering and Biotechnology	Cuba	HepB	Heberbiovac HB	12/11/2001	1- & 10-dose vial	IM	48 mo
Biological E limited	India	DTPw-HepB-Hib	DTP-HepB-Hib	11/27/2014	5- & 2-dose vial	IM	24 mo
Biological E limited	India	DTPw-HepB-Hib	DTP-HepB-Hib	08/31/2011	1- & 10-dose vial (Hib lyophilized)	IM	24 mo
Biological E limited	India	DTPw-HepB-Hib	DTP-HepB-Hib	05/18/2012	1- & 10-dose vial (liquid)	IM	24 mo
Berna Biotech Korea Corporation, a Crucell company	Republic of Korea	Hepatitis B	Hepavax-Gene	03/23/2004	1- & 10-dose vial	IM	36 mo
Berna Biotech Korea Corporation, a Crucell company	Republic of Korea	Hepatitis B	Hepavax-Gene-TF	07/31/2012	1-dose vial, thimerosal free	IM	36 mo
Berna Biotech Korea Corporation, a Crucell company	Republic of Korea	DTPw-hepB-Hib	Quinvaxem	09/26/2006	1-dose vial	IM	36 mo
Berna Biotech Korea Corporation, a Crucell company	Republic of Korea	DTPw-hepB-Hib	Quinvaxem in Cpad	12/24/2014	1-dose vial Cpad, compact prefilled autodisabled device	IM	24 mo
GlaxoSmithKline	Belgium	Hepatitis B	Engerix-B	01/01/1987	1-, 10-, & 20-dose vial	IM	24 mo
GlaxoSmithKline	Belgium	DTPw-hepB	Tritanrix-HB	04/01/1998	1-, 2-, &10-dose vial	IM	24 mo
GlaxoSmithKline	Belgium	DTPw-hepB-Hib	Tritanrix-Hib	04/16/2006	1-dose vial (+ lyophilized Hib)	IM	36 mo
GlaxoSmithKline	Belgium	DTPw-hepB-Hib	Tritanrix-Hib	10/29/2003	2-dose vial (+ lyophilized Hib)	IM	36 mo
LG Life Science, Ltd.	Republic of Korea	Hepatitis B	Euvax B	11/22/1996	1-, 2-, 6-, & 10-dose vial	IM	36 mo
LG Life Science, Ltd.	Republic of Korea	DTPw-hepB-Hib (lyophilized)	Euforvac-Hib inj.	08/24/2012	1- & 2-dose vial + lyophilized Hib	IM	36 mo
Panacea Biotec	India	DTPw-hepB-Hib	Easy Five-TT	10/02/2013	1- & 10-dose vial	IM	24 mo
Serum Institute of India, Ltd.	India	HepB	Hepatitis-B vaccine (rDNA)	11/12/2004	1- & 10-dose adult; 1-dose pediatric	IM	24 mo
Serum Institute of India, Ltd.	India	DTPw-hepB	Diphtheria, tetanus, pertussis, and hepatitis-B	07/212006	1-dose ampule & 10- & 20-dose vial	IM	24 mo

TABLE 25.3 Hepatitis B Vaccines[a] Prequalified[b] by the World Health Organization *(Continued)*

Manufacturer	Country	Vaccine Type	Brand Name	Prequalification Date	Presentation	Administration	Shelf Life at 2°–8°C[c]
Serum Institute of India, Ltd.	India	DTPw-hepB-Hib		05/26/2010	1-, 2-, 10-dose vial + lyophilized Hib	IM	24 mo
Serum Institute of India, Ltd.	India	DTPw-hepB-Hib		09/22/2010	1-, 2-, 10-dose (liquid)	IM	24 mo
Shantha Biotechnics Private, Ltd.	India	HepB	Shanvac-B	06/10/2002	1-, 2-pediatric dose vial and 1-, 6-, 10-, & 20-dose vial	IM	36 mo
Shantha Biotechnics Private, Ltd.	India	DTPw-hepB-Hib	Shan-5	04/29/2014	1-, 10-dose vial liquid	IM	24 mo

D, diphtheria; HepB: hepatitis B; Hib: *Haemophilus influenzae* type b; IM, intramuscular; P, pertussis (w, whole cell; a, acellular); SC, subcutaneous; T, tetanus.

Merck, Sharpe and Dohme (MSD) manufactures two hepatitis B–containing vaccines: Recombivax (Recombinant Hepatitis B), and Comvax (Hib Conjugate [meningococcal protein conjugate] and HepB [recombinant]). Recombivax has three presentations: pediatric single dose; adult single dose; and dialysis single dose. Comvax has one pediatric dose presentation. For both vaccines, administration is intramuscular and shelf life is 36 months at 2°–8°C. These vaccines are not prequalified by the WHO.

[a]For purchase by UNICEF and other United Nations Agencies.

[b]As of August 25, 2015. For updates, see www.who.int/immunization_standards/vaccine_quality/PQ_vaccine_list_en/en/.

[c]Shelf life is from the current licensing information for these vaccines.

contain 2.5 to 40 µg of HBsAg protein and an aluminum phosphate or aluminum hydroxide adjuvant[468]: 0.25 mg in pediatric-dosed vaccines, and 0.5 mg in adult-dosed vaccines. A new recombinant hepatitis B vaccine that is intended for adult patients with renal insufficiency uses alum and lipid A as adjuvants.[469] Since 1999, hepatitis B vaccines available in the United States for use in infants and children have not contained thimerosal as a preservative because of ill-founded concerns about possible neurodevelopmental effects of mercury.[470] However, there is no evidence of any harmful effects from the small amounts of thimerosal in hepatitis B vaccine.[471] Thimerosal continues to be used as a preservative in hepatitis B vaccines available in many countries and is particularly important in preventing bacterial contamination in multidose vials.

Recombinant DNA Vaccines

Most licensed recombinant DNA hepatitis B vaccines consist of a 226-amino-acid *S* gene product (HBsAg protein).[465] The yeast-produced vaccines, which are the most widely used, are manufactured by expression of HBsAg protein in genetically engineered yeast cells *(Saccharomyces cerevisiae)* that contain the *S* gene.[472] The expression plasmid generally contains only the 3′ portion of the *S* gene, and only the major HBsAg protein, without pre-S epitopes, is produced. As a result of biochemical and biophysical purification, there is no detectible yeast DNA and only trace amounts of yeast proteins (1–5%) in the final vaccine products.[473] Mammalian cell–derived vaccines (e.g., continuous mammalian cell line, like the mouse c127 clonal cell line) containing glycosylated pre-S1 and pre-S2 proteins, in addition to the major HBsAg protein, have also been produced in France, Germany, and Israel and are licensed in Israel, Hong Kong, India, the Philippines, and Vietnam.[35,474] Evidence exists that vaccines containing the pre-S1 and pre-S2 proteins induce a faster and higher anti-HBs response as compared to yeast derived S containing vaccines and might be effective in persons who have genetic nonresponse to the major HBsAg protein. However, the high cost of production for vaccines with pre-S1 and pre-S2 antigens limits their use and availability.[475–477]

In the production of recombinant DNA hepatitis B vaccines, working seeds are derived from a master seed of the transformed yeast. Whenever a lot of vaccine is needed, yeast from the working seed is used to start the fermentation in large vessels. The HBsAg is then purified to eliminate yeast components by various physical separation techniques, including chromatography and filtration. The expressed HBsAg polypeptide self-assembles into immunogenic spherical particles closely resembling the natural 22-nm particles found in the serum of persons with chronic HBV infection. The *a* determinant that is responsible for the most important immune response is exposed on the surface of the artificial HBsAg particle, as it is on the natural particle. The artificial particles differ from natural particles only in the glycosylation of HBsAg. In contrast to the production of yeast derived nonglycosylated small envelope antigen, mammalian cells transfected with the genes coding for two to three of the HBV envelope proteins secrete the glycosylated viral surface antigens particles into the growth medium from which they are purified by physical means.

Combination Vaccines

Several vaccine manufacturers have produced combination vaccines containing a hepatitis B vaccine component. These combination vaccines include diphtheria and tetanus toxoids and whole-cell pertussis (DTwP)–hepatitis B vaccine; DTwP-*Haemophilus influenzae* type b conjugate (Hib)–hepatitis B vaccine; diphtheria and tetanus toxoids and acellular pertussis (DTaP)–hepatitis B vaccine; DTaP–Hib–inactivated poliovirus vaccine (IPV)–hepatitis B vaccine; DTaP–IPV–hepatitis B vaccine; Hib–hepatitis B vaccine; and hepatitis A–hepatitis B vaccine. For each of these combination vaccines, the manufacturer has shown that the components remain sufficiently immunogenic to elicit protective levels of anti-HBs.[478–481]

However, a hexavalent (DTaP-IPV-Hib- hepatitis B) vaccine, licensed in Europe in 2000, was suspended from the market in 2005 because of concerns over its ability to provide long-term protection against hepatitis B. Until suspension, approximately 10 million doses were distributed worldwide, including almost 90% of the doses in Germany, Austria, and Italy. Studies

carried out 5 years after priming showed that most vaccinees that lost protective concentrations (≥10 mIU/mL) of anti-HBs had a strong anamnestic response when boosted with a single dose of monovalent hepatitis B vaccine.[482,483]

Follow-up studies carried out in teenagers primed as infants with such hexavalent vaccines are currently in progress to assess whether booster is needed to maintain long-term protection during adolescence and adulthood when risk of acquiring HBV does increase significantly.

Providers should not attempt to make extemporaneous mixtures of vaccines in the same syringe at the point of use because the results are unpredictable unless verified by the manufacturer and national disease-control authorities. Diphtheria-tetanus-pertussis (DTP)-containing hepatitis B combination vaccines from several manufacturers are increasingly used worldwide in universal immunization programs. When immunizing against HBV at birth, only monovalent hepatitis B vaccines should be used.

Stability of Vaccine

The recommended storage temperature for hepatitis B vaccine is between 2°C and 8°C. The vaccine is generally stable for 3 to 4 years from the date of manufacture if stored in this temperature range. However, the stability of hepatitis B vaccines from different manufacturers might vary considerably. Therefore, the package insert for each vaccine should be consulted for the manufacturer's recommended shelf life.

Most hepatitis B vaccines are relatively heat stable and have only a small loss of potency when stored at 20°C to 26°C for up to 1 year, at 37°C for 2 to 6 months, and at 45°C for 1 week.[484,485] No significant differences were found in seroconversion rates and levels of protective antibody between infants who received a first dose that had been stored in the cold chain and those who received a first dose stored for up to 1 month at tropical temperatures.[486] The excellent heat stability of hepatitis B vaccine allows the vaccine to be administered at birth to prevent perinatal HBV transmission during home deliveries where refrigeration is not available. In demonstration projects, use of hepatitis B vaccine in prefilled, single-use injection devices (e.g., Uniject) outside the cold chain has been reported to simplify logistics, minimize vaccine wastage, and facilitate the speed and efficiency of immunization at birth during home visits.[486,487] The outside-the-cold-chain use of hepatitis B vaccines could greatly enhance hepatitis B vaccine delivery in outreach services.[488–491]

Hepatitis B vaccine and combination vaccines that contain hepatitis B vaccine must not be frozen. The freezing of hepatitis B vaccine causes the HBsAg protein to dissociate from the alum adjuvant and thus to lose its immunogenicity/potency.[468,492,493] The freezing point of hepatitis B vaccine is approximately −0.5°C.[494]

Dosage and Route of Administration

The quantity of HBsAg protein per dose that induces a protective immune response in infants and children varies by manufacturer (range: 2.5 to 10 µg) and by composition of the envelope protein(s), and is partially related to the vaccine production processes. In general, the vaccine dosage for infants and adolescents is 50% lower than that required for adults. There is no international standard of vaccine potency expressed in micrograms of HBsAg protein, and the relative efficacy of different vaccines cannot be assessed only on the basis of differences in HBsAg content.[495]

Given the differences in the manufacturing processes, the quantity of HBsAg protein per dose that will induce a protective immune response differs among the various vaccine products depending on the recipient's age. Vaccines produced by each manufacturer have been evaluated in clinical trials to determine the age-specific dosage that achieves the maximal seroprotection rate. Persons who respond to hepatitis B vaccine (complete and correct schedule) with concentrations of anti-HBs of 10 mIU/mL or greater are protected against acute hepatitis B and chronic infection. The standard pediatric volume of administration is 0.5 mL. With lower volumes, a dose might be administered that is inadequate to produce the required immune response using standard hubbed syringes. Internationally marketed hepatitis B vaccines are considered immunologically comparable and can be used interchangeably.[496]

Hepatitis B vaccine should be administered by intramuscular injection in the anterolateral aspect of the thigh of infants and children younger than 24 months old, and in the deltoid muscle of older children, adolescents, and adults. Administration in the buttock is not recommended because of an association with decreased protective antibody levels in some studies, probably because of inadvertent subcutaneous injection or injection into deep fat tissue.[497] If hepatitis B vaccine is administered on the same day as another injectable vaccine, it is preferable to give the two vaccines in different limbs. If more than one injection must be administered in the same limb, the thigh is the preferred site of injection because of the greater muscle mass; the injection sites should be 1 to 2 inches (2.5 to 5.0 cm) apart so that any local reactions are unlikely to overlap.[460]

Although low dosages of hepatitis B vaccine administered intradermally have been shown to reduce costs of adult vaccination and to partially bypass low response,[498] this route of administration is technically difficult and currently not recommended because seroconversion rates among adults and children have been inconsistent.[499,500] This may change with the use of more standardized intradermal devices.[501–503] Data on the long-term protection afforded by the intradermal route are still limited.

No clinically significant increase in adverse reactions or interference in antibody responses has been observed when hepatitis B vaccine has been administered simultaneously with, but at a different site from, other childhood or adult vaccines.[460,478,479,504–511] Hepatitis B vaccine can be administered at the same time as other vaccines (e.g., DTP-containing, oral poliovirus [OPV], IPV, hepatitis A, Hib, measles, bacille Calmette-Guérin [BCG], and yellow fever vaccines). Hepatitis B vaccines do not interfere with the immune response to any other vaccine, and vice versa.[460] Thus, the birth dose of hepatitis B vaccine can be administered at the same time as the BCG vaccine, preferably within 24 hours of birth.[512]

Available immunogenicity data from "off-schedule" vaccination suggest that if the series is interrupted after the first dose, the second dose should be administered as soon as possible, and the second and third doses should be separated by an interval of at least 2 months; if only the third dose is delayed, it should be administered as soon as possible.[513–516] In any age group, interruption of the vaccination schedule does not require restarting the vaccine series.[517,518]

RESULTS OF VACCINATION

Vaccine Immunogenicity and Schedules

Historically, the standard three-dose hepatitis B vaccine series has consisted of two priming doses administered 1 month apart and a third dose administered 6 months after the first dose. Multiple schedules have been used successfully: at birth and at 1 and 6 months of age; at 2, 4, and 6 months of age; at 3, 5, and 11 months of age; at 8, 12, and 16 weeks and 12 or 15 months of age; and at 6, 10, and 14 weeks of age (in the WHO's Expanded Programme on Immunization [EPI]

TABLE 25.4 World Health Organization's Recommended Options for Adding Hepatitis B Vaccine to Childhood Immunization Schedules

No Birth Dose			Hepatitis B Vaccine Options[a]		
Age	**Visit**	**Other Antigens**	**I**	**II**	**III**
Birth	0	BCG (OPV0)[b]	—	HepB-birth[c,d]	HepB-birth[c,d]
6 wk	1	OPV1, DTP1	HepB1[e]	HepB2[d]	HepB2[e]
10 wk	2	OPV2, DPT2	HepB2[e]		HepB3[e]
14 wk	3	OPV3, DTP3	HepB3[e]	HepB3[d]	HepB4[d]
9–12 mo	4	Measles	—	—	—

BCG, bacille Calmette-Guérin; DPT, diphtheria-pertussis-tetanus; DTP, diphtheria-tetanus-pertussis; OPV, oral poliovirus.
[a]Option 1 is recommended in countries that are not yet targeting prevention of perinatal hepatitis B virus (HBV) transmission; options II and III are recommended in countries that are targeting prevention of perinatal HBV transmission.
[b]Only given in countries where polio is highly endemic.
[c]To prevent perinatal HBV transmission, the birth dose should be given as close as possible to the time of delivery, preferably within 12 hours.
[d]Monovalent vaccine.
[e]Monovalent or combination vaccine.

schedule) (Table 25.4).[519–525] Increasing the interval between the first and second dose of hepatitis B vaccine has little effect on immunogenicity or final antibody concentration.[517,526] Longer intervals between the last two doses result in higher final antibody concentrations but not seroconversion rates.[527] Postvaccination testing is recommended for certain persons whose subsequent clinical management depends on knowledge of their immune status, including some healthcare and public safety workers; chronic hemodialysis patients, HIV-infected persons, and other immunocompromised persons; and sex or needle-sharing partners of HBsAg-positive persons. An anti-HBs concentration of 10 mIU/mL or more measured 1 to 3 months after administration of the last dose of the primary vaccination series is considered a reliable marker of protection against infection.

Revaccination of Nonresponders

Of persons who did not respond to a primary three-dose vaccine series with anti-HBs concentrations of 10 mIU/mL or greater, 25% to 50% responded to an additional vaccine dose, and 44% to more than 70% reportedly responded to a three-dose revaccination series[528–534] or to a regimen with two vaccines (offered each in one deltoid region at the same visit) followed by the same regimen 2 months later. Better response to revaccination occurs in persons who have measurable but low (<10 mIU/mL) levels of antibody after the initial series.[528,529,532] One study identified no difference in response to revaccination in persons receiving double the standard vaccine dose.[530] Persons who do not have protective levels of anti-HBs 1 to 2 months after revaccination either are primary nonresponders or are infected with HBV. Many factors are related to HBV vaccination nonresponse, including age older than age 40 years, male gender, obesity, smoking, chronic kidney/liver diseases, HIV infection, genetic factors, impaired vaccine storage conditions, and administration of the vaccine in buttocks.[529,535–537] Recently several third-generation vaccines containing pre-S/S antigens were shown to induce an adequate anti-HBs response in the majority of primary nonresponders.[538]

Infants and Children

A variety of hepatitis B vaccine schedules have been shown to induce levels of seroprotection of greater than 95% in infants (see "Vaccine Immunogenicity and Schedules" earlier). Programmatically, there is an advantage to administering the three doses of hepatitis B vaccine at the same time as the three doses of other childhood vaccines (e.g., DTP, Hib, IPV), and these schedules will accommodate use of DTP-, IPV-, and Hib-containing combination vaccines. A hepatitis B vaccine schedule administered with DTP or Hib vaccine will prevent infections acquired during childhood as well as infections acquired later in life. However, these schedules will not prevent perinatal HBV infections because they do not include a birth dose. Therefore, concern has been expressed that increased use of combination vaccines might jeopardize perinatal hepatitis B prevention programs.[539] To prevent perinatal HBV transmission in settings where combination vaccines are used, a four-dose hepatitis B vaccination schedule is needed, with the first dose administered at birth. High titers of maternal antibodies do not interfere with neonatal immune response to vaccination.[540] Use of four-dose hepatitis B vaccine schedules, including schedules with a birth dose, has not increased vaccine reactogenicity.[542]

Certain premature infants whose birthweight was less than 2000 g might have decreased seroconversion rates after administration of hepatitis B vaccine at birth.[543] However, by age 1 month, all premature infants, regardless of initial birthweight or gestational age, have a response to vaccination that is comparable to that of full-term infants.[544–547]

WHO recommends multiple options for adding hepatitis B vaccine to existing infant immunization schedules without requiring additional visits for immunization (see Table 25.4).[468] Schedules should optimize the percentage of children completing the hepatitis B vaccine series, which can be achieved with earlier administration of vaccine.[525] The WHO-recommended minimal interval between doses one and two is 4 weeks, and the minimum interval between doses two and three is 4 weeks. Schedules with these minimal intervals (e.g., 6, 10, and 14 weeks) have seroconversion rates similar to schedules with longer intervals, albeit with lower final anti-HBs concentrations. The WHO minimal recommended intervals for infant hepatitis B vaccination are shorter than those recommended in the United States. Although data are limited regarding long-term protection for schedules with shorter intervals, alternative schedules are often not feasible. In addition, concerns about long-term protection are of less practical significance in countries of high endemicity where most HBV infections are acquired in childhood.

Adolescents

Hepatitis B vaccine schedules that have been demonstrated to induce seroprotection rates of greater than 95% in adolescents include doses administered at 0, 1, and 6 months; 0, 2, and

4 months; and 0, 12, and 24 months.[514,526,548–550] In addition, for adolescents 11 to 15 years of age, the adult dose of hepatitis B vaccine can be used for administration at 0 and at 4 to 6 months.[551,552] This two-dose schedule produces anti-HBs concentrations equivalent to those obtained with the pediatric dose administered on a three-dose schedule. After 10 years of follow-up in the Czech Republic, 85.9% of the adolescents (12 to 15 years old) vaccinated with the two-dose schedule had anti-HBs levels of 10 mIU/mL or higher, as did 85.1% of those who received three pediatric doses.[553] However, the adolescents who were vaccinated with the two-dose schedule had slightly lower geometric mean concentration 10 years after receiving the doses. In Belgium, a 5-year follow-up showed fewer adolescents (11 to 15 years old) had anti-HBs levels of 10 mIU/mL or higher if vaccinated via the two-dose schedule (79.5% vs 91.4%, vaccinated with three doses), but after a challenge dose, all subjects mounted a rapid anamnestic response, indicating immune memory.[554]

Adults

Hepatitis B vaccine induces a protective antibody response in approximately 30% to 55% of healthy adults younger than 40 years of age after the first dose, in 75% after the second dose, and in more than 90% after the third dose.[34,555] In adults older than 40 years of age, response rates decline with age, and by age 60 years, protective levels of antibody develop in only 75% of vaccinated persons.[488] Because an increased risk for HBV infection is an indication for hepatitis B vaccination and relatively high rates of seroprotection are achieved after each dose, vaccination should be initiated even if completion of the three-dose series cannot be ensured.

Host factors in addition to age that contribute to decreased vaccine response include smoking, obesity, HIV infection, genetic factors, and the presence of a chronic disease (e.g., diabetes, non-HBV chronic liver diseases, hemodialysis patients).[497,537,556–564]

Immunocompromised persons (e.g., HIV-infected persons, hematopoietic stem cell transplant recipients, and patients undergoing immunotherapy and or chemotherapy) have a suboptimal response to standard doses of hepatitis B vaccine.[557,565–568] Modified dosing regimens, including doubling of the standard antigen dose or administration of additional doses, might increase response rates. Immunization of bone marrow and stem cell donors against HBV induces anti-HBs seroconversion in recipients through adoptive transfer of immunity.[569] However, few data on the long-term protection with these alternative vaccination schedules and modified regimens are available.

Correlates of Protection

Anti-HBs is the only easily measurable correlate of vaccine-induced protection using serologic assays. An anti-HBs concentration of 10 mIU/mL or more measured 1 to 3 months after administration of the last dose of the primary vaccination series is considered a reliable marker of protection against infection: in vaccine efficacy studies, immunocompetent persons who developed anti-HBs concentrations of 10 mIU/mL or higher after vaccination had virtually complete protection against both acute disease and chronic infection for decades, even if subsequently, over time, anti-HBs concentrations declined to less than 10 mIU/mL. Indeed, the protective efficacy of hepatitis B vaccination is related to the induction of anti-HBs antibodies, but it also involves the induction of memory B and T cells.[570]

Therefore, vaccine-induced protection against HBV infection is defined as having an anti-HBs level of 10 mIU/mL or higher, measured 1 to 3 months after receipt of a complete and adequately administered vaccination course.[324,326,493,571–575] Yet, HBsAg vaccine-induced immunity protects against future infection but does not provide sterilizing immunity, as evidenced by HBcAg-specific and polymerase-specific CD8(+) T cells in vaccinated healthcare workers with occupational exposure to HBV.[576]

In immunocompromised patients who have ongoing exposure to HBV, annual anti-HBs testing is recommended, and booster doses are required to maintain anti-HBs concentrations of 10 mIU/mL or higher.[265]

Efficacy

Preexposure Vaccine Efficacy

Hepatitis B vaccine elicits development of antibody response to the *a* determinant, located in the surface antigen of HBV.[577] Anti-HBs is the serologic marker of vaccine-induced protection. The efficacy of preexposure hepatitis B vaccination has been demonstrated in randomized, double-blind, placebo-controlled clinical trials involving high-risk groups such as MSM, healthcare workers, hemodialysis staff members, and hemodialysis patients.[324,493,571,575,578–581] These studies demonstrated an overall efficacy of 80% to 100% (Table 25.5) and virtually complete protection against acute and CHB infection among persons who developed anti-HBs concentrations of 10 mIU/mL or greater, even if subsequently, over time, anti-HBs concentrations declined to less than 10 mIU/mL.

Postexposure Vaccine Efficacy

Among infants born to HBeAg-positive mothers, postexposure immunoprophylaxis with either plasma-derived or recombinant vaccine and HBIG is 85% to 95% effective in preventing chronic HBV infection (Table 25.6).[285–287,458,582–593]

In randomized, placebo-controlled clinical trials, administration of hepatitis B vaccine in a three- or four-dose schedule without HBIG, beginning less than 12 hours after birth, also prevents 70% to 95% of perinatal HBV infections among infants born to women who are positive for both HBsAg and HBeAg.[582,584,588,591,594] Population-based studies in areas with a high endemicity of HBV infection have demonstrated that active postexposure vaccination is highly effective in preventing infection when the first dose is administered soon after birth, the second at age 1 to 2 months, and the third at age 6 to 8 months (see Table 25.6).[595–597] These findings support the use of hepatitis B vaccine alone, particularly in countries where pregnant women are not screened for HBsAg and hepatitis B vaccine is routinely administered to all infants at birth.

Duration of Protection and Need for Booster Doses

After primary immunization with hepatitis B vaccine, anti-HBs concentrations decline rapidly within the first year and more slowly thereafter. Among children who respond to a primary three-dose vaccination series with anti-HBs concentrations of 10 mIU/mL or greater, 15% to 50% have low or undetectable concentrations of anti-HBs 5 to 15 years after vaccination.[598–605]

Among adult vaccinees, anti-HBs concentrations decline to less than 10 mIU/mL in 7% to 50% within 5 years after vaccination, and in 30% to 60% within 9 to 11 years after vaccination.[606–610]

The persistence of anti-HBs over time is correlated with the peak level of anti-HBs immediately achieved after primary immunization.[611] In other words, the higher the vaccine-induced concentration after the primary vaccination course, the longer the antibodies will persist. However, observational

TABLE 25.5 Randomized Clinical Studies of Preexposure Hepatitis B Vaccine Efficacy

Group (Reference)	Vaccine	Dose (μg)	Schedule (mo)	Subjects (*N*)	Attack Rate[a]		Efficacy (%)
					Vaccinated	Unvaccinated	
Men who have sex with men[324]	MSD-P	20	0, 1, 6	1402	2.3	12.6	82
Men who have sex with men[571]	MSD-P	40	0, 1, 6	1083	3.5	27.1	87
Men who have sex with men[580]	CLN-P	3	0, 1, 2	800	5.0	24.0	80
Healthcare workers[579]	MSD-P	20	0, 1, 6	865	0.5	6.0	92
Healthcare workers[578]	Pasteur-P	5	0, 1, 2	354	0	8.0	100
Hemodialysis patients[578]	Pasteur-P	5	0, 1, 2	95	2.6	27.7	91
Hemodialysis patients[581]	MSD-P	40	0, 1, 6	1311	1.0	0.7	0[b]
Hemodialysis patients[580]	CLN	3	0, 1, 2, 5	388	1.6	12.0	86

CLN, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service; MSD, Merck, Sharp & Dohme; P, plasma-derived vaccine.
[a]Attack rate (per 100 person-years) of hepatitis B virus infection defined as clinical hepatitis B and/or HBsAg-positive 3 months or more after starting vaccination.
[b]Although efficacy was not demonstrated in this study, no infections occurred among persons who developed and maintained protective concentrations of antibody to hepatitis B surface antigen.

TABLE 25.6 Studies of the Efficacy of Recombinant Hepatitis B Vaccines in Neonates Born to Hepatitis B e Antigen-Positive Mothers

Reference	HBIG at Birth	Vaccine	Dose (μg)	Schedule (age in mo)	Subjects (*N*)	HBsAg-Positive *N* (%)	Efficacy (%)[a]
Lee (1991)[287]	Yes	GSK-R	20	0, 1, 2, 12	54	4 (7.4)	92
	Yes	GSK-R	10	0, 1, 2, 12	56	1 (1.8)	98
	Yes	GSK-R	20	0, 1, 6	60	2 (3.3)	96
Pongpipat (1989)[585]	Yes	MSD-R	5	0, 1, 6	20	2 (10.0)	89
Poovorawan (1992)[587]	Yes	GSK-R	10	0, 1, 2, 12	64	1 (1.5)	98
	Yes	GSK-R	10	0, 1, 6	59	0 (0)	100
Stevens (1992)[592]	Yes	MSD-R	5	0, 1, 6 or 0, 1, 9	351	19 (5.4)	94
Stevens (1987)[285]	Yes	MSD-R	5	0, 1, 6	83	4 (4.8)	95
Assateerawatt (1993)[593]	Yes	Pasteur-R	20	0, 1, 2, 12	26	1 (3.8)	96
	No	Pasteur-R	20	0, 1, 2, 12	23	2 (8.7)	90
Milne (2002)[591]	No	MSD-R	5	0, 1, 2	82	12 (14.6)	83
Poovorawan (1989)[286]	No	GSK-R	10	0, 1, 2, 12	55	2 (3.6)	96
Poovorawan (1992)[587]	No	GSK-R	10	0, 1, 2, 12	57	1 (1.8)	98
	No	GSK-R	10	0, 1, 6	54	1 (1.9)	98
Lolekha (2002)[590]	No	MSD-R	5	0, 1, 6	50	5 (10.0)	89
	No	MSD-R	5	0, 1, 2, 12	47	7 (14.9)	83

GSK, GlaxoSmithKline; MSD, Merck, Sharpe & Dohme; R, recombinant DNA.
[a]Immunization efficacy calculation assumes 88% infection rate without postexposure prophylaxis.

studies have shown that a primary series of hepatitis B vaccine can prevent infection for more than 25 years, despite decrease or loss of vaccine-induced anti-HBs over time.[612–633]

A recent follow-up and response to a hepatitis B vaccine challenge study in Alaska confirms earlier observation of long-term protection, showing evidence of protection (anti-HBs >10 IU/L or response to challenge dose) in 94% of persons, and no chronic infections 30 years after vaccine administration.[633] This is similar to other long-term cohort studies in other parts of the world carried out through 20 to 25 years of follow up.[630,631] Indeed, in immunocompetent individuals, the specific immunity to HBsAg outlasts the presence of

vaccine-induced antibodies, conferring effective long-term protection against acute disease and development of HBsAg carriage, even in those showing waning or disappearance of anti-HBs.[634–638] Thus, a negative anti-HBs result does not necessarily indicate lack of immunity in vaccinated persons—it is immune memory that matters.[639] The mechanism for continued vaccine-induced protection is thought to be preservation of immune memory through selective expansion and differentiation of clones of antigen-specific B and T lymphocytes.[634,635]

To illustrate the prolonged duration of protection against hepatitis B, even after disappearance of antibodies, an anamnestic response was measured shortly after offering a challenge dose (the so-called boostability) in cohorts of vaccinees up to 30 years after primary vaccination (Table 25.7).[620,621,623,640–649]

Anamnestic responses have been demonstrated in 62% to more than 80% of subjects vaccinated 15 to 30 years earlier, which indicates that a high proportion of vaccine recipients retain immune memory and would develop an anti-HBs response on exposure to HBV.[633]

Recently, increasing evidence suggests that the immune memory may begin to wane after the second decade of vaccination, but this does not seem to imply increased susceptibility to clinically significant HBV disease, as no acute or chronic HBV cases were reported in any of the ongoing follow-up studies.[616,619,640,647,648] Thus absence of an anamnestic response after such booster vaccination may not necessarily mean that individuals are susceptible to HBV infection.[620,639] Further research is needed in this field.

Some long-term studies have documented breakthrough infections, illustrated by the seroconversion to anti-HBc, but virtually no clinically significant infections (acute diseases or carriage) were reported (Table 25.8).[607,622,623,642,644,648,650–654] From a public health point of view, prevention of carriage

TABLE 25.7 Studies in Different Populations in Regions With Different Endemicity During the Past Decade, Using Anamnestic Response to a Booster as a Measure for Immune Memory

Reference	Vaccinated Population	*N*	Country	Number of Doses or Schedule (mo)	Time Follow-up (yr)	% Showing an Anamnestic Response
Williams (2003)[643]	Infants	70 + 41	Samoa	3 doses, 0, 1, 6 starting at birth, booster at 5 or 9 years	5 or 9	100% after 5 yr 93% after 9 yr
Boxal (2004)[644]	Infants of carrier mothers	116	UK	3–4 doses, starting at birth	10	86%
Lu (2004)[642]	Infants of carrier mothers + infants from noncarrier mothers	78 + 113	China	4 doses, starting at birth	15	97.3–96.7%
Gabbuti (2007)[645]	Adolescents	620, 11 lost HBs antibodies	Italy	3 doses	11	91.7%
Duval (2005)[646]	Adolescents	550	Canada	3 doses	5	99%
Zanetti (2005)[641]	Infants and recruits	1212 children, 446 recruits	Italy	3 doses, 3, 5, 11 mo and 0, 1, 6 for recruits	10	97% children and 96% recruits
Jafarzadeh (2006)[623]	Infants	94	Iran	3 doses starting at birth	10	95.8%
van der Sande (2007)[621]	Adolescents	255	The Gambia	3 doses	15	92.3%
Samandari (2007)[647]	Infants, recombinant vaccine	166	Alaska	3 doses starting at birth	5–6.9	99%
	Adolescents, recombinant vaccine	138	Alaska	3 doses starting at birth	10–14.7	88%
	Adolescents, plasma derived	74	Alaska	3 doses starting at birth	11.7–14.9	71%
	Infants of carrier mothers	37	Alaska	3 doses starting at birth	15	51% after 2 wk 62% after 1 mo
Hammit (2007)[620]	Infants	872	Taiwan	3 doses starting at birth	15–21	70.3%
Lu (2008)[648]	Young adults	127	Taiwan	3 doses starting at birth	18–23	75.6% after 1 mo 20% after 1 wk
Zinke (2009)[649]	Children	186	Germany	3 doses + 1 DTPa-HBV-IPV/Hib	7–9	98.9%

Anti-HBs, anti–hepatitis B surface antibodies.
The anamnestic response was defined as: in case of detectable anti-HBs at baseline (at moment of booster vaccine administration), a fourfold increase in anti-HBs concentration from baseline to 1 week, 2 weeks, or 1 month after administration of a booster vaccine dose; in case of undetectable anti-HBs at baseline, a switch from <10 mIU/mL to >10 mIU/mL 1 week, 2 weeks, or 1 month after administration of a booster vaccine dose.
From Leuridan E, Van Damme P. Hepatitis B and the need for a booster dose. Clin Infect Dis. *2011;53:68–75.*

TABLE 25.8 Studies in Different Populations in Regions With Different Endemicities During the Past Decade, Using Measurement of Hepatitis B Markers as Markers for Breakthrough Infection in Vaccinated Populations

Reference	Vaccinated Population	Patients *(N)*	Country	No. of Doses, or Schedule	Time of Follow-up (yr)	Method	(Breakthrough) Infections: Anti-HBc Antibody-Positive and/or HBsAg
Dentinger (2005)[650]	Adolescents	334	Alaska	3 doses: birth (0–8 day), 24–103 day, 146–286 day	16	2 consecutive anti-HBc OR 1 anti-HBc and 1 HBV DNA PCR	1.8% anti-HBc of whom 0.9% HBV DNA positive
Mele (2001)[652]	Infants of carrier mothers	522	Italy	Birth + 3 doses	5–14	HBsAg, anti-HBc	3.3% anti-HBc; 3 infants carrier HBsAg
Young (2003)[653]	Infants of carrier mothers	112	China	3 doses: 0, 1, 6, or 0, 2, 8, or 0, 1, 2 mo	16	2 consecutive anti-HBc; HBsAg	8.9% anti-HBc; 3.5% infants HBsAg
Boxal (2004)[644]	Infants of carrier mothers	116	UK	3–4 doses starting at birth	15	HBsAg, anti-HBc	1.7% anti-HBc
Lu (2004)[642]	Infants of carrier mothers	191	China	4 doses starting at birth	15	HBsAg, anti-HBc	33.3% anti-HBc; 1 infant HBsAg
McMahon (2005)[651]	General population	1578	Alaska	3 doses: 0, 1, 6 mo	15	2 consecutive anti-HBc OR 1 anti-HBc + 1 HBV DNA PCR OR HBsAg	1% anti-HBc, 3 infants HBsAg, 3 infants HBV DNA positive
Jafarzadeh (2006)[623]	Infants	146	Iran	3 doses starting at birth	10	HBsAg, anti-HBc	7.5% anti-HBc
van der Sande (2006)[622]	Adolescents	1350	The Gambia	3 doses	15	HBsAg, anti-HBc	13.8% anti-HBc, 0.7% HBsAg
Lu (2008)[648]	Young adults	6156	Taiwan	4 doses	15–21	HBsAg, anti-HBc	4.1% anti-HBc
Poovorawan (2009)[654]	Infants of carrier and noncarrier mothers	204	Thailand	3 or 4 doses: 0, 1, 6 mo, or 0, 1, 2, 12 mo	15–17	HBsAg, anti-HBc	26% (53/204) anti-HBc; 2.9% (6/204) HBsAg, acquired in the first year of life

Anti-HBc, antibody to hepatitis B core antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; PCR, polymerase chain reaction.
From Leuridan E, Van Damme P. Hepatitis B and the need for a booster dose. Clin Infect Dis. *53:68–75, 2011.*

remains of utmost importance: in a 20-year evaluation of the hepatitis B universal immunization program in The Gambia, Viviani and coworkers showed a vaccine efficacy of 67% against development of anti-HBc, and 97% against HBsAg carriage.[655] In a more recent evaluation of the infant vaccination program in The Gambia a 94% vaccine effectiveness was observed in fully vaccinated infants. Many of these fully vaccinated individuals had at some time been infected and experienced a nonsignificant breakthrough infection (anti-HBc positivity of 27%); chronic active hepatitis was not common and probably a consequence of perinatal infection from the mother. The study concluded full infant HBV vaccination does protect strongly against chronic HBV infection, but less strongly against ever having HBV infection.[656] Persons who have received only one or two doses of vaccine can also have detectable anti-HBs. However, long-term protection has been demonstrated only for immunocompetent persons who have completed a licensed vaccination series and have ever had an anti-HBs concentration of 10 mIU/mL or greater.[639]

On the basis of currently available scientific evidence, the WHO as well as advisory groups in the United States and Europe do not recommend routine booster doses of hepatitis B vaccine or periodic serologic testing to monitor anti-HBs concentrations for immunocompetent persons who have responded to vaccination or in universal immunization programs.[265,617,657]

For the next decade, additional data are needed regarding the potential need for boosters and the duration of protection against infection and disease after hepatitis B vaccination, including information on the potential role of natural subclinical boosting.

Vaccine-Associated Adverse Events

Adverse events after immunization against hepatitis B are infrequent and generally mild. Except for localized pain, placebo-controlled studies reveal that reported events (e.g., myalgia and transient fever) occur no more frequently among vaccinees than among persons receiving placebo (<10% among children, 30% among adults).[658] Data from numerous long-term studies fail to causally link serious adverse events to hepatitis B vaccination.[659] Reports of severe anaphylactic reactions are very rare, and data do not indicate a causal association between hepatitis B vaccine and Guillain-Barré

syndrome or demyelinating disorders, including multiple sclerosis. However, hepatitis B vaccine has been the subject of greatest concern regarding multiple sclerosis in France. In 1998, French media revealed possible occurrence of post–hepatitis B immunization multiple sclerosis. That same year the French health authorities abruptly ended routine school-based vaccination of adolescents, while adult vaccination began to be less widespread. Several epidemiological studies have been conducted to evaluate the association between hepatitis B vaccination and multiple sclerosis, and found no link. A recent nested case-control study using data from an U.S. Health Maintenance Organization (2008–2011) showed no longer-term association between hepatitis B vaccination and the risk of multiple sclerosis or other acquired central nervous system demyelinating syndromes, which argues against a causal association.[660] Furthermore, no epidemiologic data support a causal association between hepatitis B vaccination and chronic fatigue syndrome, arthritis, autoimmune disorders, asthma, sudden infant death syndrome, alopecia, or diabetes.[661–664] The WHO's Global Advisory Committee on Vaccine Safety as well as the recent report of the Institute of Medicine confirm the excellent safety profile of hepatitis B vaccine and continue to monitor the safety of this vaccine.[665]

Indications and Goals for Hepatitis B Vaccination

Global recommendations for HBV prevention and control are set by the WHO on behalf of its 194 member states and in consultation with the Strategic Advisory Group of Experts on Immunization. In 2009, the WHO issued a position paper updating its guidance for global hepatitis B prevention through immunization.[666] The following year, member states adopted Resolution WHA63.18,[667] which designated July 28 as World Hepatitis Day and called for comprehensive prevention and control of HBV infection and development of time-specific immunization goals. The WHO's strategy for hepatitis B immunization includes the following:

- Universal vaccination of infants within 24 hours of birth
- Full immunization of infants by routine immunization programs
- Catch-up vaccination of unimmunized cohorts
- Monitoring progress
- Assessing the impact of immunization.

RECOMMENDATIONS FOR HEPATITIS B VACCINATION

Vaccination of Infants at Birth

Because perinatal and early postnatal transmission are primary causes of chronic infections globally, the first dose of hepatitis B vaccine should be given as soon as possible (<24 hours) after birth, regardless of whether a country has low, intermediate, or high HBV endemicity.[666]

Ensuring that all infants receive a dose of hepatitis B vaccine within 24 hours of birth (i.e., a "birth dose" of vaccine) requires implementation of specific programmatic measures. Increasing the number of infants born in facilities or attended by trained health staff would improve birth-dose coverage. Coordination between immunization services and maternal and child health services is important for ensuring that vaccine is available at the place of delivery or immediately after birth. Expanding vaccine management systems and innovative outreach to provide vaccine for home births[490] will ensure that hepatitis B vaccine is available wherever births take place. Efforts to develop new heat-stable and freeze-stable hepatitis B vaccines will aid these attempts. In addition, health promotion efforts aimed at parents and training aimed at providers are needed to increase awareness about the importance of administering hepatitis B vaccine within 24 hours of birth.[489]

Some countries augment universal vaccination of newborns with maternal screening for HBsAg and the administration of HBIG and a dose of hepatitis B vaccine to infants born to HBsAg-positive mothers. For example, in the United States, timing of administering the birth dose of hepatitis B vaccine varies according to the HBsAg status of the mother (Box 25.1),

BOX 25.1 U.S. Hepatitis B Vaccination Recommendations for Infants, Children, and Adolescents: Summary

MATERNAL HEPATITIS B SURFACE ANTIGEN (HBsAg) TESTING

- All pregnant women should be tested routinely for HBsAg

VACCINATION OF INFANTS

At Birth

- Infants born to mothers who are HBsAg-positive should receive hepatitis B vaccine and 0.5 mL of hepatitis B immunoglobulin (HBIG) within 12 hours of birth, administered at different injection sites. The schedule should be completed as recommended for all infants (see "After the Birth Dose," below).
- Infants born to mothers whose HBsAg status is unknown should receive hepatitis B vaccine within 12 hours of birth. The mother should have blood drawn as soon as possible to determine her HBsAg status; if she is HBsAg-positive, the infant should receive 0.5 mL of HBIG as soon as possible but no later than age 7 days, and the vaccine series should be completed according to a schedule for infants born to HBsAg-positive mothers.
- Infants born to mothers who are HBsAg-negative should receive single-antigen hepatitis B vaccine before hospital discharge.

After the Birth Dose

- All infants should receive at least three doses of hepatitis B vaccine (either single-antigen vaccine or combination vaccine).
- For infants who weigh less than 2000 g at birth, the vaccination schedule varies with the HBsAg status of the mother. Those born to HBsAg-negative mothers should receive the first dose of vaccine 1 month after birth or at hospital discharge. For those born to HBsAg-positive mothers or to mothers with unknown status, the birth dose should not be counted as part of the vaccine series; three additional doses of vaccine (for a total of four doses) should be administered beginning at age 1 month.
- For all infants, the final dose in the vaccine series should not be administered before age 24 weeks (164 days). If the hepatitis B vaccine series is interrupted, the second and third doses should be separated by an interval of at least 8 weeks.
- Infants born to HBsAg-positive mothers should be tested for HBsAg and antibody to HBsAg after completion of the hepatitis B vaccine series at age 9 to 18 months.

VACCINATION OF CHILDREN AND ADOLESCENTS

- All unvaccinated children and adolescents younger than 19 years old should receive the hepatitis B vaccine series.

and it is recommended that all pregnant women be tested routinely for HBsAg during a prenatal visit; testing should be repeated for pregnant women with ongoing risk behaviors and those with clinical signs of hepatitis.

The timely delivery of a birth dose of hepatitis B vaccine should be a performance measure for all immunization programs. To better monitor the delivery of this dose of hepatitis B vaccine and facilitate performance assessment, the birth dose should be differentiated from the two or three additional doses administered later in infancy. Full immunization performance will continue to be measured by coverage of three or four total doses.

Full Immunization of Infants by Routine Immunization Programs

To complete the primary hepatitis B vaccine series, the birth dose should be followed by two or three additional doses of vaccine administered at least 4 weeks apart. To help ensure completion of the vaccine series, doses should be given concurrently with DTP or other routine infant vaccinations.

When subsequent doses of hepatitis B vaccine are given in combination with other antigens (often referred to as Combo 1, Combo 2, and Combo 3 doses), the Combo 3 dose should contain the fourth dose of hepatitis B vaccine.

For infants who weigh less than 2000 g, the birth dose should be followed by three additional doses of vaccine administered according to the national vaccination schedule.

Administration of booster doses of hepatitis B vaccine is not warranted in routine immunization programs. Some infants born prematurely with low birthweight (<2000 g) may not respond well to vaccination at birth. However, by 1 month of chronological age, premature infants, regardless of their initial weight or gestational age at birth, are likely to respond adequately.[666,669]

For older children and adults, the primary series of three doses with appropriate intervals applies.

Catch-Up Vaccination of Unvaccinated Cohorts

Catch-up vaccination should be considered for cohorts of children with low rates of vaccination coverage. The need for catch-up vaccination in older age groups, including adolescents and adults, should be determined by the baseline epidemiology of HBV infection in the country. Vaccination efforts can be targeted toward age-specific cohorts and persons with particular risk factors for acquiring HBV infection.

In countries with intermediate or low endemicity, substantial disease burden may result from acute and chronic infection acquired by older children, adolescents, and adults born before implementation of universal vaccination recommendations. In these countries, implementation of routine infant immunization will produce broad population-based immunity to HBV infection over time, eventually preventing transmission among all age groups. However, catch-up strategies targeted at unvaccinated people in older age groups can hasten the development of population-based immunity and decrease the incidence of acute hepatitis B. Catch-up immunization typically involves age-specific cohorts (e.g., young adolescents) and populations at risk for HBV infection, such as health workers,[670] travelers to areas where HBV-infection is prevalent,[671] IDUs, MSM, and people with multiple sex partners.

Surveillance for and seroprevalence studies of HBV infection can help countries determine the populations at highest risk for acquiring infection, leading to more informed prevention efforts. In the United States, the Advisory Committee on Immunization Practices (ACIP) recommends immunization for persons at risk for HBV infection resulting from sexual, percutaneous, or mucosal exposures; travelers to areas with high or intermediate levels of endemic HBV infection; persons with certain medical conditions; and all persons seeking protection from HBV infection. In 2011, ACIP, after reviewing data regarding the risk of HBV transmission from contaminated glucose-monitoring devices and the role of immunization in infection control for this population, recommended hepatitis B vaccination for unvaccinated adults with diabetes (Box 25.2).[672]

Vaccination of Immunocompromised Persons

Immunosuppressive illnesses, including advanced HIV infection, chronic renal failure, chronic liver disease, and celiac disease, are associated with reduced immunogenicity after administration of hepatitis B vaccine. In people who are HIV-positive, several factors (e.g., viral load, $CD4^+$ cell count, sex, age, type and duration of highly active antiretroviral therapy [HAART], and the type of AIDS-defining illness) can affect immunologic response to hepatitis B vaccination. Early vaccination (before the $CD4^+$ count declines to <350 cells/μL) is recommended in HIV-infected patients to ensure adequate protection against hepatitis B infection. In one double-blind, randomized, controlled trial that compared the use of

BOX 25.2 Adults for Whom Hepatitis B Vaccination Is Recommended in the United States

PERSONS AT RISK FOR INFECTION BY SEXUAL EXPOSURE

- Sex partners of hepatitis B surface antigen (HBsAg)-positive persons.
- Sexually active persons who are not in a long-term, mutually monogamous relationship (e.g., persons with more than one sex partner during the previous 6 months).
- Persons seeking evaluation or treatment for a sexually transmitted disease.

PERSONS AT RISK FOR INFECTION BY PERCUTANEOUS OR MUCOSAL EXPOSURE TO BLOOD

- Current or recent injection-drug users.
- Household contacts of HBsAg-positive persons.
- Residents and staff of facilities for developmentally disabled persons.
- Healthcare and public safety workers with reasonably anticipated risk for exposure to blood or blood-contaminated body fluids.
- Persons with end-stage renal disease, including predialysis, hemodialysis, peritoneal dialysis, and home dialysis patients.

OTHERS

- International travelers to regions with high or intermediate levels (HBsAg prevalence of ≥2%) of endemic hepatitis B virus (HBV) infection.
- Persons with chronic liver disease.
- Persons with HIV infection.
- All other persons seeking protection from HBV infection.
- All unvaccinated persons with diabetes who are younger than 60 years of age, and those who are 60 years of age or older at the discretion of the clinician.*

*Recommended by the Advisory Committee on Immunization Practices (ACIP), October 2011.

a standard dose (20 μg) with a double dose (40 μg) of recombinant hepatitis B vaccine in 210 previously unvaccinated HIV-infected patients, a substantially higher seroconversion rate occurred with use of the double dose of vaccine, but only in the subgroup of patients with $CD4^+$ counts greater than 350 cells/μL.[673,674] Despite these data, vaccination should not be deferred for those with negative or indeterminate serologies while awaiting a rise in $CD4^+$ count to greater than 350 cells/μL.[674] Because some HIV-infected patients with $CD4^+$ counts less than 200 cells/μL *do* respond to vaccination, vaccination should be performed as previously recommended, with testing for anti-HBs done 1 to 2 months after completion of the series.

Patients experiencing chronic renal failure are at particular risk for infection with HBV. In some settings, these patients have been offered schedules containing more than three doses of vaccine, larger doses of vaccine, or both. Two metaanalyses have demonstrated that the three-dose schedule affords protection similar to that of more extensive schedules, although older age was associated with impaired immunologic response.[675] A recombinant hepatitis B vaccine prepared with a novel adjuvant system (aluminum phosphate and 3-*O*-desacyl-4′-monophosphoryl lipid A) is licensed in Europe and intended for patients older than 15 years of age with renal insufficiency. Applied in a four-dose schedule, the vaccine with the novel adjuvant is more reactogenic, but it elicits an earlier, higher, and longer-lasting antibody response than a corresponding series of four double doses of standard hepatitis B vaccine.[676,677]

TABLE 25.9 U.S. Guidelines for Postexposure Immunoprophylaxis of Unvaccinated Persons Who Are Exposed to Blood or Body Fluids That Contain Blood

Cause	Action
DISCRETE EXPOSURE TO AN HBSAG-POSITIVE SOURCE	
Percutaneous (e.g., bite or needlestick) or mucosal exposure to HBsAg-positive blood or body fluids that contain blood	Administer hepatitis B vaccine and HBIG[a]
Sexual or needle-sharing contact of an HBsAg-positive person	Administer hepatitis B vaccine and HBIG[a]
Victim of sexual assault/abuse by a perpetrator who is HBsAg-positive	Administer hepatitis B vaccine and HBIG[a]
DISCRETE EXPOSURE TO A SOURCE WITH UNKNOWN HBSAG STATUS	
Victim of sexual assault/abuse by a perpetrator with unknown HBsAg status	Administer hepatitis B vaccine[a]
Percutaneous (e.g., bite or needlestick) or mucosal exposure to blood or body fluids that contain blood from a source with unknown HBsAg status	Administer hepatitis B vaccine[a]

HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen.
[a]Immunoprophylaxis should be administered as soon as possible, preferably within 24 hours. Studies are limited on the maximal interval after exposure during which postexposure prophylaxis is effective, but the interval is unlikely to exceed 7 days for percutaneous exposures and 14 days for sexual exposures. The hepatitis B vaccine series should be completed.

POSTEXPOSURE PROPHYLAXIS TO PREVENT HEPATITIS B VIRUS INFECTION

Timely administration of hepatitis B vaccination after sexual or percutaneous exposures to HBV can prevent HBV infection. Both passive–active postexposure prophylaxis (PEP) with HBIG and hepatitis B vaccine, and active PEP with hepatitis B vaccine alone have been demonstrated to be highly effective in preventing transmission after exposure to HBV.[678–681] The major determinant of the effectiveness of PEP is early administration of the initial dose of vaccine. The effectiveness of PEP diminishes as the interval between exposure and the initial dose lengthens.[51,682,683] Studies are limited on the maximal interval after exposure during which PEP is effective, but the interval should not exceed 7 days for needlestick[348,349,681] exposures and 14 days for sexual exposures. Recommendations for PEP vary by country; in the United States, ACIP recommends hepatitis B vaccine and HBIG for persons who are exposed to HBV through a discrete, identifiable exposure to blood or body fluids (Table 25.9).

PREVACCINATION AND POSTVACCINATION SEROLOGIC TESTING

Prevaccination Testing for Susceptibility

Globally, prevaccination serologic testing is not recommended as routine practice. However, where laboratory facilities are available and it is considered cost-effective, serologic screening may reduce the number of unnecessary vaccinations of people who are already immune to HBV infection. For example, in the United States, prevaccination testing may be cost-effective in adult populations with an HBV prevalence of greater than 20% (e.g., incarcerated persons).[684] In countries with low endemicity, prevaccination testing can be directed toward persons of all ages born in countries or regions with high or moderate endemicity and children with at least one parent from these areas; household, sex, and needle-sharing contacts of HBsAg-positive persons; MSM; and IDUs.[387,684]

Prevaccination testing also provides an opportunity to refer people with CHB for care and treatment. Because persons with chronic HBV infection serve as the reservoir for new HBV infections, identification of these persons through prevaccination and other testing approaches can complement vaccination strategies for elimination of HBV transmission, allowing primary prevention of ongoing HBV transmission. Regardless of the indication for serologic testing, people found to be infected with HBV should be referred for care and protected from stigmatization and discrimination.

If warranted, prevaccination testing can be done with a single test (anti-HBc) or with a panel of tests (e.g., anti-HBs and HBsAg). When a single test is used, anti-HBc is the test of choice because it identifies all persons with previous HBV infection, including persons with chronic HBV infection. If prevaccination testing for anti-HBs is used to identify immunity after prior HBV infection, HBsAg testing must also be performed to identify persons with chronic HBV infection. In most situations, the first vaccine dose should be administered immediately after collection of the blood sample for serologic testing, unless a provider is certain the patient will return for serologic test results and that vaccination can be initiated at that time if the patient is susceptible.

Postvaccination Testing for Serologic Response

Routine postvaccination testing for immunity also is not necessary, but it is recommended for high-risk persons whose subsequent clinical management depends on knowledge of

their immune status. Populations that should be considered for postvaccination testing include the following:

- Persons at risk for occupationally acquired infection* (i.e., healthcare workers and especially those involved in exposure prone procedures) as well as public safety workers).
- Infants born to HBsAg-positive mothers.
- Chronic hemodialysis patients, persons infected with HIV, and other immunocompromised persons.
- Sex partners or needle-sharing partners of persons who are HBsAg-positive.

When postvaccination testing is indicated, it should be performed 1 to 2 months after administration of the last dose of the vaccine series using a method that allows determination of a protective concentration of anti-HBs (≥10 mIU/mL). Persons found to have anti-HBs concentrations of less than 10 mIU/mL after the primary vaccine series are considered to be nonimmune and should be evaluated further to determine if they are nonresponders. Immunocompetent persons have long-term protection and do not need further periodic testing to assess anti-HBs levels. Immunosuppressed persons might need annual testing to assess anti-HBs concentrations. The European Consensus Group on Hepatitis B Immunity recommends annual testing for immunocompromised persons,[657] and in the United States, such testing is recommended for hemodialysis patients.[684]

REVACCINATION OF THOSE WHO DO NOT SEROCONVERT

Persons at increased risk of hepatitis B found to have anti-HBs concentrations of less than 10 mIU/mL after the primary vaccine series should be revaccinated. Administration of three doses on an appropriate schedule (Table 25.10), followed by anti-HBs testing 1 to 2 months after the third dose, is usually more practical than serologic testing after one or more doses of vaccine (see "Revaccination of Nonresponders" earlier in this chapter). In countries where a pre-S/S vaccine is licensed, immunization with one or two doses of such a vaccine could be considered. Persons who do not respond to revaccination should be tested for HBsAg. If the HBsAg test result is positive, the person should receive appropriate medical management, and any household, sex, or needle-sharing contacts should be identified and vaccinated. Persons who test negative for HBsAg should be considered susceptible to HBV infection and should be counseled about precautions to prevent HBV infection and the need to obtain HBIG PEP for any known or likely mucosal or parenteral exposure to HBsAg-positive blood.

Contraindications

Hepatitis B vaccine is contraindicated only for persons with a history of allergic reactions to yeast or any of the vaccine's components. Neither pregnancy nor lactation is a contraindication for use of this vaccine. Both premature infants and HIV-positive persons can receive this vaccine.

*In the United States, healthcare and public safety workers who have documentation of a complete vaccine series but who have never had postvaccination testing do not need serologic testing for anti-HBs unless they have a percutaneous or mucosal exposure to blood or body fluids.[685] If an exposure to an HBsAg-positive source or a source that is unknown or not available for testing occurs, the exposed person should be tested for anti-HBs and PEP should be administered if the serum anti-HBs concentration is less than 10 mIU/mL.

TABLE 25.10 Hepatitis B Vaccine: U.S. Schedules for Children, Adolescents, and Adults[a]

Age (yr)	Schedule
Children (1–10)	0, 1, and 6 mo[b] 0, 2, and 4 mo[b] 0, 1, 2, and 12 mo[b,c]
Adolescents (11–19)	0, 1, and 6 mo[b] 0, 1, and 4 mo[b] 0, 2, and 4 mo[b] 0, 12, and 24 mo[b] 0 and 4–6 mo[d,e] 0, 1, 2, and 12 mo[b,d]
Adults (≥ 20)	0, 1, and 6 mo[e,f] 0, 1, and 4 mo[e] 0, 2, and 4 mo[e] 0, 1, 2, and 12 mo[d,e]

[a]Children (not vaccinated during the first year of life), adolescents, and adults may be vaccinated according to any of the schedules indicated, except as noted. Selection of a schedule should consider the need to optimize compliance with vaccination.
[b]Pediatric/adolescent formulation.
[c]A four-dose schedule of Engerix-B is licensed for all age groups.
[d]A two-dose schedule of Recombivax-HB adult formulation (10 μg) is licensed for adolescents 11 to 15 years of age. When scheduled to receive the second dose, adolescents older than 15 years of age should be switched to a three-dose series, with doses 2 and 3 consisting of the pediatric formulation administered on an appropriate schedule.
[e]Adult formulation.
[f]Twinrix may be administered to persons 18 years of age and older at 0, 1, and 6 months.

Public Health Considerations and Impact of Worldwide Hepatitis B Vaccination Programs

Elimination of HBV transmission is now an achievable public health goal,[265,686–689] particularly in light of the proven effectiveness and safety of hepatitis B vaccine. Vaccination strategies for preventing HBV are effective, feasible, and well accepted; efforts are underway to ensure universal immunization of all infants as well as populations at highest risk for HBV infection and transmission. Challenges in achieving elimination include the setting of national goals for hepatitis B control; the provision of a birth dose of hepatitis B vaccine to all infants including those born outside of health facilities; the vaccination of all persons at high risk, including those in hard-to-reach and underserved populations; and building and sustaining support for existing hepatitis B immunization policies and programs.

Several epidemiologic and etiologic factors inherent to HBV necessitate the use of immunization strategies that differ from those used for other vaccine-preventable infections. For example, because infants infected with HBV at birth or early childhood are at greatest risk for developing chronic infection, vaccination of infants should optimally begin at birth, preferably within 24 hours.[527] Among infants born to HBsAg-positive mothers, a birth dose of hepatitis B vaccine reduces the risk for perinatal HBV transmission by 72% and by greater than 90% when combined with HBIG.[294,589,666]

Also unique to HBV are the additional populations at risk for infection, including healthcare workers, MSM, IDUs, persons who have multiple sex partners as well as immune suppressed patients with malignant and auto-immune diseases who are prone to HBV infection and reactivation unless protected against HBV prior to initiation of chemo or immunotherapy. Thus, hepatitis B immunization programs tailor efforts according to risk population, educating and reaching

these populations with targeted messages and ensuring that they and their susceptible contacts are vaccinated against HBV.

Hundreds of millions of persons of all ages have been vaccinated over several decades with hepatitis B vaccines, which have proved to be safe and highly effective. No serious adverse events are associated with hepatitis B immunizations,[666] and after completion of a routine three-dose schedule of hepatitis B vaccine, protection is achieved by greater than 95% of vaccine recipients.[666,684] In addition, studies have revealed levels of protection persisting for at least 23 to 30 years after vaccination.[612–633,639–655] Breakthrough infections are infrequent and rarely result in clinically apparent disease. Based on data from recent studies, a booster dose is neither warranted nor recommended for persons who have received routine infant immunization.[690,691]

Routine infant immunization has become a longstanding practice in more than 95% of countries, providing evidence for the effectiveness of hepatitis B immunization in significantly reducing or eliminating HBV transmission.[692] In general, studies conducted in high-HBV-endemicity areas have demonstrated declines in the prevalence of chronic HBV among children to less than 2% after routine infant immunization (Table 25.11).[693]

Countries that were early to adopt and implement universal hepatitis B immunization include Taiwan (1984), Bulgaria (1989), Malaysia (1990), The Gambia (1990), Italy, Spain, the United States (1991), and Israel (1992).[694–696]

Taiwan is perhaps the best example of an area with previously high endemicity showing a substantial decrease in the burden of hepatitis B and HBV-related diseases after the 1984 mass vaccination of newborns. The HBsAg prevalence in individuals younger than 20 years old decreased from 9.8% in 1984 to 1.3% in 1994, and to 0.6% in 2004 (Fig. 25.12).[693,697,698] The annual average incidence of HCC among children between 6 and 14 years of age decreased from 0.7 per 100,000 in 1981 through 1986, to 0.36 per 100,000 in 1990 through 1994.[699] In 2004, the HCC incidence for age groups of 6 to 9, 10 to 14, and 15 to 19 years decreased to 0.15, 0.19, and 0.16 per 100,000 person-years, respectively, clearly indicating the hepatitis B vaccine as the first vaccine against a major human cancer.[700]

TABLE 25.11 Effectiveness of Hepatitis B Immunization in Protecting From HBsAg Carriage

Country	HBsAg (%)		Efficacy (%)
	Before	After	
China, rural	14.6	1.4	90.4
China (Shanghai)	11	0.63	94.3
Egypt (Alexandria)	2.2	0.8	63.6
Gambia	12	0.9	92.5
Indonesia (Lombok)	6.2	1.4	61.1
Italy (Afragola)	13.4	0.9	93.3
Japan (Iwate)	0.9	0.03	96.7
Japan (Shizuoka)	0.3	0.03	90.0
Korea	7.5	0.38	94.9
Malaysia	2.5	0.4	84.0
Micronesia	12	2.9	75.8
Polynesia	6.5	0.7	89.2
Saipan	9	0.5	94.4
Samoa	7	0.5	92.9
Saudi Arabia	6.7	0.3	95.5
Senegal	19	2	89.5
Singapore	4.1	0	100
South Africa	12.8	3.0	76.6
Taiwan (Taipei)	10	0.7	93.0
Taiwan (Hualien)	9.3	1.9	79.6
Taiwan (Taichung)	14	1.2	91.4
Thailand	4.3	0.7	83.7
United States (Alaska)	16	0	100

HBsAg, hepatitis B surface antigen.
From Chen DS. Hepatitis B vaccination: the key towards elimination and eradication of hepatitis. Br J Hepatol. *2009;50:805–816.*

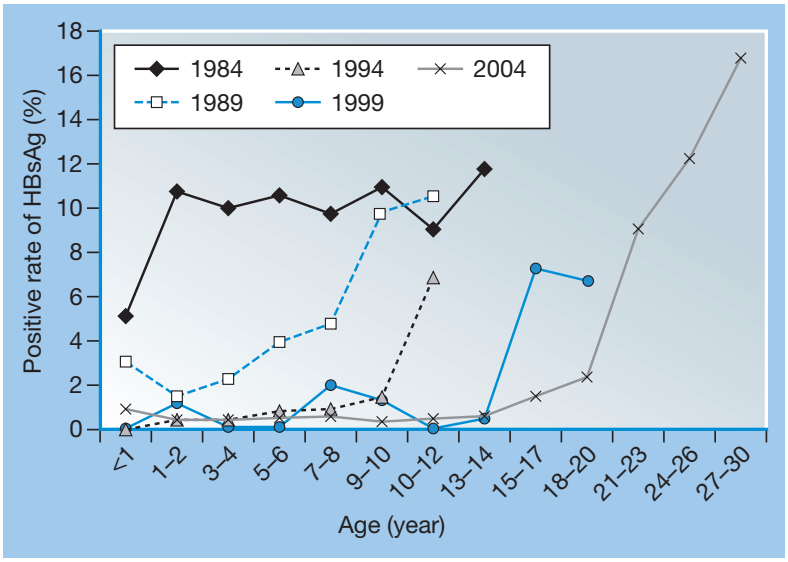


Figure 25.12. Prevalence of hepatitis B surface antigen (HBsAg) in healthy children in Taipei from 1984 to 2004. The mass hepatitis B vaccination started in July 1984. *(From Chen DS. Hepatitis B vaccination: the key toward elimination and eradication of hepatitis.* Br J Hepatol. *2009;50:805–816.)*

Likewise, in The Gambia, since the implementation of the universal infant immunization program in 1984, childhood HBsAg prevalence decreased from 10% to 0.5% in 2003.[522,603,656,701] In Malaysia, after vaccination, HBsAg seroprevalence in children from 7 to 12 years old decreased from 1.6% in 1997 to 0.3% in 2003.[702] Similar results have been achieved in other highly endemic countries, such as in South Africa (see Table 25.11).[703] Since the implementation of universal infant immunization in 1991, data from Hawaii showed a reduction of 97% in the prevalence of HBsAg. Moreover, in Hawaii, the incidence per 100,000 inhabitants of new cases of acute hepatitis B fell from 4.5 in 1990 to zero in 2004.[704] In Bristol Bay, Alaska, universal vaccination of children was introduced in 1981. Since then, a sharp decline in the incidence of acute hepatitis B (next to zero) was observed. Thanks to vaccination, generations of children and young adults are now free of chronic hepatitis infections. Moreover, a general trend toward decreasing incidence of HCC in individuals younger than 30 years of age has been observed.[705] A clear decline in HCC incidence was observed among children, from three cases per 100,000 population (1984 through 1988) to no cases since 1999.[368]

Surveillance data from Italy, where universal vaccination started in 1991 in infants as well as in adolescents, show a clear overall decline in incidence of acute hepatitis B, from 5 per 100,000 in 1990 to 0.9 per 100,000 in 2010. This decline was even more striking in individuals ages 15 and 24 years in whom the morbidity rate per 100,000 inhabitants fell from 17 in 1990 to less than 0.5 in 2010. Moreover, a generation of children and young adults (at present, 32 age cohorts in 2011) is emerging with virtually no markers of HBV infection.[640,706–710]

China, another highly endemic country, has made great strides in reducing HBV prevalence through vaccination. In 1992, national HBsAg prevalence was 9.8%, representing 120 million carriers of HBV and resulting in 300,000 deaths annually.[367] This dire situation prompted the Ministry of Health in China to recommend hepatitis B vaccination for infants. However, despite the 1992 Ministry of Health recommendations, high vaccine costs limited the uptake of recommendations nationally. In 2002, the government committed to covering hepatitis B vaccine–related costs and integrated hepatitis B vaccination into the routine infant immunization schedule, with a priority for administering a birth dose of hepatitis B vaccine to newborns within 24 hours of delivery.[711] According to a national survey, by 2006, HBsAg prevalence had declined to less than 1% among children 1 to 4 years of age.[367,712–714]

In studies conducted in Taiwan, rates of HCC among cohorts of children born after implementation of routine infant immunization in the mid-1980s declined by more than 50%.[699,715] Based on an evaluation of the hepatitis B vaccination program in China, during 1992 to 2009, hepatitis B vaccination prevented an estimated 24 million chronic HBV infections and 4.3 million future deaths from cirrhosis, HCC, and acute hepatitis.[712] In comparison, rates of HCC in older age groups and rates of other childhood cancers remained stable or increased during this period. These studies provide assurance that routine infant hepatitis B immunization is a well-conceived public health strategy that will benefit generations to come.[716]

The benefits of routine infant hepatitis B immunization have a ripple effect, extending far beyond protecting those who receive vaccine. Because infants and children tend to have high viral loads, they play a significant role in the transmission of HBV; vaccinating infants against HBV limits opportunities for horizontal transmission to susceptible household and community contacts. In addition, as cohorts of immunized children get older, vaccination programs that target newborns and infants have a substantial impact on prevalence rates in older populations. For example, in a study of university students in Taiwan, HBsAg prevalence dropped from 14.3% to 1.1% during 1995 to 2009 as a result of routine infant vaccination in that country.[717]

Hepatitis B vaccination is one of the most cost-effective public health interventions available, yielding a cost-per-life-saved of $4 to $36 in low-income countries.[718] Cost-to-benefit analysis has shown that perinatal hepatitis B prevention and routine infant hepatitis B immunization can save a net $1 in medical and work-loss costs for each $1 spent on immunization.[279] Other vaccine-based strategies, including universal adolescent vaccination and vaccination of at-risk adult populations, also have been found to be cost-effective or cost-saving.[421,719,720]

Despite the availability of hepatitis B vaccine since the early 1980s, barriers at that time impeded efforts to immunize infants and children against hepatitis B—for example, high vaccine costs, ill-founded concerns about using a plasma-derived vaccine during the first years of the AIDS epidemic, and the lack of global vaccine policies. By 1991, only 20 countries had implemented routine infant immunization against hepatitis B.[721]

Gaps in hepatitis B vaccine policy were first addressed in 1992, when the WHO called for all countries to incorporate hepatitis B vaccination into their national childhood immunization services,[721,722] stimulating broader adoption of hepatitis B immunization. By 1998, 90 countries were routinely administering hepatitis B vaccine to infants. However, by the end of 2000, only 32% of the global birth cohort had received three doses of hepatitis B vaccine, with a great majority of the unvaccinated infants living in developing countries.[723]

In the following decade, hepatitis B vaccination coverage grew rapidly (Fig. 25.13). By 2010, hepatitis B vaccination coverage among infants had reached an estimated 75% worldwide. This achievement was made possible through several developments, including removal of cost-related barriers. Throughout the 1990s, the cost of hepatitis B vaccine was $3 to $6 per dose; however, by 2010, the price had dropped to $0.185 to $0.40 per dose. Additionally, the GAVI Alliance (formerly the Global Alliance for Vaccines and Immunisation) was formed to provide targeted assistance for the countries with the lowest per capita gross national product. By 2004, 50% of the low-income countries receiving GAVI Alliance support had included hepatitis B vaccines in their routine immunization programs. China, India, and Indonesia, all of which received GAVI Alliance assistance, represented approximately one-third of the global birth cohort. In China, GAVI Alliance collaborations resulted in administration of a birth dose of hepatitis B vaccine, free of charge, to more than 25 million newborns living in the poorest and most remote provinces of western and central China; this initiative was expanded in 2002 to provide free hepatitis B vaccine to all newborns born in China. In India, a country with 1.5 million new HBV infections in the annual birth cohort, GAVI Alliance funds have improved vaccine infrastructure (e.g., increased availability of autodisposable syringes and vaccine) and supported hepatitis B vaccination in states with high-performing vaccination programs.[727] Similar collaborations have occurred worldwide. By the end of 2014, 184 countries had integrated hepatitis B vaccine into their national childhood immunization systems (Fig. 25.14). Global coverage with three doses of hepatitis B vaccines is estimated at 82% and is as high as 92% in the Western Pacific.[41]

The demonstrated progress made in global hepatitis B prevention, much of which is attributable to implementation of infant immunization programs, is commendable and can help

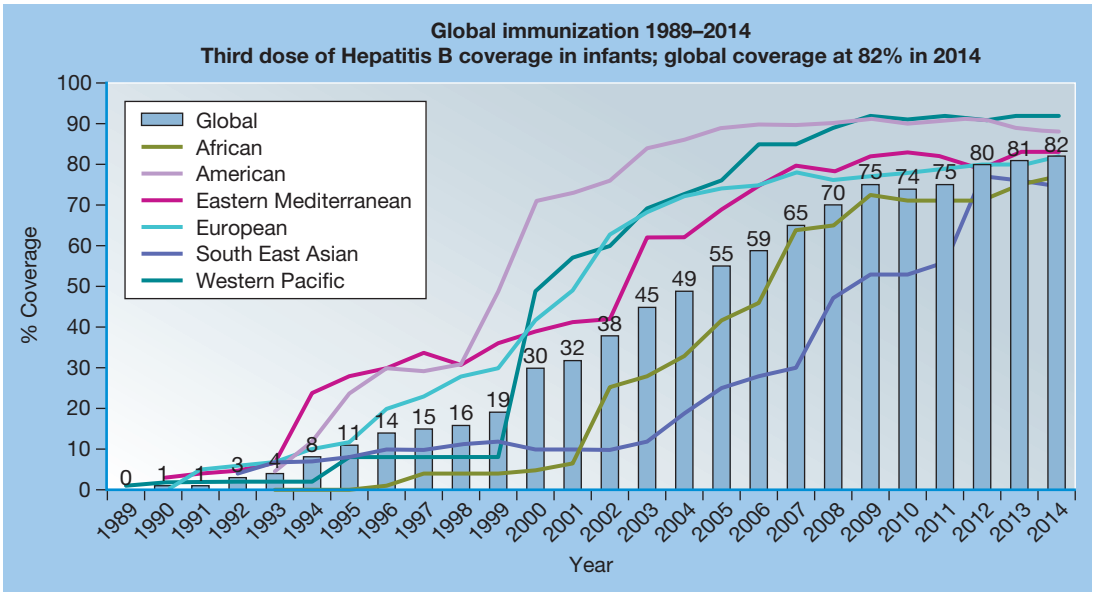


Figure 25.13. Evolution of the third-dose hepatitis B vaccine coverage in infant immunization programs, globally and by World Health Organization region (1989–2014). *(From World Health Organization. Immunization Coverage With 3rd Dose of HepB Vaccines in Infants, 2014. Available at http://gamapserver.who.int/mapLibrary/Files/Maps/global_hepb3_coverage.jpg. Data as of July 2015.)*

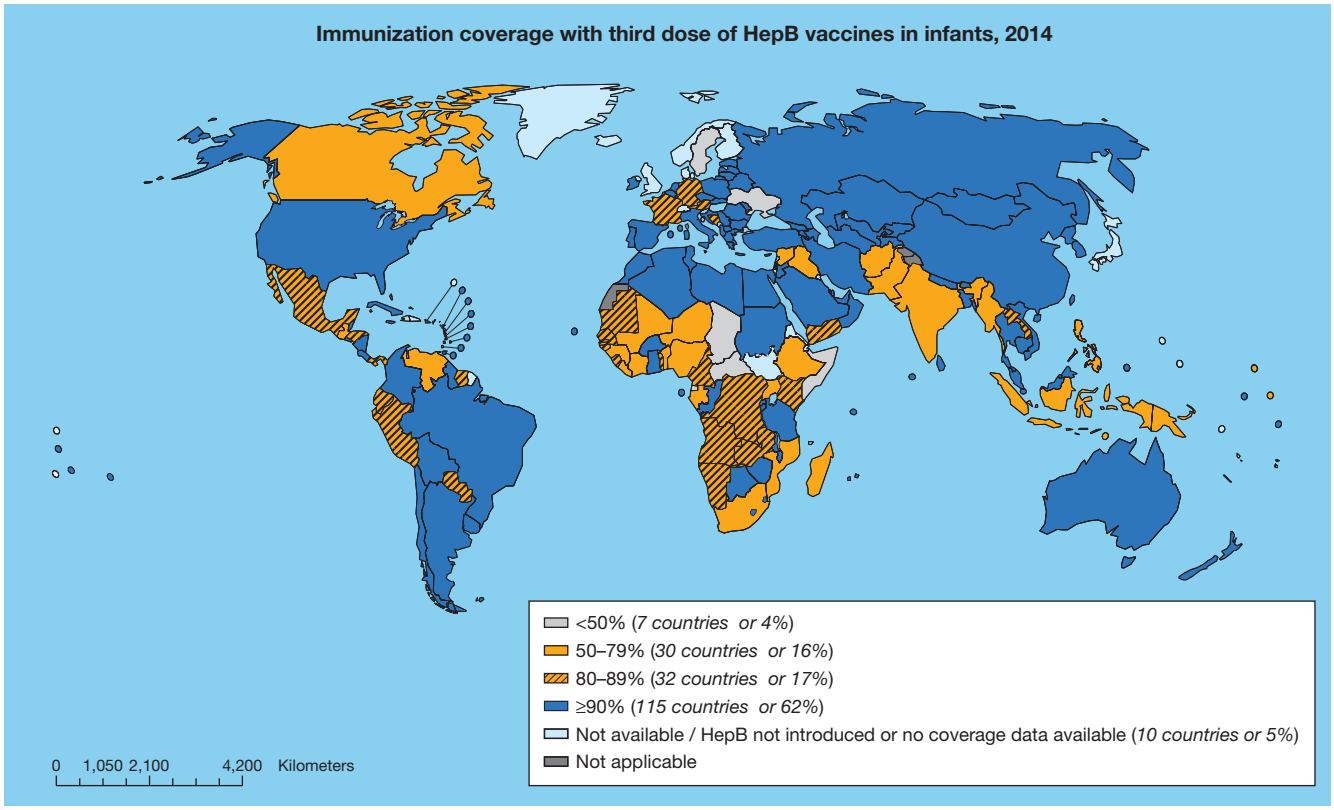


Figure 25.14. Countries using hepatitis B birth dose vaccine in their national immunization schedule, 2014. Note: The boundaries shown on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city, or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. *Dotted lines* on maps represent approximate borders, for which there may not yet be full agreement. *(From World Health Organization. Immunization Coverage With 3rd Dose of HepB Vaccines in Infants, 2014. Available at http://gamapserver.who.int/mapLibrary/Files/Maps/global_hepb3_coverage.jpg. Data as of July 2015.)*

inform policy and programming for other vaccine-preventable infections. However, the goal of HBV elimination can be achieved only by overcoming known challenges to hepatitis B vaccination and building and sustaining support for hepatitis B vaccination, particularly in developing countries with limited health resources.

To reach full potential as a public health intervention, hepatitis B vaccination must be more readily available to protect vulnerable populations, beginning with a birth dose of hepatitis B vaccine. Data from a mathematical model demonstrate that 90% coverage with a birth dose could prevent 225,000 (16%) deaths from chronic liver disease over the life of the 2000 global birth cohort[95]; in 2006, only 27% of newborns received a birth dose of hepatitis B vaccine.[490] In 2009, the WHO recommended that a dose of hepatitis B vaccine be provided within 24 hours of birth for all infants globally to maximize the potential of routine infant vaccination in preventing HBV transmission.[666] This WHO guideline can be most readily implemented in settings where most deliveries occur in healthcare facilities, as hepatitis B vaccination coverage tends to be higher for infants born in birthing facilities than those born at home or other settings. For example, in China, an increase in facility births is credited with contributing to the large increase in birth-dose coverage of hepatitis B vaccine.[367,714] However, implementation of a birth dose of hepatitis B vaccine can be a challenge for industrialized countries. In the United States during 2009, national coverage with the first dose of hepatitis B within 3 days of birth was 61%, with coverage in states ranging from 23% to 81%.[729]

In light of these data, efforts should be undertaken to encourage facility-based births. Ideally, health workers responsible for delivery would be trained to administer hepatitis B vaccination immediately after birth in collaboration with obstetric staff (e.g., obstetricians, midwives, birth attendants) and immunization service providers; furthermore, coordination between immunization and maternal child health programs should be strengthened. Potential strategies for improving provision of a birth dose of hepatitis B vaccine have been identified and include training for obstetrical staff, removing or reducing costs of in-facility births for expectant mothers, implementing the hepatitis B birth dose as a national practice standard, using standing orders for administration of hepatitis B vaccination as part of routine medical care, and ensuring record keeping to enable program evaluation.[367,730]

Regardless of the success of these efforts, not all women will deliver in a health facility; strategies are needed to ensure that hepatitis B vaccine is administered as soon as possible after birth to infants delivered at home. However, achievement of this objective is complicated by the narrow window of time associated with effective hepatitis B vaccination. To be effective, hepatitis B vaccine should be given as early as possible after birth, preferably within 24 hours, which may not be feasible in all settings. As revealed by a review of Indonesia's immunization program and confirmed by studies in Vietnam and China, strategies to achieve high vaccination coverage rates among infants born at home are complicated by insufficient vaccine policy, training for birth attendants, notification systems for pending deliveries, and community support and confidence in the safety of hepatitis B vaccine.[731–733]

The need for a ready supply of appropriately transported and stored hepatitis B vaccine is also problematic.[731] Although manufacturers recommend hepatitis B vaccine distribution and storage at 2°C to 8°C, studies have shown the WHO prequalified hepatitis B vaccines to be heat stable. These data support the feasibility of vaccinating neonates delivered at home in remote areas or at health posts lacking refrigeration, as long as vaccine is accompanied by vial monitors to measure cumulative heat exposures.[488]

To remove these barriers, hepatitis B vaccination should be integrated into maternal-infant prevention services. Several global health initiatives are in place to facilitate adoption of hepatitis B vaccination at birth as a recommended preventive service for maternal and child health programs, including the United Nations Millennium Goals to reduce the mortality rate of children younger than 5 years of age and maternal mortality, between 1990 and 2015.[723,737,738]

Large increases in hepatitis B vaccine coverage and the long interval of time required to achieve vaccine-related reductions in HBV-related morbidity and mortality can undermine support for hepatitis vaccination in the face of competing priorities; to ensure sustained support, the success associated with existing policies and programs must be measured and communicated. Efforts to document achievement in hepatitis B vaccination are already underway globally, as illustrated by the WHO's recommendation that all WHO regions and associated countries develop control goals appropriate to their epidemiologic situations.[666] To address this recommendation, countries are encouraged to use HBsAg as a measure of the impact of immunization. Data can be collected using serologic surveys of HBsAg; data from these surveys, along with active disease surveillance and mortality data, can serve as main outcome measures.

Several successes in hepatitis B vaccination have been documented as a result of this recommendation. In September 2005, the WHO Western Pacific Region, which had a population of 1.8 billion in 2007, became the first WHO region to adopt a regional goal of hepatitis B control to be met by 2012. The hepatitis B control goal for this region was to reduce the prevalence of chronic HBV infection to less than 2% in children at least 5 years old by 2012, with an ultimate goal of an HBsAg seroprevalence of less than 1% in children 5 years old and younger.[739] The Western Pacific Region has made tremendous progress toward hepatitis B control through newborn and childhood hepatitis B vaccination. Among the 37 countries in the region, 27 (comprising 87% of the region's population) are estimated to have reached the 2012 goal for children at least 5 years of age.[740]

In the WHO European Region as of 2012, 47 of the 53 European countries (89%) had implemented a universal hepatitis B vaccination program. The most recent countries to follow the recommendation were Ireland (in 2008) and the Netherlands (in 2011). Still, six countries (Denmark, Finland, Iceland, Norway, Sweden, and the UK) adopt risk-group-targeted vaccination only, instead of adding a universal vaccination program. However, changing demography, increasing immigration, and the current vaccine costs make the cost-to-benefit ratios in these remaining low endemicity countries strongly in favor of universal HBV vaccination.[741–744]

On September 17, 2014 the Regional Committee for Europe approved a resolution EUR/RC64/R5 to adopt the "European Vaccine Action Plan 2015–2020" which defines a regional vision and goals for immunization and control of vaccine preventable diseases and outlines priority actions to achieve them. One of the major European Vaccine Action Plan (EVAP) goals is to strengthen hepatitis B control through immunization. The EVAP 2015–2020 states: "The Regional Office commits itself to prepare a program and action plan for the control of hepatitis B infection and identify targets for 2020." The overriding vision of hepatitis B control in the Region is that the children in all countries in the region would be free of CHB infection, defined as an HBsAg prevalence of 0.5% or lower in children 5 to 10 years of age by 2020.[745]

Linking hepatitis B vaccination with other global health initiatives also can help sustain existing hepatitis B vaccination programs. For example, because hepatitis B vaccination prevents liver cancer and cirrhosis, immunization efforts can be

linked to activities to reduce chronic, noncommunicable diseases; the United Nations (UN) General Assembly recognizes noncommunicable disease as an emerging global epidemic that is impeding achievement of UN Millennium Development Goals.[746] In a report to the UN secretary-general, delivery of hepatitis B immunization was considered a "best buy" intervention because it is inexpensive, highly cost-effective, and culturally acceptable. Because HBV coinfection is a health issue for persons living with HIV, active chronic HBV infection is now considered to be a condition for modifying antiretroviral therapy. This will necessitate increased screening for HBV in the population of HIV-infected persons. To intensify the global response to HIV/AIDS, the UN General Assembly adopted a resolution to reduce the high rates of HIV and hepatitis B coinfection by rapidly expanding access to appropriate vaccination for hepatitis B, and to diagnostics and treatment of HIV and hepatitis coinfections.[747]

Integrating hepatitis B vaccination with screening, care, and treatment can promote a synergistic approach to hepatitis B prevention. Therapies for HBV infection, which are most effective when taken early in the course of infection, have greatly improved over the past decade, underscoring the importance of linking infected persons to care and treatment. Through screening, public health programs have an opportunity to ensure that infected persons and their susceptible contacts receive needed care, such as counseling, medical evaluation, and treatment. Likewise, providers of hepatitis B care and treatment can refer susceptible close contacts of HBsAg-positive patients for hepatitis B vaccination, and therapies can improve the effectiveness of vaccination programs. Studies reveal that providing antiviral therapy to HBsAg-positive mothers with high HBV viral loads during the third trimester of pregnancy can lower the rate of perinatal infection among exposed newborns receiving hepatitis vaccine and HBIG.[748–752]

The importance of this type of synergy is reflected in recent viral hepatitis prevention policies; for example, in 2011, the U.S. government released an action plan to promote a coordinated approach to viral hepatitis care and treatment, which can be tailored to meet the needs of other countries seeking to improve hepatitis B prevention.

In May 2014, the World Health Assembly approved a resolution to improve the prevention, diagnosis, and treatment of viral hepatitis. The importance of implementing appropriate measures to protect groups such as people who inject drugs from infection and to improve the access to screening, diagnosis, and treatment.[753] The Global Health Sector Strategy on HIV/AIDS, 2011–2015, endorsed by the World Health Assembly in 2011 (resolution WHA64.14) has ended, and several member states have requested the WHO secretariat to start a process to develop a post-2015 strategy. In a technical briefing at the World Health Assembly in May 2015, member state commitments to finish to emerging hepatitis epidemic were heard. The department of HIV and Global Hepatitis Programme at the WHO is developing three global health sector strategies to cover HIV/AIDS, viral hepatitis, and sexually transmitted infections. The strategies will cover 2016 to 2021 and were finalized at the 69th World Health Assembly in 2016. At this World Health Assembly the first "Global Health Sector Strategy on Viral Hepatitis, 2016-2021" was adopted. This strategy highlights the critical role of universal health coverage, and the targets of the strategy are aligned with those of the sustainable development goals. The strategy has a vision of eliminating viral hepatitis as a public health problem; this is now encapsulated in the global targets of reducing new viral hepatitis infections by 90% and reducing deaths due to hepatitis by 65% by 2030.[754]

In 1993 and 1999, expert committees considering targets for disease control concluded that the elimination of HBV transmission is a feasible public health goal.[686,688] Infant immunization programs successfully implemented by almost all countries provide the framework for such elimination. Universal infant vaccination beginning at birth on a global scale, along with targeted efforts to vaccinate all children and adults at risk for HBV infection, can reduce the risk for HBV infection to zero. However, elimination of HBV transmission will require high vaccine coverage in multiple generations. Expanded access to diagnostic testing and HBV care and treatment provide additional opportunities to prevent both HBV transmission and related disease. Commitment of public health resources to eliminate HBV transmission will require recognition of the large global burden of disease, demonstration of the benefits of vaccination, and patience to realize the goals of vaccine-associated disease reduction.

FUTURE VACCINES

Existing hepatitis B vaccines are highly effective, and there is no evidence that new vaccines will be needed to eliminate HBV transmission in immunocompetent populations with recommended immunization strategies. Potential uses for new hepatitis B vaccines include enhancing seroprotection in nonresponders to existing vaccines (using pre-S1 and/or pre-S2, or adding new vaccine adjuvants instead of aluminum hydroxide),[477,755–760] decreasing the number of doses required for seroprotection, and providing protection against HBV infection with S mutant viruses.[474,759,761,762] The addition of CPG 7909 to hepatitis B vaccine is promising as the immunostimulatory activity of this adjuvant can help in achieving long-term protection in hepatitis B vaccine hyporesponsive populations like HIV seropositives, hemodialysis patients, oncologic patients, the obese, and the elderly.[759,761,762a,762b] Such experimental vaccines, currently under examination for licensure in the United States, are effective in patients with renal failure and on hemodialysis, in nonresponders to conventional yeast-derived vaccines, and in immunocompetent healthy individuals receiving only two vaccine doses instead of three.[474,477,759,761,762] A two-dose schedule (4 weeks apart) was shown to trigger higher seroprotective rates compared to a classical three-dose schedule (0, 4, and 24 weeks) with a conventional hepatitis B vaccine.[762c,762d] As it has been hypothesized that a lack in vaccine efficacy might be explained by a mismatch between vaccine genotype and endemic HBV genotype (*adw* vs *ayw*), if proven, the use of a modified HBV vaccine in specific regions could be considered.[763,764]

Yet, to date, CHB is still an incurable disease and despite the tremendous progress made in developing nucleoside/nucleotide analogs that efficiently suppress HBV replication, these agents do not eliminate the covalently closed circular HBV-DNA residing in the nuclei of infected hepatocytes. Consequently, development of therapeutic immunization for persons with chronic HBV infection and restoring the defective immune tolerance to HBV remains an important goal.[765,766]

Vaccines generating strong T-cell immunity with purified or recombinant vaccine antigens, and intradermal, oral, and nasal hepatitis B vaccines, which might be easier to administer and less expensive than injections, are also under investigation.[503,660,767–770]

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26 Hepatitis C Vaccines

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Hepatitis C virus (HCV) is a leading cause of chronic liver disease worldwide. According to the World Health Organization,[1] 130 to 150 million people are chronically infected with HCV worldwide—approximately 3% of the world's population—and approximately 500,000 people die each year from HCV-related liver diseases. Importantly, 3 to 4 million more individuals are newly infected with HCV each year. A significant number of those newly infected will develop progressive liver disease, ultimately leading to liver cirrhosis and liver cancer. These scary numbers highlight the pressing need to develop vaccination strategies aimed at preventing and possibly eradicating HCV infection.

Although a prophylactic vaccine preventing HCV infection is not yet available, great progress has been made with respect to the development of increasingly more effective therapeutic regimens. Until recently, therapy for chronic HCV infection consisted of a combination of pegylated interferon-α (PegIFNα) and ribavirin. This approach, however, eradicates the virus in less than half of treated patients and is plagued with serious side effects. In recent years, the treatment paradigm for chronic HCV infection has undergone a revolution. New treatment regimens employing all-oral combinations of direct-acting antiviral agents (DAAs) lead to cure rates of greater than 90%, even in patients who failed prior interferon-based therapy.[2] The list of currently available DAAs include sofosbuvir, a pangenotypic nucleotide analog inhibitor of the RNA-dependent RNA polymerase; dasabuvir, a nonnucleoside polymerase inhibitor active against genotype 1; simeprevir, paritaprevir, and grazoprevir; NS3/4A protease inhibitors indicated for genotypes 1 and 4; and NS5A inhibitors ombitasvir, elbasvir (genotypes 1 and 4), ledipasvir (genotypes 1, 4, 5, and 6), daclatasvir, and velpatasvir (all genotypes). These drugs are used in different combination regimens, with or without ribavirin, depending on the viral genotype, liver disease staging, previous treatment history, and presence of comorbidities.[3,4] Furthermore, new drugs and treatment combinations are being developed with the objective of achieving a broader spectrum of action and possibly requiring a shorter duration of therapy.

The possibility of achieving a virological cure for chronic HCV infection is quite a remarkable feat; in fact, virus eradication is not possible in any other chronic viral infection. Thus, with the development of very effective antiviral drugs that are active on all HCV genotypes, global eradication of HCV by means of pharmacological treatment has become a theoretical possibility. In reality, however, three major challenges remain. First, in the absence of effective screening programs, HCV infection is underdiagnosed. Second, the high cost of these new therapies and the large number of HCV-infected individuals means that the healthcare systems, even in high-income countries, cannot afford to treat all patients. This limitation is even more pronounced in low- and mid-income countries. Third, reinfection remains possible even after successful curative therapy. For these reasons, an efficient HCV vaccine would still represent the most cost-effective and realistic means to significantly reduce the worldwide mortality and morbidity associated with HCV infection.

The scientific and clinical challenges that must be addressed and overcome in developing an efficacious HCV vaccine are substantial but may not be insurmountable. Among the difficulties that have hampered the development of a vaccine against HCV are its extreme genetic variability, the lack of small animal models for testing vaccines, and the fact that a cell culture system supporting the production of infectious HCV and allowing studies on virus neutralization in vitro became available only recently. Despite these issues, much progress has been made toward the identification of promising prophylactic vaccine candidates, and some of these candidate vaccines have started to be assessed in early clinical trials.

VIROLOGY

HCV was identified in 1989 as the major cause of transfusion and community-acquired non-A non-B hepatitis.[5,6] It is an enveloped, single-stranded RNA virus classified as a member of the *Hepacivirus* genus within the Flaviviridae family.[7] The 9.6-kb positive-sense RNA genome contains a single open reading frame encoding a polyprotein of approximately 3000 amino acids, flanked by highly structured 5′ and 3′ untranslated regions (UTRs), required for RNA replication and translation.[8]

Viral RNA released into the cytoplasm of the host cell is translated via an internal ribosome entry site (IRES) located in the 5′ UTR,[9] giving rise to an approximately 3000-amino-acid-long polypeptide that is cleaved into 10 different products (core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Fig. 26.1).[10]

HCV RNA replication takes place in the cytoplasm, in association with a virus-induced intracellular membrane structure termed "membranous web,"[11] and is directed by the error-prone RNA-dependent RNA polymerase encoded by the viral NS5B gene. This is a low-fidelity polymerase that introduces frequent mutations into the viral genome, generating quasispecies.[12] The extreme genetic variability of HCV, dwarfing that of HIV, provides a selective advantage for the evasion from immune control and is believed to contribute to the remarkable persistence of HCV in infected individuals.[13] Thus, the 5′ and 3′ UTRs, as well as the *core* gene are highly conserved, whereas the remaining viral genes and the corresponding proteins exhibit various but substantial levels of heterogeneity.[14] The site of greatest variability is within the N-terminal region of E2 envelope glycoprotein (hypervariable region 1 [HVR1]). This region is the major target of virus-neutralizing antibodies.[15–20] Recent structural insights into the aminoterminal domain of E1 and the core domain of E2 have revealed unexpected folds not present in glycoproteins from related viruses.[21] In vitro–produced HCV particles have been only recently visualized by electron microscopy.[22] Based on available data, HCV appears to be the most structurally irregular member of the *Flaviviridae* family: particles are spherical, with spike-like projections, and heterogeneous in size ranging from 40 to 100 nm in diameter. By analogy to related viruses, HCV virions are thought to consist of an icosahedral lattice of E1/E2 heterodimers bound to a cell-derived lipid bilayer membrane surrounding a nucleocapsid built from multiple copies of the core protein, which, in turn, package the genomic RNA. HCV is invariably found in association with low-density and very-low-density lipoproteins (LDL and VLDL), resulting in

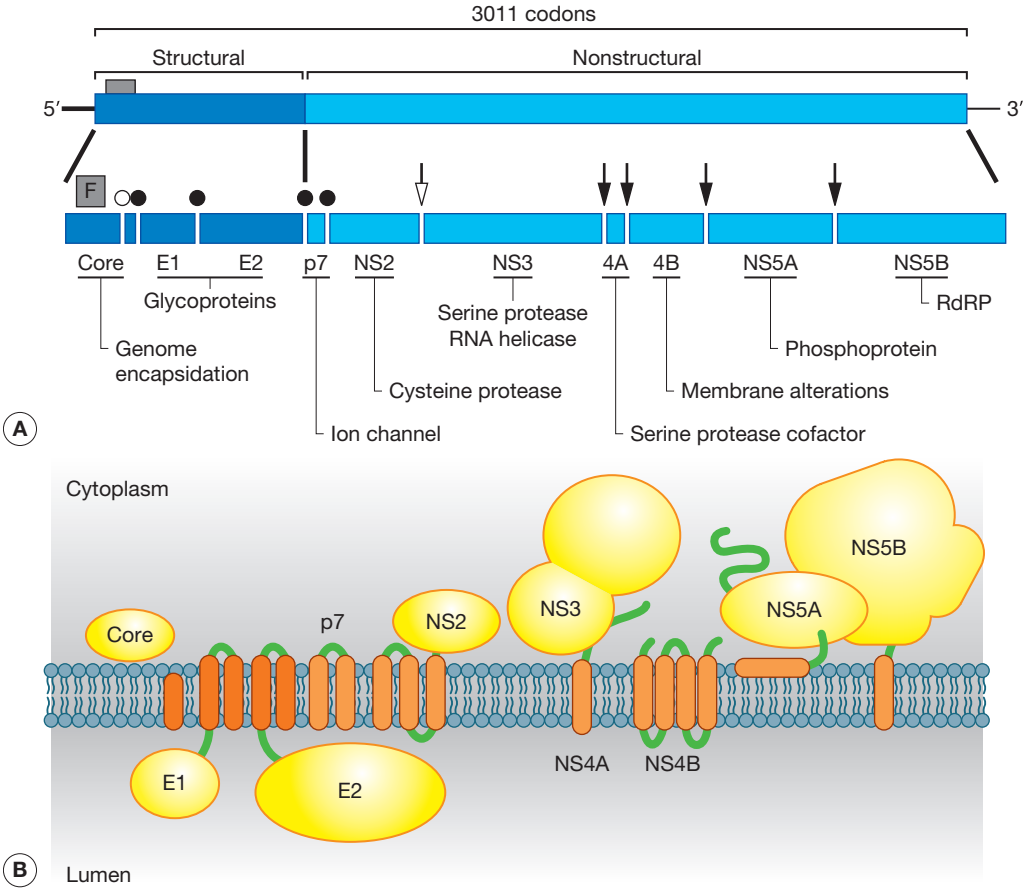


Figure 26.1. Hepatitis C virus (HCV) genes and gene products. A, The structure of the viral genome, including the long open reading frame encoding structural and nonstructural genes 5′ and 3′ untranslated regions (UTRs). The polyprotein processing scheme is shown. The C-terminal third of the polyprotein, core to p7, is processed by host cell signal peptidase, giving rise to the structural proteins, namely the N-glycosylated envelope virion glycoproteins, E1 and E2, and the core (C) protein, and to the nonstructural protein p7, an ion channel of the viroporin family. *Closed circles* refer to signal peptidase cleavage sites. The C protein is further cleaved by host signal peptide peptidase to yield the smaller species that appears to be the form found within virions circulating in the bloodstream. The *open circle* refers to the signal peptide peptidase cleavage site. A variable frameshift during translation of the C region results in the synthesis of the F protein (also called ARFP or core + 11).[193,194] This is a protein of unknown function and significance, but antibodies to these proteins circulate in patients chronically infected with HCV.[195] The remainder of the polyprotein is cleaved by two viral enzymes. The cysteine protease residing in nonstructural protein 2 (NS2) cleaves between NS2 and NS3 *(open arrow)*. The NS3 serine protease is activated by interaction with NS4A and catalyzes processing at all other sites within the NS region *(solid arrow)*. **B,** The topology of HCV proteins with respect to the cellular membrane is indicated. *(From Lindenbach BD, Rice CM. Unraveling hepatitis C virus replication from genome to function.* Nature. *2005;436:933–938.)*

the so-called lipo-viro-particles.[23] Infection of the hepatocyte by HCV depends on a complex set of receptors (CD81, scavenger receptor class B type I, claudin-1, and occludin), as well as several auxiliary molecules, facilitating adsorption of the virus to the cell (e.g., glycosaminoglycans, LDL receptor) or virus entry (e.g., epidermal growth factor receptor, Niemann–Pick C1-like cholesterol absorption receptor).[24] Of these, only the tetraspanin cellular protein CD81 and scavenger-receptor B1 (SR-B1) are believed to bind directly to the HCV envelope glycoprotein E2.[25,26]

For many years, it was impossible to propagate HCV in cell culture. Subgenomic HCV replicons were originally established for genotype 1b.[27] More recently, the panel of available replicons has expanded to include isolates from genotypes 1a, 2a, 3a, 4a, 5a, and 6a.[28–30] Because of a lack of structural proteins, however, subgenomic replicons could not be used to study immune responses against the virus. A fully permissive cell culture system supporting the production of infectious HCV only became possible with the identification of a genotype 2a isolate cloned from the serum of a Japanese patient with fulminant hepatitis, which was termed JFH1.[31] This breakthrough has allowed the generation of infectious hybrid viruses in vitro, in which the envelope glycoprotein genes of the 2a strain were replaced with equivalent genes from other HCV genotypes.[32] These key developments have opened up the field to investigations into HCV replication, virion structure, and virus-specific immune responses, as well as being valuable in the development of vaccines and new antivirals.

In addition to the above culture systems, a convenient and safe experimental model used to study HCV entry is based on the HCV pseudotype particles (HCVpp). HCVpp are obtained by exploiting a retrovirus in which HCV E1 and E2 substitute for the endogenous retroviral envelope proteins.[33–35] Because the HCVpp system can express a range of HCV glycoproteins originating from different isolates and genotypes, it represents an excellent platform for the establishment of a virus-neutralizing antibody assay.[36]

HCV can only infect humans and chimpanzees. Historically, attempts to develop small animal models of infection were based on the transplantation of human liver cells into

immunodeficient mice.[37] Whereas these have represented useful small animal models to study antiviral agents, they are unsuitable to study the immune response because of the lack of B-cell–mediated and T-cell–mediated immunity. Only very recently was a humanized mouse with a human immune system and liver tissue developed.[38] This was obtained by expressing in Balb/C Rag2(−/−) γC-null mice a fusion protein of the K506 binding protein and caspase 8 under control of a hepatocyte-specific promoter that induces liver cell death. Transplantation of human $CD34^+$ human hematopoietic stem cells and hepatocyte progenitors into the transgenic mice led to efficient engraftment of human leukocytes and hepatocytes. These animals, termed AFC8-hu HSC/Hep, supported HCV infection in the liver and generated a human immune T-cell response against HCV, and also developed hepatitis and fibrosis. An alternative method to overcome species barriers to HCV infection builds on the observation that CD81 and occludin comprise the minimal set of human factors required to render mouse cells permissive to HCV entry. Thus, transgenic mice were constructed that stably expressed human CD81 and occludin (Rosa26-Fluc mice[39]). These animals support HCV entry, but innate and adaptive immune responses restrict HCV infection in vivo.[39,40] Blunting innate antiviral immunity in these genetically humanized mice infected with HCV, however, resulted in measurable viremia over several weeks.[40] Alternatively, transgenic expression of human CD81 and occludin in an ICR (imprinting control region) mouse background yielded transgenic ICR mice that can support persistent HCV infection, with complete replication cycle and hepatopathological manifestations, without the need for targeting the disruption of interferon-stimulated genes.[41] Future work will assess the usefulness of these models in vaccine research.

HEPATITIS C VIRUS INFECTION

The major site of HCV replication is the hepatocyte. HCV has also been reported to infect other cell types, most notably B cells and dendritic cells,[42–46] but also cells in the intestine[47] and in the central nervous system.[48] However, the issue of extrahepatic HCV infection remains controversial.[49]

Historically, transmission seemed to occur primarily via direct parenteral exposure to contaminated blood. The frequent transmission of HCV that occurred in transfusion recipients before the discovery of the virus has now disappeared with the advent of serologic tests based on the detection of antibodies to viral proteins and on the direct detection of HCV RNA. As of today, intravenous drug use, unprotected sex with multiple partners, and viral exposure during invasive medical procedures (e.g., surgery, dialysis, and dental treatment) are factors associated with the highest degree of risk for HCV infection. After infection, HCV RNA appears in the plasma within a few days and usually shows a peak within the first few months. The inflammatory processes leading to initial liver injury, as assessed by serum elevation of the liver specific alanine aminotransferase (ALT) enzyme, generally occur within 1 to 3 months. The absence of correlation between HCV RNA levels and liver damage[50] suggests that HCV does not exert a direct cytopathic effect on the infected cells. At present, liver damage is postulated to be primarily caused by the infiltration of inflammatory cells, which cause extended inflammation that in turn causes hepatocyte damage and death. Although the mechanisms involved in initiating and maintaining an inflammatory response in the HCV-infected liver are not completely understood, the currently accepted model implicates the $CD8^+$ cytotoxic T-lymphocyte (CTL) T-cell response in mediating most if not all the liver damage that is associated with chronic viral hepatitis.[51] According to this model, hepatocyte injury is initiated by the cytolytic activity of HCV-specific CTLs and CTL-produced cytokines, such as IFN-γ, and IFN-γ–inducible chemokines (e.g., CXCL9 and CXCL10), recruit into the liver parenchyma large numbers of antigen-nonspecific inflammatory cells that have the potential to amplify the liver damage. Notably, work in animal models of viral hepatitis shows that platelets play a key role in T-lymphocyte–induced liver damage by facilitating the intrahepatic accumulation of CTLs.[51–53]

If infection is not resolved within the first 6 to 12 months, patients generally remain infected for life. Chronically infected patients have viral loads that typically range from 10^5 to 10^7 genomes per milliliter of serum. The virions have a rapid turnover, with a half-life of about 3 hours and up to 10^{12} viral particles per day are produced in an infected person.[54]

Natural History of Hepatitis C Virus Infection

Acute HCV infection is usually subclinical, as the symptoms are typically mild or absent. Between 15% and 50% of acute hepatitis C infections resolve spontaneously, whereas chronic infection develops in 50% to 85% of infected persons. During chronic inflammation of the liver, regeneration of tissue occurs up to a certain point, after which hepatocytes are replaced with fibrotic tissue that can subvert liver structure and function.[55] This condition can lead to cirrhosis and can, in some cases, progress to liver failure or hepatocellular carcinoma. In up to 20% of patients with chronic HCV infection, progressive liver damage develops, leading to end-stage disease, usually during a period of 10 to 30 years.[56] Owing to direct and indirect effects of HCV on lipid and glucose metabolism, respectively, chronic HCV infection is also often associated with liver steatosis and insulin resistance, which have the potential to synergize with the inflammatory liver disease.[57,58] The spectrum of severity of HCV-associated liver disease and its rate of progression show a high degree of variability between persons and are influenced by several cofactors, including age at infection, gender, host genetic variability, viral genotype, exposure to alcohol and other toxins, immune status, coinfections, and the presence of metabolic syndrome.[59] Chronic HCV infection has also been associated with extrahepatic diseases[60] such as mixed cryoglobulinemia, membranoproliferative glomerulonephritis, non-Hodgkin B-cell lymphoma, lichen planus, and porphyria cutanea tarda.

In recent years, it has become clear that an infected person's ability to clear HCV spontaneously, or following IFN-based treatment, is genetically determined. In fact, high rates of spontaneous clearance of acute HCV infection and high-sustained virological response rates following treatment with Peg-IFNα/ribavirin are associated with specific polymorphisms of the *IL28B* gene.[61] While the IL28B genotype does not have a significant value in predicting a patient response to the new IFN-free, all-oral DAA regimens, the presence of such a genetic determinant of spontaneous viral clearance is bound to have a significant impact on the host's ability to mount a protective immune response following vaccination and, therefore, may have to be considered in the design of clinical trials aimed at assessing the effectiveness of vaccines for HCV.

EPIDEMIOLOGY

A recent metaanalysis aimed at estimating the burden represented by HCV epidemics suggested that, globally, the estimated prevalence of people with anti-HCV antibodies increased from 2.3% to 2.8% between 1990 and 2005 (from >122 million to >185 million people).[62] Central and East Asia and North Africa/Middle East are estimated to have the highest prevalence (>3.5%); South and Southeast Asia, sub-Saharan Africa, Andean, Central, and Southern Latin America,

Caribbean, Oceania, Australasia, and Central, Eastern, and Western Europe have moderate prevalence (1.5% to 3.5%); whereas Asia Pacific, tropical Latin America, and North America have low prevalence (<1.5%). At the level of individual countries, Egypt is thought to have the highest prevalence of chronic HCV in the world, estimated at approximately 15% to 20%. What led to an HCV epidemic of this proportions in Egypt is the extensive use of parenteral antischistosomal therapy in a mass treatment setting, a practice discontinued only in the 1980s.[63]

Seven different major HCV genotypes and many subtypes have been distinguished phylogenetically, with 30% to 50% sequence variation among viral genotypes and 15% to 30% among different subtypes.[64,65] Globally, genotype 1 is the most common (46%), followed by genotype 3 (22%), genotype 2 (13%), genotype 4 (13%), genotype 6 (2%), and genotype 5 (1%).[66] Definitive data regarding the prevalence of genotype 7 are still lacking. Genotype 1b is the most common subtype, accounting for 22% of all infections. Significant regional and countrywide variations in genotype distribution exist. Infections in Europe, North America, and Latin America are predominantly genotype 1. North Africa and the Middle East have large genotype 4 populations, primarily as a consequence of the extraordinarily high prevalence of this genotype in Egypt. Excluding Egypt, genotype 1 becomes predominant also in this region. Asia is primarily affected by genotype 3, followed by genotype 1, with the former being very common in India, Nepal, and Pakistan. Finally, genotype 1 predominates in Australia, followed by genotype 3.[66]

Transmission of HCV seems to occur via direct parenteral exposure to contaminated blood. The frequent transmission of HCV that occurred in transfusion recipients before the discovery of the virus has disappeared with the advent of serologic tests based on the detection of HCV-specific antibodies or the direct detection of HCV RNA. At present, a history of intravenous drug use represents the major risk factor for newly acquired hepatitis C infection.[67] Other possible sources of parenteral exposure to HCV are surgical and dental procedures and folk practices such as piercing and tattoos.[68] Although the risk of sexual transmission is low, sexual partners of infected persons still account for a certain percentage of HCV infections.[69] Vertical transmission also occurs particularly in mothers with high viral load or who are coinfected with HIV.[70–72] Coinfection with HIV and HCV is common in intravenous drug users (IVDUs) of illicit drugs, and importantly the immunosuppression associated with HIV infection results in higher HCV titers and more rapid progression to cirrhosis and end-stage liver disease.[73]

IMMUNE RESPONSE TO HEPATITIS C VIRUS

HCV has adapted to escape host immune control, in most cases progressing into a persistent infection. Moreover, unlike for many other viruses, resolution of acute HCV infection does not necessarily result in sterilizing immunity upon reexposure to the virus. Nonetheless, approximately 20% of the acute HCV infections are spontaneously cleared, indicating the existence of effective adaptive immune responses. A detailed understanding of the immune mechanisms that contribute to rapid control of an acute HCV infection, after 25 years of studies in humans and chimpanzees, however, is still lacking. Moreover, there is still considerable uncertainty about the relative contribution of humoral versus cellular immune response to the resolution of infection.

Several studies show that recovery from acute HCV infection is associated with the induction of a multispecific T-helper cell type 1 (Th1)-type $CD4^+$ T-helper and $CD8^+$ CTL T-cell responses to the virus.[74] This is evidenced, for example, by the associations of class I human leukocyte antigens[75–77] and class II antigens[78] with spontaneous virus clearance, the temporal association of resolution of infection with HCV-specific IFN-γ–secreting T cells,[79] and the generation of polyfunctional, durable $CD4^+$ and $CD8^+$ T-cell subsets directed against multiple HCV antigens in spontaneous resolution.[80,81] Additionally, T-cell depletion experiments with chimpanzees confirmed the essential role of both the $CD4^+$ T-helper and the $CD8^+$ CTL T-cell responses in controlling HCV infection.[82,83]

Whereas the importance of cellular immunity in controlling HCV infection is well established, the role of the humoral immunity in recovery from infection is less clear. However, an increasing number of studies suggest that B cells and neutralizing antibodies (nAbs) could also play an active role in the spontaneous resolution of HCV infection. Antibodies produced during acute HCV infection target epitopes within both structural and nonstructural viral proteins. However, all known nAbs target epitopes within the HCV envelope glycoproteins E1 and E2, or the E1/E2 heterodimer. Most characterized nAbs are directed against regions of E2 that interact with the viral entry factors CD81 and scavenger-receptor B1 (SR-BI) (Fig. 26.2).[84]

Longitudinal analysis of HCV infection cohorts revealed a clear association between eradication of acute viremia and the presence of antibodies able to block entry of HCV pseudoparticles into cultured hepatocytes.[85,86] Additional recent evidence suggests that early induction of cross-reactive nAbs during acute infection strongly correlates with spontaneous clearance of HCV.[87–90] nAbs also play an important role in persons who have previously cleared their primary infection but become infected on reexposure to the virus.[91] In this study, nAbs were detected during the acute infection phase in 60% of reinfected participants who went on to clear the reinfection, but were not detected in two patients who progressed to chronic infection. Overall, these studies suggest that the development of a nAb response during the early phase of infection could be critically implicated in viral clearance. Even during chronic infection, nAbs may mediate some control of HCV as hypogammaglobulinemic persons experience a more severe and rapidly progressing liver disease.[92,93] Consistent with this view, passive transfer of nAbs also has been shown to protect humans and animal models against viral challenge. Passive immunization using human immunoglobulin preparations also has shown efficacy in preventing HCV transmission following blood transfusion,[94] sexual activity,[69] and liver transplantation.[95] Recently, a human monoclonal antibody targeting E2 was shown to prevent HCV infection in the chimpanzee model.[96] Antibodies targeting the HCV envelope glycoproteins also have been shown to protect against heterologous virus challenge in human liver-chimeric mouse model.[97,98]

Now that HCV can be propagated in cell culture, assays for viral nAbs are available. In fact, the availability of cell culture viruses with envelope proteins of the HCV genotypes 1 to 7 (JFH1 intergenotypic recombinants) permitted cross-neutralization studies in vitro.[32] Using these intergenotypic recombinant viruses, sera obtained following infection with HCV genotypes 1a and 4a cross-neutralized genotype 1a, 4a, 5a, and 6a viruses, but not genotype 2a and 3a viruses,[99] suggesting a clustering of genotypes that could be important for future development of passive and active HCV immunoprophylaxis strategies. In the chimpanzee model, infusion of anti–genotype 1a HCV immunoglobulins with high-titer in vitro cross-neutralizing HCV antibodies prevented the infection by genotype 1a or 6a HCV, but failed to protect the animals against genotype 4a or 5a,[100] indicating that more work is needed in this area of research.

HCV has evolved several mechanisms to persist in the human host and to evade the adaptive and innate immune

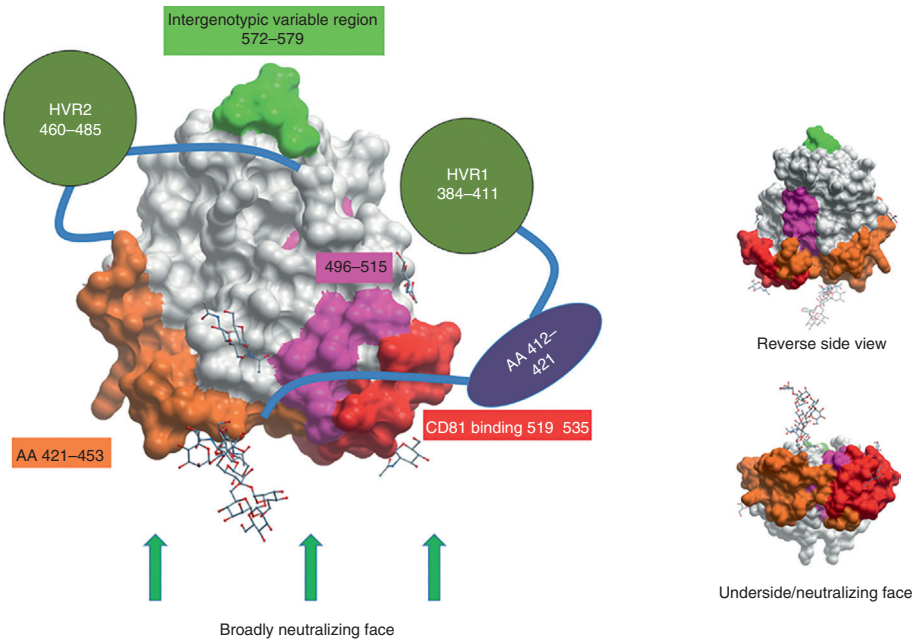


Figure 26.2. Representation of hepatitis C virus (HCV) E2 glycoprotein structure. E2 is a globular protein with three regions of hypervariability—HVR1, HVR2, and intergenotypic variable region—shown in *green*. Domains whose structures are currently unknown are depicted as shapes apart from the structure. HVR1 is predicted to mask a hydrophobic region that is sensitive to neutralizing antibodies (nAbs). E2's broadly neutralizing face is shown, where many broadly nAbs bind, comprises CD81-binding loop (in *red*), residues 421–453 (in *orange*), residues 502–520 (in *pink*), and residues 412–421 (in *purple*). The possible positions of some glycans are shown as sticks and balls. *(From Cashman SB, Marsden BD, and Dustin LB. The Humoral immune response to HCV: understanding is key to vaccine development.* Front Immunol. *2014;5:550.)*

responses. At the level of innate immune response, the virus has evolved the ability to disrupt the cellular signaling pathways leading to IFN synthesis and to block antiviral activity of IFNγ-sensitive genes.[101,102] Moreover, the binding of E2 to CD81 directly inhibits natural killer (NK) cell activity,[103,104] providing the virus with a mechanism aimed at limiting the antiviral activity of NK cells early in the infectious process.

HCV has also developed several ways to elude the adaptive immune response. First, the virus exists as a population of viral quasi-species that is sufficiently immunologically distinct to enable the emergence of variants that elude host humoral and cellular immune responses.[105] Thus, HCV produces with high-frequency escape mutants to both CTLs[106] and to nAbs directed to the envelope glycoproteins.[15,16,18,107,108] HCV has also evolved a number of indirect mechanisms to evade antibody responses. These include the association of HCV with lipoproteins,[23,109–111] possibly leading to the interference of antibody binding. The viral envelope is also heavily glycosylated and the presence of these modifications protects the virus against the development and binding of nAbs (see Fig. 26.2).[112–114] Another possible mechanism of escape from the immune response may be related to the finding that HCV infects cells not only by the direct uptake of cell-free virus following virus engagement with specific cell receptors, but also through direct cell-to-cell transmission.[115] Unfortunately, this last mode of transmission is shielded from the action of nAbs.[116] Finally, the activity of effector T cells specific for the virus can be impaired as a consequence of persistent HCV infection.[117,118] In this respect, it is worth noting that immunization of mice with adenoviral vectors expressing HCV core or F protein leads to T cells with reduced production of effector molecules such as granzyme B and IFN-γ.[119] These results reveal a potential new strategy of immune modulation by HCV-derived F and core proteins, and may have important implications in vaccine design.

Despite the strategies HCV puts in place to blunt the host immune response, when the host has the ability to elicit early and broad Th1-type $CD4^+$ and $CD8^+$ T-cell responses to the virus[79,82,83,120–127] and also has an NK receptor repertoire that facilitates innate immune clearance of virus,[128] then eradication of virus can occur (Fig. 26.3).

In conclusion, infected humans and chimpanzees who mount early, multispecific $CD4^+$ and $CD8^+$ T-cell responses to HCV proteins can eradicate the virus. In addition, many experimental studies have provided evidence that broadly neutralizing antibodies to HCV can offer some protection against heterologous viral infection in vivo.

PROTECTIVE IMMUNITY AGAINST HEPATITIS C VIRUS

Studies in the chimpanzee challenge model and of multiply exposed humans have demonstrated that there is significant natural immunity against HCV. In one chimpanzee study,[20] an animal was infected by intrahepatic administration of an infectious RNA derived from HCV strain HCV-1 (1a subtype). Following resolution of the acute infection, the animal was shown to be resistant to an intravenous rechallenge with a homologous virus. No viremia could be detected following rechallenge, indicating that sterilizing immunity was generated during the first infection. Importantly, a subsequent challenge with heterologous 1a or 1b strains induced only a low, transient viremia before disappearance of the virus from plasma and the liver.[20] Similar results were obtained in separate chimpanzee studies in which animals were challenged and then rechallenged with different infectious viral inocula.[129,130] Opposite results have been obtained in another chimpanzee study,[131] in which protection against chronic HCV infection was observed only after rechallenge with homologous, but not heterologous, genotypes. Moreover it has been

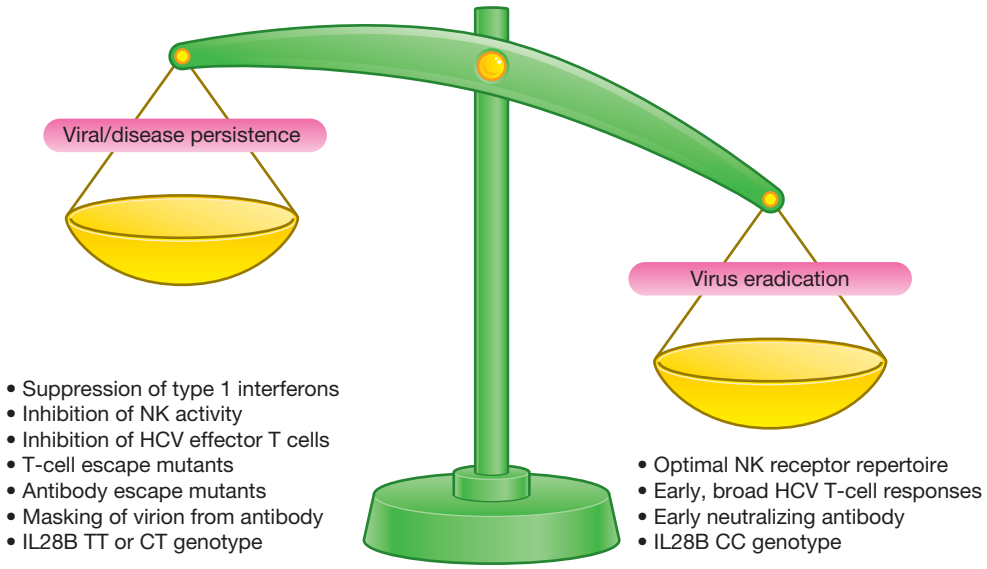


Figure 26.3. Current view about immunological correlates of protection against hepatitis C virus (HCV) infection and chronic hepatitis. NK, natural killer.

more recently reported that previously infected chimpanzees are not consistently protected against reinfection or persistent infection after reexposure to the identical hepatitis C virus strain.[132]

In humans, evidence of cross-protective immunity has been reported in a prospective study of IVDUs from the United States. Strikingly, the incidence of persistent viremia in IVDUs who had recovered from a previous infection was 12-fold lower than that in IVDUs who had not experienced a previous infection, as shown using multivariate analyses.[133] As seen also in the chimpanzee, peak viral loads were substantially higher (by almost 2 logs) in the first-time infections as compared with reinfections. In addition, HIV coinfection produced persistent HCV infection in all cases, indicating the importance of an intact immune response in recovery.[133] Interestingly, it was recently reported that the reduced risk of HCV persistence in IVDUs previously recovered from HCV infection correlated with T-cell responses, and prolonged antigenic stimulation seems to be required to maintain humoral responses.[134]

Collectively, these chimpanzee and human data provide evidence for the existence of immunity to HCV and, importantly, suggest the existence of cross-protective immunity within and between commonly occurring HCV genotypes. It is important to note, however, that not all reinfections in IVDUs are resolved without progression to chronicity,[133] indicating that natural immunity to HCV is incomplete and not as effective as that found following infection with hepatitis A and B viruses. It should also be mentioned that earlier studies in the chimpanzee model have concluded a lack of protective immunity to HCV.[135] This apparent contradiction may be a result of the earlier studies measuring immunity in terms of sterilizing immunity, rather than as the ability to prevent the development of chronic infection.

In summary, the current consensus is that the development of an early, broad, and multispecific T-cell response during acute viral infection is associated with spontaneous clearance of infection. Similarly, protection from reinfection correlates with the breadth and magnitude of a multifunctional $CD8^+$ T-cell response and also with the quality, functional potency, and cytotoxic potential of HCV-specific $CD8^+$ T cells responding against diverse HCV epitopes. It is also increasingly clear that the presence of nAbs against the virus envelope glycoproteins can be protective and is often associated with the rapid clearance of viremia. Therefore, it would be desirable for an optimal vaccine to induce a strong and broadly cross-reactive cellular immune response as well as cross-neutralizing antibodies.

STRATEGIES FOR A HEPATITIS C VIRUS VACCINE

Historically, the strategies for the development of HCV vaccines have included approaches aimed at (1) preventing initial infection (i.e., providing sterilizing immunity), (2) preventing the development of chronic viral persistence that occurs in the majority of natural infections, or (3) clearing the virus in persons who already have an established chronic HCV infection (i.e., therapeutic vaccines).[136] In consideration of the huge genetic variability of HCV, achieving broad, sterilizing immunity by vaccination could prove practically impossible. However, because the clinically relevant disease is chronic and not acute hepatitis, an HCV vaccine that would not elicit sterilizing immunity but rather prevented persistence of the virus, allowing a self-limited acute infection, would be entirely acceptable. Accordingly, the goal for the prophylactic HCV vaccines being currently developed is not prevention of infection but prevention of viral persistence.[137] Conversely, therapeutic vaccine approaches are not currently being pursued in light of the success of the new antiviral agents in providing a definitive virological cure.

To date, a number of HCV vaccine approaches, including subunit vaccines and genetic vaccines (i.e., plasmid DNA and viral vector vaccines), have been tested clinically (Table 26.1) or in the chimpanzee model. These approaches are briefly reviewed below.

Vaccines Based on Adjuvanted Recombinant Hepatitis C Virus Proteins

Historically, the first prophylactic vaccine candidate tested for HCV consisted of a C-terminally truncated recombinant E1 protein formulated with aluminium hydroxide (alum) adjuvant (T2S-918/InniVac-C[138]). This vaccine was shown to elicit anti–E1 antibodies in healthy volunteers.[138] Development of

TABLE 26.1 Summary of Outcome of Chimpanzee Vaccination/Challenge Studies Performed With Adjuvanted Recombinant gpE1/gpE2 Formulations[a]

Viral Challenge	Group	Total	Acute Infection	Chronic Infection (%)	*P* Value[b]
Homologous	Adjuvanted gpE1/gpE2	12[c]	7[c]	2 (17)	.03
HCV-1 challenges	Unimmunized control animals	10	10	7 (70)	
Heterologous	Adjuvanted gpE1/gpE2	19	19	3 (16)	.02
HCV-H77 challenges	Unimmunized control animals	14	14	8 (57)	
Total	Adjuvanted gpE1/gpE2 Unimmunized control animals	31 24	26 24	5 (16) 15 (63)	≤.001

gp, glycoprotein; HCV, hepatitis C virus.

[a]These studies were performed during the course of many years. Typically, animals were immunized with 30–80 μg gpE1/gpE2 in various oil/water adjuvants (MF59 ± MTP [muramyl tripeptide] or MF75 ± MTP or MF59 ± CpG [cytosine phosphate guanine]) at months 0, 1, and 6 approximately, followed by intravenous challenge 2–4 weeks later with 10–100 chimpanzee infectious doses of HCV-1 or HCV-H77. Both strains belong to the 1a subtype that predominates in North America. Circulating levels of viremia were measured using reverse transcription–polymerase chain reaction assays for HCV genomic RNA for at least 1 year postchallenge. Qualitative transcription-mediated amplification (TMA) assays were also performed in some animals.

[b]*P* values (Fisher exact test) refer to chronic carrier rates between control animals and vaccinees.

[c]In five vaccinees challenged with homologous HCV-1 virus, no viremia could be detected at any time postchallenge in plasma peripheral blood mononuclear cells (PBMNCs) or liver biopsy samples; thus, they were considered to have been sterilized.

From Houghton M. Prospects for prophylactic and therapeutic vaccines against the hepatitis C viruses. Immunol Rev. *2011;239:99–108.*

this vaccine candidate, however, was stopped as no effects on viremia or progression to fibrosis in individuals with chronic HCV infection were observed.[139]

In a study with a different vaccine candidate,[140] a chimpanzee was immunized with insect cell–derived recombinant E1 and E2 derived from HCV strain N2 and a peptide spanning the E2 HVR1 region from HCV strain 6. The two envelope proteins, which lacked the C-terminal transmembrane anchors, were expressed and purified separately. The HVR1 peptide was conjugated to keyhole limpet hemocyanin and immunizations were performed with Freund adjuvant. The conclusion, derived from a series of immunizations and challenges in the same animal with HCV strain 6, was that sterilization against infection was dependent on high anti-HVR1 antibody titers rather than total anti-E2 or anti-E1 titers.[140,141] The outcome of challenging with other viral strains was not reported, however.

In a more recent study,[142] the immunogenicity of E1 and E2 as separate proteins was assessed in chimpanzees using recombinant E1 and an HVR1-deleted form of E2 produced in human Vero cells (HCV 1b BE11 strain). After vaccination with either protein adjuvanted with alum, immunization of chimpanzees with either E1 or E2 alone induced antigen-specific T-helper cytokines of similar magnitude. Unexpectedly, the capacity to neutralize HCVpp in vitro was observed only with serum from animals vaccinated with E1. Furthermore, only E1-vaccinated animals exposed to a different HCV-1b inoculum (strain J4) were protected from viral persistence.

The E1/E2 heterodimer is generally considered to be a more native reflection of the HCV virion than either ectodomain alone.[143] Consequently, full-length E1/E2 heterodimers have long been considered and evaluated as candidate HCV vaccine antigens. A vaccine consisting of the recombinant E1/E2 heterodimer derived from mammalian cells has been tested extensively for efficacy in chimpanzees (Table 26.2).[144–148] Combined with oil/water-based adjuvants, this vaccine elicited antienvelope antibodies as well as T-helper cell responses to E1 and E2 in this animal model. When the vaccinated animals were challenged experimentally with homologous viral inocula, the highest responding animals (in terms of anti–E1/E2 antibody titers) were completely protected against infection. Using sensitive reverse transcription–polymerase chain reaction assays, no viremia was detected in serum or liver samples at any time after challenge in these "sterilized" animals. This apparent sterilizing immunity correlated directly with anti-E2 antibody titers that prevent the binding of E2 or the virus itself to CD81.[149] Furthermore, although lower-responding animals became infected, the majority underwent an abortive acute infection that did not result in the persistently infected carrier state.[144,147] Overall, these data showed that the progression rate to chronic infection was significantly lower in vaccinees than in unimmunized control animals.[145] These pioneering studies provided the first data supporting the feasibility of a vaccine against HCV because they indicated the capability of recombinant envelope glycoprotein immunization to either elicit sterilizing immunity or to prevent the progression to chronic infection following an acute, transient infection after viral challenge.

This work was subsequently extended to address the key question of vaccine protection against challenge with a different viral strain of the same genotype. (The vaccine strain, HCV-1, and the challenge strain, HCV-H, are both members of the 1a genotype that predominates in the United States). Although none of these animals were sterilized against this heterologous viral challenge, the majority failed to develop into persistent carriers of the virus, unlike the situation with control unvaccinated animals and for human infections (see Table 26.1).[145] Protection against viral challenge correlated with the vaccine-induced anti–E1/E2 responses. Importantly, antisera from several of the immunized chimpanzees were shown to exhibit in vitro cross-neutralizing activity.[150] Based on the encouraging preclinical data, a clinical development program was started aimed at assessing tolerability and immunogenicity of the E1/E2 complex purified from Chinese hamster ovary cell lines and formulated with the oil/water M59 adjuvant.[151,152]

Strong T-helper cell responses were elicited in the human volunteers along with substantial anti–E1/E2 antibody titers that were similar to those elicited in vaccinated and protected chimpanzees. Additional analyses showed that most vaccinees developed antibodies that could neutralize the infectivity of HCVpp bearing envelope glycoproteins derived from the homologous 1a strain, HCV-1.[151] Moreover, it was recently shown that antisera from selected vaccinated individuals—although with varying efficacy—were capable of neutralizing

TABLE 26.2 Clinical-Stage Prophylactic and Therapeutic Vaccine Candidates

Vaccine	Efficacy	Stage
PROPHYLACTIC VACCINE CANDIDATES		
Chiron/Novartis: Recombinant E1/E2 in oil/water adjuvants[144,151,152]	Protects chimpanzees against chronic infection[144]	Phase I[151,152]
Okairos/GlaxoSmithKline: Ad6/electroporated DNA prime/boost[186] Chd3NSmut/MVA-NSmut (chimpanzee adenovirus/MVA prime/boost)[188]	Protected chimpanzee from acute or chronic infection following heterologous viral challenge[186] Broad $CD4^+$ and $CD8^+$ T-cell response[188,190]	Phase II[178]
IMMUNOTHERAPEUTIC VACCINE CANDIDATES		
Innogenetics (T2S-918/InnoVac-C): Alum-adjuvanted E1 glycoprotein[138]	Boosts humoral and cellular immune responses to E1 in healthy volunteers[138] and in chronic hepatitis C patients[139]	Phase II[139] Program stopped as no effects on viremia or fibrosis progression
Intercell AG (IC41): HCV peptide cocktail with polyarginine[165,166]	Designed to boost $CD4^+$ and $CD8^+$ responses to conserved T cell epitopes using novel adjuvant	Phase II in combination with Peg-IFNα[167,169]
Globe Immune (GI-5005): Heat-killed yeast expressing core-NS3 fusion protein[171]	Primes specific $CD4^+$ and $CD8^+$ T cells in mice.[172] Moderate antiviral activity in chronic hepatitis C patients[173]	Phase II in combination with Peg-IFNα[196,197]
Tripep (ChronVac-C): DNA-based vaccination with NS3/4A-expressing plasmid)[175]	Elicits $CD8^+$ T cells that clear liver cells expressing HCV proteins in transgenic mouse model. Small viral load reduction in chronic hepatitis patients[175]	Phase Ib[175]
CIGB-230: Mixture of plasmid expressing HCV structural antigens with a recombinant HCV core protein[176]	Possible amelioration of fibrosis in chronic hepatitis patients, despite no effect on viremia	Phase Ib[177]
Transgene (TG4040): Modified Vaccinia Ankara virus expressing NS proteins (NS3-NS5B)[198]	$CD4^+$ and $CD8^+$ response in chimpanzee; viral load reduction of up to 1.5 $\log_{10}$ in chronic hepatitis C patients[181]	Phase II in combination with Peg-IFNα[182]

HCV, hepatitis C virus; Peg-IFNα, pegylated interferon-α.

chimeric cell culture-derived viruses (HCVcc) expressing the structural proteins of strains representing all seven major HCV genotypes.[153] No further progress in clinical development has been reported for this vaccine candidate.

Expression of a core-E1-E2 gene cassette in insect cells has been reported to result in the generation of 40- to 60-nm viral-like particles (VLPs) resembling the putative HCV virions.[154] After partial purification, insect-cell produced VLPs appeared to be immunogenic in mice[155] and baboons.[156] In addition, these VLPs were shown to induce specific $CD4^+$ and $CD8^+$ cell responses in chimpanzees.[157] Upon HCV challenge with a homologous HCV strain, all four animals became infected but after 10 weeks three of them cleared the virus and one continued to have intermittent viremia with low HCV RNA levels. While an increase in the magnitude of peripheral and intrahepatic T cell and proliferative response against HCV structural proteins was observed, no HCV-specific antibody response was detected. The outcome of challenges with other viral strains, or results of clinical trial using this approach have not been reported. In addition, developmental issues exist for E1/E2 vaccine. Indeed the heterodimer has to be purified as intracellular material from endoplasmic reticulum of mammalian cells, making it difficult to scale up its production.

Recombinant core protein has also been investigated as a potential component of an HCV vaccine based on the following rationale. First, broad HCV-specific $CD4^+$ and $CD8^+$ T-cell responses, including those to the core protein, are associated with recovery from HCV infection.[79,121,125–127] Second, the core protein is the most conserved HCV polypeptide and contains $CD4^+$ and $CD8^+$ epitopes that are highly conserved among the different HCV genotypes, which should therefore facilitate the generation of cross-protective immunity.[158,159] Third, recombinant core protein has been shown to self-assemble into particles[160] with concomitant high immunogenicity in animal models. Through administration of a recombinant *Escherichia coli*-produced HCV core protein formulated as "immunostimulatory complex" (ISCOM) it has been possible to prime strong $CD8^+$ CTL activity (and $CD4^+$ activity) in rhesus macaques.[161] (ISCOMs are particulate antigen delivery systems composed of antigen, cholesterol, phospholipid and saponin, while ISCOMATRIX is a particulate adjuvant comprising cholesterol, phospholipid and saponin but without antigen. The combination of an antigen with ISCOMATRIX is called an ISCOMATRIX vaccine.[162]) Based on the encouraging preclinical data, a Phase I study was performed[163] aimed at the evaluation of the immunogenicity of the HCV core/ISCOMATRIX vaccine in healthy people. Antibody responses were detected in all but one of the participants receiving the vaccine. However, $CD8^+$ T-cell responses were only detected in two of the eight participants receiving the highest dose.[163] The results of this study suggest that the development of a protein-based HCV vaccine capable of priming cross-reactive HCV-specific $CD8^+$ T-cell responses in humans will require further investigation.

Numerous $CD4^+$ and $CD8^+$ epitopes that are conserved among the different HCV genotypes reside within structural and nonstructural proteins.[158,159,164] The inclusion of a multiplicity of these epitopes could facilitate the vaccine-mediated generation of broadly cross-reactive cellular immune responses to the virus, which could then result in the elicitation of cross-protective immunity against different HCV genotypes. One such approach involves the assembly of "HCV polytope vaccines" consisting of a consecutive sequence of these conserved HCV T-cell epitopes[154,155] in the form of a recombinant

polypeptide or DNA vaccine. Focusing the immune response on a collection of highly conserved epitopes that can be presented by diverse human major histocompatibility complex (MHC) class I and class II antigens may optimize the generation of strong, cross-protective immunity and possibly avoid the "dilution" of the immune response to variable, more mutable HCV epitopes. In line with this notion, IC41, a peptide vaccine candidate,[165,166] contains five peptides encoding four HCV specific human leukocyte antigen (HLA)-A2 restricted CTL epitopes and three highly promiscuous CD4+ T-cell epitopes (from the core, NS3 and NS4 proteins) adjuvanted with poly-L-arginine to augment Th1/Tc1 (IFN-γ) responses. The sequences contained within these epitopes are highly conserved in the most prevalent HCV genotypes. The inclusion of poly-L-arginine was shown to be important for the production of functional IFN-γ–secreting T cells in a healthy volunteer Phase I trial.[165] Discouragingly, in the setting of chronic infection this vaccine caused only a moderate drop in viremia that did not correlate with the size of the T-cell response[167,168] and did not prevent virological relapses when used as late add-on to standard Peg-IFNα/ribavirin therapy.[169]

Following a different approach, Houghton and colleagues[145] vaccinated naïve chimpanzees with a yeast-derived fusion polyprotein comprising NS3, NS4, NS5, and core proteins. Following purification and formulation with the ISCOMATRIX adjuvant, a group of five chimpanzees were vaccinated at months 0, 1, and 2 before experimental challenge intravenously with the heterologous 1a strain, HCV-H77, at month 3. Vaccination resulted in the appearance of vigorous HCV multispecific CD4+ and CD8+ T-cell responses in the liver prior to experimental challenge and infection. Following challenge, both acute viremia and hepatitis were significantly ameliorated in comparison with control animals that were similarly challenged. Surprisingly, however, none of the five vaccinees went on to eradicate viremia up to 18 months post-challenge. This result contrasts starkly not only with data obtained with the recombinant E1/E2 heterodimer vaccines, but also with many other vaccine candidates tested in the chimpanzee model in which some vaccinees were always observed to eradicate acute viremia following viral challenge. Although it is difficult to make firm conclusions from a vaccine study involving just five animals, it remains a formal, disturbing possibility that this vaccine may actually have increased the rate of chronicity rather than decreasing it.[145]

The use of whole heat-killed recombinant *Saccharomyces cerevisiae* yeast that has been genetically modified to express targeted molecular immunogens (tarmogens) has also been assessed, but only in a therapeutic setting. Tarmogens are sensed by dendritic cells and stimulate both innate and specific cellular immune responses.[170] This technology has been used for the design of an HCV vaccine candidate (GI-5005) that encodes a core-NS3 fusion.[171] GI-5005 demonstrated robust immunogenicity in preclinical in vitro and in vivo models.[172] Disappointingly, in patients with chronic hepatitis C, GI-5005 monotherapy displayed only a marginal antiviral efficacy[171,173] and was not tested preclinically or clinically in a prophylactic setting.

Vaccines Based on Hepatitis C Virus Complementary DNA

Intramuscular or intradermal injection of recombinant DNA plasmids has been shown to result in effective protein expression in vivo and in the induction of an immune response in small animal models. This initial success, however, has not yet been translated into humans as the efficiency of DNA uptake and gene expression appear to sharply decrease with the size of the immunized organism.

In the chimpanzee model, only one small DNA vaccine study has been reported to date.[174] To optimize the immunogenicity, the recombinant construct was designed by fusing the ectodomain of E2 (aa 384–715) to the C-terminal, transmembrane region of CD4, to favor sequestration of the encoded E2 glycoprotein to the outer cell surface. DNA was administered using a bioinjector into the quadriceps at weeks 0, 9, and 23, followed by experimental challenge with homologous monoclonal virus 3 weeks later. Importantly, both vaccinees resolved their acute infections quickly, whereas the control, unvaccinated animal became chronically infected following viral challenge. Although humoral and cellular immune responses to the vaccine were observed in only one animal out of two, both vaccinated animals displayed lower viral titers following challenge and developed hepatitis earlier than control animals consequent to primed immunity.[174]

As of this writing, two DNA vaccine candidates have entered human experimentation, both limited to a therapeutic rather than a prophylactic setting. The first candidate, ChronVac-C, is based on a codon optimized HCV nonstructural NS3/4A DNA-gene expressed under the control of the cytomegalovirus immediate early promoter. The vaccine has entered a Phase I/IIa clinical trial in HCV genotype 1–infected patients to assess safety, immunogenicity, and therapeutic potential.[175] Following dosing of the vaccine by intramuscular electroporation, two patients had moderate viral load reductions and development of HCV-specific T-cell responses that coincided with the time of the viral load reductions.[175] However, there are no published data on the effectiveness of ChronVac-C as prophylactic vaccine.

A different group vaccinated chronically HCV infected patients in a Phase I study with a therapeutic vaccine containing a combination of a DNA plasmid expressing HCV structural antigens and recombinant HCV core protein[176] (CIGB-230). The patients, all nonresponders to previous antiviral treatment, received six doses of vaccine by intramuscular injection at 4-week intervals. nAbs directed against heterologous viral pseudoparticles and HCV core-specific T cell responses developed in the majority of patients. Despite persistent viremia, an improvement in liver histologic findings, with a reduction in fibrosis, was reported for nearly half of the vaccinated patients,[177] making the observed result difficult to interpret.

Hepatitis C Virus Vaccines Delivered by Viral Vectors

The use of a defective or attenuated viral vector to deliver vaccines can offer improved immunogenicity as a result of a wide tropism of the vector, including that of antigen-presenting cells, as well as being able to stimulate innate immune responses that, in turn, stimulate adaptive immune responses to the encoded vaccine antigens. Furthermore, the use of a vector that is already used as a vaccine itself offers a potential advantage with respect to safety, manufacturing, and distribution issues. Finally, many vectors allow the insertion of large inserts or multiple genes or both, thus facilitating the induction of a broad, cross-protective immune response, particularly useful against heterogeneous agents such as HCV.

Modified vaccinia Ankara (MVA) is an attractive vaccine vector because of an excellent safety record and pronounced immunogenicity. In particular, MVA is particularly effective as a boosting vector, broadening and increasing the magnitude of preexisting T-cell responses.[178] A DNA prime/MVA boost approach was reported by Rollier et al.[179] Briefly, they vaccinated chimpanzees with DNA plasmids encoding HCV core-E1-E2 and NS3 and subsequently boosted with recombinant MVA encoding core-E1-E2 and NS3 genes. This DNA

primed-MVA boosted immunization induced strong Th1- and Th2-cytokine responses together with strong HCV specific CD8+ T-cell response as well as high HCV-specific antibody titers. A subsequent challenge with a heterologous virus demonstrated that the DNA primed-MVA boosted immunization was associated with control of HCV viral levels in the acute stage. However, despite control of HCV in plasma and liver in the acute period, three of four vaccinated animals developed persistent infection.

TG4040 is a recombinant polyantigenic T-cell vaccine based on MVA that has advanced in clinical trials as a therapeutic vaccine. TG4040 encodes the HCV NS3, NS4, and NS5B proteins. It was originally tested in HLA31 class I transgenic mice and shown to produce strong long-lasting cross-reactive HCV-specific $CD8^+$ and $CD4^+$ T cells.[180] These responses could be readily boosted by an additional dose of vaccine given after 6 months, indicating that the vaccination protocol was able to induce effective memory T-cell responses. The safety and biological activity of TG4040 as a candidate therapeutic vaccine has been evaluated as monotherapy in a Phase I study in 15 treatment-naïve HCV patients.[181] Of 15 patients, six received three weekly injections of vaccine and the remaining patients received a fourth injection at 6 months. Vaccine-induced HCV-specific cellular immune responses were observed in five of the 15 patients. A significant decrease in circulating levels of HCV RNA was observed in eight patients. The greatest viral load reductions were observed in patients who had achieved the strongest vaccine-specific T-cell responses. TG4040 was investigated as candidate therapeutic vaccine in further clinical studies in combination with Peg-IFNα and ribavirin. TG4040 in combination with Peg-IFNα/ribavirin was relatively well tolerated, but no significant advantages where observed versus the standard of care (i.e., Peg-IFNα/ribavirin alone) in terms of number of patients who achieved a sustained virological response.[182]

Another group has used a replicating *Vaccinia* virus expressing HCV structural and nonstructural genes to immunize four naïve chimpanzees. Following experimental challenge with homologous virus, all four vaccinees resolved their acute infections without progressing to the carrier state, whereas two control animals developed chronic infection after the same challenge,[183] providing further support for the ability of prophylactic vaccination to prevent the development of the chronic, carrier state.

Adenoviral (Ad) vectors expressing HCV proteins have also been considered as prophylactic or therapeutic (or both) T-cell vaccine candidates. Ad vectors are particularly attractive for this approach because of their superior potency at T-cell priming in primates and humans.[184] The main potential drawback of Ad vectors is preexisting immunity to the vector, which can lead to its clearance before a response is elicited to the inserted immunogen. However, this issue can be overcome in part by use of low-prevalence serotypes or by using chimpanzee adenovirus as the gene vehicle.[185]

Proof-of-concept chimpanzee studies were performed in which naïve animals were first immunized twice with a replication-defective Ad6 vector encoding for the whole HCV NS region (NS3 to NS5B of genotype 1b, BK strain).[186] Subsequently, boosting of immune responses was achieved by repeated electroporation of a naked DNA vaccine harboring the same HCV sequence. This regimen elicited HCV-specific and multispecific $CD4^+$ and $CD8^+$ T cells in four of the five vaccinees. Following a delayed experimental challenge with a heterologous 1a strain, these four animals showed a clear amelioration of acute viremia and hepatitis followed by eradication of viremia. In contrast, the weak responder to the vaccine exhibited viremia and hepatitis similar to that shown in control, unvaccinated animals and this vaccinee became a chronic carrier of the virus, as in the case of four of the five control, unvaccinated animals challenged with the same 1a viral strain (Fig. 26.4).

Based on these initial encouraging data, human Ad6 and chimpanzee adenovirus 3 expressing the HCV BK NS3- NS5B gene cassette with loss-of-function mutations in the HCV polymerase gene[187] (Ad6-NSmut- and ChAd3-NSmut, respectively) were tested in a Phase I clinical trial in healthy volunteers.[188] Ten patients receiving the highest dose of Ad showed robust, multispecific and cross-reactive T-cell responses. However, boosting with heterologous Ad was not as effective as observed in preclinical species, presumably because of the high level of cross-reactive antibody against the Ads in humans.[188,189] A prime-boost regimen based on ChAd3NSmut boosted with a MVA carrying the same HCV sequence (MVA-NSmut) was assessed in a Phase I clinical trial in healthy volunteers.[190] The results of this trial showed that HCV-specific T cells induced by ChAd3 are optimally boosted with MVA, and generate very high levels of both CD8+ and CD4+ HCV-specific T cells targeting multiple HCV antigens. Sustained memory and effector T-cell populations were generated, and T-cell memory evolved over time with improvement of proliferation and polyfunctional properties after the heterologous MVA boost. This ChAd/MVA vaccine regime has progressed to a Phase II clinical trial enrolling an HCV-naïve IVDU populations at high risk for infection (ClinicalTrial.gov Identifier: NCT01436357). This study, sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), is set up to assess whether this regimen can enhance the rate of spontaneous resolution of acute HCV infection.

FUTURE DIRECTIONS

Several years ago, prospects for effective vaccination against HCV were considered remote because of the high propensity of this virus to promote chronic persistent infections, evidence that convalescent humans and chimpanzees could be readily reinfected following reexposure, and the considerable genetic heterogeneity of this virus. Today, we can be more optimistic for several reasons. First, we now know that the spontaneous eradication of virus occurs in a significant fraction of acute infections and is associated with specific immune responses to the virus. Recapitulation of such immune responses by appropriate vaccination, therefore, becomes a realistic option. Second, clear evidence for some natural immunity has emerged in humans and chimpanzees. These studies have shown that convalescent humans and chimpanzees are protected from chronic infection against reexposure to virus in the majority of cases, even against divergent viral strains.

So far, the most promising approaches to HCV vaccination include the use of recombinant envelope proteins to elicit nAbs and $CD4^+$ T cells[144–148] and defective or attenuated viral vectors to enhance priming of humoral and cellular ($CD4^+$ and $CD8^+$) immune responses to multiple HCV nonstructural gene products expressed by the vector.[186,188,190] Ideally, any future HCV vaccine should elicit broad humoral (anti–E1/E2) and cellular immune responses. Several prophylactic or therapeutic vaccine candidates have reached human clinical trials (see Table 26.2) and more approaches are in preclinical development, including for example a heterologous prime-boost immunization with Ad vectors expressing E1-E2 glycoprotein followed by recombinant E1/E1 heterodimer.[191] With the availability of a system to propagate HCV in vitro, the possibility of using an inactivated HCV vaccine has also started to be explored.[192] In the future, it will be important to better determine correlates of protection, duration of memory of vaccine-induced protection, and the ability to cross-protect against diverse genotypes and to identify optimal vaccine formulations.

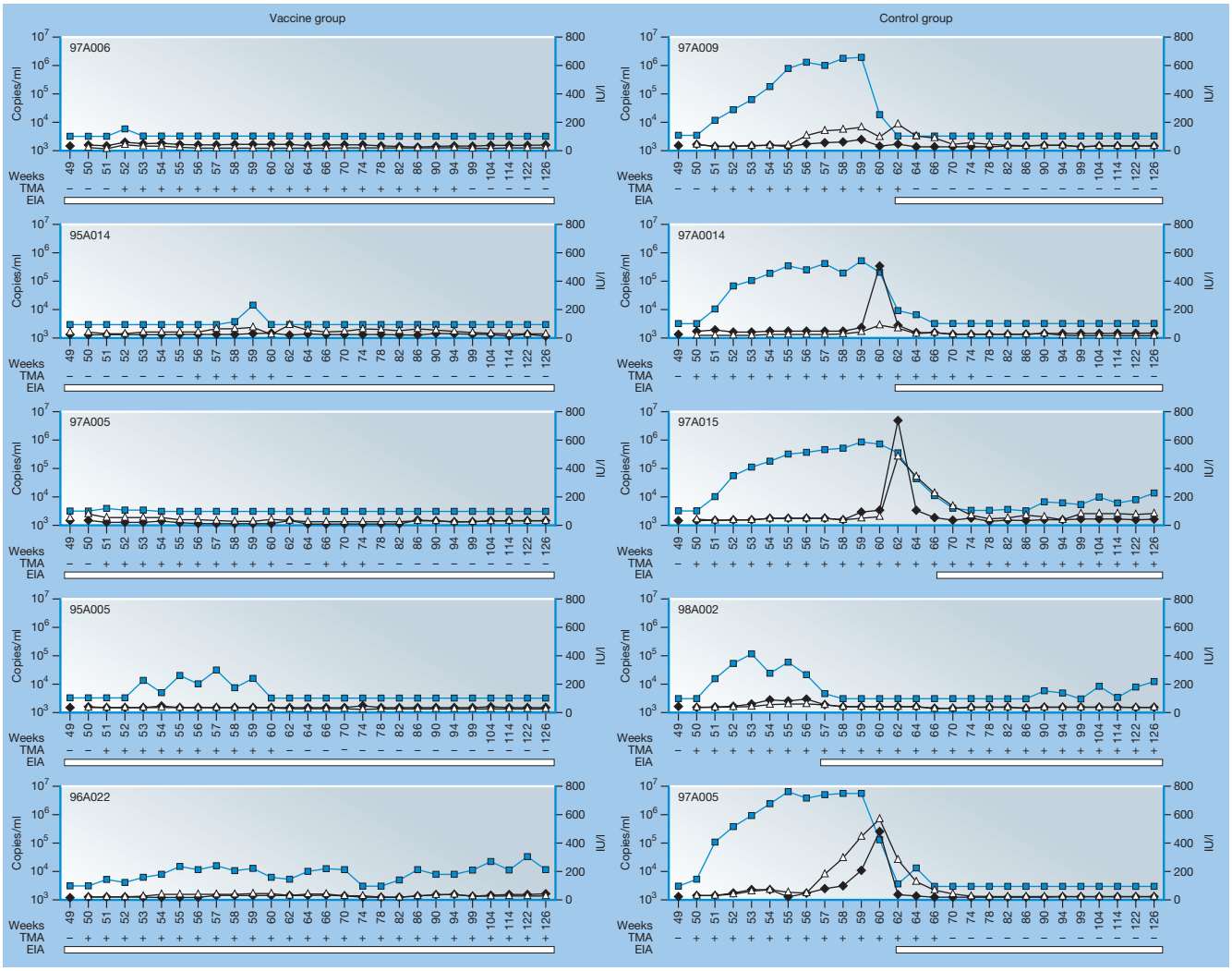


Figure 26.4. Kinetics of hepatitis C virus (HCV) RNA, alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), and HCV-specific antibody response in vaccinated and control chimpanzees. The course of acute HCV infection (18-month follow-up) is shown for vaccinated *(left panels)* and control *(right panels)* chimpanzees. *Blue squares* represent the kinetics of HCV RNA measured in plasma samples using a branched DNA assay, and *black diamonds* and *white triangles* represent kinetics of ALT and GGT liver enzymes, respectively. Data points at T = 49 weeks represent the average ALT and GGT values calculated from 10 time points before challenge. Results of the qualitative HCV RNA assay (TMA [transcription-mediated amplification]; detection limit: 50 copies/mL) are reported as + or – at the bottom of each graph. Anti-HCV seroconversion is indicated by a white bar across the bottom of each graph. *(From Folgori A, Capone S, Ruggeri L, et al. A T-cell HCV vaccine eliciting effective immunity against heterologous virus challenge in chimpanzees.* Nat Med. *2006;12:190–197.)*

Some issues surrounding clinical and preclinical development of a prophylactic HCV vaccine, however, remain to be resolved. A serious challenge to the development of a prophylactic HCV vaccine is represented by the fact that it is no longer possible to use the chimpanzee model in HCV research in Europe and in the United States. Thus, more research is needed to improve and validate small animal models of HCV infection that could be used to assess the efficacy of vaccines and to define the immune correlates of protection preclinically. Moreover, because of the relatively low incidence of new infections in the developed world, it is challenging to identify the appropriate at-risk population to enroll in an efficacy trial for a preventive HCV vaccine. Posttransfusion infection risk has been eliminated by donor screening, and other high-risk groups suitable for efficacy testing can have inherent difficulties such as lack of compliance (IVDUs), low incidence of infection (healthcare workers), lack of supporting infrastructure (in many developing countries where incidence of infection is high), or ethical issues (in prisoner populations where prevalence and incidence of infection are both high). Despite these difficulties, the first double-blinded, randomized, placebo-controlled trial of a prophylactic HCV vaccine in IVDUs is underway as of this writing in Baltimore.[178] Successful completion of this trial will determine whether a T-cell response primed with nonstructural HCV proteins is sufficient to reduce rates of chronic disease following acute infection. Moreover, it will also provide important support that vaccine efficacy trials of this kind are feasible. Taken together, the prospect of an HCV vaccine appears today as a realistic objective for the near future. Considering that chronic infection with HCV is one of the most common causes of primary liver cancer, this would mean that an important cause of global morbidity and mortality would be controlled and—possibly—eradicated.

References for this chapter are available at ExpertConsult.com.

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27 Hepatitis E Vaccines

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Hepatitis E was not recognized as a distinct disease until 1980, when waterborne epidemics of hepatitis in India, previously thought to have been caused by hepatitis A virus (HAV), were shown to have occurred in persons who were already immune to HAV.[1,2] Three years later, the etiologic agent (tentatively designated as "epidemic non-A non-B hepatitis," or "enterically transmitted non-A non-B hepatitis") was transmitted to a volunteer and to cynomolgus monkeys.[3] The viral genome was cloned and sequenced in 1990,[4] and the virus was renamed hepatitis E virus (HEV) with four genotypes (genotypes 1 to 4, HEV1 to HEV4). In retrospect, it is probable that most waterborne clinical hepatitis occurring in developing countries in the first half of the 20th century or earlier was hepatitis E and not hepatitis A.[5]

For many years, hepatitis E was thought to be largely restricted to endemic developing countries, and only of clinical relevance in developed countries in returning travelers. Over the last few years this notion was shown to be mistaken, as hepatitis E is endemic in many developed countries. In such settings, it is largely a porcine zoonosis caused by HEV genotypes 3 and 4 (HEV3 and HEV4), and human infection is surprisingly common.[6]

CLINICAL DESCRIPTION

Acute Hepatitis E

In developing countries hepatitis E is caused by HEV1 and HEV2, and mainly affects young adults. Infections are sporadic, with occasional large outbreaks involving hundreds or thousands of cases. The incubation period is approximately 40 days, and infections may range from inapparent to fulminant hepatitis, with an overall reported mortality of approximately 1%. Clinical signs and symptoms may include malaise, anorexia, abdominal pain and tenderness, nausea, vomiting, fever, and jaundice. Biochemical evidence of hepatitis includes elevated serum levels of liver enzymes and bilirubinemia; these generally return to normal within 6 weeks of onset.[7] The clinical features of hepatitis E in developed countries are similar to those seen in endemic developing nations, and are indistinguishable from those observed in any form of acute viral hepatitis. However, there are some important differences (Table 27.1), the most notable of which is that acute hepatitis E in developed countries is commonly asymptomatic and clinical illness is mostly observed in middle aged and elderly males.[6–10]

Chronic Hepatitis E

Chronic infection with HEV occurs in the immunosuppressed, including transplant recipients, patients with hematological malignancy, and individuals with HIV.[11–14] To date, chronic infection has only been documented with HEV3. The best characterized population is transplant recipients in developed countries, where up to 60% of such patients develop chronic infection after exposure to HEV. Chronically infected patients usually have no symptoms, are not icteric, and have modestly elevated serum alanine aminotransferase (ALT) (100–200 IU/L). Untreated, rapidly progressive chronic liver disease ensues, with 10% becoming cirrhotic within 2 years.[13]

Hepatic Complications

In developing countries, the mortality of acute hepatitis E is approximately 1%. This is largely from acute hepatic failure in pregnant women with associated fetal loss.[7] Acute liver failure is commonly seen in patients with underlying chronic liver disease, with an associated mortality of up to 70%.[15] In developed countries, the mortality rate ranges from 3% to 10%. Most deaths occur from acute liver failure in older males with underlying chronic liver disease. These data are an overestimate of the population based mortality, as asymptomatic cases (>90% of cases) were mostly not included in these hospital-based case series. Of transplant patients with untreated chronic infection, 10% are cirrhotic within 2 years. In such patients chronic liver failure requiring (re)transplantation commonly develops, and a number of such cases have died.[7]

Extrahepatic Complications

A number of extrahepatic manifestations have been described in association with hepatitis E, including acute pancreatitis,[16] thrombocytopenia,[8] cryoglobulinemia,[17] autoimmune thyroiditis,[18] acute myocarditis,[8] and a range of neurologic injuries.[19] Such complications have been described in both acute and chronic infections and in developing and developed countries. Neurologic complications are the most commonly described, with 8% of patients presenting with a neurological illness in one case series.[8]

The most commonly described neurologic syndromes are Guillain-Barré syndrome (GBS), neuralgic amyotrophy (NA), and meningoencephalitis. Recent studies from Europe of cohorts of GBS and NA patients showed that 5% and 10%, respectively, had evidence of acute hepatitis E (HEV3) at the start of their neurological illness.[20,21] In such patients the hepatitis was mild or asymptomatic and the neurologic symptoms and signs dominated the clinical picture. The mechanisms of neurologic injury are unknown. In some cases HEV RNA has been recovered from the cerebrospinal fluid. In one patient, HEV quasispecies compartmentalization was documented, with differences in HEV RNA found in the virus isolated from the blood and HEV isolated from the cerebrospinal fluid.[22] This suggests that, at least in some cases, HEV may be directly neurotropic.

VIROLOGY AND PATHOGENESIS

HEV is a spherical nonenveloped virus that is 27 to 34 nm in diameter. The virus circulating in the blood during acute or chronic infection is associated with lipids and is distinct from the nonenveloped virions shed in feces.[23,24] The HEV genome is a positive-stranded RNA of approximately 7.2 kb. The genome consists of a short 5′ noncoding region that is capped with 7-methylguanosine, three open reading frames (ORFs), and a short 3′ noncoding region that is terminated by a stretch of adenosines.

TABLE 27.1 Comparison of Hepatitis E Virus Genotypes 1 and 2 With Genotypes 3 and 4

	HEV 1 and 2	HEV 3 and 4
Geographical distribution	Asia (HEV1) Africa (HEV1 and HEV2) Mexico (HEV2)	Worldwide, including developed countries (HEV3) Japan, China, Europe (HEV4)
Source of infection	Obligate human pathogen	Zoonotic Blood supply
Route of infection	Oral-fecal via infected water	Oral via consumption of infected pork Parenteral, iatrogenic (blood supply)
Risk of infection from blood supply	Low	High
Outbreaks	Yes	No
Intrafamilial spread	No	No
Clinical attack rate	1:2	<1:10
Demographics	Mainly affects young adults	Mainly affects older men (median age, 63 years; M:F = 3:1)
Chronic infection	No	Yes, in the immunocompromised Rapidly progressive liver disease if untreated: 10% are cirrhotic within 2 years Viral clearance is usually achieved with a 3-mo course of ribavirin monotherapy
Can second HEV infections occur?	Yes; not well documented	Yes Not well documented for HEV3 Well documented in HEV4; more likely in women who have a milder hepatitis than that seen in primary infections
Clinical course	Self-limiting hepatitis in most	Self-limiting hepatitis in most
Pregnancy	Mortality rate of 25%	Increased mortality rate not seen
Underlying chronic liver disease	Increased mortality rate	Increased mortality rate
Neurological sequelae	Yes; poorly documented	Yes

HEV, hepatitis E virus.

ORF1

ORF1 encodes a nonstructural protein of approximately 1700 amino acids containing several functional domains.[25] These include a methyltransferase/guanyltransferase responsible for capping the viral genome, a papain-like cysteine protease, a macrodomain, a helicase exhibiting RNA 5′ triphosphatase (NTPase), RNA unwinding activity, and an RNA-dependent RNA polymerase. A variable region containing a proline-rich hinge, called the polyproline region (PPR), is located between the protease and the macrodomain. The PPR is an intrinsically disordered region where segments of human gene have been identified in vitro and in vivo.[26–30] This region could be involved in virus adaptation to the host.[31,32] A greater heterogeneity of PPR quasispecies at the acute phase of HEV infection was found in immunocompromised patients who develop chronic infection than in individuals with resolving infection.[26]

ORF2

ORF2 encodes the viral antigen corresponding to the capsid protein of 660 amino acids which form the capsid through dimerization.[33] Each monomer contains a shell domain (S), a middle domain (M), and a protruding domain (P).[33–35] During assembly, capsid monomers self-assemble into dimers and subsequently in decamers that encapsidate the viral RNA. The virion-sized capsid is a T=3 icosahedral capsid while the recombinant HEV capsid protein can self-assemble into a T=1 virus-like particle (VLP) that retains the antigenicity of the native HEV virion.[36] Immunological and structural studies of the capsid protein have contributed to the hepatitis E vaccine development.[37,38] A high heterogeneity of HEV quasispecies in the region encoding the M and P capsid domains was associated with evolution of chronic HEV infection in immunocompromised patients.[39]

ORF3

ORF3 encodes a small protein of 133 or 114 amino acids that is involved in the release of virus from cells. The ORF3 protein interacts with the capsid protein in a manner dependent upon phosphorylation of ORF3 at Ser80.[40] The ORF3 protein also contains a PSAP motif that specifies interactions with TSG 101, a host ESCRT-I protein involved in the budding of many enveloped viruses.[41,42]

Taxonomy

HEV belongs to the *Hepeviridae* family (International Committee on Taxonomy of Viruses). The classification is still evolving because of the continuing increase in the number of novel strains of the virus in various animal species and the availability of full-length genome sequences. There are several genotypes found in mammals, birds, and fish. A classification that divides the *Hepeviridae* family into two genera, *Orthohepevirus* and *Piscihepevirus*, was recently proposed.[43] Within the *Orthohepevirus* genus, four species have been designated that infect mammalian species (*Orthohepevirus A*, *Orthohepevirus C*, and *Orthohepevirus D*) and avian species (*Orthohepevirus B*). The *Orthohepevirus A* species contains HEV strains that are able to infect humans and are divided into four major genotypes (HEV1 to HEV4). HEV1 and HEV2 have been recovered only from humans whereas HEV3 and HEV4 have been recovered principally from humans and swine.[44] Other genotypes, including strains from rabbits,[45,46] wild boars,[47,48] and camels,[49]

also belong to *Orthohepevirus A* species. All genotypes of HEV infecting humans belong to the same serotype. The *Orthohepevirus B* (infecting chicken), *Orthohepevirus C* (infecting rat and ferret), and *Orthohepevirus D* (infecting bat) species and the *Piscihepevirus* (infecting trout) genera contain strains that apparently do not infect humans.[50]

DIAGNOSIS

Hepatitis E is diagnosed by detecting viral RNA (reverse transcription–polymerase chain reaction [RT-PCR]) in the serum or feces or both during the incubation period or early acute phase of disease or, more commonly, by demonstrating anti-HEV antibodies of the immunoglobulin (Ig) M class or a rising titer of anti-HEV antibodies of the IgG class in the serum during the late acute phase or convalescent phase of the illness.[51] Commercial enzyme-linked immunosorbent assays for detecting such antibodies have been developed. They vary considerably in sensitivity and specificity,[52] and are not licensed in the United States.

TREATMENT

Acute hepatitis E is usually a self-limiting illness and requires no treatment. Patients with acute hepatitis E and underlying chronic liver disease have a poor prognosis,[53,54] and a small number of such patients have been treated successfully with ribavirin.[55] Patients with chronic infection (defined as persisting viremia >3 months) should be treated. In transplantation recipients, the first step is to reduce the level of immunosuppression, if possible. This achieves viral clearance in approximately 30% of transplantation recipients. If this is not possible or unsuccessful, then a 3-month course of ribavirin monotherapy should be given. This achieves a sustained viral response in approximately 85% of cases. The duration of therapy should be longer if HEV RNA is detected in the stool at the end of 3 months of treatment.[56]

EPIDEMIOLOGY

Incidence, Prevalence, and Modes of Transmission

In developing countries, HEV1 and HEV2 are transmitted via contaminated water as a consequence of inadequate sanitation. In addition to sporadic cases occurring throughout the year, many small and occasional large-scale outbreaks also occur.[57] Outbreaks are frequent in Asia and Africa and result in high morbidity and mortality particularly when occurring in refugee camps. Every year an estimated 20 million HEV infections occur globally, resulting in more than 3 million clinical cases and 70,000 deaths.[58] Pregnant women are particularly vulnerable with the case-fatality rate reaching 30% in the third trimester of pregnancy.[59] HEV can be transmitted to the fetus resulting in an estimated 3000 stillbirths per year.[58] Anti-HEV seroprevalence can reach 80% and increases dramatically between the ages of 15 and 30 years except in Egypt where children less than 10 years of age have high levels of previous exposure to HEV.[60] The incidence of infection varies between and within countries and over time.[60,61]

In developed countries, although some sporadic cases of hepatitis E are caused by HEV1 and HEV2 in persons returning from Asia and Africa, most of the cases are caused by HEV3 (worldwide) or HEV4 (mainly in Asia) that are transmitted locally from an animal reservoir. In France, since the optimization of diagnostic tests and adaption of testing algorithms in 2012, 99% of HEV infections reported by the National Reference Center are autochthonous. An important route of infection is by consumption of uncooked or poorly cooked pork meat or game meat (wild boar, deer, rabbit).[7] It has been demonstrated that cooking temperatures of 71°C for 20 minutes are required to fully inactivate the virus.[62] Several studies from the United Kingdom and France demonstrated HEV contamination in food containing raw pork liver with frequencies ranging from 3% to greater than 30%, depending on the categories of products.[63–67] Direct contact with HEV-infected animals is another possible route of transmission of HEV. Prevalence of anti-HEV antibodies is higher in veterinarians, swine handlers and forestry workers, than in other population categories in different countries.[68–70] The waterborne route of infection may also be important for HEV3 and HEV4. HEV3 has been detected in untreated waste water, swine manure, swine slurry storage facilities, and river water.[60] HEV3 also has been detected in shellfish, and an outbreak on a cruise ship was caused by the consumption of mussels.[71] Finally, transfusion-transmitted HEV infection has been documented in Japan, the United Kingdom, and France.[72–80] Plasma, including amotosalen-treated plasma, platelets, and red blood cells can transmit HEV, but no transmission by plasma-derived products has yet been documented.

Anti-HEV seroprevalence in industrialized countries ranges from 10% to more than 50% using the same assay. In European blood donor populations, seroprevalence is 12% in England,[81] 27% in the Netherlands,[82] 29% in Germany,[83] and 52% in southwestern France.[66] Variations of seroprevalence are seen in differing geographic locations in the same country for unknown reasons. A common characteristic is an increasing seroprevalence with age indicating cumulative exposure to the virus during life.[66] The seroprevalence of HEV in the general population was 23% in China during the period 2005 to 2006,[84] and in another study from China, seroprevalence was 38% in the general population and 48% in swine farmers.[85] The seroprevalence was 6% in the United States during period 2009 to 2010.[86,87] However, because of the relatively low sensitivity of serological assays used, the true HEV seroprevalence in these countries is probably underestimated. The annual incidence of HEV infection in developed countries is 0.2% in the United Kingdom,[81] 0.7% in the United States,[88] 2% to 3% in southern France,[89,90] and 4.3% in China.[91] The frequency of HEV infections in minipools of plasma (24 to 96 samples) from blood donations is 1 in 1430 in China,[92] 1 in 2200 in France,[93] 1 in 2700 in the Netherlands,[82] 1 in 1240 to 1 in 6925 in Germany,[94–96] and 1 in 2848 to 1 in 14,520 in the United Kingdom (Tables 27.2 and 27.3).[77,97,98] Based on these data, each year there are more than 100,000 infections in France and in the United Kingdom, and more than 2 million infections in the United States.

Risk Groups

In developing countries, the group at general risk of infection is young adults. Those at increased risk of adverse outcome are pregnant females and individuals with underlying chronic liver disease. In industrialized countries, the groups at general risk are middle-aged and elderly males and recipients of blood products. Those especially at risk include the immunosuppressed and patients with chronic liver disease.

Significance as a Public Health Problem

The global burden of disease associated with HEV is unknown. An estimate from 2005 suggested there were 20 million infections during that year (see "Incidence, Prevalence, and Modes of Transmission" above[58]). However, this estimate did not include the impact of HEV in industrialized countries nor the

TABLE 27.2 Hepatitis E Virus Viremia and Seroprevalence in Blood Donors in Highly Endemic Developed Countries

Country	Blood Donors HEV RNA Positive	HEV IgG Seroprevalence	Reference
The Netherlands	1:600 1:2671	 27.0%	Zaaijer, 2015[151] Slot et al, 2013[82]
Midi-Pyrennes, southwest France[a]	1:1595	 52.5%	Gallian et al, 2014[93] Mansuy et al, 2011[66]
France[a]	1:2218		Gallian et al, 2014[93]
Germany	1:1200 1:4525	 29.5%	Vollmer et al, 2012[96] Baylis et al, 2012[94] Wenzel et al, 2013[83]
Japan	1:1781		Fukuda et al, 2004[152]
China[b]	1:1493	32.6%	Guo et al, 2010[92]

HEV, hepatitis E virus; IgG, immunoglobulin G.
"Highly endemic" has been arbitrarily defined as an incidence of HEV viremia in blood donors >1:2500 and/or seroprevalence of >20%. Seroprevalence studies have been restricted to those employing the highly sensitive and partially validated Wantai anti-HEV IgG assay, as other assays have shown to have very poor sensitivity. HEV RNA was genotype 3 in all cases, except where stated.
[a]Deconstructed solvent-detergent–treated minipools.
[b]Of those with HEV viremia, 57% were genotype 1 and 43% were genotype 4.

TABLE 27.3 Hepatitis E Virus Viremia and Seroprevalence in Blood Donors in Countries of Intermediate/Low Endemicity

Country	Blood Donors HEV RNA Positive	HEV IgG Seroprevalence	Reference
England	1:2848 1:7000	 12.0% 16.0%	Hewitt et al, 2014[77] Ijaz et al, 2012[98] Beale et al, 2011[81] Dalton et al, 2008[9]
Sweden	1:7986		Baylis et al, 2012[94]
Austria	1:8416	13.5%	Fischer et al, 2015[153]
USA	Nil Nil[b]	16.0%	Baylis et al, 2012[94] Xu et al, 2013[154]
Scotland	1:14520	4.7%	Cleland et al, 2013[97]
Australia	Nil[c]	6.0%	Shrestha et al, 2014[155]
New Zealand	NA	4.0%	Dalton et al, 2007[156]
Fiji	NA	2.0%[a]	Halliday et al, 2014[157]

HEV, hepatitis E virus; NA, not available.
"Intermediate/low endemicity" has been arbitrarily defined as an incidence of HEV viremia in blood donors of >1:2500 and/or seroprevalence <20%. Seroprevalence studies have been restricted to those employing the highly sensitive and partially validated Wantai anti-HEV immunoglobulin G assay, as other assays have shown to have very poor sensitivity. HEV RNA was genotype 3 in all cases.
[a]Healthy adults and children.
[b]Only 1939 donors tested.
[c]Only 3237 donors tested.

emerging clinical phenotype of HEV-associated disease (e.g., neurological complications) and is likely to be an underestimate. The prevalence of chronic infection in European transplant centers is 1% to 2%.[7]

PASSIVE IMMUNIZATION

While low preexisting anti-HEV antibodies (<7 WHO-units/mL) seemed not to protect HEV reinfection in immunocompromised organ recipients,[89] individuals with naturally acquired anti-HEV antibodies are protected from hepatitis E reinfection and severe hepatitis E in endemic areas.[99–101] Repeated challenges, passive immunoprophylaxis and antibody neutralization studies in monkeys have shown that antibodies to HEV can protect against HEV infection and hepatitis.[102–105] However, immunoglobulin prepared in endemic countries failed to protect, probably because of the relatively low titers of anti-HEV.[106] Whether an immunoglobulin preparation with high titer of anti-HEV could be protective remains to be studied.

ACTIVE IMMUNIZATION

Active immunization is usually achieved by prophylactic vaccination, but the choice of antigen is crucial in terms of vaccine efficacy. For example, HEV proteins encoded by ORF1 are enzymes and other cell-modulation effectors involved in HEV life cycle. Thus, the antibody against an ORF1 protein would not recognize HEV during viral infection and might only protect through cellular immune mechanisms.[107,108] Antibody to the ORF3 protein is nonneutralizing in cell models and immunization of nonhuman primates with recombinant ORF3 provides only partial protection.[109,110] In contrast, the capsid protein encoded by ORF2 appears to be the ideal candidate for a hepatitis E vaccine. Antibodies to plasmid open reading frame 2 (pORF2) cross react with all four serotypes.[111]

Antibodies to the capsid protein neutralize HEV in vitro and in vivo and protect nonhuman primates against hepatitis E and HEV infection.[110,112,113]

When the existing hepatitis E vaccines were developed there was no robust cell culture for HEV available. Although HEV cell culture system has recently been established to produce some specific strains of HEV isolated from chronic hepatitis E patients,[114–116] the virus yield is still limited for the development of attenuated or inactive vaccine. For this reason a variety of systems were applied to generate recombinant proteins as potential immunogens. Functional domains for neutralization site formation and particle assembly were studied in *Escherichia coli* or in insect cells from baculovirus vectors. Several elements for VLP assembly in insects and key regions for particle reassembly in vitro derived from *E. coli* expression system were identified by truncation mutational analysis.[117]

Native HEV virions arrange in a T=3 icosahedral lattice of pORF2 and pack HEV RNA genomes through the positive-charged capsid N-terminal. The structures of various truncates were well characterized in near-atomic resolution by X-ray crystallography and cryoelectron microscopy,[33–35,37,38,103,118] and the B-cell and T-cell epitopes were characterized by immune assay, structural biology, and virology. The crystal structures of the truncated pORF2 of aa 112–608 forming T=1 VLPs showed that the HEV capsid contains three major domains: specifically, the S domain, P1 domain, and P2 domain (also designated as E2s domain).[35,37] The outermost E2s domain seems to be the core antigenic region of pORF2 harboring most of the key neutralization sites, some of which are essential for virus–host interactions and are responsible for the induction of neutralizing antibodies.[33,37,103] Another truncated version that contains aa 14–608 form T=3 virion-sized VLPs, which assemble with the binding of RNA.[36]

Two VLP-based vaccines against HEV have been developed and entered clinical trials, both containing P2 domains (E2s domains).[119,120] A baculovirus-expressed vaccine candidate of 56 kDa (aa 112–607) was evaluated by GlaxoSmithKline (Rixensart, Belgium).[119,121–123] The other vaccine is a further truncated version of pORF2 aa 368–607, designated as HEV 239, with the trade name Hecolin (Xiamen Innovax Biotech, Xiamen, China), which was expressed in an *E. coli* system and can self-assemble into VLPs in vitro, with a diameter of 20 to 30 nm.[120,124,125] HEV 239 vaccine, with a robust manufacturing process, considerable production capacity, and well-defined quality control,[126] has been available in China since 2012. These two vaccines were subjected to clinical trials. In addition, another vaccine candidate with VLPs spanning aa 452–617 of genotype 4 HEV expressed in *E. coli* was combined with a commercial inactivated hepatitis A vaccine.[127] The individual components of the combined vaccine were immunogenic, and HEV components appear to protect nonhuman primates from hepatitis E.[128]

Several other approaches to vaccine development have been pursued. These include a DNA vaccine, chimeric virus particles, fusion proteins, an oral vaccine, and an adeno-associated virus (AAV) delivery vaccine. None of these approaches have been used in humans.[129–139]

PRECLINICAL TRIALS: SAFETY, IMMUNOGENICITY, AND EFFICACY

Different approaches to develop a recombinant vaccine using bacterial, animal, insect, and plant cells have been applied.[140] Two genotype 1–derived recombinant antigens, rHEV (recombinant hepatitis E vaccine) (baculovirus-expressed 56-kDa antigen; GlaxoSmithKline),[122] and HEV 239 (Xiamen Innovax),[124,125,141] demonstrated safety, immunogenicity, and efficacy in monkey models before being assessed in clinical trials.

Two formulations (1 μg or 10 μg antigens adsorbed to alum adjuvant at a volume of 0.5 mL) and two vaccination schedules (one dose or two doses administered 4 weeks apart) of the rHEV (GlaxoSmithKline) vaccine were tested in rhesus monkeys.[119] One month following the last dosing, the monkeys were challenged intravenously with 10,000 50% monkey infectious doses (ID_{50}) of HEV genotype 1, 2, or 3. Both the two-dose and single-dose regimens protected monkeys from hepatitis E, with an efficacy of 100% (95% confidence interval [CI], 65–100) and 78% (95% CI, 29–94), respectively. The vaccine similarly protected against challenge with homologous HEV genotype 1 and heterologous genotypes 2 and 3. Another study showed that the efficacy of protection against hepatitis E remained for at least 12 months after the second vaccine dose.[142]

The *E. coli*–expressed HEV 239 vaccine (Xiamen Innovax) was assessed similarly.[120] Two doses of 5 μg, 10 μg, or 20 μg HEV 239 adsorbed in alum adjuvant were administered 4 weeks apart and induced comparable antibody responses that peaked at week 7. The monkeys were then challenged with 10^4 or 10^7 genome equivalents of HEV genotype 1 or 4. All the formulations of HEV 239 vaccine completely protected the monkeys from symptomatic or asymptomatic HEV infection by the lower viral dose. When challenged with 10^7 genome equivalents of virus, the HEV 239 vaccinated monkeys were completely protected from acute hepatitis E while partially (75%, 95% CI 46.2–90.9) protected from HEV infection. The protective efficacy against homologous HEV (genotype 1) or heterologous HEV (genotype 4) infection was similar.

CLINICAL TRIALS: SAFETY, IMMUNOGENICITY, AND EFFICACY

Both the rHEV and HEV 239 vaccines have undergone clinical evaluation and HEV 239 was licensed and then launched in China in 2012.[143]

In an open Phase I study of the rHEV vaccine, 88 seronegative healthy American volunteers ages 18 to 50 years were enrolled and assigned to four groups to receive 1 μg, 5 μg, 20 μg, or 40 μg doses of vaccine at 0, 1, and 6 months after enrollment.[144] The vaccine was well-tolerated. The vaccine elicited detectable anti-HEV titers in at least 88% of those in the 5 μg, 20 μg, and 40 μg groups. The 1-μg formulation appeared to be less immunogenic (66% seroconversion).

Based on these encouraging data, a double-blind, placebo-controlled Phase II clinical trial of rHEV vaccine was conducted in Nepal, where homologous genotype 1 HEV is highly endemic.[122] In this trial, 2000 participants with low to negative anti-HEV titers, 99.6% of whom were men, with a mean age of 25.2 ± 6.25 years (range: 18 to 62) randomly received 20 μg rHEV or placebo at 0, 1, and 6 months and were followed up for a median of 804 days. Most of the reported adverse reactions were mild and occurred with similar frequency in the two groups. No serious adverse events were related to vaccination. Three vaccine doses elicited complete seroconversion among the participants but antibodies in approximately half of them dropped to undetectable levels 2 years later. One month after the last dose, 66 hepatitis E cases developed in the placebo group while three cases developed in the vaccine group, for a vaccine efficacy of 95.5% (95% CI, 85.6–98.6). In those who received at least one injection, the vaccine efficacy was 88.5% (95% CI, 77.1–94.2). During the period from 2 weeks after the second dose until the third dose, one case of hepatitis E developed in the vaccine group, and seven developed in the placebo group, suggesting that protection might be achieved with two doses of the vaccine, although the

difference was not statistically significant. The rHEV vaccine appeared to be as safe and efficacious; however, its further development was stopped by GlaxoSmithKline for commercial reasons.

The HEV 239 vaccine has been assessed through Phase I, Phase II, and Phase III clinical trials in China.[124,125,141,145] In the trials, the vaccine formulations contained 10 μg, 20 μg, 30 μg, or 40 μg of recombinant particulate HEV 239 antigens, which were adsorbed to 0.8 mg of aluminum hydroxide, suspended in 0.5 mL of buffered saline and given by intramuscular injection. The data from these clinical trials suggest that the HEV 239 vaccine is well tolerated and no serious adverse events (SAEs) associated with the vaccine were reported. In the Phase I trial, 44 healthy seronegative adults received two doses of 20 μg HEV 239 vaccine at months 0 and 1. The vaccine was well tolerated and serum biochemistry values showed no abnormal changes from the baseline values by day 60.[146] The Phase III clinical trial involved 112,604 participants who were 1 : 1 randomly assigned to the vaccine or the control groups. Local adverse events occurred more frequently in the HEV 239 group than in the control group. Local adverse events of grade 3 or higher, solicited systemic adverse events; unsolicited adverse events occurred at similar rates between the study groups.[125] Adverse events were mainly pain and swelling with itching at the injection site. Adverse events of grade 3 or more were reported very rarely. During the Phase III trial and the extended 4.5-year follow-up period, a comparable number of participants in both the vaccine and placebo groups experienced SAEs.[141] None of the SAEs were believed to be related to vaccination. Thirty-seven pregnant women in the HEV 239 vaccine group and 31 pregnant women in the control group were inadvertently vaccinated in the Phase III vaccine study.[147] The vaccine was well tolerated in the pregnant women with only one woman reporting grade 1 inoculation site pain. The weights, body lengths, and gestational ages of the newborns were comparable in both groups. The safety of HEV 239 was also evaluated in hepatitis B surface antigen (HBsAg)-positive persons, and the resulting data are reassuring.[148]

HEV 239 vaccine induces a robust and persistent immune response. Dose escalation and dose scheduling observations in the Phase II trial showed that 100% of the seronegative participants produced anti-HEV at month 7 after they received three doses of 10, 20, 30, or 40 μg HEV 239 vaccine.[124] Geometric mean concentrations (GMC) of anti-HEV IgG post vaccination were statistically similar between the three high dosage groups, while slightly higher than the 10 μg group; 3 doses were more potent than 2 doses. In the Phase III clinical trial, 11,165 participants were included in an immunogenicity subset; 5567 received three doses of 30 μg HEV 239 vaccine. Of the baseline seronegative participants, 99.9% seroconverted at 1 month after the last dose, and 87% remained seropositive at month 55. Geometric mean concentration (GMC) of anti-HEV IgG dropped from a peak of 14.96 WHO-units/mL (World Health Organization [WHO], WHO-units/mL, calibrated against WHO reference standard, National Institute for Biological Standards and Control, NIBSC catalog no. 95/584) at month 7, which is 25 times higher than that induced by natural HEV infection (0.6 WHO-units/mL), to 1.47 WHO-units/mL at month 19, and then slowly decreased to 0.27 WHO-units/mL by month 55.[125,141] For participants with preexisting antibodies, the antibody response was robust even after receiving only one shot.[141] The retrospective analysis of data in participants with or without HBsAg demonstrated similar immunogenicity.[148]

The HEV 239 vaccine is highly efficacious against symptomatic or overall HEV infection. In the Phase III clinical trial, 112,604 participants were randomly assigned to receive three doses of 30 μg HEV 239 vaccine or the hepatitis B vaccine (controls) at months 0, 1, and 6.[125] Symptomatic hepatitis E cases were active surveyed through 205 local sentinels, including community clinics, private clinics, and central hospitals. During the first follow-up year after the last dose, in the participants who received all three doses of the HEV 239 vaccine or the control vaccine in the requested time frames (per-protocol cohort), no HEV 239 vaccine recipients contracted hepatitis E. In contrast, 15 hepatitis E cases developed in the control group, indicating a vaccine efficacy against hepatitis E of 100% (95% CI, 72–100). For those who received at least one dose (modified intention-to-treat cohort), the efficacy was 96% (95% CI, 66–99) during months 0 to 18.[125] After receiving 2 doses and before the third dose (months 2–6), all five hepatitis E cases occurred in the control group and the efficacy was 100% (95% CI, 9–100).

In an extended efficacy study,[141] all the participants were blindly followed up for another 3 years. The long-term efficacy of HEV 239 for up to 4.5 years was 93% (95% CI, 79–98) in the per-protocol cohort (months 7–54) and 87% (95% CI, 71–94) in the modified intention-to-treat cohort (months 0–54). Of the 29 identified hepatitis E cases whose viral isolates were sequenced, 26 were infected with HEV genotype 4 (three in the vaccine group and 23 in the control group), and three were infected with HEV genotype 1 (one in the vaccine group and two in the control group).

HEV 239 vaccine appears efficacious against symptomatic and asymptomatic HEV infection. In the Phase III trial, the observed efficacy against HEV infection (indicated by anti-HEV IgG level increasing for four times or more in paired serum samples collected at 1-year intervals) in participants who had received at least one dose of vaccine was 77% (95% CI, 65–85) during the observation period up to month 31. It was estimated that a marginal antibody level (0.077 to 0.25 WHO-units/mL) may prevent infection in 74% of persons exposed to HEV.[99] Data from the Phase II clinical trial show that two doses of 20 μg HEV 239 administered at months 0 and 6 or months 0 and 1 can effectively protect from asymptomatic HEV infection with an efficacy of 85% (95% CI, 10–99) or 86% (95% CI, 13–99), respectively for at least 6 months.[124]

PROSPECTS FOR THE FUTURE

The most pressing issue for the immediate future is to make the vaccine available where it is most needed, in particular for use in large outbreaks in developing countries. For example, in the years 2012 to 2013 there was an epidemic of hepatitis E in South Sudan, with 5000 cases and more than 100 deaths, 50% in pregnant women.[149] At the time, as is currently the case, HEV 239 vaccine was only licensed for use in China. Had it been available for use, it may have prevented many cases and much morbidity and mortality. It seems likely that HEV 239 will be effective in this kind of emergency setting,[150] as its efficacy after two doses 1 month apart is 100%. The issue of vaccine availability in countries in need is complex. There are a number of issues that have impeded the use of HEV 239 in countries other than China, including lack of vaccine prequalification, insufficient data on the safety in pregnant women, and efficacy against genotype 1. These issues need urgent resolution.

In both developing and developed countries, hepatitis E has an adverse prognosis in patients with preexisting chronic liver disease. It would seem appropriate to consider vaccinating such individuals for HEV, alongside the existing recommendation that they should be vaccinated against HAV and hepatitis B virus. However, before this can be implemented, more data are required on the safety and effectiveness of HEV

239 in patients with chronic liver disease. Existing data show that it is safe and effective in carriers of HBsAg, but how many of the individuals in this study were cirrhotic is unknown.[148]

Finally, a safe and effective vaccine could potentially be used in the immunosuppressed, particularly transplant recipients, to prevent chronic infection. However, the safety and immunogenicity of the vaccine in this group of patients remain to be established.

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28 Herpes Simplex Virus Vaccines

Lawrence R. Stanberry

"O'er ladies' lips, who straight on kisses dream, Which oft the angry Mab with blisters plagues…."

William Shakespeare, *Romeo and Juliet*, Act 1, Scene 4, p. 4

More than 2500 years ago Hippocrates used the Greek term herpes, meaning "to creep," to describe spreading skin lesions.[1] Since then mucocutaneous herpes simplex virus (HSV) infections have been viewed largely as nuisance diseases as most are self-limiting. This rather uninformed view fails to consider that HSV infections can be life threatening in the newborn, the severely malnourished, and the immunocompromised host; leave survivors severely neurologically impaired; result in blindness; trigger bouts of erythema multiforme; and that genital herpes increases threefold the risk of HIV acquisition.[2]

BACKGROUND

Clinical Description

The clinical illness caused by HSV is determined by the portal of entry, the immune status of the host, and whether the infection is primary or recurrent.

Genital herpes classically presents with vesiculoulcerative skin lesions which appear on mucosal and keratinized epithelium, as well as around the anal opening, buttocks, and/or thighs. Mild infections instead lack classical findings and may not be recognized as genital herpes,[3] but may have nonpathognomonic findings, including tingling, burning, itching, erythematous patches, and small skin fissures. Greater than 80% of primary genital HSV infections are asymptomatic or unrecognized,[4] and asymptomatic recurrent infections or asymptomatic viral shedding is also more common than clinically recognized recurrences and occur both in those with a history of symptomatic infections and those lacking such history.[5] While HSV-1 and HSV-2 can cause clinically indistinguishable primary infections they differ in their ability to cause recurrent infections with HSV-1 causing significantly fewer episodes of recurrent genital infection than HSV-2,[6] while the converse is true for oral–labial HSV infections.

Common oral–facial infections are generally caused by HSV-1.[7] Herpes gingivostomatitis is characterized by oral pain, fever (40°C–40.6°C), and vesiculoulcerative lesions throughout the mouth and pharynx. Herpes pharyngitis is less severe than gingivostomatitis with lesions generally limited to the tonsils and pharynx.[8] Herpes labialis is the most common recurrent oral–facial HSV infection and often is heralded by prodromal symptoms, including tingling, itching, burning, or pain, before the development of the herpetic lesions, which typically develop on the vermilion border of the lip, although lesions sometimes occur on the nose, chin, cheek, or oral mucosa.[9]

HSV can also cause other skin infections including herpes gladiatorum or scrum pox, herpes whitlow, and potentially life-threatening HSV infections among individuals with skin disorders.[10,11]

HSV infection of the eye may be primary or recurrent and is usually unilateral and may involve the conjunctiva, cornea, or retina.[12] Conjunctivitis is the most common ocular HSV infection. The distinguishing feature of corneal infection is dendritic or geographic ulcers.

Beyond the newborn period HSV encephalitis is almost always caused by HSV-1 and typically involves the frontal and/or temporal cortex. HSV is the most common cause of recurrent aseptic (Mollaret) meningitis and aseptic meningitis is seen in up to 15% of cases of primary genital herpes.[13,14]

Perinatal HSV infections are life threatening and most commonly result from mother-to-infant transmission.[15] The clinical manifestations are variable but broadly fall into one of three patterns of disease: (a) disease localized to the skin, eyes, or mouth (SEM); (b) encephalitis; or (c) disseminated infection.

HSV infections in the immunocompromised host, particularly those with compromised cellular immunity such as seen with AIDS, may be severe and life threatening.[16,17]

Complications

There are myriad complications associated with HSV infection. Acute genital herpes infection may be associated with urological and neurological complications, and recurrent genital herpes can cause intermittent and persistent neurological signs and symptoms, significant psychological morbidity,[18] and significantly increases the risk of HIV acquisition and transmission.[2] Cutaneous infections can result in erythema multiforme. Ocular HSV infections can result in blindness. Patients surviving HSV encephalitis may have severe neurological sequelae including blindness, deafness, cerebral palsy, and profound mental retardation. Death can result from infections in patients who are immunocompromised, those with immature immune systems, and those with skin disorders. There is also growing evidence that HSV neuronal infection may play a role in the pathogenesis of Alzheimer disease.[19]

Virology and Viral Antigens

HSV contains a double-stranded DNA genome of approximately 152 kilobases that encodes at least 84 proteins. The viral genome resides within an icosadeltahedral capsid which is surrounded by an outer envelope composed of a lipid bilayer containing at least 12 viral glycoproteins (Fig. 28.1). The envelope glycoproteins are the major targets for humoral immunity; glycoprotein B (gB), glycoprotein C (gC), glycoprotein D (gD), or glycoprotein H (gH)/glycoprotein L (gL) antibodies inhibit the absorption or neutralize HSV in vitro and antibodies to gB or gD inhibit neuron-to-keratinocyte spread in a cell culture system.[20] CD4 and CD8 cellular responses can be induced by a wide array of HSV proteins including glycoproteins (gB, gC, gD, gE, and gH), structural capsid and tegument proteins (gene products of UL 7, UL21, UL25, UL26, UL29,UL47, UL48, UL49, and the proteins VP5, VP11/12, VP13/14, VP16, VP22, and ICP8), and nonstructural proteins (ICP0, ICP4, and ICP27 and the gene product of UL50).[20] HSV-1 and HSV-2 have a similar genetic composition, genome organization, and extensive DNA and protein homology. One notable difference exists between the HSV-1 and HSV-2 glycoprotein G genes. This difference forms the basis for the current type-specific serological tests that are used to discriminate

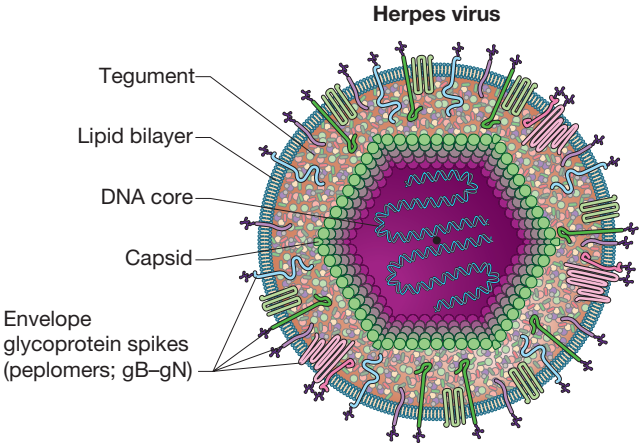


Figure 28.1. Herpes simplex virus structure.

accurately whether an individual is infected with HSV-1, HSV-2, or both.[21]

Pathogenesis as It Relates to Prevention

The pathogenesis of mucocutaneous HSV infections involves replication of virus at the portal of entry and uptake and spread via nerve fibers to regional sensory ganglia where further viral replication occurs and where latent infection is established.[22] Animal studies indicate that challenge with low doses of virus is sufficient to cause infection but that higher doses are required to cause clinically apparent illness.[23] During the initial infection innate and adaptive humoral and cellular immune responses limit viral replication and allow for control of the acute disease. Despite these responses the latent virus can periodically reactivate in sensory ganglia to produce progeny virus which is transported to the periphery where further replication occurs. This peripheral replication can result in symptomatic recurrent infection or asymptomatic shedding. It is important to note that hematogenous spread does not play an important role in the pathogenesis of infection except in the host with an immature or compromised immune system.[16] In considering the development of vaccines to control HSV infections an understanding of the pathogenesis provides three insights. First, the lack of hematogenous viral dissemination in healthy individuals means that an HSV vaccine cannot target prevention of viral spread in the bloodstream, a step that has proved key for other pathogens. Consequently, HSV vaccines may need to engender responses that protect at the portal of entry, that is, the genital or oral mucosa, or possibly sensory nerve endings and/or sensory ganglia. Second, higher doses of virus are required to cause disease than are needed to cause subclinical infection, which suggests that if a vaccine cannot prevent infection, it might be able to shift symptomatic disease to subclinical infection. Third, the ability of the virus to reactivate and cause recurrent infections despite the host having produced a variety of virus-specific adaptive responses suggests that it will be necessary for an effective vaccine to produce protective responses superior to what develops as a consequence of infection.

Diagnosis

HSV infections should be suspected based upon the history of exposure and clinical findings, particularly vesicular lesions; however, the clinical diagnosis of genital herpes is remarkably unreliable.[24] Suspicion of an HSV infection requires laboratory confirmation. Virus culture is the gold standard for mucocutaneous HSV infections. Virus typing should be done in conjunction with culture for suspected genital herpes as virus type is important in predicting the frequency of subsequent recurrent infections. Although not as sensitive as culture, direct detection of HSV antigens in clinical specimens can be done rapidly and has very good specificity.[25] The use of a polymerase chain reaction (PCR)-based assay for detection of HSV DNA is highly sensitive and specific, and even though of somewhat limited availability, it is emerging in routine clinical use and is the test of choice in examining cerebrospinal fluid in cases of suspected HSV encephalitis or meningitis.[26]

Serological tests can be used to support the clinical diagnosis of HSV infection in patients who present too late in the course of an acute illness for viral culture or viral antigen or DNA assays to be accurate. Immunoglobulin (Ig) M assays are unreliable and their interpretation made difficult by the fact that recurrent infections can trigger an anamnestic IgM response that can be misinterpreted as indicating an acute primary infection. To be helpful IgG tests should be type specific[21] and their results interpreted in the context of the disease manifestations and clinical history.

Treatment and Prevention With Antivirals

All symptomatic primary HSV infections probably warrant treatment. Acyclovir, valacyclovir, and famciclovir are the principal drugs used in the management of HSV infections. Early initiation of therapy results in maximum therapeutic benefit, but does not prevent the establishment of the latent infection. Likewise, treatment of recurrent infections does not eradicate the latent virus infection. Less commonly used drugs include foscarnet, cidofovir, trifluorothymidine, brivudin, and idoxuridine.

A landmark study determined that the chronic use of valacyclovir by patients with HSV-2 genital herpes could reduce the risk of transmission of infection to a susceptible heterosexual partner by 48%.[27]

EPIDEMIOLOGY

Seroprevalence

HSV-1 infection is generally spread through contact with contaminated oral secretions. HSV-1 seroprevalence rises rapidly in the first decade of life with 50% of European and 70% of American 10-year-old children being seropositive.[28] By the sixth and seventh decades 70% to 80% of adult Americans and Europeans are HSV-1 seropositive. Even though data on HSV-1 seroprevalence in the developing world are limited, studies suggest greater than 90% of adults in Africa and Asia are HSV-1 seropositive.[29] In contrast to HSV-1, HSV-2 seroprevalence is essentially zero in the first decade of life, rising sharply with the onset of sexual activity during adolescence, thus reflecting the primary mode of transmission. HSV-2 seroprevalence rates tend to increase steadily throughout the third and fourth decades of life among Europeans and Americans, reaching a peak of approximately 20% in Europe and 30% in the United States among generally low-risk populations, but with peak rates of approximately 45% and 60% for high-risk populations in Europe and the United States, respectively.[28] In general, Central and South America has HSV-2 seroprevalence rates similar to the United States and Europe, whereas parts of Africa generally have much higher rates and Asia tends to be lower; in any geographic area, however, there are great variations in HSV-2 seroprevalence rates among different populations.[29] Two American longitudinal studies provide some estimate of the rate of HSV-2 acquisition. In a vaccine trial involving high-risk volunteers followed prospectively, gender

was shown to influence acquisition of HSV-2 infection with a rate of 3.8 cases per 100 person-years of follow-up for men compared to 5.8 for women.[30] In this study, preexisting immunity to HSV-1 did not influence the rate of acquisition of HSV-2 infection but did increase the proportion of infections that were asymptomatic. A study of adolescent girls found an acquisition rate of 4.4 cases per 100 person-years of sexual activity for HSV-2 infection and reported that preexisting HSV-1 immunity affords girls protection against both HSV-2 infection and disease.[31] The impact of preexisting HSV-1 immunity on HSV-2 acquisition is controversial but needs to be considered in designing vaccine trials aimed at preventing HSV-2 infection or disease.

Incidence data are limited and underrepresent the magnitude of HSV infections as most HSV infections are asymptomatic.[32] Clinical studies of patients presenting with HSV disease indicate that most oral and nongenital skin infections, as well as central nervous system infections, outside the neonatal period are caused by HSV-1.[32]

ACTIVE IMMUNIZATION

History of Vaccine Development

The history of HSV vaccine development dates from the 1930s.[33] The initial work focused on therapeutic vaccines intended for the control of recurrent mucocutaneous HSV infections. Clinical assessment of prophylactic vaccines did not begin until the late 1940s.[34] Two major problems hampered early research: the lack of defined vaccine products and fatally flawed design of clinical trials. Early vaccines were crude preparations, exemplified by the 1938 vaccine developed by Frank which consisted of a formalin-treated HSV-2–infected brain emulsion prepared from rabbits dying of encephalitis.[35] These products were evaluated in open trials involving patients with oral, ocular, or genital HSV infections and using subjective patient assessment of improvement. The importance of randomized, blinded, placebo-controlled clinical trials was not appreciated until the landmark study of Kern and Schiff in 1964, which demonstrated comparable self-reported improvement in the frequency of recurrences for both vaccine and placebo recipients.[36] In the 1940s and 1950s, vaccines were made from virus grown in embryonated eggs and inactivated by physical means. By the 1960s vaccines were prepared by chemical or physical inactivation of virus grown in cell cultures. Between 1946 and 1982 more than two dozen clinical trials of experimental vaccines were reported but no vaccine was convincingly proven to be effective.[37]

The modern era of HSV vaccine development begun in the 1980s and 1990s has been largely unsuccessful (summarized in Table 28.1). Two studies, however, conducted in the 1990s were successful in establishing the feasibility of developing therapeutic and prophylactic HSV vaccines.

Therapeutic Genital Herpes Vaccine

Using a recombinant HSV-2 glycoprotein D (gD2) vaccine, Stanberry and colleagues developed the first experimental evidence supporting the concept of therapeutic immunization to control recurrent genital herpes.[45,46] Straus and colleagues[47] subsequently conducted a double-blind, placebo-controlled clinical trial investigating the effect of the vaccine on the frequency of symptomatic outbreaks in patients with genital herpes. Ninety-eight patients with documented genital herpes who reported four to 14 recurrences per year were randomized to receive deltoid injections of either 100 μg gD2 in alum or alum alone (placebo) at 0 and 2 months. Clinically recognizable recurrences were documented for 1 year. The vaccinees experienced fewer monthly recurrences than placebo recipients (mean: 0.42 ± 0.05 vs 0.55 ± 0.05), had significantly fewer virologically confirmed monthly recurrences (mean: 0.18 ± 0.03 vs 0.28 ± 0.03), and had a significantly lower median number of recurrences for the study year (4 [range: 0–17] vs 6 [range: 0–15]). The overall effect appeared greater during the first 4 months of the trial. By the nature of the study all participants were HSV-2 seropositive but the gD2 vaccine boosted neutralizing HSV-2 antibodies fourfold and gD2-specific titers sevenfold over baseline levels. Even though the overall effect was transient and modest, the results did establish the feasibility of treating an established HSV infection with a therapeutic

TABLE 28.1 Herpes Simplex Virus Vaccines Not Proven Effective in Clinical Trials

Vaccine	Developer or Manufacturer	Application	Comment	Reference
Cell-culture derived inactivated HSV-1	Skinner	Prophylaxis genital herpes	Clinical trials used historic controls, efficacy not established	37,38
Cell-culture derived inactivated HSV-1	Skinner	Therapeutic genital herpes	No clinical benefit in controlled trials	39
Egg-derived inactivated HSV-1 (Lupidon H) or HSV-2 (Lupidon G)	Bruschettini	Therapeutic mucocutaneous herpes	Patients with oral and genital herpes included in same study making interpretation of results impossible	40
HSV-2 deletion mutant	Sanofi-Pasteur	Prophylaxis genital herpes	Minimally immunogenic in Phase I trial	41
Cell-culture derived HSV-2 glycoproteins	Merck	Prophylaxis genital herpes	Shown to be ineffective in a well-designed clinical study	42
Recombinant HSV-2 glycoproteins B and D with MF59	Chiron (now Novartis Vaccines)	Prophylaxis genital herpes	Shown to be ineffective in a well-designed clinical study	29
Recombinant HSV-2 glycoproteins B and D with MF59	Chiron (now Novartis Vaccines)	Therapeutic genital herpes	Shown to be ineffective in a well-designed clinical study	43
Glycoprotein H–deleted HSV-2 mutant	Glaxo-Wellcome/Cantab	Therapeutic genital herpes	Shown to be ineffective in a well-designed clinical study	44

HSV, herpes simplex virus.

vaccine. A follow-up Chiron study using recombinant HSV-2 gB and gD combined with MF59, a squalene oil-in-water emulsion adjuvant, failed to demonstrate a therapeutic benefit in patients with recurrent genital herpes.[44] It is possible that the failure was the result of use of lower amounts of the HSV glycoproteins in the second trial (10 μg each of gB2 and gD2) compared to the first trial (100 μg gD2); however, it has also been postulated that the failure of the second vaccine might have been caused by the selection of the MF59 adjuvant, which principally augments antibody responses.[48] Important questions remain unanswered including these: What were the key immunological mediators of the effect (e.g., cellular versus antibody responses)? What was the critical site of the effect (e.g., genital skin or latently infected sensory ganglia)? Could therapeutic vaccines impact asymptomatic shedding and transmission as well as symptomatic recurrences? Animal studies indicate that induction or enhancement of HSV-specific cellular immune responses are required for a therapeutic effect and that therapeutic vaccines can reduce clinically recognizable recurrences as well as viral shedding.[49,50]

Prophylactic Genital Herpes Vaccines

In the 1980s, Chiron developed and tested a prophylactic HSV-2 vaccine consisting of truncated HSV-2 glycoproteins gD2 and gB2 with the adjuvant MF59. The vaccine was shown to be highly immunogenic but was ineffective in two randomized, double-blind, placebo-controlled studies.[30] The first study (Partners Trial) enrolled 531 HSV-2 seronegative sexual partners of HSV-2–infected persons, while the second study (STI Clinic Trial) enrolled 1862 high-risk individuals from sexually transmitted infection (STI) clinics. The trials were placebo controlled, with a 0-, 1-, and 6-month immunization schedule and an 18-month study duration. The primary outcome measure was acquisition of HSV-2 infection as assessed by seroconversion to HSV-2 antigens not present in the vaccine. The data from the two studies were combined for the analysis and while the overall efficacy of the vaccine was only 9% during the entirety of the studies, in the first 5 months the rate of HSV-2 acquisition was 50% lower among vaccine recipients compared to placebo recipients. A post hoc analysis of the data suggested there might be a difference in efficacy by gender with an efficacy rate of 26% in women compared with 4% in men. It may be worth noting that there appeared to be a trend toward protection in the Partners Trial in the vaccinated women compared to the placebo recipients (4.0 vs 8.8 cases per 100 person-years, respectively); however, the same trend was not observed in women enrolled from STI clinics (5.5 cases per 100 person-years for vaccine recipients vs 5.4 cases per 100 person-years for placebo recipients) (Table 28.2). The reasons why the adjuvanted subunit vaccine failed despite inducing high titers of HSV-2–specific antibody are unclear and, likewise, the explanation for transient protection in women is uncertain. The apparent trend toward protection in vaccinated women in the Partners Trial but not the STI Clinic Trial suggests that these two populations of women may respond to vaccines differently and that women in HSV-2 discordant relationships, who are repetitively exposed to subinfectious doses of virus, may be immunologically primed and hence respond more favorably to immunization.

In parallel with the Chiron HSV vaccine program, GlaxoSmithKline Biologicals was developing a subunit vaccine containing a CHO cell derived truncated gD2 antigen (20 μg) combined with alum (500 μg) and the potent adjuvant 3-de-0-acylated monophosphoryl lipid A (MPL) (50 μg). In a Phase I evaluation, the vaccine was shown to be well tolerated and capable of inducing humoral and cellular immune responses superior to gD2 and alum alone.[51] In the 1990s, the vaccine was evaluated in two multinational, double-blind, randomized, placebo-controlled consort studies.[52] The first study (007 Trial) enrolled 847 HSV-1– and HSV-2–seronegative partners (268 women) of HSV-2–infected individuals; the second study (017 Trial) enrolled 1867 HSV-2–seronegative partners (710 women) of HSV-2–infected individuals. The vaccine or control (alum-MPL in first study and alum in the second study) was administered intramuscularly at 0, 1, and 6 months. The study duration was 19 months. The primary outcome measure was protection against HSV-2 disease; in the first study the primary outcome was assessed in all participants (men and women) whereas in the second study the primary outcome was assessed in women only, with assessment in men being a secondary outcome measure. In the first study the HSV-2–infected partners were required to abstain from using suppressive antiherpes therapy, but were allowed to do so in the second study.

Overall, in both studies, the protection against HSV-2 disease offered by the vaccine and alum control were similar: the efficacy of the vaccine in the first study (007 Trial) was 38% in HSV-1– and HSV-2–seronegative men and women, whereas in the second study (017 Trial) it was 42% in HSV-2–seronegative women participants (see Table 28.2). In both studies there was a nonsignificant trend toward protection of HSV-seronegative women against HSV-2 infection (vaccine efficacy of 46% in the first study and 39% in the second study; $P = .08$ in each study). However, the gD2-alum-MPL vaccine afforded significant protection (vaccine efficacy of 73%–74%) against genital HSV-2 disease in women who had no preexisting antibody to either HSV-1 or HSV-2. There was no evidence that the vaccine was protective in women who were initially HSV-1–seropositive or in men. In the Phase III trials the vaccine was safe, generally well tolerated, and induced gD-specific neutralizing antibodies and a T-helper–1 (Th1) cell-mediated immune profile. Because neither study met its predetermined primary outcome (i.e., for the 007 study, prevention of genital herpes disease in HSV-2–seronegative men and women; for the 017 study, prevention of genital herpes disease in HSV-2–seronegative women), a third study (the HERPEVAC Trial for Women) was planned and undertaken.

During the course of the HERPEVAC study the results of a previously initiated multinational safety and immunogenicity trial became available. The study, involving 7460 volunteers (4968 in the vaccine group and 2492 in the placebo group), found the vaccine to be generally safe but did find significantly more local reactions, especially soreness, compared to placebo.[53] The reactions tended to be mild to moderate in severity, lasting less than 3 days, although severe local reactions were noted in 7.0% of participants receiving vaccine doses and in 1.2% of participants receiving placebo doses. The rate of local reactions did not increase with subsequent vaccine administrations or in subjects with preexisting HSV immunity. Interestingly, women receiving either the vaccine or placebo reported significantly more local and systemic adverse reactions than did men. With regards to immunogenicity, the vaccine induced higher titers of HSV gD-specific antibody than occurs following HSV infection.

The third Phase III trial (the HERPEVAC study) was cosponsored by GlaxoSmithKline and the National Institutes of Health and differed from the previous two Phase III trials in that the participants were young, HSV–seronegative women who were more representative of the general population as compared to participants in the previous trials who were at risk of genital HSV-2 infection because they were in a relationship with a sexual partner who had a history of recurrent genital herpes.[54] The randomized, double-blinded trial was conducted in 8323 women ages 18 to 30 years. The primary end point was reduction in genital disease caused by either

TABLE 28.2 Attack Rates and Vaccine Efficacy in Four HSV Vaccine Trials

	Chiron[a] gB/gD-MF59 Vaccine				GlaxoSmithKline[b] gD-AS04 Vaccine			
	Partners Trial		STI Clinic Trial		007 Trial[c]		017 Trial[d]	
	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control
Overall attack rate[e]	3.4	4.6	4.4	4.6	3.5 (1.7 to 5.3)[f]	6.0 (3.7 to 8.5)	3.0 (1.8 to 4.2)	3.6 (2.3 to 4.9)
Overall vaccine efficacy					38% (–18 to 68)		11% (–20 to 34)	
Attack rate in men	2.8	1.1	4.1	4.3	3.7 (1.5–6.0)	3.7 (1.4–5.9)	2.8 (1.4 to 4.2)	2.7 (1.3 to 4.1)
Vaccine efficacy in men					–11% (–161 to 53)		–10% (–127 to 47)	
Attack rate in women	4.0	8.8	5.5	5.4	3.1 (0.1 to 6.1)	11.6 (5.9 to 17.4)	3.3 (1.1 to 5.4)	5.0 (2.6 to 7.5)
Vaccine efficacy in women					73% (19 to 91)		42% (–31 to 74)	
Attack rate in HSV-1–negative participants	3.5	6.3	5.1	4.4	Same as overall	Same as overall	3.4 (1.2 to 5.6)	8.3 (4.8 to 11.8)
Attack rate in HSV-1–positive participants	3.4	3.5	4.1	4.7	NA	NA	ND	ND
Attack rate in HSV-1–negative women	ND	ND	ND	ND	As above for women	As above for women	3.5 (0 to 7.4)	13.3 (6.3 to 20.3)
Vaccine efficacy in HSV-1–negative women					As above for women		74% (9 to 93)	
Attack rate in HSV-1–negative men	ND	ND	ND	ND	As above for men	As above for men	3.3 (0.7 to 6.0)	5.3 (1.7 to 8.9)
Vaccine efficacy in HSV-1–negative men					As above for men		32% (–95 to 76)	

HSV, herpes simplex virus; NA, not applicable; ND, not determined.
[a]Chiron trials' primary outcome measure was prevention of HSV-2 infection.
[b]GlaxoSmithKline trials' primary outcome measure was prevention of HSV-2 disease.
[c]007 Trial participants were HSV-2 and HSV-1 seronegative.
[d]017 Trial participants were HSV-2 seronegative but could be HSV-1 seropositive or seronegative.
[e]Attack rates are per 100 person-years.
[f]Values in parentheses are the 95% confidence intervals. Confidence interval data not given for Chiron trials.
Data from Corey L, Langenberg AG, Ashley R, et al. Recombinant glycoprotein vaccine for the prevention of genital HSV-2 infection: two randomized controlled trials. JAMA. *1999;282:331–340; and Stanberry LR, Spruance SL, Cunningham AL, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes.* N Engl J Med. *2002;347:1652–1661.*

type 1 or type 2 HSV, but the vaccine did not meet this end point. Overall, vaccine efficacy was 20% confidence interval (CI) (–29, 50) against genital herpes disease; however, type-specific efficacy was found against HSV-1 but not HSV-2. After three doses of vaccine, the vaccine efficacy against culture-positive genital HSV-1 disease was 82% CI (35, 95). Vaccine efficacy against HSV-1 infection (with or without disease) was 35% CI (13, 52), but efficacy against HSV-2 infection was not observed (–8% CI [–59, 26]).

A substudy examined whether immunized women who became infected had comparable patterns of asymptomatic viral shedding to those observed in unimmunized HSV-infected women or whether they shed less virus,[54] as was shown in experimental animal studies.[55] The issue of asymptomatic shedding has important public health implications.[56] The substudy enrolled 43 participants (30 HSV vaccine recipients and 13 control subjects) who acquired HSV-2 infection during the trial. The women collected anogenital swabs on 60 consecutive days for quantitation of HSV-2 genome beginning 3 to 6 months after disease onset (24 women: 15 in the HSV vaccine group and nine in the control group) or seroconversion (19 women: 15 in the HSV vaccine group and four in the control group). Although the rate of viral shedding was higher among the HSV vaccine recipients compared with controls, 29% versus 17%, respectively (relative risk [RR] = 1.55, 95% CI [1.28, 1.86]), the mean quantity of HSV DNA on days with shedding did not differ between the two groups.

The lack of vaccine efficacy against HSV-2 in the HERPEVAC trial is puzzling in view of the previous two gD2 vaccine studies in discordant couples that found efficacy against HSV-2. The distinguishing feature of discordant couples is that they are a highly select group in which one uninfected partner is potentially repeatedly exposed to HSV by an infected partner. Attack rates of HSV-2 genital disease in the prior GlaxoSmithKline gD2 vaccine studies in discordant couples were high in the uninfected women (13.9% for 19 months or 8.4% per year) and were reduced significantly by vaccine (VE = 73% and 74% in the two trials; $P < .05$ in each case). For

the 007 and 017 trials, the primary end point was the occurrence of genital HSV disease in all subjects in Study 007 and in HSV-2–seronegative female subjects in Study 017. Vaccine efficacy was defined as the percentage reduction in the frequency of the end point among recipients of vaccine compared with the frequency among recipients of control injections; it was calculated as 1 − (attack rate among recipients of vaccine ÷ attack rate among recipients of control injections), with two-tailed 95% CIs, according to the Cochran-Mantel-Haenszel method.[52] Likewise, the Chiron gB2/gD2 vaccine appeared to have some effect in vaccinated women in the discordant couples (Partners) trial, but not in women who were recruited from STI clinics.[30] Potential reasons why the gD2 vaccine might protect women who were in a long-term relationship with an HSV-2–infected male partner (mean duration of relationship was 23 months) but not women in other high-risk relationships might include (a) an undefined immunologic priming event from chronic sexual exposure to HSV-2 antigens from the infected partner[57] that could be boosted by the subunit vaccine; (b) selection bias for a population of relatively HSV-2–resistant women who developed enhanced resistance from the gD2 vaccine; or (c) women in a long-term relationship have less frequent,[58] and perhaps less intense or less traumatic, exposure to HSV-2 because of the long-term nature of the relationship.

Vaccine efficacy against genital HSV-1 infections was demonstrated in the HERPEVAC trial but not in the original two GlaxoSmithKline discordant couple studies as too few cases of HSV-1 genital disease occurred in the original studies. As the HERPEVAC study recruited women from the general population it was anticipated that approximately 30% of the incident cases of symptomatic genital herpes would be caused by HSV-1. The gD2 antigen is derived from HSV-2 but shares significant homology with HSV-1 gD, which may explain the protection against HSV-1. The finding that the gD2 vaccine protected women recruited from the general population against HSV-1 genital infection but not HSV-2 genital infection might indicate some important difference in the immunobiology of the two viruses, and type-specific immune responses to vaccine antigen may reveal differences in antibody activity versus HSV-1 or HSV-2 or whether HSV-1 is more easily neutralized by vaccine-induced antibodies. It is also possible that the vaccine efficacy is influenced by the mode of virus transmission. It is generally believed that most cases of genital HSV-1 infection result from oral–genital sex rather than penile–vaginal sex.[59] Perhaps oral–genital sex results in exposure to lower concentrations of virus; possibly microabrasions associated with penile–vaginal sex facilitates transmission; or perhaps the cervical epithelium reached during penile–vaginal sex is more susceptible to HSV than the cells of the vaginal introitus, the cells most generally exposed during oral–genital sex. At this time, we can only speculate on the biological basis for the efficacy against HSV-1 genital infection but not against HSV-2 genital infection.

In comparing the results of the Chiron and GlaxoSmithKline vaccine programs, it may be worth noting that a major difference in the two vaccines was the adjuvant. The Chiron vaccine contained MF59 whereas the GlaxoSmithKline vaccine contained alum and MPL. The partial effectiveness of this glycoprotein vaccine compared to the failure of the Chiron glycoprotein vaccine suggests that adjuvant may be critical in facilitating the induction of important protective immune responses. More research is needed to understand the adjuvant effects as well as the unexpected gender-specific protection seen with the gD2-alum-MPL vaccine. Another important unanswered question is whether a vaccine that can protect against genital herpes can afford protection against nongenital HSV diseases.[60]

Correlates of Protection

The critical immune responses required of an effective prophylactic or therapeutic HSV vaccine are unknown although it is widely held that they are likely to be different for the two types of products.[61,62] Because HSV has evolved several strategies to evade the immune system[61] and because HSV infection in one anatomic site provides, at best, limited protection against HSV infection at a second site,[63] an evaluation of host responses to infection may not be a useful strategy for identifying protective immune responses. Animal studies suggest that neutralizing antibody is essential for prophylactic vaccination and a recent animal study demonstrated the importance of mucosal T cells in protecting against experimental genital herpes,[64] while the results of clinical trials suggest that robust T-cell responses will be necessary for effective prophylaxis and induction of effective immune responses will likely require some balance between innate and adaptive responses.[62,65] It is likely that the correlates of protection for prophylactic HSV vaccines will be both neutralizing antibody and T-cell responses greater than those seen in infected individuals. At this point it is unclear whether $CD4^+$ or $CD8^+$ T cells will be more important in affording protection against infection. Regarding therapeutic vaccines, it is clear that antibody is not a correlate of protection, and most evidence currently suggests that HSV-specific $CD8^+$ T-cell responses will be the key to protection. Recent studies on HSV CD8 T cells in animal ganglia suggest that latent infection may lead to CD8 T-cell exhaustion and hence compromise the ability of therapeutic vaccines to engender robust CD8 T-cell augmentation.[66]

Public Health Considerations

HSV infections are a global public health problem both as a consequence of HSV-induced disease and indirectly by enhancing the acquisition and transmission of HIV.[28,29] The United States is experiencing an ongoing epidemic of genital HSV infections.[4] The potential impact of the partially effective GlaxoSmithKline prophylactic vaccine will be limited by its gender-specificity and its lack of efficacy in HSV-1–seropositive women, a particular problem for the developing world where most individuals acquire nongenital HSV-1 infections in early life. Nevertheless, mathematical modeling of universal immunization of young women in the United States suggests that a partially effective vaccine could have a significant impact on the ongoing genital herpes epidemic.[67]

FUTURE VACCINES

Currently there are several companies with vaccines in early clinical development; all these candidates are intended for use as therapeutic vaccines.

The HerpV plus QS21vaccine being developed by Agenus, Inc., consists of a recombinant human heat shock protein (HSP)-70 complexed with 32 HSV-2 peptides and a proprietary QS21 adjuvant system (ClinicalTrials.gov Identifier: NCT01687595). Their Phase II results indicated the vaccine was well tolerated, more than half of the vaccine recipients developed a robust anti-HSV cytotoxic T-cell immune response, and in those patients there was a statistically significant 75% reduction in viral shedding ($P < .001$; CI: 46.2–88.6%).[68,69]

The GEN-003 plus Matrix-M-2 product being developed by Genocea Biosciences is a subunit vaccine containing HSV-2 ICP4 and gD proteins plus a proprietary saponin-based adjuvant system. In a Phase I/II clinical trial (ClinicalTrials.gov Identifier: NCT01667341) the vaccine was reported to be well tolerated and immunogenic, boosting antibody and T-cell responses for at least 12 months. At 6 months, the

group receiving a 30-μg dose of the vaccine showed a 65% reduction in the rate of recurrences and a 40% decrease in viral shedding. At 12 months the mean viral shedding rate had reverted to baseline levels.[70] A dose-ranging Phase II clinical trial has been initiated (ClinicalTrials.gov Identifier: NCT02114060).

VCL-HB01 and VLC-HM01 with Vaxfectin, developed by Vical, are plasmid DNA vaccines encoding one or two HSV-2 proteins in a proprietary cationic lipid-based adjuvant formulation. Results of a Phase I/II clinical trial showed that neither the monovalent nor the bivalent vaccine met the primary end point of viral shedding rate reduction from baseline. However, the bivalent vaccine achieved statistically significant reduction in a prospectively defined secondary end point of genital lesion rate at 3 months versus baseline (−49%; $P = .031$) with persistent of the effect at the 9-month time point (−57%; $P = .009$). The company announced that it plans to advance the bivalent vaccine to a Phase II trial in 2016.[70a]

Coridon/Admedus Vaccines, an Australian company, has in development an HSV-2 polynucleotide (DNA) vaccine.[71] The vaccine was reported to be safe and to engender a T-cell response in a Phase I study[72]; a Phase II trial reportedly began in April 2015.[73]

Sanofi Pasteur is developing HSV-529 vaccine, a replication-defective HSV-2 mutant deleted of two essential genes U_L5 and U_L29 and grown on a complementing cell line.[74] The vaccine is being tested in an ongoing National Institutes of Health–sponsored Phase I safety/immunogenicity study in healthy volunteers with or without HSV infection; the estimated completion date is October 2016 (ClinicalTrials.gov Identifier: NCT01915212). There are plans to combine the replication defective mutant with a glucopyranosyl lipid adjuvant from Immune Design with a goal of developing an effective therapeutic vaccine. A single deletion, replication defective vaccine was shown to safe and immunogenic but ineffective as a therapeutic vaccine in the 1990s.[75] No replication defective HSV vaccine candidate has been investigated in clinical trials for use as a prophylactic vaccine.

Ongoing research continues to explore interesting new vaccine strategies. Examples of recent preclinical studies include a DNA prime-formalin-inactivated-HSV-2 boost vaccine strategy,[65] a HSV-2 glycoprotein E deletion mutant being evaluated as a replication-competent, attenuated live virus vaccine candidate,[76] a two-step "prime-and-pull" strategy that uses conventional parenteral vaccination (prime), followed by vaginal chemokine application to "pull" activated T cells to the genital track, new genetically engineered virus constructs including a nonreplicating dominant-negative HSV-2 recombinant viral vaccine,[77] ICP0 deleted HSV-1 and HSV-2 mutants,[79,80] and a vaccine containing four HSV proteins designed to stimulate both humoral and T-cell specific HSV immune responses.[81]

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29 Human Immunodeficiency Virus Vaccines

Marc P. Girard and Wayne C. Koff

Although the disease was most likely present in Africa for many decades earlier, it only was in the summer of 1981 that AIDS was first recognized in the human population as an outbreak of *Pneumocystis carinii* pneumonia among homosexual men in the United States.[1,2] There is evidence that AIDS initially spread from Central Africa to Haiti and then to the United States, then globally.[3,4] The disease is caused by the HIV, a lentivirus that is transmitted through sexual intercourse and through injection of infected blood or blood-derived products, and from mother-to-child.[5] It is characterized by the progressive depletion of $CD4^+$ helper T cells, which are the preferred target of the virus, and by a chronic activation of the immune system. This results in an immunodeficiency syndrome that paves the way to opportunistic infections, including pulmonary tuberculosis (a frequent complication), *P. carinii (Pneumocystis jiroveci)* pneumonia, *Mycobacterium avium-intracellulare* infections, toxoplasmosis, candidiasis, cryptosporidiosis, and a variety of viral infections (cytomegalovirus, hepatitis C, herpes simplex) as well as cancers, such as Kaposi sarcoma and non-Hodgkin B-cell lymphomas. Since 1981, an estimated 70 million people have been infected with HIV, and approximately 36 million people have died.[6] AIDS is notably the number one killer of young women of reproductive age in sub-Saharan Africa.

Several modalities can reduce HIV-1 infection rates in persons at risk of exposure, including screening of blood donors, awareness and counseling campaigns, monogamy and the use of condoms, expanded treatment of sexually transmitted infections, and male circumcision.[7–10] The use of a tenofovir vaginal gel, before and after sexual intercourse, has also been found to lower HIV-1 incidence rates among sexually active South African women after 2.5 years of use by 39%. Efficacy actually reached 54% among "high adherer" women who used the microbicide as instructed.[11–13] Preexposure prophylaxis (PrEP) using oral antiretrovirals such as Truvada pills (a combination of tenofovir and emtricitabine) demonstrated substantial efficacy in reducing the risk of acquiring HIV in high-risk individuals, including men who have sex with men (MSM).[14] Immediate postexposure antiretroviral therapy (ART) is another way to prevent transmission.[15,16] Antiviral treatment of HIV-infected adults was associated with a 92% reduction in HIV transmission to their uninfected partner.[17]

Transmission of HIV-1 from mother to child can also be successfully prevented by treating the infected mother with antiretroviral drugs.[18–21] Although full implementation of these preventive measures have the potential to drastically reduce the spread of the epidemic, as was observed in a few field settings such as Uganda,[22] in practice there are many obstacles, including scale-up difficulties, costs of the drugs and healthcare, and profound resistance to behavioral change. The best chance to curb the HIV epidemic, therefore, lies in the development of an effective vaccine. This remains the greatest unmet need and a public health priority as the epidemic continues.[23]

The development of an effective HIV vaccine is indeed hampered by the very properties of the virus (Table 29.1). The antiviral immune mechanisms that are induced in response to HIV infection, whether initial innate immune responses or later cell-mediated or specific antibody responses, fail to clear the virus. One reason is that HIV integrates as a provirus into the chromosomes of long-lived reservoir memory T cells where it can persist in a latent state.[24–26] The virus seeks refuge in follicular helper $CD4^+$ T-cells (T_{FH}) within the B-cell follicles, where it remains in a latent form[27–29]; the same phenomenon is observed with the simian immunodeficiency virus (SIV) in macaques.[28,30] The earlier the initiation of antiviral therapy, especially in children, the smaller is the size of the viral reservoir.[31–33] Reversing HIV-1 latency in HIV-infected persons has become, at this time, a major research objective, but it still remains an unmet goal.[34–36]

Another reason it has been so difficult to develop an effective HIV vaccine is the fast rate of replication of the virus, which, coupled with a high rate of mutation and recombination, rapidly leads to the emergence of quasispecies of viral clones that are able to thrive as escape mutants and evade both the cell-mediated and the humoral immune responses of the host.[37–41] At the same time, HIV-1 infection is accompanied by a downregulation of the major histocompatibility complex (MHC) class I antigen presentation at the surface of the infected cell, allowing for escape from antiviral cytotoxic T-lymphocyte (CTL) responses.[42,43] It has also been suggested that overwhelming virus replication at the time of primary HIV infection, together with chronic immune activation, lead to exhaustion of virus-specific CTLs and gradual impairment of cellular immunity, progressively leading to AIDS.[44–46]

How to elicit protective immune responses with a vaccine under such conditions remains a daunting challenge. Since 1987, more than 150 candidate HIV-1 vaccines or analogous vaccines against SIV have been developed and shown to elicit varying degrees of protective responses in nonhuman primate (NHP) models.[47–49] Many have also advanced to human clinical trials.[50–53] However, with the sole exception of the RV-144 Phase III trial, which evaluated a recombinant Canarypox (ALVAC) vector prime and recombinant HIV-1 envelope gp120 subunit protein plus alum boost and provided a modest 31% protection against HIV infection,[54,55] none of the vaccine candidates tested so far has shown efficacy in preventing HIV-1 infection or in controlling virus replication and delaying progression of disease in humans.

VIROLOGY

There are two well-defined etiological agents of AIDS, **HIV-1**[56–58] and **HIV-2**,[59] and both cause disease, but HIV-2 appears to be less aggressive and has a more restricted geographic spread. HIV-1 can further be subdivided into pandemic group M *(Major)* and less prevalent groups O *(Outlier)*,[60] N *(non-M, non-O)*[61] and P.[62] Only 15 cases of HIV-N infections and two cases of HIV-P infections have been reported to date, whereas HIV-O has been identified in approximately 1% of HIV infections in Cameroon, and in a few cases in Western countries.[63,64] HIV-1 group M can further be subdivided into subtypes or clades (see "Virus Variability" below).

HIV belongs to the genus lentivirus in the family *Retroviridae.* Lentiviruses are enveloped RNA viruses that typically produce slow, progressive infections after long periods of latency and persist in the host despite the host's active immune response. Their replication depends on the presence of an

active reverse transcriptase (RT), which transforms the RNA genome into a proviral DNA copy that integrates into the host cell chromosomes. Other lentiviruses are the simian, the feline, and the bovine immunodeficiency viruses; the Visna virus of sheep; the caprine arthritis-encephalitis virus; and the equine infectious anemia virus.

The genome of HIV is a single-stranded positive-sense RNA molecule, 9.5 kb in length, which encodes the typical retrovirus structural proteins Gag, further cleaved into $p18_{gag}$ (Matrix), $p24_{gag}$ (Capsid), and $p7_{gag}$ and $p9_{gag}$ (Nucleocapsid) proteins; Pol, itself cleaved into $p12_{pol}$ (Protease), $p68_{pol}$ (RT), and $p31_{pol}$ (Integrase); and Env, a 160-kDa glycoprotein eventually cleaved into a $gp120_{env}$ external subunit and a $gp41_{env}$ transmembrane subunit that together form trimeric spikes on the surface of the virion. In addition, the genome encodes a variety of nonstructural proteins, such as the regulatory proteins Tat and Rev and the accessory proteins Nef, Vif, Vpr, and Vpu (Fig. 29.1). Viral replication is initiated by the action of RT, which makes a proviral DNA copy of the RNA genome.

TABLE 29.1 Obstacles to the Development of HIV Vaccines

Absence of spontaneous recovery from natural infection
Antigenic diversity and hypervariability of the virus
Transmission of the disease by mucosal routes
Transmission of the virus by infected cells
Integration of the viral genome into host cell chromosomes
Persistence of the virus in a latent state in resting memory T cells
Rapid emergence of viral escape mutants in the host
Downregulation of major histocompatibility complex class I antigen presentation
Masking of the broad neutralization epitopes on virion spikes
Lack of an ideal HIV-1 animal model, requiring long and expensive human clinical trials

The provirus migrates into the host cell nucleus where it integrates into host cell genes at the periphery of the nucleus, in close correspondence with the nuclear pore[65] and in contact with various nucleoporins. The integrated provirus is then eventually transcribed into a set of messenger RNAs (mRNAs) with various splicing patterns that encode the various viral proteins, or into full-length progeny viral RNA that is transported to the cytoplasm where it will be encapsidated into progeny virions.

Virions emerge from the infected cell by budding as immature, noninfectious virus particles in which the Gag polyprotein is still intact and arranged in a radial fashion.[66] Gag is eventually cleaved by the viral protease, resulting in a dramatic morphological rearrangement that leads to the formation of mature virus core and acquisition of infectivity. Assembly and egress of new virus particles most often occur at sites of transient contact between an infected cell and a naïve target cell, resulting in the formation of "virological synapses" in which the membranes of the two cells form protrusions that come together like interlocking fingers.[67–70] Virological synapses allow the virus to directly spread to the uninfected target cell and shield progeny virions from neutralizing antibodies.[71] However, many synapses appear to be porous and antibodies can enter.[72]

The HIV virion consists of an internal cone-shaped capsid built from 1200 molecules of capsid protein ($p24_{gag}$) and surrounded by the viral lipid envelope, wrapped on a scaffold of matrix protein ($p17_{gag}$), and spiked with some 15 trimers of the highly glycosylated protein gp160 (gp120 + gp41) (Fig. 29.2).[73–75] There is evidence that the envelope of the virus also contains numerous molecules of type II human leukocyte antigen (HLA)-DR and β_2-microglobulin borrowed from the host cell in which the virus last replicated, together with cell membrane proteins, including CD43, CD44, CD55, CD59,

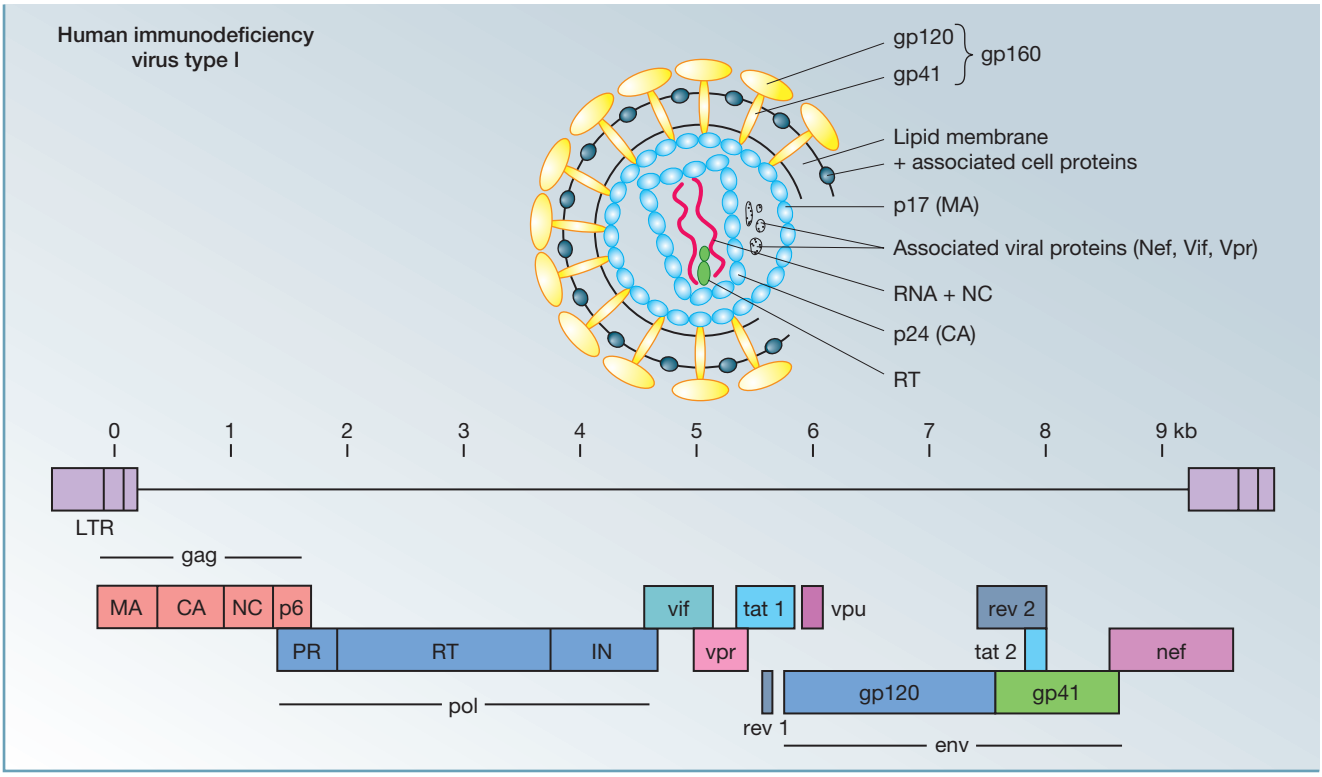


Figure 29.1. HIV-1 genomic organization and structure of the virion. CA, capsid; IN: integrase; MA, matrix; NC, nucleocapsid; PR, protease; RT, reverse transcriptase.

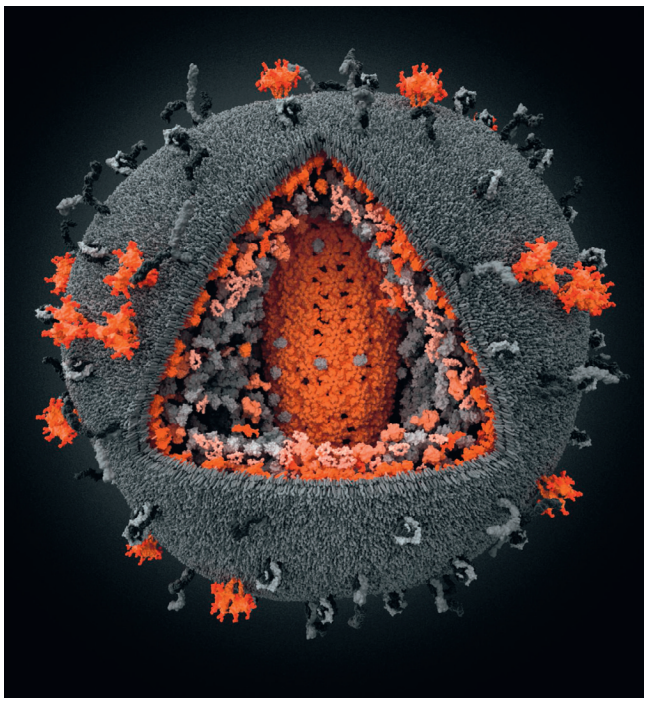


Figure 29.2. Reconstruction of the three-dimensional appearance of an HIV-1 virion. *(Courtesy Visual Science, www.vsci.us.)*

CD63, or CD71; leukocyte function–associated antigen (LFA)-1; and intercellular adhesion molecule (ICAM)-1.[76] This explains why the virus can be neutralized by antibodies to HLA-DR, LFA-1, β_2-microglobulin, or ICAM-1.[77]

The virus capsid contains two copies of the RNA genome associated with the nucleocapsid protein ($p7_{gag}$), together with the transfer RNA molecules that serve as primers for their reverse transcription. The capsid also contains internal viral proteins p1, p2, and p6, copies of viral proteins Vpr, Nef, and Vif, which play a role in the early events of HIV replication, and a few host proteins such as cyclophilin A, Tsg101, calmodulin, actin, cofifin, ezrin, and moesin.[78] Cyclophilin A binds tripartite motif (TRIM)-5α, an important mediator of innate antiretroviral immunity which is the basis for the resistance of Old World monkeys to HIV-1 infection and an important factor in the susceptibility of macaque monkeys to SIV infection.[79–83]

The ***env* gene** codes for the HIV-1 envelope gp160 glycoprotein that forms the trimeric spikes at the surface of the virion. gp160 folding, cleavage in the Golgi into noncovalently associated subunits gp120 (the external chain) and gp41 (the transmembrane chain), trimerization, glycosylation, and transport to the cell surface have been studied in detail.[83–85] Target cell tropism and attachment of the virus are determined by the gp120 moiety, which binds sequentially to the CD4 receptor then to the CCR5 or CXCR-4 coreceptors, whereas gp41 anchors the viral envelope spikes in the viral envelope and maintains their trimeric organization.[86] There is evidence that the $\alpha_4\beta_7$ integrin, the gut mucosal homing receptor, which is in a complex with CD4 in metabolically active $CD4^+$ T-cells, also serves as coreceptor.[87,88] The specific affinity of gp120 for $\alpha_4\beta_7$ provides a mechanism for HIV-1 to target activated T-cells in the gut-associated lymphoid tissue (GALT).[89] The membrane-proximal external region (MPER) of gp41 contains the receptor-binding site to the mucosal HIV-1–receptor galactosyl ceramide, as well as a calcium-binding site, and is one of the principal sites for binding of broadly neutralizing antibodies (bNAbs) (see below).[90,91] gp120 is heavily glycosylated at 25 to 35 glycosylation sites[92] with a mixture of high-mannose and complex sialic acid-containing carbohydrates,[73] that provides a 50 kDa "glycan shield" to neutralization epitopes.[93]

The ***gag* gene** is transcribed into either a *gag* or a *gag-pol* mRNA, whose translation yields $Pr55_{gag}$ and $p160_{gag\text{-}pol}$, respectively. $Pr55_{gag}$ is eventually cleaved to generate the $p17_{gag}$ matrix protein, the $p24_{gag}$ capsid protein, and the $p7_{gag}$ nucleocapsid protein. The $p7_{gag}$ nucleocapsid protein, which remains in tight association with the RNA genome, contains two zinc finger motifs that bind a zinc ion in a tetrahedral conformation necessary for packaging of viral RNA and infectivity.[94,95]

The ***pol* gene** encodes key viral enzymes, RT, which is associated with an RNase H activity, integrase, which allows the integration of the provirus DNA into the host T-cell genome, and the aspartyl proteinase, a homodimer of two subunits, which each contributes the amino acid triplet DTG to form the active site of the enzyme. The viral protease is required for virus maturation.

The ***tat* gene** encodes a *trans*-activating protein that prevents premature termination of provirus transcription through binding to a 59 residue stem-loop at the 5′ end of the nascent viral mRNA molecules called the *Tat-responsive element*, increasing production of viral mRNA several hundred-fold. The process involves the Tat-cdk9/cyclin T1 phosphorylation of RNA polymerase II.[96] Tat also plays a role in AIDS pathogenesis: free Tat molecules are released by HIV-1–infected cells[97,98] and activate, at a distance, uninfected $CD4^+$ T-cells through interaction with cell membrane lipid rafts and internalization followed by transport to the cell nucleus.[99] Anti-Tat antibodies are associated with long-term nonprogression.[98]

The ***rev* gene** encodes a protein that binds to a specific sequence element, the Rev-responsive element, which is present on the unspliced or single-spliced viral mRNAs that encode Gag, Pol, and Env. This allows their transportation from the nucleus to the cytoplasm and permits the expression of structural proteins Gag, Pol, and Env. Rev thus controls the switch from an early phase of the virus replication cycle, when only regulatory proteins Tat and Rev and accessory proteins Nef, Vif, Vpu, and Vpx are synthesized from multispliced mRNAs, to a late phase, when structural proteins are made and virions assembled.[100]

The ***nef* gene** encodes a major virulence factor, the myristoylated Nef protein, which contributes to the maintenance of high viral loads and promotes activation of resting T cells by selectively binding the p21-associated kinase 2.[101–104] Extracellular Nef also contributes to the depletion of $CD4^+$ T cells by interacting with the CXCR-4 receptor and inducing Fas ligand–mediated apoptosis of uninfected bystander $CD4^+$ T cells.[105,106] Intracellular Nef downmodulates cell-surface expression of both CD4 and class I MHC (HLA) antigens, thus providing infected cells with a mechanism for evading the host cellular immune response.[42,43,107,108] Transgenic mice that express the SIV *nef* gene in their $CD4^+$ T cells develop an AIDS-like disease with $CD4^+$ T-cell depletion, activation, and apoptosis.[109,110] As a corollary, SIV with a deletion in the *nef* gene (SIV Δnef) is attenuated and can serve as a live attenuated virus vaccine in macaque monkeys.[111–115] The Nef protein also increases viral infectivity by inhibiting the incorporation into progeny virions of two host-cell antiviral proteins, SERINC3 and SERINC5.[116,117]

The precise role and importance of the accessory proteins Vif, Vpr, and Vpu are still not completely deciphered (Table 29.2). Vpr binds cyclophilin A and induces cell-cycle arrest in G_2 and apoptosis[118,119]; it also enhances HIV replication in macrophages and nondividing cells by allowing entry of the

TABLE 29.2 Role of HIV-1 Accessory Proteins

Gene	Principal Role
Nef	Activation of resting CD4⁺ T cells Downmodulation of human leukocyte antigen Apoptosis of bystander CD4⁺ T cells
Vif	Inactivation of APOBEC3G
Vpr	Arrest of cell cycle in G_2
Vpu	Blockage of tetherin
Vpx	Blockage of TRIM5α and SAMHD1

SAMHD1, sterile alpha motif and histidine-aspartate–domain-containing protein 1; TRIM, tripartite motif.

viral preintegration complex into the nucleus of the cell.[119] Vpu forms ion channels in the cell membrane, which promotes the release of progeny virions from infected cells. It also antagonizes the action of tetherin (CD317/BST2), which opposes the release of fully formed virions at the surface of infected cells,[120,121] a role played by Nef in NHP lentiviruses,[122,123] as well as in HIV-1 group O virus strains.[124,125] Vpu also binds CD4 molecules and targets them to the proteasome, leading to their degradation. HIV-2 and SIV lack a *vpu* gene but carry another accessory gene, *vpx*, which appears to be a duplication of *vpr*[126–128] and counteracts the restriction factor SAMHD1 (sterile alpha motif and histidine-aspartate–domain-containing protein 1).[129]

Vif prevents HIV inactivation by APOBEC3G (A3G), which can edit the HIV-1 early reverse transcripts through cytidine deamination, leading to degradation of the provirus DNA.[130–132] Vif connects A3G to a polyubiquitination complex that leads to its proteasomal degradation.[133–135] A3G expression is one of the key determinants of innate immunity that counter HIV infection in humans and SIV infection in macaques.[136–143] The Vif protein also associates with cytoskeleton intermediate filaments and helps transport incoming virus to the cell nucleus.

VIRUS–CELL INTERACTIONS

Entry of HIV-1 into cells is a multistep process in which the Env glycoprotein interacts with attachment factors and engages the CD4 receptor then the CCR5 or CXCR-4 coreceptor. Thus, virus entry can be efficiently prevented by targeting the CD4 binding site on gp120 using either a composite molecule such as the eCD4-Ig[144] or a broadly neutralizing monoclonal antibody such as VRC01 or 3BNC117.[145] Adhesion of the virions to target cells results from nonspecific interactions between gp120 and charged groups at the surface of the cell, such as heparin sulfate proteoglycans. Major HIV attachment factors are syndecans[146] such as DC-SIGN, a mannose-binding lectin at the surface of dendritic cells (DCs) and macrophages.[147] Immature DCs capture the virus by their surface DC-SIGN, internalize it by endocytosis into multivesicular endosomal bodies, and transmit it to T cells by exocytosis into the extracellular medium in association with vesicular exosomes. Transmission from DCs to T cells thus does not require a second round of infection and provides HIV an avenue for escaping immune responses.[148]

HIV-1, HIV-2, and SIV use as their main receptor, the CD4 (OK-T4) molecule, which is present on the surface of T-helper lymphocytes (CD4⁺ T cells), monocyte-macrophages, lymph node follicular DCs, Langerhans cells in the skin, and microglia in the central nervous system.[149,150] The virus can also infect CD4⁻ cells such as glial cells, natural killer (NK) cells, brain endothelial cells, gut epithelial cells, and probably epithelial cells in the genital tract by using the glycolipid galactosyl ceramide as a receptor.[151]

The CD4-binding domain of the gp120 molecule is a complex conformational motif comprising a recessed pocket flanked by variable regions with considerable glycosylation.[152,153] Monomeric gp120 seems to be a highly flexible molecule in which neither the receptor (CD4)-binding nor the coreceptor (CCR5 or CXCR-4)-binding sites are properly formed.[154–156] Binding of gp120 to CD4 triggers conformational changes in the molecule, including the displacement of the V1V2 hypervariable loops, which results in the exposure of the coreceptor binding site (Fig. 29.3). The choice of the CCR5 or CXCR-4 coreceptor is largely dependent on the amino acid sequence of the V3 loop, which thus determines which type of cells the virus can infect.[157–159]

Binding of gp120 to the coreceptor in turn triggers conformational changes in the gp41 ectodomain, resulting in exposure of the highly hydrophobic, glycine-rich, N-terminal fusion peptide, which needs to be inserted into the target cell membrane to initiate virus–cell fusion (see Fig. 29.3).[160–162] The rod-like trimeric structure of gp41 comprises three parallel N-terminal α-helices ("heptad repeats") assembled as a coil in the center with three antiparallel C-terminal α-helices packed on the outside.[163–165] Binding of gp120 to the cell surface triggers a first change of conformation of gp41 which adopts an extended conformation referred to as the "prehairpin intermediate."[166,167] This allows the gp41 N-terminal fusion peptide to be projected into the target cell membrane. Refolding of the gp41 heptad repeats in to a hairpin conformation reconstitutes the six-helix bundle and places the fusion peptide and the transmembrane segment of gp41 at the same end of the molecule, bringing the viral and cell membranes together and initiating fusion. This process is very reminiscent of the virus–cell fusion mechanism mediated by the influenza virus HA molecule.[168,169] It has been suggested that several HIV-1 envelope trimers must cooperate and assemble into an aster to create a fusion pore needed for virus core entry.[165] The postfusion form of gp41 probably presents distinct antigenic motifs to the immune system.[170]

Most HIV-1 isolates obtained at the time of primary infection and during the asymptomatic period of the disease use the CCR5 coreceptor and for that reason are called **R5.** R5 HIV-1 strains are macrophage-tropic **(M-tropic)**, replicate in peripheral blood mononuclear cells (PBMCs) and in effector memory CD4⁺ CCR5⁺ T cells, but do not form syncytia in culture nor infect transformed CD4⁺ T-cell lines.[171] Emerging late in infection in association with progression to AIDS are T-lymphotropic **(T-tropic)** isolates, called **R5-X4,** which primarily use the CXCR-4 coreceptor, can replicate in immortalized T-cell lines, and can form syncytia in CD4⁺ T-cell cultures.[172] A subset of T-tropic HIV-1 strains has been adapted to grow in CD4⁺ T-cell lines and has become T-cell line-adapted **(TCLA),** losing their ability to grow in PBMCs and to use the CCR5 coreceptor. They are for that reason, called **X4.** Unlike HIV-1, both M-tropic and T-tropic SIV isolates use CCR-5 but not CXCR-4.[173]

A remarkable resistance to HIV-1 infection was observed in individuals with a 32-bp deletion in both alleles of the CCR5 gene, which encodes the CCR5 coreceptor.[174–179] The deletion is thought to have occurred approximately 4000 years ago in Northern Europe, where its frequency today can reach up to 10% to 15% in some populations, whereas it is totally unknown in the African and Asian populations.[180,181] The frequency of the Δ32 homozygosity, which is 1% to 3% in the general white population, reaches up to 25% in uninfected hemophiliacs.[182] A few individuals who are

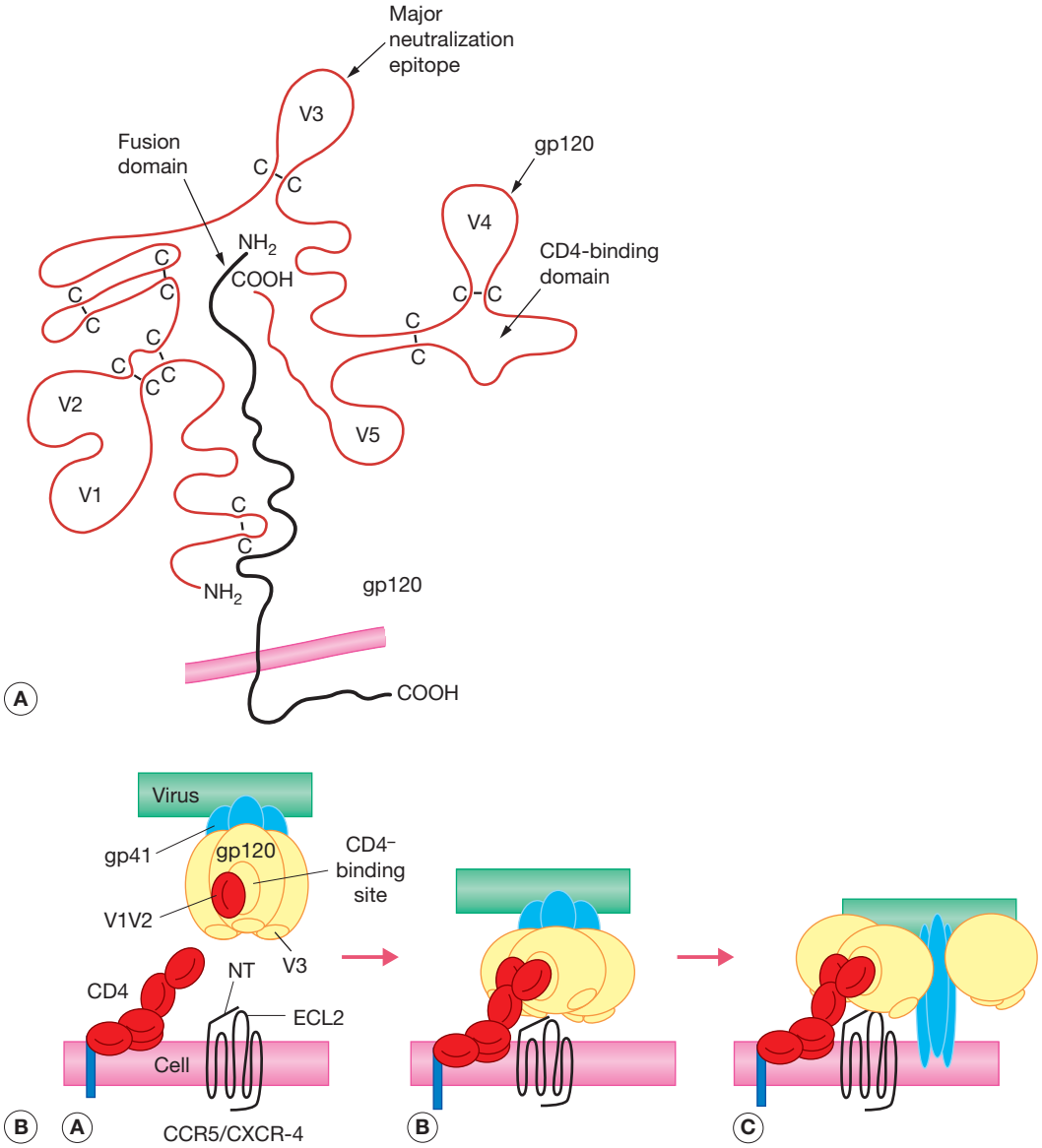


Figure 29.3. A–B, Interaction of the Env spike with target cell receptor and coreceptor.

homozygous for the Δ32 mutation have nevertheless been found to be infected with HIV-1, suggesting they probably were infected with an X4 virus strain.[183] Other genetic polymorphisms in the chemokine receptors that can affect host resistance or susceptibility to HIV-1 infection have also been identified.[184,185]

Penetration of HIV-1 through the mucosal barrier of the genital or the intestinal mucosa can involve uptake of the virus by intraepithelial Langerhans cells or DCs, as is observed in pluristratified squamous epithelia such as those of the vagina and exocervix, the anus, the oral cavity, and the esophagus. It can also happen by translocation of the virus across simple columnar epithelia such as those of the endocervix, rectum, and intestinal tract by the mechanism of transcytosis.[186]

CLINICAL DISEASE

AIDS can be viewed as a "tale of two infections"—(a) an acute infection in which the virus massively depletes mucosal lamina propria and intraepithelial $CD4^+$ T cells in the genital and GALTs, and (b) a chronic infection in which the wounded immune system progressively dies.[187,188] The major site of HIV replication is the GALT and mesenteric lymph nodes,[189] because these tissues are rich in highly activated $\alpha_4\beta_7^+$ $CCR5^+$ $CD4^+$ memory T cells that are the primary target of the virus.[171,190] Average content of HIV-1 proviral DNA per million $CD4^+$ T cells is on average five times higher in the gut than in the peripheral blood.[191] Both viral RNA and proviral DNA remain concentrated in the gut of patients receiving ART even though their viral loads have become undetectable.[192]

HIV-1 replication first occurs in a small number of resting $CD4^+$ T cells in the genital mucosa in the case of sexual transmission.[193] In 80% of sexual transmission cases, a single (founder) R5 virus clone establishes productive clinical infection, even though multiple sequence variants may be transmitted.[194–198] Within 1 day after atraumatic SIV vaginal exposure, plasmacytoid DCs line the endocervical submucosa and express numerous inflammatory markers, including

chemokines that attract $CD4^+$ $CCR\text{-}5^+$ T cells within the endocervix, thus initiating an inflammatory cascade leading to an influx of potential target T cells. At the same time the founder virus undergoes rapid mutations, eventually yielding a swarm of related molecular clones that form a "quasispecies"[199] and allow for optimal selection of variants which readily escape the host immune response.[37,39–41] Ultradeep sequencing has revealed that during the first weeks of infection, the virus explores an extraordinary array of potential escape routes in the process of evading the earliest $CD8^+$ T-cell responses, with dramatic shifts in the frequencies of epitope variants, probably because of a complex interplay between viral fitness and immune escape.[41,200] In contrast to heterosexual transmission, injection drug use seems to be associated with the transmission of multiple founder viruses.[201]

The initial viral eclipse phase lasts for only a few days and is followed by an explosive viral expansion that leads to unabated viral replication in activated $CD4^+$ T cells especially in the GALT,[89] constituting the primary, acute phase of infection. The patient experiences an acute febrile phase with disseminated lymphadenopathy, rash, and arthralgia, reminiscent of an episode of infectious mononucleosis. A transient decrease in total lymphocyte count and an inversion of the CD4-to-CD8 ratio are observed. This is accompanied by Gag p24 antigenemia and high plasma viral loads.

HIV-1 infection rapidly leads to the onset of a generalized chronic immune activation with increased T-cell turnover, increased polyclonal B-cell activation, increased frequencies of T cells with an activated phenotype ($CD38^+$, $HLA\text{-}DR^+$), and increased production of proinflammatory cytokines and chemokines.[26] Chronic immune activation is thought to result from increased permeability of the intestinal epithelium to bacterial products such as lipopolysaccharides,[202–204] the concentration of which in the blood correlates positively with viral load and negatively with $CD4^+$ T-cell count in both HIV-1– and HIV-2–infected humans.[205] Similar correlations are observed in SIV infection in macaque monkeys, but not in SIV-infected sooty mangabeys, which do not develop AIDS. The influx of microbial products and the increased amount of inflammatory cytokines in the blood of infected individuals induce the upregulation of PD-1 (programmed death-1) on lymphocytes and monocytes that results in interleukin (IL)-10 production and impairment of T-cell responses.[206–209] It also results in apoptosis of noninfected bystander $CD4^+$ T cells, which are readily found in HIV-1–infected humans or SIV-infected macaques[210–212] and show strongly upregulated expression of the PD-1 marker.[213] PD-1 expression was found to correlate with HIV viral load and inversely correlate with T-cell counts.[214] The degree of systemic immune activation is today considered a better predictor of disease progression than plasma viral loads.

Studies of pathogenic and nonpathogenic lentiviral infections in NHPs suggest a major role for T-helper (Th)17 cells in controlling the infection, as Th17 cells secrete IL-17, a cytokine that helps maintain the integrity of the mucosal barrier.[215–218] Early destruction of Th17 cells is explained by the fact that they express the CCR5 coreceptor and thus can serve as preferential targets for virus replication.[219,220] Pathogenic SIV infection in macaques, as well as HIV-1 infection in humans, is associated with loss of Th17 cells and an increase in the frequency of $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells (**Tregs**).[221–225] Foxp3+ Treg cells secrete transforming growth factor-β and IL-10, and dampen the CTL response to HIV infection.

The primary, acute phase of infection ends when the cellular immune system responds. A long asymptomatic period of clinical latency follows, corresponding to the chronic phase of infection (Fig. 29.4). During that phase a slow-but-steady

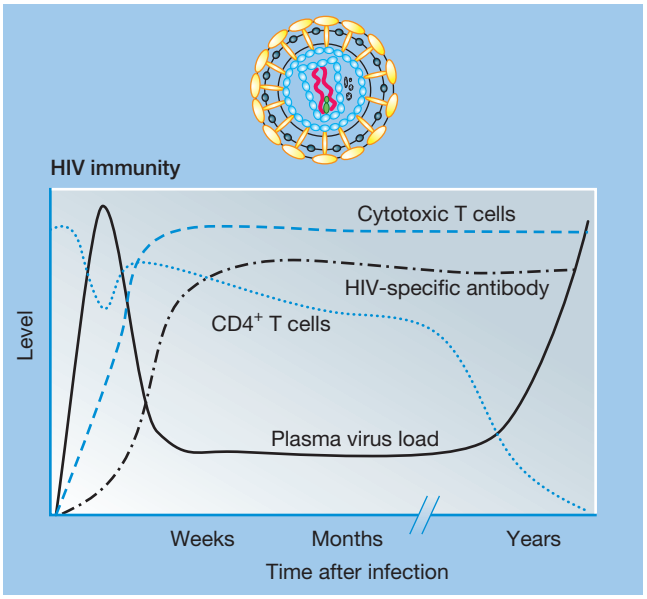


Figure 29.4. Diagrammatic representation of the evolution of virological and immunological markers during the course of a typical HIV-1 infection.

decline in $CD4^+$ T cells number is observed. A humoral immune response occurs and the patient becomes seropositive, except in very rare cases of rapid progression to AIDS[226]; the patient is less infectious than during the primary phase of the disease because viral replication continues at a more restrained pace. The major site of virus replication again is the GALT, essentially the colon and rectum.[227] The plasma viral load, which peaked during the acute phase of infection at values of 10^6 to greater than 10^7 viral RNA copies per milliliter, declines and remains at a relatively stable plateau (the **set-point**) during chronic infection. The set-point differs from patient to patient and serves as an indicator of disease progression. It remains high in **fast progressors,** who rapidly progress to AIDS, whereas it falls to relatively low values in **long-term nonprogressors,** who contain viremia spontaneously and progress to disease slowly. Thus, patients with more than 10^5 HIV RNA copies per milliliter of plasma at set-point are 10-fold more likely to progress to AIDS during the following 5 years than those with less than 10^5 copies/mL. In a few seropositive persons, called **elite controllers** or **elite suppressors,** viral loads spontaneously fall below the limit of detection by standard clinical assays (<50–75 viral RNA copies/mL).[228,229] However, even in these patients, virus replication continues at a low pace, as judged from the observation that the *rt* and *env* genes of the resident HIV-1 strain undergo sequence changes over time.[230,231]

HIV-1 rapidly evades neutralizing antibody responses during the course of infection in an individual.[38,93] The virus also readily escapes specific CTL responses.[232–239] Of note is the fact that some viral escape mutations in response to immune pressure come at a fitness cost to the virus,[240] which eventually reverts to wild-type sequences after transmission to a new host.[241–243] Selective pressures within microenvironments of different anatomic compartments result in the emergence in various tissues of distinct, independently evolving replication-competent quasispecies.[244]

The asymptomatic phase of the infection can last from a few months to more than 25 years, after which symptoms occur, following onset of opportunistic infections. At times, HIV infection can also manifest itself as a purely cachectic

disease, to which the name *slim disease* has been given in Africa. It can also cause a neurological syndrome such as acute encephalitis or progressive dementia (the "AIDS dementia complex"). The symptomatic phase of the disease is accompanied by a return of *gag* p24 antigenemia, an accelerated decrease in the number of $CD4^+$ T cells, and a greatly increased virus load. The number of peripheral T cells producing HIV-1 can reach 1 in 10, with production of up to 10^{10} virions per day.[245–248]

Direct killing of $CD4^+$ T cells by HIV-1 cannot however explain the full spectrum of AIDS pathogenesis. Chimpanzee-adapted strains of HIV-1 are cytopathic for chimpanzee $CD4^+$ T cells, grow in chimpanzee macrophages-monocytes, and replicate efficiently in chimpanzees, but usually do not cause AIDS in the animal.[249,250] Similarly, SIVmac infection in African NHP such as mandrills or sooty mangabey monkeys remains chronic and almost never progresses to AIDS.[251–255] Natural NHP hosts that are infected by SIVs in the wild, show no evidence for destruction of their $CD4^+$ T cells, although they maintain high plasma viral loads[256–258] and do not exhibit superior cellular immune control of viremia.[259] SIV-infected sooty mangabeys do lose $CD4^+$ T cells but develop double-negative ($CD3^+$, $CD4^-$, $CD8^-$) T cells that are able to maintain efficient immune responses and prevent the appearance of AIDS.[260]

Interestingly, these SIV-infected African monkeys show no evidence for persisting chronic immune activation[261] and no evidence for apoptotic $CD4^+$ T lymphocytes, in contrast to HIV-1–infected humans or SIVmac-infected macaques.[218] These observations strengthen the likely role of chronic immune activation being caused by microbial translocation in the eventual collapse of the T-cell compartment in the course of infection.[202,262–264] Hyperactivation overwhelms the immune system and gradually causes a collapse of immune-protective mechanisms, leading to AIDS. This is observed even in "elite controllers," who show abnormal T-cell activation levels leading to progressive $CD4^+$ T-cell loss in the absence of detectable viremia.[265] In addition, other mechanisms have been suggested to account for the depletion of $CD4^+$ T cells, such as cytolytic attack by HIV-1–specific CTLs of bystander $CD4^+$ T cells that have bound virus-shed circulating gp120 molecules; apoptosis of bystander $CD4^+$ T cells mediated by circulating Tat or Nef proteins, molecular mimicry between the ectodomain of gp41 and IL-2, leading to the induction of anti–IL-2 antibodies that could block the interaction of the cytokine with its receptor[266]; and apoptosis of bystander T cells triggered by incomplete reverse transcripts during abortive HIV infection.[267,268] Abortive HIV replication leads to $CD4^+$ T-cell death by pyroptosis and drives the $CD4^+$ T-cell depletion in HIV-infected persons.[268] The death of uninfected bystander $CD4^+$ T cells has also been linked to the expression of the ligand to the NK cell receptor NKp44, which makes $CD4^+$ T cells sensitive to NK lysis.[269,270] The mechanism involves a gp41 motif located between residues 618 and 623, the 3S motif, which is able to elicit the expression of the cellular ligand of NKp44 (NKp44L) through binding to the receptor of the C1q complement factor gC1qR. Indeed, anti-3S antibodies inhibit NKp44L expression on $CD4^+$ T cells and elicit protection against NK-cell–mediated $CD4^+$ T-cell loss in NHP models.[271,272]

AIDS patients are anergic to recall antigens, as seen by lack of delayed-type hypersensitivity (skin test) in vivo and by lack of T-helper cell proliferation in vitro. The unresponsiveness to recall antigens has been interpreted as the consequence of HIV infection of follicular DCs, which severely impairs the ability of these cells to present antigens in vitro and which triggers the secretion of IL-10.[273,274] Anergy was indeed found to correlate with destruction of the lymph node follicles.[275,276] Patients with AIDS also show increased levels of tumor necrosis factor (TNF)-α, IL-1, IL-2, and IL-6, interferon (IFN)-α and IFN-β, β_2-microglobulin, and neopterin, an interleukin produced by macrophages in response to TNF-α.[277]

Not all exposures to HIV, however, lead to infection and not all HIV infections lead to AIDS. Only one-third of infants born to infected untreated mothers acquire infection, heterosexual transmission occurs approximately between 1 out of 100 and 1 out of 1000 exposures,[278–281] hemophiliacs exposed to infected blood products do not consistently get infected,[282] and some commercial sex workers and seronegative partners in serodiscordant couples appear to remain uninfected despite repeated extensive exposure to HIV-1.[283,284] These **highly exposed persistently seronegative (HEPS)** persons, also simply called **exposed uninfected (EU)** persons, have been the object of numerous studies to try to decipher possible correlates of protection that could be used for the development of protective HIV vaccines (see below).

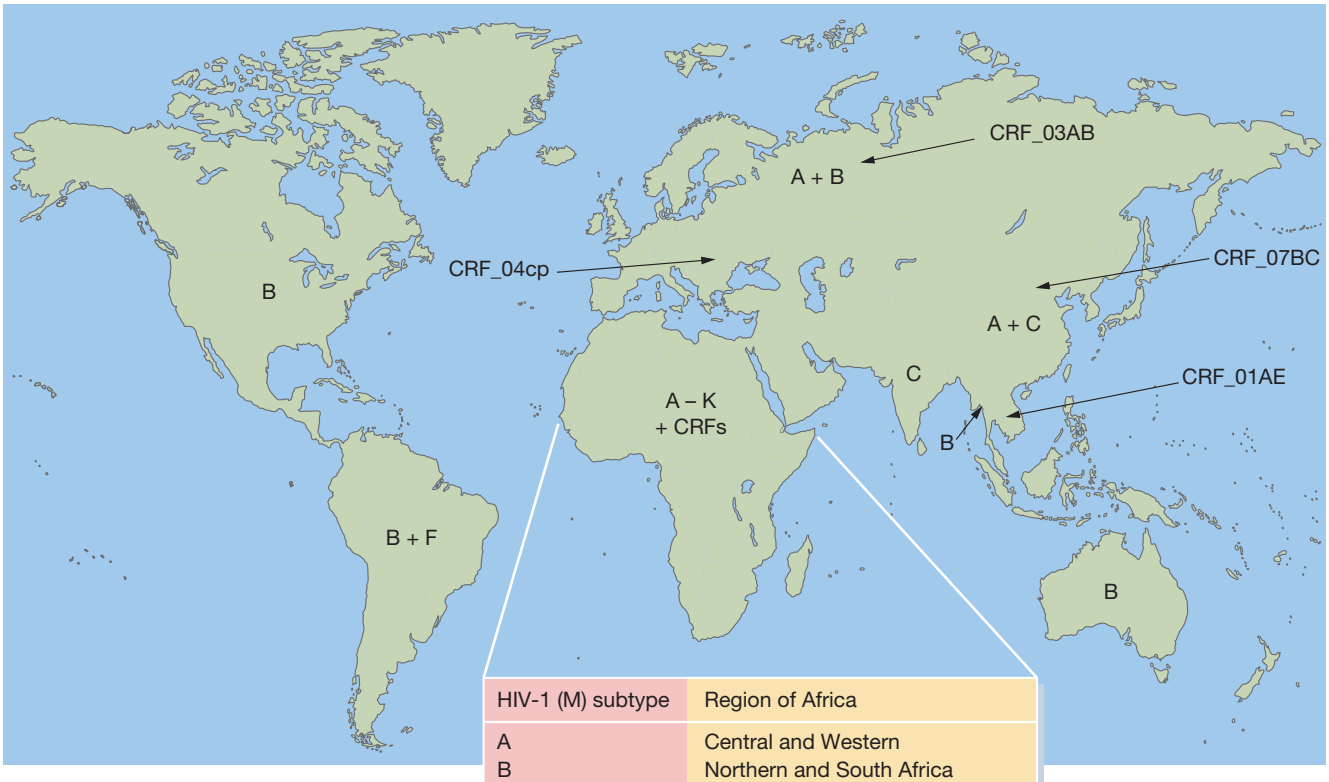
VIRUS VARIABILITY

HIV-1 exhibits a remarkable degree of genetic variability in the host that results from the error-prone nature of RT (approximately 3×10^{-5} mistakes per replication cycle), together with high rates of virus production (up to 10^{10} particles per day per infected person during the acute early phase of infection) and high frequency of recombination (seven to 30 crossover's per genome, per round).[245,285] In general, genetic diversity among virus clones within an individual is approximately 2% to 3%.

HIV-1 strains in group M are found worldwide and show major diversity. They have been divided into 10 **subtypes**, or **clades**, designated by letters A to K,[285,286] which are approximately equidistantly related and exhibit 25% to 35% amino acid sequence difference in their Env protein. HIV-1 subtype B predominates in industrialized countries, as well as in Latin America and the Caribbean, but causes less than 12% of all global infections.[287,288] HIV-1 subtype C, in contrast, accounts for nearly half (48%) of all global infections and predominates in Southern Africa, Eastern Africa, and India. Subtypes A and D are more common in Central Africa, with subtype A appearing to have followed heroin trading routes into Eastern Europe.[289] East African HIV-1 infections are predominantly caused by subtype C and to a lesser extent by subtypes A and D.

In addition, numerous HIV-1 interclade recombinant viral strains have been detected, an indication that superinfection by a second HIV-1 strain is not uncommon.[290–295] Recombinant virus strains which contain genetic material from more than one subtype and which are circulating widely in the population have been designated **circulating recombinant forms (CRFs)**.[285,296] The greatest diversity of HIV-1 subtypes and recombinants is found in Central Africa where all clades and many CRFs are present (Fig. 29.5). In West Africa, all clades are also detected with the dominant variants being CRF02_AG and subtype G. All major subtypes and many CRFs and unique recombinant forms are similarly found in Western and Central Europe. CRF03_AB is prevalent in Russia and Central Asia, whereas in East Asia, the epidemic is dominated by CRF07_BC, CRF08_BC, and CRF01_AE, and in South-East Asia by CRF01_AE-often wrongly called *subtype E*.[287]

The biological significance of such a high degree of variability remains unknown. There are no clear genotype–serotype relationships between subtype or CRF sequences and neutralizing antibody patterns. Presumably, a recombinant virus strain that becomes predominant in an individual has some type of selective advantage over the parental strains, either because of enhanced fitness or immune evasion. When



HIV-1 (M) subtype	Region of Africa
A	Central and Western
B	Northern and South Africa
C	Central, Eastern and South Africa
D	Central and Eastern
CRF_01AE	Central
F	Central
CRF_02AG	Central and Western
H	Central
J	Central

Figure 29.5. Geographic distribution of major HIV-1 M group subtypes.

such a recombinant emerges as the predominant strain in an epidemic in a population, this also must be the result of significant selective advantages. The fear that an HIV strain would readily escape from too narrow an immune response induced by a vaccine is in favor of the development of vaccines that target multiple HIV antigens and if possible multiple viral subtypes, so as to provide as broad as possible a protection. This has also led to the development of fully synthetic genes derived from theoretically defined **consensus** or ancestral HIV envelope sequences,[297–299] which were found to be superior to many wild-type immunogens for inducing cross-subtype T-cell and B-cell immune responses.[300,301] Similarly, **mosaic antigens** have been developed with sequences derived from in silico algorithms that closely match the sequences of natural HIV-1 strains worldwide and maximize the overlap between them.[302–305]

There is convincing evidence that HIV originated in NHPs.[306,307] Several independent crossing of the species barrier from chimpanzees living in Western Central Africa led to HIV-1 groups M, N, and O in humans, whereas multiple independent transmissions of SIV from West African sooty mangabeys led to HIV-2.[4,308–311] Thus, HIV-1 groups M, N, and O are phylogenetically close to SIVcpz, a commensal virus in chimpanzees *Pan troglodytes*, whereas HIV-2 is closely related to SIVsmm, a commensal virus in sooty mangabey monkeys *(Cercocebus atys)*; the SIV, which causes AIDS in Asian rhesus macaques *(Macaca mulatta)*, is similarly closely related to SIVsmm from sooty mangabeys. Recently identified HIV-1 group P is related to SIVgor, a commensal virus in gorillas.[62,312]

All these viruses belong in the same group of lentiviruses that infect a wide range of NHP species in Africa,[313–315] where multiple cross-species transmissions in the wild have been documented.[316–318] Molecular clock estimates indicate that HIV-1 group M probably established itself in the human population at the beginning of the 20th century (1884–1924),[319] group O in the 1920s (1890–1940),[320] and group N in the 1960s (1948–1977).[4] In fact, SIVs have been present in African primates for more than 30,000 years, suggesting that humans most probably had many chances of sporadic encounter with these viruses for millennia.[321] Humans are still naturally exposed today to infection by diverse SIVs, as illustrated by the high frequency and diversity of lentiviruses that can be encountered in primate bush meat in Southeastern Cameroon.[322,323]

EPIDEMIOLOGY AND DISEASE BURDEN

HIV transmission occurs essentially through homosexual or heterosexual intercourse, through injection of blood or blood-derived products, and from mother-to-child transmission during pregnancy, at delivery, or through breastfeeding.[5] Recently infected subjects are potentially more infectious than infected individuals in the chronic phase of the disease: it has

been suggested that up to 50% of new HIV-1 infections are acquired from newly infected individuals.[279,324–326] The importance of the acute infection period for subsequent transmission events is related to both the level of plasma viral load during the early phase of disease and to virus-specific properties.[278] Thus, virions isolated from SIV-infected macaques at the time of primary infection are more infectious than those from the chronic phase of infection,[327] possibly because a greater number of defective virus particles are circulating during the chronic than during the acute stage of disease[198] and/or because lack of protective antibodies at primary infection stage substantially increases virus infectivity.[328] The difference in sexual behaviors makes MSM add to their risk of infection because receptive anal intercourse has a relative risk of 1.43%, which is approximately 10 times higher than that of receptive vaginal intercourse.[329]

Since 1981, more than 36 million people have died of AIDS. HIV-1 is the fourth largest infectious killer in the world with an annual toll of approximately 1.2 million deaths,[330,330a] the great majority of which occurs in sub-Saharan Africa. As of 2014, UNAIDS estimated that 36.9 million people were living with HIV-1 infection worldwide, including 2.5 million children younger than 15 years of age. Of these 36.9 million people, 25.8 million lived in sub-Saharan Africa and approximately 5 million in Asia. Approximately 2 million people become infected each year, 95% of whom live in developing countries.[330,330a] The number of new infections is equally distributed among men and women, but infection rates in young African women are close to three times higher than those among young men, reflecting the degree to which gender inequities are driving the epidemic. This is also true in Asia, as young girls are frequently steered toward sex work by their families.

The HIV-1 epidemic has expanded at an alarming rate in the 1990s in sub-Saharan Africa, which hosts 80% of females living with HIV in the world. Highest infection rates are found among commercial sex workers, truck drivers, and seasonal migrant workers. Countless cases of mother-to-child transmission have been documented. In some African countries, overall prevalence in the adult population can be greater than 10%, with figures reaching up to 28% in some areas. Most severely hit are South Africa, with more than 5.5 million infected people, Botswana, Mozambique, Zimbabwe, Tanzania, and Ethiopia. In addition, sub-Saharan Africa faces numerous wars and civil conflicts, producing large numbers of refugees who are at heightened risk of contracting HIV. Through increased awareness campaigns, education and wider access to ART, many African countries are now seeing their incidence rates stabilize or even decrease. The incidence rate in African children has also started to decline, a result of the systematic use of antiviral therapy in pregnant women, as exemplified by programs implemented in South Africa, Swaziland, Botswana, and Namibia.

The estimated number of people living today with HIV-1 in Asia and the Pacific region is 5 million, but the accuracy of the figure is questionable, in view of the fast pace at which the epidemic has been expanding. Increasing sex trade, use of illicit drugs, and rates of sexually transmitted infections contributed to an increased vulnerability in the region. Improper blood donation practices in China and unsafe injection practices in healthcare settings in India and surrounding countries have resulted in hundreds of thousands of infections. Substantial transmission also occurs in MSM, with prevalence rates of 14% to 20% reported in male homosexual communities in India, Cambodia, and Thailand. Gender inequities play a major role in the epidemic. The female commercial sex industry facilitates transmission in Thailand, Cambodia, India, Nepal, China, Vietnam, and several other countries.

The estimated number of adults and children living with HIV-1 in Latin America and the Caribbean at the end of 2014 was 1.7 million. While in some countries HIV infections remained concentrated mainly in MSM and people who inject drugs, others were experiencing increasing rates of heterosexual transmission.

Since the mid-1990s, the Eastern European countries continue to experience a sharp increase in the number of new HIV-1 infections, most of which occur among people who inject drugs, commercial sex workers, and MSM. The number of HIV-infected persons in the region nearly tripled between 2000 and 2009, rising from 500,000 to 1.4 million, of which an estimated 940,000 were living in the Russian Federation and 440,000 in Ukraine.[331]

An estimated 2.3 million people are living with HIV-1 in industrialized countries (1.5 million in North America alone). In France, The Netherlands, and Spain, from one-third to three-fourths of new HIV-1 infections are concentrated among migrants. For the general population, highly active antiretroviral treatment (HAART) has considerably reduced disease progression to AIDS and transformed HIV infection from a deadly disease to a somewhat manageable chronic disease. However, success in treatment and care is not being matched by progress in prevention and new evidence of rising HIV-1 infection rates is emerging, particularly in marginalized communities. For example, in 2010, 1 in 16 black men in Washington, D.C. were HIV-infected, as were 1 in 10 MSM and 1 in 8 were people who inject drugs in New York City.[332] In several U.S. urban areas, the prevalence among MSM was as high as 30%. It is estimated that half a million Americans became infected with HIV in the 2000–2010 decade, including more than 55,000 in 2009 alone.[333] Washington, DC has been beset by an HIV-1 epidemic that rivals some in developing countries,[334] with rates in particular subsets of the heterosexual population as high as 5.2% (6.3% in women vs 3.9% in men).[335,336] HIV-1 infection rates among young people in Western nations are three times higher than in the early 2000s. HIV thus remains a major health threat in these countries.

IMMUNE CORRELATES OF PROTECTION

The immune correlates of protection against HIV infection in humans are still unknown,[337,338] mostly because of the lack of natural protective immunity against HIV. Most available data have been drawn from the study of chronically infected individuals, which does not imply that they would be associated with protection from infection if elicited by a vaccine.[339] Other data arose from the study of vaccine protection of macaques against experimental SIV infection. Possible immune correlates of protection can be of four types: neutralizing antibodies, cellular immune responses, nonneutralizing antibodies, and innate immune responses.

Neutralizing Antibodies

Neutralizing antibody responses to HIV-1 are unusually late, arising several weeks after infection.[340,341] In addition, most antibodies elicited during the course of HIV infection are nonneutralizing or neutralize only a narrow range of circulating viral strains,[93] and many target the highly variable loops in gp120 that act as antigenic decoys, such as the V1 loop,[342] the V2 loop,[343] or the V3 loop.[344–348] Broad neutralization epitopes actually exist on both gp120 and gp41,[348] and at the junction of gp120 and gp41, but they remain poorly accessible to the immune system because of conformation-dependent exposure on the molecule, hindrance by the "glycan shield," and masking by the hypervariable loops in gp120.[349] Although a subset of individuals infected with HIV-1 develop bNAbs a few

years after infection, only a small percentage of those subjects develop Abs that are both potent and broad.[350]

HIV-1 Env: The Target for Broadly Neutralizing Antibodies

HIV-1 has developed several strategies that together enable evasion of neutralization. First, there are relatively few native spikes of HIV-1 Env on the virion, whose surface is primarily composed of denatured spikes and host cell antigens, providing additional decoys for immune responses.[73,74] Second, variable regions of gp120 are highly immunogenic and provide additional decoys focusing immune responses away from conserved epitopes.[344,345,347,351,352] Third, conserved regions of HIV *env* are poorly immunogenic because of masking by a glycan shield over gp120,[93,353] and for other reasons, including steric hindrance,[156,354] and in some cases transient appearance.[170] Gp120 is heavily glycosylated at 25 to 35 glycosylation sites, and antigenically the majority of the glycans are similar to host cell carbohydrates. Last, it has been suggested that some epitopes have homologies with host cell proteins, and immune responses to these epitopes may be polyreactive and thus immunogenicity compromised by tolerance mechanisms.[355,356]

Until recently, the field was focused on four broadly neutralizing monoclonal antibodies identified several years ago. The first HIV-1 broadly neutralizing monoclonal antibody identified was b12, which binds to gp120 at the CD4 binding site.[357–360] The structure of b12 in complex with gp120 demonstrated that b12 binds to a conformationally invariant surface that overlaps a distinct subsurface of the CD4 binding site.[361] Two broadly neutralizing monoclonal antibodies, 2F5 and 4E10, have also been identified that target the MPER of gp41.[362–366] 2F5 binds the ELDKWA sequence in the heptad repeat 2 region of gp41, and 4E10 to the NWFDIT sequence at the point where gp41 inserts into the lipid bilayer.[367,368] These antibodies also attach to the viral membrane via their hydrophobic CDR H3 loops, which is a prerequisite for their neutralizing activity.[369,370] Furthermore, 2G12, a broadly neutralizing monoclonal antibody, targets the high-mannose cluster on the glycan shield of HIV-1,[371–373] and has a unique V(H) domain-exchange structure, with a multivalent binding surface that includes two primary glycan binding sites.[374]

Since 2009, application of novel technologies in recombinant human monoclonal antibody isolation have led to the identification of a large number of new, broader, and more potent HIV-1 specific neutralizing monoclonal antibodies than previously described. These new tools have revolutionized the HIV-1 vaccine field by providing new targets on HIV-1 Env which may now be exploited for vaccine design. The first of these advances came with the identification of PG9 and PG16, broad and potent neutralizing monoclonal antibodies targeting an epitope preferentially expressed on trimeric HIV-Env protein which spans conserved regions of variable loops of gp120.[375] These antibodies were identified by using a high-throughput neutralization screen of antibody, containing supernatants from approximately 30,000 memory B-cells from an HIV-1–infected donor.

Shortly following the identification of PG9 and PG16, antigenically resurfaced glycoproteins, that is, molecularly engineered glycoproteins designed to focus the immune response, were used as probes to identify sera from donors with neutralizing antibodies to the CD4-binding site. Harnessing immunoglobulin genes from B-cells from such donors led to the identification of VRC01 and related monoclonal antibodies which were also very broad and potent[376] and targeted the CD4-binding site. In addition, another monoclonal antibody termed HJ16 was identified that targets a novel epitope proximal to the CD4-binding site and is comparable in breadth to b12.[377]

Finally, a limited number of antibody specificities were found to mediate broad and potent serum neutralization from elite neutralizers, individuals identified by the breadth and potency of their serum for neutralizing standard panels of HIV-1 isolates.[378] Using this information, several additional broad and potent monoclonal antibodies have been identified from other elite neutralizers, with studies currently underway to identify their binding sites on HIV-1.[379] Shown in Fig. 29.6 are a structural model of the HIV-1 Env trimer and binding sites for these monoclonal antibodies, which now represent the principal targets for vaccine design.

These sites include the CD4-binding site on gp120 (the **VRC01** family), the glycan shield and the V1V2 region of gp120 (the **PG9** and **PG16** families), the V3 region of gp120 and the glycans centered on N332 (the **PGT121** family), the base of the spike including elements from both gp120 and

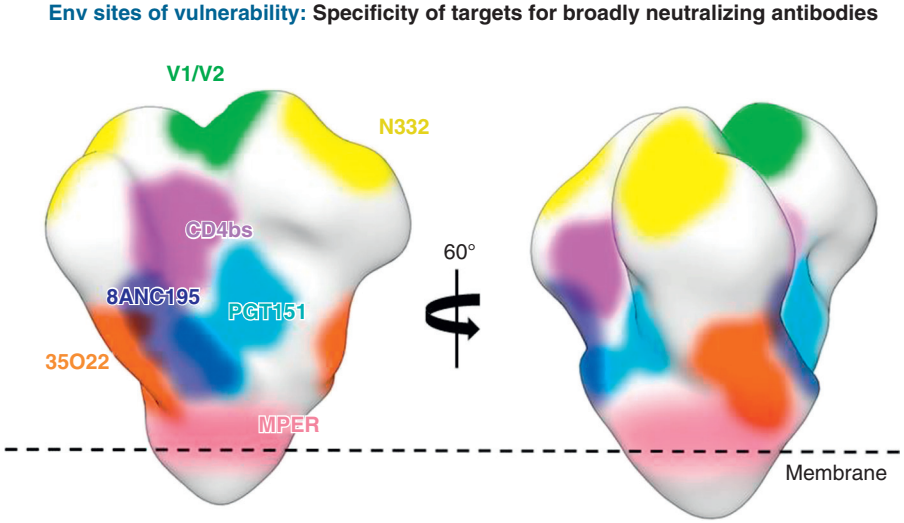


Figure 29.6. Env sites of vulnerability: specificity of targets for broadly neutralizing antibodies. *(From Ward AB, Wilson IA. Insights into the trimeric HIV-1 envelope glycoprotein structure.* Trends Biochem Sci. *2015;40[2]:101–107.)*

gp41 (the **35O22** bNAb), and the MPER of gp41 (the **2F5, 4E10, and 10E8** family).[380] Still another type of bNAb, **PGDM 1400**, was recently isolated from the plasma of a chronically infected donor using the HIV-Env trimer BG505 SOSIP-664 as an affinity reagent.[381] PGDM 1400 is exceptionally broad and potent, with a cross-clade coverage of 83% and a median IC50 of 0.003 μg/mL; it targets a three-dimensional epitope at the apex of trimeric gp120.

Recently, antibodies to a modified 3S gp41 motif with a W614A mutation were found to be able to neutralize a broad array of cross clade HIV-1 strains,[382] implying that the 3S region in gp41 could both be used as a bNAb epitope and elicit protection against $CD4^+$ T-cell depletion.[270,271]

Additionally, there has been a dramatic expansion in the number, breadth, and potency of bNAbs identified against HIV, as biomolecular tools such as single cell B-cell cloning and next generation sequencing become more refined. The site on the HIV-1 gp120 glycoprotein that binds the CD4 receptor is recognized by several bNAbs, many of which neutralize more than 90% of HIV-1 strains. The repertoire for effective recognition of this CD4 supersite comprises antibodies with paratopes arrayed about two optimal geometric orientations, one achieved by CDR H3 ontogenies and the other achieved by VH-gene-restricted ontogenies.[383] A new, broad, and potent neutralizing antibody, which targets the gp41 MPER 10E8,[384] is relatively unique as it does not bind phospholipids, is not autoreactive, and binds the cell-surface envelope. The structure of 10E8 in complex with the complete MPER has revealed a site of vulnerability comprising a narrow stretch of highly conserved gp41-hydrophobic residues and a critical arginine or lysine just before the transmembrane region. These findings have reinvigorated vaccine design efforts targeting the highly conserved MPER region of HIV-Env.

bNAbs targeting the trimer apex of HIV envelope are also a favored candidate for vaccine design because of their great neutralization breadth and potency, as characterized by a subset of the PGT series of antibodies.[385–387] However, methods of isolating bNAbs against this site have been limited by the quaternary nature of the epitope region.

The N-332 site, characterized by antibodies binding to the high-mannose patch, is also another major target for vaccine design[378] and from delineation of antibody recognition in polyclonal sera from patterns of HIV-1 isolate neutralization.[388] Antibody recognition of the N332 region is complex due in part to variability in neighboring loops and glycans critical to the epitopes. Recently, two classes of bNAbs within a single lineage directed to the high-mannose patch of HIV envelope were identified.[389,390] One branch, characterized by bNAb PGT128, has a six-amino-acid insertion in CDRH2 that is crucial for broad neutralization. Antibodies in this branch favor a glycan site at N332 on gp120, whereas, a second branch characterized by bNAb PGT130 lack the insertion and appear to favor the N-334 glycan site. PGT 128 complexed with a fully glycosylated gp120 outer domain at 3.25 angstroms revealed that the antibody penetrates the glycan shield and recognizes two conserved glycans as well as a short β-strand segment of the gp120 V3 loop, accounting for its high binding affinity and broad specificity.[391] Interestingly, in screening plasma of R5 SHIV infected macaques, one monkey was identified with bNAbs and mutagenesis studies mapped the epitope to the N-332 glycan site.[392,393]

Most recently, three groups have identified trimer-specific antibodies that represent novel sites of vulnerability and offer potential to serve as reagents for purification of HIV-Env trimers.[394–396] Monoclonal antibody PGT151 recognizes a glycan-dependent epitope on the prefusion conformation of gp41 and distinguishes cleaved from uncleaved Env trimers.[395] Monoclonal antibody 35022 also binds to a conserved epitope that stretched across gp120 and gp41,[394] and a monoclonal antibody termed 8ANC195 binds to a site that includes portions of gp41 and N-linked glycans adjacent to the CD4-binding site on gp120.[396]

The identification of these new broad and very potent neutralizing monoclonal antibodies has led to further characterization of the antibodies and the observation that most have high levels of somatic mutations and long heavy-chain complementarity determining region 3 (HCDR3), suggestive of extensive affinity maturation.[397–401] For example, the mean number of mutations in an immune response to influenza is approximately 5%, whereas variable heavy-chain mutations for PG9/PG16 and VRC01 range from 11.9% to 31.2%, respectively.[52] The HCDR3 lengths for the broadly neutralizing monoclonal antibodies range from 14 to 30 amino acids, and it has been suggested that B cells' long HCDR3s may be eliminated at the naïve B-cell stage, a hypothesis proposed to account for the difficulties in eliciting such antibody responses.[402,403]

Indeed, bNAbs usually appear on average after 2.0 to 2.5 years of infection, probably because of a need for antibody affinity maturation[404,405] and in persons with higher viral plasma load, suggesting a need for chronic antigen exposure.[406–410] They can nevertheless also be found in infected infants.[411] The development of bNAbs also seems to be driven by immune escape and superinfection mechanisms.[412–414] Up to 50% of subjects ultimately develop neutralizing antibodies with a degree of breadth, but only approximately 1% of the donors, designated as **elite neutralizers**, show unusually potent neutralizing serum activity against a majority of clades.[350]

Experiments in the macaque model have shown that SIV or simian-human immunodeficiency virus (SHIV) infections can be prevented by bNAbs, whether administered by passive immunization, or through vector delivery using an adeno-associated virus (AAV) vector able to continuously express in vivo immunoadhesins derived from bNAbs.[415] Similar protection results by bNAbs expressed from an AAV vector against HIV infection were obtained in a humanized mouse model.[416,417] Similarly, neonatal macaques could be protected from oral SHIV challenge by passive immunization with bNAbs b12, 2G12, and 2F5.[418,419] The therapeutic efficacy of bNAbs was also demonstrated in SHIV-infected adult macaques.[420,421] bNAbs are therefore not associated with control of viremia in the host,[407,422–424] but they can be protective against live virus challenge. Fc-γ-receptor (FcγR)-mediated effector function contributes substantially to the capacity of bNAbs to block virus entry, suppress viremia and confer therapeutic activity.[425]

The major challenge for HIV vaccine development at this time is to translate these findings into the design of immunogens capable of eliciting bNAbs, which could be used as a vaccine (see below)[354,401,426–432]

Cell-Mediated Immunity

Multiple studies have shown that $CD8^+$ T-cell responses control SIV or SHIV replication and the level of the set-point viral load in infected rhesus macaques. The best evidence came from the demonstration that experimental depletion of $CD8^+$ T cells resulted in a rapid and dramatic increase in viremia and accelerated the lethal outcome of the disease in the animals.[433–435] Accidental reemergence of virus and progression to AIDS in long-term protected, DNA-MVA (modified vaccinia virus Ankara) vaccinated macaques was observed at 4 years after challenge with $SHIV_{89.6P}$, coincident with a loss of T-cell function together with appearance of escape mutations in CD8 epitopes.[436] Transient depletion of $CD8^+$ T cells

in rhesus macaques that controlled the replication of SIVmac for 1 to 5 years resulted in 100-fold to 10,000-fold increases in viremia; control was reestablished when the $CD8^+$ T cells returned, at which time some subsets of subdominant $CD8^+$ T cells expanded more than 2500-fold above predepression level.[437] Infection of female macaques with nonpathogenic $SHIV_{89.6}$ used as an attenuated, live vaccine generated protection against uncontrolled SIV replication following vaginal challenge,[438] but depletion of $CD8^+$ T cells at the time of the challenge again completely abrogated protection, demonstrating that it was the $CD8^+$ T cells in the genital tract that controlled viral replication and delayed progression to AIDS.[439]

Efficient control of SIV infection after repeated low-dose rectal challenge with SIVmac239 was also observed in approximately 50% of rhesus macaques vaccinated with a replicating cytomegalovirus (CMV) vector expressing Gag, Rev, Tat, Nef, and Env that efficiently induced effector memory $CD4^+$ and $CD8^+$ tissue-resident T cells. Protection was observed whether the animals had been challenged by the intravenous, intravaginal, or intrarectal route. Complete elimination of the challenge virus after 1-year follow-up was documented in 13 out of 24 monkeys, none of which showed evidence for neutralizing antibodies, but all of which developed $CD4^+$ and $CD8^+$ T cells in lymph nodes and mucosal tissues.[436,440,441] The persistence of the vaccine vector seems to be the key to the long-term maintenance of virus-specific effector memory T cells (T_{EM}) able to eliminate all infected cells. However, the difference between the 50% of vaccinated monkeys controlling SIV and the 50% of monkeys not capable of controlling SIV infection remains unexplained.

Efforts at elucidating the potentially protective immune response of individuals who remain resistant to HIV-1 infection,[442,443] have also helped shed light on the role of cell-mediated immunity in protection.[444] **Elite controllers** or **elite suppressors** appear to completely control viral replication for up to 30 years, maintain viral loads below the level of detection by standard assays, and most often do not show signs of disease progression.[445] Elite suppressors rarely develop bNAbs,[409,446,447] but they all show potent cellular immune responses.[448] Upon stimulation, their HIV-specific $CD4^+$ T cells proliferate and secrete multiple cytokines, including IL-2, whereas cells from chronic progressors do not proliferate and secrete only IFN-γ. The presence of strong virus-specific $CD4^+$ T-cell responses also distinguishes nonpathogenic HIV-2 infections from HIV-1 infections.[449,450]

Even more strikingly than $CD4^+$ T cells, HIV-specific $CD8^+$ T cells appear to play a key role in the control of viral replication in elite suppressors. This is demonstrated in part by the overrepresentation of certain HLA alleles such as HLA-B*57 and HLA-B*27 in elite suppressor cohorts,[451–453] and that macaque monkeys with the MHC class I Mamu-B*08 allele achieve the elite suppressor status. The central role of HLA class I in controlling HIV-1 infection,[454–456] was highlighted by the International HIV Controllers Study, which followed a large cohort of HIV patients and showed that HLA types B*57:01, B*27:05, and B*14 were protective, whereas types B*35 and C*07 were associated with progression to AIDS.[457] Allele combinations like HLA-B*57:01-Cw0602, HLA-B*27:05-Cw0102, or HLA-B*38:01-Cw1203 have the strongest impact on nonprogression.[458]

While HIV-specific $CD8^+$ T cells from patients with progressive disease typically secrete only IFN-γ, those from elite suppressors are multifunctional, secrete multiple cytokines,[459–461] express CCL3 (macrophage inflammatory protein [MIP]-1α) and/or CD107a, undergo degranulation,[462] and proliferate in response to stimulation.[463,464] They are also much more efficient at granzyme B delivery and killing of infected $CD4^+$ T cells.[465,466] Moreover, they express perforin immediately upon antigen-specific stimulation, without the need for prior proliferation or the addition of exogenous cytokines.[467] HIV Gag-specific T-cell responses, especially those directed at conserved regions in $p24_{gag}$[430] and $p7_{gag}$, have been found to dominate in rectal mucosa of HIV controllers, in magnitude as well as breadth,[468] and often are more abundant in gastrointestinal mucosa than in blood.[469] Long-term nonprogressors have more polyfunctional responses, higher-magnitude and broader $p24_{gag}$-specific proliferation, and higher levels of IL-2 and TNF-α production than do progressors.[470]

HIV-specific helper T cells and $CD8^+$ CTL responses to multiple HIV epitopes have also been demonstrated in EU persons.[471,472] Some of the Nairobi EU sex workers seroconverted after they took a break from sex work, which was associated with a loss of HIV-specific CD8 responses, and suggested a waning of HIV-specific immunity following reduced antigenic exposure,[473] thus the need for a continuous antigenic stimulation to maintain the effectiveness of the CD8 T-cell response.

The conclusion that T cells, especially $CD8^+$ T cells, can limit virus replication and control viral set-point viremia also stems from vaccine experiments in the macaque/SIV model. Indian rhesus macaques vaccinated intracolorectally with a peptide prime/MVA boost adjuvanted with Toll-like receptor (TLR) agonists and IL-15 controlled virus replication after high-dose intrarectal SIV challenge. In addition to a strong stimulation of A3G (see below), protection correlated with antigen-specific polyfunctional $CD8^+$ T cells.[142] Correlation was observed only above a threshold of approximately 2% $CD8^+$ T cells, below which no effect of $CD8^+$ T cells on viral load was observed.

A vaccine that would generate effector memory T cells at mucosal target sites and high avidity, polyfunctional CTLs able to inhibit viral replication through granzyme B–mediated killing of infected target T cells would likely elicit efficient long-term control of HIV infection.[474–482]

Nonneutralizing Antibodies

Nonneutralizing antibodies that bind to HIV antigens on the surface of HIV-infected cells by their Fab fragments can recruit by their Fc fragment innate immune cells that possess an FcγR, including antigen-presenting cells, NK cells, or monocyte/macrophages. Such FcγR-mediated recruitment of innate immune cells can lead to either killing of the infected cell, referred to as **antibody-dependent cellular cytotoxicity (ADCC)**, or to inhibition of viral replication, referred to as **antibody-dependent cell-mediated virus inhibition (ADCVI)**.[483,484]

Studies in animal models have shown that protection of newborn monkeys against oral SIV infection by passive immunization with a nonneutralizing anti-SIV serum correlated with ADCVI activity in the serum.[485] Immunization with a replicating recombinant adenovirus followed by boosting with an Env subunit vaccine partially protected rhesus macaques against mucosal challenge with SIV or pathogenic SHIV that result from ADCC and ADCVI responses in the absence of a neutralizing antibody response.[486–489] Although unable to afford protection against productive clinical infection, nonneutralizing antibodies that bind HIV-Env are able to limit the number of transmitted/founder viruses involved in transmission.[490] In addition, there is evidence that reduced viral loads at both the acute and chronic stages of infection significantly correlate with both ADCC and ADCVI.[491–493] Both ADCC and ADCVI activities are, in turn, directly correlated with antibody affinity, suggesting the importance of antibody maturation for functionality. Thus, Fc-dependent effector

functions of antibodies elicited by vaccination may be a major factor in effective protection against infection. It was shown that immunization of mice with HIV-Env alone elicited an Env-specific antibody response with reduced Fc effector functions, whereas immunization with virus-like particles (VLPs) containing Gag-Pol and Env led to a balanced Env-specific humoral immune response with a pronounced inflammatory Fc glycan profile.[494]

Most anti-gp41 antibodies are of the nonneutralizing type and are directed either at the immunodominant C-C loop in gp41 (cluster I) or at epitopes located in the MPER segment (cluster II). Cluster II antibodies mediate ADCC and other Fc-mediated antiviral activities.[495–497] A recent study in which gp41-specific antibodies were cloned from the memory B-cell compartment of HIV-1–infected individuals showed that clones targeting cluster II epitopes accounted for 49% of all anti-gp41 reactive B cells.[498] The gp120-depleted gp41 stumps observed on the surface of HIV virions are probably in the triggered, six-helix bundle form and may be the chief source of this type of antibody response.[499,500]

Anti–HIV-1 bNAbs may also be mediators of ADCC, as suggested for antibody b12 whose infusion in the native form protected eight of nine monkeys against high-dose vaginal SHIV challenge, whereas the same dose of a mutated version of b12 with no capacity to bind to FcγRs protected only four of nine macaques.[501,502] Another bNAb, 2G12, seems to afford better protection in vivo than would be expected from its neutralization potency in vitro.[373,503] Modification of Fc fragment glycosylation of 2G12 was capable of improving antiviral activity by enhancing ADCC and ADCVI, as shown by comparing various glycoforms produced in wild-type and glycoengineered plants.[504] Similarly, the in vitro neutralization potency of antibodies 2F5 and 4E10 was substantially increased when the cell line used for assessment of HIV-1 neutralization was engineered to express FcγRI,[497,505] or to a lesser extent, FcγRIIb.[506] 2F5 at a few mg/mL concentrations elicited ADCC against both X4- and R5-HIV–infected monocytes or monocytic cell lines.[507] Usually, enhancing the Fc receptor function of bNAbs much improves their protective efficacy against SHIV mucosal challenge.[425,508]

In humans, the appearance of ADCVI in acutely infected subjects occurs as early as the CTL response; that is, much earlier than the neutralizing antibody response.[340,483] Elite controllers exhibit more potent ADCC activity than viremic individuals, whereas neutralizing antibody activity tended to paradoxically be higher in viremic subjects.[509] HIV-1 Env-specific ADCC was detected in a number of early studies,[496,510] as well as in human clinical trials, where its presence correlated with protection against infection.[511,512] ADCC was also detected in breast milk.[513,514] Polyfunctionality of the Fc receptor depends on immunoglobulin (Ig) G subclass selection. Thus, in the moderately successful RV-144 clinical trial (see below), nonneutralizing antibodies with high Fc-mediated effector functions were elicited through the selective induction of short-lived IgG3; whereas in the unsuccessful VAX003 trial, the gp120 vaccine elicited IgG_4 responses with monofunctional Fc functions.[515]

Another important function of some nonneutralizing antibodies, especially IgAs, appears to be the inhibition of virus transcytosis. Mucosal transcytosis-blocking IgAs have been detected in the cervicovaginal secretions of HEPS women in discordant couples[516] and in sex worker cohorts.[517,518]

HEPS persons, also known as EU persons,[519,520] show no HIV-1–specific plasma IgG,[521–523] but exhibit HIV-1–specific mucosal IgGs and IgAs,[521,524–527] with IgAs targeting gp41, especially the Gal/Cer binding site in the MPER of gp41.[528,529] A mucosal gp41-specific Fab IgA library was constructed and cervical B cells from these women were used to generate libraries of gp41 MPER-specific monoclonal IgAs that were able to inhibit HIV-1 transcytosis across an epithelial cell membrane in vitro.[530] gp41 MPER-derived immunogens were able to elicit transcytosis-inhibiting, nonneutralizing IgAs and to provide mucosal immunity in NHP models.[531,532] IgAs with HIV-1–neutralizing activity could also be recovered from plasma and saliva from HEPS persons.[533,534] Mucosally administered dimeric IgAs were shown to elicit efficient protection against mucosal SHIV challenge in macaque monkeys.[535,536]

Innate Immunity

Viral infections usually result in strong activation of the innate immune system that precedes the development of adaptive immune responses.[537,538] Lentiviral infections are no exception. Innate immunity responses are among key determinants of the outcome of HIV-1 infection.[136,139,140,539]

SIV infection of African green monkeys or sooty mangabeys was found to induce a strong but rapidly controlled innate response.[540,541] The activation of innate immune cells, essentially plasmacytoid DCs, proceeds through recognition of HIV-1 or SIV by Toll-like receptors (TLRs), especially TLR7 and TLR8,[542,543] and leads to the production of antiviral cytokines[538,544] such as TRIM22 and type I IFN.[545–548] In turn, IFN-α upregulates intrinsic host restriction factors, such as tripartite motif (TRIM) proteins, including TRIM5α,[549] and human APOBEC3G (hA3G), which can edit the HIV-1 early reverse transcripts through cytidine deamination, leading to degradation of the provirus DNA.[130,132]

A striking inverse correlation was found between A3G mRNA levels and HIV viral loads, and a positive correlation between A3G mRNA levels and CD4 cell counts in HIV-1–infected subjects.[550,551] Mucosal immunization of macaques against SIV led to persistent expression of A3G,[137] and elevated A3G was demonstrated to mediate resistance of monocyte-derived DCs to HIV-1 infection.[138,552] The use of TLR agonists and IL-15 as vaccine adjuvants in macaques had a remarkable effect on protection against mucosal SIV challenge: the adjuvants alone without vaccine antigen elicited enhanced A3G mRNA expression in DCs, monocytes/macrophages, and $CD4^+$ T cells, resulting in significant decreases in the set-point plasma and colon tissue viral loads following rectal SIV challenge.[142]

Another member of the APOBEC3 family that might be involved in innate immunity against HIV-1 is APOBEC3B, as judged by the fact that a naturally occurring deletion in the APOBEC3B gene was significantly associated with increased susceptibility to HIV-1 infection, progression to AIDS and viral set-point.[553]

Acute HIV-1 infection also results in the activation and expansion of $CD3^-$ NK T cells,[554,555] which, in combination with appropriate HLA class I ligands, are associated with better control of HIV-1 replication and slower disease progression.[556–559] NK cells make up half of the IFN-γ–producing cells in HIV-1 peptide-stimulated PBMCs from HIV-infected persons.[560] NK activity is increased in "long-term nonprogressors"[561,562] and in EU subjects.[562,563] The expression of certain alleles of killer immunoglobulin receptors (KIRs) on NK cells, such as KIR3DS1 or 3DL1, in combination with HLA-Bw4801, was associated with the slowest rate of progression to AIDS in large cohort studies.[557]

NK cell activity was found to be enhanced in EU subjects in the absence of target stimulation, providing these persons with an improved immune response to resist HIV infection.[559] NK activity is also increased in acute SIV infection of African NHP, but not in rhesus macaques, which develop full-blown disease.[564] The variety of NK cells and NK cell receptors makes their study complex but the discovery of innate memory in

NK cells may offer interesting perspectives for vaccine development.[55,565] Cytokines such as IL-21 or IL-15 may help induce or improve NK cell responses.[566]

Several other factors of innate immunity also play a role in the control of lentivirus infection, such as p21, an inhibitor of cyclin-dependent kinase present in $CD4^+$ T cells[567,568]; T-bet, a master transcription factor that promotes the expression of IFN-γ, granzyme B, and perforin by $CD8^+$ T cells[569]; IL-27[570]; and TRIM5α, which controls macaque susceptibility to SIVsmE660[81,83] and was found to be associated with protection from HIV-1 infection in some sex worker cohorts.[571] The p21 inhibitor appears to reduce the susceptibility of activated $CD4^+$ T cells and human macrophages to HIV-1 replication through inhibition of transcription of the proviral DNA. It also can block reverse transcription. The expression of both p21 and T-bet is upregulated in $CD4^+$ T cells from elite controllers and experimentally knocking out p21 substantially increases cell susceptibility to HIV-1.[572] Another antiviral innate immunity factor is TRIM21, which neutralizes virus–antibody complexes inside the cell by binding to antibodies that remain attached to virus particles and targeting the virus–antibody complex to the proteasome, thus mediating the intracellular disabling of the virus.[573]

It is now evident that innate immune mechanisms contribute to control of HIV, but it is not clear if and how the response could be harnessed through vaccination.[140,442,574] Ongoing systems biological approaches to SIV immunization studies in macaques are focused on identifying innate signatures that may predict adaptive immune responses, and thus might facilitate efforts to modulate innate immunity to more effectively enhance adaptive immunity.

CANDIDATE HIV VACCINES: PRECLINICAL DEVELOPMENT

The development of an HIV vaccine is a formidable challenge. An effective HIV vaccine should be able to induce durable immunity and prevent the acquisition of infection and/or reduce virus replication in infected persons so as to slow down disease evolution and reduce viral transmission.[574–578] The first Phase I trial of an HIV vaccine was conducted in the United States in 1987 using a gp160 subunit vaccine. Since then, more than 170 vaccines have been tested in more than 250 Phase I/II clinical trials, involving more than 27,000 healthy human volunteers.[50,51,579] HIV-1 vaccines that were developed and tested in humans were first based on induction of neutralizing antibodies, then on induction of cellular immune responses.[54,580–584] Studies show that robust mucosal immunity, high-avidity polyfunctional T cells, and bNAbs are potentially important factors in protective immunity against HIV-1,[585,586] but the studies also highlight the need to design vaccines that can induce more potent immune responses than those that are elicited by natural infection, as the latter provide very poor protective immunity.[49,52,587] As classical vaccine strategies based on attenuated live virus or whole inactivated virus show severe limitations because of safety considerations and HIV variability, most efforts to develop HIV vaccines have focused on newer vaccine approaches. This section discusses vaccine development efforts in animal models; human vaccine trials are reviewed in a later section.

Most of the efforts to develop and evaluate HIV vaccines are borne by public sector research agencies (e.g., the National Institutes of Health [NIH], the Centers for Disease Control and Prevention [CDC], and the Walter Reed Army Institute of Research [WRAIR] in the United States; the Agence Nationale de Recherches sur le SIDA et les Hépatites Virales (ANRS) in France; the Karolinska Institute in Sweden); international development agencies (e.g., United States Agency for International Development [USAID]; United Kingdom Department for International Development [UK-DFID], and the European Union agencies and development donors); and nongovernmental organizations (NGOs) (e.g., International AIDS Vaccine Initiative [IAVI], New York, NY; Bill and Melinda Gates Foundation [BMGF], Seattle, Washington); together with initiatives in the World Health Organization (WHO) and UNAIDS. The HIV Vaccine Trials Network (HVTN) established by the National Institute of Allergy and Infectious Diseases (NIAID) in 2000, with 25 clinical sites on four continents, represents a major resource for clinical HIV vaccine research. Complementary clinical trials partnerships have been developed by the U.S. Military HIV Research Program in Africa and Thailand, and by IAVI in Africa and India. The European Union has created the European and Developing Countries Clinical Trials Partnership (EDCTP) with the aim of helping developing countries build their capacity for testing the efficacy of new drugs, microbicides, and vaccines. Major efforts have also been made by the vaccine industry in developing and testing candidate HIV vaccines, including Phase IIb/III clinical trials.

In 2003, recognizing the enormity of the scientific challenge, leading investigators and major funding organizations formed the Global HIV Vaccine Enterprise, an alliance of independent organizations committed to working together to accelerate the development of an HIV vaccine.[588] A strategic scientific plan was drafted in 2005 and updated in 2010.[589] The plan aims at accelerating clinical trials of promising vaccine candidates, bridging the gaps between basic and clinical research, facilitating exchange of reagents and data, enhancing global collaborations, and fostering the development of young scientists and clinicians in the field of HIV vaccine research.

Animal Models

HIV-1 can be transmitted to chimpanzees[590,591] and pig-tail macaques *(Macaca nemestrina)*, but neither of these models can reproduce the pathology of human AIDS. HIV-1 also can replicate in immunodeficient transgenic mice engrafted with human fetal lymphoid tissues ("SCID-hu" mice) or with adult human peripheral blood leukocytes ("hu-PBL-SCID" mice)[592] or in humanized immunodeficient mice transplanted with human cord blood hematopoietic stem cells.[593–595] More recently, humanized mice have been used to study efficacy of vector-delivered bNAbs in prevention of HIV infection.[596,597]

The SIVmac is easily transmitted to and readily causes AIDS in rhesus macaques, especially in those from India.[598,599] Indeed, infection of macaque monkeys with SIVmac recapitulates the pathogenic effects of HIV-1 in humans and provides the most reliable animal model for testing antiviral therapies and candidate vaccines against HIV-1 available today.[600–603] As with HIV-1, the first cellular targets for SIV are the $CD4^+$ $CCR-5^+$ memory T cells in the lymphoid tissue of the genital tract and in the GALT, which has unique anatomical and functional features that makes it a major reservoir for virus sequestration, persistence, and ongoing replication.[227] The percentage of $CD4^+$ T cells in the lamina propria of the gut of SIV-infected macaques dramatically decreased from 67% in uninfected animals to 6% at 21 days postinfection.[189,604–609] Mucosal transmission of HIV can also be modeled in the SIV-rhesus macaque system, including cell-associated virus transmission.[610–612]

Plasma viral loads at the peak of primary SIV infection and at the set-point during chronic SIV infection in macaques parallel those observed in HIV-1–infected humans.[611] Some animals maintain high viral loads and quickly progress to AIDS, as human **fast progressors,** while others contain viremia spontaneously and progress to disease slowly, as HIV-1–infected human **long-term nonprogressors,** or those who

maintain barely detectable viral loads without treatment, such as human **controllers.**[613] Several monkey MHC class I haplotypes, such as Mamu-A*01, Mamu-B*08, and Mamu-B*17, have been identified which correlate with elite control of viral loads and relative resistance of the animals to progression to SIV-induced AIDS,[614,615] as has been observed in humans with haplotypes HLA-B*27, HLA-B*57, or HLA B*58, in HIV controllers and elite controllers.[452,616–622]

There is only, however, a limited number of SIV isolates that can be used to test protection across genetically diverse virus strains, a major obstacle for any field test of an HIV vaccine. Also, because of a complete difference between the SIV and HIV-1 Env antigenicity, the SIV/macaque model does not allow evaluation of the role of HIV-specific neutralizing antibodies in vaccine-induced protection.[623] To alleviate this difficulty, replication-competent chimeric SIV/HIV viruses called **SHIVs** were engineered that combine the *env-tat* and *rev* genes from HIV-1 with the *gag-pol-vif* and *nef* genes from SIVmac and replicate to high titers in cynomolgus and rhesus monkeys.[624,625] Serial passages of these hybrid viruses in monkeys can lead to the emergence of stable pathogenic SHIV strains able to induce $CD4^+$ T-cell depletion and AIDS in the animals, such as $SHIV_{89.6P}$, an X4 virus,[626,627] or $SHIV_{SF162P3}$, an R5 virus.[628] The relative transmissibility of an R5 clade C SHIV ($SHIV\text{-}1157_{ipd3N4}$) via different mucosal routes was found to parallel the relative risk of sexual transmission in humans, rectal challenge requiring the least virus, followed by vaginal and then oral routes,[629] as observed in humans.[630–633]

As a result, the SHIV/macaque model is widely used for preclinical testing of candidate HIV vaccines. Paradoxically, however, highly virulent X4 SHIVs, such as $SHIV_{89.6P}$, are relatively easy to control by vaccination, casting doubts on their validity as a model for HIV vaccine efficacy, especially when the end point is control of infection.[226,602,623,634]

Monkey models suffer from the fact that a very high dose of virus is typically used to challenge the animals in vaccine protection efficacy experiments so as to achieve 100% take of the virus in the placebo group after a single exposure. These doses are higher than the amount of virus in natural exposures in humans.[635,636] The difficulty was eliminated by using repeated low-dose mucosal challenges, after it was shown that low-dose SIV challenges (10–50 $TCID_{50}$ [median tissue culture infective dose]) resulted in the same viral and immunological kinetics of infection as a high-dose challenge.[637] More recently, protection in macaques with analogous candidates to an HIV vaccine evaluated in clinical trials was shown to be dependent on the dose of SIV at challenge exposure.[638]

One variable in the HIV vaccine field related to the use of SIV and SHIV models that would benefit from standardization is the definition of "protection" in the context of vaccine and challenge studies. Although it is clear that complete prevention of infection is synonymous with protection, it is less clear when the measurement of protective efficacy is suppression of viral load, or reduction in per-exposure acquisition risk following repetitive viral challenges.[639] Another limitation of monkey models stems from the usually low number of animals that can be used in vaccine experiments, whose results therefore often lack sufficient statistical significance.

Live Attenuated Vaccines

The observation that *nef*-deleted mutants of SIV were attenuated and could serve as a live vaccine to elicit protection against challenge with pathogenic SIV in rhesus macaques served as a model for live attenuated HIV vaccines.[111,113,640] The result was confirmed by using as a vaccine a molecular clone of SIVmac that had been attenuated by a 12-base pair deletion in the *nef* gene, C8, which provided complete protection against infection with a full-length, pathogenic molecular clone, J5.[112] Deletion of *nef*, alone or in combination with deletions in *vpr* and in the long terminal repeat region,[641] showed no drastic effect on virus growth in cell culture.

SIVΔ*nef* was actually found to establish a lifelong, persistent low-grade infection that protects monkeys against disease following challenge with pathogenic virus but generally does not protect them from infection. The actual correlates of protection remain incompletely deciphered,[115] but evidence is in favor of the induction of a long-lasting CTL response.[642–644] It was recently demonstrated that the degree of SIVΔnef-mediated protection against intravenous wild-type SIVmac239 challenge strongly correlates with the magnitude and function of SIV-specific, effector-differentiated T cells in the lymph node but not with the responses of such T cells in the blood or with other cellular, humoral and innate immune parameters.[477] However, other potential correlates of protection have been suggested, including local antibody production and concentration in the path of virus entry, and immune complex–inhibitory Fc receptor interactions that reduce target cell availability.[645,646]

Similarly, nonpathogenic $SHIV_{89.6}$ established persistent infection in monkeys that, following mucosal challenge with SIV, provided protection against uncontrolled virus replication, but not against infection. A clear role for $CD8^+$ T cells in protection was documented.[439,647] Macaques previously exposed to low doses of HIV-2 also demonstrated a virus-specific cellular immune response and were protected against uncontrolled virus replication when challenged intrarectally with SIV.[648]

SIVΔ*nef* is not totally attenuated and may still cause AIDS when administered orally to infant monkeys.[649] It also could revert to full-size *nef* virulence in some animals.[650] Additional deletions or mutations could further attenuate its virulence but at the expense of protective efficacy. Efforts to make the virus safer included the construction of replication-competent SIVΔ*nef* viruses that expressed the human IFN-γ gene[651] or the herpes simplex thymidine kinase gene.[652]

Yet, the prospect of using a live attenuated HIV vaccine in humans has enormous safety concerns owing to the high mutation rate of the virus, and thus the approach was not tested in humans. A few individuals infected with HIV-1 for long periods of time without any sign of AIDS were found to be infected with *nef*-deleted viruses[653,654] but these persons eventually experienced late decline in their CD4 T-cell counts[655,656] and slowly developed AIDS.[657] As a result, there is no development of live attenuated HIV vaccines as an ongoing strategy for the prevention of HIV-1 infection.

Whole Inactivated Vaccines and Virus-Like Particles

The best way to inactivate HIV or SIV without destroying the antigenicity of the viral envelope glycoproteins was mild oxidation with aldithriol-2 to remove the essential Zn^{2+} ions from the Gag and Int proteins,[94] alkylation with *N*-ethyleneimine,[658] or through UV inactivation of the viral RNA with the help of psoralen.[659] The immunogenicity of the resulting preparations was modest, perhaps as a result of the low number of glycoprotein spikes per virion.[75] Immunization with an inactivated SIV vaccine did not protect monkeys against SIV infection, but the animals showed decreased levels of viremia after challenge.[660]

When coexpressed in insect cell cultures using a baculovirus vector or in mammalian cell cultures using a poxvirus vector, the HIV or SIV Gag and Env proteins spontaneously assembled to form VLPs that were made of only the viral core and envelope proteins.[661,662] SIV VLPs were tested as a vaccine

in NHPs by a variety of routes,[663] but their immunogenicity was found to be modest. No bNAb was elicited by HIV-1 VLPs in mice or guinea pigs, even when the sequence of gp41 or that of the gp41 MPER was included in the construction.[664]

The success of the human papillomavirus (HPV) and hepatitis B vaccines, both of which are VLPs, has led many investigators focused on eliciting bNAbs to evaluate different VLP platforms. Strategies aimed at dampening nonneutralizing epitopes, or using prime-boost approaches with viral vector–VLP combinations, are currently being evaluated.[665,666]

Hybrid VLPs were also engineered by fusing the MPER from gp41 to the N-terminus of the hepatitis B surface antigen,[667] or to bovine papillomavirus (BPV)-L1 capsid protein, which spontaneously forms VLPs.[668] Peptides with the sequence of the gp120 V3 loop or of a known CTL epitope, P18, were also fused to BPV-L1 VLPs.[669] None of the resulting chimeric VLPs were found to elicit more than low titers of HIV-specific antibodies and none could elicit HIV-1–neutralizing antibodies.

Subunit Vaccines

Env Subunit Vaccines

Subunit protein vaccines were initially developed based on monomeric HIV-1 gp120 or gp140 (gp160 deleted of the transmembrane and intracytoplasmic domains of gp41) that were presented either in their soluble form or as related synthetic peptides (V3 loop), using alum as an adjuvant. These vaccines were found to induce neutralizing antibodies that targeted the V3 loop in gp120 and protected chimpanzees against challenge with a homologous or near-homologous HIV-1 X4 strain, but not against challenge with a distant virus strain nor with a primary R5 HIV-1 isolate.[670–675] Passive immunization with a monoclonal antibody targeting the V3 loop was also shown to protect chimpanzees against homologous X4 virus challenge.[676] Similarly, in the SHIV/macaque model, vaccines based on gp120 were able to provide protection against homologous X4 SHIV challenge,[677] but not against challenge with a heterologous SHIV with a different envelope specificity.[678] In contrast, immunization of macaques with R5 SIVmac gp120 formulated with a variety of adjuvants, liposomes, or immunostimulating complexes[679] was unsuccessful at providing protection against SIV challenge.[680]

Recombinant soluble HIV-1 envelope proteins have been the target of intensive investigation, as one of the major obstacles in HIV vaccine development remains the inability of current immunogens to elicit bNAbs. Numerous approaches have been explored[681,682] including:

- Trimeric gp140 molecules (gp120 + the ectodomain of gp41) stabilized by the addition of heterologous trimerization domains at the C-terminus of the gp41 sequence[683,684] and similar trimers with a deletion of the hypervariable V2 loop, so as to better expose the neutralization epitopes that overlap the CD4-binding site.[343,685,686] Systemic immunization or a combination of systemic and mucosal immunization with the **ΔV2 gp140** subunit vaccine emulsified with water-in-oil adjuvant MF59 protected female rhesus macaques against intravaginal challenge with a homologous SHIV.[687]
- Trimeric gp140 molecules internally stabilized by a disulfide bond between gp120 and gp41 (**"SOS" proteins**). Their immunogenicity was tested using priming with a DNA plasmid encoding the SOS protein and repeated boosting with the purified SOS trimers. This regimen resulted in high titers of neutralizing antibodies against virus strains of the same clade but only low levels of cross-clade neutralizing antibodies.[688,689] More recently, using a bioinformatics approach to identify HIV isolates sensitive to bNAbs, a most-recent-common-ancestor (MRCA) sequence for the HIV-Env was determined, and the Env from clade A viral isolate BG505 was found to have the highest degree of homology to the MRCA.[690] Using the sequence of BG505 as a template, a cleaved HIV-Env SOSIP trimer was stabilized and analyzed by X-ray crystallography and cryoelectron microscopy, and found to be antigenically near native.[691–693] HIV-1 neutralizing antibodies induced by this native-like trimer gave higher titers of autologous neutralizing antibody than the monomeric gp120, but failed to elicit bNAbs.[694]
- Oligomeric gp140 molecules covalently coupled to synthetic mimics of the CD4 receptor, or "single-chain gp120 derivatives" covalently linked to the first two domains of the human or simian CD4 molecule, so as to induce in the Env molecule the same conformational changes that take place at the time of virus entry and to expose potential neutralization epitopes that overlap the coreceptor-binding site.[695,696] The **single-chain** antigen was indeed found to elicit in macaques neutralizing antibodies to CD4-induced epitopes,[25,697] which were able to accelerate control of viremia upon challenge with $SHIV_{162P3}$.[698] Efforts to elucidate the correlates of protection suggest that antibody-dependent cellular cytotoxicity with limited antigen-specific T-cell activation played an important role.[699]
- A cocktail of Env immunogens derived from globally prevalent HIV strains, or more simply from representatives of each of the different clades[700–702] in the hope that **multivalent Env** vaccines will broaden the spectrum of neutralizing antibodies.[703,704]
- Fully synthetic genes derived from theoretically defined **consensus** or **ancestral** HIV envelope sequences[297–299] that were found to be superior to many wild-type immunogens for inducing cross-subtype T-cell and B-cell immune responses and antibodies that neutralized a spectrum of HIV-Env pseudoviruses.[300] The M-group "CON-S" and a clade B-based "CON-B" Env protein[705,706] each elicited antibodies with good titers and breadth of responses against tier-1/HIV-1 viruses, but not against isolates that are more difficult to neutralize.[301]
- **Mosaic antigens** with sequence derived from in silico algorithms to closely match the sequences of natural HIV-1 strains worldwide and maximize overlap between them.[302,303] An HIV-1 mosaic M trimer was recently generated, but also failed to elicit bNAbs.[707]

Despite these many efforts and of unabated attempts at designing mimics of the viral envelope spike or specifically tailored Env preparations,[349] the quest for immunogens able to efficiently elicit high-titer bNAb has remained unsuccessful so far,[86,354,428,430,431,708] and continued efforts to find an optimal immunogen design and delivery are ongoing (see below). The formulation of gp120 or gp140 under the form of immunostimulating complexes with saponin derivative Quil A, or in combination with water-in-oil emulsions containing QS21 or muramyl dipeptide derivatives, or with specific adjuvant formulations such as MF59, resulted in the induction of high gp120-binding antibody titers with neutralizing activity against closely matched X4 virus strains, but not against primary HIV-1 isolates.[709–713]

Induction of 2F5- or 4E10-like fusion-blocking antibodies by immunization with recombinant oligomeric gp41 molecules or formulations of the gp41 MPER has similarly been mostly a failure. Displays of constrained 2F5 epitope in the context of various protein scaffolds induced high antibody titers against the primary amino acid sequence of the epitope, but failed to elicit neutralizing antibodies against HIV-1

isolates,[664,713,714] confirming the need to adequately mimic the native conformation of the epitope,[715,716] as well as the likely importance of the lipid membrane and hydrophobic context for the binding of 2F5 and 4E10 antibodies to their respective epitopes.[717–719] The challenges of eliciting bNAbs against HIV are further detailed below.

Tat Vaccines

Subunit vaccines based on the accessory protein Tat have also been developed in the hope that immune responses directed at antigens such as Tat that are expressed very early in the virus replication cycle might lead to containment, if not abortion of infection.[720,721] Eight genetic variants of Tat have been identified as of this writing.[722] Both anti-Tat antibody and Tat-specific CTLs correlate with reduced viremia and slow progression to AIDS.[723,724] Conflicting results have been reported following immunization of rhesus macaques with Tat protein, formalin-inactivated Tat ("Tat toxoid"), or Tat peptides. These vaccines elicited little or no protection against challenge with SIVmac239, $SHIV_{33}$, or $SHIV_{89.6P}$.[725–728] In contrast, immunization of cynomolgus macaques with Tat protein or DNA encoding Tat elicited control of infection following challenge with $SHIV_{89.6P}$.[729–732] A Tat-Nef fusion protein that was combined with a gp120 subunit vaccine in AS02 adjuvant was found to prevent the development of disease in rhesus macaques for at least 2.5 years following challenge with $SHIV_{89.6P}$, especially when presented as a DNA vaccine formulation.[733,734] HIV Tat vaccines might thus show better efficacy when combined with other HIV antigens.[735–739]

Nucleic Acid Vaccines

In view of the lack of success of vaccines to elicit bNAbs for prevention of HIV-1 infection,[424] early HIV vaccine development focused on vaccines that would induce T-cell responses, especially a $CD8^+$ CTL response aimed at controlling HIV infection. Vaccines that stimulate the T-cell arm of the immune response are not expected to protect against infection, but rather to protect against appearance of disease by reducing viral load, slowing down $CD4^+$ T-cell decline and preventing, or at least retarding, the occurrence of symptoms.[740] Naked DNA vaccines and live vectored recombinant vaccines have been developed based on this strategy.[50–52,741] Reduction of viral loads in HIV-infected individuals should also result in lower probability of virus transmission to seronegative partners.

DNA vaccines (also referred to as genetic vaccines) are bacterial plasmids that carry the gene(s) encoding one or several desired antigens, and which, once injected into the muscle or the dermis of an animal, express the desired antigen(s) in situ, leading to induction of an antigen-specific cellular immune response.[742–745] However, the immune potency of DNA vaccines in humans and NHPs has generally been mediocre, inducing weak helper T-cell responses and little, if any, antibody and $CD8^+$ T-cell responses.[746,747] Improved immunogenicity was obtained through the use of synthetic genes with optimized codons,[748] the insertion of engineered B7 costimulatory molecules, the coexpression of cytokines such as IL-12, IL-15, or granulocyte-macrophage colony-stimulating factor (GM-CSF) to serve as vaccine adjuvants, and the simple repetition of vaccine injections three successive times,[749–753] which resulted in greatly enhanced expansion of polyfunctional memory $CD8^+$ T cells. A DNA vaccine expressing mosaic sequences of potential T-cell epitopes from HIV-1 M group Env was also found to induce $CD8^+$ T-cell responses with significantly greater breadth than similar vaccines with natural sequences.[304,305,480] DNA vaccines also have been employed to elicit broad cell-mediated immune responses to conserved antigens.[754]

Among strategies for further improvement of DNA vaccine potency[755] are novel means of vaccine delivery, such as needleless injections (the Biovector device),[756,757] formulation of the DNA onto microparticles, as was done using cationic microparticles,[758] or on gold beads administered by gene gun,[759,760] delivery of the DNA transcutaneously with the Nanopatch microprojection device,[761–763] or the use of electroporation and an electroporation device.[764–768] Combined effects of IL-12 and electroporation greatly enhanced the potency of DNA vaccination in macaques.[765] Optimized plasmid DNAs encoding the majority of SIV proteins and delivered to macaques by electroporation, elicited strong cellular and humoral responses against the vaccine Gag, Pol, Env, Nef, and Tat antigens, and decreased viremia in both the acute and chronic phases of infection following challenge with SIVmac.[769] Similar protection results were observed against a SHIV clade C challenge using DNA plasmids expressing SIV Gag and Pol and HIV-1 clade C Env that were delivered by electroporation.[770] Moreover, enhanced immunogenicity of HIV-1 DNA was obtained by delivery of the DNA via electroporation through both intramuscular and intradermal routes.[771]

A topical plasmid DNA vaccine formulated in a nanoparticle with polyethylenimine-mannose and glucose, DermaVir, was developed to deliver DNA-encoded antigens to Langerhans cells.[772,773] DermaVir formulated with HIV-1 Gag plasmid in the presence of a IL-15–encoding plasmid significantly enhanced Gag-specific central memory T cells, as measured by IFN-γ ELISPOT(enzyme-linked immunospot).[774]

DNA vaccines have also been coadministered with protein, a strategy that yielded enhanced humoral immune responses in mice and macaques.[775] Still, the best immunogenicity results with DNA vaccines have been obtained using a prime-boost strategy, that is, delivering the DNA vaccine as a prime, later to be followed by a live recombinant vaccine booster immunization.[776] Thus, immunization of macaques with a DNA-Env vaccine followed by a polyvalent Env formulation was able to elicit a more diverse antibody response, with higher antibody avidity and improved cross-clade neutralizing activity, albeit only against relatively easy to neutralize isolates of HIV-1, together with cross-subtype T-cell responses.

With the limited success of DNA vaccines in humans, greater attention is now focused on the potential of mRNA-based vaccines.[777,778] These vaccines provide the flexibility of DNA vaccines, but with the potential for enhanced immunogenicity and safety. One of the keys to realizing the potential of mRNA based vaccines is the efficient delivery of the mRNA to the cell's cytoplasm to enable expression of the encoded antigenic protein. Self-adjuvanted mRNA has advanced to clinical trials for cancer immunotherapy. It is well tolerated and immunogenic,[777] and is the first HIV-based mRNA vaccine candidate expressing a native-like trimer of HIV-Env. Self-adjuvanted mRNA will likely enter clinical trials in 2016–2017 by IAVI and CureVac Inc. Self-assembled nanomicelles based on polyethyleneimine-stearic acid copolymer delivered HIV-1 gag encoding mRNA to Balb/c mice and induced antigen-specific humoral and cellular immune responses.[778]

mRNA-based vaccines can be further enhanced by engineering as self-amplifying RNA vaccines.[779–784] Potent immune responses in rhesus macaques were induced by nonviral delivery of self-amplifying RNA vaccines expressing HIV-1 envelope with a cationic nanoemulsion.[779] Moreover, preclinical proof of concept with this approach has also been achieved for influenza, respiratory syncytial virus, rabies, Ebola, CMV, and malaria.[782]

Live Recombinant (Vectored) Vaccines

Live recombinant vaccines are made of a live viral or bacterial vector that is engineered to express a variety of exogenous antigens in the cytoplasm of targeted T cells, in this case HIV-1 genes. The antigens are eventually broken down in the proteasome of the transduced cell and presented as externalized peptides in the context of MHC class I molecules, thus priming for a $CD8^+$ T-cell response. Alternatively, antigen-presenting cells can phagocytose transduced cells that become apoptotic and present antigenic peptides to CTLs through cross-presentation. If the vaccine contains too few antigens, virus escape mutants with mutations in T-cell epitopes can readily emerge.[614] Vaccine impact on control of viral loads has been the best with vaccines that express multiple HIV-1 proteins including Env.[784,785]

Numerous vectors have been engineered into live recombinant vaccines (Table 29.3).[786–789] Most of these vectors had previously been made replication-defective by deletion of essential genes, such as the E1A gene in adenoviruses, or a series of genes in the New York vaccinia virus strain (NYVAC). Other vectors are spontaneously unable to replicate in primates, such as Canarypox (ALVAC), Fowlpox, or the attenuated vaccinia virus strain MVA. Replication defectiveness allows for greater safety, but may result in decreased immunogenicity of the candidate vaccine, necessitating a considerable increase in the dose of vaccine (number of particles of recombinant).

One of the major challenges for live recombinant vaccine immunogenicity is the subsequent immune response to the vector, which dampens the take of the vaccine and subsequent expression of the vectored antigens: this applies both to the difficulty with using the same vectored vaccine for repeated immunizations and to the situation of pre-existing immune responses to the vector in the general population. This is especially true for adenovirus serotype 5 (Ad5), against which the human population shows a high prevalence of neutralizing antibodies.

TABLE 29.3 Vectors Used for the Engineering of Live Recombinant Vectored HIV or Simian Immunodeficiency Virus Vaccines

Bacillus Calmette Guérin (BCG; NIH, Japan)[1007–1009]
Salmonella (IAVI, University of Maryland)[1010,1011]
Other bacterial vectors: *Lactobacillus, Streptococcus, Listeria*[1012]
Poxvirus vectors (MVA, NYVAC, Fowlpox, and Canarypox [ALVAC] viruses)[1013]
Human ΔE1A replication-defective adenoviruses Ad5, Ad11, Ad24, Ad26, and Ad35[1014–1016]
Replication-competent human Ad5 *hr* mutants[486]
Nonreplicating chimpanzee adenoviruses AdC68, AdC6, and AdC7[1017–1019]
Picornavirus vectors (Poliovirus, Rhinovirus)[805,1020]
Venezuelan equine encephalitis virus (VEEV; Alphavax)[799]
Adeno-associated virus (AAV; IAVI/Targeted Genetics)[1021]
Sendai virus (SeV; NIH, Japan)[824,1022]
Vesicular stomatitis virus (VSV; Yale University/Wyeth; IAVI)[804,1023,1024]
Newcastle disease virus (NDV; Mount Sinai, New York, and Kyoto University, Japan)[1025]
Measles virus (MV; Pasteur Institute/GlaxoSmithKline)[1026,1027]
Rhesus cytomegalovirus (RhCMV; Oregon Health and Science University)[436,440,441,803]
Live attenuated rubella vector (FDA)[1028]

FDA, U.S. Food and Drug Administration; IAVI, International AIDS Vaccine Initiative; MVA, modified vaccinia virus Ankara; NIH, National Institutes of Health; NYVAC, New York vaccinia virus strain.

Live recombinant vaccines mostly elicit T-cell responses, especially $CD8^+$ T-cell responses, but different vaccine vectors delivering the same antigen can elicit $CD8^+$ T cells with different fine specificities.[790] It was recently observed that multiple innate immune pathways may contribute to the immunogenicity of the Ad5-vectored vaccines.[791] MVA recombinants were found to induce in NHPs potent CTL responses able to partially control virus loads after challenge with pathogenic SHIV or SIV.[792–794] A NYVAC vector that expressed the *gag, env,* and *pol* genes of SIV provided protection against challenge with SIVmac by the rectal route but not by the intravenous route.[795] An Ad5 vector expressing SIV-Gag was similarly found to attenuate infection and mitigate disease progression following challenge with a pathogenic SHIV,[796] but the same or similar constructs provided a lower and only transient level of protection against SIV infection.[434,797,798] More recently, two novel NYVAC-based HIV vaccine candidates expressing HIV 1 clade C soluble trimeric gp140 and gag as VLPs were developed and are being tested in preclinical studies.[666]

Replication-defective alphavirus "replicons" are attractive vectors because they undergo enormous amplification in the host. Also, they have the potential to target DCs, resulting in efficacious antigen presentation. The most developed replicon system is that of the Venezuelan equine encephalitis virus (VEEV),[799,800] which was shown to elicit potent protective immune responses in macaques.[801] In addition, the Venezuelan equine encephalitis replicon particles have the capacity to act as strong adjuvants to other vaccines, including the induction of mucosal immune responses.[802]

A replication-competent vector that has recently shown great promise is rhesus CMV (RhCMV), which elicited immune responses capable of immediate viral control upon challenge with SIVmac, in 13 of 24 vaccinated monkeys.[436,440] In fact, 12 macaques maintained undetectable plasma viral loads for a year with occasional transient blips of detectable virus. This was characterized by predominantly undetectable cell-associated viral load in blood and lymph node mononuclear cells; no depletion of effector-site $CD4^+$ memory T cells; no induction or boosting of SIV Env-specific antibodies; and induction and then loss of T-cell responses to an SIV protein (Vif) not included in the RhCMV vectors. Protection correlated with the magnitude of the peak SIV-specific $CD8^+$ T-cell responses in the vaccine phase, and occurred without anamnestic T-cell responses. Moreover, SIV protein-expressing RhCMV vectors elicit SIV-specific $CD8^+$ T cells that recognize unusual, diverse, and highly promiscuous epitopes, including dominant responses to epitopes restricted by class II MHC molecules.[803] Remarkably, protected rhesus monkeys lost signs of SIV infection, showing a consistent lack of measurable plasma- or tissue-associated virus using ultrasensitive assays. Extensive ultrasensitive quantitative polymerase chain reaction (PCR) and quantitative PCR with reverse transcription analyses of tissues from RhCMV/SIV vector-protected monkeys necropsied 69 to 172 weeks after challenge did not detect SIV RNA or DNA sequences above background levels. Moreover, replication-competent SIV was not detected in these monkeys by extensive coculture analysis of tissues or by adoptive transfer of 60 million hematolymphoid cells to naïve rhesus macaques, compared with SIV-infected elite controllers whose cells infected naïve macaques following adoptive transfer.[441] Recently, replication-competent vesicular stomatitis virus (VSV) expressing functional Env[804] protected against heterologous SHIV challenge in rhesus macaques (C.L. Parks, personal communication, 2015).

Live recombinant vaccines can also be used to elicit neutralizing antibodies, as was done for the HIV-1 2F5 epitope. A combinatorial library of human rhinoviruses displaying the 2F5 epitope (ELDKWA) was constructed and screened with the

2F5 monoclonal antibody. The best 2F5-binding chimeras were shown to elicit antibodies capable of neutralizing HIV-1 pseudoviruses of diverse subtypes and coreceptor usage, albeit, as with other neutralizing-antibody approaches, only neutralization-sensitive isolates.[805] Molecular characterization of the chimeras will hopefully clarify how to improve the presentation of the 2F5 epitope in future vaccine designs.[806] More recently, with new information on the HIV trimer, vectors are being designed to display conformationally intact functional HIV-1 envelope trimers.[804]

PRIME-BOOST IMMUNIZATION REGIMENS

DNA Prime-Live Recombinant Vaccine Boost

Multiple comparative studies in NHPs have demonstrated that the immunogenicity of live vectored vaccines was significantly improved when administered as a boost after initial priming with a DNA vaccine.[434,807–810] As an example, DNA-Ad5 prime-boost improved by sevenfold the magnitude of HIV-1 Env-specific T-cell responses as compared to Ad5 recombinant vaccine alone, resulting in induction of multifunctional CD127+ central and effector memory T cells, and increased by 100-fold the Env-binding antibody response.[811] Such a synergy between the DNA and the live recombinant vaccines was observed in NHPs whether the live vaccine used for boosting was MVA,[812,813] NYVAC,[814] VSV,[815] or Ad5.[816] Priming with DNA followed by a recombinant viral vector boost, was shown to be an effective approach for inducing immune T-cell responses and eliciting control of viral loads in SHIV or SIV challenge studies in macaques.[434,435,815,817–820]

A study of T-cell correlates of vaccine efficacy at controlling viral replication after low-dose heterologous mucosal challenge with SIVsmE660 was carried out in macaques immunized with a DNA-Ad5 prime-boost regimen that expressed SIVmac Gag, Tat, Pol, Rev, Vif, Vpr, and Vpx.[821] The vaccinated animals showed a 1.9 log reduction in peak viral loads, a 2.6 log reduction in set-point viral loads, and intact $CD4^+$ T-cell counts 1 year after challenge,[819] which correlated with the breadth of the T-cell responses directed against Gag and Vif as measured by IFN-γ ELISPOT assays. Surprisingly, the ability of prechallenge peripheral blood-derived $CD8^+$ T cells to suppress viral replication in vitro did not predict the control of viral loads in vivo, perhaps reflecting the fact that the assay was not run with T cells present in mucosa-associated lymphoid tissues where viral replication is concentrated.

Prime-boost immunization of rhesus macaques using a DNA expressing the SIV *gag*, *pol*, *vif*, and *vpx* genes for priming followed by boosting with a live Sendai virus (SeV) expressing SIV *gag*, resulted in long-term control of virus replication and preservation of central memory $CD4^+$ T cells after SIVmac challenge.[822–824] In contrast, DNA vaccine priming did not seem to improve the protective efficacy of a live chimeric Ad5 hexon hypervariable region 48 (Ad5HVR48) vector expressing SIV Gag, Pol, Nef, and Env against SIV challenge in rhesus monkeys.[825] Topical priming immunization with Gag-DNA and Env-DNA plasmids using DermaVir followed by boosting with gp140 resulted in control of viral load set-point in four of five macaques upon challenge with pathogenic R5 $SHIV_{162P3}$.[826]

Several DNA-MVA prime-boost studies have also been done in NHPs.[404,827–830] The DNA-MVA vaccine regimen was found to result in efficient control of viral loads and to significantly slow down $CD4^+$ T-cell depletion in monkeys challenged with pathogenic $SHIV_{89.6P}$.[807] The association of a single intradermal or intramuscular inoculation of GM-CSF DNA with the DNA prime enhanced the avidity maturation of non-neutralizing anti-Env IgG in the plasma and the presence of long-lasting antiviral IgA in rectal secretions of rhesus monkeys, but had no effect on the elicited T-cell responses. Upon rectal challenge with $SHIV_{89.6P}$, the animals with the highest avidity anti-Env antibodies and an enhanced IgA response had up to a 1000-fold reduction in peak viremia, demonstrating the importance of avidity for nonneutralizing antibody-mediated control of viral infection.[831,832] Similar observations were made with DNA-MVA prime-boost vaccination against clade B (strain ADA) or clade C (strain IN98012) HIV-1, followed by challenge with R5 $SHIV_{SF162P3}$, which showed inverse correlation between the avidity of the anti-Env antibodies and peak postchallenge viremia.[833] DNA-MVA prime-boost immunization was shown to induce in rhesus macaques high-frequency virus-specific $CD4^+$ $CD28^+$ central memory T-cell responses with a polyfunctional repertoire[834] that were associated with a survival advantage following challenge with SIVmac251.[835] More recently, CD40L-adjuvanted DNA/MVA SIV vaccine was found to enhance protection against neutralization-resistant mucosal SIV infection.[836] Alphaviral replicon DNA expressing HIV antigens has also been shown to be an effective prime for MVA or with a protein subunit boost.[837] A broadened immune $CD8^+$ T-cell response was obtained by using mosaic HIV vaccines in a DNA prime-vaccinia virus boost regimen, resulting in responses to more CTL epitopes of each viral protein and to more variant sequences of each epitope than did consensus immunogens.[305]

Prime-Boost Using Two Different Live Recombinant Vaccines

Another type of prime-boost vaccine regimen was developed using two different live recombinant vectors expressing the same antigen, such as an Ad5 vector followed by a poxvirus vector[838]; or two successive adenovirus vectors, such as Ad11 followed by Ad35,[839] or Ad26 followed by Ad5[435,840] or by Ad35; or two successive poxvirus vectors. Responses of rhesus monkeys to an Ad5-poxvirus (MVA or ALVAC) prime-boost regimen were significantly greater than those elicited by homologous regimens with the individual vectors. Similar findings were obtained with a bacille Calmette-Guérin (BCG) prime-recombinant adenovirus boost.[840]

Ad26 prime/Ad5 boost elicited cellular immune responses with augmented magnitude, breadth, and polyfunctionality as compared to the homologous Ad5-only regimen. After viral challenge, the Ad26/Ad5 vaccinated animals showed a 1.4 log reduction of peak and a 2.4 log reduction of set-point viral loads, as well as reduced AIDS-related mortality, as compared to control animals.[435] In another experiment, Ad26 and MVA vectors expressing the SIV Gag, Pol, and Env antigens were compared in a series of parallel prime-boost vaccination regimens in rhesus macaques. The Ad26/MVA and MVA/Ad26 regimens were more potent inducers of Gag-specific and Env-specific binding antibody responses and Gag-, Pol-, and Env-specific $CD8^+$ and $CD4^+$ T-cell responses than DNA/MVA or MVA/MVA. They also induced the best control of infection after repeated intrarectal challenge with SIVmac.[841]

Adenovirus/poxvirus and adenovirus/adenovirus vector-based vaccines, expressing HIV-1 mosaic Env, Gag, and Pol, afforded a significant reduction in the per-exposure acquisition risk following repetitive, intrarectal $SHIV_{SF162P3}$ challenges. Protection against acquisition of infection correlated with vaccine-elicited binding, neutralizing, and functional nonneutralizing antibodies.[639]

Live Vectored Vaccine Prime-Protein Boost

Quite a different prime-boost regimen strategy was developed using a combination of a live vectored vaccine as a prime

followed by a protein subunit vaccine as a boost, in the hope to stimulate both the humoral and the cellular arms of the immune system. For instance, rhesus monkeys were immunized with an ALVAC-SIV prime followed by SIV gp120 boosts. Of the 12 animals challenged rectally with repeated low doses of SIVmac, three were completely protected, two of which showed good ADCVI responses.[842,843] The same type of prime-boost was used in the RV-144 trial in humans, using an ALVAC-HIV prime and an HIV-1 gp120 boost (see "Human Efficacy Trials of HIV-1 Vaccines" below).

Priming systemic immune responses with intramuscular immunization with ALVAC-SIV vaccines, followed by intravaginal HPV pseudovirions (HPV-PsV-SIV/gp120) boosting, expanded and/or recruited T cells in the female genital tract. High-avidity antibody responses to the envelope gp120 V1/V2 region correlated with delayed SIVmac251 acquisition, whereas virus levels in mucosal tissues were inversely correlated with anti-ENV $CD4^+$ T-cell responses. In addition, $CD8^+$ T-cell depletion in animals with controlled viremia caused an increase in tissue virus load in some animals, suggesting a role for $CD8^+$ T cells in virus control.[844]

A nonneutralizing antibody response was induced in rectal secretions by a replicating Ad5hr-SHIV prime/gp120 protein boost that provided partial control of virus replication after SHIV challenge in macaque monkeys.[486,487] Improved control of infection against intrarectal SIVmac challenge was similarly provided to macaques by immunization with replication-competent Ad5hr recombinants expressing SIV Env and SIV Gag followed by boosting with SIV gp120[845]; in both experiments, ADCC and transcytosis-blocking antibody activities were found to correlate with reduction in chronic viral load after challenge, suggesting that nonneutralizing mucosal antibodies helped control cell-to-cell viral spread during the course of infection.[491,492]

More recently, NYVAC was found to be a more effective vector than ALVAC in stimulating HIV-specific cellular and humoral immune responses in rhesus macaques in prime-boost studies in combination with gp120 clade C protein, and is now advancing to human trials.[846] Heterologous prime-boost regimens with a recombinant chimpanzee adenoviral vector and adjuvanted F4 protein elicited polyfunctional HIV-1–specific T-cell responses in macaques, suggesting that regimens combining adjuvanted protein and low-seroprevalent chimpanzee adenoviral vectors represent an attractive vaccination strategy for clinical evaluation.[847]

Finally, rhesus monkeys primed with Ad26 vectors expressing SIVsmE543 Env, Gag, and Pol and boosted with AS01B-adjuvanted SIVmac32H Env gp140 demonstrated complete protection in 50% of vaccinated animals against a series of repeated, heterologous, intrarectal SIVmac251 challenges that infected all controls. Protective efficacy correlated with the functionality of Env-specific antibody responses. Comparable protection was also observed with a similar Ad/Env vaccine against repeated, heterologous, intrarectal $SHIV_{SF162P3}$ challenges[848]; these strategies are now advancing toward clinical trials.

DNA Vaccine Prime-Protein Boost

Still another type of prime-boost immunization regimen has been studied, combining DNA vaccine for priming with an *env* protein subunit vaccine for boosting, and provided successful protection from SHIV challenge in macaques.[849,850] Using a mixture of *env* DNA plasmids for priming and a mixture of rgp120 proteins from different HIV-1 clades for boosting[851] neutralizing antibodies were generated against primary HIV-1 isolates belonging to different virus subtypes. Immunization of rhesus macaques using this type of prime-boost regimen and enhancing the immunogenicity of the rgp120 boosts by the addition of a QS21 adjuvant protected four of six monkeys against rectal challenge with an R5 SHIV.[852]

More recently, studies have been undertaken using a DNA+vector prime, plus an HIV-Env protein boost. Studies with DNA+recombinant NYVAC prime, followed by an HIV-Env protein boost comparing transmitted-founder B clade Env (B.1059), a global group M consensus Env (Con-S), and a global trivalent mosaic Env protein in rhesus macaques, found that in silico–designed global HIV-1 immunogens were capable of not only eliciting T-cell responses, but also of eliciting potent anti-Env antibody responses.[853] In addition, DNA and virus particle vaccination protects against acquisition and confers control of viremia upon heterologous SIV challenge.[854] Lastly, an enhanced synthetic multiclade DNA prime using a single HIV-Env consensus sequence or multiple constructs expressing HIV subtype-specific Env consensus sequences induced improved cross-clade reactive functional antibodies when combined with an adjuvanted protein boost in NHPs.[855]

Synthetic Peptide Vaccines

Initial attempts to use synthetic peptide immunogens focused on the V3 loop of gp120, including presentation as a multiple antigenic peptide system.[856] Multiepitopic combinations of peptides, fusion proteins, and long lipopeptides are at an early stage of clinical development, either alone[857,858] or in prime-boost combinations with live vector-based recombinant vaccines.[859,860] Peptide mimetics of the gp41 prehairpin fusion intermediate were able to elicit neutralizing antibodies against HIV-1 isolates.[861] HIV-1 lipopeptides with the sequence of Gag, Pol or Nef CTL epitopes have the capacity to elicit a $CD8^+$ T-cell response[862] and are being developed and tested as therapeutic vaccines.[863–865]

Synthetic long peptide (SLP) booster immunization in rhesus macaques primed with replication competent NYVAC-C induced a balanced CD4/CD8 T-cell and antibody response against the conserved regions of HIV-1.[866] Moreover, the humoral responses induced by a fusion gene expressing conserved regions of the HIV genome (HIVconsv) administered to macaques were assessed using six different vaccine modalities: plasmid DNA, human Ad5, Chimpanzee adenovirus serotype 63, MVA, Semliki Forest virus replicons, and adjuvanted overlapping SLPs. It was observed that only the SLP formulation induced antibodies recognizing linear HIVconsv epitopes.[867]

Mucosal Immunity

The genitourinary, rectal, or oral mucosa is the site of entry of HIV-1 in sexually acquired infections. Although oral transmission of HIV-1 is far less common than vaginal and rectal transmissions, infections through the oral route do occur through oral sex as well as vertically, from mother to child. Therefore, there is great interest and demand for vaccines that target mucosal sites of infection and would prevent virus entry and/or the establishment and dissemination of infection.[868–870] Oral vaccination usually gives an immune response in the gastrointestinal tract and in breast milk, while nasal immunization can promote strong IgA responses in both the female genital tract and rectum.[587,870–872] Thus, intravaginal inoculation of mice with an MVA recombinant expressing HIV-1 *env* was not immunogenic, whereas intranasally it induced a significant immune response in the genital mucosa, especially when combined with cholera toxin as an adjuvant.[873] A vaccination regimen including oral–intranasal–intratracheal priming with an adenovirus-SIV recombinant followed by boosting with gp120 in monophosphoryl lipid A emulsion

elicited rectal anti-gp120 IgA and IgG responses that were protective against rectal SIV challenge,[874] and oral immunization alone or oral and nasal immunizations with the same prime-boost regimen was significantly associated with protective mucosal immune responses.[875] Nonneutralizing mucosal antibody activities may thus be important contributors of immune protection.

A gp41-based vaccine candidate was manufactured by inserting trimeric rgp41 and a gp41 MPER peptide into virosomes (liposomes spiked with influenza virus hemagglutinin and neuraminidase [NA] glycoproteins). The vaccine was administered to female rhesus macaques by the intramuscular route followed by the intranasal route, which resulted in induction of transcytosis-blocking IgAs and ADCC-associated IgGs in cervicovaginal secretions, but no systemic neutralizing antibodies. The presence of transcytosis-blocking IgAs, moreover, correlated with full protection of the vaccinated females against repeated intravaginal challenge with R5 $SHIV_{SF162P}$ and partial cross-clade protection against vaginal challenge with a clade C SHIV. Altogether, the vaccine candidate prevented SHIV acquisition and/or sustained infection in 8 of 11 vaccinated animals,[531] with ongoing studies aimed at reproducing the protection observed by this vaccine regimen. These findings underscore the conclusion that protection may occur at mucosal sites in the absence of neutralization in serum as detected by conventional methods.[876]

Mucosal priming followed by systemic boosting with a fusion protein consisting of the cholera toxin B subunit and a gp41 MPER peptide similarly elicited mucosal anti-MPER IgA and IgG antibodies with significant transcytosis-blocking properties.[877] The induction of humoral immunity at mucosal sites could significantly benefit from specific mucosal adjuvants such as Eurocine L3.[878]

Mucosal adjuvants have also been used to enhance mucosal immune responses to HIV immunogens. α-Galactosylceramide (α-GalCer), a synthetic glycolipid agonist of NK T cells, was shown to enhance immunogenicity of vaccine antigens delivered using viral vectors by mucosal routes in murine and NHP models. Significant improvement in adaptive immune responses in systemic and mucosal tissues was demonstrated by including α-GalCer adjuvant for intranasal immunization of mice with VSV vector-expressing HIV Env and Gag antigens. Activation of NK T cells in systemic and mucosal tissues along with significant increases in adaptive immune responses were observed in rhesus macaques immunized by intranasal and sublingual routes with protein or adenovirus vectored antigens when combined with α-GalCer adjuvant.[879]

CHALLENGE OF ELICITING BROADLY NEUTRALIZING ANTIBODIES AGAINST HIV-1

The unprecedented variability of HIV-1 suggests that induction of bNAbs, for example, antibodies that target conserved regions on HIV-1 Env, will be an important component of a successful HIV-1 vaccine. However, immunization of humans with HIV-1 Env, either as subunit proteins or in vector-subunit prime-boost regimens, have failed to induce bNAbs. A major obstacle in the development of a safe and globally effective HIV-1 vaccine is the challenge of eliciting broad, potent, and durable neutralizing antibodies capable of preventing infection by diverse isolates of HIV-1 circulating worldwide.

Strategies to Elicit Broadly Neutralizing Antibodies Against HIV-1

Longitudinal studies of HIV-infected subjects have begun to reveal important insights in the evolution of bNAbs that is informing vaccine design.[880] CH103 neutralized 55% of HIV-1 isolates, with virus and antibody gene sequencing revealing both virus evolution and antibody maturation, as evolution of neutralization breadth was preceded by extensive viral diversification.[399] The evolution of a bNAb targeting the V1V2 apex revealed rapid development following initial selection of B cells with a long HCDR3 region and limited subsequent somatic hypermutation.[414] Lastly, in a recent lineage analysis, two B-cell lineages cooperated to induce the development of bNAbs.[881]

Several vaccine strategies (for reviews see ref. 681) have been used in attempts to elicit bNAbs against HIV-1, and to date, all have failed. There currently is not a single candidate vaccine that has successfully elicited bNAbs in clinical trials. These strategies can be divided into the following categories: trimer mimic, epitope mimic, consensus antigens and cocktails, and antiidiotypes.

Trimer Mimic

This approach aims to mimic the native trimer on the surface of the HIV-1 virion. Trimeric gp140 molecules (gp120 + the ectodomain of gp41) stabilized by the addition of heterologous trimerization motifs at the C terminus of the gp41 sequence,[683,684] and similar trimers with a deletion of the hypervariable V2 loop have been designed with the aim of better exposing the neutralization epitopes that overlap the CD4-binding site.[343,685,686] Similarly, trimeric gp140 molecules internally stabilized by a disulfide bond between gp120 and gp41 (SOS proteins) have been designed and screened preclinically.[688,689] Immunogenicity studies with these trimer mimics and other related trimer mimics have generally showed modest improvements in immunogenicity when compared with monomeric gp120.[694]

With the recent identification of broader and more potent neutralizing monoclonal antibodies, high throughput screens have been developed aimed at identifying HIV-1 Env proteins that preferentially bind these antibodies and using these proteins as the basis for the next generation of trimer mimic designs. The immunogenicity of HIV-1 envelope glycoprotein oligomers has been reviewed.[882]

Insights into the trimeric HIV-1 envelope glycoprotein structure by cryoelectron microscopy and X-ray crystallography, offer potential for iterative improvements in trimer design.[692,693,883] The recent structure of the BG505 trimer captured in a mature closed state by antibodies PGT122 and 35O22 revealed the prefusion conformation of gp41, and further defined parameters of immune evasion and immune recognition.[380] To systematically explore how immunization might be tailored to produce bNAbs, human Ig knock-in mice were generated with mice expressing the predicted germline or mature heavy chains of a potent bNAb to the CD4 binding site on HIV-1 Env.[884] To date, however, native-like HIV-Env trimers failed to activate B cells expressing such germline antibodies. Most recently, well-ordered trimers were generated by negative selection and display native-like properties.[885]

Mixtures of trimers, administered as cocktails or sequentially, are also being assessed to determine if increased breadth of neutralization can be obtained. A quadrivalent cocktail of clade C trimers elicited a greater magnitude of neutralizing antibodies against a panel of tier 1A and 1B viruses than any single clade C trimer alone, demonstrating that the mixture had an advantage over all individual components of the cocktail.[886]

Epitope Mimic

This approach takes a reverse vaccine engineering strategy, by focusing on the binding sites of bNAbs and attempting to

mimic such binding sites. Structural biology studies have now identified the binding sites for 2F5, 4E10, 2G12, and VRC01, with the 2F5 and 4E10 epitopes being linear, the 2G12 epitope focused on a distinct pattern of high-mannose carbohydrates, and the VRC01 epitope being discontinuous at the CD4 binding site, along with structures of trimer-specific antibodies described above.[883,887,888] Coupling linear epitopes to carriers, expressing such epitopes in nucleic acid or viral vectors, or placing these epitopes in carrier scaffolds are among the approaches being tested to elicit neutralizing antibodies to these epitopes.[354] One interesting approach that has generated modest improvements in neutralization breadth is the use of nonglycosylated outer-domain-derived HIV-1 gp120 immunogen.[889] The first epitope mimic now in early development for clinical trials is the engineered outer domain multimerized on nanoparticles.[890] This computational biological approach to structure-assisted vaccine design is similar to the successful proof of principle for epitope based design of a vaccine candidate against respiratory syncytial virus.[891] Most recently, the engineered outer-domain immunogen-induced antibodies with characteristics of VRC01-class bNAbs, including a short CDRL3 (light-chain complementarity-determining region) in knock-in mice.[892]

Synthetic peptide approaches are also in development as experimental HIV vaccines.[893] Synthetic, homogeneously glycosylated peptides that mimic hypervariable loops V1V2 and bind avidly bNAbs PG9 and CH01 have been designed, and V1V2 glycopeptide dimers bearing Man5GlcNAc2 glycan units bind with apparent nanomolar affinities to unmutated common ancestor B-cell receptors on naïve B cells, suggesting that these glycopeptide constructs may be attractive immunogens for targeting subdominant HIV-1 envelope V1V2-neutralizing antibody-producing B cells.[894] Computational designs of high-affinity epitope scaffolds by backbone grafting of a linear epitopes is also a strategy being employed to elicit bNAbs to MPER epitopes.[895] In addition, peptide mimetics of the gp41 prehairpin fusion intermediate provides for a starting point for immunogens to elicit bNAbs against the gp41 prehairpin intermediate.[861]

Consensus Antigens and Cocktails

In silico analysis of sequences from globally diverse isolates of HIV-1 has led to the development of consensus antigens for use in attempting to elicit bNAbs.[300] In addition, cocktails of HIV-1 Env, either as protein subunits, DNA prime + protein boost, or incorporation into viral vectors (e.g., DNA prime + Ad5 boost), have also been attempted[700–704] without much success. Algorithms to identify amino acid sequence motifs required for recognition by HIV-1 neutralizing monoclonal antibodies[896–898] may help in the design of improved consensus antigens.

Antiidiotypes

Fab fragments from CD4-binding site-directed human IgGs from HIV-1–infected long-term nonprogressors were used as immunogens in mice for hybridoma generation. Two hybridomas, P1 and P2, were shown to recognize the idiotype of the bNAb b12, and when used as immunogens in rabbits elicited strong anti-gp120 responses, with modest HIV-1–specific neutralizing antibody activity in the first preclinical proof-of-concept study of the antiidiotype approach.[899]

Summary and Future Directions

Induction of bNAbs against HIV-1 remains the greatest challenge in HIV-1 vaccine development. Recent identification of new targets on the HIV-1 virion for broadly neutralizing monoclonal antibodies has revitalized the field and offered several promising avenues for vaccine design. However, several key questions still remain including: Why does it take 2 to 3 years in HIV-infected subjects to elicit bNAbs? Why does this occur in only a subset of HIV subjects?[405] To address these questions, acute infection cohorts have been established and scientific consortia set up to probe the evolution of bNAbs.[880] Not much is known as to why HIV isolates fall into categories of neutralization-sensitive versus neutralization-resistant isolates at the molecular level. Understanding how HIV-Env differs from these sensitive and resistant isolates may help with vaccine design to elicit bNAbs. Interestingly, envelope glycans of HIV virions are almost entirely oligomannose antigens, in contrast to recombinantly generated gp120.[900] There is now a greater recognition of the role of glycans as key components of epitopes at supersites of bNAb recognition, including the V1V2 apex and N-332 sites, respectively.[901] Lastly, as more antigens emerge capable of binding the bNAbs, formulation of such antigens with novel carriers and adjuvants may potentiate the induction of bNAb responses.

PASSIVE IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY

Although passive administration of monoclonal antibodies is effective in a variety of disease settings, there are no monoclonal antibody products that are licensed for use for immunoprophylaxis or immunotherapy for prevention or treatment of HIV infection.[902] While practical considerations may preclude the use of monoclonal antibody preparations as a prophylactic passive immunization strategy for prevention of HIV-1 infection in large populations of the developing world, it is possible to consider passive immunoprophylaxis in the context of prevention of mother-to-child transmission of HIV-1,[903] or potentially in a postexposure immunotherapy setting. However, it should be noted that efforts are ongoing to significantly improve half-life of antibodies, and thus future engineered antibodies may be utilized as for PrEP.[904] The recent advances described above, and the development of more broad and potent neutralizing monoclonal antibodies against HIV-1, has reinvigorated studies on passive immunoprophylaxis and immunotherapy.

Preexposure Immunoprophylaxis

One of the considerations for the use of bNAbs in a passive immunoprophylaxis setting is to provide proof-of-concept in humans that such antibodies can mediate protection against HIV-1. Such studies might also provide an indication of the levels of antibody required for protection, which would be helpful for HIV-1 vaccine development. However, the strategy would be more feasible for broader applications if the potency and longevity of the antibodies could be optimized. A variety of molecular engineering strategies have been developed that can increase the potency and half-life of antibodies in circulation.

Potency of ADCC can be enhanced through the FcRγIII receptor.[905,906] Half-life of antibodies can be significantly enhanced through modification of the neonatal Fc receptor.[907] Enhanced neonatal Fc receptor function improved protection against primate SHIV infection with bNAbs directed against the CD4 binding site of HIV-Env.[508] In addition, it was demonstrated that neutralizing 2F5 IgG at the portal of infection protected against vaginal SHIV challenge, whereas 2F5 Fab was not protective.[908] Moreover, modification of the glycoforms of monoclonal antibodies can be exploited to provide maximum efficiency for a given disease indication.[909]

More recently, passive transfer of modest titers of potent and broadly neutralizing anti-HIV monoclonal antibodies were shown to block infection in macaques, whereas no protection was observed following passive transfer of polyclonal highly functional low-dose neutralizing antibodies.[910,911] Somewhat surprisingly, the protective efficacy of bNAbs directed to the HIV-Env–versus–SHIV challenge was greater than antibodies directed against the CD4 receptor, despite comparable or greater neutralization potency in vitro by the anti-CD4 antibody.[912] Lastly, combinations of bNAbs provided improved neutralization profiles in vitro compared with individual bNAbs, suggesting that combinations of bNAbs should be considered for clinical use.[913] Future studies should also assess the protective efficacy of bNAbs against cell-associated mucosal transmission.[914]

Vector-Mediated Gene Delivery

As a result of the practical limitations for product development and large-scale application of passive immunoprophylaxis against HIV-1, other strategies are being explored, including the long-term expression of antibody by gene transfer, that is, vector-mediated gene delivery. Recombinant adeno-associated virus (rAAV) vectors have been shown to successfully deliver HIV-1 bNAb IgG1b12 to mouse muscle, with significant levels of HIV-1 neutralizing activity maintained in the sera of mice for more than 6 months after a single intramuscular administration of the rAAV vector.[915] These studies led to the recently successful demonstration of long-lived neutralizing activity and protection against SIV in monkeys.[415] To fully exploit this technology, iterative clinical trials aimed at optimizing the rAAV subtype and selection of genetic inserts will likely be required. At the time of this writing, a prototype rAAV-1 expressing the bNAb PG9 is currently being assessed in clinical trials for levels of expression and duration of PG9 neutralizing antibody (P.R. Johnson, personal communication, 2016).

AAV8-based vectors are also being evaluated as delivery vehicles for bNAbs and will soon be entering clinical trials.[417] An AAV8 vector encoding a simianized form of the CD4bs bNAb, VRC07 expressed antibody in macaques for 16 weeks at levels up to 66 μg/mL, although immune suppression with cyclosporine was needed to sustain expression. Gene-delivered simian VRC07 protected against SHIV infection in monkeys 5.5 weeks after treatment.[916] Successful implementation of AAV-mediated prophylaxis of HIV infection will require improvements in manufacturing processes for AAV, optimization of vector and insert for expression and durability, and demonstration in efficacy trials of prevention of HIV infection. Most recently, an engineered antibody like molecule eCD4-Ig, a fusion of CD4-Ig with a small CCR5-mimetic sulfopeptide, bound avidly and cooperatively to the HIV-1 envelope glycoprotein (Env) and was more potent at preventing infection than the best bNAbs. Moreover, rhesus macaques inoculated with an AAV vector stably expressed 17 to 77 μg/mL of fully functional rhesus eCD4-Ig for more than 40 weeks and these macaques were protected from several infectious challenges with SHIV-AD8.[144] Thus, in addition to delivering bNAbs, AAV may be considered as a delivery vehicle for viral inhibitory molecules, and therefore may be a platform for prevention of other viral infections beyond HIV.[917]

Lentiviral vectors for engineering of human hematopoietic stem cells are also being evaluated as a strategy for eliciting bNAbs. By transducing human cord blood CD34+ cells with lentiviral vectors encoding a monoclonal anti-HIV antibody, b12 (IgG_1), human B cells were programmed to produce in vitro up to 1.5 μg/mL of this bNAb.[918] Similarly, inhibition of in vivo HIV-1 infection of humanized nonobese diabetic (NOD)/severe combined immunodeficiency (SCID)/γ(c) (null) mice was observed by gene therapy of human hematopoietic stem cells with a lentiviral vector encoding the broadly neutralizing monoclonal antibody 2G12.[919] More recently, mutated self-cleaving 2A peptides were designed as a molecular rheostat to direct simultaneous formation of membrane and secreted anti-HIV immunoglobulins in lentiviral transgenesis studies.[920]

Postexposure Immunoprophylaxis and Immunotherapy

Passively transferred neutralizing antibodies can block lentivirus infection, but their role in postexposure prophylaxis is poorly understood. Immune escape from a single antibody would be expected, thus cocktails of monoclonal antibodies or polyclonal sera might be more beneficial. In this context, it was shown that postinfection passive immunotherapy with polyclonal immunoglobulin with high neutralizing titers against SIVsmE660 significantly controlled SIV infection in rhesus monkeys and accelerated de novo production of neutralizing antibodies.[921]

Preadministration of high-affinity humanized anti–HIV-1 monoclonal antibody KD-247 by passive transfer provides sterile protection of monkeys from heterologous SHIV infection, and postinfection passive transfer of KD-247 protected against SHIV-induced $CD4^+$ T-cell loss in macaque lymphoid tissue.[922]

There have been very few postinfection trials of HIV-1 bNAbs in HIV-infected subjects. Mixtures of 2G12, 2F5, and 4E10 were evaluated in six acutely and eight chronically infected subjects undergoing interruption of ART. Only 25% of the chronically infected individuals showed evidence of a delay in viral rebound, and only 2G12 appeared to be important for the in vivo effect.[923] Administration of the neutralizing monoclonal antibodies also led to an immediate, high activation of the complement system, even in the absence of viremia in the 14 participating HIV-1–infected individuals.[924]

More recently, however, owing to the greater potency and breadth of newly identified monoclonal antibodies, there has been a resurgence of interest in the potential of these bNAbs to provide therapeutic benefit and potentially be components of functional cure strategies. Administration of a cocktail of HIV-1–specific bNAbs, as well as the single glycan-dependent monoclonal antibody PGT121, resulted in a rapid and precipitous decline of plasma viremia to undetectable levels in rhesus monkeys chronically infected with the pathogenic $SHIV_{SF162P3}$.[421] Similarly, two new antibodies, directed to the CD4-binding site and the V3 region (3BNC117 and 10–1074, respectively), were assessed for their ability to block infection and suppress viremia in macaques infected with the R5 tropic SHIV-AD8, and were found when administered together to rapidly suppress plasma viremia for 3 to 5 weeks in some long-term chronically SHIV-infected animals with low $CD4^+$ T-cell levels.[420] These studies led to a first-in-man dose escalation Phase I clinical trial of 3BNC117, in uninfected and HIV-1–infected individuals, and showed that 3BNC117 infusion was well tolerated, and demonstrated favorable pharmacokinetics. A single 30 mg/kg infusion of 3BNC117 reduced the viral load in HIV-1–infected individuals by 0.8 to 2.5 log10 and viremia remained significantly reduced for 28 days.[145]

CLINICAL TRIALS OF HIV VACCINES

Since 1987, when the first preventive HIV-1 vaccine candidate entered clinical trials, several vaccine candidates have been evaluated in safety and immunogenicity trials, and four vaccine candidates have completed efficacy trials. Table 29.4

TABLE 29.4 Summary of Candidate HIV-1 Vaccines Evaluated in Recent Clinical Trials

Vaccine	Immunogenicity[a]	Comment
SUBUNIT PROTEINS, PEPTIDES, AND ADJUVANTS		
gp120: Alum, MF-59; QS21 MPL, MDP, MTP-PE, Liposome/lipid A, AS02a	gp120: ELISA; ADCC; CD4-binding Abs: neutralizing Abs against TCLA isolates; CD4 lymphoproliferation	Phase III efficacy trials of gp120 in alum showed no efficacy[581,582]
F4/AS01 (gag-pol-nef fusion protein)	Polyfunctional CD4 responses[971]	
Lipopeptides	$CD4^+$ and $CD8^+$ cellular immune responses[973]	
Trimeric gp140		
DNA PLASMIDS		
Naked DNA	Cellular immune responses (CD4>CD8), after multiple immunizations[1029]	As standalone candidates, naked DNA vaccines have generally been less immunogenic in humans, compared to preclinical small animal or NHP studies
Adjuvanted DNA: CRL005, IL-15, IL-12	No significant enhancement with adjuvants/ cytokines detected[820]	
Delivered by electroporation	Electroporation enhances immunogenicity[1030]	
Consensus/mosaic DNA		
VIRAL VECTORS		
Adenovirus types 5, 35, 26, 5/48 chimera, ChimpAd63 consensus, Ad mosaic	Adenovirus type 5 elicited cellular immune responses (CD8>CD4), with responses suppressed with antivector immunity[583]	Phase IIb trials: STEP trial in the Americas and Phambili trial in the Republic of South Africa (Ad5–HIV-1 trivalent vaccine)[584,932] showed no efficacy
Pox viruses: *Vaccinia*; MVA, MVA consensus/ mosaic; NYVAC; Fowlpox; Canarypox	Pox vectors: CD4 cellular immune responses >CD8 cellular immune responses	
Venezuelan equine encephalitis (VEE)	First generation VEE, AAV candidates weakly immunogenic	
AAV		
REPLICATING VECTORS		
Tiantan *Vaccinia*	Multiple replicating viral vectors have been demonstrated to be safe and immunogenic, with evidence of efficacy in NHP. First in-class clinical trials underway	
Measles (Schwarz)		
Vesicular stomatitis virus		
Sendai		
Adenovirus types 4 and 26		
PRIME-BOOST REGIMENS		
Vector prime + subunit boost • Canarypox + gp120 • Ad35 prime + gag–pol–nef fusion protein in AS01B	Canarypox (gag, env, prot) + go120 elicit binding Ab, ADCC, neutralizing Ab against tier 1 isolates, and CD4 lymphoproliferative responses	Phase III efficacy trial: RV-144 trial in Thailand (ALVAC-HIV vCP1521+AIDSVAX gp120 B/E)[54] 31% efficacy of Canarypox + gp120 against HIV-1 acquisition
DNA prime + vector boost • DNA + Ad5 • DNA + Ad35 • DNA + MVA • DNA + NYVAC • DNA prime + Ad35 + MVA • DNA prime + ChimpAd63 + MVA	DNA + Ad5 induce both $CD4^+$ and $CD8^+$ multifunctional T cells[1031]	Phase IIb efficacy trial HVTN 505 in men and male-to-female transgender persons who have sex with men (VRC-HIVDNA016–00-VP; VRC-HIVADV014–00-VP) showed no efficacy[945]
DNA prime + subunit protein boost • DNA + gp120	DNA + gp120 elicit polyfunctional T-cell responses and neutralizing antibodies against Tier 1 isolates[969]	
DNA prime + replicating vector • DNA + Tiantan vaccinia • DNA + VSV		
Vector prime + vector boost • Ad26 prime + Ad35 boost • Ad35 prime + Ad5 boost • Ad35 prime + MVA boost • MVA + Fowlpox • ChimpAd63 prime + MVA	DNA+NYVAC or MVA induce multifunctional $CD4^+ > CD8^+$ T cells[958,1032]	
Replicating vector prime + vector boost • Sendai + Ad35		

AAV, adeno-associated virus; Ab, antibody; Ad, adenovirus; ADCC, antibody-dependent cellular toxicity; ELISA, enzyme-linked immunosorbent assay; HIV, human immunodeficiency virus; IL, interleukin; MVA, modified vaccinia Ankara; NHP, nonhuman primate; NYVAC, New York vaccinia virus strain; TCLA, T-cell line-adapted; VEE, Venezuelan equine encephalitis.

[a]See HIV vaccine clinical trials database, www.iavi.org.

summarizes the vaccine concepts that have been tested in clinical trials and the references for further information on these concepts. This section summarizes advances made in the past few years and lessons learned from clinical trials of HIV-1 vaccines (for additional review, see reference 925), from the database maintained by the IAVI (www.iavi.org).

Human Efficacy Trials of HIV-1 Vaccines

gp120

The first HIV-1 vaccine concept to complete Phase III efficacy trials was the recombinant gp120 vaccine developed by VaxGen. Two gp120 proteins based on subtype B (MN, GNE8, termed AIDSVAX B/B) in alum were used for the first trial that enrolled 5403 volunteers who were at high risk for sexually transmitted HIV-1. The volunteers were randomized to receive either vaccine or placebo. The vaccine was shown to be safe in Phase I and Phase II trials, elicited neutralizing antibodies against T-cell laboratory-adapted neutralization-sensitive viruses, and protected chimpanzees from infection in preclinical studies. Participants from the United States, Canada, and the Netherlands received seven injections at 0, 1, 6, 12, 18, 24, and 30 months of study duration, and were seen for a final visit at 36 months. The AIDSVAX B/B vaccine appeared to be safe, but was not effective in preventing infection or altering the course of infection.[580,581,926,927] Further analysis of the magnitude and breadth of neutralizing antibody responses was conducted, and showed that most vaccines mounted potent neutralizing antibody responses against laboratory-adapted HIV-1 isolates such as HIV-1 MN, but very weak responses against circulating primary isolates of HIV-1.[928]

A related HIV-1 vaccine efficacy trial of two gp120 proteins based on subtypes B and CRF01A_E (AIDSVAX B/E) was the first to be conducted in a developing country (Thailand) and paved the way for the RV-144 trial (see below). The trial enrolled 2545 injection drug users, and the results concurred with the other trial—that is, the vaccine was safe but had no effect on prevention of HIV-1.[929] Taken together, the failure of the two AIDSVAX vaccines to prevent HIV infection or suppress virus load in vaccinated subjects who subsequently became HIV-1 infected served to accelerate clinical efforts to assess vaccine candidates that elicited cell-mediated immunity, and to spur basic research and preclinical efforts to design vaccines to elicit bNAbs (described above).

Adenovirus-5

The first HIV-1 vaccine concept aimed at eliciting cellular immune responses against HIV-1 that advanced to efficacy trials was the replication-defective Ad5-Gag-Pol-Nef vaccine developed by Merck. The rationale for the advancement of this vaccine concept to efficacy trials was based on data from Phase I trials and from NHP protection studies using the SHIV model. In Phase I trials, the Ad5-Gag-Pol-Nef vaccine was well tolerated and elicited cell-mediated immune responses based on ELISPOT assays in the majority of immunized subjects, although responses were diminished in subjects with high levels of preexisting immunity to Ad5.[930] In monkeys, analogous vaccines controlled SHIV infection but had limited effect against pathogenic SIV challenge (see above).[931]

A double-blind Phase IIb test-of-concept efficacy trial in 3000 subjects (the STEP Trial) was conducted to assess the efficacy of the Ad5-Gag-Pol-Nef vaccine. Subjects were randomized into either placebo or vaccine arms and received three immunizations of MRKAd5 HIV-1 Gag-Pol-Nef vaccine at 0, 1, and 6 months. The study was stopped early because it unexpectedly met prespecified futility boundaries at the first interim analysis, which suggested that continuation of the clinical trial was unlikely to demonstrate the benefit of the vaccine. The vaccine elicited cell-mediated immune responses in approximately 75% of a random sample of subjects, but did not prevent infection or reduce viral load. In an exploratory analyses of all study volunteers, irrespective of baseline Ad5 antibody titer, the hazard ratio (HR) of HIV-1 infection between vaccine and placebo recipients was higher in Ad5 seropositive men (HR 2.3 [95% confidence interval (CI) 1.2–4.3]) and uncircumcised men (HR 3.8 [95% CI 1.5–9.3]), but was not increased in Ad5 seronegative (HR 1.0 [95% CI 0.5–1.9]) or circumcised (HR 1.0 [95% CI 0.6–1.7]) men.[584]

A second Phase IIb trial of the same vaccine was conducted in South Africa (Phambili Trial). While the STEP Trial enrolled men and women in the Americas, Caribbean, and Australia, the Phambili Trial enrolled men and women in South Africa, where the modes of sexual transmission and HIV-1 risk, subtypes of HIV-1, and background Ad5 seroprevalence differed. Vaccinations were stopped after the first interim efficacy analysis of the STEP study crossed predetermined nonefficacy boundaries.[932] At time of stopping, the Phambili Trial also showed no efficacy for prevention or control of HIV-1 infection. Post hoc analyses of men enrolled in the STEP study showed a larger number of HIV infections in the subgroup of vaccinated men who were Ad5-seropositive and uncircumcised compared with a comparable placebo group. Unblinded, long-term follow-up of subjects from the Phambili study showed that 63 vaccine recipients (16%) had HIV-1 infections compared with 37 placebo recipients (9%), and that risk of HIV-1 infection did not differ according to the number of vaccinations received, sex, circumcision status, or Ad5 serostatus.[933]

Further analysis was undertaken on samples from the STEP Trial, in an effort to understand why the vaccine had no efficacy, and also to attempt to determine mechanisms for the observed enhanced number of infections in subgroups of vaccinees compared with placebo. Using ELISPOT and intracellular cytokine assays, high response rates (90% in male vaccinees) were observed, along with the finding that 66% of responding T cells expressed two or more antiviral cytokines, and responses persisted for more than a year.[583] Thus, although these assays were useful in screening immunogenicity, they were not predictive of efficacy. Recent sequence analyses indicated that the vaccine exerted immune pressure, that is, that sequences from infected vaccinees differed from those in placebos at specific sites, reflective of escape from vaccine-induced cellular immune responses.[934] However, vaccinees who responded positively in ELISPOT assays only recognized a median of one to two epitopes compared with more than 10 epitopes recognized in situations where virus control is observed in monkeys.[819] Thus, the lack of efficacy of the vaccine may have been from insufficient potency and/or breadth of epitope recognition. The mechanisms underlying increased HIV-1 acquisition in Ad5-seropositive uncircumcised subjects remain unclear.[935,936]

Canarypox Prime + gp120 Boost (RV-144)

Preclinical studies have shown that priming with one vaccine candidate, and boosting with another candidate, often yields greater levels of cellular and humoral immune responses than repeated immunization with a single vaccine (see above and Table 29.4). One of the first prime-boost concepts to be evaluated was the use of a Canarypox vector prime + Env protein boost, which advanced to efficacy trials in the RV-144 trial conducted in Thailand. Phase I trial data of this concept demonstrated that the vaccines were well tolerated, and stimulated T-cell–proliferative responses, binding antibodies, and ADCC,

as well as low levels of neutralizing antibodies primarily toward tissue culture laboratory-adapted isolates of HIV-1.

In a community-based randomized, multicenter, double-blind, placebo-controlled efficacy trial, the concept was assessed using two priming injections of recombinant Canarypox vector vaccine (ALVAC-HIV [vCP1521]) expressing subtype B gag/pro and subtype E env, developed by Sanofi Pasteur, at 0 and 1 month, plus two booster injections of ALVAC-HIV [vCP1521] and recombinant gp120 subunit vaccine (AIDSVAX B/E) developed by VaxGen at 3 and 6 months. A total of 16,402 healthy men and women in the Rayong and ChonBuri provinces of Thailand participated in the trial. In the modified intent-to-treat analysis, the vaccine efficacy in preventing HIV-1 infection was 31.2% (95% confidence interval [CI] 1.1 to 52.1, $P = .04$). In contrast, vaccination had no effect on the degree of viremia or the $CD4^+$ T-cell count in subjects in whom HIV-1 infection was subsequently diagnosed[54]; this was the first time a candidate HIV-1 vaccine had demonstrated efficacy in prevention of HIV-1 infection, and thus had a significant impact on the future directions for the field.

Since the demonstration of modest efficacy in the RV-144 trial, there has been extensive ongoing work in the search for an immune correlate of protection, but a correlate has yet to be confirmed.[55,937] Preliminary analysis of subjects from the trial indicated that nearly all subjects elicited binding antibodies to subtype B and E gp120s, and that these antibodies waned significantly after 6 months. ADCC responses were also prevalent in greater than 90% of subjects early postimmunization, and also waned over 6 months. In addition, in post hoc analysis at 6-month intervals, vaccine efficacy reached 60% through 12 months after initial immunization and then rapidly waned, and participants classified as high risk based on self-administered questionnaires had limited efficacy compared with those classified as low risk.[938] Most recently, a statistical evaluation of the RV-144 trial concluded that there was moderate evidence of low-level positive vaccine efficacy but still a greater than 22% chance existed to show no efficacy.[939]

For RV-144, analysis of immune correlates of risk showed that antibodies directed against the V1V2 region of gp120, in particular the IgG_1 and IgG_3 subclass mediating ADCC, seemed to play a role in protection, along with an inverse correlation with plasma Env specific IgA antibodies.[940,941] In addition, studies revealed that the ALVAC-HIV/AIDSVAX vaccine induced V2-specific antibodies that cross-react with multiple HIV-1 subgroups and recognize both conformational and linear epitopes.[942] More recently, using a computational framework for unbiased combinatorial polyfunctionality analysis of antigen-specific T-cell subsets (COMPASS), $CD4^+$ antigen-specific helper T cells were also identified as a potential correlate of protection in the RV-144 trial.[943] While a V1/V2-targeted comparison of the genomes of HIV-1 breakthrough viruses identified two V2 amino acid sites that differed between the vaccine and placebo groups, extension of the V1/V2 analysis to the entire HIV-1 genome identified additional signature sites that were not preferentially restricted to the vaccine immunogens. These data suggested that few of the genetic differences in breakthrough viruses were strongly linked to the RV-144 vaccine-induced immune pressure.[944]

Neutralizing antibodies elicited in this trial were less potent than in earlier clinical trials of the gp120 vaccine alone that failed to show efficacy. Cellular immune responses were weak and irregular, consistent with those observed in Phase I trials of this prime-boost vaccine combination. Plans are being implemented for additional preclinical and clinical studies aimed at focusing in on potential correlates of protection, as well as preliminary planning for a next series of efficacy trials to include licensure trials in Thailand of the same or closely related regimen, and for related trials in South Africa using poxvirus prime and subunit boost candidates developed against the prevalent clade C subtype circulating in southern Africa.

DNA Prime + Ad5 Recombinant Boost

Building on the prime-boost theme, a Phase IIb test-of-concept efficacy trial was conducted to assess the ability of the DNA prime + Ad5 boost vaccine approach to provide control of HIV-1 infection, for example, by decreasing the viral load end point, in subjects where HIV-1 infection is subsequently diagnosed postimmunization. The DNA vaccine included HIV-1 genes *gag-pol-nef* from subtype B, and the *env* gene from subtypes A, B, and C. The Ad5 booster has a similar set of genes as the DNA, with the exclusion of the *nef* gene.

The Phase IIb clinical trial (HVTN 505) enrolled 2504 men or transgender women who have sex with men to receive the vaccine (1253 participants) or placebo (1251 participants). The six-plasmid DNA vaccine was administered at 0, 4, and 8 weeks; the Ad5 vector boost was administered on week 24. The DNA/rAd5 vaccine regimen did not reduce either the rate of HIV-1 acquisition or the viral load set-point in the population studied. The primary analysis showed that week 28+ infection was diagnosed in 27 participants in the vaccine group and in 21 participants in the placebo group (vaccine efficacy: −25.0%; 95% CI, −121.2 to 29.3; $P = .44$), with mean viral load set-points of 4.46 and 4.47 HIV-1 RNA log 10 copies/mL, respectively. Analysis of all infections during the study period (41 in the vaccine group and 31 in the placebo group) also showed lack of vaccine efficacy ($P = .28$).[945]

Since the iPrEx (Iniciativa Profilaxis Pre-Exposición) study reported that PrEP with daily tenofovir disoproxil fumarate/emtricitabine reduced HIV infections by 44% among MSM and subsequent trials corroborated efficacy among heterosexual men and women, HVTN 505 was the first HIV vaccine efficacy trial where PrEP was offered. Most subjects (91%) believed taking PrEP would not change their willingness to stay in the vaccine trial. As new prevention technologies emerge, HIV vaccine efficacy trials will need to take into account their potential impact on HIV incidence in powering sample size and in development of primary end points.[946,947]

Future Phase I/II Safety and Immunogenicity Trials of HIV-1 Vaccines

The next two concepts likely to advance to efficacy trials will be heterologous prime-boost trials with adenovectors plus HIV-Env boost and DNA prime/poxvirus prime + HIV-Env boost.

Heterologous Adenovectors

Preexisting immunity to Ad5 diminishes immune responses to vaccines which use Ad5 as a vector. Alternate adenovirus serotypes as vaccine vectors may overcome this problem, particularly in the developing world where seroprevalence of Ad5 antibodies exceeds 80%. Preclinical studies show that combinations of two adenovirus vectors results in greater protection against a SIV challenge of rhesus monkeys than administration of individual vectors.[304] Moreover, heterologous vector combinations of Ad26 + MVA or Ad26 + protein also provided better protection.[639,848] In small, exploratory Phase I trials of Ad5 and Ad6 in a prime-boost regimen, it was concluded that combining two adenovirus vectors may be a useful vaccine strategy for circumventing isolated immunity to a single adenovirus serotype.[948]

The leading heterologous prime-boost program assessing adenovector as the prime is being conducted by a consortium of partners led by Johnson & Johnson, including Harvard-Beth Israel Deaconess Medical Center, NIAID HIV Vaccines Trials Network, Ragon Institute, and the IAVI, and focuses on the assessment of combinations of Ad26, MVA, and a trimeric gp140 subunit boost. Ad26 expressing HIV-Env was found to be safe and immunogenic, induced antigen-specific humoral and cellular immune responses, and was not accompanied by a detectable increase in mucosal inflammation.[949–951] Preclinical studies with Ad26 expressing mosaic HIV-1 antigens expanded the breadth and depth of cellular immune responses in rhesus monkeys.[952] Currently, the Johnson & Johnson consortium is assessing combinations of Ad26 and MVA expressing mosaic internal antigens and Env of HIV, and gp140 subunit boosts in Phase I clinical trials to determine the best regimen to consider advancing to efficacy trials (H. Schuitemaker, personal communication, 2015).

Ad35, chAd63 (chimp adenovirus 63), and chimeric Ad5/48 have also been assessed as vectors for HIV vaccines, and Phase I trials have been initiated with mucosally delivered Ad4 and replication-competent Ad26. Ad35 as an HIV vector was found to be safe and immunogenic alone and when combined with a subunit boost.[953,954] Interestingly, coadministration of Ad35 + subunit boost provided robust immunogenicity, and may be a novel strategy for simplifying immunization regimens. Ad63 provided significant priming for an MVA boost of a T-cell–based immunogen (HIVconsv) from functionally conserved regions of the HIV proteome.[955] The chimeric Ad5/48 expressing HIV-Env (Ad5HVR48.ENVA.01) was safe and immunogenic, with vector-elicited neutralizing antibody responses greater for Ad48 than Ad5, confirming that Ad-specific neutralizing antibodies in humans are primarily, but not exclusively, directed against the hexon hypervariable regions.[956]

DNA Prime + Poxvector Boost

Several groups are pursuing the development of prime boost approaches using DNA prime + poxvector boosts. GeoVax is advancing a DNA + MVA candidate, which is currently in Phase II trials. In Phase I trials, the vaccine was found to be well tolerated, with 77% and 42% of subjects eliciting $CD4^+$ and $CD8^+$ responses, respectively. In contrast, response rates for humoral immunity were more robust after three immunizations with MVA when compared with the prime-boost regimen.[957,958]

Sandstrom and colleagues[959] evaluated the safety and immunogenicity of another DNA + MVA candidate. Volunteers were immunized with DNA plasmids expressing gp160 of HIV-1 subtypes A, B, and C; Rev B; p17/p24 Gag A and B, and RTmut B by use of a needle-free injection system. The vaccine was administered with or without recombinant GM-CSF, and boosted with a heterologous MVA expressing Env, Gag, and Pol of CRF01A_E. The vaccine was well tolerated, and 92% of vaccinees elicited HIV-specific IFN-γ responses and 55% $CD8^+$ and 100% $CD4^+$ T-cell responses. The MVA vaccine was also tested independently of DNA priming. MVA or placebos were administered intramuscularly or intradermally at months 0, 1, and 3 to 48 healthy volunteers who were at low risk for HIV-1 infection. Cell-mediated immune responses were moderate in magnitude (median IFN-γ ELISPOT of 78 SFC/10^6 PBMC), but high in response rate (70% ^{51}Cr-release–positive; 90% ELISPOT positive; 100% intracellular staining–positive). Binding antibodies against gp120 and p24 were detectable in all vaccination groups, with ADCC detectable at the highest dose (40% positive).[960] The impact of preexisting immunity to vaccinia virus on the induction of HIV-specific responses after immunization of healthy volunteers with a HIV-1 DNA prime-MVA boost vaccine was also evaluated: preexisting immunity did not reduce the proportion of individuals who responded to HIV-1 antigens in the vaccine, but did lower the magnitude of responses.[961] More recently, broad and potent cellular and humoral immune responses were induced following a second immunization of MVA expressing HIV antigens in subjects previously immunized with DNA + MVA.[962] Lastly, a DNA + MVA multigenic combination based on an HIV Indian clade C was similarly found to be safe and immunogenic.[963,964]

A DNA + NYVAC prime-boost regimen was also found to be well tolerated and immunogenic. Intramuscular DNA priming at weeks 0 and 4 followed by NYVAC boosting at weeks 20 and 24 elicited ELISPOT responses, primarily to Env, in 90% of volunteers.[965] The DNA + NYVAC regimen also induced $CD4^+$ and $CD8^+$ HIV-specific immune responses in intestinal mucosa[966]; 83% of NYVAC-specific CD4 T cells expressed $\alpha_4\beta_7$ integrins and the HIV coreceptor CCR-5. These results suggested that the experimental DNA-C/NYVAC-C HIV vaccine regimen induced the homing of HIV-specific CD4 and CD8 T cells in the gut. It was also noted that the order of prime-boosting with NYVAC was important, with Ad5 prime + NYVAC providing greater immune responses than NYVAC prime + Ad5 boost.[967]

A DNA + replication-competent Tiantan *Vaccinia* virus vector expressing Env, Gag, Pol, and Nef is also under study in China, with Phase I trials completed. The Tiantan *Vaccinia* virus was based on the virus used in the smallpox eradication campaigns in China and further attenuated as an HIV-vaccine vector. The vaccines were well tolerated and elicited cellular immune responses.[968] Three immunizations of DNA followed by a boost with Tiantan *Pox* elicited high-levels of cellular immune responses based on intracellular cytokine staining to Env, Gag, and Pol (10/11 subjects) with lower levels (4/11 subjects) to Nef.

DNA + Protein

Priming with DNA followed by boosting with subunit protein is also being considered as a vaccine strategy for HIV-1 vaccines.[810] In Phase I trials, a regimen composed of a DNA prime formulation expressing five-valent Env and monovalent Gag, followed by a five-valent env protein boost elicited polyfunctional T-cell responses, as well as neutralizing antibody responses to primarily neutralization sensitive HIV-1 isolates.[969] However, adverse reactions were observed with this combination of DNA + protein in QS21 adjuvant, including delayed-type hypersensitivity reactions at prior DNA inoculation sites in 43% of subjects, and two cases of systemic vasculitis temporally related to inoculation with the protein + QS21 boost.[970]

Subunit Protein

As noted above, since the failure of the gp120 efficacy trial, preclinical efforts have focused on the development of subunit proteins capable of eliciting bNAbs; but as of this writing, no candidates have advanced to clinical trials. In addition, in the aftermath of RV-144, additional HIV-Env subunit proteins are being developed for combination prime-boost studies with poxvirus primes. At the time of this writing, the first of these HIV-Env–based subunit vaccines, based on HIV-1 subtype C had just entered Phase I trials in South Africa.

Subunit proteins in novel adjuvants are also being advanced, with the aim of eliciting cellular immune responses. A fusion protein (F4) containing four HIV-1 clade B antigens (Gag p24, Pol RT, Nef, and Gag p17) adjuvanted with AS01, a liposome-based vaccine adjuvant containing

3-*O*-desacyl-4′-monophosphoryl lipid A and the saponin QS-21 developed by GlaxoSmithKline has been assessed in Phase I trials. Two doses of the recombinant F4 protein (10, 30, or 90 μg/dose), adjuvanted with AS01 or reconstituted with water for injection, were administered 1 month apart to 180 healthy volunteers. The candidate was well tolerated and induced polyfunctional $CD4^+$ T-cell responses with the highest responder rates seen in the 10 μg F4/AS01 group (100% to three HIV-1 antigens and 80% to all four HIV-1 antigens). Vaccine-induced $CD4^+$ T-cell responses were broadly cross-reactive to all four antigens derived from HIV-1 clades A and C.[971] This candidate has been evaluated in coadministration with prime-boost trials with Ad35.[954]

Lastly, infant HIV type 1 gp120 vaccination (given between 0 and 20 weeks of age) was shown to be safe and to elicit robust and durable anti-V1V2 IgG responses and only rare envelope-specific IgA responses.[972]

Lipopeptides

The French National Agency for Research on AIDS and Viral Hepatitis's HIV-LIPO-5 vaccine includes five HIV-1 peptides, containing multiple CD8 and CD4 T-cell epitopes, coupled to a palmitoyl tail. In Phase I/II clinical trials, the lipopeptides were well tolerated, and stimulated $CD4^+$ and $CD8^+$ cellular immune responses in approximately 60% of subjects.[973] More recently, studies with these lipopeptides, in prime-boost regimens with ALVAC Canarypox vectors were halted because of myelitis potentially related to the lipopeptide vaccines. Only 42% of enrolled subjects received all four immunizations. No appreciable cell-mediated immune responses were detected in this study.[974]

Nucleic Acid and Viral Vectors

Electroporation of DNA, in combination with plasmid IL-12, was found to enhance immunogenicity, with nearly 90% of subjects developing cellular immune responses after three immunizations.[975] Most recently, additional replication competent vector based HIV vaccines have entered clinical trials, including adenoviruses (types 4 and 26), measles, Sendai, and VSV (see Table 29.4), with additional vectors based on CMV likely to enter clinical testing soon.

Summary of the Clinical Pipeline for HIV-1 Vaccines

Following the demonstration of modest efficacy in the RV-144 trial of ALVAC + gp120, efficacy trials of related and improved regimens are likely to begin in 2016 or 2017. In addition, heterologous adenovirus vectors expressing mosaic antigens, and DNA prime + poxvector boost approaches will also likely enter efficacy trials in the coming years. We also anticipate seeing additional replication competent vectors entering into Phase I trials, and hopefully the first HIV-Env subunit proteins to elicit bNAbs.

THERAPEUTIC VACCINES AND FUNCTIONAL CURE

Because HIV-1 infection progresses slowly and cellular immune responses have been temporally associated with initial control of acute infection, there has been significant interest in the development of therapeutic HIV-1 vaccines. However, studies with the first generation of such candidates—including protein subunits, inactivated virus, and other strategies—have shown short-term safety but poor or limited immunogenicity in HIV-infected individuals, and no conclusive clinical benefit.[976,977] More recently, strategies have focused on improving immunogenicity and evaluating the potential of these candidates in the context of antiviral therapy, or in situations of analytic treatment interruptions. Moreover, recent advances in understanding how anti-HIV immune responses are elicited and regulated prompted a surge of interest in harnessing these responses to reduce the HIV "residual disease" that is present in ART-treated HIV-infected individuals, through the use of cell-mediated immune-based vaccines and/or bNAbs.[978] The following section briefly summarizes the approaches for therapeutic intervention with HIV-1 vaccines.

Inactivated Virus

A therapeutic vaccine applied to the tonsils in SIV-infected rhesus monkeys was shown to maintain low viral loads after cessation of ART. Animals received 40 weeks of ART initiated 9 weeks after rectal SIVmac239 infection. During ART, animals were vaccinated (or not) with AT-2 inactivated SIVmac239 using CpG-C ISS-ODN (C274) or polyICLC as adjuvants. The lower viral loads observed in the vaccinated animals provide support for the potential benefit of mucosally delivered vaccines in therapeutic immunization strategies for control of HIV-1 infection.[979] A therapeutic vaccine, combining ALVAC and Remune, an inactivated HIV vaccine without gp120, or ALVAC alone followed by treatment interruption at 24 weeks were monitored to week 48 median time to a viral load of more than 50copies/mL were longer in each of the vaccinated groups compared to placebo, but the subsequent viral load setpoint was not different between the groups.[980]

Subunit Proteins and Peptides

A subunit HIV-1 gp160 vaccine was assessed in Phase I therapeutic immunization protocols, and repeated administration of doses at 0, 1, 2, 3, 4, 6, 9, and 12 months was shown to increase $CD4^+$ central memory cells.[980]

The native HIV-1 Tat protein was chosen as a therapeutic HIV-1 vaccine candidate, with the hypothesis that Tat-specific immune responses might provide control of infection. The vaccine was well tolerated and elicited anti-*tat* antibodies as well as T-helper responses.[981,982] Thirty HIV-positive volunteers receiving a stable regimen of ART with CD4 counts higher than 400 were recruited for the safety evaluation of LFn-p24C, a detoxified anthrax-derived polypeptide fused to the subtype C HIV *gag* protein p24. The vaccine was well tolerated and HIV-specific responses were associated with increases in CD4 counts following immunizations.[983]

Preliminary studies with an adjuvanted protein therapeutic HIV-1 vaccine showed a clinically acceptable reactogenicity and safety profile, and induced polyfunctional HIV-1–specific $CD4^+$ T-cell responses in ART-experienced and ART-naïve subjects.[984]

Another candidate vaccine, Vacc-4x, is being developed by Bionor Immuno AS as a therapeutic vaccine. The vaccine is comprised of four synthetic peptides sequences within the highly conserved HIV core protein p24, which are thought to induce T-cell epitopes. Vacc-4x was assessed in 51 patients infected with HIV-1 and found to be safe and immunogenic, inducing cell-mediated immune responses. This candidate is now undergoing additional, Phase IIa trials to assess the effect of the therapeutic vaccination on viral load compared with placebo.[984,985] More recently, a Phase II randomized double blind placebo controlled trial with this therapeutic vaccine showed that it was safe and immunogenic, and elicited a threefold reduction of viral load.[986]

Another peptide-based therapeutic HIV vaccine was evaluated in Phase I clinical trials (Opal-HIV-gag). The vaccine was administered intravenously after ex vivo incubation with autologous white blood cells. The study was terminated after a serious, potentially life-threatening event of hypotension and the product will not be further developed.[987]

Lipopeptides were also used as a therapeutic vaccine to study immunological responses and assess treatment interruption in HIV-1–infected patients. In a prospective open pilot study, 24 HIV-1–infected HAART-treated patients with undetectable plasma viral loads and CD4 T-cell counts above 350/mm were immunized at weeks 0, 3, and 6 with a candidate vaccine consisting of six HIV lipopeptides. New specific CD8 responses to HIV-1 epitopes were detected after immunization in 13 (57%) of 23 patients. At week 24, subjects were taken off HAART and monitored until 96 weeks. By 96 weeks, 8 of 24 patients had still not resumed HAART having maintained low viral loads, suggesting that a future randomized study with control arm would be warranted.[865]

Pulsed Dendritic Cells

The ANRS HIV-lipopeptide vaccine was also used to pulse monocyte-derived DCs. The vaccine was composed of five lipopeptides from Gag, Nef, and Pol. Monocytes were enriched from HIV-1–infected HAART-treated patients and were cultured for 3 days with GM-CSF and IFN-α. Once pulsed with the lipopeptides, and reinjected into their host, the cells were capable of eliciting HIV-1 antigen-specific responses. The safety and immunogenicity of this DC vaccine was evaluated in a Phase I/II clinical trial in chronically HIV-1–infected patients on HAART. The vaccine was found to elicit polyfunctional HIV-specific T-cell immunity associated with a decrease in viral load.[988]

Therapeutic immunization with HIV-1 peptide-loaded DCs was well tolerated in Phase I trials of HIV-1–infected subjects. Eighteen seropositive subjects on ART underwent leukophoresis to obtain PBMCs for DC generation. The DCs were pulsed with Gag, Env, and Pol peptides and influenza A matrix peptide, and administered either intravenously or subcutaneously at the dose of 3 or 10 million cells. Immunization significantly increased frequency of HIV-1 peptide-specific IFN-γ–positive cells.[989]

Targeting Canarypox-HIV vaccines to DCs elicited anti–HIV-1 immune responses in vitro, and this approach was advanced to Phase I/II trials to evaluate whether adding DCs to Canarypox-HIV vaccine improved virologic control during analytic treatment interruption. The vaccine was well tolerated, but did not lower viral load set-point during treatment interruption compared with the Canarypox vaccine alone, suggesting that additional methods to enhance the immunogenicity of DC-pulsed vaccines will need to be developed.[990]

The hypothesis that therapeutic vaccination against HIV-1 can increase the frequency and suppressive function of Treg cells, thereby masking enhancement of HIV-1–specific $CD8^+$ T-cell response, was assessed by immunization with autologous DCs loaded with HIV-1 Gag peptides. Immunization with a DC-based vaccine against HIV-1 caused a modest increase in Treg cell frequency and a significant increase in HIV-1-specific, Treg suppressive function. The Treg suppressive effect masked an increase in the vaccine-induced anti–HIV-1–specific polyfunctional responses.[991]

Autologous DC vaccination was also attempted as a strategy for therapeutic immunization. A decrease in HIV-1–specific $CD8^+$ T-cell responses associated with a partial control of viral replication occurred in 12 HIV-1–infected patients during autologous DC vaccination.[992]

Autologous RNA-electroporated DCs were evaluated in 10 HIV-1–positive subjects successfully being treated with ART. Autologous monocyte-derived DCs were electroporated with RNA encoding CD40L and HIV antigens (Gag, Vpr, Rev, and Nef) and injected monthly in combination with ART. Mild adverse events included flu-like symptoms, fatigue, and injection-site reactions. No evidence of autoimmunity, changes in viral load, or significant changes in absolute $CD4^+$ and $CD8^+$ T-cell counts were observed.[993,994] This novel concept is being considered for additional trials at this time.

Viral Vectors

HIV-1–infected volunteers who were receiving ART were given a replication-defective Ad5 HIV-1 Gag vaccine in a randomized, blinded therapeutic vaccination study. HIV-1–infected vaccine or placebo recipients underwent analytical treatment interruption for 16 weeks. The vaccine was generally safe and well tolerated, but significant control of viral load was not observed.[995]

The safety and immunogenicity of recombinant MVA and Fowlpox vectors expressing multiple HIV-1 proteins was evaluated in 20 HIV-infected persons with suppressed viral replication on HAART. The vaccines were well tolerated, and increased $CD4^+$ proliferative responses suggested further studies be undertaken to assess viral load.[996]

An interesting observation was made on the effect of therapeutic immunization on the extent of sequence diversity. HIV sequences from chronically infected subjects before and after vaccination with MVA-Nef were assessed, and MVA-Nef reduced Nef sequence variability, in contrast to no changes in Vif or Gag sequence diversity.[997]

In some cases, however, negative effects of viral vector based therapeutic vaccines have been observed, as with ALVAC–HIV-1 vaccine. A multicenter, double-blind, placebo-controlled Phase II clinical trial was conducted in which 65 antiretroviral-treated patients were randomized to receive an HIV-1 recombinant Canarypox vaccine (vCP1452, ALVAC) or placebo, followed by analytical treatment interruption. Patients exposed to vaccine had higher levels of viral replication and more rapid time to treatment resumption. A significant increase in HIV-specific CD4 T cells producing IFN-γ and IL-2 was observed in the vaccine arm compared to the placebo arm following vaccination. In contrast, no difference was observed following vaccination in the phenotype and functional capacity within the CD8 T-cell compartment. These findings suggest that a vaccine-induced transient activation of HIV-specific $CD4^+$ but not $CD8^+$ T cells may have a detrimental effect on HIV outcomes.[998]

NYVAC immunization induced polyfunctional HIV-specific T-cell responses in chronically infected, ART-treated HIV patients and may be further evaluated for therapeutic immunization.[999]

Nucleic Acid Vaccines

DNA vaccines have also been evaluated in the context of therapeutic immunization. A pediatric trial designed to analyze safety and immunogenicity of a therapeutic DNA vaccine was evaluated in 20 HIV-1 vertically infected children in a Phase I trial. Children (6–16 years of age), on stable antiretroviral treatment for at least 6 months with HIV-1 RNA of less than 50 copies/mL and stable CD4 counts (>400 cells/mm^3 or 25%) over 12 months of follow-up, were recruited into the study. The vaccine, a multiclade multigene HIV-DNA vaccine, was well tolerated, and immunogenicity assessments are ongoing.[1000]

A Phase I/II, randomized, placebo-controlled, double-blinded trial was conducted to evaluate the safety and immunogenicity of an HIV-1 DNA vaccine (VRC-HVDNA 009–00-VP) in subjects treated with ART during acute/early HIV-1 infection. Twenty healthy HIV-1–infected subjects who were treated with ART and had HIV-1 RNA of less than 50 copies/mL were randomized to receive either vaccine or placebo. Postvaccination, subjects interrupted antiretroviral treatment, and set-point HIV-1 viral loads and CD4 T-cell counts were determined. No evidence of differences in immunogenicity in subjects receiving vaccine versus placebo, and no significant differences in set-point HIV-1 viral loads or CD4 T-cell counts were detected following treatment.[1001]

Lastly, DermaVir, a plasmid DNA therapeutic vaccine expressing 15 antigens, induced predominantly central memory responses in patients receiving combination ART; studies are continuing with this candidate.[1002]

Summary of Therapeutic Vaccines

Despite several novel approaches being assessed in clinical trials, there is limited evidence for efficacy with therapeutic vaccines against HIV-1 infection. This is likely because of the suppression of key elements of the host immune system conferred by HIV-1 infection. Novel vaccine strategies will soon be entering into clinical trials in HIV-infected subjects, with new and more potent adjuvants, with the aim of eliciting immune responses capable of providing long-term control of infection.

The recent advances in understanding the HIV reservoir, coupled with advances in histone deacetylase inhibitors as potential agents to stimulate the provirus from the reservoir, have led to a reinvigoration of efforts on therapeutic vaccines and antibody based strategies for functional cure.[1003] Several challenges remain however, including development of biomarkers for the reservoir and clinical trials with both histone deacetylase inhibitors and immune interventions.

SUMMARY AND FUTURE DIRECTIONS IN HIV VACCINE DEVELOPMENT

The HIV vaccine field is currently undergoing a renaissance[1004,1005] as a result of three recent advances. First, the demonstration of modest vaccine efficacy in RV-144 has led to clinical research programs focused on correlates of protection, and plans to repeat and expand the observations of the RV-144 efficacy trial. At the time of this writing, plans are being developed for an efficacy trial in South Africa of related immunogens.

Second, a number of new vaccine candidates have recently entered, or will soon be entering clinical trials, which in preclinical studies have outperformed earlier candidates currently in trials, on the basis of either control of infection or in some cases prevention of acquisition of infection in monkeys. The field will also gain knowledge as these candidates progress, and prioritization of the most promising candidates will lead to future efficacy trials.

Finally, the recent identification of broad and potent neutralizing monoclonal antibodies against HIV-1 has led to the identification of new targets for vaccine design, with the aim of eliciting bNAbs. As incremental progress in this field continues, one would expect new and improved candidate vaccines, capable of eliciting such bNAbs, emerging soon.[1006]

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30 Human Papillomavirus Vaccines

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Human papillomavirus (HPV) infections, which are very common, produce epithelial lesions of the nongenital and genital skin and mucous membranes. Although most infections are benign and self-limited, persistent infection by a subset of the more than 150 HPV genotypes (types) can lead to a variety of epithelial malignancies.[1,2] The oncogenic potential of HPVs was shown first in the 1970s by Orth and colleagues for epidermodysplasia verruciformis, a rare condition of widespread nongenital warts that sometimes progress to squamous cell cancer.[3] In the 1980s, zur Hausen and colleagues identified HPV DNA in the majority of cervical cancers,[4] and subsequent epidemiologic studies confirmed that most cases of this cancer can be attributed to the sexual transmission of approximately 12 oncogenic, high-risk HPV types, especially HPV16 and HPV18.[5–7] Cervical cancer is the third leading cause of cancer in women, with an estimated 530,000 new cases and 275,000 deaths annually worldwide, numbers that are predicted to continue increasing over time.[8,9] More than 80% of the cases and deaths occur in the developing world.[10]

The causal link between HPV infection and cervical cancer established the public health importance of this group of viruses. In addition, it is now recognized that infection by HPV16 and HPV18, and infrequently other high-risk types, accounts for a substantial percentage of other anogenital and oropharyngeal cancers (Fig. 30.1).[10] Although cytologic screening is an effective approach to cervical cancer prevention in some developed countries, it has traditionally been too difficult and costly to implement in most developing countries.[11,12] No effective screening strategies have been defined for the other HPV-associated cancers.

The availability of vaccines designed to prevent disease caused by the most oncogenic HPV types, HPV16 and HPV18, in the first-generation vaccines and, more recently, by other oncogenic types, is therefore a significant step toward control of these endemic and potentially lethal infections. As detailed below, the vaccines are effective in preventing new infections and dysplasia, but do not appear to alter the natural history of prevalent infection by HPV16 or HPV18.[13,14] As women and men are at very high risk of developing HPV infection soon after initiating sexual activity, HPV vaccination would have the greatest impact if given before they become sexually active.[11,15]

Most of the attention in HPV vaccines has focused on cervical cancer prevention because of the high incidence of this disease and its strong association with HPV infection. While the incidence of cervical cancer remains high in many developing countries, its incidence has been decreasing in the developed world as a result of effective cervical cancer screening programs.[10] However, the incidences of HPV-associated anal cancer and, especially, HPV-associated oropharyngeal cancer have been increasing in many developed countries.[16] HPV vaccination should also have the potential to reduce the incidences of these HPV-associated cancers, as they also appear to be predominantly sexually transmitted.[17]

The prices of the current commercial HPV vaccines are relatively high and do not target some of the high-risk HPV types. Efforts are therefore underway to develop second-generation vaccines that can prevent infection by a broader range of high-risk HPVs and/or reduce the cost of vaccination.[18] Candidate therapeutic vaccines are also under study, as preexisting persistent infections remain very common, and the preventive vaccines are not effective against them.

CLINICAL DESCRIPTION

Genital HPV infection may be subclinical or produce benign lesions such as external genital or anal warts (condylomas), and flat cervical condylomas or low-grade dysplasias of the cervix, anus, vulva, vagina, or penis.[19] Most infections are self-limited, with the majority of low-grade cervical lesions and infections clearing in 6 to 12 months.[20] In some patients, however, HPV infection persists. If caused by a high-risk HPV type, persistent infection may be associated with increased dysplasia that can eventually progress to invasive cancer. Women who are immunosuppressed, as occurs with HIV infection, are at increased risk of persistent cervical infection and malignant progression to cancer.[21] Increased risk for persistent infection and, especially, anal cancer is also seen in HIV-positive men. There is a good correlation between HPV genotype and clinical phenotype (Table 30.1).

Cervical cancer tends to strike relatively young women.[7] In many developed countries, there is a steady rise in incidence from the late-20s to the mid-40s, after which age the rates tend to remain relatively constant. Most cervical cancers occur at the junction between squamous epithelium of the exocervix and the columnar epithelium of the endocervix, called the transition zone. They arise from premalignant neoplastic lesions that most often present first as mild dysplastic lesions that progress to high-grade dysplasias and carcinoma in situ before becoming malignant (Fig. 30.2). Most high-grade dysplasias may arise from infection of a distinct population of transition zone epithelial cells that have maintained embryologic characteristics.[22] In the cervix, mild cellular abnormalities are termed low-grade squamous intraepithelial lesions (LSILs) by cytology, while on biopsy they are referred to as cervical intraepithelial neoplasia grade 1 (CIN1).[23] More significant cellular abnormalities, referred to as high-grade squamous intraepithelial lesion (HSIL) on cytology and CIN2/3 on biopsy, may be present within a low-grade cervical lesion as early as 12 months after primary infection.[24] It was recently suggested that the CIN1–3 terminology be replaced with a two-tier LSIL or HSIL histologic designation, but this review retains the former convention because it is used in most of the cited literature.[25] The entire process from infection to invasive cancer usually takes 1 to 3 decades.[20]

HPV infections by so-called low-risk (nononcogenic) HPV types are not generally associated with malignant potential. Approximately 90% of anogenital warts are caused by low-risk types HPV6 and HPV11.[26] As with infection by high-risk types, the majority of lesions are self-limited, although some warts may persist for several years, and persistence is more common in patients with impaired cell-mediated immunity. Nononcogenic HPVs can also cause recurrent respiratory papillomatosis (RRP) a rare condition characterized by recurrent papillomas in the upper respiratory tract. Based on age of onset, RRP is classified as juvenile-onset recurrent respiratory papillomatosis (JORRP) or adult onset. RRP occurs less commonly than

genital warts but is caused by the same HPV types.[27] The condition is more likely to develop in infants and young children, where the papillomas may cause hoarseness and substantial morbidity because of partial airway obstruction and their recalcitrance to treatment. JORRP is believed to arise mainly by transmission from a mother with genital HPV infection to her child during labor.

The natural history of infection with high-risk HPVs at anogenital sites other than the cervix and in the oropharynx is less-well understood, although premalignant lesions of the anal verge, termed *anal intraepithelial neoplasia 3 (AIN3)*, and of the vulva (VIN3) and vagina (VaIN3) are well recognized and have some potential for malignant transformation.[28,29] The risk of malignant transformation of high-grade anogenital lesions at anogenital sites other than the cervix is not as clearly defined as in the cervix, although the lower incidence of HPV-associated carcinomas at these sites suggests the risk may be lower.

HPV-positive oropharyngeal cancer is believed to arise following long-term persistent infection at this site.[17,30] Consistent with this hypothesis, seropositivity for HPV16-specific antibodies was associated with an increased risk of oropharyngeal cancer even when the antibodies were detected more than 10 years prior to cancer development.[31] The tumors, which are squamous cell carcinomas that often involve the tonsillar area, frequently arise in patients who lack of history of tobacco use. By contrast, cigarette smoking and alcohol abuse are important risk factors for HPV-negative oropharyngeal cancers. Premalignant stages for the HPV-associated infections have not been well defined, and there is no approved screening test for at-risk individuals.

HPV16 and HPV18 are associated with approximately 70% of cervical cancer in all regions of the world. The two are found in an even greater percentage of the HPV-associated vulvar/vaginal, anal, and oropharyngeal cancers worldwide.

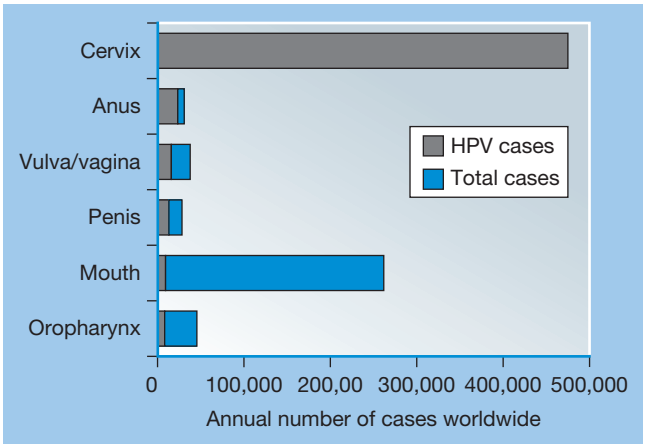


Figure 30.1. Worldwide annual incidence of human papillomavirus (HPV)-associated cancers and fraction attributable to HPV infection. *(Modified from Orth G. Host defenses against human papillomaviruses: lessons from epidermodysplasia verruciformis.* Curr Top Microbiol Immunol*. 2008;321:59–83; and Chaturvedi AK. Beyond cervical cancer: burden of other HPV-related cancers among men and women.* J Adolesc Health*. 2010;46[4 Suppl]:S20–S26.)*

VIROLOGY

Papillomavirus (PV) virions are composed of nonenveloped icosahedral capsids approximately 55 nm in diameter that contain a double-stranded circular DNA genome of approximately 8 kb (Fig. 30.3).[2] PVs are found in many vertebrate species and replicate in the nuclei of stratified squamous epithelia. They are highly species-specific and are named according to the host species they infect: HPV for human PV, BPV for bovine PV, CRPV for cottontail rabbit (Shope) PV, etc. PVs are

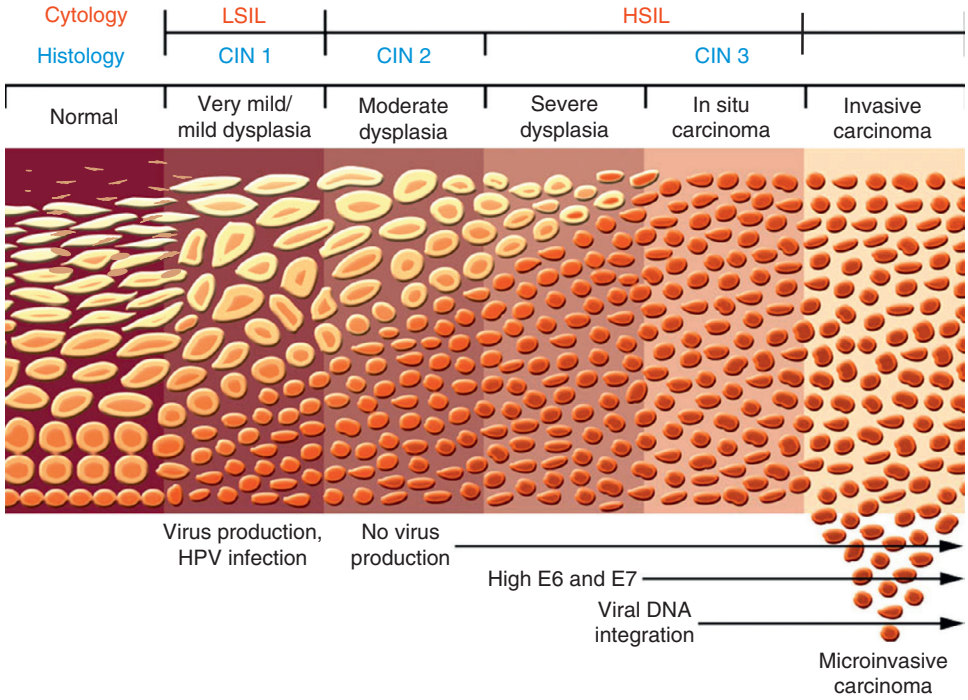


Figure 30.2. Progression of human papillomavirus (HPV) infection to cervical cancer. A schematic of the cervical stratified squamous epithelium is depicted. Cytological and histological classifications are shown at the top and virologic events are shown at the bottom. CIN, cervical intraepithelial neoplasia; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion. *(From Lowy DR, Schiller JT. Prophylactic human papillomavirus vaccines.* J Clin Invest*. 2006;116[5]:1167–1173.)*

TABLE 30.1 Broad Classification of Human Papillomavirus Genotypes

Group	Site of Infection	Lesions	Cancer Association	Common Examples	Genus
Benign skin	Keratinized epithelium	Palmar/plantar warts	No	HPVs 1, 2, 4	Mu, alpha, gamma
EV associated	Keratinized epithelium	Flat warts or subclinical	Yes in EV	HPVs 5, 8	Beta
Genital warts	Genital epithelium and mucosa	Warts	No	HPVs 6, 11	Alpha
High-risk genital	Genital epithelium and mucosa	Flat warts or subclinical	Yes	HPVs 16, 18, 31, 45	Alpha

EV, epidermodysplasia verruciformis; HPV, human papillomavirus.

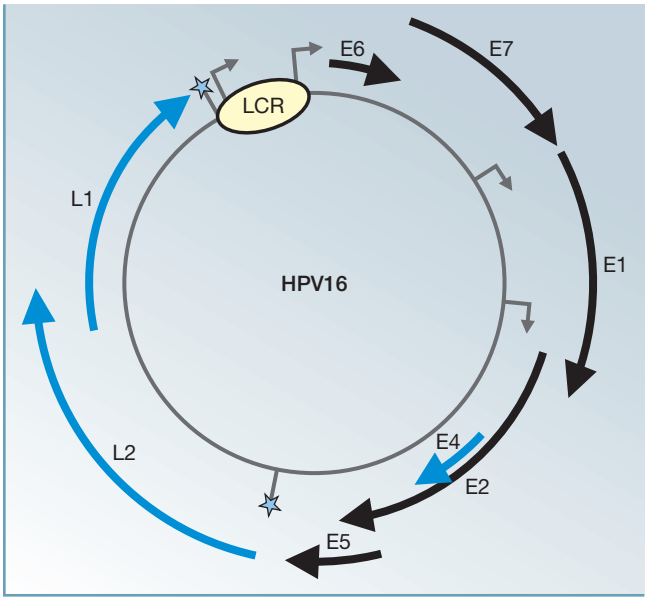


Figure 30.3. Schematic of the 7.9-kb double-stranded DNA genome of human papillomavirus type 16 (HPV16). *Large arrows* indicate open reading frames (ORFs; protein coding regions) and direction of transcription. *Black arrows*, ORFs expressed in the lower epithelial layers; *blue arrows*, ORFs expressed only in the upper epithelial layers; *gray arrows*, transcription start sites; *blue stars*, polyadenylation sites. LCR, long control region.

classified phylogenetically into genera (alpha, beta, gamma, etc), species (1, 2, 3, etc.), types, subtypes, and variants.[32] They are classified according to the comparative homology of their L1 open reading frame, which encodes the major capsid protein and is the most conserved PV open reading frame.[33] PVs tend to have similar phylogenetic relationships when homologies between other regions of the genome are compared, as these viruses appear to have arisen primarily via point mutations scattered throughout the genome, rather than by recombination between PVs. Most of the more than 150 HPV types belong to the alpha, beta, gamma, or mu genera. The alpha HPVs include the high-risk (α5, α6, α7, α9, and α11) and low-risk (such as α10 HPV6 and HPV11) HPVs that cause genital infections, as well as most of the HPVs that cause nongenital common warts (Fig. 30.4). In many people, the beta HPVs may be asymptomatic commensal agents, although they cause lesions in patients with epidermodysplasia verruciformis. The gamma and mu HPVs tend to be asymptomatic or cause foot warts (see Table 30.1).[2] Some of these genera also include animal PVs.

PVs encode two structural (L) proteins, the major (L1) capsid protein and the minor (L2) capsid protein (Fig. 30.5). They are designated major and minor, respectively, because the capsid contains more molecules of L1 than L2. L1 monomers spontaneously form pentamers that can self-assemble to form virus-like particles (VLPs) that, as in authentic virions, have 72 pentameric capsomers and possess the conformation-dependent immunodominant neutralization epitopes, which are predominantly type-specific.[34] The commercialized preventive HPV vaccines discussed later in the chapter are composed of L1 VLPs from several HPV types. In virions, up to 72 L2 molecules are found beneath the axial lumen of the L1 capsomeres.[35] L2 has several functions required for efficient infection.[36] Its aminoterminus contains cryptic epitopes that are broadly cross-neutralizing.[37]

PVs also encode several nonstructural (E) proteins present in infected cells but not the mature virion (see Fig. 30.3). E1 binds the viral origin of DNA replication and possesses a helicase function, whereas E2 is a transcription and replication regulator that binds to E2 binding sites present in several locations in the viral genome.[38] E4 can disrupt the keratin intermediate filament network.[39] E5, E6, and E7 are the main viral oncogenes.[40] E5 from high-risk HPVs appears to contribute to oncogenesis through an interaction with epidermal growth factor receptor.[41] E6 and E7 from high-risk HPVs encode multifunctional oncoproteins.[42] Their best-known biochemical activities are the ability of E6 to inactivate the p53 tumor-suppressor protein by inducing its ubiquitin-dependent degradation,[43] and of E7 to inactivate the retinoblastoma protein tumor suppressor.[44] The viral genome also has a region, the long control region, which does not contain open reading frames but instead includes the viral origin of replication and numerous cis-regulatory elements (see Fig. 30.3).

PVs have a complex life cycle that is adapted to the processes of epithelial wounding and differentiation (Fig. 30.6).[2] In stratified squamous epithelia, cell proliferation is limited to basal cells, with suprabasal cells becoming more differentiated and eventually being sloughed into the environment. Consequently, to induce the long-term infection characteristic of PVs it is necessary to infect and chronically parasitize basal epithelial cells. Evidence from a mouse genital tract PV challenge model suggests that PVs initiate their life cycle by binding to the basement membrane that separates the dermis and epithelium, after exposure of the basement membrane by microtrauma.[45] The cryptic cross-neutralization epitope(s) on L2 become exposed while the virion is still on the basement membrane, where it remains until it transfers to the basal keratinocyte.[46]

The viral life cycle in the keratinocyte can be divided into three phases: viral genome maintenance, production of virion components, and virion assembly (see Fig. 30.6).[47,48] The maintenance phase takes place in the basal cells, which maintain the viral genome as a low copy number episome. E1, E2, E6, and E7 all contribute to this phase. The second phase, which includes viral genome amplification and expression of the capsid proteins, takes place in suprabasal layers. Virion

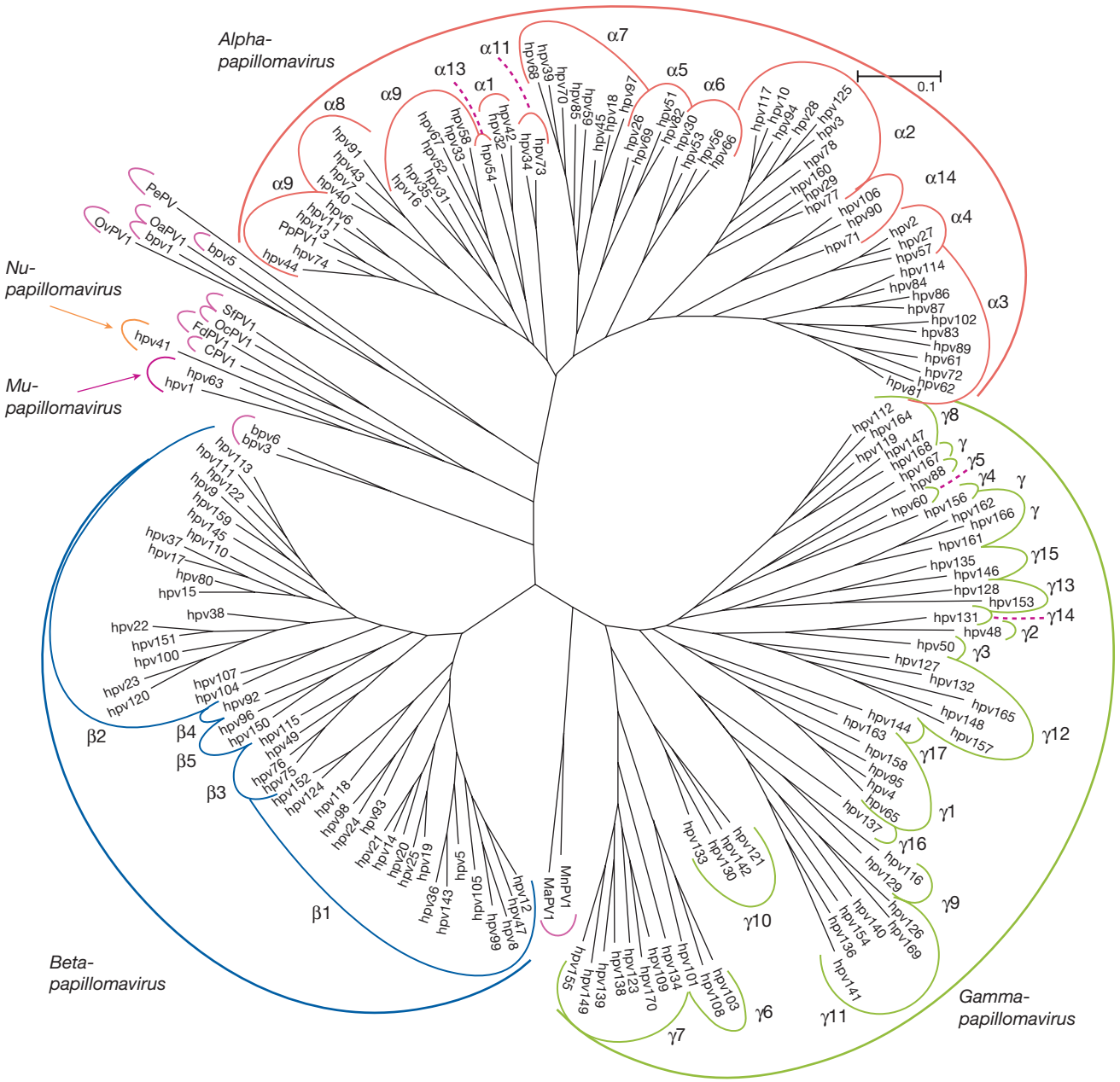


Figure 30.4. Taxonomy of papillomaviruses. The major clades are divided into subclades according to sequence homology within the L1 gene. In general, closely related genotypes produce similar pathology and share some immunoreactivity. *(From de Villiers EM. Cross-roads in the classification of papillomaviruses.* Virology. *2013;445[1–2]:2–10.)*

assembly occurs even closer to the upper epithelial cell layers and leads to eventual shedding of virus.

PATHOGENESIS

The pathogenesis of genital infection in women has been examined in greatest detail.[20,23,40] As noted earlier, genital HPV infection is sexually transmitted and is often acquired soon after sexual initiation. If infected with a low-risk HPV type, such as 6 or 11, the patient may develop genital warts, which do not have malignant potential. If the infection is with a high-risk HPV type, such as 16 or 18, the acute infection may be accompanied by a period of low-grade cytological abnormalities. Most of these infections clear spontaneously, but HPV becomes persistent in a small percentage of cases, and the virus is not cleared from the cervical transformation zone. It is these cases that are at risk of progression to high-grade dysplasia and, in some, to invasive cancer.

The long interval between infection and cancer suggests that although viral infection may be necessary for cancer to develop, it is not sufficient. Other changes in the cell presumably collaborate with the viral genes to produce cancer. The major genetic and environmental determinants of risk of persistence are, with the exception of immunocompetence, not well documented. Cigarette smoking, use of oral contraceptives, high parity, and early age of sexual intercourse all seem to add slightly to the risk of persistent HPV infection and cancer.[49]

Neoplastic changes in cervical epithelium can now be understood in light of the HPV infections that cause them

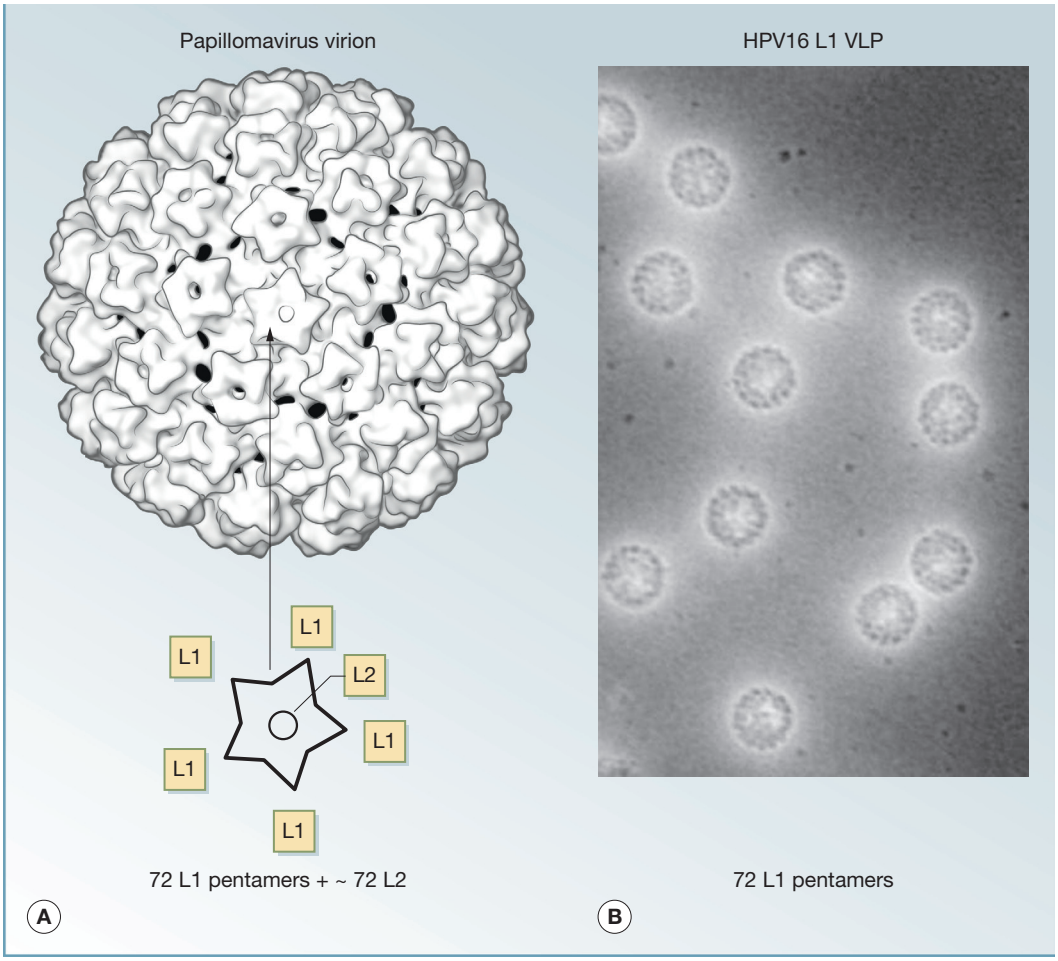


Figure 30.5. A, *Top*, Computer-generated three-dimensional image of a papillomavirus virion; *bottom*, schematic of an L1 pentamer with the location of L2 indicated by the arrow. **B,** Electron photomicrograph of negatively stained HPV16 L1 virus-like particles (VLPs) purified from L1 recombinant baculovirus infected insect cells.

(see Fig. 30.2). The low-grade cervical dysplasias identified in Papanicolaou (Pap) screening tests are simply productive HPV infections. In contrast, high-grade dysplasias and cancers do not produce virions because they do not undergo normal squamous differentiation. Consequently, the L1 and L2 proteins are expressed at low or undetectable levels. The viral genome remains episomal in most high-grade lesions, but is integrated into the host genome in a substantial percentage of invasive cancers.[50] For reasons that are not understood, the frequency of viral DNA integration varies with the HPV type. Integrated viral DNA is found in almost all cancers that contain HPV18 or the closely related HPV45, in about two-thirds of HPV16-positive cancers, and in a minority of cancers that contain HPV31 or HPV33. When the viral DNA is integrated, part of the viral genome is usually deleted, but the E6 and E7 genes are preferentially retained and expressed. Methylation of viral genes, particularly L1, is often associated with progression.[51,52]

E6 and E7, which encode the main viral oncoproteins, function together to destabilize the host genome, leading to aneuploidy and contributing to oncogenic progression. E6 and E7 seem to be required for maintenance of the tumorigenic phenotype. In cervical cancer cell lines, their inhibition leads to apoptosis. There is some evidence that E5 also plays a role in carcinogenesis, especially in tumors where the viral DNA remains episomal.[53] Destabilization of the host genome is associated with a variety of genetic and epigenetic changes in the genome of the host cell that presumably contribute to carcinogenic progression by collaborating with the viral oncogenes.

Among the high-risk HPV types, their relative oncogenicity varies.[54,55] HPV16 is the most oncogenic, in that a higher proportion of HPV16 infections persist and induce high-grade cervical dysplasia or worse when compared with other HPV types, and infections with this HPV type also tend to progress faster than those caused by other types. The next most oncogenic appear to be HPV18, HPV31, and HPV33. The molecular basis for these differences is unclear.

The immune response to HPV infection is incompletely understood.[56] HPVs have clearly evolved multiple mechanisms to evade both innate and adaptive immune responses. For instance, activities of E6 and E7 impair interferon and proinflammatory responses, interfere with antigen presentation, and dysregulate local antigen presenting cells, thereby inhibiting cell-mediated immune responses.[57,58] The development of neutralizing antibodies, which is often delayed for months after initial infection, probably helps prevent reinfection, but is not thought to contribute to clearance.[59,60] As noted earlier, defective cell-mediated immunity is associated with an increased risk of persistent HPV infection and progression to invasive cancer. This observation implies that the host T-cell responses mediate clearance of infection and that infections persist because of an ineffective induction of a host immune response to the infection, or ineffective trafficking of the induced T cells into the infected epithelium.[61] However,

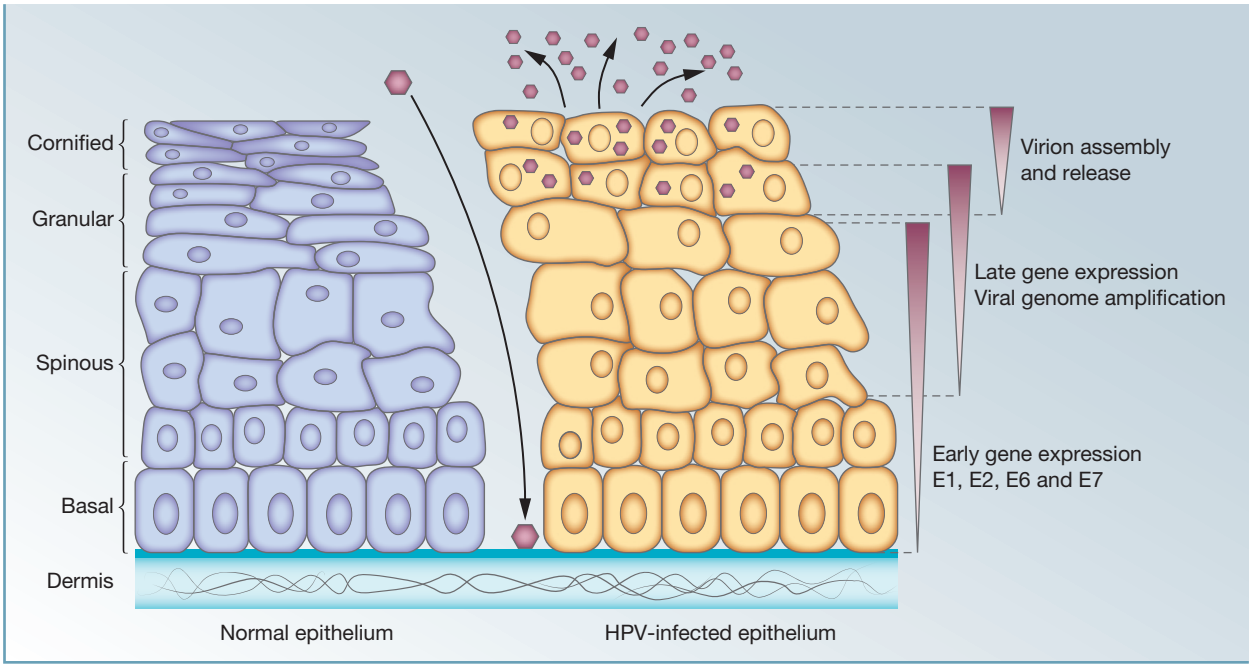


Figure 30.6. Human papillomavirus (HPV) life cycle. Uninfected stratified squamous epithelium is shown on the left and infected epithelium on the right. Binding of the basement membrane (shown in *blue*) at sites of trauma initiates infection of basal keratinocytes, resulting in the establishment of the viral genome as an autonomous replicon in the nucleus. Subsequent events in the viral life cycle are indicated on the far right. *(From Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation.* Nat Rev Cancer. *2010;10[8]:550–560.)*

the principal effector mechanisms and target antigens involved in immune-mediate regression of benign PV lesions are not well understood.

EPIDEMIOLOGY AND TRANSMISSION

The epidemiology of infection with genital HPVs has been examined in greatest detail with large-scale epidemiologic studies of cervical infection.[15,23,62,63] Genital HPV infection is spread through sexual contact. It is estimated that more than 80% of sexually active men and women in the United States will acquire at least one genital HPV infection during their lifetime, of which one-half of the infections will be a high-risk type.[64] Women are at especially high risk of becoming infected soon after initiating sexual activity, with more than 40% becoming infected during the first 2 years. The risk of infection increases with the number of sexual partners, and with the number of sexual partners of a current partner. However, infection is so common that even a first sexual relationship conveys substantial risk.[65]

It is difficult to directly compare the prevalence of cervical HPV infection in international studies because of differences in collection procedures and HPV DNA test procedures, and the paucity of population-based studies. However, in almost all studies, point prevalence peaks at an early age (<25 years) and declines thereafter. In middle-aged women (35–50 years), prevalences tend to be in the range of 15% to 20% globally. Prevalences plateau or decrease in older women in most studies, but there are notable exceptions, particularly in some Central and South American counties, where a second peak is seen in women older than age 40 years and in some countries in sub-Saharan Africa, where there appears to be no decline in women in their 30s.[66]

Most HPV infections are subclinical, with no evident abnormality on inspection, but these individuals are able to transmit infection. Simultaneous infection with more than one HPV type is common. Most infections are self-limited, with about half clearing in 6 to 12 months and approximately 90% clearing within a few years.[67,68] The rate of acquisition decreases once women reach their mid-20s. Consequently, the point prevalence of HPV infection tends to be higher among younger sexually active women, and decreases progressively once women reach their mid-20s. The percentage of seemingly cleared infections, as defined by loss of HPV DNA detection by sensitive polymerase chain reaction (PCR)-based methods, that are retained in a latent state and the cancer risk associated with reactivation from latency are uncertain.[69] Male circumcision is associated with a lower risk of HPV transmission and cervical cancer.[70,71] The regular use of condoms may reduce the risk of transmission,[72] but HPV infection can occur beyond the area covered by condoms.

Genital HPV infection in males has been examined less extensively.[73–75] The sexually associated risk factors for acquisition of HPV infection in females appear to be similar in males. Men who have sex with men (MSM) have a high prevalence of HPV infection and HPV-related disease.[76–78] Among heterosexual males, infection may be even more likely to be asymptomatic than in females. Recent data suggest that the rate of HPV infection in men may be stable throughout their lives, in contrast to the decrease in women as they get older.[73]

Although cervical/vaginal infection has been most studied, anal infection in women also occurs commonly. Genital HPV infection, HIV infection, and a history of receptive anal intercourse are associated with an increased risk of anal infection and neoplastic disease.[79] Among men, MSM have the highest prevalence of anal HPV infection and disease, particularly those who are HIV-positive.[80] However, most cases of anal cancer occur in women, many of whom lack a history of receptive anal intercourse.

Oral HPV infection is detected less commonly than genital infection.[81] However, most oral infections appear to be transmitted by sexual contact, with increased risk being associated

with the number lifetime oral or vaginal sex partners, and recent oral sex partners and open-mouthed kissing.[82] High numbers of lifetime vaginal sex partners and oral sex partners are also associated with an increased risk of HPV-positive oropharyngeal cancer.[83] JORRP is associated with transmission of low-risk HPVs, especially HPV6 and HPV11, from the birth canal of infected mothers to infants during delivery.[27]

DIAGNOSIS

Genital HPV infection is usually diagnosed by detection of the viral DNA genome.[84] For research purposes, PCR assays are generally used. They employ consensus or degenerate primer sets corresponding to conserved regions of the genome, followed by specific type determination using blot hybridization technologies. Clinical tests (discussed below) are mostly based on a signal amplification technology designated "hybrid capture" that involves detection of HPV DNA/RNA hybridization products by a monoclonal antibody specific to the hybrids. More recently, other HPV nucleic acid–based assays have been clinically validated and commercialized.[85] The value of serologic assays in detection of HPV infection has been evaluated. L1 VLP-based enzyme-linked immunosorbent assays (ELISAs) and in vitro neutralization assays (discussed below) are type-specific, but relatively insensitive, measures of HPV infection. Only about 50% of women with genital tract infections detected by PCR-based assays are seropositive to the corresponding types and seroconversion takes more than 6 months on average.[86] Serological responses to the nonstructural proteins are seldom detected during cervical infection except in invasive cancer patients, where antibodies to E6 and E7 can often be detected.[31,87]

The diagnosis of genital or nongenital warts usually relies on the clinical appearance. For HPV-associated dysplasia and cancer, diagnosis is by cytology and histologic confirmation. For anogenital cancers, the demonstration of HPV DNA in the tumor does not usually have major implications for clinical management or prognosis. However, in oropharyngeal cancers, HPV-positive tumors tend to respond better than HPV-negative tumors to most treatments and to have a better prognosis.[88]

The success of repeated Pap smears in identifying the majority of women with precancerous lesions of the cervix, coupled with the ability to eradicate these lesions, has led to an emphasis on the routine use of cervical cancer screening to prevent cervical cancer before it occurs.[15,89] High-grade premalignant lesions determined cytologically and confirmed by biopsy can be eradicated by local destruction, which is generally greater than 95% successful at eliminating the lesion and the risk of subsequent cancer. Although a single Pap smear has limited sensitivity (~50%) for detecting HSIL, the specificity is high, and repeated cytologic examinations increase the probability of detecting high-grade lesions. Given the generally long interval between the development of premalignant lesion and progression to invasive cancer, a Pap smear program can offer a high probability that most cervical cancers will be avoided if there is a good call/recall system to ensure that women at risk are screened at regular intervals. However, broadly based cervical cytologic screening programs are not available to most of the developing world.

To increase the sensitivity of a single screening test, the use of cervical swabs for HPV detection has been evaluated as an adjunct to, or as replacement for, cervical cytology. Assay of HPV nucleic acids can be automated and is less operator-dependent. Because acute infection with high-risk HPVs is common, specificity of a positive test result for HPV DNA for premalignant change is overall lower compared with cytologic analysis. However, the specificity of DNA testing is highly age-dependent and is lowest in younger women because they are more likely to have a recent infection that will clear. Results of prospective trials have shown that HPV testing is substantially more sensitive than cytology, with acceptable specificity in women in their late 20s or older.[90] In 2014, one HPV test was approved by the U.S. Food and Drug Administration (FDA) for primary cervical cancer screening.[91] Given that Pap smear screening is more sensitive for HPV16 than for other HPV types, HPV-based screening is likely to have even greater utility than Pap-based screening in HPV-vaccinated populations.[92] A clinical trial carried out in rural India demonstrated the ability of a single HPV DNA test to reduce the risk of death from cervical cancer by approximately half.[93] It is interesting to note that no reduction in mortality was seen in the groups screened once by cytology or by visual inspection of the cervix with acetic acid. An alternative use of HPV DNA testing is for triage of abnormal cytologic changes of uncertain significance, where an HPV-negative result is associated with the same low risk of a future HSIL as a women who is cytologically negative, and where an HPV-positive result has a similar positive predictive value as a low-grade cytological abnormality.[90]

PROPHYLACTIC VACCINES

Preclinical Development

The identification of a virus as the primary cause of a substantial number of human cancers provides an exceptional opportunity for cancer prevention through vaccination. However, certain aspects of PV biology and its relationship to cervical cancer created challenges to the development of effective vaccines. Pioneering work in the 1980s demonstrated that cattle could be protected against BPV infection by immunization with inactivated purified BPV virions[94]; similar observations were subsequently made using canine oral PV-induced oral lesions in beagle dogs.[95] In the beagle dogs, neutralizing antibody was shown to be necessary and sufficient for protection against viral challenge, demonstrating that vaccines to protect against PV infection would likely be conventional, working by induction of neutralizing antibody, and hence would require presentation of conformational "neutralizing" epitopes to provide protection. Prophylactic vaccines based on live attenuated or inactivated virions have proven to be very successful for other viruses, such as polio and measles. This strategy could not be adopted for HPV vaccines for two reasons. First, difficulties in propagating the viruses preclude large-scale production.[96] Second, the viral genomes contain oncogenes.[42] The possibility of carcinogenesis induced by a live or inactivated HPV vaccine would be considered too great for a vaccine designed for use in healthy young people, the vast majority of whom are not destined to acquire cervical cancer in the absence of vaccination. Therefore, the design of prophylactic HPV vaccines has centered on subunit approaches.

The current commercial prophylactic HPV vaccines are based on VLPs, which self-assemble spontaneously from pentamers of the L1 major capsid protein (see Fig. 30.5). PV VLPs can be produced in a variety of cells,[34,97–101] and procedures for production of clinical grade VLPs were first developed using L1-recombinant baculovirus-infected insect cells and *Saccharomyces cerevisiae* (Table 30.2).[102,103] Because the VLPs are produced from a single virion protein, they are noninfectious and nononcogenic. The VLPs are purified by column chromatography and purification involves dissociation into pentamers followed by reassembly into VLPs. This process increases structural regularity, stability, and immunogenicity, and also permits removal of nucleic acids that are encapsidated during intracellular assembly.[103,104] These VLPs are not only morphologically similar to authentic virus, but critically, they also induce high titers of virion neutralizing antibodies after

TABLE 30.2 Comparison of Commercial Human Papillomavirus Virus-Like Particle Vaccines

	Cervarix	Gardasil	Gardasil-9
Manufacturer	GlaxoSmithKline	Merck	Merck
VLP types and dosage	HPV16: 20 μg HPV18: 20 μg	HPV6: 20 μg HPV11: 40 μg HPV16: 40 μg HPV18: 20 μg	HPV6: 30 μg HPV11: 40 μg HPV16: 60 μg HPV18: 40 μg HPV31: 20 μg HPV33: 20 μg HPV45: 20 μg HPV52: 20 μg HPV58: 20 μg
Producer cells	*Spodoptera frugiperda* SF9, *Trichoplusia ni* Hi 5	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
Adjuvant	500 μg aluminum hydroxide, 50 μg 3-*O*-deacylated-4′-monophosphoryl lipid A	225 μg aluminum hydroxyphosphate sulfate	500 μg aluminum hydroxyphosphate sulfate
Injection schedule	0, 1, 6 months	0, 2, 6 months	0, 2, 6 months

HPV, human papillomavirus; VLP, virus-like particle.

low-dose parenteral vaccination.[34,105,106] In contrast to VLPs, neither denatured monomeric L1 nor L1 peptides induce neutralizing antibodies (reviewed in ref. 107).

Studies in rabbits, dogs, and cattle demonstrated excellent protection from experimental challenge with high-dose homologous virus after VLP vaccination.[108–110] Strong protection from experimental challenge with high-dose virus was seen in the rabbit model even 1 year after vaccination.[111] Protection could be passively transferred via serum, indicating that neutralizing antibodies are sufficient to confer protection. Although certainly encouraging, the relevance of these results for human vaccine development was limited by the fact that protection mediated by HPV VLP vaccination could not be directly assessed in preclinical animal models because HPVs do not productively infect animals. Also, none of the animal challenge models involved genital infection or venereal transmission, the route by which the HPV infections that cause cervical cancer are acquired. However, it was demonstrated that mice or rabbits vaccinated with HPV VLPs produce high titers of antibodies that prevented infection of cultured cells or human skin chips in a mouse xenograft model.[105,106,112–114] Unfortunately, the neutralizing activity of the VLP antibodies was predominantly genotype-specific. This observation implied that if an HPV vaccine were to confer protection in people, and if neutralizing antibodies accounted for most of the protection, the protection was likely to be predominantly genotype-specific.

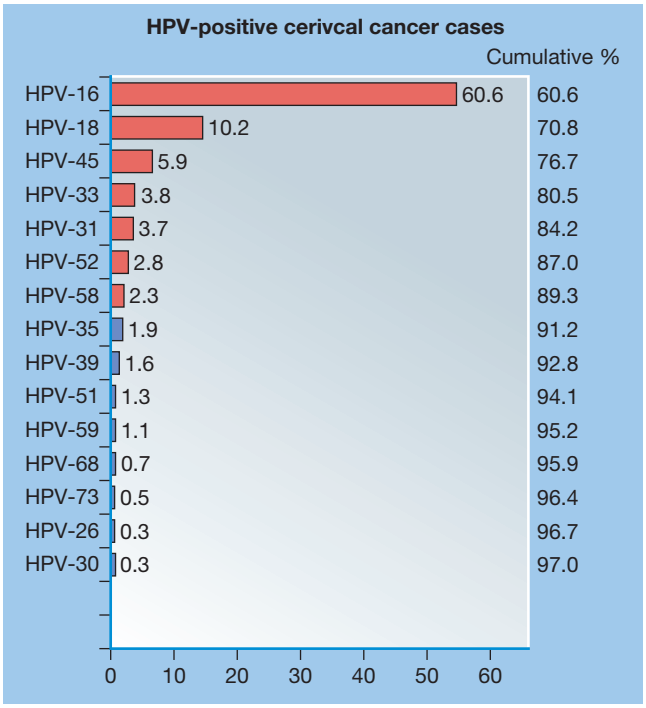


Figure 30.7. Human papillomavirus (HPV) genotypes in cases of invasive cervical cancers that were positive for HPV DNA. The seven high-risk HPV types included in Merck's nonavalent vaccine Gardasil-9 are indicated in red. *(Modified from data on 8977 cases worldwide as reported in de Sanjose S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study.* Lancet Oncol. *2010;11[11]:1048–1056.)*

CURRENT VACCINES

Formulation

The preclinical trials outlined above induced two companies, GlaxoSmithKline and Merck, to undertake commercial development HPV VLP vaccines.[103] Even though both companies based their vaccines on self-assembled L1 particles, the vaccines differed in several respects (see Table 30.2). The GlaxoSmithKline vaccine, Cervarix, is bivalent, composed of the VLPs of HPV types 16 and 18, which together cause approximately 70% of cervical cancers worldwide (Fig. 30.7). It is produced in L1 recombinant baculovirus-infected insect cells and employs the proprietary adjuvant AS04, composed of aluminum hydroxide plus 3-deacylated monophosphoryl lipid A (MPL, a detoxified form of lipopolysaccharide, that is a Toll-like receptor 4 agonist). The original Merck vaccine, Gardasil, is quadrivalent and is produced in the yeast *Saccharomyces cerevisiae*. It also contains the L1 VLPs of oncogenic types 16 and 18, as well as L1 VLPs of types 6 and 11, which cause approximately 90% of genital warts but are considered nononcogenic. Recently Gardasil-9, an expanded version of Gardasil containing L1 VLPs of five additional oncogenic types—31, 33, 45, 52, and 58—was licensed. This vaccine has

the potential to protect against approximately 90% of cervical cancer worldwide (Fig. 30.7).[115] The Merck vaccines contain a simple aluminum salt adjuvant (aluminum hydroxyphosphate sulfate). None of the vaccines contain a preservative. The vaccines are delivered by intramuscular injection in three doses over 6 months, although the timing of the second dose differs (see Table 30.2). Before initiating Phase III trials, promising safety, immunogenicity, and preliminary efficacy results were obtained with monovalent HPV16 and HPV11 vaccines and subsequently with the bivalent and quadrivalent vaccine formulations in Phase I/II trials.[102,116–120]

Phase III Trial Design

Phase III efficacy trials of cervical neoplasia were independently initiated by Merck and GlaxoSmithKline.[121–123] There was considerable discussion concerning the appropriate end points before the Phase III efficacy trials of Gardasil and Cervarix were undertaken. Cervical cancer was universally rejected as an end point because women in trials with active follow-up would need to receive generally effective ablative therapy of their premalignant lesions before they progressed to cervical cancer. Advisory panels to the World Health Organization (WHO) and national regulators (e.g., the U.S. FDA and the European Medicines Agency [EMA]) considered protection against a combination of CIN2 or worse (CIN2+) and cervical adenocarcinoma in situ (AIS) caused by incident infection of the vaccine-targeted types to be a reasonable end point for obtaining an indication for cervical cancer prevention.[124] Formally, cervical cancer was also included in the combined end point, but, as expected, no incident cases were seen in the trials. This surrogate end point for cancer was acceptable because the relation of cervical HPV infection to CIN and cervical cancer was so well established in natural history studies.[5] Also, CIN2+ usually triggers ablative therapy. Prevention of external genital lesions was also a primary end point in one of the Gardasil trials (Table 30.3).[122]

With a CIN2+ end point in mind, the efficacy trials had a similar design (see Table 30.3). The randomized trials each involved several thousand young women (age range: 15–26 years) followed over approximately 4 years. The controls received a simple aluminum salts adjuvant (Gardasil) or a licensed hepatitis A vaccine (Cervarix). Clinical end points were based on colposcopy-directed biopsy and histologic determination of CIN/AIS by an expert panel. Type-specific cervical HPV infection was determined by sensitive PCR-based assays. Because the main object of the trials was prevention of disease from incident infection, the number of lifetime sexual partners was limited to no more than four or six so as to limit the enrollment of women with prevalent infection (see Table 30.3). The Merck and GlaxoSmithKline trials involved multiple trial sites around the world.

An independent trial of Cervarix, the Costa Rica vaccine trial (CVT), was undertaken by the U.S. National Cancer Institute in cooperation with the Costa Rican government. It was community based and centered in the province of Guanacaste.[125] Persistent type-specific HPV infection and high-grade CIN were used as primary end points in the Costa Rican trial. Persistent infection is a more desirable end point than incident infection because persistent, but not transient, infection is on the causal pathway to cancer. Also, detection of HPV DNA at least twice is more likely to distinguish true infections from transient contaminations. Persistence was operationally defined as detection of the same HPV type DNA in consecutive cervical samples taken approximately 6 or 12 months apart.

Because Gardasil and Cervarix were licensed and widely recommended prior to the initiation of the Phase III trial of Gardasil-9, it was considered unethical to employ a placebo control in an efficacy trial of this second generation vaccine. Therefore the participants were randomized to receive either Gardasil-9 or Gardasil, as an active comparator. The primary disease end point was high-grade cervical, vulvar, or vaginal dysplasia associated with a combination of the five oncogenic types (31, 33, 45, 52, and 58) not included in Gardasil. In addition, noninferiority of the antibody responses to the four VLP types common to the two vaccines was considered a critical criterion.

TABLE 30.3 Outline of Phase III Vaccine Efficacy Trials in Young Women

Characteristic	PATRICIA	FUTURE I	FUTURE II	Costa Rica	V503
Vaccine	Cervarix	Gardasil	Gardasil	Cervarix	Gardasil-9
Control	Hepatitis A vaccine	225 μg aluminum hydroxyphosphate sulfate	225 μg aluminum hydroxyphosphate sulfate	Hepatitis A vaccine	Gardasil
No. of participants	18,644	5455	12,167	7466	14,215
Mean age (y) (range)	20 (15–25)	20 (16–24)	20 (15–26)	21 (18–25)	22 (16–26)
Lifetime no. of sex partners	≤6	≤4	≤4	Unrestricted	≤4
Screening frequency (months)	12	6	12	12	6
Mean duration of follow-up (months)	35[a]	42[b]	42[b]	48	54
Primary efficacy end point	HPV16/18 CIN2+	HPV6/11/16/18 CIN1+ and external genital lesions	HPV16/18 CIN2+	Persistent HPV16/18 infection	HPV 31, 33, 45, 52, or 58 CIN2+,VIN2+, VaIN2+

[a]Interim analysis of 48-month trial.
[b]For combined FUTURE I/II analyses.
CIN, cervical intraepithelial neoplasia; CIN1+, CIN grade 1 or worse; CIN2+, CIN grade 2 or worse; FUTURE, Formulation of Bosentan in Pulmonary Arterial Hypertension; PATRICIA, Papilloma Trial Against Cancer in Young Adults; VaIN+, vaginal intraepithelial neoplasia grade 2 or worse; VIN2+, vulvar intraepithelial neoplasia grade 2 or worse.

Immunogenicity

For reasons outlined below, immunogenicity assessment in the VLP clinical trials has focused on antibody responses. Because the vaccines are administered via intramuscular injection, most analyses have been on serum titers. It has been difficult to compare immunogenicity results of Gardasil and Cervarix because of substantial differences between Merck's and GlaxoSmithKline's main serologic assays.[126,127] GlaxoSmithKline predominantly used a VLP ELISA, which is sensitive but does not distinguish neutralizing from nonneutralizing VLP-binding antibodies.[128] Merck predominantly used a Luminex-based assay involving competition for binding of a type-specific neutralizing monoclonal antibody to the VLPs (chemiluminescent assay [cLIA]).[129] The monoclonal antibodies were specifically chosen because they do not cross-react with VLPs of other types, so the cLIA is exceptionally type specific. However, the cLIA may only measure a subset of potentially neutralizing antibodies in the sera, because VLP-induced multivalent antibodies are known to bind to multiple distinct sites on the VLPs.[130,131]

Despite the assay differences, it is clear that both vaccines induce robust antibody responses in young women, with virtually 100% seroconversion against the HPV types in each vaccine. Geometric mean titers (GMTs) increased after each dose, peaking 1 month after the third dose. There was then a rather steep 1- to 2-log drop in titers over the next 2 years followed by a distinct stabilization in titers (Fig. 30.8). Plateau titers are generally well above those seen after natural infection.[132] Similar responses were observed in adolescent girls and boys (i.e., those younger than 16 years of age), although their serum titers were higher than those 16 years of age or older.[133–137]

An interesting head-to-head immunogenicity trial comparing Cervarix and Gardasil was conducted in women ages 18 to 45 years.[138] Using an in vitro neutralization assay based on pseudovirions transferring a marker gene,[113] Cervarix was found to induce 24-month GMT titers that were, depending on age group, 2.4- to 5.8-fold higher against HPV16 and 7.7- to 9.4-fold higher against HPV18.[139] VLP ELISA titers were similarly higher for Cervarix. GMTs continued to be higher for Cervarix in sera taken at 60 months.[140] Although higher neutralizing titers would, in principle, seem desirable, it remains to be determined whether they will translate into longer-term protection against vaccine-targeted or -related types.

There has been some concern expressed over the finding that Gardasil-induced antibody responses to HPV18 VLPs fell below the level of detection in a substantial minority of women (35%) by 4 years. However, most of these women were shown to maintain detectable VLP antibody responses in a less epitope-restricted bind assay and also an in vitro neutralization assay, suggesting that their immunodominant responses to HPV18 VLPs may be to epitopes that do not overlap those of the competing monoclonal antibodies.[141,142] There is no indication that protection to HPV18 is preferentially waning in Gardasil-vaccinated women.[143]

The immunogenicity of fewer than three doses of Cervarix was evaluated in a post hoc analysis of the CVT. HPV16 and HPV18 antibody responses, as measured by VLP ELISA and in vitro neutralization assays, were noninferior for young women receiving two doses 6 months apart, compared with those who received the proscribed three doses during the 6-month period. Surprisingly, all subjects who received only a single dose seroconverted and maintained a durable response through the 4 years of follow-up. In another unexpected result, GMT titers in single-dose recipients plateaued at levels that were only fourfold lower than those of women receiving three doses and ninefold higher than observed following natural infection (Fig. 30.9).[143a]

In young women, Gardasil-9 was judged to be noninferior to Gardasil in the induction of L1 antibodies to the four VLP types included in both vaccines.[144] The antibody responses in type-specific cLIA assays developed to the five addition types were also robust. These results indicate that increasing the valency, and also dosage of HPV6, HPV16, and HPV18, of the vaccine did not lead to excessive antigen competition among the VLP types. Similar results were reported in an

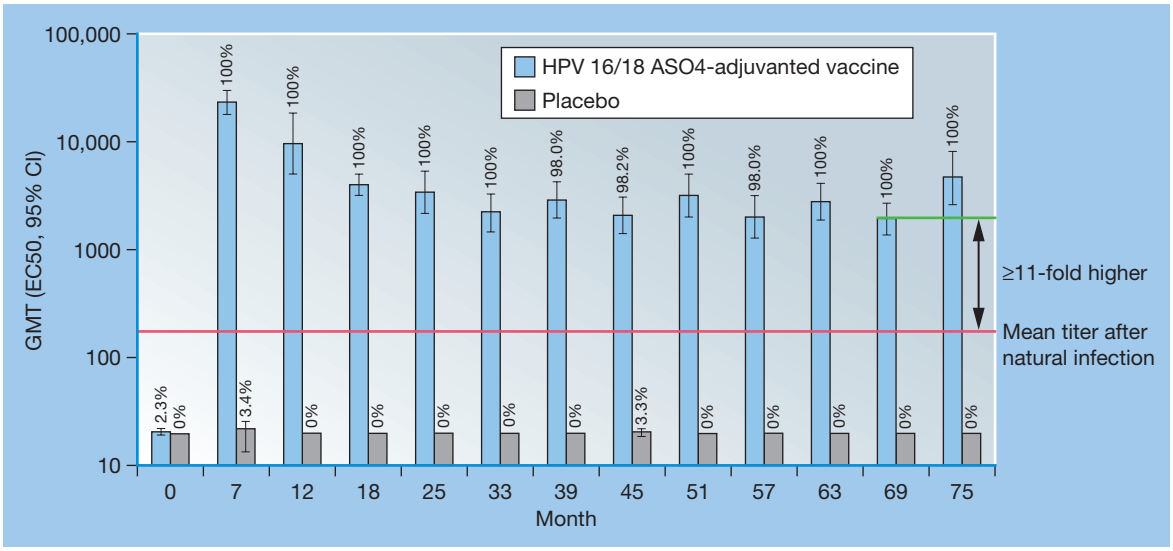


Figure 30.8. Durability of Cervarix-induced human papillomavirus type 16 (HPV16) neutralizing antibodies as measured in an in vitro pseudovirion-based assay. Percent seropositive is shown above the bars. The *red line* indicates the geometric mean titer (GMT) of unvaccinated seropositive women who cleared a natural HPV16 infection. The *green line* indicates the lowest GMT recorded in vaccinated women. Month 7 was 1 month after the third vaccine dose. CI, confidence interval; EC50, median effective concentration. *(From Romanowski B, de Borba PC, Naud PS, et al. Sustained efficacy and immunogenicity of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine: analysis of a randomised placebo-controlled trial up to 6.4 years.* Lancet. *2009;374[9706]:1975–1985.)*

immunogenicity trial comparing Gardasil in 9- to 15-year-old girls.[145]

Efficacy in Women

The primary efficacy analyses involved the Per Protocol (PP) cohorts, which are also called According To Protocol (ATP).

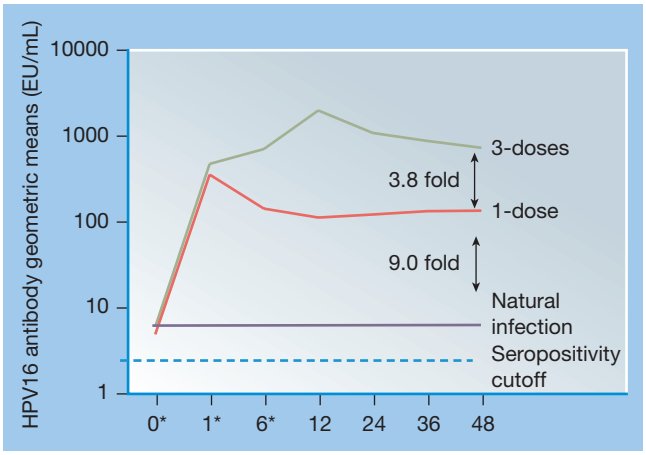


Figure 30.9. Stabilization of antibody responses to human papillomavirus type 16 (HPV16) after a single dose of Cervarix. Geometric mean virus-like particle enzyme-linked immunosorbent assay titers using sera for one- and three-dose Cervarix recipients in the Costa Rica Vaccine Trial are shown. The *x*-axis depicts number of months since first vaccination. Asterisks indicate times of vaccination in three-dose recipients. EU/mL, enzyme-linked immunosorbent assay unit per milliliter. *(Data from Safaeian M, Porras C, Pan Y, Kreimer A, et al. Durable antibody responses following one dose of the bivalent human papillomavirus L1 virus-like particle vaccine in the Costa Rica Vaccine Trial.* Cancer Prev Res (Phila)*. 2013;6[11]:1242–1250.)*

The PP/ATP cohorts included young women who were cervical cytology–negative, cervicovaginal HPV DNA and VLP antibody-negative (at least for the types in the vaccine) throughout the period of vaccination, and received all three doses within prescribed time limits. At the other end of the spectrum are the Total Vaccinated (TVC) or Intention To Treat (ITT) cohorts, which included all randomized women who receive at least one dose of vaccine, regardless of HPV or disease status at enrollment. TVC/ITT analyses best estimate the impact of the vaccine in vaccination programs in this age group, although the limitation on the number of sexual partners (see Table 30.3) limits generalizations to some extent. HPV-naïve or TVC-naïve cohorts included women receiving at least one dose and who, at baseline, were cervical HPV DNA–negative and serologically negative for vaccine types, as well as cervical DNA-negative for nonvaccine genital types. These cohorts were examined because they best approximate the primary target of prophylactic HPV vaccines, adolescent girls before they become sexually active. Adolescent girls were not enrolled in the efficacy trials, primarily because it would take too long to accrue a sufficient number of infections/lesions.

End-of-study results for the Merck Gardasil trials and GlaxoSmithKline and National Cancer Institute CVT trials of Cervarix have been published.[146–150] For all the trials, high efficacy in the PP/ATP analyses was reported. Remarkably, efficacy against vaccine type CIN2+ approached or equaled 100% for both vaccines (Table 30.4). Because simultaneous infection with multiple types was common in the enrolled women, it was often difficult to unambiguously assign lesion causality to a specific type. Assignment methods involved favoring persistent over newly detected types (GlaxoSmithKline) and PCR-based evaluation of HPV DNA in biopsy sections (Merck). Vaccine efficacy against CIN1 and persistent infection was also high for vaccine-targeted types in PP/ATP and TVC-/HPV-naïve analyses. Even transient infection was infrequently detected by sensitive PCR-based methods in vaccinees, suggesting that most of them had acquired sterilizing immunity.

TABLE 30.4 Phase III Efficacy of L1 Virus-Like Particle Vaccines Against Intermediate Grade Dysplasia or Worse by Vaccine-Targeted Types

	Cervarix				Gardasil			
	No. Women: Placebo/ Vaccine	Placebo Rate[a]	Vaccine Rate	Efficacy (95% CI)	No. Women: Placebo/ Vaccine	Placebo Rate	Vaccine Rate	Efficacy (95% CI)
CIN2+								
ATP[c]/PP[d]	7312/7344	0.32	0.02	92.9 (79.9–98.3)[b]	7865/7864	0.5	0.0	98.2 (93.3–99.8)
TVC/ITT[e]	8682/8668	0.39	0.02	52.8 (37.5–64.7)	8860/8823	1.0	0.5	51.5 (40.6–60.6)
TVC-naïve[f]	5436/4549	NR	NR	98.4 (90.4–100)	4680/4616	0.3	0.0	100 (91.9–100)
CIN3								
ATP[c]/PP[d]	7312/7344	0.06	0.01	80.0 (0.3–98.1)[b]	7865/7864	0.3	0.0	96.8 (88.1–99.6)
TVC/ITT[e]	8682/8668	0.09	0.01	33.6 (–1.1–56.9)	8860/8823	0.6	0.3	45.1 (29.8–57.3)
TVC-/HPV-naïve[f]	5436/4549	NR	NR	100 (64.7–100)	4680/4616	0.2	0.0	100 (90.5–100)

[a]Rates per 100 person years at risk.
[b]For CIN2+: 98.1 (88.4–100); for CIN3: 100 (36.4–100) if HPV16/18 is in the specimen preceding lesion detection.
[c]ATP (according to protocol): received three vaccinations, seronegative/DNA-negative to respective HPV types at day 1; DNA-negative to respective HPV types at month 6; normal or low-grade Pap test at day 1; case counting began 1 day after vaccine dose 3.
[d]PP (per protocol): received three vaccinations, seronegative/DNA-negative to vaccine HPV types; remained DNA negative through 1 month post dose 3; case counting started 1 month after dose 3.
[e]TVC (total vaccinated cohort) and ITT (intention to treat): received at least 1 vaccination, regardless of baseline HPV-related infection or disease; case counting began after day 1.
[f]TVC-naïve and HPV-naïve: received at least 1 dose; PCR negative at entry for HPVs 16, 18, 31, 33, 35, 39 45, 51, 52, 56, 58, and 59 (and 66 and 68 for TVC-naïve); seronegative for vaccine types and Pap cytology normal at day 1; case counting after day 1.
CIN2+, cervical intraepithelial neoplasia 2 or worse; CIN3, cervical intraepithelial neoplasia 3; HPV, human papillomavirus; NR, not reported; Pap, Papanicolaou; PCR, polymerase chain reaction.
Data from references 89, 92, and 95.

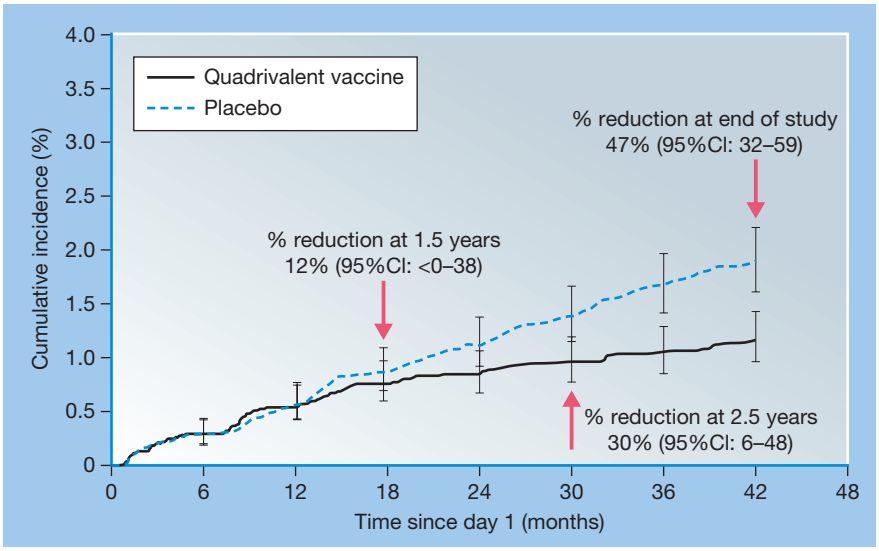


Figure 30.10. Kaplan-Meier time-to-event curve (estimates and 95% confidence intervals [CIs] at 6-month intervals) for cervical intraepithelial neoplasia 3 or adenocarcinoma in situ related to human papillomaviruses (HPV) 6, HPV11, HPV16, and/or HPV18 in the combined Intention To Treat cohorts of the FUTURE (Formulation of Bosentan in Pulmonary Arterial Hypertension) I/II trials of Gardasil (quadrivalent vaccine). *(From Munoz N, Kjaer SK, Sigurdsson K, et al. Impact of human papillomavirus (HPV)-6/11/16/18 vaccine on all HPV-associated genital diseases in young women.* J Natl Cancer Inst. *2010;102[5]:325–339.)*

As expected, the calculated vaccine efficacy in the TVC/ITT cohorts was considerably lower (see Table 30.4). Many of the CIN cases in these types of cohorts are expected to arise from prevalent, rather than incident, infection in trials of relatively short duration, and rates of prevalent infection were similar in vaccinees and controls because the trials randomized subjects. It is important to note that in these types of trials vaccine efficacy is not an absolute outcome, rather it is a time-dependent variable. Efficacy increases with time since vaccination, as disease from prevalent infection is gradually cleared in both arms and disease from incident infection is preferentially prevented in the vaccine arm. This phenomenon is most clearly seen in time-to-event curves (Fig. 30.10).[151] As a corollary, a vaccine that is equally effective at reducing the rate of CIN in two cohorts could exhibit different vaccine efficacies in this analysis if the cohorts differ in the extent of prevalent infection at enrollment. Therefore, reduction in disease rate may be a relevant measure of the potential population impact of an HPV prophylactic vaccine, in addition to the more widely reported efficacy values (see Table 30.4). These considerations are especially important for TVC/ITT analyses, as they usually include many women with prevalent infection. However, they may also be relevant to PP/ATP analyses, as at least a small proportion of women in the PP/ATP cohorts are expected have occult HPV infection at enrollment that will manifest itself during the course of the trial. This situation likely explains why "breakthrough infections" in vaccinees were more often detected toward the beginning of the follow-up phase rather than later in the trials.[143,150]

In the CVT trial, the efficacy of Cervarix for protection against 6 months of persistent cervical HPV16/18 infection after 4 years was 93.6% (95% confidence interval [CI] 88.0–96.9) in the ATP analysis and 52.3% (95% CI 44.3–59.3) in ITT.[150] However, efficacy in the ITT analysis was highly age-dependent, falling from 68.9% (95% CI 53.1, 79.9) in 18 to 19 year olds to 21.8% (95% CI −16.9, 47.9) in 24- to 25-year-olds. This trend reflects the combined effects in the cohort of an increase in prevalent infections with age and a decrease in attack rates.

Vaccine efficacy against nonvaccine types was examined for both vaccines. Efficacy was overall low against infection by nonvaccine oncogenic types (Table 30.5). There was evidence for partial protection against infection by some specific types closely related to the vaccine types. In both PATRICIA (Papilloma Trial Against Cancer in Young Adults) and CVT, Cervarix demonstrated protection against HPV31 and HPV33, two types that are closely related to HPV16, and also protection against HPV45, a type closely related to HPV18.[150,152] For Gardasil, relatively few women were evaluated for cross-type protection, and significant protection was only observed for HPV31 and HPV33. Interestingly, there was no trend for protection against HPV45. Neither vaccine protected against HPV52 or HPV58. Therefore the two vaccines are considered type-restricted with regard to protection against infection, as they provide a high degree of protection against vaccine types and partial protection against a limited number of additional oncogenic types.

Given the type-restricted protection from infection, CIN3+ efficacy irrespective of the type of HPV DNA in the lesion was notably high for Cervarix in the PATRICIA TVC-naïve cohort (91.4% [95% CI 65.5–99.0]).[152] Protection from CIN3+ irrespective of type was more modest in the HPV-naïve cohorts of the Gardasil trials (43% [95% CI 13.0–63.2]).[153] The reasons for these differences are unclear, but could include differences between the vaccines or differences between the trials in study methodology such as inclusion criteria, laboratory assays, and/or study population.[154]

Protection from cervical infection was evaluated in CVT subjects who for various reasons received fewer than the intended three doses. Unexpectedly, protection from HPV16/18 infection after 4 years was as high in women who received two vaccine doses, or even a single dose, as in women receiving the full course of three injections.[155] Similar findings were recently reported in a post hoc analysis of the GlaxoSmithKline's PATRICIA trial (Table 30.6 provides a combined analysis).[156] Interestingly, partial protection from HPV31/33/45 was detected in subjects receiving two doses at a 6-month interval but not in subjects receiving a single dose or two doses

TABLE 30.5 Vaccine Efficacy Against Incident Infection by Nonvaccine Oncogenic Types That Persists at Least 6 Months

	Cervarix: PATRICIA-ATP	Cervarix: CVT-ATP	Gardasil: HPV-Naïve
HPV31	78.7 (7.2–85.2)	64.7 (42.6–78.9)	46.2 (15.3–66.4)
HPV33	45.7 (25.1–60.9)	32.1 (–41.1–68.2)	46.2 (15.3–66.4)
HPV45	63.0 (18.4–84.7)	73.0 (45.3–87.8)	7.8 (–67.0–49.3)
HPV52	7.8 (–8.7–21.8)	19.6 (–8.1–40.4)	18.4 (–20.6–45.0)
HPV58	1.8 (–26.0–23.4)	2.8 (–48.0–36.2)	5.5 (–54.3–42.2)
HPV31/33/45/52/58	30.2 (21.5–38.1)	NR	25.0 (5.0–40.9)
Any HR HPV type except 16/18	12.1 (4.7–19.0)	NR	NR
Any HR HPV type	25.0 (18.9–30.6)	16.2% (4.3–26.3)	NR

Values are given as efficacy (95% CI).
ATP, according to protocol; CI, confidence interval; CVT, Costa Rica vaccine trial; HR HPV, high-risk human papillomavirus types; NR, not reported; PATRICIA, Papilloma Trial Against Cancer in Young Adults.
Data from Paavonen J, Naud P, Salmeron J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet. *2009;374(9686):301–314; Herrero R, Wacholder S, Rodriguez AC, et al. Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica.* Cancer Discov. *2011;1(5):408–419; and Brown DR, Kjaer SK, Sigurdsson K, et al. The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-naive women aged 16–26 years.* J Infect Dis. *2009;199(7):926–935.*

TABLE 30.6 Efficacy of Less Than Three Doses of Cervarix Against Incident Human Papillomavirus 16/18 Infection That Persists for a Least 6 Months[a]

No. Doses	No. Women	No. Events	Rate/100 Person-Years (95% CI)	Vaccine Efficacy (95% CI)
THREE				
HPV	6634	38	0.15 (0.10–0.20)	93.6%
Control	6660	567	2.28 (2.10–2.48)	(91.2–95.5)
TWO				
HPV	273	2	0.18 (0.03–0.59)	87.9%
Control	276	16	1.47 (0.87–2.33)	(54.0–98.1)
ONE				
HPV	138	0	0.00 (0.00–0.53)	100%
Control	100	8	1.99 (0.92–3.77)	(67.4–100)

[a]Combined CVT (Costa Rica vaccine trial) and PATRICIA (Papilloma Trial Against Cancer in Young Adults) Total Vaccine Cohort-naïve.[156]

at a 1-month interval. The programmatic implications of these results must be interpreted with caution because the subjects were not formally randomized by number of doses and the long-term durability of the protection after fewer than three doses needs to be assessed.

In 2010, a randomized trial of two and three doses of Gardasil was suspended by the Indian government, leaving many young women with fewer than the intended three doses.[157] At 18 months after vaccination, the GMT VLP antibody titers in girls (ages 10–18 years) receiving one dose or two doses 1 month apart were inferior to the titers in the girls receive three doses, whereas the titers in the girls receiving two doses 6 months apart were noninferior. The frequency of HPV6/11/16/18 incident infection was similar irrespective of number of doses received.[157a] Continued follow-up of the project participants should provide additional data on the relative efficacy of one, two, and three doses of Gardasil, although lack of randomization by dose will be a concern.

In the CVT, protection from anal and oral HPV16/18 infection was evaluated at the exit visit 4 years after initial vaccination. In a restricted cohort that was negative for cervical HPV16/18 DNA and HPV16/18 serum antibodies at entry, the efficacy against anal infection was 83.6% (95% CI 66.7–92.8), compared with 87.9% (95% CI 77.4–94.0) efficacy against cervical infection in the same cohort.[158] HPV16/18 DNA detection was much lower in the oral than cervical or anal specimens, just 0.5%. Nevertheless, in a similar analysis of oral HPV16/18, vaccine efficacy in the restricted cohort against oral infection at the exit visit was 91.7% (95% CI 52.3%–99.6%).[159] Although the analyses were limited by the inability to assess oral and anal infections at entry, it appears that Cervarix affords similar protection against oral and anal HPV16/18 infections as it does against cervical infection.

Gardasil contains HPV6 and HPV11, which are associated with 90% of genital warts, so this vaccine was also evaluated for protection against external genital lesions.[122] End-of-study efficacy for the HPV-naïve cohort was greater than 95% for genital warts and vulvar/vaginal intraepithelial neoplasia grades 1 to 3 (VIN1–3 and VaIN1–3, respectively) (Table 30.7). Efficacy was also relatively high in the ITT analyses, more than 75%, perhaps reflecting a relatively rapid appearance of external genital lesions after incident infection.[153,160]

Unfortunately, there is no evidence that the HPV VLP vaccines act therapeutically.[161] In the Costa Rican trial, Cervarix did not influence the rate of clearance of cervical HPV infections present at the time of vaccination.[14] Neither Gardasil nor Cervarix prevented progression of infection to CIN2/3 among young women who were HPV DNA–positive at the time of vaccination.[121,148] These findings underscore the importance of vaccination before exposure to the viruses, that is, before sexual debut. However, there is evidence, at least for Gardasil, that vaccination can prevent new HPV-associated genital disease in women who were previously treated for an HPV-induced neoplasia.[162] This activity is presumably the result of prevention of new infections, not inhibition of outgrowth of preexisting infections.

Gardasil was evaluated in an efficacy trial of mid-adult women ages 24 to 45 years.[151,163] Unlike the trials in young women, there was no restriction on the lifetime number of sexual partners. In the PP analysis, efficacy was 90.5% (95% CI 73.7, 97.5) for a combined primary end point of incident vaccine type–related persistent infection, CIN1+, and external genital lesions. Protection against HPV16/18–related end

points was 83.1% (95% CI 50.6, 95.8). Vaccine efficacy was similar in 24- to 34-year-old and 35- to 45-year-old women. In ITT analyses, the efficacy against all vaccine types was 30.9% (11.1, 46.5), but was not significant for HPV16/18-specific end points, 22.6% (−2.9, 61.7).[151] The low efficacy in the ITT analyses reflects the relatively high rates of prevalent infection/disease in the older women together with the relatively low rate of new infections, compared with younger women. These limitations were presumably influential in the FDA's decision to not approve Gardasil for mid-adult women. High efficacy against CIN1+ was also recently reported in an interim analysis of the ATP cohort of a Cervarix trial in mid-adult women.[164]

The results of the pivotal Phase III trial of Gardasil-9 were recently published.[144] In comparison to the control group vaccinated with Gardasil, the young women vaccinated with Gardasil-9 exhibited a 97% reduction in combined high-grade cervical, vulvar, and vaginal disease related to the five types—HPV31, HPV33, HPV45, HPV52, and HPV58—uniquely targeted by Gardasil-9 (Table 30.8). Similarly, the efficacy of the vaccine against infection by these types was 96%. As expected, the number of HPV6/11/16/18–associated events was low, and not significantly different, for the two vaccines. These efficacy results, coupled with the noninferior antibody responses to the shared types, led to the recent regulatory approval of Gardasil-9 by the FDA and EMA.

TABLE 30.7 Phase III Vaccine Efficacy of Gardasil Against External Genital Lesion by Vaccine-Specific Types

	No. Women: Control/ Vaccine	Placebo Rate	Vaccine Rate	Efficacy (95% CI)
HPV Naïve				
Genital warts	4735/4689	0.8	<0.1	96.4 (91.4–98.8)
VIN1/VaIN1	4735/4689	0.1	<0.1	95.2 (70.0–99.9)
VIN2+/ VaIN2+	4735/4689	0.1	<0.1	95.4 (71.5–99.9)
Intention to Treat				
Genital warts	8702/8689	1.0	0.2	79.5 (73.0–84.6)
VIN1/VaIN1	8702/8689	0.2	<0.1	76.0 (54.2–88.3)
VIN2+/ VaIN2+	8702/8689	0.1	<0.1	78.5 (55.2–90.8)

CI, confidence interval; HPV, human papillomavirus; VaIN, vaginal intraepithelial neoplasia; VIN, vulvar intraepithelial neoplasia.

Data from Munoz N, Kjaer SK, Sigurdsson K, et al. Impact of human papillomavirus (HPV)-6/11/16/18 vaccine on all HPV-associated genital diseases in young women. J Natl Cancer Inst. *2010; 102(5):325–339; and Kjaer SK, Sigurdsson K, Iversen OE, et al. A pooled analysis of continued prophylactic efficacy of quadrivalent human papillomavirus (types 6/11/16/18) vaccine against high-grade cervical and external genital lesions.* Cancer Prev Res (Phila). *2009;2(10):868–878.*

Efficacy in Men

Only one efficacy study in men has been conducted. The end-of-study results of a trial of Gardasil in 4065 young men (ages 16–26 years) have been published.[165,166] The combined primary clinical end point was vaccine type–related external genital lesions, that is, genital warts; penile, perianal, or perineal intraepithelial neoplasia; or cancers at these sites. Most events were HPV6/11–related genital warts. Observed efficacy was 90.4% (95% CI 69.2, 98.1) for PP and 65.5% (95% CI 45.8, 78.6) for ITT. A relatively high efficacy of 60.2% (95% CI 40.8, 73.8) was observed in the ITT cohort against external genital lesions, irrespective of HPV type, which is consistent with the high proportion of external genital lesions in men that are caused by one of the vaccine-targeted types.

The subset of MSM in the above study were concurrently enrolled in a study of anal infection and associated AIN. PP efficacy against HPV6/11/16/18–related AIN of any grade and AIN2+ was 77.5% (95% CI 39.6, 93.3) and 74.9 (95% CI 8.8, 95.4), respectively.[166] The relatively wide confidence intervals reflect the small trial size (300 per arm) and relatively short follow-up (3 years). PP efficacy against persistent anal infection by the vaccine types was 94.9% (95% CI 80.4, 99.4).

Safety Evaluations in the Clinical Trials

The safety data compiled in the Phase III and other clinical trials established that both vaccines, although they were highly immunogenic, were well tolerated in both females and males.[122,135,148,165,166] Common side effects attributable to the vaccine included short-term local pain, swelling, and erythema at the injection site, low-grade fever, and headache. As would be expected from such large trials, several serious adverse events were recorded; however, no pattern attributable to the vaccine was discerned for any of them. An evaluation of Cervarix did not identify any increased risk of miscarriage in pregnancies that occurred during the vaccination period or

TABLE 30.8 Efficacy of Gardasil-9 Against High-Grade Cervical, Vulvar, and Vaginal Disease and 6-Month Persistent Infection by the Vaccine-Targeted Types in the Per-Protocol Population

	Gardasil-9		Gardasil		Risk Reduction
End Point	Cases/Total	Rate[a]	Cases/Total	Rate[a]	(95% CI)
CIN/VIN/VaIN2+					
Related to HPV 31, 33, 45, 52, 58	1/6016	0.1	30/6017	1.6	96.7 (80.9–99.8)
Related to HPV 6, 11, 16, 18	1/5883	0.1	3/5898	0.2	66.6 (–203.0–98.7)
6-Month Persistent Infection					
Related to HPV 31, 33, 45, 52, 58	35/5939	2.1	810/5953	52.2	96.0 (94.4–97.2)
Related to HPV 6, 11, 16, 18	59/5812	3.6	80/5830	5.0	26.4 (–4.3–47.5)

[a]Cases per 1000 person-years.

CI, confidence interval; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; VaIN, vaginal intraepithelial neoplasia; VIN, vulvar intraepithelial neoplasia.

afterward.[167] In head-to-head comparisons, Cervarix and Gardasil-9 were both somewhat more likely to be associated with injection site adverse events than Gardasil, but these minor differences did influence completion rates of the three-dose vaccination schedule.[138,144]

IMMUNOGENICITY BRIDGING TRIALS

Immunobridging studies were conducted to extend vaccine licensure to groups for which there are no efficacy data, in particular adolescent girls and boys. All licensed vaccines proved to be safe and highly immunogenic in 9- to 15-year-olds, generating two- to threefold higher GMTs to each of the types in the vaccines than they did in the 16- to 26-year-old women enrolled in the efficacy trials.[133–136] These findings led to regulatory approval for vaccination of adolescent girls, and, in some countries, adolescent boys. Similarly, a safety/immunogenicity bridging trial of Gardasil-9 demonstrated noninferior antibody responses in 9- to 15-year-old girls and boys in comparison to 16- to 26-year-old women. Seroconversion was greater than 99% for all nine types, and GMTs were two- to threefold higher in the adolescents than in the young women.[168]

Among adults, GMTs decreased with increasing age at vaccination for all the HPV vaccines. A safety/immunogenicity study of Cervarix in middle-age women reported high rates of seroconversion but somewhat lower VLP antibody titers that than those detected in young women.[169] Similar trends were seen in the Gardasil trial of middle-age women.[151] In a head-to-head comparison of Cervarix and Gardasil in 18- to 45-year-old women, serum neutralizing titers against HPV16 and HPV18 were 2.0- to 12.8-fold higher for Cervarix at 48 months, but the clinical significance of this difference is unknown.[170]

A 0-1-12–month dosing schedule for Cervarix was found to be noninferior in eliciting VLP-binding antibodies compared with the standard 0-1-6–month dosing schedule.[171] Similarly, delaying the second, third, or both doses of Gardasil did not reduce the antibody responses to the vaccines. In fact, delaying the third dose significantly increased responses. Taken together with the results of several additional studies (reviewed in reference 172) the findings indicate that extending the vaccination period over at least a year is not detrimental.

Both vaccines have been evaluated for compatibility with other vaccines administered to adolescents.[173] Coadministration of either Cervarix or Gardasil with a diphtheria-tetanus-acellular pertussis–inactivated polio vaccine in opposite arms of adolescents was safe and generated antibody responses to each of the antigens that were noninferior to the responses to the two vaccines administered separately.[174,175] Coadministration of Gardasil with a meningococcal polysaccharide vaccine (Menactra) was also judged safe and noninferior to separate administration.[176] Gardasil was also safe and immunogenic when coadministered with a hepatitis B vaccine.[177] Similarly, the antibody responses to Cervarix were judged non-inferior when co-administered with GlaxoSmithKline's hepatitis B virus and hepatitis A virus vaccines.[178]

Gardasil has been evaluated in HIV-infected children ages 7 to 12 years and in HIV-infected adult males.[179–182] The safety profile was similar to that of the placebo controls and/or that of vaccinees in other trials. Vaccination did not alter CD4 counts or plasma HIV RNA levels. Seroconversion was greater than 95% for all four antigens. However, GMTs in the HIV-infected children were 30% to 50% lower than age-similar historic controls. Cervarix has been evaluated in HIV-infected women ages 18 to 25 years. All seroconverted, but GMTs were lower than in HIV-uninfected women in the trial.[183] In a direct comparison of Gardasil and Cervarix in HIV-positive men and women, neutralizing titers against HPV18 were higher for Cervarix, whereas titers against HPV16 were similar for the two vaccines.[184]

Two- and three-dose regimens of Gardasil were compared for the induction of neutralizing antibodies. A two-dose regimen (0 and 6 months) in Canadian adolescent girls (ages 9–13 years) was noninferior to the standard three-dose regimen in young women (ages 16–26 years), both in the induction of peak titers at 7 months and plateau titers at 36 months.[185,186] Similarly, the antibody responses to HPV16 and HPV18 in girls ages 9 to 14 years who were receiving two doses of Cervarix at 0 and 6 months were noninferior to the responses induced in young women ages 15 to 25 years who received the vaccine at 0, 1, and 6 months, both at the 7-month peak and the plateau phase at month 48 and month 60.[187,188] These findings have led to a WHO Strategic Advisory Group of Experts (SAGE) recommendation that two doses of Cervarix or Gardasil be administered at least 6 months apart to girls who start the vaccination series younger than 15 years of age, but that the standard three-dose schedules be retained for older recipients. A similar recommendation was recently made by the EMA. The potential risks of adopting this recommendation, in the absence of data on the durability of protection from disease end points, were recently reviewed.[172]

A recently reported trial compared the immune response to Gardasil-9 in young men and young women 16 to 26 years of age. The antibody responses at month 7 were noninferior for all nine types in heterosexual men compared with women. GMTs were numerically lower for MSM, but seroconversion rates were greater than 99% for all types. These findings were used to support the bridging of the efficacy of Gardasil-9 in young women to young men.[189] GMTs to HPV31/33/45/52/58 in Gardasil-9 were robust, but lower, in young women previously vaccinated with Gardasil than in subjects who had not previously been vaccinated, presumably because of "orginal antigenic sin."[190]

MECHANISM OF PROTECTION

An immune correlate of protection has not yet been established in the efficacy trials, at least in part because of the remarkably small number of vaccine failures to date. Given the low failure rate, it is quite possible that many of the "breakthrough infections" actually represent the emergence or reactivation of prevalent infection rather than being true incident infection. To date, "breakthrough" infections have not correlated with antibody titer.[143] Nevertheless, there are several reasons to believe that neutralizing antibodies are the primary, if not the exclusive, immune effectors for the VLP vaccines. First, neutralizing antibodies are thought to be the principal effectors of many other prophylactic viral vaccines.[191,192] Second, passive transfer of sera from a VLP vaccinee can protect against experimental virus challenge in animal models.[108,110,193] Third, the type-restriction of protection afforded by the vaccines largely mimics the type-restricted in vitro neutralizing activity of serum antibodies from animals and people immunized with VLP vaccines.[106,113,194] It is very unlikely that antibody-dependent cellular cytotoxicity plays a role in protection, because HPV virions are naked icosahedrons that assemble in the nucleus.[96] Consequently, L1 is not displayed on the cell surface, in contrast to the virion spike proteins of many enveloped viruses. It is unclear whether memory B-cell responses play a role in protection, as the superficial exposure to virions from an infected partner may be insufficient to trigger an anamnestic response. It is fortunate that long-lived plasmid cells that maintain stable serum antibody levels indefinitely are apparently generated after HPV VLP vaccination.

There are also several reasons to suspect that cell-mediated immunity may not be directly involved in protection after VLP vaccination, even though HPV VLP vaccines generate robust T-cell responses to L1.[195–197] First, L1 is not detectably expressed in the basal epithelial cells in which the infection is thought to be maintained (see Fig. 30.6).[96] Second, there is no indication, in either animal models or clinical trials, that VLP vaccination induces regression of established infections.[14,108,121] Third, Gardasil induces a predominantly T-helper cell type 2 response, and Cervarix induces a predominantly T-helper cell type 1 response because of the differences in adjuvant in the two vaccines.[198,199] Nevertheless, both vaccines are highly protective against HPV infection and disease.

If antibodies are indeed the primary effectors of protection, it raises the question of how they get to the site of infection. The HPV infections involved in cervical carcinogenesis are largely confined to the epithelia of the genital tract and do not arise via systemic spread.[96] Therefore, only antibodies in the genital tract mucosa would be mediators of protection from the infections that cause cervical cancer. Both Gardasil and Cervarix are delivered by intramuscular injection, and this route of immunization does not induce appreciable amounts of secretory immunoglobulin (Ig) A, which is usually the predominant type of antibody on mucosal surfaces. Fortunately, cervical mucus secretions in women also contain large amounts IgG, much of which is apparently transudated from the serum via the neonatal Fc receptor.[200,201] In a clinical trial comparing serum and mucosal antibodies after intramuscular vaccination of women with HPV16 VLPs, the titers of VLP-specific IgG in cervical secretions were generally found to be about one-tenth those in the serum.[202] However, in women with normal menstrual cycles, both total and vaccine-specific IgG dropped an additional 10-fold during ovulation.

It is also likely that protection is mediated by the direct exudation of serum antibodies at sites of trauma that are believed to be the primary entry point for infection of basal keratinocytes.[45] That Gardasil is very effective in both men and women at preventing external genital warts, which often arise in epidermis that is not bathed in mucosal secretions,[122,165] suggests that the vaccine is able to protect exclusively via the latter mechanism. Exudation-dependent protection would be advantageous because levels of protecting antibodies would not be expected to vary across the menstrual cycle.

As noted earlier, studies of HPV infection in a mouse cervicovaginal challenge model indicate that a required initial step in infection is binding the acellular basement membrane that separates the dermis from the epithelium,[46] thus explaining the requirement for trauma or permeabilization of the epithelium for infection (see Fig. 30.10). Virion binding to the basement membrane is prevented by high levels of L1 VLP antibodies, either induced by vaccination or passively transferred from vaccinated to naïve animals.[193] Lower levels of VLP antibodies, which are nevertheless sufficient to prevent genital infection, permit basement membrane binding but prevent transfer of the virion to the keratinocytes (Fig. 30.11). The series of conformational changes on the basement membrane needed to expose the keratinocyte-specific receptor-binding site on L1 takes several hours.[46] This extended period provides an exceptionally long time for neutralizing antibodies to bind the capsid, which may help explain the remarkable prophylactic efficacy of HPV VLP vaccines. Levels of passively transferred VLP antibodies that are approximately 100-fold lower than the level that can be detected in an in vitro neutralization assay are able to protect mice from cervicovaginal challenge, suggesting that there exists a potent mechanism of infection inhibition in vivo that is not measured in the in vitro neutralization assays.[203]

DURATION OF PROTECTION

As expected for a relatively recently licensed vaccine, the duration of protection afforded by the VLP vaccines is unknown. There are several reasons to be optimistic about continued protection from these vaccines. First, solid protection against CIN2+ in follow up of randomized trials has been observed for up to 9.4 years with Cervarix,[204–206] for 7 years with Gardasil, and up to 9.5 years for Merck's HPV16 VLP vaccine.[207] Gardasil vaccination of adolescent boys and girls also appears to protect from infection after initiation of sexual activity for up to 8 years.[137]

Antibody titers for the licensed vaccines largely plateaued after 2 years, both in young adults and adolescents (see

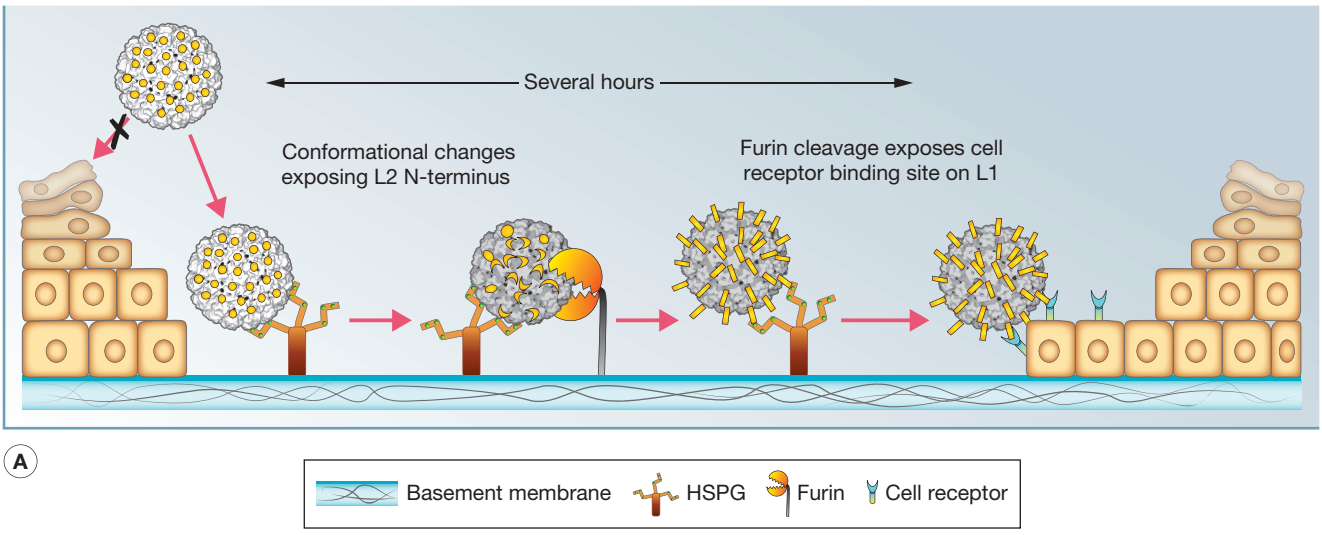
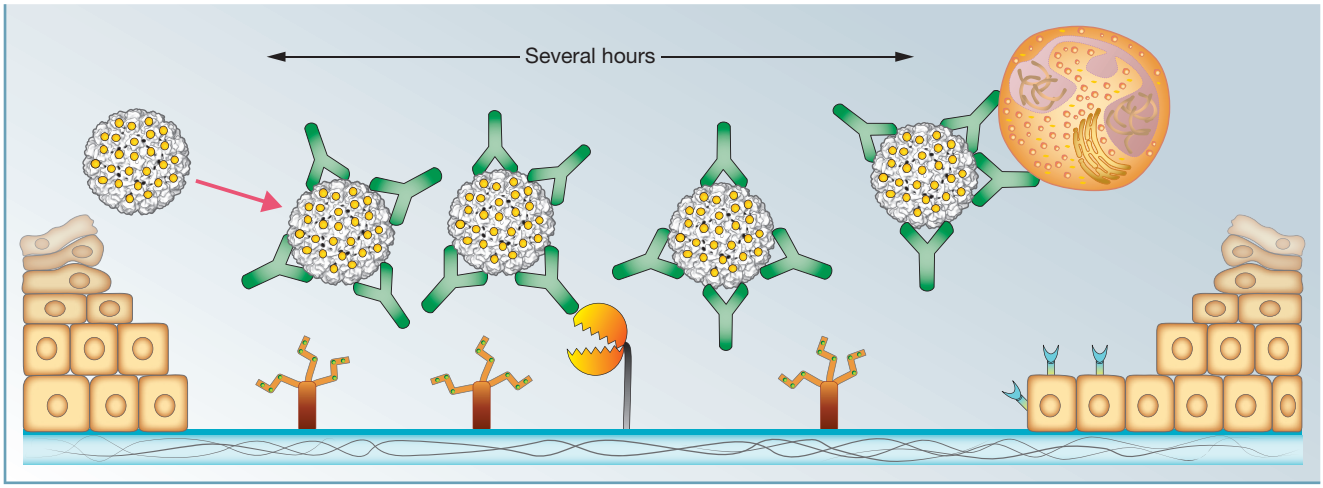
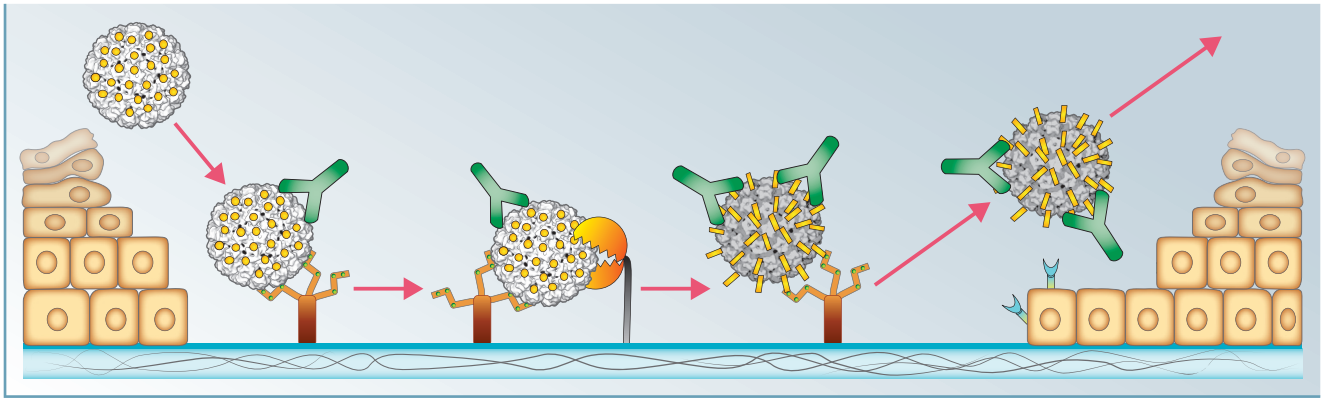


Figure 30.11. Model of in vivo cervicovaginal human papillomavirus infection and the mechanisms of infection inhibition by L1 virus-like particle (VLP) and L2 vaccine-induced antibodies. L1 virion surfaces are depicted in *gray* and L2 virion surface-exposed segments are depicted in *yellow*. **A,** Virions can associate with the keratinocyte surface only after binding basement membrane heparan sulfate proteoglycans (HSPGs) and undergoing a conformational change that result in furin cleavage of L2.

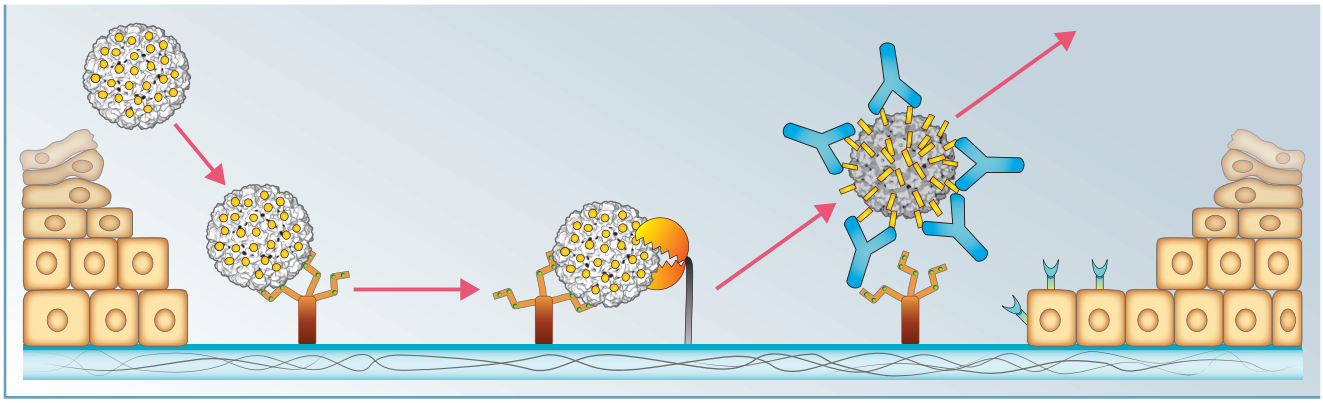
Continued



B



C



D



Figure 30.11, cont'd B, High levels of L1 VLP antibodies (shown in *blue*) prevent infection by blocking association with basement membrane HSPGs. **C,** Low levels of L1 VLP antibodies (shown in *green*) prevent infection by blocking transfer from basement membrane HSPGs to the keratinocyte surface receptors. **D,** N-terminal L2 neutralizing antibodies (shown in *blue*) also prevent infection by blocking transfer from basement membrane HSPGs to the keratinocyte surface receptors.

Fig. 30.8).[132,137,141] For instance, cLIA titers 9 years after Gardasil vaccination remained at 70% to 93% of their year 4 values for the four types in the vaccine.[208] There were similar findings for Gardasil-9.[144] This stabilization is reminiscent of the very slowly decaying antibody titers induced by live attenuated virus vaccines such as MMR (measles, mumps, rubella) and vaccinia.[209] It is possible that polymeric B-cell receptor engagement with the closely spaced repetitive neutralizing epitopes on the VLP surface might, together with the adjuvant, send exceptionally strong activating and survival

signals to B cells, signals that rival those induced by live virus infection.[210]

Second, the GMT plateau titers are generally higher than GMT titers seen after natural infection (see Fig. 30.9).[132,141] There is growing evidence that virion antibody responses to natural infection protect from reinfection.[60,211] As noted above, it is difficult to distinguish new infection from reactivation of latent infection, and individuals with low titers in serologic assays might be false-positives, given the relatively arbitrary nature of positive/negative cut points. The fact that HPV genotypes have clearly evolved to be distinct serotypes argues strongly that virion antibodies induced by infection play an inhibitory role in the natural history of the virus.

Third, the titers of passively transferred serum needed to protect against mouse cervicovaginal challenge with high dose HPV virus are at least 500-fold lower than the plateau levels achieved after VLP vaccination.[193] However, because titers of cross-neutralizing antibodies are at least 1 or 2 logs lower than type-specific titers,[194,212] it is possible that protection against nonvaccine types will be of shorter duration.

INDICATIONS

Young Women

Cervarix and Gardasil are licensed in most countries worldwide. Licensure has been based on efficacy and safety data from the pivotal efficacy trials and bridging immunogenicity and safety data in younger age groups discussed above. In 2006, Gardasil was approved by the FDA and by the EMA for prevention of HPV6/11/16/18–related external genital lesions, CIN, AIS, and cervical cancer in females 9 to 26 years of age. Cervarix was approved by the EMA in 2007 and by FDA in 2009 for prevention of HPV16/18–related CIN, AIS, and cervical cancer in females ages 9 to 25 years of age.[213] The latter approval is noteworthy in that it is the first FDA-approved vaccine that contains a specific Toll-like receptor ligand. Gardasil-9 was licensed by the FDA in December 2014 and has the same indications in females as the quadrivalent HPV vaccine.[214]

Young Males

Gardasil and Gardasil-9 are licensed for use in males in the United States. Data on Gardasil efficacy in males were not available in 2006 at the time of first licensure of the vaccine in females in the United States and other countries. In 2009, based on safety data and clinical trial results showing an efficacy of 89% for protection against HPV vaccine-type–related genital warts,[165] Gardasil was licensed by FDA for use in males ages 9 through 26 years for prevention of genital warts.[215] Data from the substudy in MSM, demonstrating efficacy for prevention of anal precancer lesions,[166] convinced the FDA to extend the indication for the quadrivalent vaccine to include prevention of HPV16- and HPV18-related AIN and anal cancers in males and females, ages 9 to 26 years in 2010.[216] Extension of the indication to females was based on the argument that anal HPV infection and associated AIN are biologically indistinguishable in men and women. In December 2014, Gardasil-9 was licensed by FDA in males based on immunobridging data and has indications for prevention of the same outcomes in males as does the quadrivalent HPV vaccine.[214]

Older Women

HPV vaccines are not licensed for women older than 26 years of age in the United States; however, they are so licensed in other countries.[217] Gardasil and Cervarix are effective at preventing incident infection and disease from the vaccine types in clinical trials of among women ages 24 to 45 years.[151,163,164] However efficacy in the ITT analyses was low, because of the relatively high rate of prevalent infection and relatively low rate of new infection in this age group, compared with younger women. In Europe and some other countries, Gardasil and Cervarix have an indication for use in girls and women starting at age 9 years.[217,218]

RECOMMENDATIONS

Target Groups

Recommendations for HPV vaccination vary by country, but the target group for routine vaccination is generally preadolescent females. The U.S. Advisory Committee on Immunization Practices (ACIP) recommended HPV vaccination routinely for girls at age 11 or 12 years in 2006 and for boys in 2011. The vaccination schedule can be started at age 9 years.[219,220] The target age group of 11 or 12 years was selected to target vaccination prior to sexual initiation. This age also allows HPV vaccine to be incorporated into the adolescent vaccination schedule in the United States. Other vaccines are recommended for children age 11 or 12 years, including meningococcal conjugate vaccine and tetanus-diphtheria-acellular pertussis vaccine (Tdap). ACIP also recommends HPV vaccination of girls and women ages 13 through 26 years and of boys and men ages 13 through 21 years who have not been previously vaccinated or who have not completed the full vaccination series. Vaccination of MSM and of immunocompromised men is recommended through age 26 years. Although ideally the vaccine should be administered before potential exposure to HPV through sexual contact, sexually active people who have not been infected with the HPV vaccine types would receive benefit from vaccination. Prevaccination assessments (such as Pap testing and screening for HPV DNA or HPV antibody) to establish the appropriateness of HPV vaccination are not recommended at any age.

HPV vaccines can be administered to people who are immunosuppressed as a result of disease or medications. While studies have found the vaccines to be well tolerated and immunogenic in HIV-infected persons, some found that GMTs were lower among HIV-infected persons compared with those uninfected.[179–183] It is unclear if there will be differences in HPV vaccine efficacy between immunocompromised and immunocompetent persons; ongoing studies are evaluating HPV vaccine efficacy and long-term protection in these populations.

Dosing Schedule

In the United States, HPV vaccines were recommended to be administered in a three-dose schedule in all targeted age groups until 2016, with the second dose administered 1 to 2 months after the first dose and the third 6 months after the first. In 2016, based on immunogenicity studies showing noninferior antibody response after two doses administered 6 to 12 months apart in 9- to 14 year-olds compared with three doses in young women in the age group in which efficacy was demonstrated,[214] ACIP changed its recommendation.[220a] A two-dose schedule is now recommended for persons who initiate vaccination at ages 9 through 14 years. The second dose is administered 6 to 12 months after the first dose. A three-dose schedule is recommended if the vaccination series is initiated at age 15 years or older and for most persons with immunocompromising conditions.[220a] If the vaccine schedule is interrupted, the vaccine series does not need to be restarted.[219] The number of recommended doses is based on age at administration of the first dose. In 2014, WHO had changed its

earlier recommendation for a three-dose schedule[221] and recommended a two-dose HPV vaccination schedule for adolescent girls if the vaccination series is initiated before age 15 years. A three-dose schedule is recommended for those age 15 years.[222]

PRECAUTIONS AND CONTRAINDICATIONS

Hypersensitivity or Allergy to Vaccine Components

Because Gardasil and Gardasil-9 are a recombinant vaccines produced in *S. cerevisiae* (baker's yeast) they are contraindicated for persons with a history of immediate hypersensitivity to yeast or to any vaccine component. Cervarix is contraindicated for persons with a history of immediate hypersensitivity to any vaccine component. The prefilled syringes of bivalent HPV vaccine should not be used in persons with anaphylactic latex allergy because syringes have latex in the rubber stopper. Cervarix single-dose vials contain no latex.

Vaccination During Pregnancy

HPV vaccines are not recommended for use in pregnant women. However, the vaccines have not been causally associated with adverse outcomes of pregnancy or adverse events in the developing fetus.[167,223,224] Initiation of the vaccine series should be delayed until after completion of the pregnancy. If a woman is found to be pregnant after initiating the vaccination series, the remainder of the three-dose regimen should be delayed until after completion of the pregnancy. The pregnancy registries for bivalent and quadrivalent vaccines have been closed with concurrence from FDA[220]; a new registry has been established to collect data on exposure to Gardasil-9 vaccines during pregnancy.

SYNCOPE AFTER VACCINATION

Vaccine Safety

Prelicensure trials for Gardasil included more than 20,000 females ages 9 to 26 years; the Cervarix trials included more than 30,000 females age 10 years and older; and the Gardasil-9 trials included more than 15,000 persons ages 9 to 26 years.[220,225] The most common injection site reaction was pain. There were no differences in serious adverse events, new onset autoimmune and chronic diseases or deaths between vaccine and control groups for any of the vaccines. Substantial data from postlicensure safety monitoring of bivalent and quadrivalent vaccines are available[223,226–234] Data from passive systems are available from several countries, but have limitations, including reporting of events occurring coincidentally after vaccination and incomplete reporting.

The most commonly reported adverse events after vaccination (AEFIs) have been injection-site reactions, dizziness, and headache.[223,226,234] Pain was modestly increased in young females receiving Gardasil after receiving concomitantly administered vaccines.[235]

One of the most common adverse events during the first few years after introduction of the quadrivalent HPV vaccine reported in the Vaccine Adverse Events Reporting System (VAERS) was syncope, with a rate of 8.2 per 100,000 doses distributed.[226] It has also been noted in the passive reporting systems in the United Kingdom and the Netherlands where Cervarix was introduced.[223] Syncope is a well-described preventable adverse event following an injection, particularly in adolescents.[236] Most syncopal episodes occur immediately or within 15 minutes of vaccine administration. To avoid serious injury related to a syncopal episode, recipients should sit or lie down for up to 15 minutes after receiving an injectable vaccine.

Active postlicensure safety evaluations have evaluated specific adverse events and provide the ability to investigate possible associations between vaccination and AEFIs. In the United States, an evaluation by the Vaccine Safety Datalink of approximately 600,000 doses of quadrivalent HPV vaccine administered to girls and women showed no statistically significant increased risk of the prespecified end points (Guillain-Barré syndrome, stroke, venous thromboembolism, appendicitis, seizures, syncope, allergic reactions, and anaphylaxis).[228] A study conducted as part of a manufacturer postlicensure commitment to the FDA included a general safety assessment and an assessment of autoimmune disorders among approximately 189,000 females receiving at least one dose of the quadrivalent vaccine. Same-day syncope and skin infections in the 2 weeks after vaccination were identified as having a likely association with vaccination.[230] Rates of autoimmune disorders were also evaluated; the study identified no autoimmune safety concerns in women who received quadrivalent HPV vaccine.[227] Safety data using national registries are available from Demark and Sweden. In one study, more than 997,000 girls were evaluated, of whom 296,826 had received quadrivalent vaccine.[237] There was no consistent evidence supporting causal associations between exposure to quadrivalent vaccine and conditions evaluated. Further studies using the registries evaluated venous thromboembolism among 1.6 million girls and women[232] and multiple sclerosis or other demyelinating diseases among almost 4 million girls and women[233] and did not find any association with quadrivalent vaccination. A nested case-control study conducted in a managed care organization in the United States also found no association between quadrivalent HPV vaccine and demyelinating disease,[231] and a case-control study conducted in France found none with autoimmune disorders.[229] Available safety data were reviewed by the Institute of Medicine in 2011[238] and by the WHO's Global Advisory Committee on Vaccine Safety (GACVS) at multiple meetings.

Despite the large amount of safety data available, concerns about HPV vaccine safety remain because of anecdotal reports or events temporally associated with vaccination. Antivaccine groups have capitalized on hesitancy and the debate about HPV vaccine in some countries, and have been active in advancing misconceptions about HPV vaccinations.[240] Some countries have seen dramatic declines in vaccination as a result of safety concerns. In 2013, Japan suspended its recommendation for HPV vaccination because of reports of complex regional pain syndrome following vaccination, while investigating further reported adverse events.[240,241] Although a review concluded that there was no evidence to suggest a causal association with HPV vaccine, the recommendation for HPV vaccine was not reinstated. In France, concerns about adverse events, potentially based on unfounded concerns related to other vaccines, has impacted its HPV vaccine uptake, which is one of the lowest in Western Europe.[242]

CERVICAL CANCER SCREENING AMONG VACCINATED FEMALES

Cervical cancer screening is still recommended for vaccinated women to prevent cancers caused by other oncogenic types.[220,243,244] Furthermore, females who were sexually active before vaccination could have been infected with a vaccine type HPV before vaccination. In the United States, cervical cancer screening recommendations have not changed for females who receive HPV vaccine. The introduction of Gardasil-9 will likely require a reassessment of screening

recommendations, particularly in countries with high vaccine coverage.[244a]

High coverage of Gardasil or Cervarix is expected to eliminate, or at least significantly reduce, the incidence of precancerous and cancerous lesions associated with HPV types 16 and 18 and possibly some related types. Coverage with Gardasil-9 would further reduce lesions caused by other types. Elimination of HPV16/18-related lesions in the population will result in lower positive predictive values for both cytology- and HPV-based tests.[245,246] Establishing different guidelines for vaccinated individuals in the United States will be challenging given the low vaccine coverage in 11- and 12-year-old female cohorts, and the incomplete protection in females recommended for catch-up vaccination but who may be vaccinated after exposure to HPV types in the vaccines. Future screening guidelines will have to consider vaccination coverage in the population and the effects on test performance.

PUBLIC HEALTH CONSIDERATIONS

Vaccine Introduction and Cost Effectiveness

Through 2014, HPV vaccines had been introduced in more than 60 countries worldwide.[247] While all country HPV vaccination programs target young adolescent girls, specific target age groups differ, as do catch-up vaccination recommendations.[248,249] Two countries have included males in their national vaccination programs—the United States in 2011 and Australia in 2013.[250] Some provinces in Canada have also introduced HPV vaccine for males.[251] Austria has recommended HPV vaccine for males since 2011 but without funding.

In 2009, when WHO issued recommendations for HPV vaccination, guidance stated that HPV vaccine be included in national immunization programs provided that prevention of cervical cancer or other HPV-related diseases, or both, constitutes a public health priority; vaccine introduction is programmatically feasible; sustainable financing can be secured and the cost-effectiveness of vaccination strategies in the country or region is considered.[221] The recommended primary target age group is a single year age cohort between 9 and 13 years. WHO also stated that HPV vaccine introduction should not divert resources from effective cervical cancer screening programs and that the vaccine should be introduced as part of a coordinated strategy to prevent cervical cancer and other HPV-related disease.

Various issues have been considered in the decision-making process for vaccination introduction, including cost-effectiveness. HPV vaccine modeling is complex because of the natural history of HPV infection and gaps in our understanding of type-specific transmission and progression rates, as well as assumptions about sexual behaviors that are included in the modeling. Multiple different models have been developed to evaluate cost effectiveness[252,253]; most models have found that adding bivalent or quadrivalent HPV vaccination of preadolescent girls to a cervical cancer screening program is cost-effective in developed countries, with estimates ranging from approximately $3000 to $45,000 per quality-adjusted life-year.[254–262] Cost-effectiveness of vaccinating older age groups varies across models, and all models show decreasing cost-effectiveness with increasing age at vaccination because more females would have already been exposed to vaccine HPV types and attack rates are generally lower in older women.[252] Models have found that the nine-valent vaccine would be cost saving compared with a quadrivalent HPV vaccination program provided the difference in price between the two vaccines does not exceed $13.[263]

For vaccine to be affordable and cost-effective in low resource settings, one economic analysis found that the cost per vaccinated girl would need to be less than $10 to $25.[219] Countries that have not introduced cervical cancer screening need to consider the cost-effectiveness of vaccine introduction and cervical cancer screening and the operational challenges of each of these prevention strategies.

The cost-effectiveness of adding males to a female only vaccination program depends on the coverage among females and cost of vaccine.[253] Early cost effectiveness analyses only considered prevention of cervical cancers.[252] With the addition of other HPV-associated cancers to the models, vaccination of males appears more cost-effective, particularly in countries where the rate of HPV-associated oropharyngeal cancer (a predominately male disease) is increasing. However, including males in a vaccination program may not be cost effective if there is high coverage in females.[252,264] When considering vaccination priorities, it is important to note that transmission models of HPV infection suggest that high vaccine coverage of one gender should be sufficient to prevent disease in women and heterosexual men.[265] The overall medical and economic importance of cervical cancer makes vaccination of females prior to their becoming sexually active the highest priority. Models have also examined targeted vaccination of MSM, who have high rates of anal cancer and who would probably have little benefit from a female vaccination program. Vaccination of this population in the United States appears cost effective through the licensed age range, even accounting for previous exposure to HPV.[266]

United States

HPV vaccine was introduced in 2006 for girls and women and in 2011 for boys and men. The target age group is 11 to 12 years, and vaccine is being delivered by primary care providers in both the public and private sectors. Coverage with HPV vaccine in the United States is lower than that for other vaccines recommended for adolescents. Coverage with at least one dose of HPV vaccine among 13- to 17-year-old girls increased from 25.0% in 2007 to 62.8% and among 13- to 17-year-old boys from 8.0% in 2011 to 49.8% in 2015. In 2015, coverage with three doses for girls and boys was 41.9% and 28.1%, respectively. There are wide variations in coverage by state, with vaccine initiation among girls ranging from a low of 47.7% to a high of 87.9% in 2015.[267] A provider recommendation is strongly associated with receipt of HPV vaccine but physician surveys have suggested that some providers delay strongly recommending HPV vaccine until older adolescence, and many parents of unvaccinated girls report no provider recommendation.[268] Multifaceted interventions with clinicians and other immunization stakeholders are ongoing to increase awareness, to encourage strong provider recommendations, and to prevent missed opportunities to improve HPV vaccination coverage.

Australia

In Australia, a publicly funded program started in 2007 using Gardasil for a school-based vaccination. The target age group is 12 to 13 years. From 2007 through 2009, there was also a catch-up program for girls 12 to 17 years of age delivered through schools and for women up to 26 years of age through community providers. Since 2009, the continuing school program routinely vaccinates girls in the first year of high school (12 to 13 years of age); boys of the same age were included in the routine HPV immunization program in 2013.[269,270] Through the school-based vaccination program in Australia more than 70% of girls in the targeted age groups have received three doses of HPV vaccine.[226] Australia was the first country to report an early positive impact of the HPV vaccination program.[271]

Canada

In Canada, while national recommendations are for vaccination through publicly funded programs to girls in at least one of grades 4 to 8 (approximately ages 9 to 13 years); target age group and other aspects of the vaccination program vary by province.[272] In 2009, Quebec implemented a two-dose schedule, with the doses administered 6 months apart for 9- to 10-year-olds with an option to give the third dose 5 years later. Quebec changed to a two-dose schedule with Gardasil for girls vaccinated in grade 4, keeping a three-dose schedule in older age groups. British Columbia changed from a three-dose schedule to an extended three-dose schedule for girls in grade 6 (approximately 11 to 12 years of age) in 2010 and then to a two-dose schedule for the same age group in 2014.[273] Several provinces have introduced HPV vaccine for males.

European Countries

As of 2015, most countries in Europe had introduced HPV vaccination, including 16 with organized programs with efforts in place to systematically offer vaccine to targeted age groups; 11 additional countries have less organized vaccination efforts.[248] Recommendations currently target adolescent girls and some provide for catch-up to varying degrees for older age groups. Among 16 countries with organized programs, 46% have school-based vaccination delivery, 20% have physician- or health center–based delivery, and others have mixed delivery systems. Countries in Europe without school-based programs have generally achieved lower vaccine coverage to date. In the United Kingdom, HPV vaccine was included in the routine immunization program in 2008, using Cervarix delivered through a school-based vaccination program. The age group targeted was 12- to 13-year-old females. There was a catch-up program for females through age 17 years. After the first year of the program, 80.1% of females 12 to 13 years of age who were eligible to receive the HPV vaccine routinely had completed the three-dose series. The United Kingdom changed to the quadrivalent vaccine in their national program in 2012 and in 2014 to a two-dose schedule.[274] Denmark is one of the few countries to achieve high coverage rates in a largely clinic-based vaccination program, with greater than 80% three-dose vaccination rates in 12-year-old girls.[275]

Middle- and Low-Resource Countries

The first middle-income countries to introduce vaccine (2008) into national immunization programs were Panama and Mexico.[249] By mid-2014, 18 countries in Central and South America offered the HPV vaccine through public immunization programs.[276] The first country in Africa to introduce HPV vaccination nationwide was Rwanda.[277] Vaccine was obtained through an industry donation in 2011. Using a school-based program, 93% three-dose coverage was achieved among girls in grade 6. Bhutan introduced vaccine through a donation program in 2010 and also delivered vaccination through schools[278]; coverage was 99% in the target age group in the initial year of the vaccination program.

Gavi, the Vaccine Alliance, prioritized HPV vaccine in 2009 and in 2012 made HPV vaccines available to Gavi-eligible countries.[279] There are two pathways for countries to apply for HPV vaccines: (a) national introduction if there is demonstrated ability to deliver a multidose series to at least 50% of the target vaccination or (b) demonstration projects if countries cannot meet this requirement.[280] In the first 2 years of HPV vaccine Gavi funding, no country introduced a national program. Most countries with demonstration projects chose to implement vaccination through school-based programs. This strategy means that girls who do not attend are less likely to receive vaccine, a challenge that needs to be addressed going forward.[281]

EPIDEMIOLOGIC IMPACT OF VACCINATION

Evidence of population level HPV vaccine impact on HPV-associated disease and infection became available within a few years of vaccination introduction.[282,283] HPV type-specific prevalence,[270,284–288] genital warts,[275,289] and cervical lesions[271,290–293] are among the early and intermediate HPV-associated outcomes being monitored. Data on vaccine impact on HPV-associated cancers will not be available for many more years. Monitoring impact of HPV vaccination is challenging in countries without national registries for vaccine and early HPV-associated outcomes. WHO has stated that monitoring HPV-associated disease or infection is not a prerequisite to vaccine introduction.[294]

Australia was the first country to observe an impact from quadrivalent vaccine introduction. An ecologic evaluation showed a sharp decrease in new genital warts in young women seeking care at a clinic for treatment of sexually transmitted diseases within 2 years after vaccine introduction.[289] Within 5 years of vaccine introduction, the proportion of women younger than age 21 years and 21 to 30 years diagnosed with genital warts decreased by 93% and 73% (Fig. 30.12).[295] A substantial decrease was observed for heterosexual men, suggesting impact from herd immunity. Declines in genital warts among targeted age groups have also been observed in other countries using Gardasil, including the United States and Belgium, where lower coverage has been achieved.[282,296,297]

Declines in vaccine-type HPV prevalence between the prevaccine and vaccine era have been reported from both England and Scotland, where high coverage was achieved using the bivalent vaccine. In England, HPV16/18 prevalence decreased from 19.1% to 6.5% among the vaccine-eligible 16- to 18-year-olds while no decrease was observed in 22- to 24-year-olds, an age group not targeted for vaccination.[286] In Scotland, a decrease was observed in HPV16/18 prevalence from 28.8% to 16.7% among 20- to 21-year-old females attending cervical cancer screening between 2009 and 2012.[284] Data also suggested some cross-protection against types 31, 33, and 45. In the United States, analysis of data from the National Health and Nutrition Examination Survey (NHANES), a nationally representative survey, found a 56% decrease in population prevalence of vaccine-type HPV in self-collected cervical–vaginal samples from females 14 to 19 years of age in the 4 years postvaccine introduction, but no decreases were observed in older age groups.[285]

Although some countries with cervical cancer screening and vaccine registries have demonstrated declines in precancerous lesions (Fig. 30.13),[271,290,293] monitoring of cervical precancers is challenging in the United States. Not only is there incomplete reporting of adolescent vaccination to state-based immunization registries and lack of national cervical cancer screening registries, but most importantly, cervical cancer screening practices have been changing.[298,299] Over the past decade, the recommended age for initiating cervical cancer screening has been raised to 21 years, HPV-based screening has been increasingly used, and screening intervals have been increased. Nevertheless, data show a decrease in the percentage of precancer lesions caused by HPV16/18 among vaccinated women and suggest some population impact of vaccination on precancers while at the same time highlighting challenges in examining the ecologic effect of HPV vaccination on incidence trends during a time of concurrent changes to screening recommendations.[300,301]

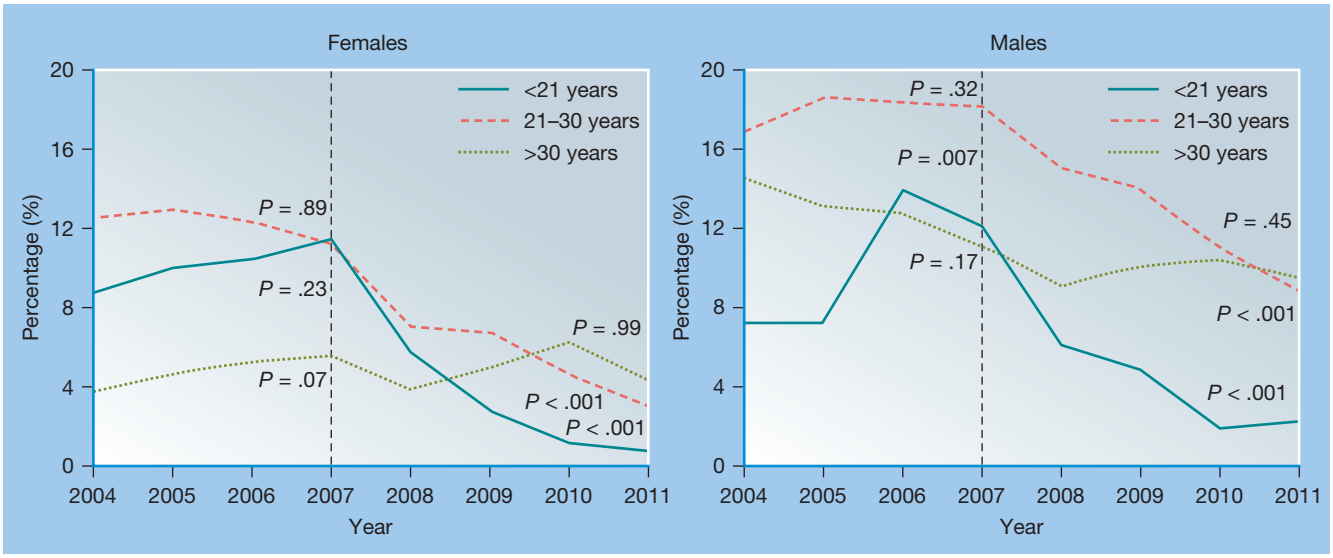


Figure 30.12. Decline in genital treatments of genital warts in young Australians after initiation of Gardasil immunization program. Vertical dashed line indicates the year that the program was initiated.[295]

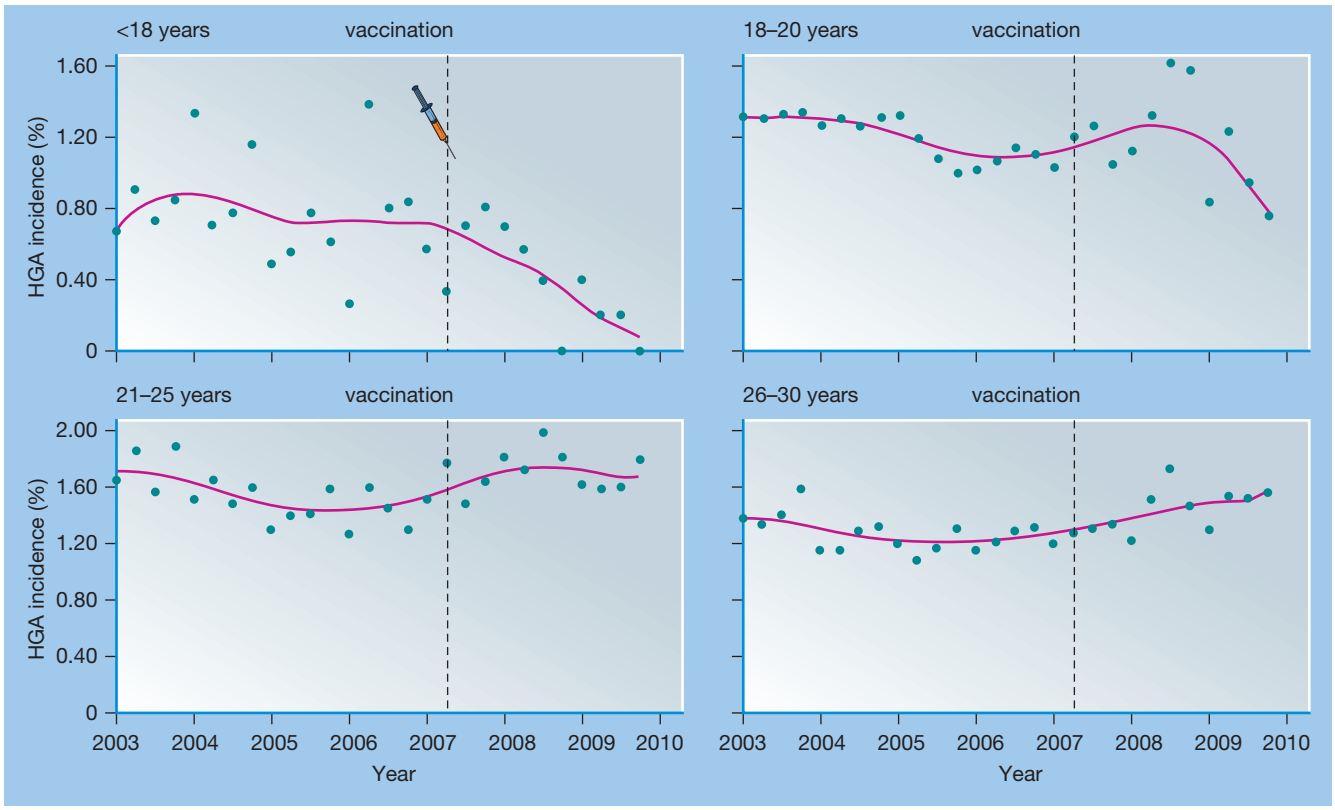


Figure 30.13. Reduction in cervical intraepithelial neoplasia grade 2+ in Australia after the initiation of Gardasil vaccination. HGA, high-grade cervical abnormalities. *(From Brotherton JM, Fridman M, May CL, et al. Early effect of the HPV vaccination programme on cervical abnormalities in Victoria, Australia: an ecological study.* Lancet. *2011;377[9783]:2085–2092.)*

Postlicensure evaluations of vaccine effectiveness for prevention of prevalent infection, genital warts, and cervical lesions have been conducted in a variety of settings.[285,302–305] The highest effectiveness has been observed among those vaccinated at youngest age. For example, Leval and colleagues found effectiveness against genital warts in Sweden of 93% among those vaccinated before age 14 years compared with only 48% among those vaccinated at age 18 to 22 years.[303] Some effectiveness studies have found protection against non–vaccine-targeted, but related, HPV types (i.e., cross-protection).[270,282,284] Other studies have examined effectiveness by number of vaccine doses.[306] The possibility of confounding because of prevalent infection disproportionally affecting vaccinees receiving fewer than three doses has been raised.[307,308]

Long-term monitoring of duration of protection will also be important because vaccination is given in adolescence but

protection is needed for the many years during which exposure may occur. Both vaccine manufacturers are conducting long-term follow-up in the Nordic countries to determine duration of protection[309,310] and further postlicensure effectiveness studies will be important.

SECOND-GENERATION PROPHYLACTIC VACCINES

A number of second-generation prophylactic vaccines, in addition to Gardasil-9, have been generated and evaluated to varying degrees.[18,311] They seek to address some limitations of the current vaccines, including type-restriction, relatively high cost of production and implementation, and the lack of therapeutic activity.

To generate a vaccine with a lower cost of production, Xiamen Innovax Biotech has generated HPV VLPs in *Escherichia coli*, using patented L1 mutants. Results of Phase I and Phase II safety/immunization trials of a bivalent HPV16/18 vaccine, trademarked "Cecolin," were recently reported.[312–314] Encouragingly, consistent induction of high titers of neutralizing antibodies for both types was observed 1 month after the last intramuscular injection at 0, 1, and 6 months. A formulation of 40 μg HPV16 and 20 μg HPV18 VLPs in aluminum hydroxide adjuvant was selected for further development.[314] A Phase III efficacy trial is now underway in China (ClinicalTrials.gov NCT01735006). Several companies have undertaken programs to produce VLPs in yeast species that may produce higher yields than *S. cerevisiae* including Serum Institute of India in *Hansenula polymorpha*, and Indian Immunologicals, Instituto Butantan, and Shanghai Zerun in *Pichia pastoris*. However, only Shanghai Zerun has announced the initiation of a Phase I trial (NCT01548118).

The only other second-generation vaccine approach for which a clinical trial has been published is an academic trial involving the upper respiratory tract delivery of purified VLPs in young women.[315] In a small pilot study, the VLP antibody responses in one-half of the women receiving two aerosolized 50-μg aqueous doses without adjuvant, delivered via a cold ultrasound nebulizer, were similar to the responses in women receiving two 50-μg doses by intramuscular injection. All the women receiving 250 μg to the respiratory tract responded well. Thus, the VLPs appear to be quite immunogenic by the upper respiratory route, which can generate secretory IgA in the female genital tract in addition to serum IgG.[200] However, improvement in formulation or delivery mechanics may be needed to make aerosol delivery of HPV VLPs a practical alternative to injection, and commercial interest in this approach has been limited.

Numerous preclinical studies of more novel approaches for second generation L1 vaccines have been published but only a few appear to be moving toward clinical evaluation, often with academic/commercial sector partnerships.[316,317] Two vaccine candidates are designed to add HPV infection prevention to existing live microbial vaccines. Zydus Cadila has generated an HPV16 L1 recombinant of the Moraten-Berna vaccine strain of measles virus. Vaccination of transgenic mice expressing the human measles virus receptor induced similar levels of measles virus antibodies as the parental vaccine strain, and titers of HPV16-neutralizing antibodies that were comparable to those induced after injection of purified VLPs.[318] If protection from measles is not compromised in humans, the recombinant vaccine might be able to add protection from HPV infection to MMR vaccination programs, which have been widely implemented, even in low-resource settings, potentially at no additional cost. Indian Immunologicals is developing a live HPV16 L1 recombinant *Salmonella typhi* vaccine based on the attenuated strain Ty21a. Encouraging immunogenicity results were obtained in a mouse model.[319] This vaccine holds promise for low-cost production and efficient delivery via the oral route.

Pentameric subunits of L1 can induce neutralizing antibodies, although they had lower intrinsic immunogenicity than VLPs in preclinical studies.[320,321] Pentamers can be readily produced in *E. coli*, raising the possibility of low-cost production.[322] In one variation, a peptide of the E7 oncoprotein was genetically fused to L1 to increase the therapeutic potential of the pentamer-based vaccine.[323] This type L1–E7 fusion protein is expected to undergo clinical testing by BioSidus in the near future.[316]

Vaccines based on the L2 minor capsid protein are also under development. Early studies with animal PVs demonstrated protection from type-specific challenge in rabbits and cattle with L2 protein vaccines.[324,325] The L2 protein contains cryptic neutralizing epitopes that induce appreciable antibody responses only when L2 is taken out of its normal context in the virion or VLP.[326] In contrast to L1, short L2 peptides retain the ability to induce neutralizing antibodies.[327] Antibodies to some L2 epitopes are remarkably broadly cross-neutralizing against an array of mucosal and cutaneous types.[37] In cervicovaginal tissue, these N-terminal L2 peptides are exposed only after basement binding induces a conformational change in the capsid that permit removal of the dozen N-terminal amino acids by the cellular protease furin (see Fig. 30.11).[46] Unlike cryptic neutralizing epitopes of some other viruses, they are exposed for hours prior to internalization of the capsid by the cell.[46] L2 vaccines can protect against cervicovaginal challenge by heterologous types in rabbit cutaneous and oral models and mouse cutaneous and cervicovaginal challenge models, indicating that broad cross-neutralization is not an in vitro artifact.[193,328–330] In the mouse genital model, L2 antibodies permit basement membrane binding but prevent transfer of the capsids to keratinocytes (see Fig. 30.10).[193] Therefore, their in vivo activity is superficially similar to that of low levels of L1 neutralizing antibodies.

Type-specific neutralizing titers induced by L2 vaccines in standard in vitro neutralization assays are generally much lower than the titers induced by L1 VLP vaccines.[326] However, these assays appear to underestimate the potency of L2 vaccines, as L2 vaccinated mice with undetectable serum levels of in vitro neutralizing antibodies are often fully protected from cervicovaginal challenge with the same type. The likely explanation for this discrepancy is that the primary attachment factor, heparan sulfate proteoglycans, and the internalization receptor are topologically separated in vivo.[331] The former is on the basement membrane and the latter on the keratinocyte. In the in vitro assays, both are on the cultured cell membrane, reducing the time that the L2 cross-neutralizing epitopes on the intermediate form of the capsid are exposed to antibodies before the secondary receptor can be engaged.[332] These insights into the mechanism of HPV infection led to the development of an in vitro neutralization assay that better mimics in vivo infection and is 100- to 10,000-fold more sensitive at detecting L2-specific neutralizing antibodies in the sera of L2-vaccinated mice.[333]

Multimeric fusion proteins containing the corresponding L2 peptide of five to eight diverse HPV types can be generated in *E. coli* and appear to be exceptionally potent at inducing cross-neutralizing antibodies.[334,335] These vaccines hold the promise of broad protection against mucosal and cutaneous HPV infections by an inexpensive monovalent vaccine. Other attractive L2 vaccine strategies include fusion to immunogenic proteins (e.g., thioredoxin or flagellin)[328,336] or display on virion surfaces (e.g., adenoassociated virus-2 or bacteriophages).[337,338]

There is an emerging consensus among most experts in the field, based on the results of previous vaccine efficacy trials and current understanding of HPV infection, that the demonstration of efficacy against high-grade dysplasia should not be required for licensure of second-generation HPV prophylactic vaccines.[339] Immunological noninferiority in an in vitro neutralization assay would be sufficient for licensure of alternative dosing schedules or route of administration for the licensed vaccines and for new vaccines that are essentially biosimilars of the current ones. A virologic end point (e.g., protection from an infection that persists for at least 6 months) was recommended as the primary efficacy end point for more novel vaccine candidates. Postlicensure surveillance could be used to confirm the effectiveness of the new vaccines. The recommended changes in primary end points would allow for smaller size and shorter duration of the pivotal trials for licensure and thereby help stimulate continued and more rapid vaccine development.

THERAPEUTIC VACCINES

Disease Targets

HPV-induced proliferative diseases are attractive targets for immunotherapeutic interventions. Two viral oncoproteins, E6 and E7, are continuously expressed from initial infection to malignant carcinoma (albeit at low levels initially), and the virus is apparently unable to evade immune responses by downregulation or loss of these proteins.[42] Because E6 and E7 are non–self-antigens, they may be less subject to mechanisms of immune tolerance than cell-derived tumor antigens.

The stage of HPV-induced neoplasia that should be targeted by therapeutic vaccines has been debated. The most important public health goal is clearly to prevent HPV-induced cancer deaths, which could be accomplished either by successfully treating the cancers or by eliminating the precancerous lesions/infections before they become malignant. Targeting premalignant disease could be considered immunoprophylaxis from the point of view of cancer, but will be considered immunotherapy herein because it involves the elimination of preexisting viral infection.

Cancers will almost certainly be the most difficult stage of the HPV-induced disease spectrum for which to develop an effective immunotherapy. Several of the initial clinical trials of HPV-targeted immunotherapies were safety and immunogenicity studies in cervical cancer patients.[340,341] Clinical responses were not observed, and T-cell responses to vaccination were not always detected. These results are consistent with the limited success, until recently, of immunotherapy against other solid tumors. However, the recent advances in our understanding of the immunology of the tumor microenvironment coupled with developments in adoptive T-cell therapies, combination immunotherapies (particularly involving checkpoint inhibitors such as CTLA-4, PD-1, and PD-L1 monoclonal antibodies), and combination of chemotherapy or radiation therapy with immunotherapy has led to increasing success in treatment of with other solid tumors, notably melanoma.[342–344] Given that HPV-associated cancers harbor viral tumor-restricted antigens to target, it is likely that these conceptual and practical advances will be translated into improved treatments of HPV-associated cancer in the near future. A promising example is the complete response of two of nine patients with metastatic cervical cancer after infusion of ex vivo amplified E6 and E7 reactive T cells.[345]

Most academic and commercial interest is currently focused on the treatment of premalignant HPV-induced neoplasia. In addition to its greater potential for success, the far greater incidence of cervical premalignant disease than cancer also makes it a more attractive commercial target. For example, there are approximately 10,000 cervical cancer cases annually in the United States, compared with an estimated 300,000 high-grade cervical dysplasias, 2,000,000 low-grade dysplasias, and 10,000,000 subclinical infections. However, unless they were used in mass immunization campaigns, therapeutic vaccines targeting premalignant disease are likely to have little impact in developing countries, where more than 80% of cervical cancers occur[8,346] because few women in these countries have access to the screening programs that would identify the premalignant lesions for treatment.

Several aspects of premalignant cervical neoplasias make them attractive candidates for therapeutic vaccines. First, cervical cancer arises from well-defined precursor lesions that normally take many years to progress.[20] Thus, there is a relatively long window of opportunity to intervene in the treatment of premalignant disease. Second, the precursor lesions are routinely identified in screening programs, providing a ready pool of candidates for vaccine trials and eventually for licensed vaccines. Third, effective treatment exists for cervical dysplasias, so women in clinical trials who fail to clear their infection after vaccination should not be placed at undo risk of progressing to cancer.

Most of the recent therapeutic HPV vaccine trials have targeted the intermediate stages of cervical disease, CIN2 and CIN3. Unlike lower-grade lesions, CIN3 is universally considered a premalignant condition that should be treated. However, the overall effectiveness (>90%) and tolerability of current ablative treatments makes the bar for alternative treatments rather high. In addition, the availability of an effective alternative treatment has hampered compliance in some vaccines trials targeting high-grade dysplasias.[347] Decisions regarding whether to treat or follow CIN2 can be difficult, especially in young women.[348] Hence, it would be useful to have a nonablative management alternative. Many clinical trials have included both CIN2 and CIN3 patients. This mixture can complicate the interpretation of results, as the end point encompasses a rather broad biological spectrum of HPV disease, from relatively recently acquired infections with somewhat greater dedifferentiation than normal to longstanding carcinoma in situ.[63] Better responses might be expected among the more recently acquired lesions. Both CIN2 and CIN3 may regress spontaneously, making it difficult to interpret uncontrolled trials.

With the move toward HPV DNA–based primary screening for cervical cancer, there will be an increasing number of women diagnosed with persistent oncogenic HPV infection without clinical lesions.[15] Although these women are at a substantially elevated risk of developing premalignant lesions and ultimately cancer, there are currently no reasonable treatment options for them. A well-tolerated and effective therapeutic vaccine against persistent oncogenic HPV infection would likely have a large future market.

In addition to cervical dysplasias, HPV-induced vulvar, vaginal, and anal dysplastic lesions provide similar opportunities for therapeutic vaccination.[74] They may be less common and not as routinely identified, but in many cases are more difficult to treat by current ablative methods. A substantial number of oral cancers, particularly those of the tonsils and oropharynx, contain HPV DNA, especially type 16, and also express E6 and E7.[349] However, the precursor lesions for these cancers have not been unambiguously identified. Consequently, immunotherapy of HPV-associated oral lesions would currently be limited to cancers.

Genital warts are an attractive target for therapeutic intervention because of their high incidence, considerable morbidity, and frequent recurrence following conventional treatments.[350] There is also the possibility for long-term

follow-up in vaccination studies without alternative intervention in wart patients, in contrast to CIN2/3 patients, where nonresponders receive conventional ablative therapy after no more than 6 months to prevent progression to cancer.

Animal Models

The current animal models for investigating immunotherapeutic approaches against genital HPV infection are limited and have several deficits. HPVs do not cause hyperproliferative diseases in animals, and none of the animal PVs used in established models induce anogenital neoplasia in immune competent animals. Most studies of HPV therapeutic vaccines have used mouse-transplantable tumors expressing the HPV oncogenes. Subcutaneous injection of TC-1 cells, which express HPV16 E6 and E7 in a H-2Db background, is the most commonly used model.[351] A similar murine line that presents E6/E7 peptides in the context of human HLA-A.2 and forms tumors in A.2 transgenic mice has also been used.[352] Protection from tumor challenge and induction of tumor regression is primarily CD8 cytotoxic T-lymphocyte–mediated in these models. These rapidly growing subcutaneous tumors are biologically quite different from the slow growing in situ epithelial tumors that are the clinical targets of most HPV vaccine studies, and it is currently uncertain whether CD8 or other cell-mediated responses are primarily responsible for regression of the latter.[353] In addition, the models appear to have rather low stringency, as several therapeutic vaccination strategies that have proven to be successful in these models have not generated encouraging result in clinical trials.[354,355]

A orthotopic mouse model involving implantation of TC-1–derived tumor cells into the vaginal epithelium may provide a more biological relevant setting for evaluation of HPV therapeutic vaccines.[356] Alternatively, induction of effective immune responses to in situ HPV gene-expressing lesions has been studied in a skin transplantation model, involving grafting onto syngeneic mice the skin of mice transgenic for an HPV16 E7 gene expressed from a basal keratinocyte-specific promoter. The grafts are not spontaneously rejected in immune-competent recipients or in recipients vaccinated with some therapeutic vaccine candidates that lead to rejection of transplantable tumors. Several treatment regimens have induced graft rejection in this model, with overcoming the local immunosuppressive microenvironment a central component to many of them (see reference 357 for a review).

Preclinical studies of therapeutic wart vaccines have primarily employed either CRPV infection of domestic rabbits or canine oral PV (COPV) infection of dogs. The COPV model is attractive because it involves a mucosal infection. However, it can be difficult to study immunotherapies in this model because of the rapid rate of spontaneous regression.[358,359] In contrast, CRPV-induced lesions of the epidermis tend to persist, and most immunotherapeutic approaches have not consistently induced regression of established CRPV warts.[360–362] The principal lessons from these studies are that E2 appears to be the most effective target for immunotherapy and that inclusion of more viral protein targets increases the frequency of regression.

Clinical Trials

Many different strategies have been tested clinically to induce T-cell responses and regression of HPV-induced neoplasia, including peptides, fusion proteins, naked DNA, viral vectors, and dendritic cell–based approaches (see references 354, 355, 357, and 363 for reviews). Several Phase I/II studies in CIN2/3 patients have been published to date. In general, they detected antigen-specific T-cell responses in most vaccinees, but most lacked a placebo control arm and/or sufficient size to critically evaluate clinical responses. However, some encouraging results have been obtained in several relatively larger and/or placebo-controlled trials.

Zycos (then MGI Pharma, now Eisai) conducted a placebo-controlled trial in CIN2/3 patients using a DNA-based vaccine composed of HPV16 and HPV18 E6 and E7 epitopes encapsidated in biodegradable polymer microparticles. A significant clinical response was detected, but only in the subset of 43 women younger than 25 years of age, in whom 70% of lesions regressed, compared with 23% of controls.[365]

An attenuated modified vaccinia Ankara (MVA) expressing mutated HPV16 E6 and E7 and interleukin-12 was developed by Transgene. A clinical response, defined as no CIN2/3 at 6 months, was observed in 10 of 21 (48%) HPV16-positive CIN2/3 patients receiving three doses of the vaccine.[366] The expected rate of spontaneous regression of HPV16 related CIN2/3 is approximately 20%. Eight of the 10 clinical responders were HPV16 DNA–negative at the end of the study.

Stressgen (then Nventa, now Akela) tested a mycobacterial heat shock protein 65–HPV16 E7 fusion protein in several uncontrolled Phase I/II trials.[367–369] The bacterial heat shock polypeptide was included as a molecular adjuvant to activate dendritic cell induction of innate immune responses. Some evidence of efficacy was obtained. For example, 6 months after 58 CIN2/3 patients received three injections, 13 (22%) had a complete pathological response (CIN1 or less), 32 (55%) had partial responses (lesion regression >50%), and 11 (19%) had stable disease.[368] There was no difference in the rate of regression in women infected with HPV16 compared with those infected by other types.

Medigene sponsored a placebo-controlled trial of an HPV16 chimeric VLP in which a portion of HPV16 E7 was fused to L1, thus creating a vaccine with both prophylactic and therapeutic potential. Regression rates in HPV16 DNA–positive CIN2/3 patients at 6 months after intramuscular injection without adjuvant was 39% and 25% in chimeric VLP and placebo vaccines, respectively, a nonsignificant difference.[370] E7 T-cell responses were modest and did not correlate with regression.

Another, quite distinct, MVA-based vaccine has also been investigated. It involves injection of an MVA recombinant expressing bovine PV type 1 E2 directly into the uterus of CIN patients.[371,372] All the vaccines discussed above involve intramuscular or subcutaneous injection of E6/E7-based vaccines. By contrast, this vaccine in theory would function primarily through (a) induction of E6/E7 immune responses that would indirectly result from E2-mediated apoptosis of the HPV E6/E7 expressing tumor cells (E2 inhibits transcription of the viral oncogenes) and (b) the MVA vector inducing a proinflammatory environment at the tumor site. Clinical regression was often observed over 14 weeks in a large, single-arm Phase III study involving 1176 women and 180 men with HPV-induced intraepithelial lesion. Complete regression was seen in 95% and 73% of previously untreated low-grade and high-grade dysplasias in women, respectively, and in 100% of previously untreated urethral and anal condyloma in men.[373] While these results are certainly encouraging, the role of E2 in inducing the responses remains unclear, as the parental MVA was not evaluated. It is also unclear whether the presumably vaccine-induced regression primarily involves tumor antigen-specific or antigen-independent responses.

High-grade VIN is a chronic disorder with a low spontaneous regression rate that often recurs after ablative therapy and is, therefore, an attractive target for HPV-directed immunotherapy. Complete regression of VIN3 was observed, after a 12-month follow-up, in nine of 19 (47%) HPV16-positive

VIN3 patients treated with three or four doses of a mixture of HPV16 E6 and E7 long peptides (25–35 amino acids) in incomplete Freund adjuvant.[374] While there was no control arm in the trial, the response rate was considerably higher than the expected spontaneous regression rate of 2% in VIN3 patients. Interestingly, complete clinical responses correlated with small lesion size, systemic E6/E7-specific interferon-γ–associated CD4 and CD8 T-cell responses and lower numbers of CD25(+)/Foxp3(+) T-regulatory cells in the lesions.[375]

Another trial in VIN2/3 patients involved activation of local innate immune responses, via an 8-week course of topical imiquimod (a Toll-like receptor 7 ligand), followed by induction of systemic HPV immunity by three intramuscular injections of an HPV16 E6/E7/L2 fusion protein vaccine (delivered without adjuvant).[376] Complete regression was observed in 12 or 19 (63%) of patients after 12 months of follow up. As in the previous study, regression correlated directly with antigen specific T-cell responses and inversely with the levels of T-regulatory cells. However, the role of the vaccine component of this combined therapy remains uncertain, because a similar rate of VIN2/3 regression was seen in a study involving 16 weeks of topical imiquimod treatment without vaccination.[377]

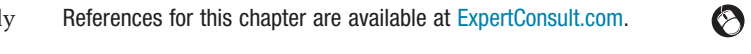
Several uncontrolled Phase I studies suggested that therapeutic vaccines might promote regression of anogenital warts. However, in the one large placebo-controlled trial reported to date, therapeutic vaccination with an HPV6 L2-E7 fusion proteins plus GlaxoSmithKline's AS02 adjuvant (monophosphoryl Lipid A and QS21 in an oil-in-water emulsion) failed to increase the efficacy of conventional therapy in a study of 320 patients infected with HPV6 or HPV11.[378]

WHY THERAPEUTIC HUMAN PAPILLOMAVIRUS VACCINES HAVE HAD MODEST SUCCESS

Several factors may have contributed to the limited success thus far of therapeutic HPV vaccines. First, except for the E2 recombinant MVA vaccine, systemic immune responses have been generated and monitored in the trials (with one notable recent exception[379]), whereas local mucosal or epidermal cell-mediated immunity responses are likely to be critical for clearing HPV infected lesions.[353] There is evidence that T cells generated in response to systemic exposure to an antigen do not efficiently migrate into mucosal epithelium, particularly in the absences of local proinflammatory signals.[380–382] The generation of a higher density of T cells in mucosal/epidermal tissues might be needed to produce more effective therapeutic HPV vaccines. This goal might be achieved by local induction of immunity[382] or recruitment of systemic immune responses by topical application of immune response modifiers (e.g., Toll-like receptor agonists or chemokines) after vaccination.[383,384] Second, persistent HPV infection can result in immune tolerance and/or active suppression to the viral antigens because of the activity of the viral oncogenes and long-term exposure of the immune system to low doses of the antigen in a noninflammatory epithelial setting.[58] The transition from normal epithelium to CIN and eventually cancer is associated with a decrease in interferon-γ and increases in interleukin-10, Foxp3+ T cells, indoleamine 2,3-dioxygenase (IDO)+ cells, and macrophages—all indicative of the evolution of a suppressive microenvirionment.[363] The inverse correlation between the number of lesional T-regulatory cell and clinical response supports the importance of altering the local immune environment for vaccine effectiveness. Third, cervical cancers and high-grade dysplasias are genetically unstable, and therefore often evolve to evade immune induction and recognition. The majority of cervical cancer and many CIN3 lesions have defects in major histocompatibility complex class I presentation, often through downregulation of specific class I alleles or defects in processing peptides for class I presentation.[385,386] The failure to detect a significant clinical response in older women in the Zycos trial cited above may have been a result of the longer mean duration of infection in older women and, therefore, the greater probability that immune tolerance/suppression to E6/E7 and/or evolution of immune evasion by the lesions had occurred.

Despite the limited success of therapeutic HPV vaccines to date, there are several reasons to be optimistic about future developments. Our increasing knowledge of the molecular mechanisms involved in the induction and suppression of cell-mediated immunity responses and their targeting to specific tissues should contribute to the rational design of future vaccines. In addition, the apparent continuing interest in academia and industry in these vaccines and the number of therapeutic clinical trials being conducted bodes well for future breakthroughs, particularly with respect to therapy of persistent infection and low-grade disease.

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31 Inactivated Influenza Vaccines

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HISTORY AND IMPORTANCE

History of the Disease and Vaccines

Influenza is a leading cause of morbidity and mortality worldwide, occurring in annual epidemics and in occasional global pandemics. Five global pandemics of influenza have been well-described, the first beginning in 1889, with subsequent pandemics occurring in 1918, 1957, 1968, and 2009.[1] Each of these pandemics was associated with high attack rates and substantial disease impact. However, influenza pandemics and epidemics have likely occurred for millennia, with early mentions of what might have been epidemics by the 12th century and a pandemic of influenza in the 16th century.[2–4] Isolation of the influenza A virus in 1933[5] rapidly led to the identification of influenza viruses as the cause of epidemics and pandemics of respiratory disease. While the severity and timing of influenza activity was found to vary from year to year, the identification of the influenza virus as the cause of seasonal epidemics (and past pandemics) led quickly to the development, testing, and first use of influenza vaccines in the 1930s and 1940s.[6–9]

Early influenza vaccines were developed for and used primarily in military populations. In 1960, following the 1957–58 pandemic, the U.S. Public Health Service first recommended seasonal influenza vaccines be given to nonmilitary persons who were known to have a high risk of influenza complications.[10] From the beginning, influenza vaccination programs were based on principles that remain relevant for modern vaccine programs. First, for the most part, the strategy of targeting high-risk groups for immunization against influenza became the standard paradigm for influenza vaccination programs and policies globally. With the notable exception of the United States' decision to expand influenza vaccine policy to recommend annual vaccination for all persons older than 6 months of age (i.e., "universal vaccination"),[11] national influenza programs generally target specific persons who are at elevated risk of severe or complicated disease based on age, underlying disease, or pregnancy status.[12,13] Second, until 2003 when the first live attenuated vaccine was approved,[14] influenza vaccines have been produced from inactivated, purified influenza viruses grown in embryonated eggs inoculated with influenza viruses, and administered intramuscularly. Although approaches to influenza immunization have expanded to include additional modes of delivery (e.g., mucosal delivery of live attenuated vaccines, intradermal injection) and approaches (e.g., recombinant protein vaccines),[15] the majority of influenza vaccines given worldwide remain inactivated injected vaccines.[16] Third, because of continual genetic and antigenic changes in influenza viruses, influenza vaccines are continually updated with contemporary strains, and vaccine programs must target recipients annually to ensure protection against current strains. The requirement for annual vaccine development and production and for annual immunization campaigns makes influenza vaccines unique and challenging.

Why the Disease Is Important

Seasonal influenza epidemics are associated with substantial disease burden each year worldwide in persons of all ages. Each year, an estimated 5% to 15% of persons have influenza, although significant variability in rates of illness occur from year to year.[17] Most infections lead to self-limited disease that does not result in healthcare visits; however, severe complications resulting in hospitalization and deaths may occur, particularly in persons with certain underlying health conditions and among young children and the elderly.[18–23] Each year in the United States, for instance, influenza is estimated to be associated with thousands to tens of thousands of deaths,[24,25] and hundreds of thousands of hospitalizations.[22,26,27] As a result, influenza-associated illnesses are a substantial cause of lost work and school time, overwhelmed hospitals and regional medical care systems, and a large economic burden to health systems and families.[28–31] Few other infectious diseases have adversely affected the health and economies of global populations as consistently and extensively as influenza.

Because of the importance of influenza in terms of global disease burden, vaccination against seasonal influenza viruses has been long promoted as the optimal method of influenza prevention. However, the capacity of influenza A and B viruses to undergo continual antigenic change in their two surface antigens, the hemagglutinin (HA) and neuraminidase (NA), complicates vaccination against the disease. This type of antigenic change, which is known as *antigenic drift*, results from the accumulation of point mutations in the RNA genes encoding H and N proteins and leads to the emergence of new variant influenza strains (Fig. 31.1).[32] As the antibody prevalence to older variants increases in the population, the circulation of the older, previously dominant variants is suppressed, allowing new antigenic variants to become predominant. When antibody prevalence to the new variant increases within a population, yet another antigenic variant emerges and the cycle continues. This ongoing process of antigenic drift ensures a continually renewed pool of susceptible hosts and the repetitive occurrence of epidemics. The occurrence of antigenic drift requires robust global surveillance systems that can monitor for the emergence of new strains, and careful review of these data contribute to semiannual updates to influenza vaccine formulations.[33–39]

Finally, the importance of influenza lies also in its capacity to cause global pandemics. Influenza pandemics are the result of "antigenic shift," when an influenza A virus with an H or an H-N combination that has never or not recently infected humans gains the ability to infect, cause disease, and transmit efficiently among humans (see Fig. 31.1).[32] In this case, the population immunity to the new subtype is sufficiently low to facilitate person-to-person transmission and increase severity of illness among those infected. As a result, pandemics are generally associated with higher rates of illness and death compared with seasonal influenza.[40] Four pandemics have occurred in the past century: 1918 (when a new H1N1 emerged), 1957 (H2N2), 1968 (H3N2), and 2009 (H1N1).

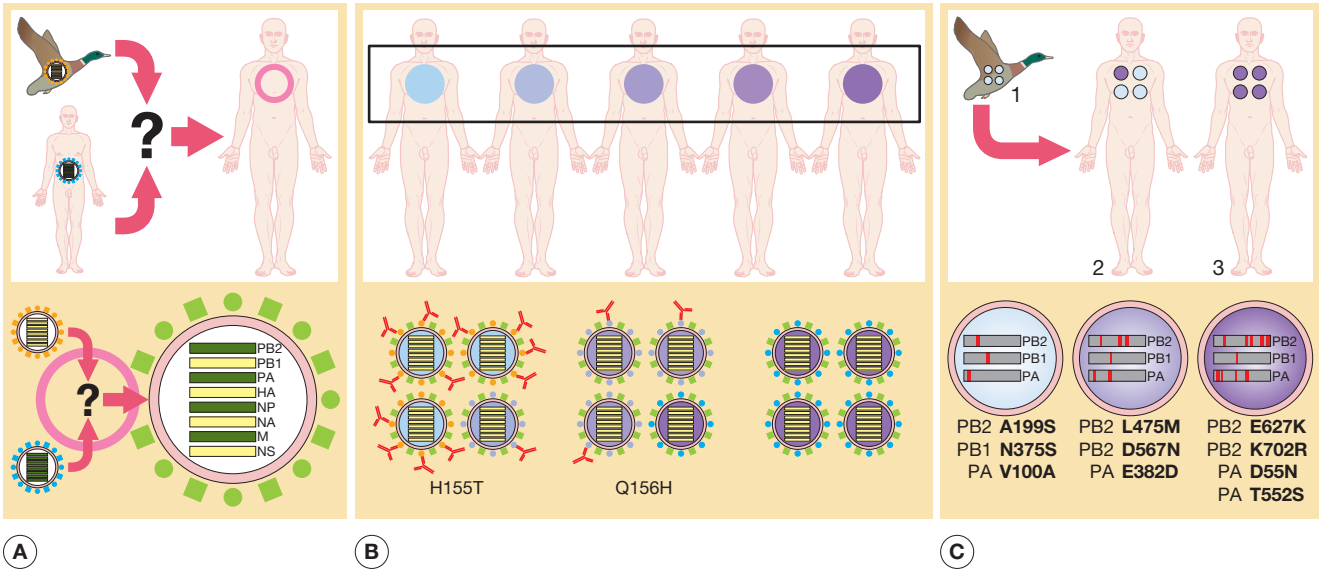


Figure 31.1. Classical mechanisms of influenza A virus variation. A, Antigenic shift, representing what is thought to have occurred in the 1957 pandemic, in which a human H1N1 virus reassorted with an avian H2N2 virus. **B,** Antigenic drift, the process by which circulating influenza viruses in the human population mutate under pressure from antibody to hemagglutinin. **C,** Changes shown experimentally to be associated with adaptation of influenza viruses to mammals or to affect receptor binding or virulence. *(From Doherty PC, Turner SJ, Webby RG, et al. Influenza and the challenge for immunology.* Nat Immunol. *2006;7:449–455.)*

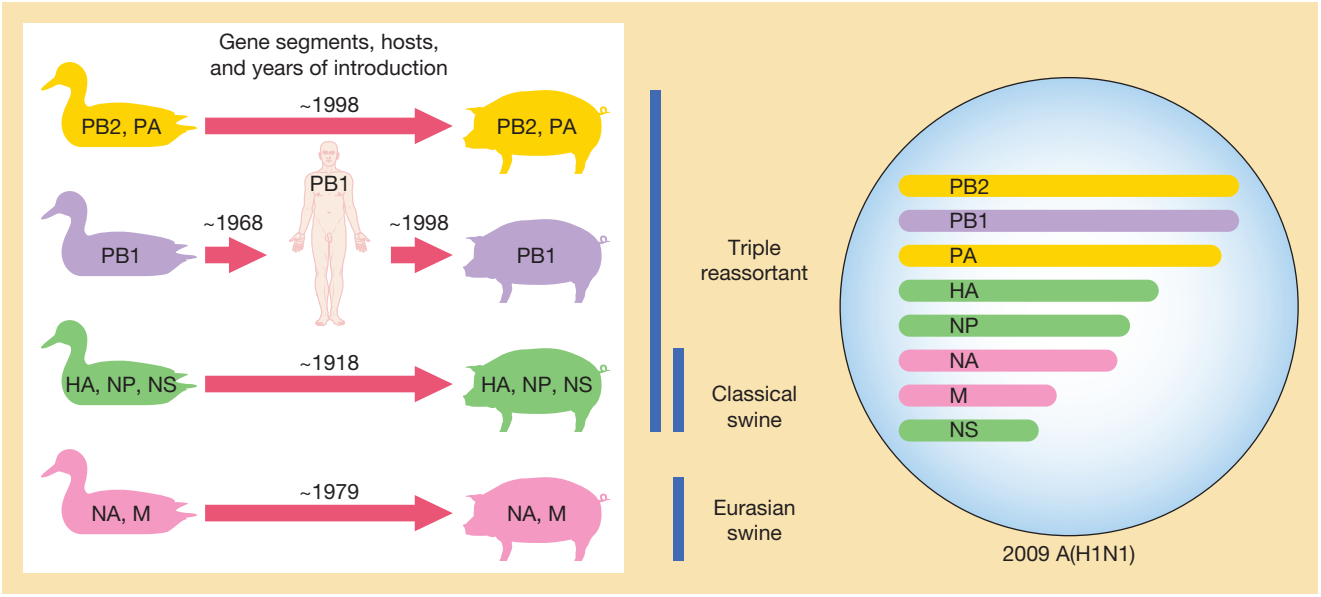


Figure 31.2. Host and lineage origins for the gene segments of the 2009 A(H1N1) virus. Color of gene segments in the circle indicates most recent and prior originating hosts. Genes: HA, hemagglutinin; M, matrix; NA, neuraminidase; NP, nucleoprotein; NS, nonstructural; PA, polymerase acidic; PB1, polymerase basic 1; PB2, polymerase basic 2. *(From Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A[H1N1].* Science. *2009;395:197–201.)*

Pandemics, such as the "Spanish flu" pandemic of 1918, can be catastrophic.[41,42] However, as demonstrated during the 2009 (H1N1) pandemic, an H subtype that is sufficiently antigenically different from those in currently circulating strains can be enough to cause a pandemic; that is, a new subtype is not necessary if a large segment of the population lacks immunity to the new virus (Fig. 31.2).[43] Pandemic influenza viruses are always influenza A viruses because of the presence of a large reservoir of influenza viruses in animal populations, particularly in avian and swine populations, to which humans have no immunity. The continued circulation and evolution of animal influenza viruses present a constant threat that one of these viruses will acquire the potential to cause a pandemic.[44] The emergence of highly pathogenic avian influenza A(H5N1) viruses in Asia in 1997 with their rapid spread and capacity to cause severe human illnesses,[25,26] led to intensive global and national efforts to prepare for pandemic influenza.[45] Even though influenza B viruses do not pose a pandemic threat, continued antigenic "drift" among these viruses also occurs, with resulting substantial interpandemic disease burden.[46] Since 1997, substantial investments have been made in expanding global surveillance for new emergent

viruses, creating more robust pandemic response plans, proactive pandemic vaccine and antiviral development and stockpiling, and in forming more coordinated international partnerships.[37,39,47–50] These efforts produced real benefits during the 2009 response, but there is a recognition that continued work is required for more effective responses to future pandemics.

Although the quality, availability, and use of inactivated influenza vaccine have increased substantially, influenza vaccines are underused in most countries, and influenza remains a poorly controlled infectious disease.

BACKGROUND

Clinical Description

Influenza is characterized by respiratory symptoms, such as cough and sore throat, along with constitutional symptoms, such as fever, headache, myalgias, chills, fatigue, and anorexia.[19,23,37,38,51–54] In uncomplicated influenza cases, signs and symptoms have abrupt onset and generally persist for approximately 7 days, but resolve with nonspecific therapies. The presence of cough and fever are the best predictors of influenza illness in adults and children during periods of influenza circulation.[54–57] Fever usually ranges between 38°C and 40°C, but may be higher, and usually lasts for 3 to 5 days.[52] Cough is usually unproductive of sputum, but rhinorrhea is common. Substernal tenderness and photophobia occur less frequently. Gastrointestinal symptoms, such as vomiting, diarrhea, and abdominal pain, are less common, but more likely to occur in children. Rash has occasionally been described with influenza B infections.[58] Young infants may present only with unexplained fever or sepsis-like syndrome, without prominent respiratory symptoms. Because fevers are often higher in children; influenza can present as febrile seizures in this age group.[19] Fever may be absent in the elderly, while the presenting signs may include anorexia, lassitude, or confusion.[59,60] Illness typically improves within a week, but cough and malaise may persist for 2 or more weeks. A minority of patients may experience fatigue for months.[61]

Asymptomatic influenza infections are likely common, and may account for greater than 30% of all influenza infections,[62–67] although estimates have varied considerably depending on methods used to confirm influenza infection and study design.[68] Asymptomatically infected persons can shed detectable influenza virus and may transmit influenza to others.[69]

Complications of Influenza

Although any influenza illness can result in severe disease and complications, the risk of complications from influenza is elevated in persons with certain underlying health conditions,[18] young children, and elderly persons compared with healthy nonelderly adults.[22,24,70–76] In addition, pregnant women have had higher rates of hospitalizations associated with seasonal influenza,[77] especially those with underlying health conditions.[77,78] Even though increased mortality caused by seasonal influenza among pregnant women has not been consistently observed, increased complications and risk of death in this population has been documented during pandemics.[79–82] During the 2009 pandemic, pregnant women accounted for 6% to 10% of all patients who required hospitalization or died.[80,81,83,84] In addition, risk factors for severe disease highlighted during the 2009 pandemic included morbid obesity[85–87] and aboriginal ethnic or racial origin (e.g., American Indian, Alaskan Native, First Nation peoples, Australian aboriginal peoples).[88–90] Although risk factors for complications are well known, severe influenza complications and deaths can also occur each season among persons who have no known risk factors.[72,91–93]

The most common serious complications of influenza include exacerbation of underlying chronic pulmonary and cardiopulmonary diseases.[22,73] Increased hospitalizations and clinic visits associated with influenza have been reported among persons with chronic obstructive pulmonary disease,[59,94] asthma,[76,92,95–97] and congestive heart failure.[98,99] In addition, some persons with other chronic diseases also have higher rates of disease complications during influenza illnesses. Children with underlying neurologic diseases have increased risks of hospitalization and deaths, likely because of preexisting difficulties handling respiratory secretions or restrictive lung disease.[100] Exacerbations of diabetes,[98–101] renal diseases,[98,99] and hemoglobinopathies[100,102] have also been reported. Persons with immunocompromising conditions, including cancer, hematopoietic and solid-organ transplant recipients, and HIV have a higher risk of severe or prolonged disease and chronic viral shedding associated with influenza.[99,100,103–108]

In addition to exacerbations of underlying respiratory conditions, influenza can cause lower respiratory tract disease as a result of primary viral pneumonia or from secondary bacterial pneumonias. Influenza has been associated with approximately 10% of hospitalized lower respiratory infections in studies from developed and developing countries that have been conducted for at least 1 full year, with higher proportions observed during periods of influenza circulation.[19,27,31,109–111] Primary viral pneumonia can range from mild disease with radiographic evidence of pneumonia to severe disease with respiratory failure.[52] Primary viral pneumonia and pneumonitis can occur during any influenza infection, but is more commonly observed during pandemics, such as in 2009, where severe cases with shock and respiratory failure were reported.[30,96,112–115] Primary viral pneumonias are also reported among infections with novel influenza A viruses, such as avian influenza A(H5N1).[26] Influenza cases can lead to pneumonias due to secondary bacterial infection, most commonly associated with *Streptococcus pneumoniae and Staphylococcus aureus*.[76,113,114,116–120] Bacterial pathogens have been detected in approximately 20% to 40% of pediatric influenza-associated deaths.[117,118]

More common respiratory tract complications of influenza include otitis media and laryngotracheobronchitis in children and bronchitis in adults. While less common, influenza is associated with nonrespiratory complications that affect almost all other organ systems.[23,121] Neurologic complications include febrile seizures in children, encephalitis/encephalopathy, aseptic meningitis, transverse myelitis, and Guillain-Barré syndrome.[122,123] Reye syndrome, an encephalopathy with hepatic inflammation, associated with influenza has been reported primarily in children associated with salicylate ingestion,[124–127] but the incidence of Reye syndrome has decreased dramatically in the United States after warnings were issued in the 1980s regarding the use of aspirin to treat children.[125,126,127a] Myocarditis and pericarditis were reported in association with influenza, especially with influenza B infections in children. Myositis with rhabdomyolysis and myoglobinuria has been reported, but is uncommon.[128,129] Influenza can present as sepsis-like syndromes in infants and has been associated with hematologic abnormalities, including lymphopenia and disseminated intravascular coagulation.[23]

Virology

Taxonomy and Nomenclature

Influenza viruses, together with Thogoto-like viruses, form the family *Orthomyxoviridae*.[125] Influenza viruses are further

divided into three genera, *influenzavirus A*, *influenzavirus B*, and *influenzavirus C*, based on antigenic differences in two major structural proteins, the nucleoprotein (NP) and the matrix protein (M). Influenza A viruses are further classified into subtypes according to the properties of their major membrane glycoproteins, HA and NA (see Fig. 31.1).[125] Eighteen HA subtypes and 11 NA subtypes have been identified among influenza A viruses.[130] The nomenclature of human influenza viruses includes the type of isolate, the geographic location where it was isolated, the year of isolation, a laboratory identification number, and, for influenza A viruses, the subtype of the HA and NA (e.g., A/Panama/2007/99 [H3N2]). For animal influenza viruses, the type of animal from which the sample was obtained is also included (e.g., A/chicken/Hong Kong/220/1997 [H5N1]).[125]

Natural Hosts

Influenza A viruses naturally infect humans. Influenza A viruses of the H1N1, H3N2, and (rarely) H1N2 subtypes are currently circulating among humans whereas H2N2 viruses circulated among humans in the mid-1900s. However, 16 of the 18 influenza A HA subtypes and 9 of the 11 NA subtypes have been isolated from wild aquatic birds, which serve as a natural reservoir and a source of novel genes for pandemic influenza viruses. Influenza A viruses also infect poultry, pigs, horses, dogs, and occasionally sea mammals.[125] Interspecies transmission of influenza A viruses has occurred and can result in severe illness, as demonstrated by the transmission of influenza A(H5N1) viruses from poultry to humans.[131–134] In 2009, a novel influenza A(H1N1) virus was the cause of a worldwide pandemic. This virus possessed a unique combination of gene segments, including genes that originated from swine, avian, and human influenza viruses that were established in pigs in North America and Europe (see Fig. 31.2).[43] Widespread circulation of the 2009 H1N1 virus occurred in humans before infections in swine were documented, and humans infected with the 2009 H1N1 virus can infect swine.[135] The highly pathogenic influenza A(H5N1) virus also causes illness and death in many different animal species not previously known to be infected with influenza A viruses, such as cats.[136] Recently, two new subtypes of influenza were identified in bats (H17N10 and H18N11); neither of these subtypes have been detected in wild birds.[137–139] Although influenza B virus infection of seals has been documented,[140] humans are the primary host for influenza B viruses. Influenza C viruses infect only humans and pigs and cause sporadic cases or localized outbreaks of mild upper respiratory tract infection.

Virus Structure

Influenza viruses are enveloped and contain segmented RNA-negative sense genomes (Fig. 31.3).[125] Segmentation of the viral RNA allows exchange of genes (i.e., genetic reassortment) among influenza viruses of the same type (see below). The segmented RNA genome is associated with NP and three viral polymerase proteins (PB1, PB2, and PA) within helical nucleocapsids that are surrounded by the matrix protein and the virus envelope derived from the host cell membrane. The spherical, often pleomorphic 80- to 120-nm virus particles have a surface layer of spike-like projections, 10 to 14 nm long, consisting of HA and NA proteins. HA is the major antigen against which the host's protective antibody response is directed and is responsible for attachment of influenza viruses to oligosaccharide-containing terminal sialic acids on the cell surface during early stages of infection (Fig. 31.4). HA of avian influenza viruses preferentially bind sialic acid (SA) residues attached by $\alpha_{2,3}$ linkage to galactose ($\alpha_{2,3}$Gal) while

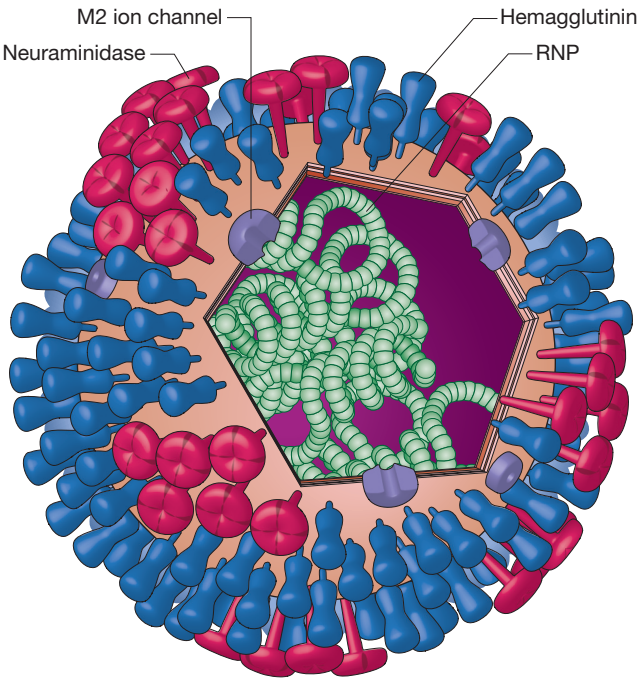


Figure 31.3. Schematic of the influenza A virus depicting some of the key components of virus structure. Hemagglutinin (HA) and neuraminidase (NA) are the two most important surface glycoproteins for host immune response. Protective antibodies that are formed after exposure to influenza viruses (by natural infection or vaccination) are mainly directed at the HA. NA inhibitor medications (oseltamivir and zanamivir), which can treat or prevent infection, block the function of neuraminidase. M2 ion channels control flow of substances into the viral core. Adamantane medications (amantadine and rimantadine) can inhibit M2 ion channel function of influenza A viruses, but are not currently recommended for use because of high levels of resistance among circulating influenza A viruses. RNPs are ribonucleoproteins, which consist of viral genetic material joined with nucleoproteins.

human viruses bind predominantly to those having $\alpha_{2,6}$Gal; however, swine viruses tend to bind both $\alpha_{2,3}$Gal and $\alpha_{2,6}$Gal providing the molecular basis for the observations that pigs serve as a mixing vessel for both avian and human viruses thus facilitating the generation of new influenza viruses.[141] Furthermore, the distribution of SA residues in the respiratory tract varies among the birds, humans, and pigs. While SA $\alpha_{2,6}$Gal is expressed predominantly in the trachea in humans, $\alpha_{2,3}$Gal is expressed on nonciliated cuboidal cells in the region between the bronchioles and alveoli, as well as on alveolar cells, indicating the reasons as to why humans are infected sporadically with avian influenza viruses. Furthermore, the distribution of $\alpha_{2,3}$Gal SA in the lower respiratory tract of humans limits human-to-human transmission of avian influenza viruses via aerosols.[142] In addition, the presence of multiple basic amino acids at the cleavage site in avian HAs and its cleavage by endoprotease furin is attributed to their pathogenicity in susceptible species.[143] Because furin is widely distributed in the body in various tissues and organ systems, avian influenza viruses containing polybasic amino acids are able to infect a wide range of cell types leading to severe disease. The HA is a homotrimeric transmembrane protein consisting of a globular head (HA1) and membrane-anchoring α-helical chain stem (HA2). The error-prone nature of RNA polymerase coupled with immune selection pressure leads to hypervariability in the globular head region. Phylogenetic differences of the HA led to grouping influenza viruses into Group 1 or 2. While H1,

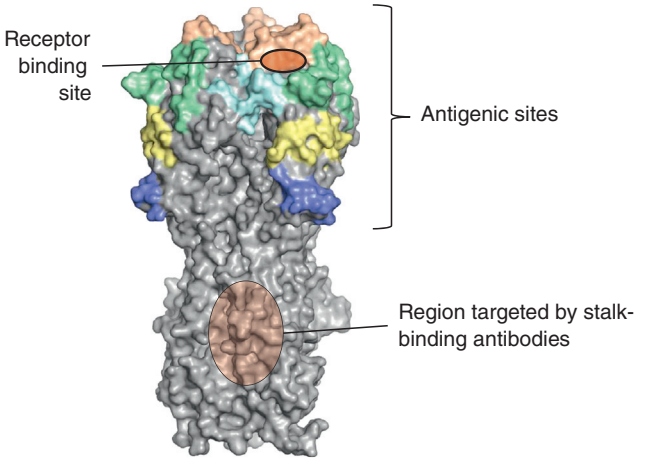


Figure 31.4. Influenza A (H3N2) hemagglutinin illustrating the receptor binding site in the variable head region and the more conserved stalk region. *(Courtesy James Stevens, Centers for Disease Control and Prevention, based on Popova L, Smith K, West AH, et al. Immunodominance of antigenic site B over site A of hemagglutinin of recent H3N2 influenza viruses.* PLoS One. *2012;7:e41895; Daniels RS, Douglas AR, Skehel JJ, et al. Analyses of the antigenicity of influenza haemagglutinin at the pH optimum for virus-mediated membrane fusion.* J Gen Virol. *1983;64(Pt 8):1657–1662; and Stray SJ, Pittman LB. Subtype- and antigenic site-specific differences in biophysical influences on evolution of influenza virus hemagglutinin.* Virol J. *2012;9:91.)*

H2, H5, H6, H8, H9, H11, H12, H13, H16, H17, and H18 HAs are placed in Group 1, HAs H3, H4, H7, H10, H14, and H15 are placed in Group 2.[144] While the immunodominant immune response consists of strain-specific antibodies directed against the globular head (HA1), broadly neutralizing antibodies directed to the highly conserved conformational stem (HA2) region can be induced (see "'Universal Vaccines" later). These broadly neutralizing antibodies neutralize many strains within Group 1 or Group 2 influenza viruses.[145] NA is less abundant on the viral surface and facilitates release of mature virus from infected cells. It is homotetrameric protein with a mushroom head and is anchored with a short stem. Similar to HA, nine NA types of influenza viruses are grouped into two groups. Group 1 contains the N1, N4, N5, and N8 subtypes, and Group 2 consists of N2, N3, N6, N7, and N9 subtypes.[146] NA types 10 and 11 are genetically distinct from other NAs. Antibody to NA is believed to restrict virus spread and reduce severity of the influenza infection. By definition, the HA and NA of different subtypes are serologically distinct.[125] Although some serological cross-reactivity may exist among HA or NA of the same influenza subtype but from different species, the extent of cross-protection afforded by such responses is not well understood. During the 2009 H1N1 pandemic, most studies showed that persons who had previously been vaccinated against or likely to have been infected with circulating seasonal influenza A(H1N1) strains appeared to have no significant protection against severe cases with the novel H1N1 virus; some studies performed in Canada suggested that previous vaccination with seasonal influenza vaccines might have increased risk for symptomatic infection with the 2009 H1N1 virus.[147–152]

The influenza A virus envelope also contains matrix (M1) and transmembrane (M2) proteins. M1 protein is located inside the viral membrane and is thought to add rigidity to the lipid bilayer, whereas the M2 protein functions as a pH-activated ion channel.[125] A nonstructural gene encodes two proteins: the multifunctional NS1 which is found only in virus-infected cells and nuclear export protein (NEP), also known as NS2, which is a minor virion component involved in nuclear export of the nucleocapsids containing viral RNA.[125]

Pathogenesis as It Relates to Prevention

Infection and viral replication of seasonal influenza viruses occur primarily in the columnar epithelial cells of the respiratory tract,[153,154] but viral replication can occur throughout the respiratory tract. After infection, epithelial cells become vacuolated, lose cilia, and become necrotic. Regeneration of epithelium takes approximately 3 to 4 weeks, during which time pulmonary abnormalities can persist. Influenza viruses are rarely isolated outside of the respiratory tract, even though infection can result in signs and symptoms referent to several other organ systems and is associated with pronounced constitutional manifestations. Viremia is rarely observed with seasonal influenza virus infections,[155–159] although it has been detected more frequently in human infections with avian influenza A(H5N1) viruses.[160]

Consequently, influenza viruses are spread through exposure to respiratory secretions containing virus from an infected person. The most likely modes of transmission are through large or small droplet aerosols produced by the coughing, or perhaps sneezing, of infectious individuals. The relative contribution of large droplet versus aerosol transmission is not completely clear, but influenza viruses have been detected in large and small droplets, and recent data indicate that aerosol transmission of fine droplets are a significant source of transmission.[161,162] Transmission of virus by direct contact with infected respiratory secretions can also occur, including transmission through fomites.[163] No evidence for transmission through bloodborne or sexual routes has been described.

The incubation period for influenza is commonly 2 days, but ranges from 1 to 4 days.[164] Virus can be detected in the upper airway for up to a day before symptoms and approximately 3 to 5 days after illness in adults. The peak of viral shedding, 1 to 3 days following illness onset,[165–167] likely coincides with the period during which most transmission occurs. Children may shed virus for up to 2 weeks, and have higher virus titers in their upper airway,[168] which is one factor that promotes transmission in school settings. Severely immunocompromised persons can shed virus for months.[169–171]

The transmissibility or reproductive number (Ro) for influenza is estimated to be approximately 1.5 to 2, and the serial interval (the interval between the onset of symptoms in a case patient and the onset of symptoms in the contacts who were infected by that patient) is usually estimated to be 2 to 3 days.[172–176] However, given the typical short incubation period for influenza, influenza outbreaks may be explosive, especially in highly susceptible populations as can occur in a pandemic.[172,177–179]

Diagnosis

Infections by other pathogens, including respiratory syncytial virus (RSV), adenovirus, parainfluenza virus, rhinovirus, numerous other respiratory viruses, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae,* and *Legionella pneumophila*, can result in individual illnesses similar to influenza. Nonetheless, certain epidemiological features of influenza outbreaks and epidemics can greatly aid in the clinical diagnosis of influenza. For example, communitywide or institutional outbreaks of febrile respiratory illness cases during winter and spring months are characteristic of influenza, although RSV also can exhibit a similar epidemiological pattern. In most instances, individual cases of influenza are difficult to identify reliably

by clinical examination and routine laboratory findings alone, and the appropriate use of influenza diagnostic tests is helpful. Such tests include virus isolation (culture) and identification, direct detection of influenza virus in clinical specimens, rapid point of care tests, molecular methods, and serological tests.

Isolation of influenza viruses in cell culture or eggs followed by hemagglutination inhibition (HI) testing to identify the virus was the "gold standard" for influenza diagnosis, but has been largely replaced by reverse-transcriptase polymerase chain reaction (RT-PCR). Isolation of influenza viruses remains critical for identifying viruses for use in influenza vaccines. The sensitivity of viral culture depends on when in the course of illness the specimen is collected and its quality.[180] Results usually are not available for at least 3 days, although some rapid culture methods allows virus to be detected within 18 to 24 hours.[181] While state health department laboratories and some hospital laboratories can type and subtype viral isolates, further antigenic characterization generally is conducted in specialized laboratories.

A variety of sensitive and specific radioimmunoassays, fluoroimmunoassays, and enzyme immunoassays can detect viral antigens in clinical samples. Although these assays can produce a result within a few hours, they are often less sensitive than standard virus isolation and they require specialized laboratory equipment and reagents.[180]

There are many commercially available "point-of-care" tests for rapid diagnosis of influenza. Tests may use immunoassays that detect influenza viral proteins or may detect viral NA activity in specimens. Some tests detect influenza A and influenza B viruses but do not distinguish between them, while others detect only influenza A viruses, or detect and distinguish between influenza A and influenza B. In general, these diagnostic tests are useful for determining rapidly if influenza is the cause of outbreaks in institutions or other settings and for documenting circulation of influenza viruses in populations of patients. These tests generally have a sensitivity of 20% to 100% and a specificity of 52% to 100%, with sensitivity estimates in the higher range derived from reports from manufacturers.[182–184] During the 2009 H1N1 pandemic, sensitivity of point-of-care tests was 11% to 70%, and negative test results were viewed as providing insufficient information to help in making treatment or infection control decisions.[185–187] These tests are also not sensitive for detecting avian influenza virus infections in humans.[188] Collection of additional specimens for viral isolation or RT-PCR testing is strongly recommended to confirm rapid antigen test results for human influenza infections. However, for persons suspected of having H5N1 avian influenza infection, viral isolation should not be done except in laboratory facilities under Biosafety Level 3 enhancements. Influenza H5N1-specific RT-PCR testing conducted under Biosafety Level 2 conditions is the preferred method for diagnosis of human infection with H5N1. In the United States, all state public health laboratories, several local public health laboratories, and the Centers for Disease Control and Prevention (CDC) are able to perform influenza H5N1 RT-PCR testing, and are the recommended sites for initial diagnosis.[189]

Molecular methods are now commonly used to diagnose influenza virus infections and have supplanted virus culture as the "gold standard" for virus detection. Methods using RT-PCR are more rapid and sensitive than virus culture.[180] A new molecular approach uses DNA microarrays containing nucleic acid probes to type and subtype influenza viruses.[190,191]

Influenza virus infections can also be detected by measuring increases in influenza-specific antibody between acute and convalescent serum samples.[192] Techniques for measuring antibody against influenza in sera include HI, virus neutralization, enzyme immunoassay, and complement fixation. In general, these tests are little used outside of a research setting, and may underestimate infections among recently vaccinated individuals.[193]

Drugs Active Against Influenza Viruses

Two classes of prescription medications, adamantanes and NA (NA) inhibitors, have been approved in the United States for use against influenza virus infections. The adamantane derivatives, amantadine hydrochloride and rimantadine hydrochloride, inhibit viral replication by blocking the proton channel formed by the M2 protein of influenza A viruses. The channel activity is necessary for viral particle uncoating during early and late stages of viral replication.[194] A single substitution at one of five residues (26, 27, 30, 31, 34) of the M2 protein is associated with resistance to all adamantane derivatives.[195,196] Widespread emergence and now predominance of adamantine resistance among human influenza A(H3N2) and influenza A(H1N1) viruses and among animal influenza viruses have severely limited the usefulness of these medications for influenza treatment or prophylaxis.[197–200]

The NA inhibitors, zanamivir, oseltamivir, peramivir, and laninamivir inhibit the NA of both influenza A and influenza B viruses. Blocking the active site of the NA results in viral aggregation at the host cell surfaces and fewer viruses released from infected cells.[201–204] Resistance to NA inhibitors can occur from substitutions in the active site of the NA or its proximity that reduce drug binding affinity.[205,206] Cross-resistance to multiple NA inhibitors can occur with single-residue substitutions, often with varying degrees of resistance.[206–208] In addition, multiple NA substitutions that result in resistance to multiple NA inhibitors have been reported.[209–212] In 2008, widespread and unexpected circulation of seasonal influenza A(H1N1) viruses that were resistant to oseltamivir and peramivir because of NA substitution H275Y occurred; however, susceptibility to zanamivir was maintained.[213–215] In 2009, the H275Y containing influenza A(H1N1) viruses were replaced by influenza A(H1N1)pdm09 viruses that were susceptible to all NA inhibitors. Currently, it is rare to find NA inhibitors resistance in circulating human or animal influenza viruses.[211,212] Although not common, NA inhibitors resistance can also occur during or after drug use.[209,210,212,216,217]

EPIDEMIOLOGY

Seasonality

Influenza occurs in seasonal peaks in much of the world. In temperate climates, influenza activity typically occurs during the late autumn and winter months—between October and April in Northern hemisphere temperate countries, and between May and September in Southern hemisphere tropical countries.[218] Although in tropical climates influenza transmission occurs year-round, one or two annual peaks can often be discerned in many countries.[219–225] In large countries, differences in seasonal occurrence of influenza can be observed within the country.[220,226] Despite a vaccine nomenclature indicating "Southern" and "Northern" hemisphere formulations, as data from subtropical and tropical countries accumulate, it is clear that seasonal peaks of influenza don't fall neatly into two seasonal patterns—one Northern and one Southern.[218] Data demonstrating a summertime peak in influenza in Central American[12,218] and southeast Asian countries[223] resulted in several countries changing vaccine recommendations to use the Southern hemisphere formulation, despite residing in the Northern hemisphere.

Sporadic cases and institutional outbreaks can occur at any time of the year, including the summer months. Outbreaks

associated with large groups of international travelers suggest that large outbreaks of influenza may begin to occur more frequently during unusual times of the year.[227,228] Within communities, epidemics or outbreaks typically last 6 to 8 weeks or longer. Reasons for the seasonality of influenza are not completely understood and are likely multifactorial and include the role of temperature and humidity on virus survival,[229] and seasonal differences in behavior (indoor crowding in winter months and school attendance).[230–232]

Even in locations where seasonal peaks occur each year, the start, peak, duration, and size of individual seasons vary substantially from year to year and are not predictable. In the United States, approximately 80% of seasons have peaked in January to March during the past 30 years, but peaks in December have been observed in three recent seasons. The timing, size, and impact of epidemics reflect the interplay of several factors, including the extent of the virus' antigenic variation, virulence, and transmissibility; the extent of immunity in the population; and the specific population groups that are affected.

Incidence of Disease

Influenza attack rates vary by season, by geographic location, by setting (e.g., institutional, closed settings versus community settings), by predominant subtype/type, and by age group. In addition, reported attack rates may vary considerably depending on the methods used to document infection (e.g., viral isolation, RNA detection, antibody detection, etc.). In general, virus isolation is considerably less sensitive than RT-PCR or serologic assays for documenting infection; however, not all persons with infections documented by culture will have documented seroconversion and vice versa. However, approximately 30% to 50% of persons with serologic evidence of infection after an influenza season are either asymptomatically infected or have very mild illness.[66,233] Similar rates of mild or asymptomatic infections were observed during the 2009 pandemic.[67]

Among community studies, one study of families in Seattle showed annual attack rates of 19% and 20%, respectively, for serologically confirmed influenza A and B infections between 1965 and 1969.[234] In the same study in 1978–79, rates of influenza A(H1N1) infection soon after its reemergence in 1977 were approximately 31% among persons born after 1956.[235] In Michigan, a community study during 1966–71 found infection rates of 17% and 8% using serology for influenza A and influenza B infections, respectively, but rates of only 1.4% and 1.5%, respectively, using[236] virus isolation.[17,236] The attack rates by age group, based on serology for influenza A, were approximately 15% to 24% in children younger than 5 years, approximately 17% to 21% in children 5 to 19 years of age, and approximately 12% to 18% in persons older than 19 years of age. Rates were lower for influenza B infections. In Houston, between 1976 and 1984, estimated attack rates ranged from approximately 36% to 45% in children 5 years of age and younger, from approximately 40% to 48% in children 6 to 17 years of age, and from 21% to 23% in persons 18 years of age and older.[236]

During the 2009 H1N1 pandemic, the attack rate, as measured by fourfold increases in specific antibody titer among other household members after laboratory confirmation of an index household member, was 20%; 13% of contacts were shown to have viral shedding by RT-PCR. Serial cross-sectional seroprevalence data obtained before and after the summer-fall 2009 H1N1 pandemic wave indicated that the overall attack rate was 11%, including 43% in persons 5 to 14 years of age, 16% in persons 15 to 19 years of age, 12% in persons 20 to 29 years of age, and 5% in persons 30 to 59 years of age.[237] Attack rates among institutionalized populations can be much higher. In military and boarding school populations, attack rates up to 87% and 90%, respectively, have been reported. One review of outbreaks in nursing home populations estimated an average attack rate of 43%,[238] although higher rates have also been found.[239] Influenza outbreaks also have been described in hospitals[240–242] and aboard cruise ships.[227,228]

Current Epidemiology as It Relates to Vaccination

Vaccination strategies for influenza have evolved over time to address the improved understanding of risk groups, changing epidemiology of influenza viruses, and to maximize efficacy in specific target populations. Importantly, vaccine strategies have largely focused on targeting specific groups found to be at increased risk of influenza complications. The first U.S. vaccine policy recognized key high-risk groups for which vaccine was recommended.[10,243] These groups, including elderly persons, those with underlying chronic disease, and pregnant women, remain the foundation for most national influenza immunization strategies[12,244] as well as global recommendations.[245] Even so, over time, additional high-risk groups have been added to many national policies as compelling data were produced demonstrating relatively high risk in these groups. A notable example are policies that target young children, who have a higher risk than older children or non-elderly adults of hospitalizations and deaths associated with influenza. During the 2009 pandemic, data indicated that persons with morbid obesity[85,87,246] and persons from indigenous populations[246–248] were at higher risk of hospitalization and were added to targeted high-risk groups. Finally, because the risk of influenza is universal, even while the risk of severe disease varies among populations, vaccination policy in the United States expanded to recommend influenza vaccine for all persons in 2010.[147] This expansion of policy recognized the value of preventing not only severe disease and deaths,[243] but increasingly preventing milder disease and healthcare visits, with associated costs.

Early monovalent vaccines were replaced with multivalent vaccines, acknowledging the cocirculation of influenza A(H3N2), influenza A(H1N1), and influenza B viruses, the need for type or subtype-specific immunization, and the unpredictability of strain prevalence from year to year.[243] More recently, the emergence and cocirculation of two distinct lineages of influenza B with limited cross-reactivity led to the development and approval of quadrivalent vaccines containing antigens representative of each B lineage.[11] Models have estimated a modest public health benefit of including representative viruses of both B lineages[249]; this benefit may be observed to a greater extent in children, who may generate less robust heterotypic antibody responses to influenza B vaccinations, than among adults.[250]

While multivalent vaccines are used globally in seasonal immunization programs, monovalent influenza vaccines have been used for responding to specific influenza threats. Most notably, monovalent vaccines have been prepared and used for each of the recent pandemics.[8,251–255] The most recent example is during the 2009 H1N1 pandemic. In April 2009, the first cases of human infection with a novel influenza A(H1N1) virus were identified,[256] and the virus spread quickly over the next several months to cause disease worldwide.[257] The 2009 H1N1 pandemic strain possessed a unique combination of gene segments including genes that originated from swine, avian and human influenza viruses that had been circulating among in pigs in North America and Europe.[43] Antibodies to contemporary seasonal influenza A(H1N1) viruses at that time engendered by previous infection or vaccination

offered little or no cross-protection against 2009 pandemic H1N1 viruses.[258] As a result, a monovalent vaccine based on one of the original isolates of the virus was produced and found to be effective in preventing disease associated with the virus[255]; vaccination with the multivalent vaccine produced using the seasonal H1N1 strain was found to confer no protection.[149] Similarly, pandemic strain-specific vaccines were produced for pandemic responses in 1957–58 and 1968–69. A monovalent vaccine also was produced to respond to the emergence of the antigenically drifted strain of H1N1 in 1986.[259,260] Finally, a monovalent vaccine was produced and administered in the United States based on the emergence of a novel H1N1 swine origin virus in 1976, based on the fears that the virus was a pandemic threat.[261] The emergence of a drifted H3N2 virus in 2014–15 with resulting low vaccine effectiveness has renewed interest in developing the ability to produce monovalent or multivalent vaccines rapidly to respond to seasonal threats and to respond to pandemic threats with more rapid vaccine production.

Pandemics of Influenza

Pandemics of influenza have occurred unpredictably and relatively infrequently. Influenza pandemics are caused by the appearance and spread in humans of a new influenza A virus bearing either a novel HA or novel HA and NA combination against which the human population has little or no immunity and which cause illness in humans. The other requirement for a pandemic influenza virus is that it readily spreads from person to person.[262] Influenza viruses that have not circulated in humans in recent years are considered "novel" and arise through antigenic shift or through direct transmission of a novel influenza A virus into the human population from animals if the virus is adapted to human hosts.

Antigenic shift among influenza A viruses can occur in at least two ways. In the first mechanism for shift, the simultaneous infection of a host by influenza A viruses of two different subtypes allows corresponding viral genes to be exchanged (or to reassort) between the infecting viruses. For example, infection of a pig simultaneously by both an avian influenza A virus and a human influenza A virus can allow genes from the virus normally in circulation among birds to reassort with genes of the influenza A viruses circulating among humans. The resulting "reassortment" of human and avian influenza genes could produce new influenza viruses containing many different combinations of genes. Some of the new progeny viruses will contain HA or HA-NA genes from the avian influenza viruses. Pigs may serve as "mixing vessels" for genetic reassortment among influenza A viruses because they are susceptible to infection by avian, swine, and human influenza A viruses since respiratory epithelial cells in pigs possess both SA $\alpha_{2,3}$ receptors for which avian viruses have a preference, and SA $\alpha_{2,6}$ receptors, which are preferred by human influenza viruses.[156] Avian species are considered to be the primary reservoir of influenza A viruses because viruses bearing almost all known HA subtypes circulate among wild birds.[44] The viruses that caused the 1957, 1968, and 2009 pandemics showed evidence of genetic reassortment between avian and human influenza viruses.[4,256]

The second mechanism involves direct transmission of avian viruses to humans with subsequent adaptation to the new host. The 1918 pandemic influenza virus is thought to have arisen in this way.[263] Fears of pandemic influenza have arisen because of the emergence of avian viruses, such as H5N1 and H7N9, that have demonstrated the ability to infect and cause severe disease in humans.[264–267] Since the emergence of avian influenza A(H5N1) in 1997 in Hong Kong,[131] more than 600 human infections have been documented, with a case fatality rate of approximately 60%.[134,160] Similarly, avian influenza A(H7N9) viruses emerged in China in 2013, and since have infected more than 500 persons, almost all in China, of whom approximately 37% have died.[268] Swine-origin influenza A viruses have also been occasional causes of illness in humans in the United States, mostly resulting in mild respiratory illnesses.[269,270] Aside from occasional human-to-human spread of these avian and swine-origin viruses, almost all human infections have been by direct transmission from infected poultry or swine to humans[270–272]; none of these viruses have as yet demonstrated the capacity for sustained human-to-human spread. However, the viruses are geographically widespread in poultry and swine populations, with recent H5 containing viruses identified in poultry in the United States that might allow for more efficient human-to-human transmission.[273]

Pandemics are of great public health significance because of their capacity to result in substantial mortality and morbidity. In addition, because pandemics spread quickly, affect entire countries and regions in a short time, and may have more than one wave, they have the capacity to cause disruptions in healthcare delivery and other societal functions. Between the spring of 1918 and the spring of 1919, three waves of "Spanish flu" caused by influenza A(H1N1) viruses swept around the world leading to more than 550,000 deaths in the United States and more than 20 million deaths worldwide.[262] Three subsequent pandemics, beginning in 1957 (the "Asian flu"), in 1968 ("Hong Kong flu"), and in 2009 were associated with the emergence of the influenza A(H2N2) virus, the influenza A(H3N2) virus, and a new H1N1 virus, respectively, but resulted in lower mortality rates compared with the 1918 pandemic.[262] In fact, the most recent pandemic, which was characterized by relatively low attack rates among persons older than 60 years of age, is estimated to have resulted in fewer deaths than many annual seasonal epidemics in the United States.[274,275] However, during pandemics, severe infections were much more common in children and younger adults compared to typical winter influenza seasons, in which more than 90% of deaths and approximately 60% of hospitalizations occur among persons 65 years of age or older.[73,275] During 2009, the first year after the emergence of the virus, an estimated 61 million illnesses, 274,000 hospitalizations, and 12,400 deaths associated with the 2009 H1N1 virus occurred in the United States.[276] During this period, the highest estimated attack rates occurred in children and young adults, the highest estimated hospitalization rates occurred in children younger than 5 years of age, and 90% of estimated deaths occurred in persons younger than 65 years of age. In one estimate, the mean age among persons whose deaths were attributed to the 2009 H1N1 influenza pandemic was 37 years, compared to a mean age of 76 years among deaths attributed to seasonal influenza A(H3N2).[277] Because of the potential for the development of a new pandemic strain with higher case-fatality rates, preparedness planning has been a priority for most countries since the emergence of H5N1 viruses in 1997. Critical to preparedness has been the development of plans for developing monovalent pandemic vaccines, hoping to circumvent the problem that pandemic vaccines may be available only after the pandemic has begun, mitigating their effectiveness.[49] These plans were effective in responding to the H1N1 pandemic in 2009, and while vaccine was delivered relatively late, it was demonstrated to be effective.[49,255,278] Efforts are underway to speed the process for pandemic vaccine development and delivery, including the continual updating of candidate vaccine virus strains, and work toward more broadly reactive vaccines that might increase the value of prepandemic stockpiled vaccines.

Significance as a Public Health Problem

Influenza is a significant public health threat because of relatively high annual attack rates, coupled with the capacity to cause large numbers of severe illnesses requiring medical care. During influenza seasons, an estimated 5% to 15% of the U.S. population can develop influenza,[17,279] but attack rates of 40% to 50% within institutions such as nursing homes are not unusual.[280,281] In communities, influenza cases often appear first among school-age children, among whom attack rates usually are the highest.[62,235,279,282,283] While attack rates among the elderly are generally lower as a result of differences in mixing patterns and preexisting immunity,[284] rates of serious disease during most seasonal influenza epidemics are highest among the elderly, the very young, and those with certain underlying chronic conditions.[22,24] Influenza epidemics are generally associated with increases in physician visits, hospitalizations, and deaths, as well as increases in school and workplace absenteeism.[65,285,286] The World Health Organization has estimated that between 250,000 and 500,000 deaths occur each year as a result of influenza.[245] While this burden has been well-documented in developed countries,[24,26,287] increasing data from middle-income and low-income countries have confirmed that the burden of severe disease is perhaps even higher in these settings, possibly a consequence of higher rates of underlying conditions such as HIV.[110,288,288a] The large numbers of medical care visits generated by influenza can overwhelm medical care systems, requiring hospitals and emergency services to divert patients.[28] As a result, substantial medical care and societal costs are incurred each season.[29] Pandemic influenza has the potential to cause even greater disease burden and stress on medical care systems. For instance, in 2009, one study found that mechanical ventilation was required by 72% of patients admitted to intensive care units (ICUs), and extracorporeal membrane oxygenation was used for 7% in one series.[82] The high attack rates during that pandemic also prompted school and workplace closures in many areas.

Influenza-associated severe disease cases (such as those leading to hospitalization or death) are usually not confirmed using influenza-specific laboratory tests because of low testing rates or because a person presents for care late in the course of illness, when testing may be less sensitive. As a result, influenza-associated mortality and hospitalizations are usually estimated using models. The most commonly employed models estimate "excess" events, where the number of influenza-related deaths or hospitalizations is calculated by subtracting the number of deaths that would be expected in the absence of influenza (i.e., the "baseline") from the total number of deaths that occurred during an influenza epidemic from the same time period.[72,92,289,290]

Using these methods, in the United States during 1976–2007, the estimated annual number of excess deaths resulting from circulatory or respiratory causes and associated with influenza varied from 3300 deaths to 48,000 deaths.[24] Estimates of the number of deaths using a variety of methods have been similar.[291] Increases in the annual estimated number of deaths caused by influenza since the 1970s have been attributed to the disproportionate growth in the population 65 years of age or older, who account for 90% of deaths caused by influenza. For many of these deaths, influenza is the catalyst of complications resulting from other underlying diseases and might not be recognized as an important cofactor.[21,72] The estimated annual number of deaths related to influenza is typically higher in years where influenza A(H3N2) viruses predominate.[24,289] In the United States, the average annual number of hospitalizations during 1979–2001 that were associated with influenza has been estimated at 226,000 (range: 55,000 to 431,000).[22]

Recently, U.S. estimates of hospitalizations (and outpatient visits) have been calculated using a model in which rates of influenza-confirmed hospitalizations collected from hospital-based active surveillance systems are extrapolated to national figures and then used to estimate less-severe outcomes based on known multipliers. Data from 2005–13 have also estimated substantial numbers of cases and healthcare visits associated with influenza, often estimating higher disease burden than models based on calculation of excess events from non-laboratory confirmed outcomes.[26] According to this model, the United States experienced approximately 400,000 hospitalizations, 14.6 million healthcare visits, and 35 million cases of influenza in the 2013–14 season.[27]

PASSIVE IMMUNIZATION

Passive immunization consists of transfer of ready-made antibodies in serum from an immunized or infected and recovered individual to a naïve individual to protect and/or treat against the disease. During the 19th century, either hyperimmune or convalescent homologous or heterologous polyclonal sera were successfully used both prophylactically and therapeutically against several infectious diseases, including, rabies, tetanus, diphtheria, and influenza, before the advent of antibiotics and antivirals.[292] During the 1918 influenza pandemic, convalescent human serum transfer reduced absolute risk for death in patients from 26% to 8%, if administered within 4 days of the onset of symptoms. However, delayed treatment had a reduced effect on the favorable outcomes.[293] After the identification of the causative agent, in their landmark study Francis and Magill demonstrated that transfer of sera from either influenza virus-infected ferrets and mice or virus-immunized rabbits into naïve mice protected the naïve mice against homologous viral challenge, thus setting the stage for a series of animal studies as well as medical intervention strategies against epidemic influenza.[294] Administration of equine hyperimmune serum or human serum in the respiratory tract for influenza prophylaxis in children or adults reduced influenza-related complications during an epidemic. In addition, passive immunization of sera in patients diagnosed with influenza reduced not only the severity but also the duration of disease.[295]

Despite the demonstrated clinical utility of passive immunization for prophylaxis and treatment against influenza from earlier studies, this approach did not receive much attention because of safety issues associated with the use of human convalescent sera and hyperimmune sera generated in heterologous species. However, an impending avian influenza pandemic and technological advances in generating monoclonal antibodies and polyclonal sera using a variety of approaches along with elucidation of the structural details of HA, reinvigorated interest in exploring passive immunization strategies against pandemic and epidemic influenza.[296–302]

Administration of human or humanized monoclonal antibodies or polyclonal sera against HA or conserved extracellular M2e protected animals against lethal challenge and reduced viral replication. These antibodies vary in their ability to confer protection when given 24 to 72 hours prior to and up to 72 hours postchallenge. Furthermore, intranasal administration is more effective and dose sparing when compared to other routes and a cocktail of monoclonal antibodies is superior and prevents the generation of escape mutants rather than using a single monoclonal antibody.[303–314] Antibodies against the highly conserved stem region of the HA protein have been shown to be broadly neutralizing and/or protective across HA

subtypes as well as within the subtype by inhibiting the fusion of virus and through the function of antibody dependent cell-mediated cytotoxicity function, by lysing virus-infected cells via Fc receptor–expressing macrophages and/or natural killer cells. In contrast, antibodies that are specific to the receptor binding site of the globular domain of the HA inhibit a narrow range of virus subtypes.[145,315–317] Apart from these animal studies exploring the potential utility of passive immunization, a recent case report demonstrated the clinical utility of passive immunization by administrating convalescent plasma from a patient recovered from H5N1 infection to an H5N1 infected individual which resulted in reduced viral load by 10-fold within the first 8 hours of administration and to undetectable levels within 32 hours leading to the patient's recovery.[318]

ACTIVE IMMUNIZATION

History of Vaccine Development and Current Non-Live Vaccines

Efforts to develop influenza virus vaccines began soon after influenza A and influenza B viruses were recognized as the etiologic agents of clinical influenza.[5,319–323] The first commercial vaccines were approved for use in the United States in 1945, based on efficacy studies performed in military recruits and college students using whole-virus inactivated influenza vaccines.[324,325] An influenza vaccine was of particular interest to the U.S. military during World War II, in part because of the devastation caused in both military and civilian populations by the 1918–19 influenza pandemic during the late stages of World War I. The ability to grow large quantities of influenza viruses in eggs, elucidation of the physical properties of the viruses, and development of the principles of chemical inactivation made it possible to prepare millions of vaccine doses.[326]

The processes used to make the current commercially available inactivated influenza virus vaccines share certain key features. All the influenza viruses are replicated individually in substrates of animal origin and are harvested in liquid form. The majority of influenza vaccine viruses are replicated in the allantoic cavities of embryonated hens' eggs, but vaccines for commercial use also are now being produced using mammalian cell lines (MDCK [Madin-Darby canine kidney] or Vero).[327–331] For preparation of the monovalent intermediate vaccines, the harvested influenza viruses are inactivated by controlled treatment using either formalin or β-propiolactone, and several purification steps reduce nonviral proteins and other materials introduced during the manufacturing process. The monovalent vaccines are combined to formulate the final multi-valent bulk vaccines, which then are filled into containers.

Although whole-virus vaccines are still in use in some countries and are highly effective, most vaccines manufactured since the 1970s have been subvirion (sometimes referred to as "split") preparations. These vaccines retain the immunogenic properties of the viral proteins but are associated with greatly reduced reactogenicity.[332–335] Subvirion vaccines are prepared by using a solvent (such as ether or a detergent) to dissolve or disrupt the viral lipid envelope. Additional purification steps can be taken to reduce the amounts of viral proteins (predominantly influenza matrix protein and NP), resulting in vaccines referred to as subunit or purified surface antigen preparations. It is also possible to produce purified influenza virus HA and NA vaccines using recombinant DNA technology,[336–339] and one vaccine is currently licensed using this technology.[340,341]

The original inactivated whole-virus influenza vaccines were crude preparations, and the purity of influenza virus vaccines has steadily increased,[328] aided by the introduction of centrifugation and chromatographic steps to reduce residual egg materials. Because dissolution of the lipid envelope allows retention of immunogenicity with reduction in reactogenicity, "splitting" influenza viruses to produce subvirion preparations has become routine. An intact viral membrane is essential for infectivity of enveloped viruses. Therefore, disruption of the viral envelope adds assurance of viral inactivation. Subvirion vaccines were first prepared using ethyl ether and polysorbate 80, but a variety of detergents, including deoxycholate, tri-*N*-butyl phosphate, Triton X-100, Triton N101, and cetyltrimethylammonium bromide, are now used for commercial vaccine preparation.

The development of "high-growth" influenza A viruses suited to maximal replication in eggs has helped increase vaccine production. Since the early 1970s, influenza virus A/Puerto Rico/8/34 (PR8), a strain very well adapted to replication in eggs, has been used to develop influenza A virus reassortants that combine the HA and NA from wild-type viruses with the high growth properties of the PR8 donor virus.[342,343] Influenza A virus reassortants derived from PR8 often are more uniformly spherical than wild-type viruses,[343] which may facilitate recovery of virus during the various process steps. The growth characteristics of reassortants vary because the HA and NA also affect the adaptation and replication capabilities of the viruses in growth substrates. The overall advantage provided by strains that replicate well in eggs has meant that most, if not all, influenza vaccines prepared in eggs over the last 35 years have been prepared using a "high growth" influenza A virus reassortant. An area of continuing investigation is development of reliable high-growth donor strains of influenza B viruses suited to vaccine production.

Vaccine Constituents, Including Antibiotics and Preservatives

Hemagglutinin is the main immunogen in inactivated influenza vaccines (and the only immunogen in the licensed recombinant vaccine[344]), and its level (i.e., potency) is standardized by single radial immunodiffusion (SRID).[345–347] Although inactivated influenza vaccines contain NA, M, and NP in varying amounts, depending on the process methods, their levels are not specifically quantified. Antibody to NA can add to the protective effect of vaccines, as increased levels of anti-HA and NA antibody in serum and secretions correlated with reducing pH1N1 influenza virus infection and illness in healthy young adults during 2009 pandemic.[348] Furthermore, anti-NA and anti-HA antibodies are found to be independent predictors of immunity to influenza infection.[349] The immune responses to other viral proteins (e.g., M2)[350] are being investigated, but their relative contributions to protection appear much less than the effects of antibodies directed against HAs. Therefore, HA content continues to be the primary concern in preparing inactivated vaccines.

The amount of HA, as measured by SRID, has been correlated with the immunogenicity of subvirion and whole-virion inactivated influenza virus vaccines.[333,334,351] To test commercially prepared influenza vaccines, most authorities favor SRID performed with influenza strain-specific antigens and antisera because other methods have correlated less well with immunogenicity. Because SRID requires strain-specific HA antigen and antibodies, using SRID as a necessary step in the regulatory approval process and is time-consuming. Other methods, such as mass spectrometry,[352] enzyme-linked immunoassays,[353] and chromatography,[354] have been investigated to

speed up this process. Accelerating vaccine production timelines is a key goal in pandemic preparedness plans.

Antibiotics are not added to inactivated influenza virus vaccines as active ingredients. Aminoglycoside antibiotics are used in some production schemes to reduce bacterial growth in eggs during processing steps. Antimicrobial agents associated with anaphylactic-type hypersensitivity responses are strongly discouraged for use at any phase of production. Antibiotics that are used in the production process are reduced to trace or undetectable amounts during purification of the viral proteins and dilution of the concentrated vaccine components during formulation of the final multivalent vaccine. Minimal amounts of the detergents or solvents used for virus disruption may remain in the final vaccine preparation, but purification and dilution steps often reduce the amount to the limits of detection.

Thimerosal, an organic mercury-containing compound with broad, highly effective antimicrobial properties, is present in inactivated influenza virus vaccines produced for multidose presentation. Thimerosal is used to reduce bioburden (the total amount of bacteria and fungi) during production of influenza vaccines in eggs and/or as a preservative to prevent growth of bacteria and fungi. Although vaccines in single-dose containers are formulated to contain no thimerosal or in amounts too low to have a preservative effect (such vaccines may be called "preservative free"), multidose containers are designed to be entered several times, which raises the possibility of introduction and growth of bacteria or fungi.[355] Alternative preservatives such as 2-phenoxyethanol and phenol have been used in other vaccines, and are being evaluated as part of a continuing trend to limit the amount of mercury present in vaccines of all kinds, but have not been found to be an ideal replacement for thimerosal.[356,357]

Either formalin or β-propiolactone is used in influenza vaccine to inactivate the viruses. If processed properly, β-propiolactone is chemically degraded to levels below the limits of detection.[358] Although detectable quantities of formaldehyde persist in inactivated vaccines when formalin is used, the steps used for purification also reduce the amount of free formaldehyde, which, if it remains in high concentration, can reduce the potency of vaccines or interfere with SRID measurements of potency.[359–362]

Adjuvants, which are substances that are intended to augment immune responses to vaccine antigens, have been used with seasonal, pandemic, and avian influenza vaccines. One seasonal influenza vaccine containing an oil-in-water adjuvant (MF59) has been approved for use in persons age 65 years and older in the United States.[355,362a] In addition, an avian influenza A(H5N1) vaccine was approved by the U.S. Food and Drug Administration (FDA) in 2012 that contains AS03 as an adjuvant (AS03 is an oil-in-water emulsion).[363] Influenza vaccines that contain AS03 and MF59 as well as other adjuvants (virosomes and aluminum phosphate), are approved for use in some European countries and Canada.[364] Additionally, some governments have stockpiled adjuvants (such as AS03 and MF59) for use with possible pandemic vaccines against avian influenza viruses to augment immune responses when given to a naïve human population. Adjuvanted vaccines are of particular interest for use in populations that may respond less well to unadjuvanted inactivated vaccines, such as elderly persons and young children. They were used widely in many countries during the H1N1 pandemic.[253]

Because most inactivated influenza vaccines are produced with egg-grown viruses, traces of egg proteins are present in final vaccine formulations, generally measured as ovalbumin content.[355] Because cell-culture vaccines also use egg-grown seed strains, they also may contain traces of egg protein, but concentrations are expected to be exceedingly low. Because recombinant antigen vaccines are produced without eggs, they do not contain egg proteins.[365]

Manufacture of Vaccine

The requirements of national authorities for influenza vaccines generally reflect the guidelines published by the World Health Organization (WHO),[366] but may include items that are specific to individual control authorities. To expedite and standardize clinical studies that are intended to support licensure of new influenza vaccines, both the U.S. FDA and the European Medicines Agency (EMA) have developed guidelines for industry.[367–369] Recommendations for the antigenic composition of influenza vaccines are made by WHO semiannually (once for the Northern hemisphere formulation and once for the Southern hemisphere formulation) to ensure that current influenza vaccines are effective against recently circulating strains in both the Northern and Southern hemispheres.[370,371] Data for these decisions are created through WHO's Global Influenza Surveillance Response System (GISRS). GISRS is a network of surveillance systems and laboratories in more than 120 countries that send data to WHO and share specimens and isolates with five WHO Collaborating Laboratories that provide antigenic and genetic characterization of viruses for use in determining optimal vaccine strains.[371,372] The surveillance system monitors for antigenic and genetic changes in circulating influenza viruses. Surveillance has made it clear that antigenic changes occur not only by point mutations (antigenic drift), but also by way of antigenic shift (at irregular and unpredictable intervals). National regulatory authorities then make national recommendations for influenza vaccine composition, generally based on the WHO recommendations.

Influenza vaccine viruses usually are isolates obtained through GISRS. The WHO and various national authorities recommend use of certain strains based primarily on the antigenic characteristics of their HAs and NAs. Except for recombinant protein vaccines, original isolates are passaged in eggs or primary chick kidney cultures to develop reference strains that are distributed to manufacturers to develop seed viruses. Often the original wild-type strains grow relatively poorly in eggs, so considerable effort is expended examining several antigenically similar strains for several qualities needed to maximize virus yield during large-scale production, including their potential for development of high-growth reassortants, their growth characteristics, and their optimal incubation conditions. Because the available production time for multivalent influenza vaccine is limited by the need to distribute vaccine each autumn, the total amount of vaccine that can be produced is limited by the least-productive strain.[370]

Because most influenza surveillance laboratories are not prepared to handle tissue cultures in a way necessary to prevent introduction of extraneous agents, a concern is that the possible introduction and amplification of extraneous agents during subsequent passages could compromise the safety of vaccines.[373] However, replication of influenza viruses in eggs might provide a partial barrier to extraneous agents that might originate in the clinical source material. Interest in producing vaccines in mammalian cell systems stems from concern that the availability of eggs could be reduced by an event requiring destruction of the egg-producing hens (e.g., an outbreak of avian influenza or Newcastle disease virus), and also from concern that the HAs of viruses grown in eggs may exhibit antigenic alterations.[374–377] Therefore, strategies are being developed to directly isolate influenza viruses in mammalian tissue culture to provide suitable starting seed viruses.

Regardless of the growth substrate used, the prevention of the introduction of extraneous agents is paramount. When

using hen eggs, the main concern is the introduction of extraneous agents, such as avian leucosis virus, that originate in the flocks of chickens providing the eggs. However, extraneous agents could be introduced from the original human host to mammalian tissue cultures, from the tissue cultures used by the laboratory recovering the influenza virus, from the cell substrate used for manufacturing, or from materials used to support the growth of the cell substrate.[378–381] For inactivated vaccines produced either in eggs or mammalian tissue cultures, these concerns are reduced provided that the process used to inactivate influenza viruses will inactivate other microorganisms effectively.

After replication of influenza viruses, a number of manufacturing steps are taken to increase the concentration of the active immunizing ingredients of the vaccines (mainly the viral HAs) and to reduce other (mainly nonviral) materials. For inactivated influenza virus vaccines produced using eggs or mammalian tissue cultures, removal and reduction of egg or tissue culture proteins occurs throughout the manufacturing process, beginning with concentration of virus by means of centrifugation through a sucrose gradient or passage of the virus-containing allantoic fluid over a chromatographic column. The resulting fluids may be additionally purified by dialysis or diafiltration, and the concentration of residual egg or cell proteins is reduced further during the final formulation when the HA concentration is adjusted to achieve final target levels.

Disruption of the lipid envelope permits further purification of the viral proteins and, in particular, the HAs and NAs. These proteins form rosette-like structures in which the hydrophilic heads of the proteins are on the exterior and the hydrophobic tail portions of the molecules are buried internally. The disruption process varies in efficiency, so that HA and NA still can be attached to lipid but in pieces smaller than the original virion. Dissolution of the viral envelope and removal of additional viral components results in vaccine products with reduced reactogenicity.[332–335,382–384]

Chemical inactivation steps are used to minimize the microbial load in the raw viral harvest from the growth substrate, and careful handling and use of component reagents and buffers also facilitate inactivated vaccine sterility. However, filtration steps ensure elimination of undesirable microbes. Although the sterile filtration step does not eliminate endotoxins, which may have been formed previously by bacteria in the product, some of the purification steps can help reduce endotoxin levels.[383] Because endotoxins contribute to febrile responses to injected vaccines, the limits for endotoxin levels on finished product are set well below clinically established thresholds for reactions.[384]

A recombinant HA vaccine is available that is produced in a continuous insect cell line in which genes encoding HA proteins for each of the vaccine strains to be included in the vaccine are inserted into a baculovirus vector, which leads to expression of the HA proteins.[341] Purification steps for this vaccine are similar to egg and cell-based vaccines. Because no eggs are used in the production of this vaccine, no traces of egg antigens are present in the final product.

Producers

Several manufacturers produce inactivated influenza vaccines either for global distribution or for national demand. Each manufacturer has a somewhat different, proprietary process, but all the processes result in vaccines standardized to ensure the immunogenicity of the HA. Global supply of inactivated influenza vaccines has increased significantly in the last decade as demand has increased, in part as a byproduct of pandemic preparedness efforts that have highlighted the burden of seasonal influenza and the value of developing seasonal vaccination programs as a foundation for pandemic response.[16,386,387] As an example, annual inactivated influenza vaccine distribution in the United States increased substantially in the last two decades, with the expansion of target groups during that time. In 2014–15, more than 147 million doses were distributed in the United States.[388] WHO estimated that the world's manufacturing capacity increased from 500 million doses of trivalent vaccine in 2006 to 1 billion doses by 2010.[389] While most vaccines are produced by multinational manufacturers, WHO's Global Action Plan has catalyzed growth of manufacturing capacity in Asia, Eastern Europe, Latin America, and Africa, which holds the promise of increasing global supply and lowering vaccine prices in the future.[387] Even so, more than 80% of seasonal influenza vaccine doses produced in 2009–10 originated from seven large manufacturers that are located in the United States, Canada, Australia, Western Europe, Russia, China, and Japan. Sufficient supply during a pandemic and for newly emerging markets will require greater production capacity with likely growth in emerging suppliers.[390] Information on influenza vaccine manufacturers available by country can be obtained from national regulatory agencies.

Preparations Available

All vaccines mentioned in this chapter are inactivated vaccines produced from influenza viruses grown in eggs or cell culture,[355] with one exception (Table 31.1). A vaccine that contains HA protein recombinantly expressed in baculovirus-infected insect cells was licensed in the United States in 2013.[341] Because this vaccine does not have a production step that includes live virus, there is no inactivation step required. This type of vaccine is included in this chapter. While most approved vaccines are manufactured using embryonated eggs, cell culture vaccines are available in the United States and Europe.[364] Development of vaccines produced in cell culture has been promoted in part for pandemic preparedness, both as a tool to meet increased vaccine demand during a pandemic and as a hedge against the possibility that a pandemic strain derived from avian viruses might endanger birds used to produce the eggs for production.[386]

Monovalent, bivalent, trivalent, quadrivalent, and even pentavalent influenza vaccines have been produced. Multivalent vaccines have generally included both influenza A and influenza B virus components. In recent years, monovalent vaccines have been used only in unusual circumstances to provide protection against a circulating strain substantially antigenically different from those used in the seasonal vaccine formulation. Examples include the creation and distribution of a supplemental A/Taiwan/21/86 (H1N1) vaccine in 1986[260,391] and, more recently the development of the monovalent H1N1 pandemic in 2009.[392] Since 1978, most vaccines have been trivalent and have incorporated influenza A(H1N1) and A(H3N2) subtype viruses and an influenza B virus. During the 1990s, influenza B viruses diverged into two antigenically distinct lineages based on the HA.[393] This led to the development of quadrivalent vaccines that incorporate two B strains (one from each lineage)[394]; quadrivalent vaccines have occupied an increasing proportion of the global market since their introduction and likely will continue to become more common.[395]

Inactivated vaccines can be categorized based on their production methods into three types: whole cell (virions), subvirion (or "split virus"), and purified subunit vaccines.[396] Whole-cell vaccines have become less common because of relatively high rates of local reactions and fevers associated with them.[335,382,397] For subvirion vaccine, the viruses are

TABLE 31.1 Approved Influenza Vaccines in the United States, 2016–17 Influenza Season[a]

Trade Name	Manufacturer	Presentation	Age Indications	Route	HA/Antigen/ Dose (μg)
INACTIVATED, IIV4, EGG-BASED					
Fluarix Quadrivalent	GlaxoSmithKline	0.5 mL SD PFS	≥3 y	IM	15
FluLaval Quadrivalent	ID Biomedical Corp. of Quebec (distributed by GlaxoSmithKline)	5.0 mL MDV 0.5 mL SD PFS	≥3 y ≥3 y	IM	15
Fluzone Quadrivalent	Sanofi Pasteur	0.25 mL SD PFS 0.5 mL SD PFS 0.5 mL MDV 5.0 mL MDV	6–35 mo ≥36 mo ≥36 mo ≥6 mo	IM IM IM IM	7.5 15 15 15
Fluzone Intradermal Quadrivalent	Sanofi Pasteur	0.1 mL SD PFS	18–64 y	ID	9
Fluad	Seqirus	0.5 mL SD PFS	≥65 y	IM	15
INACTIVATED IIV3, EGG-BASED					
Afluria	Seqirus	0.5 mL SD PFS 5.0 mL MDV	≥9 y ≥9 y via needle; 18–64 y via jet injector	IM IM	15 15
Fluvirin	Seqirus	0.5 mL SD PFS 5.0 mL MDV	≥4 y ≥4 y	IM IM	15 15
Fluzone	Sanofi Pasteur	5.0 mL MDV	≥6 mo	IM	15
Fluzone High-Dose	Sanofi Pasteur	0.5 mL SD PFS	≥65 y	IM	60
INACTIVATED IIV4, CELL-BASED					
Flucelvax	Seqirus	0.5 mL SD PFS	≥4 y	IM	15
Recombinant PROTEIN, RIV3					
FluBlok	Protein Sciences	0.5 mL SDV	≥18 y	IM	45
LIVE ATTENUATED, LAIV4					
FluMist Quadrivalent[c]	MedImmune	0.2 mL SD prefilled intranasal sprayer	2–49 y	IN	[b]

[a]Updated list of influenza vaccines approved in the United States is available at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6430a3.htm.
[b]Dose of LAIV4 is $10^{6.5}$–$10^{7.5}$ plaque-forming units per antigen per dose.
[c]Not recommended for use during the 2016–17 season.
ID, intradermal; IIV3, trivalent inactivated vaccine; IIV4, quadrivalent inactivated vaccine; IM, intramuscular; IN, intranasal; MDV, multidose vial; RIV3, trivalent recombinant vaccine; SD PFS, single-dose prefilled syringe; SDV, single-dose vial.
Modified from Grohskopf LA, Sokolow LZ, Olsen SJ, et al. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices, United States, 2015–16 influenza season. MMWR Morb Mortal Wkly Rep. *2015;64:818–825.*

treated with a solvent that disrupts the lipid envelope while retaining the immunogenic potential of the viral proteins. They are less reactogenic compared with whole cell preparations.[332–335,383,384,397] Subunit vaccines are further purified to reduce the amount of non-HA protein, such as structural proteins.[396]

Adjuvants

While most approved inactivated influenza vaccines (IIVs) are nonadjuvanted, some products contain adjuvants designed to improve the immunogenicity of the vaccine, either in terms of magnitude or breadth of immune response.[364,398] The components of influenza viruses themselves (including possibly the lipid envelope) play a role in producing reactions.[384] Because whole-virus preparations already include cellular lipids in the viral envelope, their increased immunogenicity in some studies may relate to an adjuvant effect of the lipid envelope. Adjuvanted products are generally targeted to populations that might respond suboptimally to standard vaccines, such as in the case of pandemic vaccines given to an immunologically naïve population or vaccines targeted to the elderly or very young children.[399] Adjuvanted vaccines have been used or investigated for decades,[400] and a wide variety of adjuvants have been tested.[364,401–416] While most adjuvants have combined a lipid or fatty acid with a bacterial cell wall or bacterial protein, other materials such as chitosan, polyinositol, aluminum, and cytokines also have been tried. Three types of adjuvants have been approved in some countries in seasonal or 2009 H1N1 pandemic vaccines: oil-in-water emulsions, aluminium phosphate, and virosomes.[364] Two proprietary oil-in-water emulsions, AS03 and MF59, are incorporated into licensed vaccines; AS03 is approved for use in a pandemic H1N1 vaccine and an H5N1 vaccine (for pandemic use), while MF59 is contained in seasonal and pandemic vaccines.[328] Two vaccines use immunopotentiating, reconstituted influenza virosomes.[417] One whole-cell vaccine that uses alum is approved in Hungary for seasonal use.[364,418]

Generally, adjuvants improve vaccine responses but may increase reactogenicity. Increased rates of local reactions, including pain at the injection site, tenderness, and erythema, have been noted in some clinical trials.[419] Elderly recipients of an MF59 adjuvanted influenza vaccine developed geometric mean HI antibody titers that were 1.5 to two times higher against each of the three influenza vaccine components than those of recipients of standard subunit vaccine without

adjuvant.[406,420] In addition, fourfold titer rises were also increased, and there was evidence of broader serological protection against drifted strains that circulated 1 and 2 years after vaccination.[406,410,419,421–424] Adjuvanted vaccines were used in many countries during the 2009 H1N1 pandemic,[253,425] and immunogenicity data indicated that adjuvanted 2009 H1N1 vaccines produced high antibody titers after a single dose.[425–431] Studies using experimental vaccines made with influenza A virus subtypes including H5 and H9 have also demonstrated that higher immune responses were obtained with the vaccine containing MF59 or AS03 as an adjuvant.[413,432–435] However, some experiences suggest that responses of the immunologically naïve may not always be improved by incorporation of an adjuvant in influenza vaccines,[436] particularly an aluminum-based adjuvant system.[435,437] Systems other than the classical adjuvant method are being pursued as means to increase the immunogenicity and possibly effectiveness of IIVs. Several systems being explored combine a number of influenza viral proteins (virus-like particles [VLPs], which contain HA in combination with M or NP) or incorporate viral proteins in presentation systems with other agents or compounds to evoke strong cellular and humoral immune responses (such as inducers of innate immune responses).[438–441]

For the virosome vaccines, influenza virus is inactivated, followed by extraction of the HA and NA proteins which are then associated with lecithin to produce a conformation in which the influenza glycoproteins are exposed in a lipid particle. The vaccine produced comparable or modestly higher immune response compared with inactivated vaccines among elderly persons.[403,442,443] A small study in children with cystic fibrosis suggested that the virosome vaccine might be more immunogenic than subunit vaccine after one dose, but the differences were not striking[416]; immune responses were not superior to unadjuvanted vaccine in a small group of elderly subjects. No clinical efficacy studies comparing standard IIVs with adjuvanted vaccines have been published.

Timeline for Influenza Vaccine Production

Production of influenza virus vaccines follows a similar schedule each year, reflecting the need to produce and administer vaccine before each influenza season (Fig. 31.5). The foundation for influenza vaccine production is global influenza virus surveillance that continues throughout the year in both hemispheres. Information from global surveillance is used to inform manufacturers about trends in antigenic changes in influenza viruses, particularly trends that might signify the need to make changes in vaccine composition. Strains showing antigenic changes are examined and when warranted, high-growth reassortant viruses are produced.

Because several months are required for production of annual influenza virus vaccines, the time allotted for collecting and assessing surveillance data to ensure the best possible recommendations for vaccine composition must be balanced against the time needed by manufacturers to produce all components of the vaccine. If recommendations are made too early, significant antigenic changes may be missed. However, if recommendations are made too late, the total vaccine output can be hampered. In addition to collecting and analyzing viral surveillance data, time is needed to allow experience to be gained handling the vaccine candidate strains and to produce the reagents needed to standardize the new vaccine component viruses.

Manufacturing of monovalent vaccine components begins as early as possible and continues until sufficient material is available to produce the manufacturer's final targeted number of doses. The potency of monovalent vaccine components must be known before formulation of the multivalent vaccines can begin. Currently, the potency of monovalent components and trivalent or quadrivalent vaccine is established using SRID.[346,347] This technique requires both a HA-specific antigen and a HA-specific antiserum. The antiserum is produced by immunizing animals with preparations of purified HA and takes at least 3 to 6 weeks.

Once the potency of all three components can be determined, formulation of the trivalent vaccine begins, followed by its filling into containers, labeling, packaging, and distribution. Despite increases in vaccine production and distribution of multimillions of doses, all manufacturing steps must be completed within 6 to 8 months because administration of vaccine typically begins and largely is completed during October to December in the Northern hemisphere and during April to July in the Southern hemisphere. The time needed for production of a monovalent vaccine against a new pandemic strain would require approximately 4 to 6 months from identification of a candidate vaccine virus to the time when the first vaccine doses would be available. During the 2009 H1N1 pandemic, these timelines were shown to be approximately correct. However, widespread availability of the 2009 pandemic H1N1 monovalent vaccine lagged well behind the peak pandemic wave, again illustrating the urgent need to more rapidly identify candidate viruses for vaccine production,

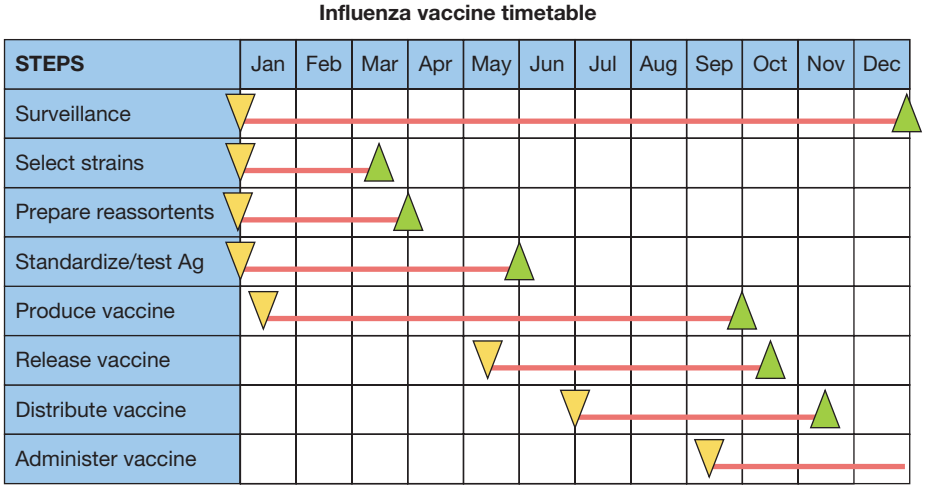


Figure 31.5. Approximate production timetable for influenza vaccines manufactured for the Northern Hemisphere. Southern Hemisphere vaccine production timetable would start in approximately October of each year. Ag, antigen.

develop vaccine virus candidates, and scale up production of a safe and immunogenic vaccine.[390]

Dosage and Route

IIVs have been given by intramuscular, subcutaneous, intradermal, intranasal, and oral routes. Most licensed unadjuvanted IIVs given by intramuscular injection contain 15 µg of each HA antigen per dose (45 µg of total antigen per dose for trivalent vaccines and 60 µg of total antigen per dose for quadrivalent vaccines; based on the SRID content).[355] For children 6 months to 35 months of age, vaccines are produced with 7.5 µg of each HA, which are intended to be provided in a two-dose schedule to ensure adequate immune responses in this naïve population.[355] The IIV dosages are based on extensive clinical studies undertaken when influenza A(H1N1) viruses were reestablished in human populations during the late 1970s.[333,334,382] These studies demonstrated that children and adults who were unexposed to influenza through vaccine or natural infection required two doses of inactivated vaccine to achieve maximal antibody titers, whereas persons with some degree of preexisting immunity needed only a single dose. Subsequent studies conducted with more contemporary vaccines confirmed the need for two doses in previously unvaccinated children younger than 9 years of age to optimize the immune response and vaccine effectiveness.[444–447] The quantity of antigen in each dose is chosen by balancing the desirability of inducing a maximum immune response in an individual (a higher dose increases the probability of achieving a maximum antibody titer) against the desirability of maximizing population coverage (a smaller dose will allow more individuals to be immunized, which might induce some degree of herd immunity) and the desirability of minimizing adverse reactions (a larger dose has a greater probability of causing immediate local and systemic reactions).

Vaccine formulations with higher and lower HA antigen contents are available, often targeted to specific populations. In 2009, a trivalent high-dose vaccine was licensed for use in persons 65 years of age and older. The vaccine contains 60 µg of HA antigen for each vaccine strain, for a total of 180 µg of antigen per dose, based on studies of dose responses in elderly persons that suggested that increased doses of modern subvirion vaccines can increase antibody responses without substantially increasing reactogenicity.[201,448–450] One large clinical trial demonstrated superior efficacy of high-dose vaccine compared with standard-dose vaccine among persons 65 years of age or older.[451,452] In this study, the rate of laboratory-confirmed influenza was 1.9% among persons who received standard-dose vaccine, compared with 1.4% of persons who received the high-dose formulation (a relative efficacy of 24.2%). A similar level of increased relative effectiveness against influenza-associated hospitalizations was observed in a large observational study of national electronic medical claims data[453]; however, a superior effectiveness was only found among persons older than 85 years of age in a large study in a Veterans Administration study.[454] A recombinant antigen vaccine is available that contains 45 µg of total antigen per dose (135 µg of total antigen per dose), and is approved for adults older than 18 years of age based on studies demonstrating comparable immunogenicity and efficacy to standard-dose vaccines.[452,455,456] Lower antigen content is available in intradermally administered preparations (e.g., 9 µg of total antigen per dose) using a prefilled microinjection devices, in which they have been found to provide immune responses comparable to standard dose vaccines, but an increased rate of local reactions.[202,203,457–462] The varying antigen contents of products are designed to produce adequate immune responses and protection (or superior protection in the case of high-dose inactivated vaccine), while minimizing reactogenicity. Although formulations with varying antigen contents are available, most countries do not express a preference for any product over another, as long as the product is appropriate for the person vaccinated (e.g., approved for the age of the person). As vaccine availability increases globally, further studies may be warranted to determine whether vaccine effectiveness can be improved simply by increasing the amount of HA in vaccines given to other specific populations that respond poorly to standard dose vaccine, such as HIV-infected persons.[463]

While IIVs are generally administered using single-use sterile needle and syringes, vaccines have been administered transdermally through jet injector devices, and one such formulation was approved by FDA in 2014.[204,355] The device is a reusable spring-powered device that injects the vaccine through a single-use sterile syringe into the deltoid muscle. In prelicensure evaluation, subjects who were vaccinated using the jet injector had higher rates of injection-site adverse events (tenderness, swelling, pain, and redness), but had noninferior immune responses compared with those vaccinated using needle and syringe.[204]

The intranasal administration of IIV has not been studied as extensively as other routes of delivery. A commercially available nasally administered formulation was withdrawn because of its association with cranial nerve palsies.[442] The ability of oral administration to induce protective antibody responses has been studied for several decades. Recent studies demonstrate adequate immune responses and safety, but this route often requires very large doses or an adjuvant to promote mucosal and systemic immune responses.[464–466]

Vaccine Stability

Vaccine manufacturers in the United States and elsewhere have ongoing programs to assess the stability of all vaccine products. Stability of a product is influenced by the specific formulation of the product, the addition of stabilizing compounds (such as gelatin or polysorbate), the compatibility of the product with the intended container and closure and the preparative treatments needed to reduce adsorption or chemical interaction of the vaccine components with the container, and the vaccine's specific temperature limits. Stability assessment programs usually also examine sterility, pH, and the measurable content of preservatives and other chemical ingredients.

Experience indicates that IIVs are generally sufficiently robust to maintain full potency above the minimum release specifications for more than 1 year, when stored at 4°C to 8. However, the potency of individual influenza virus vaccine components can decline at varying rates. For example, in 1996, the influenza A(H3N2) component (but not the influenza A[H1N1] or influenza B components) in the vaccine from one manufacturer lost potency faster than expected[467]; accelerated potency loss was noted in several vaccine products during the 2009 H1N1 pandemic,[468] and in 2015, vaccine components from two companies fell below potency thresholds before the end of the influenza season.

IMMUNE RESPONSE TO VACCINATION AND CORRELATES OF PROTECTION

Measurement of Antibodies

Influenza vaccination induces primarily antibodies against the major surface glycoproteins, the HA and NA, although in some cases, antibody to the NP and M1 proteins may also be induced owing to the presence of those components in some of the vaccines.[469] While the HI and neutralization tests both

measure levels of strain-specific antibodies, the HI test[180] is most commonly used for measuring the antibody response to human IIVs. In the HI test, antibodies present in an immune serum compete with red blood cells to bind the viral HA. In the absence of antibodies, the viruses agglutinate the red blood cells. Red blood cells from chickens or turkeys are used most often; however, guinea pig or human erythrocytes can be substituted in circumstances where avian red blood cells do not bind well to the influenza viruses in question. The HI assay can be complicated by nonspecific inhibitors of hemagglutination in human and animal serum. The inhibitors can be removed by pretreating serum with receptor-destroying enzyme from *Vibrio cholerae* or with periodate. An HI titer of 40 represents a 50% protection rate against influenza viral infection in a population and is used as a gold standard as a seroprotective titer.[470]

The neutralization test is an alternative method for the detection of strain-specific antibodies to influenza viruses and in some cases may be more sensitive than the HI test and has the added advantage of measuring functional antibody capable of preventing virus infection. Neutralization-enzyme immunoassay tests, or the microneutralization assay, measure neutralizing antibodies in small quantities of serum.[471,472] When detecting serum antibody responses to human influenza strains, titers of neutralizing antibody measured using this method correlate well with titers obtained by HI.[472] The seroprotective HI titer of 1:40 corresponds to an MN titer of approximately 1:160.[473] In addition, neutralizing antibodies may be present when there are low or undetectable levels of HI antibodies. For example, HI antibodies bind to the globular head of the HA. But influenza viruses can also be neutralized with antibodies directed against the HA stem, which would not be detected with an HI assay.[474]

The neutralization or microneutralization assay is preferred when detecting antibody responses to avian influenza viruses, as the traditional HI test using avian RBC is not sufficiently sensitive.[475] A modified HI assay that uses horse RBC, which express a predominance of SA $\alpha_{2,3}$ receptors to which most avian influenza viruses preferentially bind may also be used as an alternate or confirmatory assay for the detection of antibodies against avian influenza virus HA.[476] Finally, enzyme immunoassays are typically performed to measure virus-specific immunoglobulin (Ig) G, IgM, or IgA antibodies in paired pre- and postvaccination serum or respiratory samples. Such immunoassays perform optimally when concentrated and/or purified preparations of viral antigen are used to detect antibodies and may correlate with neutralizing antibodies.[477]

Antibody Responses

The robustness of the serum antibody response to inactivated vaccines is dependent on age and preexisting antibody levels. Thomas Francis noted that individuals exposed sequentially to influenza virus variants with shared epitopes tend to generate antibody responses preferentially to the previously exposed virus and diminished responses to the current strain and termed this phenomenon as "original antigenic sin." [478] Original antigenic sin would potentially have a major negative impact on the immunogenicity of vaccines and vaccine efficacy when the vaccine strains are closely related to previous strains; however, in animal studies administering influenza vaccine with adjuvant overcomes original antigenic sin.[479] In previously primed individuals; the response is predominantly anti-HA IgG, but in young, unprimed children, systemic IgM antibody may be more evident.[480,481] In primed individuals, antibodies that are specific for the vaccine strain or cross-reactive with earlier, antigenically related strains can be detected using the HI assay. Numbers of influenza virus–specific antibody-secreting cells in peripheral blood peak approximately 1 week after vaccination, whereas serum antibody levels peak 2 to 4 weeks after vaccination in healthy, previously primed individuals, but may peak 4 weeks or later in unprimed individuals and the elderly.[480,482–485] The magnitude of antibody-secreting cells responses correlate with serum antibody responses and the expanded B-cell clones use specific immunoglobulin genes.[485] Furthermore, females tend to develop higher antibody titers and vaccine efficacy than males and the molecular basis for these differential responses are not known.[486] In children 9 years of age and younger, and in unprimed individuals, two doses of inactivated vaccine are required to induce an optimal serum antibody response.[444–447,487] IIVs delivered intramuscularly may also induce local influenza virus–specific IgA in oral and respiratory fluids, with responses being more substantial in individuals primed by natural infection.[483] In addition, besides inducing subtype-specific neutralizing antibodies, IIV3 also induces in some individuals broadly neutralizing antibodies that react against the stem region of HA.[311]

Multiple immunogenicity studies conducted with both unadjuvanted and adjuvanted 2009 H1N1 monovalent vaccines demonstrated excellent immunogenicity in healthy persons in all age groups.[427,428,431,488–492] However, adjuvanted but not unadjuvanted 2009 H1N1 vaccine overcame poor responses in individuals who received IIV3 seasonal vaccine earlier.[493] For children younger than 9 years old, immunogenicity of the H1N1 vaccines matched or exceeded what is typically seen with seasonal influenza vaccines. However, higher rates of seroconversion and higher postvaccination antibody levels were achieved in children younger than 11 years old who received either two doses of 15 μg of HA without adjuvant or one dose of 7.5 μg of HA with MF59 adjuvant.[427,491] Increasing the amount of influenza HA per vaccine dose above the currently recommended level of 15 μg generally results in a dose-related increase in serum antibody response, with only modest increases in the occurrence of injection site reactions.[487] In the elderly, increasing dosages of inactivated trivalent vaccine resulted in significantly higher and more frequent serum antibody responses and may offer one approach to enhancing the protective effect of inactivated vaccines in this high-risk population.[448–450] Multiple studies show that increasing the influenza HA fourfold (60 μg per strain) results in significantly higher anti-HA IgG, and one high-dose vaccine has been licensed in the United States for adults 65 years old and older.[11] High-dose vaccine enhanced geometric mean HI titers by 1.8-fold when compared to those from standard vaccine recipients in persons 65 years of age and older with a relative increase in clinical efficacy of 24.2%.[451] Studies are underway to determine whether higher titers predict better vaccine effectiveness. The monovalent 2009 H1N1 vaccines adjuvanted with oil-in-water emulsions or aluminium preparations elicited high HA titers using doses similar to, or two- to fourfold less, than doses used in unadjuvanted vaccines.[426,431,494] In addition, whole virion monovalent 2009 H1N1 vaccines were used in some countries with immunogenicity data demonstrating satisfactory antibody responses after a single dose in older children and adults.[428] In a randomized controlled trial comparing the immunogenicity of a whole virion 2009 H1N1 vaccine (7.5 μg HA) versus an AS03-adjuvanted vaccine (3.8 μg HA), the adjuvanted vaccine was more immunogenic based on antibody responses. On day 21 after one dose of adjuvanted AS03 or whole-virion vaccine, the percentage of persons who developed titers of 1:40 or greater decreased with age for both vaccines, ranging from 94% and 71%, respectively, for those 18 to 44 years of age to 51% and 32%, respectively, for those 65 years of age or older. On day 42 (21 days after the second dose), 100% and 73% of participants 18 to 44 years of age who received adjuvanted versus whole virion vaccine had HI titers of 1:40 or greater, respectively.

However, only 76% and 36% of vacinees who were 65 years of age or older had titers of 1 : 40 or greater to adjuvanted versus whole-virus inactivated vaccines, respectively.[494] Vaccine-induced antibody responses measured by HI or MN have have been found to be similar in pregnant women compared with nonpregnant women, and a recent study has found increased plasmoblasts in vaccinated pregnant women compared with controls.[494a,494b]

Among persons with chronic medical or immunocompromising conditions, serologic response rates are typically lower. IIV3 or pH1N1 vaccine produces adequate antibody concentrations against influenza among vaccinated HIV-infected persons who have no or minimal AIDS-related symptoms, including those who have successfully responded to highly active antiretroviral therapy.[495–499] Among persons who have advanced HIV disease and low $CD4^+$ T-lymphocyte cell counts, IIV3 or pH1N1 vaccine might not induce protective antibody titers[498,500]; a second dose of vaccine does not improve the immune response in these persons.[500–502] A randomized, placebo-controlled trial determined that IIV3 was highly effective in preventing symptomatic, laboratory-confirmed influenza virus infection among HIV-infected persons with a mean of 400 $CD4^+$ T-lymphocyte cells/mm^3; however, a limited number of persons with $CD4^+$ T-lymphocyte cell counts of less than 200 were included in that study.[501] A nonrandomized study of HIV-infected persons determined that influenza vaccination was most effective among persons with greater than 100 $CD4^+$ cells and among those with less than 30,000 viral copies of HIV type 1/mL.[105] Initial studies indicate that the response to 2009 H1N1 antigen is also reduced. For example, a study among HIV-infected persons on highly active antiretroviral therapy who received the 2009 H1N1 monovalent vaccine showed similar poor immunogenicity with an unadjuvanted vaccine,[503] improved immunogenicity with an adjuvanted vaccine (68% with postvaccination HI titers of ≥1 : 40) and excellent immunogenicity after two doses of an adjuvanted vaccine (92% with postvaccination HI titers of ≥1 : 40).[504] Furthermore, a two-dose regimen of adjuvanted pH1N1 vaccine not only induced seroprotective titers that persisted 12 months postvaccination, but also enhanced memory responses to subsequent vaccination with nonadjuvanted seasonal vaccine.[505]

Immunogenicity among persons who received solid-organ transplants, as measured by the proportion who developed seroprotective antibody levels (HI titers ≥40), varies according to transplant type and time since transplantation. Persons with kidney or lung transplants have responses that are similar to or moderately reduced compared to healthy persons; however those with heart or liver transplants respond poorly, especially if vaccination occurs within 4 months after the transplantation procedure.[506–510] Improved humoral immune responses are achieved in patients receiving either a high dose or a booster dose.[511,512] Alternate routes of administration such as intradermal route, booster doses of unadjuvanted or adjuvanted vaccines, or high-dose vaccines may help induce protective immune responses in immunocompromised populations. Furthermore, passive transfer of hyperimmune sera or a cocktail of monoclonal antibodies may help those individuals who do not respond to vaccination.

Cellular Immune Responses

$CD4^+$ and $CD8^+$ T cells also play an important role in immunity to influenza, and in contrast to the strain-specific response of antibodies, tend to be more cross-reactive among subtypes, recognizing more conserved epitopes on the surface proteins and/or internal viral proteins.[223] The ability to recognize a given T-cell epitope is dependent on the HLA (human leukocyte antigen) phenotype of an individual. $CD4^+$ T cells provide help for the antibody response and the induction of $CD8^+$ T cells, whereas $CD8^+$ cytotoxic T lymphocytes (CTLs) have been associated with accelerated clearance of virus and recovery from infection.[224,513] The CTL responses in humans who have received IIVs have not been studied as extensively as the humoral immune response. Immunization of healthy adults with inactivated whole-virus vaccine resulted in enhanced CTL responses in peripheral blood, whereas immunization with subunit vaccine resulted in a poor CTL response, unless the vaccine is formulated to facilitate cytoplasmic delivery of antigens for processing and presentation with class I major histocompatibility complex to induce CD8 T cells.[351,513–515] The duration of the response varied greatly among individuals, persisting for several months to 1 year. In one study, an oil-in-water emulsion adjuvant (AS03) appeared to induce increased numbers of antigen-specific memory B cells compared to unadjuvanted vaccine. Cross-reactive and polyfunctional H5N1-specific CD4 T cells to conserved sequences in HA protein of clade 2 H5N1 HA were identified after a single dose of adjuvanted vaccine and amplified by a second vaccination.[434] CD4 T-cell responses induced by AS03-adjuvanted (3.8 μg HA) 2009 H1N1 vaccine strongly enhanced both antibody and CD4 T-cell responses in adults as well as in older adults.[494,516,517]

The number of influenza virus–specific $CD8^+$ T cells decreases in elderly individuals compared with younger adults.[518–520] Nevertheless, influenza vaccination may transiently enhance CTL responses[514] in this population. There is growing evidence that cell-mediated immune responses may be a component in protecting the elderly from influenza disease.[521,522] In adults 60 years of age and older with congestive heart failure, postvaccination production of granzyme B, a key mediator of cytolysis of virus-infected cells, was significantly lower in subjects with influenza compared with those without laboratory-confirmed influenza.[521] These results suggest that the evaluation of cell-mediated immune responses to vaccination, particularly in older adults, warrants further investigation.

Correlates of Protection

Strain-specific virus-neutralizing antibody directed against the HA is the primary immune mediator of protection against infection and clinical illness, while antibody directed against the NA may reduce the severity of disease through enhancing viral clearance.[523] Based on serological studies of influenza using human serum, an HI titer range of 1 : 32 to 1 : 40 is often referred to as the "protective titer." However, this range of titers represents the level at which approximately 50% of individuals will be protected, and there is no titer value that can guarantee protection from infection.[235,470,524,525] Nevertheless, elevated levels of serum anti-HA antibody generally are correlated with resistance to influenza infection, whereas lower antibody levels are associated with increased risk of illness among persons exposed to influenza viruses,[251,525–527] although elderly individuals with HI titers of greater than or equal to 1 : 40 may still be susceptible to influenza disease.[528] Because of the inherent sensitivity issues with the HI test, given the high degree of sensitivity and correlation of the MN assay with HI test, the MN assay warrants consideration for serological assessment of responses to influenza. Challenge studies using live virus show that protection against influenza is also associated with local neutralizing antibody and secretory IgA found at mucosal surfaces.[481] Although antibodies to NA do not efficiently neutralize influenza infection, they restrict virus release from infected cells, reduce the intensity of infection, and enhance recovery.[529]

EFFICACY AND EFFECTIVENESS

The measurement of influenza vaccine efficacy and effectiveness can be affected by virus and host factors as well as study methodology. Thus, published vaccine efficacy/effectiveness point estimates have varied between studies and comparison of results requires consideration of multiple factors. In years with circulation of viruses that are antigenically drifted from the vaccine virus, vaccine benefit is likely to be lower. However, the degree of antigenic drift from vaccine viruses and proportion of circulating drifted viruses can vary such that during some seasons reported to have circulation of drifted viruses, the overall vaccine effectiveness is still beneficial.[455,530–532] Although multiyear studies are often needed to achieve adequate sample size, the differences in viruses and vaccines from year to year can complicate the interpretation of vaccine efficacy/effectiveness estimates from multiyear studies.

In addition to virus factors, host factors such as age, underlying medical conditions, history of prior infections and prior vaccinations can affect the response to vaccine and vaccine effectiveness.

An important contributing factor to vaccine effectiveness is the level of antigenic match or the antigenic correspondence between the viruses in the vaccines and the circulating influenza viruses during a given influenza season. Influenza vaccines are most effective when the viruses in the vaccines are antigenically indistinguishable from those that are circulating. However, different levels of antigenic divergence between vaccine and circulating viruses have occurred as a result of ongoing antigenic variation of viruses, issues related to egg adaptation and, occasionally, the late emergence of a variant strain that has drifted significantly from the vaccine virus after vaccine recommendations have been made. While the latter occurs only once every 6 to 10 years, it is possible that no benefit from the vaccine may be seen for that particular vaccine component.

The level of antigenic match between vaccine and circulating viruses is measured in the laboratory by using postinfection ferret serum in hemagglutination-inhibition assays and/or neutralization assays. Ferret serum is used for these assays because these serum samples typically have been found to be very sensitive in detecting low levels of antigenic divergence. Importantly, prevaccination and postvaccination human serum are also tested to determine if vaccines have induced good levels of antibody to currently circulating viruses, including any antigenic variants that may have emerged recently. Circulating influenza viruses also undergo genetic sequencing so that the degree of genetic divergence can be ascertained. It must be noted that specific single mutations can confer a greater level of antigenic divergence that multiple individual mutations in other areas of the hemagglutinin.

Young vaccine-naïve children require a second, boosting dose, administered a minimum of 4 weeks apart, for optimal immune response to vaccine.[533–537] Several studies suggest that the time interval between the first and second dose may range from 4 weeks to up to 1 year. Adults older than 65 years of age may have changes in innate immunity that can affect vaccine immunity over time and prior infections could influence susceptibility to infections because of original antigenic sin.[538] In addition, underlying medical conditions, particularly immunosuppression, can affect the ability to respond immunologically to the vaccine.[539,540]

Placebo-controlled randomized clinical trials measure vaccine efficacy with the least bias and offer the strongest evidence for vaccine benefit. In recent years, the number of countries with annual influenza vaccine recommendations for older adults, persons with underlying conditions, pregnant women, and children has increased. Thus, placebo-controlled randomized clinical trials are increasingly difficult to perform, and often impossible for groups recommended for vaccination. When a placebo cannot be used, vaccine effectiveness can be estimated with observational studies; however, biases related to receipt of vaccination need to be appropriately minimized and/or controlled. Recently, the test-negative design has become an increasingly popular study design. In this method, subjects with acute respiratory illness or influenza-like illness are enrolled during a medical encounter and respiratory specimens from all participants are tested with a sensitive diagnostic assay such as RT-PCR; cases are influenza test-positive and controls are test-negative. The test-negative design minimizes selection bias by allowing for the selection of controls with similar healthcare-seeking behaviors as cases. The method has been validated for assumptions common in ambulatory care settings.[541–543]

Finally, the specificity of the outcome measured in the vaccine efficacy/effectiveness study is important. Vaccine efficacy/effectiveness from studies with nonspecific outcomes, such as clinical syndromes (e.g., influenza-like illness or pneumonia), are generally lower than estimates from studies with laboratory confirmed outcomes.[544] For these syndromes, influenza infection accounts for only a proportion of possible causative etiologic agents.[545,546] However, some outcomes are rare or present after detection of influenza viruses is not possible, such as death or cardiovascular events, such that it is not possible to confirm influenza infection. In these situations studies with nonspecific outcomes can still be informative.[546,547] However, care must be taken to minimize selection biases that can bias final vaccine efficacy/effectiveness results. For example, older persons who are generally healthier, and less likely to die or be hospitalized, may be more likely to be vaccinated whereas those who are frailer and less healthy are less likely to be vaccinated.[548–551]

Laboratory-confirmed influenza virus infection is generally the most specific outcome for vaccine efficacy/effectiveness studies. Virus isolation and RT-PCR are specific diagnostic assays to detect influenza virus infection; RT-PCR is currently the most sensitive and specific assay that is widely available. Serologic assays to detect influenza infection (i.e., fourfold rise in antibody titers against circulating viruses) were used frequently to detect influenza infections prior to wide availability of RT-PCR. However, if preinfection antibody levels are high (as is common after receipt of IIVs), infection may not be associated with a fourfold rise in antibodies[193,233,552]; a similar phenomenon has been noted with serologic response to vaccination and prior vaccination.[553–555] Thus, some vaccinated persons who are subsequently infected may be misclassified as negative infections (biasing observed vaccine efficacy/effectiveness estimates higher than the true estimate). Limited data suggest that the degree of misclassification may vary and may depend on infecting and vaccine viruses, as well as the type of prior vaccine.[233,552]

Evidence From Randomized Clinical Trials Measuring Vaccine Efficacy Against Laboratory-Confirmed Influenza-Associated Illness

Older Adults

Among older adults, no placebo-controlled randomized clinical trial measuring vaccine efficacy against RT-PCR– or culture-confirmed influenza infection has been published. One randomized clinical trial (participants 60 years of age or older) relied on serology for laboratory confirmation of influenza

infection and reported a vaccine efficacy of 58% (95% confidence interval [CI]: 26% to 77%) for symptomatic serologically confirmed illness.[394] However, it is unknown if infections were missed by serology among vaccinees. Two randomized clinical trials evaluated the superiority of new vaccines meant to boost the immune response in older adults, adjuvanted and high-dose (60-μg) IIVs, compared to standard dose (15-μg) nonadjuvanted inactivated vaccines (Table 31.2).[451,556] However, only the high-dose inactivated vaccine achieved statistically significant higher relative efficacy (22%) compared to standard-dose inactivated vaccine.[451] In a post hoc analysis, the AS03 adjuvanted inactivated vaccine had a relative efficacy of 24% (95% CI: 5.68 to 35.49) against H3N2 virus infections compared to the nonadjuvanted vaccine.[556]

Adults

Among healthy adults younger than 65 years of age, several placebo-controlled trials measuring vaccine efficacy of IIVs (egg-grown and cell grown) and a recombinant trivalent HA protein vaccine against RT-PCR– or culture-confirmed influenza infection have been published (see Table 31.2).

TABLE 31.2 Summary of Selected RCTs Evaluating Vaccine Efficacy (VE) or Relative Efficacy (RE) for Seasonal and Pandemic Inactivated Influenza Vaccines and Recombinant Protein Vaccines Against Symptomatic RT-PCR–Confirmed or Virus Culture-Confirmed Influenza

Reference	Vaccine Comparisons	Diagnostic Assay	Ages	Study Years (Location)	VE/RE Results (95% CI)	Comments
Seasonal Vaccines						
Older Adults						
556[a]	AS03 adjuvant vs nonadjuvant IIV3	RT-PCR	65+ y	2008-09, 2009-10 (3 North American, 10 European countries, Russia, Thailand)	RE A/B: 12.11 (–3.4–25.29)	Failed to meet FDA regulatory requirements for superiority
451[a]	High-dose IIV3 vs standard-dose IIV3	RT-PCR	65+ y	2011-12, 2012-13 (U.S., Canada)	RE A/B: 24.2 (9.7–35.6) RE H1N1: 11.1 (–159–70.2) RE H3N2:23.2 (6.0–37.5) RE B:25.5 (–15.7–52.4)	Met FDA regulatory requirements for superiority
Adults						
530[b]	IIV3 vs placebo (LAIV vs placebo), IIV vs LAIV	RT-PCR or culture	18–46 y, healthy	2004-05 (U.S.)	IIV VE A/B: 75% (42–90) LAIV VE A/B: 48% (–7–74) IIV vs LAIV RE A/B: 53% (–5–80)	Reported H3 moderately drifted and two lineages of B
552[b]	IIV3 vs placebo, (LAIV vs placebo), IIV vs LAIV	RT-PCR or culture	18–48 y, healthy	2005-06 (U.S.)	IIV VE A/B: 16% (–171–70) LAIV VE A/B: 8% (–194–67) IIV vs LAIV RE A/B: 9% (–110–60)	Most infections were H3N2, not drifted; B lineage mismatch (low attack rate)
557[a]	IIV3 vs placebo	Culture	18–49 y, healthy	2005-06, 2006-07 (U.S.)	Both seasons VE A/B: 49% (LCI20)	Reported limited/no H3 drift; B lineage mismatch; low attack rate
559[a]	IIV3 vs placebo	Culture	18–64 y, healthy	2005-06 (Czech Republic)	VE A/B: 22% (–49–59) VE B: NS 22% (–65–62)	Reported no virus drift; low attack rate
558[a]	IIV3 vs placebo	Culture	18–64 y, healthy	2006-07 (Czech Republic)	VE A/B: 62% (46–73)	
531[b]	IIV3 vs placebo, (LAIV vs placebo), IIV vs LAIV	RT-PCR or culture	18–49 y, healthy	2007-08 (U.S.)	IIV VE A/B: 68% (46–81) LAIV VE A/B: 36% (0–59) IIV vs LAIV RE A/B: 60% (33–77)	Reported no H3 drift; B virus different lineage than vaccine
329[a]	MDCK cell-culture grown inactivated trivalent (CCIV3) vs placebo, IIV3 (egg-based) vs placebo	Culture	18–49 y, healthy	2007-08 (U.S., Finland, Poland)	IIV3 VE A/B: 63% (LCI 47) CCIV3 VE A/B: 70% (LCI 55) IIV3: VE H1N1: 82 (LCI 61) CCIV3 VE H1N1: 89 (73) IIV3 VE H3N2: 49 (LCI –9) NS CCIV3 VE H3N2: 77 (35) IIV3B VE: 53% (LCI 22) NS CCIV3 B VE: 50 (LCL 18) NS	B virus different lineage than vaccine
455[a]	Recombinant trivalent HA protein vaccine vs placebo	Culture	18–49 y, healthy	2007-08 (U.S.)	VE A/B: 44.6% (18.8–62.6)	Reported virus drift

TABLE 31.2 Summary of Selected RCTs Evaluating Vaccine Efficacy (VE) or Relative Efficacy (RE) for Seasonal and Pandemic Inactivated Influenza Vaccines and Recombinant Protein Vaccines Against Symptomatic RT-PCR–Confirmed or Virus Culture-Confirmed Influenza *(Continued)*

Reference	Vaccine Comparisons	Diagnostic Assay	Ages	Study Years (Location)	VE/RE Results (95% CI)	Comments
560[b]	Vero cell culture-grown inactivated trivalent vs placebo	Culture	18–49 y, healthy	2008-09 (U.S.)	VE A/B: 72 (55–82) VE H1N1: 75% (55–86) VE H3N2: 50% (–173–91) VE B 72% (55–82)	Matched A/B VE: 78% (61, 88)
495[b]	IIV3 vs placebo	RT-PCR	18–55 y HIV+ on ART	2008 (South Africa)	VE A/B: 75.5% (9.2%–95.6%)	Vaccine naïve; CD4 >100 cells/mL
Children						
563[b]	IIV3 vs monovalent influenza B vaccine	Culture	1–15 y, healthy	1986-87, 1987-88, 1988-89, 1989-90 (U.S.)	IIV3 H1N1 years (86–87 and 88–89): VE 91% (64–98) IIV3 H3N2 years (87–88 and 89–90): VE 77% (20–94)	Vaccinees received H1/H3 bivalent IIV in 1985; reported virus drift 1986–87, 1987–88
564[a]	IIV3 vs placebo	Culture	6–24 mo, healthy	1999-00, 2000-01 (U.S.)	1999–00: VE A/B: 66% (34%–82%) 2000–01: VE A/B: –7% (–247%–67%)	Low attack rate in year 2; all vaccinated received 2 doses
570[a]	MF59 adjuvanted IIV3 vs control vaccine; nonadjuvanted IIV3 vs control vaccine; adjuvanted IIV3 vs nonadjuvanted IIV3	RT-PCR	6–71 mo, healthy	2007-08, 2008-09 (Germany and Finland)	MF59 VE A/B: 86% (74–93) IIV3 VE A/B: 43% (15–61) MF59 vs IIV3 RE A/B: 75% (55–87) *6–24-month subset:* MF59 VE: 77 (37–92) IIV3 VE: 11 (–89–58) MF59 vs IIV3 RE: 73 (29–90)	Vaccine-naïve children; reported no drift for 85% of H3
565[b]	IIV3 vs placebo	RT-PCR	6–17 y	2009-10 (Hong Kong)	VE B: 66% (31–83)	Vaccine did not include circulating A viruses (H1N1pdm09)
567[a]	IIV4 vs control vaccine	RT-PCR and virus culture	3–8 y, healthy	Dec 2010 –Oct 2011 (3 Asian, 3 Central American, 2 Middle Eastern)	VE A/B: 59.3 (45.2–69.7) VE H1N1: 55.6 (21.3–74.9) VE H3N2: 55.6 (21.2–74.9) VE B Vic: 47.2 (12.4–68.2)	Too few B Yamagata to assess
886[b]	IIV3 vs placebo	RT-PCR	6–35 mo, HIV+ on ART	2009 (South Africa)	VE A/B: 17.7% (<0–62.4%)	H3N2 dominant season; sequencing suggested some drift; booster given
Pregnant Women						
887[b]	IIV3 vs placebo	RT-PCR	18–36 y; 20–36 weeks' gestation; HIV and HIV+	2011, 2012 (South Africa)	HIV-neg mothers: VE A/B: 50.4% (14.5–71.2) HIV-pos mothers: VE A/B: 57.7% (0.2–82.1) Infants of HIV-neg mothers: VE A/B: 48.8% (11.6–70.4)	Unable to assess infants of HIV+ moms (small numbers)
Monovalent 2009 Pandemic Vaccine						
Children						
571[a]	AS03 adjuvant monovalent inactivated vs nonadjuvanted monovalent inactivated vaccine	RT-PCR	6 mo to 10 y	Feb 2010–Aug 2011 (Australia, Costa Rica, Mexico, 2 South American, 3 Asian countries)	RE A/B: 76.8% (18.5–93.4)	Booster given

[a]Pharmaceutical funding.
[b]Funded by a grant from government or foundation.
CI, 95% confidence interval; IIV, inactivated influenza vaccine; LAIV, live attenuated influenza vaccine; LCI, lower confidence interval; MDCK, Madin-Darby canine kidney; NS, nonsignificant (noted only if full CI not reported); RCT, randomized controlled trial; RE, relative efficacy; RT-PCR, reverse-transcription polymerase chain reaction; VE, vaccine efficacy.

Depending on the year, reported point estimates for vaccine efficacy against all influenza viruses (A and B) have varied from significant estimates of 75% during 2004–05 in the United States and 2008 in South Africa to a nonsignificant estimate of 16% during 2005–06 in the United States.[329,455,495,530,531,552,557–560] During 2005–06, low influenza infection attack rates may have contributed to lower and nonsignificant efficacy estimates.[552,557,559] Reports of antigenic drift among circulating viruses compared to vaccine viruses did not consistently correlate with vaccine efficacy estimates.[455,495,530,531]

During 2007–08, vaccine efficacy of a recombinant trivalent HA protein vaccine was 45% compared to placebo among healthy adults.[455] Two placebo-controlled trials measured efficacy of cell-based IIVs, one during 2007–08 and the other during 2008–09, and reported point estimates of 70% to 72%, respectively.[329,560] One cell-based IIV (Flucelvax) is currently licensed in the United States. Clinical trials during 2004–05, 2005–06, and 2007–08 with three arms—IIVs, live attenuated influenza vaccines, and placebo—suggested that live attenuated influenza vaccines may be inferior to IIVs in adults and are discussed in detail in Chapter 32.[530,531,552]

A metaanalysis that included data from placebo-controlled clinical trials of licensed IIVs with outcomes of RT-PCR– or culture-confirmed influenza infection among adults, and published in February 2011, reported a pooled vaccine effectiveness of 59% (95% CI: 51 to 67).[561] Several placebo-controlled randomized clinical trials measuring efficacy of IIVs used serology to confirm influenza infection; efficacy estimates ranged from 47% to 73% (1983–88, and 1997–98) and equal to or greater than 70% (1940; 1985–90).[7,66,233,285] A metaanalysis that included data from randomized clinical trials of IIVs with serologic, RT-PCR, or culture-confirmed influenza infections among adults reported a pooled vaccine effectiveness of 70% (95% CI: 55 to 80) for matched viruses and 55% (95% CI: 42 to 65) for unmatched strains.[562]

Children

Among children, only a few placebo-controlled randomized clinical trials measuring vaccine efficacy of nonadjuvanted IIVs with an outcome of RT-PCR– or culture-confirmed influenza infection have been published (see Table 31.2). During 1986 through 2000, a randomized trial evaluated vaccine efficacy of a trivalent IIV against culture-confirmed influenza A virus vaccine infection and used a monovalent influenza B virus vaccine as the comparison vaccine.[233,563] A vaccine efficacy of 91% during two seasons when A(H1N1) viruses predominated (1986–97 and 1988–89) and an efficacy of 77% during two A(H3N2) seasons (1987–88 and 1989–90) was reported. A separate study enrolled only young children, 6 to 24 months of age, and reported a vaccine efficacy against all influenza A/B viruses of 66% during 1999–2000, and a nonsignificant vaccine efficacy of –7% (CI, 247% to 67%) during the second year, 2000–01.[564] No antigenic drift in circulating viruses compared to vaccine viruses was reported either season, however, the attack rate during the second year was very low. During 2009–10 in Hong Kong, a vaccine efficacy of 66% was reported against influenza B virus infection among children 6 to 17 years of age[565]; no evidence for a reduction in the severity or duration of symptoms among vaccinated children was reported.[566] During 2010–11, the vaccine efficacy of a quadrivalent IIV among children 3 to 8 years of age was 59% against infection with influenza A/B viruses with influenza-like illness symptoms, compared to a control vaccine, and 74% (95% CI: 51.5% to 86.2%) against influenza A/B virus-associated "moderate to severe" infections; moderate to severe infections were defined as any of the following: fever greater than 39°C (53 of 66 [80%] RT-PCR–confirmed illnesses) or physician-confirmed acute otitis media (1.5%), lower respiratory tract illness (18%), or extrapulmonary complications (0%).[567] Several studies of nonadjuvanted IIVs included serologically confirmed influenza infections as an outcome and reported vaccine efficacy of 45% (3–9-year-old daycare attendees, 1996–97), 56% (3–19-year-old children, 1986–87), and 70% (school-age boys, 1972–73).[391,568,569]

Two randomized clinical trials suggested that adjuvanted IIVs may have superior efficacy in young children compared to nonadjuvanted IIVs (see Table 31.2).[488,570] One study compared a MF59 adjuvanted inactivated vaccine to a nonadjuvanted inactivated vaccine among vaccine-naïve children 6 to 71 months of age.[570] During 2008–09, the vaccine efficacy was 86% for the MF59 adjuvanted vaccine and 43% for the nonadjuvanted vaccine, and the relative efficacy of the adjuvanted vaccine compared to the nonadjuvanted was 75%. Among the subset of children 2 to 24 months of age, the relative efficacy of the adjuvanted to nonadjuvanted vaccine was 73% (95% CI: 29 to 90). During the 2009 pandemic, an AS03 adjuvanted monovalent inactivated vaccine was compared to a nonadjuvanted inactivated monovalent vaccine in children 3 to 8 years of age.[571] The adjuvanted vaccine had a relative efficacy of 77% compared to the nonadjuvanted vaccine.

Three randomized clinical trials that compared live attenuated influenza vaccine to nonadjuvanted IIV in young children suggested that live attenuated influenza vaccine had superior efficacy compared to IIV.[572–574] These studies are discussed in detail in Chapter 32.

A cluster-randomized trial in children ages 6 months to 15 years from Hutterite communities in Canada reported a combined direct (vaccine efficacy against RT-PCR–confirmed infection in enrolled children) and indirect (efficacy against RT-PCR–confirmed infection in nonvaccinated community members in clusters with vaccinated versus unvaccinated children) estimate of 59% (95% CI: 5 to 82) for trivalent IIV during 2008–09; a hepatitis A vaccine was the comparison vaccine.[575]

Evidence From Observational Studies Measuring Vaccine Effectiveness Against Laboratory-Confirmed Influenza-Associated Illness

Observational studies are often the only option to measure vaccine effectiveness against more-severe, less-common influenza outcomes, such as hospitalizations. Many observational studies have assessed vaccine effectiveness to prevent RT-PCR–confirmed or culture-confirmed influenza-associated hospitalizations in adults, a few are summarized here. A 3-year study in Tennessee using a test-negative design (e.g., hospitalized patients who tested negative for influenza were the controls and those who tested positive were cases) during 2006–07 through 2008–09, reported a vaccine effectiveness of 61% (95% CI: 18 to 83) among adults 50 years of age and older.[576] The same group of researchers added two additional seasons to this analysis, including 2006–12 (excluding 2009–10) and reported a vaccine efficacy of 58% (95% CI: 8% to 81%) for persons 65 years of age or older for the five seasons combined.[577] During the 2010–11 season, with a high proportion of A(H1N1)pdm09 viruses, a Spanish cohort study reported a significant vaccine effectiveness of 58% (95% CI: 16 to 79) among hospitalized adults 60 years of age and older or persons with chronic conditions.[578] Results were similar when data were analyzed with a test-negative design and from a separate Spanish study using a test-negative design.[579] In Australia during the 2010 season, a vaccine effectiveness of 49% (95% CI: 13% to 70%) against H1N1pdm09 infection-associated with hospitalization was reported; a test-negative design was

used but testing was clinician-directed.[580] In contrast, during 2012–13, two European Hospital Networks reported vaccine effectiveness against influenza A/B virus infection-associated hospitalization of 33% (95% CI: 11 to 49) and 21% (95% CI: −25 to 50); influenza A(H3N2), influenza A(H1N1)pdm09, and influenza B viruses cocirculated.[581,582] Mid-season, interim results from Canadian studies enrolling hospitalized adults and using a test-negative design during the 2014–15 influenza season, a season with a high proportion of circulating drifted A(H3N2) viruses, failed to find effectiveness against hospitalizations associated with A(H3N2) infection.[583,584] A cohort study in Spain reported a relative vaccine effectiveness of 33% (95% CI: 15% to 48%) among adults 65 years of age and older for intradermal inactivated trivalent vaccine compared to virosomal inactivated trivalent vaccine against influenza-associated hospitalizations during 2011–12.[585]

Few observational studies have reported vaccine effectiveness estimates against RT-PCR– or culture-confirmed influenza-associated hospitalizations in children. One study, enrolled severely ill children admitted to pediatric ICUs in the United States during 2010–11 and 2011–12. They reported a vaccine effectiveness of 74% (95% CI: 19 to 91) using a test-negative design method and 82% (95% CI: 23 to 96) using controls selected from similar clinics as cases.[586] A study from Hong Kong during 2009–13 among children 6 months to 17 years of age reported a vaccine effectiveness against hospitalizations associated with influenza A/B virus infections of 62% (95% CI: 43 to 74).[587] The estimated vaccine effectiveness estimates against hospitalizations associated with A(H3N2) virus infections was 37% (95% CI: −25 to 68), with A(H1N1)pdm09 virus infection was 72% (95% CI: 39 to 87), and with influenza B virus infections was 69% (95% CI: 42 to 83). During 2010–12 in the United States, among persons of all ages, a vaccine effectiveness of 57% (95% CI: 32% to 73%) against RT-PCR–confirmed influenza-associated community-acquired pneumonia hospitalizations was reported; age group–specific estimates were not significant.[588]

During the 2009 pandemic, several studies attempted to measure vaccine effectiveness of the monovalent pandemic vaccine against A(H1N1)pdm09 virus infection-associated hospitalizations. Infection attack rates had already started to decline rapidly when vaccine became available in 2009 and many studies identified a small number of vaccinated cases; therefore, it is possible that residual confounding related to calendar time and/or sparse data bias may exist.[589] A U.S. case-control study enrolling RT-PCR–confirmed hospitalized cases 6 months of age and older, and comparing to community controls, reported a vaccine effectiveness of 50% (95% CI: 13 to 71) for nonadjuvanted monovalent pandemic vaccine.[590] A U.S. study comparing hospitalized children 6 months to 9 years of age with RT-PCR–confirmed A(H1N1)pdm09 infection to matched controls from an immunization registry reported a vaccine effectiveness of 82% (95% CI: 0 to 100) for a single dose of nonadjuvanted inactivated or live attenuated monovalent vaccine.[591] Among hospitalized children in Canada 6 months to 9 years of age, an estimated vaccine effectiveness of 85% (95% CI: 61 to 94) was reported against RT-PCR–confirmed infections for a single dose of an AS03 adjuvanted monovalent inactivated pandemic vaccine.[592]

Since 2003, many observational studies using the test-negative design have reported vaccine effectiveness estimates against RT-PCR– or culture-confirmed influenza-associated medically attended illness; most studies enrolled participants from ambulatory care settings, while some recent studies have reported effectiveness against hospitalizations.[593–605,605a] Several networks in Australia, Canada, Europe, New Zealand, the United Kingdom, and the United States collect data from many partners/sites to report annual vaccine efficacy estimates, and often report mid-season interim estimates to inform communication efforts during the season, and may inform vaccine strain selection.[606–612] Increasingly, influenza A subtype–specific and influenza B lineage–specific vaccine effectiveness estimates have been published, allowing more direct comparison with individual vaccine components. Annual vaccine effectiveness estimates from different geographic locations and both Northern and Southern hemisphere regions have highlighted the importance of vaccine virus characteristics, such as substitutions induced during culture in eggs and antigenic and genetic drift of circulating viruses away from the vaccine strain on vaccine effectiveness estimates.[250,609–611,613–616] In addition, annual vaccine efficacy estimates have reported effect modification of prior season vaccination on current season vaccine effectiveness; the effect on current season vaccine effectiveness varies across seasons and studies.[250,595,613,617,618] Conflicting evidence on the effect of prior vaccination on current season vaccine efficacy was also reported from limited randomized trials.[66,619] Finally, networks capable of collecting annual data have allowed the comparison of vaccine effectiveness for live attenuated influenza vaccines and IIVs in children by season and by subtypes, including effectiveness against illness associated with A(H1N1)pdm09 viruses that were not circulating during randomized clinical trials comparing these vaccines.[595,596,620]

During 2010–11 through 2013–14, reported vaccine effectiveness estimates against medically attended influenza A(H1N1)pdm09 infections have ranged from 80% (95% CI: 52 to 92) in Canada during 2011–12 to 48% (95% CI: 16 to 67) in Europe during 2013–14; neither vaccine viruses nor circulating viruses had had significant antigenic changes since 2009.[593,596,598,605,613,614,618,623–626] During these seasons, vaccine effectiveness estimates against medically attended A(H3N2) virus infections have ranged from nonsignificant estimates of 13% (95% CI: −20 to 36) in Australia during 2011 to 51% (95% CI: 10 to 73) in Canada during 2011–12.[250,593,595,596,603,613,614,616,618,623,627–629] Several studies have reported influenza B lineage–specific vaccine efficacy estimates. In North America, vaccine effectiveness against B Victoria lineage during 2011–12, when it was included in the trivalent vaccine, was 52% (95% CI: 8 to 75) and 71% (95% CI: 40 to 86); vaccine effectiveness against the less frequent, nonvaccine B Yamagata lineage was reported as 66% (95% CI: 58 to 73) and 27% (95% CI: −21 to 56).[595,618] During the subsequent season, 2012–13, when a B Yamagata virus was included in the trivalent vaccine, vaccine effectiveness estimates against B Yamagata and B Victoria virus infections were similar: 51% (95% CI: 36 to 63) to 75% (95% CI: 29 to 91), respectively.[250,613]

A metaanalysis used test-negative design observational studies with vaccine effectiveness estimates for adults 60 years of age and older against RT-PCR–confirmed influenza infection. They reported a significant vaccine effectiveness during epidemic seasons or regional outbreaks when the vaccine viruses matched circulating viruses (52% [95% CI: 41 to 61]; 58% [95% CI: 30 to 70], respectively) and when a mismatch was reported (36% [95% CI: 22 to 48]; 43% [95% CI: 21 to 59], respectively).[630]

Vaccine Efficacy and Effectiveness Against Laboratory-Confirmed Influenza for Pregnant Women and Their Infants

A randomized controlled trial conducted in South Africa reported a vaccine efficacy of 50% against RT-PCR–confirmed influenza A/B infection among HIV-negative pregnant women vaccinated during the third trimester during 2011 and 2012

(see Table 31.2).[631] Vaccine efficacy among HIV-infected pregnant women (under medical care) was 58%. An observational study in the United States during 2010–11 and 2011–12, using a test-negative design, reported a vaccine effectiveness against influenza A/B infection of 44% (95% CI: 5 to 67) among pregnant women.[632]

Infants younger than age 6 months are not recommended for vaccination, although some studies suggest that immune responses among infants are relatively good.[633,634] Passive transfer of antiinfluenza antibodies to neonates after maternal vaccination during pregnancy has been documented.[631,635,636] The South African study that measured vaccine efficacy among women vaccinated during pregnancy also measured efficacy of maternal vaccination on influenza infection among infants until 24 weeks postpartum. A vaccine efficacy of 49% was reported for infants of HIV-negative women (see Table 31.2)[631]; the study was unable to assess vaccine efficacy among infants of HIV-infected women.[636a] A randomized controlled trial conducted in Bangladesh that provided influenza or pneumococcal vaccination to pregnant women during the third trimester reported that infants born to influenza-vaccinated women had a 63% (95% CI: 5 to 85) reduction in rapid influenza diagnostic test–confirmed influenza illness during the first 6 months of life[637]; a laboratory-confirmed influenza outcome was not used for pregnant women. A large randomized controlled trial among of inactivated influenza vaccine among pregnant women in Mali found that vaccine efficacy against laboratory-confirmed influenza illness in infants during during the first 6 months of life was 33.1% (CI: 3.7 to 53.9). In this study, VE was highest in the first 4 months of life (67.9% [CI: 35.1–85.3]).[637a] Similarly, in the South African trial, protection of the infant was found only in the first 8 weeks following delivery.[637a]

Several studies have been conducted to estimate the effect of maternal immunization on birth outcomes, such as the risk of prematurity or the risk of being small for gestational age. A randomized trial in Bangladesh found that babies born to mothers vaccinated during pregnancy with IIV3 were significantly less likely to be born small for gestational age and had a mean birth weight 200 g higher than babies born to unvaccinated mothers.[638] Some observational studies in developed and developing countries found a lower risk of prematurity or low birth weight in babies born to vaccinated mothers, but the effect has not been consistently demonstrated.[638–643a] A recent randomized trial in South Africa found maternal immunization had no effect on infant birth weight.[631]

Duration of Immunity and Protection

Inactivated vaccine induces a rapid systemic and local immune response in healthy young adults.[217] It has been shown that up to 90% of normal subjects develop serum HI titers of 1 : 40 or greater within 2 weeks of vaccination and that second doses provide little or no further increase in titers. Peak serum antibody levels develop within 4 to 6 weeks after vaccination[219] and then antibody levels wane by as much as a 50% within 6 months.[333]

Elderly subjects generally respond less well to influenza vaccines than young healthy adults, and those with chronic debilitating medical conditions generally respond less well than healthy subjects of similar age. Up to 50% of elderly vaccinees may fail to respond to IIV with a fourfold increase in HI antibodies.[311] In addition, antibody responses in the elderly may be somewhat delayed compared to those in younger individuals.[644] Although some studies have noted that antibody responses return toward the baseline more rapidly than in young adults,[645,646] the relevance of these observations to prevention of influenza illness is uncertain.[647]

The duration of protection from illness after influenza vaccination has been studied during several clinical trials. In 1968, a group of schoolchildren was vaccinated with the A/Hong Kong/68 vaccine and was observed over three successive influenza epidemics caused by the A/Hong Kong/68 virus. Three years after vaccination, the vaccine was still 67% effective in preventing influenza.[282] In randomized trials conducted among healthy college students, immunization with trivalent inactivated vaccine before the 1982–83 epidemic provided 92 and 100% efficacy against influenza H3N2 and H1N1 infection–related illnesses, respectively, during the first year, and a 68% reduction against H1N1 infections during the second year without revaccination.[648] Because elderly persons who have received repeated influenza vaccinations develop lower peak HI titers, and these antibody levels return to baseline faster than in young healthy adults receiving influenza vaccine for the first time, immunity may be of shorter duration in this target group than in the young adults studied above.

Several observational studies of influenza vaccine effectiveness have reported decreased vaccine protection within a single season, particularly against influenza A(H3N2) and older adults.[648a–648e] However, this effect has not been observed consistently across age groups and seasons, and the observed decline in protection could be attributable to other factors, such as increased circulation of antigenically drifted variants over the course of the influenza season. Finally, differences in methods used to measure intraseasonal changes in vaccine effectiveness could contribute to inconsistencies. The inconsistent evidence for intraseason waning of influenza vaccine protection makes drawing conclusions difficult, and further evaluation of this effect in larger studies and different seasons is needed.

Although protection may persist for longer than a year after vaccination in young healthy adults, annual immunization with inactivated vaccine is recommended because one or more vaccine antigens usually are updated each year and because reductions in serum antibody levels have been well documented during the year after immunization. In particular, annual immunization of those 65 years of age and older close to the influenza season will help maximize antibody levels and protection in this important target group.

In contrast to the relatively short-lived antibody response to vaccination, immunity to HA can persist for decades after natural infection. During 1977 and 1978, influenza A(H1N1) viruses similar to those circulating in 1950 reappeared and spread throughout the world. Individuals born before 1950 were not affected, indicating that substantial immunity remained after more than 20 years. In addition, studies of the immunogenicity of vaccines against the H1N1 virus found high rates of preexisting immunity among those born before 1957.[261] In contrast, disease occurred in individuals younger than 20 years of age, regardless of previous infection by influenza A(H3N2) viruses, and antibody studies confirmed that these persons were much more likely to require two doses of H1N1-containing vaccine to demonstrate an immune response as would be expected with an immune naïve population showing that intersubtypic immunity in humans is weak. The ability of natural infection to engender long-lasting immunity was dramatically demonstrated during the 2009 influenza A(H1N1) pandemic, where approximately one-third of adults older than 60 years of age had cross-reacting antibodies to the pandemic strain, presumably based on exposures to antigenically similar strains 50 or more years previously.[258] In addition, the surprisingly low burden of severe illness in older populations worldwide suggested that unmeasured partial or full immunity after natural infection was retained in an even larger proportion of older persons.[649,650]

Effectiveness of Vaccination for Decreasing Transmission to Contacts

Influenza vaccination has been shown in certain settings, such as nursing homes and schools, to provide indirect benefits to contacts who remain unvaccinated,[251,568,651,652] a finding that is consistent with models of vaccine-induced herd immunity. Furthermore, mass immunization among those who are most likely to acquire and transmit influenza (usually school-age children) might reduce the overall occurrence of influenza in the community, including in unvaccinated persons.[251,653] The community-level protective effect of vaccinating children was shown during the 1968 pandemic where vaccination of greater than 5% of schoolchildren in a community resulted in lower illness rates among all age groups compared to another unvaccinated community.[251,654] Several recent studies have evaluated the benefits of vaccinating school-age children with influenza vaccine. Overall, studies reported decreases in influenza-related illness in the children, and a modest benefit to adults, either in the household or in the community.[286,575,653,655] However, community impact of childhood vaccination on morbidity and mortality outside of the household effect is difficult to quantify and more studies are needed to assess the costs and benefits to children and the larger communities of school-based or universal vaccination programs.[656] (For further discussion, see Chapter 75.) Community-level, multiyear ecologic studies have also shown population-level impact. A retrospective ecologic study suggested that high rates of vaccination of schoolchildren in Japan had reduced excess mortality rates among older adults and that discontinuation of this vaccination program led to increases in excess mortality rates among elderly Japanese.[657] This study remains controversial because aging of the population and other variables might not have been adequately controlled.[658,659] The largest study to examine the community effects of increasing overall vaccine coverage was an ecologic study that described the experience in Ontario, Canada, which is the only province to implement a universal influenza vaccination program beginning in 2000. On the basis of models developed from administrative and viral surveillance data, influenza-related mortality, hospitalizations, Emergency Department use, and physicians' office visits decreased substantially more in Ontario after program introduction than in other provinces, with the largest reductions observed in younger age groups.[660] In addition, influenza-associated antibiotic prescriptions were substantially reduced compared with other provinces.[661]

Decreasing transmission of influenza from caregivers and household contacts might reduce influenza-like illness and complications among persons at high risk. Observational studies have demonstrated that vaccination of healthcare personnel in a nursing home facility is associated with decreased deaths among patients.[240,662] More recently, controlled trials have shown decreases in mortality and influenza-like illness in facilities where higher vaccine coverage levels among staff were achieved.[663,664] A comprehensive review concluded that vaccination of healthcare personnel in settings in which patients also were vaccinated provided significant reductions in deaths among elderly patients from all causes and deaths from pneumonia.[665]

Safety

Common Adverse Events

Among children and adults, local inflammatory reactions resulting in soreness or erythema at the injection site is the most frequently reported adverse event following vaccination with an IIV. Among adults in placebo-controlled studies, soreness at the vaccination site was most common and reported among 10% to 64% of subjects; soreness generally lasted less than 2 days.[432] These symptoms were mild and rarely interfered with usual daily activities. A review of Vaccine Adverse Event Reporting System (VAERS) data among adults in the United States found the most commonly reported adverse events during this period were injection-site reactions, pain, fever, myalgias, and headache.[666] This review did not identify any new safety concerns. Placebo-controlled trials have found that receipt of trivalent inactivated vaccines are not associated with higher rates for systemic symptoms, such as fever, malaise, myalgia, or headache, compared with placebo injections in older adults and healthy young adults.[333,382,432]

Among children, fever, myalgia, arthralgia, and headache are noted in less than 15% of vaccinees, most often in very young children and among those exposed to influenza virus vaccines or to one of the antigens in the vaccine for the first time.[333,335,351,383,667,668] In a study of 791 healthy children 1 through 15 years of age, postvaccination fever was noted among 12% of those 1 through 5 years of age, 5% among those 6 through 10 years of age, and 5% among those 11 through 15 years of age. However, such systemic side effects have not been observed in more recent randomized trials of inactivated vaccines where the only differences between placebo and vaccine groups have been sore arm and redness at the injection site.[667] In young children, whole-virus influenza vaccines and larger vaccine doses appear to produce more systemic reactions, including fever, than subvirion vaccines.[335,382] Data have not demonstrated significant increases in local or systemic adverse events among recipients of quadrivalent vaccines compared with trivalent vaccines (nor among recipients of monovalent vaccines, such as the 2009 unadjuvanted pandemic H1N1 vaccine[488,489,669]). Large postlicensure studies in the United States among children enrolled in health maintenance organizations strongly support the safety of IIVs. Among approximately 250,000 children included in one study, there was no increase in vaccine-associated medically attended events during the 2 weeks postvaccination compared with the 2 weeks before vaccination and days 15 to 28 following vaccination.[670] A study among approximately 45,000 children 6 to 23 months of age found no statistically significant association of vaccination with any medical events other than a small increase in gastritis and duodenitis (typically self-limited nausea and vomiting). However, statistically significant decreases in upper respiratory illness, asthma, and otitis media were also observed.[671] A subsequent Vaccine Safety Datalink study of 66,283 children 24 through 59 months of age noted diagnoses of fever, gastrointestinal tract symptoms, and gastrointestinal disorders to be significantly associated with IIV (incidence risk ratios of 1.71, 1.18, and 7.70, respectively, compared with time before vaccination and after a postvaccination risk period). No evidence of serious medically attended adverse events was noted in this study.[672]

Regarding higher-dose vaccines, recipients of IIV containing 180 μg of HA antigen had higher rates of injection-site reactions and fever after vaccination compared with those who received standard-dose vaccines (45 μg); however, these reactions were typically mild and transient. In a large trial, 915 (36%) of 2572 persons who received high-dose vaccine reported injection-site pain compared with 306 (24%) of those who received standard-dose vaccine; similarly, 1.1% of high-dose recipients reported moderate to severe fever compared with 0.3% of standard-dose IIV.[449] No differences in rates of serious adverse events were noted between high-dose and standard-dose vaccine recipients in a second randomized controlled trial.[673] Intradermally administered IIVs are associated with higher rates of injection-site reactions compared with intramuscularly administered influenza vaccines among

nonelderly adults, including erythema, induration, swelling, and pruritus; however, rates of injection-site pain were not significantly different nor were rates of systemic symptoms, such as headache, myalgia, or fever.[674]

Adjuvanted inactivated vaccines may be associated with higher rates of local reactions. For instance, among recipients of vaccine containing MF59 there were more local reactions (soreness was six times more frequent and erythema two times more frequent after the first dose of vaccine containing MF59 compared to vaccine without MF59 in one study of the elderly[194]) as well as more systemic reactions such as malaise or myalgia.[422,424] Even so, safety monitoring during the 2009 H1N1 pandemic among countries that used vaccines that included oil-in-water emulsions as adjuvants, generally found that adverse events were rare and typically nonserious, consisting mostly of local reactions, fever, and/or malaise.[675,676] The notable exception was the association of narcolepsy with an AS03-adjuvanted monovalent pandemic vaccine (see below).

Serious Adverse Events

Influenza immunizations can be associated with serious adverse events, although they are uncommon. Two reviews of national data have found that rates of serious adverse events reported following vaccination (not necessarily attributed to influenza vaccination) were 3.4 reports per million in the United States,[666] and less than 1 per million in South Korea.[677] The most common serious adverse event reported in both reviews was Guillain-Barré syndrome (GBS). GBS was first associated with receipt of IIV during the 1976 swine (H1N1) influenza vaccine campaign, when one additional case of GBS was estimated for every 100,000 persons vaccinated[678] (above an estimated annual incidence of 10 to 20 cases per 1 million adults).[679] Most commonly GBS cases are associated with antecedent gastrointestinal or respiratory illness, including influenza infection.[679–683] Studies conducted since the 1976–77 influenza vaccine campaign have failed to detect a significant risk associated with seasonal influenza vaccine in many years[684–686]; however a small but significantly increased risk of GBS among vaccinees has been detected in some studies.[687,688] As a result, the estimated risk for GBS has been approximated as one additional case of GBS per 1 million persons vaccinated.[689] Similar rates were estimated for those who received the H1N1 pandemic vaccine in 2009.[690–692] Most vaccine-associated cases will occur in the second week following vaccination. While a small risk of vaccine-associated GBS has been appreciated, one study has estimated that the risk of GBS associated with influenza infection to be substantially higher, perhaps indicating that the vaccine provides a net reduction of GBS risk among those vaccinated.[683] Because persons with a history of GBS are at higher risk for subsequent cases, influenza vaccination is usually not recommended for persons with a previous history of influenza vaccine–associated GBS, and providers should consider the risks and benefits of vaccinating persons with GBS from any cause. Even so, an 11-year, large, population-based study found no cases of GBS among 107 persons vaccinated who had had a previous case of GBS, including two with previous vaccine-associated cases.[693]

In 2010, a high rate of febrile seizures (nine cases per 100 children vaccinated) was observed among young children who had received a southern hemisphere IIV product.[694,695] Febrile seizures had not previously been associated with IIV.[670–672] After safety signals were observed for febrile seizures from surveillance among U.S. children receiving IIV during the 2010–11 influenza season,[696,697] an increased risk of less than one case per 1000 children vaccinated was detected from further studies in 2010–11 and 2011–12.[697,698] The increased risk was among children 6 months through 4 years of age within 1 day of vaccination; no risk was observed for children older than 4 years of age. The risk was higher when children received concomitant pneumococcal conjugate vaccine (PCV13) (i.e., when the two vaccines are given at the same healthcare visit) and peaked at approximately 16 months of age. No increased risk was observed in the 2012–13 influenza season. It was determined by the CDC's Advisory Committee on Immunization Practices that the benefits of influenza vaccine were greater than the risk of febrile seizures, and no change in policy was recommended.[699]

Other neurologic diseases have been reported following inactivated influenza vaccination, including optic neuritis, transverse myelitis, Bell palsy, multiple sclerosis, and tics. However, a causal association between receipt of vaccine and these diseases has not been established.[666,677] Narcolepsy has been associated with an adjuvanted monovalent pandemic H1N1 vaccine that was used in 2009–10. Following pandemic vaccination programs conducted in late 2009 and 2010, cases of narcolepsy among recipients of a monovalent AS03-adjuvanted H1N1 vaccine were reported, with the risk greatest among children.[700] Epidemiologic studies conducted in several European countries where this vaccine was used revealed a consistent and strong association with receipt of the vaccine.[700–704] The attributable risk was estimated to be between one in 57,500 and 52,000 vaccine recipients in the United Kingdom,[701] and one in 16,000 in Finland.[702] A similar vaccine using AS03 adjuvant produced in Canada was possibly associated with a much lower risk of approximately one extra case of narcolepsy per 1 million vaccinees; although the association in this study could not rule out that the association was confounded by H1N1 seasonality.[705] No association has been noted with other unadjuvanted H1N1 vaccines, nor with pandemic vaccines that used the oil-in-water adjuvant MF59.[706,707] No association has been noted between seasonal influenza vaccines and narcolepsy. Although the mechanism for the risk is not fully known, some data indicate that autoantibodies to hypocretin or human brain ganglioside proteins may play a role in genetically predisposed hosts.[708–710] A recent study found that antibodies developed against the influenza NP in the vaccine associated with narcolepsy cross-react with hypocretin, and that the NP content in the vaccine was higher than that in other adjuvanted pandemic vaccines, possibly explaining the association with a specific product.[708]

Oculorespiratory syndrome (ORS), an uncommon, acute, self-limited reaction to IIV, was first reported during the 2000–01 influenza season in Canada.[711] Affected patients reported red eyes, cough, wheeze, chest tightness, difficulty breathing, sore throat, or facial swelling[712] that began between 2 and 24 hours after receiving vaccine and resolved within 48 hours. In the initial outbreak, ORS was associated with a particular vaccine preparation,[712] and following manufacturing changes in this vaccine, the rates of ORS reports declined substantially.[713] However, other clusters since this episode have been reported.[714,715] Studies suggest that the reaction is not IgE mediated,[716] although symptoms reported have some similarities with immediate hypersensitivity reactions. The risk for ORS recurrence with subsequent vaccination appears to be low,[717,718] but guidance on revaccinating persons with suspected ORS are available.[698]

Special Populations

Pregnant Women. Inactivated vaccines have been found to be comparably safe among pregnant women compared with healthy adults; no special safety concerns have been identified.[78,719,720] No differences in local or mild systemic adverse events or pregnancy outcomes have been noted in this population.[721] Reviews of studies pertaining to seasonal and

monovalent 2009(H1N1) have demonstrated no evidence of harm to the fetus from receipt of IIVs in pregnancy; however, data on safety of influenza vaccination on the fetus when given in the first trimester is somewhat limited.[640,719,720,722–727] A case-control study conducted in healthcare organizations detected no significant increase in the risk of pregnancy loss in the 4 weeks following seasonal influenza vaccination.[728] Similarly, although no increased risk of fetal death was associated with vaccination of pregnant women with pandemic H1N1 vaccine in Norway, researchers did note an association with pandemic 2009(H1N1) infection and fetal loss.[729] Between 2000 and 2003, 20 adverse events among women who received IIV3 were reported to VAERS during a period when an estimated 2 million pregnant women were vaccinated; these included nine injection-site reactions, eight systemic reactions (e.g., fever, headache, and myalgia), and three miscarriages.[730]

Immunocompromised Persons. Available data on the safety of IIV among HIV-infected persons demonstrate no evidence of a clinically significant impact on HIV infection or immunosuppression.[698] While early studies suggested transient increases in replication of HIV-1 in the plasma or peripheral blood mononuclear cells of HIV-infected persons following vaccination,[731–733] more recent studies with superior designs have not documented a significant increase in HIV replication,[734–737] change in $CD4^+$ T-lymphocyte cell counts, or progression of HIV disease after IIV receipt among HIV-infected persons compared with unvaccinated HIV-infected persons.[498,738] Data are limited regarding the safety of IIV among persons with other immunocompromising conditions. IIV vaccination did not affect allograft function or cause rejection episodes in small numbers of recipients of kidney transplants,[508,739] heart transplants,[506] or liver transplants.[510,740] Reports of corneal graft rejection after IIV have been made, but a causal association is not clear.[741–743]

Indications for Vaccine

Influenza vaccination is indicated for anyone 6 months of age or older who wishes to reduce the risk of influenza and its complications.[355] In most countries, national policies focus recommendations on either persons at elevated risk of severe influenza or complications should they become infected, or persons who are at risk of transmitting influenza to high-risk persons in the workplace or at home (Table 31.3).[12,245,744,744a] Policies that target high-risk groups focus on persons at each end of the age range (frequently children younger than 5 years of age and persons older than 60 or 65 years of age), those with specific underlying diseases found to increase the risk of complications, either from influenza or from the underlying diseases, and pregnant women. In addition, many countries recommend vaccines for healthcare workers to prevent transmission from infected workers to patients in their care with high-risk conditions. Although the magnitude of this risk has been difficult to measure, healthcare worker–to–patient infections have been documented.[745–747] In 2012, WHO's Strategic Advisory Group of Experts (SAGE) issued recommendations for influenza that focused on five target groups: children younger than 5 years of age (especially those younger than 2 years of age), elderly persons, persons with underlying diseases, pregnant women, and healthcare workers.[245] In these recommendations, WHO recommended pregnant women be vaccinated as the highest priority, given the finding that they are at high risk of complications and hospitalization, the finding that the vaccine is safe, data indicating that vaccination may also protect their young infant from influenza (an age during which vaccine is not approved for administration), and the finding that vaccination of pregnant women may be programmatically feasible in many countries compared with some of the other target groups. This recommendation has catalyzed influenza vaccine policy development and vaccine uptake in middle- and low-income countries.[12,748]

TABLE 31.3 Groups at Risk for Severe Influenza or Complications From Influenza Infection

- Children <5 y (especially children <2 y)
- Adults aged ≥65 y
- People who have medical conditions including:
  - Asthma
  - Neurological and neurodevelopmental conditions (including disorders of the brain, spinal cord, peripheral nerve, and muscle such as cerebral palsy, epilepsy [seizure disorders], stroke, intellectual disability [mental retardation], moderate to severe developmental delay, muscular dystrophy, or spinal cord injury)
  - Chronic lung disease (such as chronic obstructive pulmonary disease [COPD] and cystic fibrosis)
  - Heart disease (such as congenital heart disease, congestive heart failure, and coronary artery disease)
  - Blood disorders (such as sickle cell disease)
  - Endocrine disorders (such as diabetes mellitus)
  - Kidney disorders
  - Liver disorders
  - Metabolic disorders (such as inherited metabolic disorders and mitochondrial disorders)
  - Immunocompromising condition resulting from disease or medication (such as people with HIV or AIDS, or cancer, or those on chronic steroids)
  - People <19 y who are receiving long-term aspirin therapy
- People who are morbidly obese
- Residents of long-term care facilities
- Indigenous populations
- Pregnant women

Modified from Centers for Disease Control and Prevention (CDC). Influenza (Flu): People at High Risk of Developing Flu-Related Complications. Available at: http://www.cdc.gov/flu/about/disease/high_risk.htm.

The United States adopted a "universal" vaccination recommendation in 2010,[147] in which all persons older than 6 months of age are recommended to receive annual influenza vaccination.[355] This strategy, which has not been adopted by other countries, was the result of steady expansion of recommendations for target groups, especially between 2000 and 2009, by which time, an estimated 84% of persons in the country were in at least one recommended target group. The universal recommendations recognized that all persons are at risk for influenza and even serious disease, and that a universal recommendation may simplify messages regarding who should be vaccinated, resulting in higher vaccine coverage in high risk groups.[11]

Contraindications and Precautions

IIVs should not be given to persons known to have an anaphylactic hypersensitivity to eggs or egg antigens or to influenza vaccine until they are evaluated by a physician. In addition to egg antigens, it is possible (as with any vaccine) that other components may be sensitizing. Information about vaccine components is located in package inserts from each manufacturer. The contraindication to vaccines among those with anaphylaxis to egg antigens extends to cell-grown vaccines that are produced using egg-grown seed strains.[355] However, recombinant influenza vaccine may be given to persons with known egg allergies since no eggs are used in their production.[355]

Severe allergic reactions to IIVs are rare. While other components of influenza vaccines may be associated with allergic reactions, most attention has been on egg allergies, as most inactivated vaccines use embryonated eggs in production. Many manufacturers measure and report ovalbumin content in their products; those that do provide ovalbumin content report maximum amounts that are equal to or less than 1 μg/0.5 mL dose.[18] While a threshold below which a vaccine would not expected to cause reactions is unknown, vaccines containing as much as 0.7 μg of ovalbumin per 0.5 mL have been tolerated in children.[749,750] A large study that followed 4172 patients with egg allergies found no cases of anaphylaxis associated with influenza vaccination.[751] Although those with a history of anaphylaxis to eggs should not receive influenza vaccine, most persons with egg allergies can safely receive influenza vaccines, if specific safety measures are followed.[355] The CDC recommends that persons with a history of hives associated with egg allergy should receive any licensed vaccine. Persons with more-severe reactions to eggs (e.g., angioedema, respiratory distress, light-headedness, or recurrent emesis) and those who have required epinephrine or another emergency medical intervention, especially if within minutes to hours after egg exposure, are at higher risk of serious systemic or anaphylactic reaction upon reexposure to egg proteins. As a result, this group can also receive any licensed vaccine, but the vaccine should be administered in an inpatient or outpatient medical setting, and be supervised by a health care provider who is able to recognize and manage severe allergic conditions.

The risk-to-benefit ratio of vaccination needs to be assessed carefully for other types of allergic reactions. For example, allergic reactions to thimerosal are characterized by a delayed-type hypersensitivity that most often results in a local inflamed or indurated lesion similar to that seen with positive reactions to tuberculin skin tests used to document infection with tuberculosis.[752] Even though the reaction may progress to vesiculation or ulceration, the reaction is not life threatening and can be managed with application of topical steroids. Current "preservative-free" influenza vaccines may contain trace amounts of thimerosal, but influenza vaccines containing no thimerosal are now available in many countries.

Vial stoppers or syringe plungers used for influenza vaccine can contain latex in the rubber composition, and concerns have been raised about reports of allergic reactions to latex rubber.[753] The contact surfaces of the rubber-containing parts are treated (usually with silicone) to reduce the possible introduction of latex rubber antigens into the vaccine. Nonetheless, latex antigens might still enter influenza vaccine by way of repeated needle insertions through the stoppers of multidose containers, because a needle puncture could entrain small bits of the stopper. In recognition of these concerns, some manufacturers use alternate elastomeric products that do not contain latex, and others are making changes. The risk of exposure and sensitization to latex in vaccines is very low, and this risk needs to be weighed against the potential benefit of vaccine.[754] Information regarding the latex content of container components can be found in the package insert written specifically for each vaccine.

Precautions for vaccination with influenza vaccination include moderate or severe acute illness with or without fever[755] and GBS within 6 weeks following a previous dose of influenza vaccine.

OTHER DIRECTIONS FOR INFLUENZA VIRUS VACCINES

IIVs are very safe and moderately efficacious. However, developing influenza vaccines that provide broader protection against heterotypic strains and more durable protection would substantially improve influenza prevention programs.

Live attenuated (cold-adapted) influenza vaccines are described in detail in Chapter 32. These vaccines were developed to elicit an immune response, including production of secretory IgA antibodies in the mucosal tissues of the respiratory tract that would be similar to the response that develops after natural infection while minimizing the symptoms associated with viral replication. Live attenuated influenza vaccines (LAIVs) have been used extensively in countries of the former Soviet Union since the 1980s. Use of LAIV in the United States has increased gradually since licensure in 2004, and has been particularly useful in immunization programs that focus on providing vaccination in schools and other mass vaccination settings.[11] Recently additional countries have approved the use of LAIVs and incorporated them into their immunization portfolios. Additionally, manufacturers in China, India, and Thailand have developed or are developing LAIVs, which should further expand their use.[387]

The use of mammalian cells to replicate influenza viruses for use in inactivated vaccine might provide several advantages, including the potential availability of a product for use by those who are allergic to eggs or egg proteins. Antigenic selection of viruses more adapted to avian cells often occurs when human influenza viruses are passaged in eggs but not when the same viruses are passaged solely in mammalian tissue culture. Therefore, it is also possible that human influenza viruses replicated only in mammalian tissue cultures will produce vaccines that are antigenically more similar to circulating viruses, increase production yield, and increase vaccine effectiveness.[328,331,375,377] Unlike other vaccines, influenza vaccine generally must be available for administration within 9 to 12 months after identification of a new vaccine virus, so that there is little time for investigating (and documenting) whether the seed virus is free of extraneous agents. The time pressure associated with the production and distribution of influenza vaccine, combined with the lack of field facilities that can maintain tissue cultures under conditions to prevent the introduction of extraneous agents are currently obstacles to using tissue culture isolates in influenza vaccine production. Because antigenic changes occurring in eggs appear to be irreversible, influenza vaccines produced in tissue cultures using seed viruses originally recovered in eggs are not expected to improve vaccine effectiveness. An infrastructure capable of supporting use of influenza viruses passaged only in mammalian tissue cultures is required to realize the full theoretical benefits. Thus far, clinical studies demonstrate that the immunogenicity and effectiveness of cell culture–derived vaccines can equal that of egg-derived vaccines and meet FDA immunogenicity criteria for licensure.[329,756,757] However, there remains uncertainty about whether cell culture–derived vaccines can substantially shorten production time, necessitate changes in potency testing and other licensure requirements, or present an unforeseen risk for vaccine contamination.[328,373]

High-growth influenza virus reassortants play a central role in facilitating the production of large quantities of influenza vaccine. However, it has not always been possible to produce high-growth reassortants with the desired characteristics using classical reassorting procedures, because these procedures rely on coinfection of two different viruses followed by selection of viral clones with specific genotypic and phenotypic traits. Reverse genetics methods provide a powerful tool to improve the ability to produce reassortants with the desired properties.[758,759] Recent reverse genetic systems relying only on transfection of cells with all genes needed to make a complete virus are now available, so that influenza A and influenza B reassortant viruses can be tailored specifically to growth substrates

such as eggs and mammalian tissue cultures. Reassortant viruses using reverse genetics techniques have been necessary for producing influenza A(H5N1) vaccine virus candidates, and these techniques are currently being used in the production of LAIVs.

Other strategies for producing influenza vaccine have taken advantage of recent advances allowing rapid sequencing of influenza virus genes as well as adjuvants and formulation that can deliver antigens for CD8 T-cell induction. For example, vectors containing influenza HA or NA genes have been used to infect tissue cultures in which the viral protein of interest (HA or NA) is produced in massive amount and can be purified for use as a vaccine. Clinical trials of purified HA or NA vaccines produced by using a baculovirus vector have demonstrated that such preparations can be standardized to produce immunogenic vaccines with reactogenicity profiles similar to the profiles of vaccines produced by viral replication.[337–339] Clinical studies demonstrated that baculovirus vector vaccines produce a similar immunogenicity profile as subvirion vaccines in adults,[340,760] and was approved by the FDA for adults 18 through 49 years of age.[761] The immunogenicity of Flublok was significantly lower compared to standard preparations in young children, although the immunogenicity and seroconversion and seroprotection rates were not inferior to egg-derived split vaccine in those 65 years of age and older.[452,762]

Noninfectious VLPs, consisting of key antigens such as HA and NA expressed in recombinant viral vectors and contained within particles expressed in insect or plant cell cultures that resemble wild-type viruses, have been shown to be immunogenic in animal and Phase I and Phase II clinical studies.[328,763–771] In healthy adults, pH1N1 VLPs without an adjuvant met all primary and secondary end points and showed seroconversion in 62.2% and 70.3% of participants, respectively, on day 21 and day 42. However, VLPs with Alhydrogel adjuvant showed seroconversion rates of 25.6% and 51.2% on days 21 and 42, respectively, and the reasons for the suppression could be because of altered HA antigen structure postadsorption or poor stability of HA with Alhydrogel.[772] Similarly, 2009pH1N1 VLP vaccine without an adjuvant induced seroconversion in 79% and 85% of recipients on days 14 and 36 postvaccination, respectively.[773] While these data suggest that VLPs vaccines may not require adjuvants to enhance their immunogenicity, results from a recent study with H7N9 VLPs indicate the requirement for an adjuvant as seroconversion rates of 64.9% and 80.6% were achieved with 5 μg of antigen together with 30 or 60 units of immunostimulating complex (ISCOM) Matrix on day 35 when compared to 9.5% seroconversion rate achieved with 45 μg of antigen alone in healthy adults.[774] Results from a Phase II dose-ranging clinical study assessing safety and immunogenicity of a quadrivalent VLP seasonal vaccine as compared to a standard IIV3 are awaited.

DNA vaccines, which are based on plasmids grown in bacteria containing the relevant viral gene(s), also have been investigated. Unlike the mammalian DNA, bacterial DNA contains unmethylated cytosine phosphate guanine (CpG) motifs which are known to stimulate the mammalian innate immune system via Toll-like receptor (TLR) 9. In animal models, DNA vaccines can stimulate both humoral and cellular immune mechanisms.[775] Although DNA vaccines could be relatively easy and inexpensive to produce and have been shown to have protective effects in animal models[775,776] and one small clinical study, progress has been slow on DNA vaccines for humans, in part because the immune response in humans has been relatively poor.[777,778] Specific CpG motifs in plasmid DNA have differential effects on the immune response, but additional work is needed to identify optimal motifs for immunogenic and protective effects in humans.[779–782] In a double-blind, randomized, placebo-controlled Phase Ib clinical trial, healthy adult subjects received 4 μg of trivalent influenza DNA vaccine and 2 μg of trivalent influenza DNA vaccine plus DNA-encoded heat labile toxin from *Escherichia coli* or placebo by epidermal route with a device. The subjects were then challenged on day 56 postvaccination with a H3N2 virus and were monitored for virus shedding by quantitative polymerase chain reaction (qPCR). The vaccine efficacy for the 4-μg dose was 41% and 53% for any illness with or without fever and upper respiratory tract infection, respectively.[778] In a double-blind, placebo-controlled Phase I study, healthy adults received two intramuscular injections of Vaxfectin-adjuvanted H5 HA or H5HA,NP and M plasmid DNA vaccine or placebo 21 days apart, either with a needle or with a needle-free device. No consistent enhancement with a needle-free device was observed. Seroconversion occurred in 47% to 67% of subjects reaching HI titers equal to or greater than 40. H5-specific T-cell responses were also detected in 75% to 100% of vaccine recipients.[783] Although these data are encouraging, improvements in plasmid vector design for enhanced expression in vivo, as well as efficient delivery methods with formulations, will take DNA vaccine strategies forward.

Replication-incompetent and replication-competent human and nonhuman adenoviral vectors have been investigated as an egg-independent strategy for seasonal and pandemic influenza in preclinical and Phase I/II clinical studies.[439,784–791] Adenovirus-vectored HIV, cancer, and Ebola vaccines have shown excellent safety profiles in human clinical trials.[786,792–794] This approach either alone or as a prime-boost strategy offers several advantages as they can be grown to very high titers in vaccine-certified cell lines, can be delivered by parenteral, mucosal or transdermal routes, and overcome poor immunogenicity of antigens.[439,785,793] In addition, HA and NA from multiple strains can be delivered with a single vector to generate a broader immune response against multiple strains.[795] Unlike the conventional split or recombinant protein antigens, adenovirus-vectored vaccines induce effective humoral and cellular immune responses against the antigens of interest. Hence, adenovirus-vectored influenza vaccines containing conserved internal proteins or epitopes is a potential approach for developing universal influenza vaccines. Preexisting adenovirus-vector immunity can be overcome by alternate delivery routes or use of nonhuman adenovirus-vectors.[790,793] A recent Phase I clinical study with a replication competent adenovirus-vectored vaccine delivered orally primed vaccinees for a subsequent boost with a H5N1 vaccine; this prime-boost approach induced broadly cross-reactive humoral and cellular immune responses against other clades.[786,796]

"Universal" Vaccines

The diversity of influenza virus subtypes and the time pressures for production of vaccines based on HA content have prompted a renewed effort to develop vaccines with broad protective effects by way of innate and adaptive immune responses against the conserved influenza viral proteins. Approaches to develop universal influenza vaccines primarily concentrated on determining the conserved proteins/epitopes of influenza that are accessible to immune recognition so that humoral and/or cellular immune response can confer protection against severe disease and death.

Universal influenza vaccines in development include those targeting the influenza NP, M1, and HA proteins.[145,328,797] The ectodomain of M2 (M2e) is very highly conserved among influenza A strains. It has been shown that passive transfer of monoclonal antibody against M2 reduced viral replication

against challenge in mice but did not prevent infection.[798] In subsequent preclinical studies, mice immunized with M2e with hepatitis B core protein as a particle or synthetic peptides or VLPs with or without adjuvant induced antibody responses against M2e and protected mice against death, but not from infection and illness in virus-challenge models.[799–802] Because M2e is expressed in very low amounts on the surface of viral particles but is abundantly expressed in virus-infected cells, M2e vaccines may not be able to prevent infection, but could aid in reducing morbidity as well as assist in viral clearance via antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-mediated death mechanisms. Indeed, a requirement for Fc receptors and alveolar macrophages for M2e antibody-mediated protection has been reported.[803] In a Phase I clinical trial, participants received two doses, on days 0 and 28, of 0.3, 1.0, and 3 μg of recombinant fusion protein consisting of four tandem copies of M2e attached to flagellin, a TLR5 ligand that is known to function as an adjuvant. All these doses were found to be safe and well tolerated, although the 10 μg dose of candidate vaccine tested originally was found to be reactogenic. After one dose, the seroconversion rates (fourfold increase) ranged from 63% to 72% in subjects who received 0.1 or 1 μg of antigen, which increased to 75% and 100% after two doses, respectively.[804] However, it is not clear if the antibody levels induced are protective.

Influenza NP protein is well conserved and NP-specific CD8 T-cell responses cannot prevent virus infection, but do aid in viral clearance and death.[805] In a number of preclinical studies, NP– DNA vaccine, Adeno-NP, or modified vaccinia virus (MVA)-NP generated NP-specific CD8 T-cell responses that conferred protection against challenge with heterosubtypic viruses.[806–814] In a series of Phase I clinical trials both in healthy adults and older adults, MVA-based influenza vaccine candidates expressing the internal proteins NP+M1 were shown to be safe and well tolerated and to induce/recall potent CD8 T-cell responses.[815,816] When challenged with A/Wisconsin/67/2005 virus, 2 of 11 vaccinees and 5 of 11 control subjects developed influenza symptoms and virus shedding; however, the symptoms were moderate and of shorter duration in vaccinees when compared to the control subjects.[817] Furthermore, in both preclinical and Phase I clinical studies, coadministration of MVA-NP+M1 with seasonal influenza vaccine enhanced antibody responses to the seasonal vaccine and enhanced CD8 T-cell responses.[818,819] In a preclinical study, mice were immunized with an MVA-vector expressing NP, an internal protein that is well conserved among influenza A viruses, the stem region of HA, matrix proteins 1 and 2, the viral polymerase basic protein 1, or the HA stem fused to a quadrivalent matrix protein 2 extracellular domain. When challenged with a lethal dose of H5N1, H7N1, or H9N2 viruses, only MVA-NP conferred significant protection, which correlated with the induction of CD4 and CD8 T-cell responses. Other conserved proteins did not provide substantial protection when administered alone.[820]

Synthetic recombinant peptide vaccines[821–823] consisting of conserved sequences from HA, NP, and M1, which were delivered with adjuvants such as flagellin, as monomers or multimers, induced cross-reactive antibody and T-cell responses and conferred protection against heterosubtypic challenge in animal models.[821–826] In a Phase I clinical study, 60 subjects received either 250 or 500 μg of synthetic recombinant vaccine containing conserved linear epitopes from the HA, NP, and M1 proteins of influenza type A and influenza type B strains twice at 21-day intervals with or without Montanide, a mineral oil–based adjuvant. The vaccine was well tolerated and sera from vaccinees lysed MDCK cells infected with several strains of influenza viruses in the presence of rabbit complement, indicating that the sera from vaccinees bound to the viral antigens expressed on the infected MDCK cells that were subsequently lysed through complement-mediated death. Furthermore, vaccine-specific T-cell proliferative responses and cytokine secretion was observed in peripheral blood mononuclear cells from vaccinees.[827] In a subsequent randomized, placebo-controlled study, the priming ability of this vaccine in the induction of immune response to IIV3 was evaluated in 120 older-adult participants. Priming with synthetic recombinant vaccine with or without alum resulted in enhanced seroconversion for all three IIV3 strains as well as for strains not contained in the IIV3, compared to priming with placebo. In addition, synthetic recombinant vaccine induced significant levels of T-cell responses that were further augmented following incubation in vitro with IIV3.[828] These findings suggest that this vaccine candidate has the potential to be used as a standalone universal influenza vaccine as well as together with IIV3 to broaden immune responses against several influenza viruses of different HA types in addition to the three strains contained in the vaccine, as the vaccine candidate contains conserved common linear influenza epitopes of both A and B viruses.

Unlike the globular head domain (mainly HA1) of HA which is subject to antigenic drift, the stalk domain, consisting of a part of HA1 and all of HA2 is highly conserved. Furthermore, antibodies with specificity against this region have shown crossreactivity against several heterologous HAs.[145,311,474,829] Passive transfer of these antibodies conferred protection against lethal challenge in animal models.[311,830] These antibodies may function by preventing membrane fusion and through ADCC activity and complement-mediated lysis. Hence, this region has attracted attention for developing a universal influenza vaccine. Indeed, mice immunized twice with plasmid DNA expressing headless stalk and subsequently boosted with VLPs containing recombinant stalk domain conferred protection against death in a lethal challenge model. However, the mice did exhibit morbidity, suggesting that antistalk antibodies may not prevent infection, but reduce disease severity.[831] Antistalk antibodies are rarely found in humans and are not generally induced by vaccination.[832] However, antistalk antibodies are found in humans who were vaccinated with 2009pH1N1 or H5N1 vaccine, suggesting that a unique head-and-stalk combination generates antistalk antibodies that are broadly cross-reactive.[833,834] This observation was confirmed in animal studies in which mice were immunized sequentially with unique heads from H9, H6, and H1, but the same stalk from Group 1 HA resulting in stalk-specific antibody responses, which conferred protection against challenge with H5N1 virus that belongs to Group 1 HA.[835] A human clinical trial with influenza vaccines that have chimeric HA from H5 and H8 with the same stalk is currently planned using a prime-boost immunization regimen.[836]

Pandemic Vaccines and Novel Influenza A Vaccines

In addition to annual seasonal influenza vaccine development and production to address the risk of antigenically drifted seasonal viruses, a public health imperative exists to prepare vaccination strategies to address the emergence of novel influenza A viruses with pandemic potential.[837–839] Pandemic vaccine preparedness faces several challenges. First, the inherent unpredictability of influenza viruses together with the need to elicit subtype specific immunity through vaccination, makes creation of inactivated pandemic vaccines in advance of a pandemic risky. While vaccine stockpiles have been proposed and created by several governments that are meant to speed delivery of vaccine to key target groups early in a pandemic, continued evolution of avian and swine influenza A

viruses that pose the greatest threat, make stockpiled vaccine potentially less useful, as the risk of mismatch between vaccine and circulating strains grows over time.[50,840] For instance, stockpiled vaccines prepared using Clade 1, 2.1, 2.2, and 2.3 avian influenza A(H5N1) viruses early following their emergence, are increasingly antigenically different from contemporary H5N1 viruses.[386] This challenge has been addressed both by continual risk assessment and updating of vaccine strains using contemporary strains and by planning for the use of adjuvants to enhance immune responses to potentially heterotypic exposures. A second challenge is the production time required to produce adequate doses of vaccines to meet the rapid spread of pandemic viruses. Because inactivated vaccines require several months to produce, they might have limited utility to prevent illness during the initial wave of disease in many countries. This was evident during the 2009 influenza A(H1N1) pandemic, during which a safe and effective vaccine was rapidly developed, and hundreds of millions of vaccine doses were manufactured and administered within 8 months of the identification of the first human infections with the novel H1N1 virus.[255,390] Even so, most countries experienced the peak of their pandemic wave before the vaccine was widely available. Prepandemic stockpiles of vaccines are one solution to a rapid vaccine response. In addition, streamlining current inactivated vaccine production steps, preparedness plans that accelerate policy, regulatory and distribution hurdles, and new vaccine technologies (e.g., recombinant antigen vaccines) are active areas of work and collaboration between public health and industry partners. Finally, effectively immunizing humans who are immunologically naïve to novel strains requires either higher doses of vaccine, more than one dose, or adjuvants to generate protective immune responses. This challenge has highlighted the need for greater production capacity to meet higher dosing needs and global needs, the value of adjuvant development and approvals, and potentially new vaccine approaches.[386,839]

Finally, the manufacture of vaccines from pathogenic avian influenza viruses by traditional methods is not feasible for safety reasons as well as technical issues. Strategies adopted to overcome these include the use of reverse genetic systems to generate modified recombinant strains,[841] the use of baculovirus-expressed HA or related nonpathogenic avian influenza strains and the use of adjuvants to enhance immunogenicity.[328] Following the emergence of highly pathogenic avian influenza H5N1 viruses in humans in 1997, two vaccine strategies were developed to overcome these limitations. One approach was to use a "surrogate" apathogenic H5 vaccine strain that was antigenically related but not identical to the highly pathogenic avian influenza H5N1 strain, overcoming the need to grow and purify a vaccine under high containment conditions. A surface-antigen vaccine based on the apathogenic A/duck/Singapore/97 (H5N3) virus, which possessed an HA that was antigenically similar to A/Hong Kong/156/97 (H5N1), was administered with or without MF59 adjuvant in two doses of 7.5, 15, or 30 μg of H5 HA given 3 weeks apart.[413] Although both vaccines were well tolerated, the nonadjuvanted vaccine was poorly immunogenic, with only 36% of persons demonstrating a fourfold rise in antibodies after two 30-μg doses of vaccine. Individuals who received the H5N3 vaccine formulated with MF59 adjuvant achieved significantly higher antibody responses with a majority of individuals showing seroconversion to the vaccine strain. Follow-up revaccination 16 months later, using the same vaccine formulation substantially boosted antibody titers in those receiving the adjuvanted, but not the nonadjuvanted, vaccine.[842] Boosting with the adjuvanted 1997 H5N3 vaccine, but not the nonadjuvanted vaccine, also induced cross-reactive antibodies against H5N1 strains isolated from humans in 2004.[843] These results suggested that the use of adjuvants may not only be beneficial for dose-sparing of vaccine, but also for enhancing the cross-reactivity of the antibody response. Another approach relied on production of a baculovirus-expressed purified H5 HA protein based on the HA gene cloned from the prototype H5N1 strain, thus overcoming the need to grow large quantities of virus in eggs. Previous clinical trials had determined that responses to doses of 15 to 45 μg of recombinant H1 and H3 antigens in primed populations were similar to those induced by licensed inactivated vaccines.[844] However, in an unprimed population, even the highest dose (two doses of 90 μg) of the baculovirus-expressed recombinant H5 vaccine elicited seroconversion in only 52% of subjects as measured by a microneutralization assay.[845]

When H5N1 viruses reemerged in humans in 2003–04, a surrogate low pathogenicity H5 subtype virus that was antigenically closely matched with the circulating H5N1 strains was not identified. Therefore, seed viruses for the production of experimental H5N1 vaccines were generated from human H5N1 isolates using the plasmid rescue system, also known as reverse genetics technology.[846] This process had first been used to generate reassortant vaccine candidates based on the HA and NA genes from H5N1 strains isolated from humans in 1997 and either the internal protein genes of the A/Ann Arbor/6/60 *ca* vaccine donor virus[847] or PR8, the high-growth donor for inactivated vaccines.[848] In this process, the H5 HA gene is genetically modified to remove the multibasic amino acid motif associated with pathogenicity for chickens, such that the resulting reassortant vaccine strain is avirulent for experimentally infected chickens, and is attenuated in mammalian species.[849] Neither of the 1997 H5N1 reassortant vaccine candidates was evaluated in humans. A plasmid rescue-derived subvirion vaccine based on A/Vietnam/1203/2004 (H5N1) virus was evaluated in 450 healthy adults receiving two doses of 7.5, 15, 45, or 90 μg of antigen. The frequency of serum antibody response was highest among subjects receiving two doses of 90 μg; 54% of individuals receiving this vaccine dose achieved neutralizing antibody titers of 1 : 40 or greater and did not suffer severe side effects.[48] In another study, an inactivated split vaccine based on A/Vietnam/1194/2004 (H5N1) and administered in two doses of 7.5, 15, and 30 μg, with or without aluminum hydroxide adjuvant, was evaluated in 300 healthy adults. The adjuvanted 30-μg formulation was safe and induced the highest rate of response (67%) after two vaccinations (compared with 52% among persons who received the 30 μg dose without adjuvant).[437]

In a study of a lipid-based adjuvanted H5N1 vaccine, using a recombinant reverse-genetics engineered seed virus, 400 adults 18 to 60 years of age were randomized to receive two doses of either 3.8, 7.5, 15, or 30 μg antigen vaccine with or without the adjuvant. The immune response was substantially higher in the group who received the adjuvant even at the lowest antigen dose; 66% and 86% of those who received the adjuvanted 3.8-μg dose vaccine seroconverted after one and two doses compared with 14% and 22%, respectively, among those who received the nonadjuvanted 3.8-μg dose, and 57% and 65% among those who received the nonadjuvanted 30-μg dose vaccine. However, reactogenicity (arm soreness and redness, fatigue, and headache) were significantly more common in those receiving the adjuvanted vaccine.[850] In clinical studies, humoral responses against the adjuvanted 3.8-μg dose vaccine fulfilled licensure criteria for immunogenicity for influenza vaccines for adults and 3- through 8-year-old children,[851,852] and the 1.9-μg dose was immunogenic in 6- to 23-month-old children.[429] Studies using aluminium or MF59 adjuvanted vaccines have also demonstrated potential for dose sparing and broad immune responses that include antibodies that might provide better cross-protection against heterotypic

viruses compared to unadjuvanted vaccines.[853,854] Adjuvanted vaccines also appear to promote broad and persistent cross-clade immunity, which could make them candidates for a prepandemic vaccine.[855] Taken together, these studies confirmed previous findings suggesting that very high doses of unadjuvanted H5 vaccine are needed to elicit antibody responses in a majority of subjects, and that addition of an adjuvant may allow for some level of dose-sparing, cross-protection against other subtypes, and possibly a reduction in the number of doses required. In addition, persons primed with the adjuvanted vaccine exhibited much better response to a heterologous antigen (different H5N1 clade) when boosted 14 months later.[856] An MF59-adjuvanted H5N1 Clade 1 vaccine used to provide a booster dose to persons who had received an H5N3 Clade 0 vaccine 6 years earlier was able to elicit a substantial increase in antibody levels to both H5N1 Clade 1 and Clade 2.2 antigens. The booster response was higher among subjects who had been primed with the adjuvanted H5N3 vaccine.[857] A recent study found that persons previously vaccinated with a Clade 1 strain who were then boosted with a Clade 2 strain had higher HI antibody titers against the Clade 2 virus than subjects who received two doses of the Clade 2 strain vaccine.[857a]

Currently, two H5N1 vaccines are approved by the FDA. Both were prepared under contracts with the U.S. Department of Health and Human Services for stockpiling in advance of a pandemic. One is an unadjuvanted inactivated vaccine based on a Clade 1 H5N1 virus (A/Vietnam/1203/2004) and is two 90-μg doses 28 days apart.[858] The vaccine elicited both a fourfold or greater rise in antibodies and an HI titer of greater than 1:40 in 43% of recipients compared with 0% among placebo recipients.[858] The second, approved in November 2013, contains 3.75 μg of the HA of the influenza virus strain A/Indonesia/05/2005 (H5N1) and the AS03A adjuvant (11.86 μg tocopherol).[859] The vaccine is given intramuscularly in two doses 21 days apart and approved for persons 18 years of age and older who are at increased risk of exposure to the influenza A virus H5N1 subtype contained in the vaccine. In an immunogenicity study of this vaccine, 92% of subjects had an HI titer of greater than 1:40 and 87% had a fourfold or greater rise in antibodies against the vaccine strain 21 days postvaccination.[860] At 6 months postvaccination, 62% of the subjects had titers of at least 1:40. These results were similar to antibody responses found in nonplacebo controlled trials.[851,861,862] Heterologous responses (to currently circulating strains and strains in vaccine development) vary greatly.[860] The safety data from these studies demonstrated no severe adverse events; the studies were too small to yield data on uncommon disorders such as narcolepsy.[851,860–862] In addition, 29 vaccine candidate viruses have been developed and four more are in development, highlighting the great diversity and continuing evolution of these viruses.[863] Neither approved vaccine is currently recommended for interpandemic use, either to prevent H5-associated disease among exposed persons (e.g., laboratory workers) or as a priming dose for future exposure to similar pandemic viruses. Data on vaccine efficacy are not available for either vaccine. In 2013, WHO's SAGE Working Group on Influenza Vaccines and Immunizations developed global recommendations for use of licensed H5N1 vaccine during interpandemic periods. In these recommendations, vaccine is strongly recommended for laboratory workers involved in certain high-risk activities, such as large-scale production with virus. Vaccine is also recommended for first responders and healthcare workers managing patients, but not recommended for persons who may only potentially come into contact with infectious animals, workers in areas where the virus is enzootic, or the general population.[840]

In addition to H5N1 vaccines, substantial work has focused on H7N9 vaccines following the emergence of this virus as a human threat in 2014 in China. Immunogenicity studies of inactivated vaccines and VLP vaccines with and without adjuvant have been conducted. Like previous avian influenza vaccines, the studies have demonstrated that unadjuvanted vaccines are poorly immunogenic and that two doses are required to maximize immune responses. In a U.S. study, most subjects had a significant immune response, with better immune responses among those that received vaccine with AS04 adjuvant than with MF59.[864] The responses were not dose-dependent and 86% of subjects that received two 3.75-μg doses had a fourfold rise in antibodies against the vaccine strain measured by HI. Similar findings were observed in another multicenter trial in the United States, in which MF59-adjuvanted vaccines generated seroconversion in 59% of participants.[865] Finally, a recent study of a H7N9 VLP vaccine candidate using ISCOMATRIX adjuvant observed seroconversion in more than 60% of subjects, but, again, poor immune responses in the absence of adjuvant.[774]

Avian influenza H9 and other H7 subtypes have also caused disease in humans, as have swine viruses (especially H3N2 variant viruses), leading to vaccine development for several of these strains.[432,866–869] In addition, the 2009 H1N1 pandemic demonstrated that the appearance of antigenic variants of HA subtypes that currently circulate in humans, or subtypes that circulated before a substantial fraction of the current population was born, can cause a pandemic. For example, because H2N2 viruses circulated in humans from 1957 until 1968, individuals born after this time lack immunity to the H2 subtype and are therefore a susceptible population for reintroduction of the H2 subtype.[870] Because of the continually changing and emerging group of zoonotic influenza viruses and the accompanying potential risk to humans, an iterative process to assess the risk associated with novel influenza viruses has been established among global partners, that results in updated candidate vaccine development and vaccine trials.[871] In addition, rapid models to understand the possible impact of novel influenza A vaccines have been developed that will aid in decision making regarding investments and policy.[872] Vaccines will continue to be a central part of global pandemic plans.

PUBLIC HEALTH CONSIDERATIONS

Global Vaccination Efforts

Substantial progress has been made in the past decade toward expansion of the use of seasonal influenza vaccines worldwide. Several factors have contributed to this progress. First, the emergence of avian influenza A(H5N1) viruses in Asia and their westward expansion provided the impetus among countries to develop plans for pandemic vaccine use. These plans have been facilitated by WHO's Global Action Plan for Influenza Vaccines (GAP).[387] The GAP strategic plan was based on the principle that pandemic vaccination implementation must be founded on a robust national seasonal vaccination program. Second, the 2009 pandemic was both an illustration of the progress in national program development and the work still to be done in most countries. Finally, the updating of the WHO SAGE recommendation in 2012 served as a catalyzing force for low-income and middle-income countries.[245] This is evidenced in the Americas, which has observed an expansion of vaccination programs.[12] While fewer low-income countries outside the Americas have begun vaccination, the example of Lao People's Democratic Republic (Laos) in introducing vaccine as a collaboration with the Partnership for Influenza

Vaccine Introduction has provided a possible pathway for other low-income countries.[748]

Further expansion of vaccine use globally will require lower cost or subsidized vaccines, greater data on the value of vaccines in low-income settings, information on the optimal timing of vaccination in tropical and subtropical countries,[872a] data on vaccine performance in some key populations (such as immunocompromised persons), compelling data on cost effectiveness, and methods to overcome programmatic challenges in targeting noninfant populations. New vaccines that need not be given each year and which might elicit more broadly reactive immune responses, will expedite willingness of many countries to expand vaccination.

Vaccination Coverage Levels

Worldwide

Although WHO has recommended influenza vaccination for high-risk groups and healthcare workers in all countries,[245] national programs for influenza vaccination are primarily in place in high-income and middle-income countries. WHO has urged member states with influenza vaccination policies to increase vaccination coverage of all people who are at high risk, setting goals in 2003 that were not reached, including vaccination coverage of elderly people of at least 50% by 2006 and 75% by 2010.[873] Few countries have achieved these goals. Even so, expansion of vaccine target groups and increases in vaccine coverage levels have been made in many countries in the past decade. In Latin America, national influenza immunization programs were present in 35 countries in the region in 2008, compared with 13 countries in 2004.[12] In addition, these countries expanded vaccination to cover pregnant women and children as well as the traditional target groups of the elderly and healthcare workers. Finally, the region used enhanced surveillance data to switch several Central American countries from the Northern hemisphere formulation to Southern hemisphere formulation to better fit the seasonality of influenza in the region. Growth in policies and target groups have also been reported in the Asian Pacific region, although less so among the low-income countries in the region.[16,244]

As countries expand policies for use of seasonal vaccines, global production has increased as well, as have the number of manufacturers who make or plan to make vaccine. Growth of vaccine production capacity was estimated to have increased from 876 million doses of trivalent influenza vaccine in 2009 to 1.42 billion doses in 2011; although substantially fewer (1.029 billion) were produced that year.[874] An industry survey estimated that doses of vaccine distributed increased by 87% between 2004 and 2011.[16] In this study, only 3.7% of doses were distributed in the WHO regions representing many of the low-income countries in Africa, South Asia, and the Middle East. However, a follow-up study demonstrated a slowing expansion of vaccine distribution. Between 2008 and 2013, a 12% increase in doses distributed was observed; during this period, however, a 32.5% decrease in doses distributed was observed in Europe.[875] Nearly all the world's vaccine production capacity is contained in the Western Europe, Western Pacific, and North American WHO regions, but the WHO GAP program might expand production in other regions.[389,390]

Vaccination programs capable of delivering annual influenza vaccination to a broad range of the population could potentially serve as a resilient and sustainable platform for delivering vaccines or other medical assistance, and monitoring outcomes for other urgently required public health interventions. Seasonal vaccination programs have also been promoted as a tool for pandemic preparedness.

United States

In the 2015–16 influenza season, overall vaccine coverage in the United States was estimated to be 46%; 42% among adults and 59% among children 6 months to 18 years of age.[876] Rates of vaccination in the United States have steadily risen in the last decade, particularly among children. Between 2004 and 2012, vaccination rates among children rose from 16% to 47%, with the expansion of vaccination recommendations from young children to all children during this time.[877] Rates are highest among children 6 to 23 months of age, among whom 75% are vaccinated.[876] No gender differences in vaccination rates have been observed in children, but rates remain lower among socioeconomically disadvantaged populations.[876,878]

Among adults, rates are highest among persons 65 years of age and older. Between 1989 and 1999, vaccination rates in this group rose from approximately 33% to 66% and have remained stable except for a small decrease noted during the 2015–16 season.[11] In the 2015–16 influenza season, 63% of this group was vaccinated, compared with 36% of 18- to 64-year-olds.[876] Among nonelderly adults, persons with at least one high-risk condition are more likely to be vaccinated (46%) than those without a high-risk condition (34%), a trend seen in previous years as well.[879] Like children, racial differences in influenza vaccination rates remain.

Vaccination of specific risk groups have made some gains in recent years. Among pregnant women, rates increased from 11% in 2001 to 38% to 50% in 2011, in large part because of the recognition of risk of severe outcomes during the 2009 pandemic.[880] The most recent estimate in this group from the 2014–15 influenza season was 50%[881]; few gains have been made since the pandemic year. Increases in vaccination rates in healthcare workers have been observed in recent years, in some part because of greater access to vaccination in the workplace and to workplace requirements in some healthcare settings. In 2014–15, 77% of healthcare workers were vaccinated, with highest rates among physicians, nurses, and pharmacists.[882]

Efforts to increase vaccine uptake in the United States have included efforts to ensure that persons coming into clinics during influenza season receive an offer of vaccine, use of client reminder/recall systems,[883,884] and expanding access in pharmacies, workplaces, and schools.[885] In addition, communicating the impact of influenza vaccination on reduction of severe disease risk and overall number of hospitalizations may help put the value of influenza vaccine in context.[26]

Disease Control Strategies

In contrast to control efforts for some diseases, the primary goal of influenza vaccination programs is to reduce the number of serious complications, including deaths, resulting from influenza infections. In most countries, the primary focus of prevention strategies has been to vaccinate those at highest risk for complications as well as their close contacts, reflecting knowledge that influenza exerts a disproportionately severe impact on the elderly, those with chronic medical conditions, and the very young. Even in the United States, where all persons 6 months of age and older are recommended to receive vaccine, one rationale for universal recommendations was that it would be simpler to communicate than a risk-based policy, thereby increasing vaccine coverage among vulnerable populations. In addition, until recently there were practical limitations (e.g., vaccine supply and program infrastructure) on trying to vaccinate entire populations annually. As part of pandemic preparedness and also in response to the 2009 H1N1 pandemic, these barriers have been reduced, with

large-scale investments in vaccine development, manufacture, and, to a lesser extent, immunization programs. However, there is increased interest in achieving these goals both through vaccination of persons at risk and by reducing community transmission through increased overall vaccine coverage among all persons. In addition, the identification of newly recognized risk factors for illness (e.g., morbid obesity, aboriginal persons such as Native Americans) and a growing appreciation of the burden of illness among healthy older children and younger adults has led to a recommendation to vaccinate all persons in the United States, and incrementally expanding recommendations in many other countries.

The recommendation to vaccinate persons 6 months of age or older annually in the United States in 2011 has led to some gains in vaccine coverage, and resulting reduction in disease outcomes.[27] Other governments have attempted annual vaccination of large portions of the population, including the Japanese vaccination programs conducted between 1962 and 1987 that focused on immunizing children,[657] and more recent efforts in Ontario, Canada to provide universal influenza vaccination to its population.[660] Although it might be assumed that high vaccine coverage within communities would lead to reduced community transmission and reductions in severe influenza-related outcomes, no large prospective study has yet convincingly demonstrated that vaccinating entire populations, or epidemiologically important subpopulations such as school-age children, will provide significant protection against influenza-related complications (e.g., death or hospitalization) for other groups. Showing significant reductions in influenza-related complications has been difficult because of the yearly variability of influenza epidemiology, the infrequent use of specific laboratory testing that could serve as the source of reliable surveillance data, the need to study a large population to capture enough severe outcomes, and the relatively low coverage achieved in areas where universal vaccination has been recommended.

Eradication or Elimination, If Feasible

Influenza cannot be considered an eradicable disease for several reasons. Most important, avian species are the natural host to influenza A viruses, and certain other animal species, particularly swine, also can support the circulation of some influenza A virus subtypes. Eradicating circulation of influenza A viruses among all animal species capable of hosting them is impossible. Furthermore, the ability of influenza viruses to rapidly change their antigenicity, the need to update vaccines frequently, and the level of effectiveness of influenza vaccine are insurmountable practical barriers to ever administering enough vaccine quickly enough to eliminate influenza virus circulation among human populations.

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32 Influenza Vaccine—Live

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Influenza viruses cause recurrent and widespread disease in humans. Vaccination is the most effective means of preventing influenza infection. The use of inactivated influenza virus vaccine (IIV) in humans has been the subject of numerous studies, with an emphasis on providing an effective vaccine with minimal reactogenicity. An alternative approach to influenza immunization using live attenuated virus administered by nasal spray has been successful. This chapter reviews the research that led to the licensing of the live attenuated influenza vaccine (LAIV) in the United States, and the clinical experience with LAIV since licensure. In this chapter, inactivated influenza vaccines are generally referred to as IIV or in specific instances as trivalent inactivated vaccine (TIV). More extensive background information on influenza can be found in Chapter 31 on inactivated influenza vaccines.

WHY THE DISEASE IS IMPORTANT

Influenza viruses are the most common cause of lower respiratory tract infections. The number of cases of influenza that occur varies depending on the susceptibility of the population to the virus and the infectiousness of the virus during an outbreak. In addition, influenza seasons vary in length and severity. In the United States, an estimated average of 36,000 deaths were associated with influenza epidemics between 1990 and 1999, and approximately 226,000 hospitalizations between 1979 and 2001[1,2]; approximately 90% of the deaths attributed to influenza occurred among persons age 65 years or older.[3,4] In 2010, the Centers for Disease Control and Prevention (CDC) released revised estimates for influenza-associated deaths from 1976 to 2007 that ranged from approximately 3000 to 49,000 per year.[4] Influenza type A infection occurs most frequently and is responsible for the greatest amount of morbidity and mortality. Although influenza B does not cause pandemics, it is responsible for regional epidemics that are generally less severe than influenza A epidemics.

Influenza incidence rates vary with the nature of the epidemic virus strains and the population affected. Rates calculated from surveillance of persons with upper respiratory illness suggest that influenza is responsible for roughly 10% to 20% of all respiratory illnesses per epidemic year,[5,6] with rates for influenza A being somewhat higher than those for influenza B. Most studies have found infection rates in preschool- and school-age children to be much higher than in adults, particularly during influenza B epidemics.[5–8] Consequently, families with school-age or younger children suffer disproportionately from influenza.[7,9] These findings support the ideas that influenza infects persons with the lowest prior immunity and that children are a common source of influenza in the community.

INFLUENZA

Influenza viruses are spread from person to person primarily via coughs and sneezes of infected persons. The incubation period for influenza is 1 to 4 days, with an average of 2 days.[10] Adults and children are typically infectious from the day before symptoms appear, until approximately 5 days after illness onset. Children can be infectious for a longer period, and very young children can shed virus for up to 6 days before symptom onset. Severely immunocompromised persons can shed virus for weeks.

Uncomplicated influenza illness is characterized by the abrupt onset of systemic and respiratory signs and symptoms (e.g., fever, myalgia, headache, severe malaise, nonproductive cough, sore throat, and rhinitis). Respiratory illness caused by influenza is difficult to distinguish from illness caused by other respiratory pathogens on the basis of symptoms alone. Sensitivity and predictive value of clinical definitions can vary, depending on the degree of cocirculation of other respiratory pathogens and the level of influenza activity.

Influenza illness typically resolves after a few days, although cough and malaise can persist for more than 2 weeks. Influenza can exacerbate underlying medical conditions (e.g., pulmonary or cardiac disease); lead to primary influenza viral pneumonia, or to secondary bacterial pneumonia; or occur as a coinfection with other viral or bacterial pathogens. Influenza infection is also associated with encephalopathy, transverse myelitis, Reye syndrome, myositis, myocarditis, and pericarditis.

Multiple mechanisms provide resistance to influenza in humans.[11,12] Acquired immunity to influenza is mediated by serum antibodies, antibodies at mucosal surfaces, and influenza-specific T cells. Antibody alone is sufficient for protection, and T cells play a role in recovery. Innate immune responses, such as production of interferon or other antiviral factors by macrophages, may also contribute to resistance to influenza infection. Immunity after natural infection provides long-lived protection against influenza virus infection and disease in the absence of antigenic shift or drift. This effect was illustrated during the 1977 influenza season, when H1N1 viruses reemerged after a 20-year absence. Epidemiologic surveys revealed that, compared with persons born after 1955, persons born before 1955 had a lower influenza attack rate in 1977. It is presumed that prior exposure to H1N1 strains in the 1950s conferred immunity to the nearly identical strains that appeared in 1977. In 2009, when the swine-origin H1N1 pandemic influenza virus (H1N1pdm09) emerged, older adults who had prior exposure to antigenically related H1N1 influenza viruses were found to have cross-reactive antibodies to the pandemic virus,[13] and disease was not as severe in this age group as in younger adults.[14,15] The H1N1pdm09 strain has replaced previously circulating seasonal H1N1 viruses, so in this chapter, H1N1 viruses isolated after 2009 are referred to as H1N1pdm09. Immunity acquired by natural infection can also provide some protection against influenza strains that have undergone antigenic drift.

VIROLOGY

Because of the complexity of the subject, it is beyond the scope of this chapter to address all aspects of influenza virology. For a comprehensive review of the biology of influenza viruses, the reader is directed to other texts.[16,17]

Orthomyxoviruses have segmented, single-stranded, negative-sense RNA genomes. Influenza A and B viruses have

TABLE 32.1 Products of Influenza A and B Virus Genes

RNA	Gene Products	Functions
1	PB2	Viral polymerase component, binds to cellular 5′ host premessenger RNA cap
2	PB1	Viral polymerase component with RNA transcription and replication activities
	PB1-F2	Viral protein produced by overlapping reading frame with proapoptotic functions
3	PA	Viral polymerase component with RNA and DNA endonuclease activity
4	HA	Virion surface attachment and fusion glycoprotein; major antigenic target
5	NP	Major component of ribonucleoprotein and type-specific antigen
6	NA	Virion surface glycoprotein with sialic acid cleavage activity, major antigenic target
	NB	Glycoprotein membrane ion channel found only in type B influenza
7	M1	Membrane matrix protein and type-specific antigen
	M2	Nonglycosylated membrane ion channel, found only in type A influenza
8	NS1	Viral nonstructural protein that modulates cellular host responses
	NS2/NEP	Nonstructural protein involved in nuclear export of viral RNA

eight RNA segments that encode at least 10 proteins (Table 32.1): hemagglutinin (HA), neuraminidase (NA), three polymerase proteins (PA, PB1, and PB2), nucleoprotein (NP), nonstructural protein 1 (NS1), nuclear export protein (NEP), and matrix proteins (M1 and M2 in influenza A; M1 and BM2 in influenza B). A second open reading frame in the PB1 gene encodes the PB1-F2 protein. The protective antibody response is directed at the two major surface glycoproteins, HA and NA. Influenza A viruses exhibit both antigenic drift[18] and antigenic shift,[19] whereas influenza B viruses show only antigenic drift.[20] Antigenic drift is caused by point mutations in the HA and NA genes that are driven by immune pressure and the infidelity inherent in the replication of RNA genomes. Although not subject to antigenic change, other genes also undergo mutational drift.[20] The rate of drift can vary among genes and viral subtypes.[21–24] Antigenic shift, however, requires complete replacement of one or both of the surface glycoprotein genes and is a consequence of the segmented genome of influenza viruses. During mixed infections, gene segments from two parent viruses can reassort to generate a virus with a novel gene constellation.[25,26] New antigenic changes, occurring by either mechanism, allow the virus to overcome existing immunity in previously infected hosts.

PATHOGENESIS AS IT RELATES TO PREVENTION

The pathology caused by influenza virus has been reviewed extensively.[27–30] Epithelial cells in the upper airway are most likely the primary site of infection.[31] Bronchitis and tracheitis are common,[32] and there may be some lung involvement.[33] Otitis media is common in children. Viremia has been reported only sporadically[34,35] amid numerous negative reports.[36–38] Influenza B demonstrates pathology similar to that of type A and is common in children. Influenza in humans is usually restricted to infection primarily of the upper respiratory tract and trachea, although, during severe influenza, lung lesions may occur.[39] The amount of virus present probably determines the extent and severity of symptoms.[40,41]

The most precise diagnosis of influenza is obtained by reverse transcriptase–polymerase chain reaction (RT-PCR) or by virus isolation in embryonated eggs or in cell cultures of nasopharyngeal or tracheal aspirates. Serologic diagnosis can be made by demonstrating four-fold rises in hemagglutination-inhibiting (HAI) antibodies.

LIVE ATTENUATED VACCINES

The segmented genome of orthomyxoviruses that permits genetic reassortment has been employed to generate vaccine strains containing the genes for the HA and NA of newly emergent wild-type (wt) viruses, while retaining other genes from attenuated strains. Attenuated master donor strains must be shown to not cause significant illness in humans and to reproducibly pass the property of attenuation on to reassortants through genes other than the HA and NA. Approaches to developing live attenuated vaccines for influenza include host-range mutants[42–48] temperature-sensitive (ts) mutants,[49] cold-adapted (ca) mutants,[50] NS1 deletion mutants,[51] M2 cytoplasmic tail mutants,[52] replication-deficient viruses,[53] and codon pair deoptimized influenza viruses,[54] but this chapter will focus on the cold-adapted LAIV that is licensed for prevention of seasonal influenza in the United States and several other countries (see below).

HISTORY AND DEVELOPMENT OF COLD-ADAPTED INTRANASAL LIVE ATTENUATED VACCINE

The ca A/Ann Arbor/6/60 (H2N2) (A/AA ca) and ca B/Ann Arbor/1/66 (B/AA ca) viruses that are the backbones of the LAIV licensed in the United States were developed at the University of Michigan.[50,55–57] The A/AA ca virus was derived in primary chick kidney (PCK) cells from a virus originally isolated from an ill child. This virus was grown at successively lower temperatures in PCK cells followed by seven serial plaque-to-plaque purifications. This virus had the following three properties: ts, which implies that the vaccine virus replication was reduced at the higher temperature (39°C); ca, which implies that the virus grew well at a reduced temperature (25°C) compared with permissive temperature (33°C); and att, which implies attenuated behavior in ferrets. The B/AA ca vaccine donor strain was developed in the same manner, although it took fewer intermediate passages to achieve the final ca variant for type B virus than for type A.[56] The wt B/AA/1/66 influenza virus was restricted for growth at 36°C at the time of isolation, and therefore the ts phenotype is defined by a reduction in replication titer at 37°C compared with the achievable titer at 25°C or 33°C.[55,56] Changes in the nucleotide sequence for all eight genes of the attenuated A/AA ca virus, compared with the nucleotide sequence of its wt counterpart, have been identified. Four major loci at base positions 1195 and 1766 of the PB1 gene, 821 of the PB2 gene, and 146 of the NP gene were identified as determinants of the ts phenotype by reverse genetics. Position 2005 of the PB1 gene was also found to contribute to the ts phenotype.[58,59] The ts, attenuation (att), and ca phenotypes of the B/AA ca donor strain are also conferred by multiple genes: PA and NP for ts; PA, NP, and M for att; and PA, NP, and PB2 for ca.[60–62] The concept underlying the use of ts viruses as live attenuated respiratory virus vaccines is that the vaccine virus will replicate in the

cooler upper respiratory tract and induce immunity but will be restricted in replication and will therefore be attenuated in the warmer, core body temperature of the lower respiratory tract.

The reassortant vaccine viruses included in LAIV were previously generated by classical reassortment, achieved by coinfection with vaccine donor viruses and wt influenza viruses, but they are now derived using plasmid-based reverse genetics as discussed later (Fig. 32.1). The resulting recombinant reassortant virus contains six internal gene segments of the vaccine strain combined with the two antigen-encoding gene segments, HA and NA, and it is referred to as a 6:2 reassortant. Numerous reassortants have been made using the A/AA ca virus and B/AA ca donor viruses, and, in all cases, attenuation, antigenicity, and genetic stability have remained unaltered and consistent.[56,57,63,64] Influenza A and B ca donor strains were also developed for a LAIV in Russia. The current Russian influenza A donor strain, A/Leningrad/134/47/57, contains changes in at least one of the polymerase genes as well as in the HA, NA, NP, and M genes.[65,66]

REVERSE GENETICS

The influenza virus genome comprises eight negative-sense virion (v) RNA segments that cannot function as messenger RNAs (mRNAs) to produce proteins; to initiate replication of the viral genome, viral polymerase proteins carried in with the virion must transcribe mRNAs from the viral gene segments to produce mRNAs. Several systems have been developed that allow recombinant influenza viruses to be produced entirely from cloned plasmid DNAs.[67–69] Hoffman and colleagues further refined these techniques and developed an eight-plasmid system.[68] In this method, each viral complementary (c) DNA is inserted into a dual-promoter bidirectional plasmid

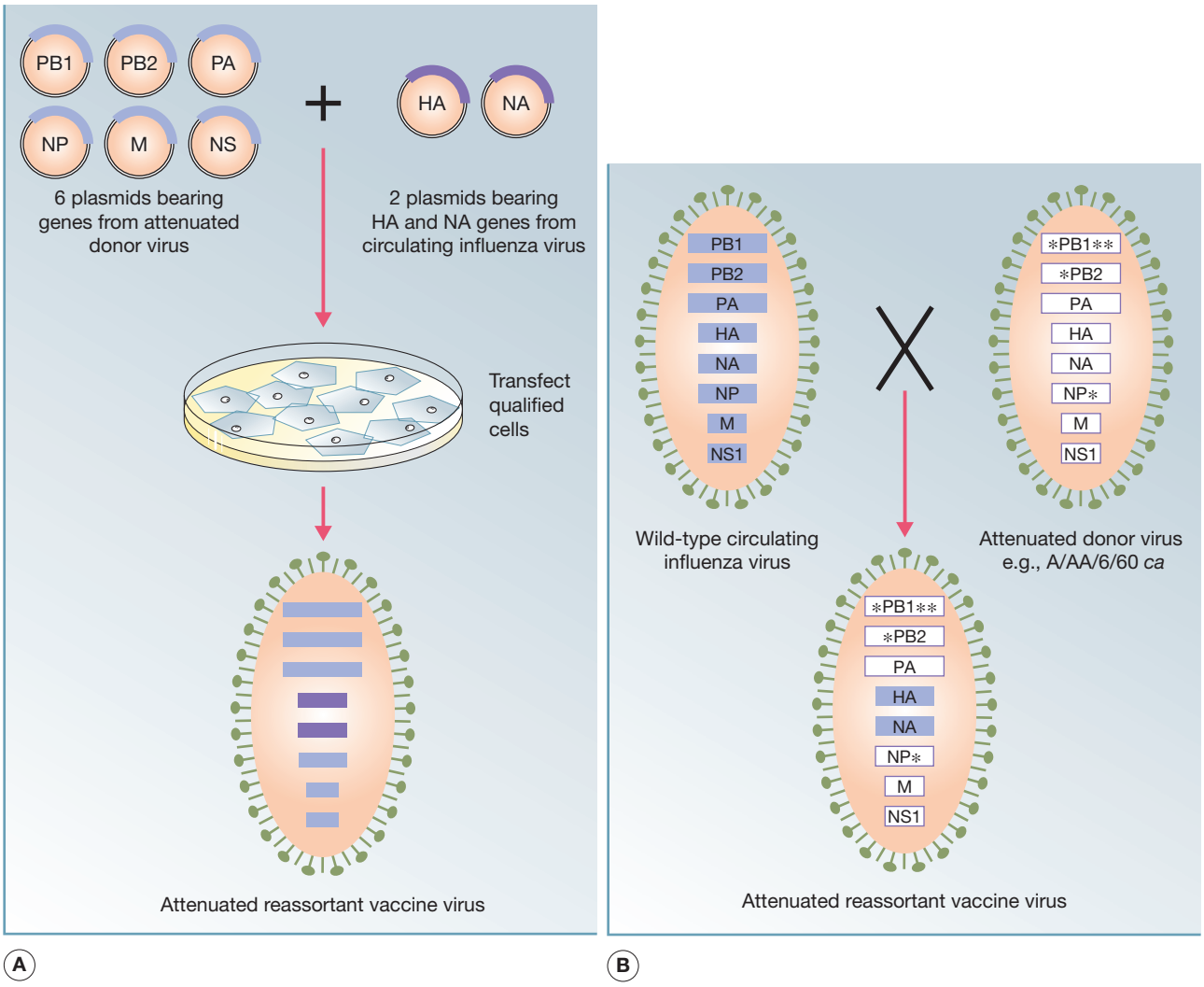


Figure 32.1. A, The eight-plasmid reverse-genetics system to generate recombinant live attenuated influenza vaccines. Six plasmids encoding the internal protein genes of the attenuated donor virus (three polymerase proteins [PA, PB1, and PB2], nucleoprotein [NP], nonstructural protein [NS], and matrix protein [M]) are mixed with two plasmids encoding the hemagglutinin (HA) and neuraminidase (NA) genes of the circulating influenza virus. Qualified cells are transfected with the plasmids, and the attenuated reassortant virus is isolated. **B,** Generation of live attenuated influenza vaccine viruses with the six internal protein genes from the attenuated donor viruses (the five major attenuating mutations for the A/AA cold-adapted [ca] donor virus are indicated by an asterisk), and the HA and NA genes from the circulating influenza virus by classical reassortment. The 6:2 reassortants generated by this method are selected in the presence of antiserum specific for the HA and NA of the attenuated donor virus. *(Modified from Luke CJ, Subbarao K. Vaccines for pandemic influenza.* Emerg Infect Dis. *2006;12:66–72.)*

where the viral gene is flanked by RNA polymerase I (pol I) and RNA polymerase II (pol II) promoters. Thus, eight negative-sense viral RNAs and positive-sense mRNAs are generated from the eight plasmids.

This system is now used routinely to generate the seed virus for seasonal LAIV in the United States. The HA and NA gene segments of the predicted epidemic strain are cotransfected into a cell with plasmid DNAs encoding the other six gene segments of the vaccine donor strain such as A/AA ca (see Fig. 32.1A). The resulting recombinant virus contains six internal gene segments of the vaccine strain, combined with the two gene segments encoding the surface glycoproteins, HA and NA. Another application of reverse genetics is exemplified by the construction of live attenuated pandemic vaccine viruses by plasmid rescue.[70,71] Virulence determinants, such as the polybasic cleavage signal between the HA1 and HA2 subunits of the H5 HA, can be modified or removed by recombinant DNA techniques.[72] Plasmid rescue techniques have advanced basic research on influenza virus as well as vaccine development.

CHARACTERISTICS AND FORMULATIONS OF LIVE ATTENUATED INFLUENZA VACCINE

LAIVs based on the A/AA ca and B/AA ca master donor strains were first licensed in the United States in 2003[73] as a trivalent vaccine. The need for a quadrivalent seasonal influenza vaccine to provide coverage against the two lineages of influenza B that circulate in the human population was a matter of debate for several years. The influenza B epidemic strain was difficult to predict, and there was often a mismatch between the vaccine strain and the circulating influenza B virus. For example, between 2001 and 2010, the influenza B component of the vaccine was of the correct lineage five out of the 10 influenza seasons.[74] Studies by the CDC determined that production of quadrivalent seasonal influenza vaccines would be possible, and would have added benefit in protection against influenza B disease.[75] The clinical trials conducted with the trivalent formulation of LAIV also supported licensure of a quadrivalent LAIV (Q/LAIV), because both vaccines were manufactured using the same process, the dose of each of the component strains is the same, and both formulations are administered by the same route, in the same diluent. In addition to the clinical experience with trivalent LAIV, bridging noninferiority clinical trials were conducted in support of licensure of Q/LAIV.[76,77] Q/LAIV had a similar safety profile to the trivalent LAIV in adults and children and was shown to be noninferior to trivalent LAIV in terms of immunogenicity (see "Noninferiority Studies to Support Licensure of Quadrivalent LAIV" below). In early 2012, Q/LAIV was approved by the U.S. Food and Drug Administration (FDA). Q/LAIV, marketed in the United States as FluMist Quadrivalent, was available for the first time in the 2013–14 influenza season, and contains two influenza B components, so that viruses representative of both the B/Victoria and B/Yamagata lineages are included. Q/LAIV contains $10^{6.5}$ to $10^{7.5}$ focus forming units (FFU) of the four component viruses and is administered in the same manner and volume as the trivalent LAIV. Q/LAIV has replaced trivalent LAIV. Q/LAIV is manufactured and marketed by MedImmune in the United States. It is also licensed in Canada, the United Kingdom, Austria, Finland, Germany, Norway, Spain, Sweden, and Israel. In Canada, Q/LAIV is marketed as FluMist Quadrivalent; elsewhere it is marketed under the trade name Fluenz Tetra.

LAIV is sprayed into the nose using a simple syringe-like device that delivers a 0.1-mL volume of a large-particle aerosol into each nostril for a total volume of 0.2 mL (Fig. 32.2). Vaccination of children requires minimal cooperation. The device is easy to use, and the vaccine is readily accepted and preferable to many over a parenteral injection by needle and syringe. Studies demonstrated self-administration of LAIV was successful, and may be an option for situations where mass immunization is necessary.[78,79]



Figure 32.2. Transilluminated illustration of large-particle aerosol generated for intranasal administration. The tip of the applicator is inserted into the anterior nares, and the plunger is depressed to administer the live attenuated vaccine to a nostril. Removing the flange on the plunger allows a second spray to be administered into the other nostril.

The component influenza virus strains in LAIV are updated each year as recommended by national health authorities. Monovalent bulk vaccine is produced for each reassortant 6 : 2 vaccine strain in specific pathogen–free hen's eggs. The allantoic fluids are clarified by centrifugation and stabilized by the addition of stabilizing buffer: each 0.2-mL dose contains 0.188 mg/dose monosodium glutamate, 2.00 mg/dose hydrolyzed porcine gelatin, 2.42 mg/dose arginine, 13.68 mg/dose sucrose, 2.26 mg/dose dibasic potassium phosphate, and 0.96 mg/dose monobasic potassium phosphate. Each dose contains residual amounts of ovalbumin (<0.24 μg/dose) and may also contain residual amounts of gentamicin sulfate (<0.015 μg/mL) and ethylenediaminetetraacetic acid (EDTA) (<0.37 μg/dose). It is free of preservatives; however, because the vaccine is produced in specific pathogen-free hen's eggs, it should not be administered to persons with egg allergies. Monovalent bulk preparations of each of the four strains in the formulation are combined along with the stabilizing buffer to achieve a titer of $10^{6.5}$ to $10^{7.5}$ FFU/dose and filled into the sprayer device. FluMist Quadrivalent is a refrigerator-stable (i.e., 2 °C to 8 °C) formulation of Q/LAIV. The shelf life of Q/LAIV is 18 weeks.

Genetic and Phenotypic Stability of Live Attenuated Influenza Vaccine

LAIV strains are genetically and phenotypically stable during the vaccine manufacturing process and after replication in humans.

Stability During Vaccine Manufacture

The low-fidelity RNA-dependent RNA polymerase of influenza viruses results in an estimated error rate of 10^{-4} to 10^{-5} misincorporations per nucleotide position per genome replication.[80] Because the manufacturing process of LAIV requires sequential amplification of virus seeds in embryonated hen's eggs, it is possible that mutations that alter the biological properties of the vaccine virus could accumulate, possibly resulting in loss of attenuation or change in antigenicity.

Analysis of the genetic sequence of monovalent ca LAIV revealed a high level of genetic homogeneity in vaccine viruses at several stages of the manufacturing process.[81] A total of six nucleotide changes resulting in amino acid substitutions were identified in the internal protein genes of four influenza A and five influenza B vaccine viruses compared with their respective ca donor viruses, and these changes were generally found to occur in the manufacturing steps upstream of amplification in eggs—that is, during reassortment and clonal isolation. None of the changes occurred at positions that confer the ts and att phenotypes of the cold-adapted viruses.[58–60,62] A total of 14 nucleotide changes were observed in the HA and NA genes of the four influenza A and five influenza B vaccine viruses compared with their respective wt parent viruses (one to two changes per virus for six of the viruses), but the antigenic properties of the HA were not affected.

Genotypic and Phenotypic Stability After Replication in Humans

During the early evaluation of LAIV, the stability of the ts and ca phenotypes were monitored as surrogates for genetic stability. As sequencing technology advanced and the genetic bases for the phenotypes of the LAIV donor virus were determined, genetic stability and phenotypic stability were evaluated in virus isolates from vaccine recipients. The ts and ca phenotypes of several monovalent AA ca-based LAIV were found to be stable after replication in humans.[82] In one study, an AA ca reassortant virus was recovered that had partially lost its ts phenotype after replication in children, but the virus retained the att phenotype in ferrets.[83]

Genetic stability of the trivalent ca influenza virus vaccine, containing ca influenza A H1N1, H3N2, and ca influenza B reassortant viruses, was reported by Cha and colleagues.[84] Virus isolates from nasal and throat swab specimens obtained from 17 children after vaccination were subtyped by multiplex RT-PCR and genotyped. All viruses detected during the 2-week postvaccination period were vaccine viruses, and all maintained the 6:2 reassortant genotype, as well as the ca and ts phenotypes.

Two studies of the genetic and phenotypic stability of trivalent LAIV in children were undertaken in a daycare setting. In one study of children younger than 3 years, it was found that the ts and ca phenotypes were preserved in all recovered viruses tested ($n = 135$).[85] One placebo recipient in this study became infected with an influenza B virus that was a component of the LAIV. This virus retained the ts, ca, and att phenotypes of the vaccine strain and had the same genetic sequence as the influenza B vaccine virus isolated from a vaccine recipient in the same playgroup. In another study, Buonagurio and colleagues[86] examined 56 independent isolates obtained from children after vaccination. Between one and seven nucleotide changes per virus were observed in 80% of isolates compared with the vaccine viruses, but none resulted in changes in the ts, ca, and att phenotypes.

Safety of Live Attenuated Influenza Vaccine

In the United States, LAIV is approved for use in healthy adults and children from 2 to 49 years of age who do not have a history of egg allergies or asthma. Numerous studies have been performed before and after licensure to evaluate the safety of LAIV vaccine in both children and adults. These studies assessed the risk of fever, runny nose and congestion, cough, bronchitis, headache, fatigue, vomiting, and serious adverse events (SAEs) that resulted in hospitalization. LAIV is generally well tolerated in adults, and only runny nose and congestion have been observed at a greater frequency in LAIV recipients than in trivalent inactivated influenza vaccine (TIV) or placebo recipients.[87] Children had a significant increase in runny nose, congestion, and fever after one dose of LAIV, but these symptoms did not occur as often following the second dose.[88–95]

Some studies also reported an increased rate in children vaccinated with LAIV compared with placebo, of SAEs such as wheezing, upper respiratory tract infections, and increased hospitalization, that are likely to have resulted from vaccination.[88,90,91] Although the rates of some of these events were not statistically different between vaccinated children and placebo recipients, a post hoc analysis of one study determined that the reported SAEs occurred exclusively in children younger than 24 months of age.[88] The vaccine is known to be safe in 2- to 17-year-old children, and is not licensed for use in children younger than 2 years of age.[96]

Postlicensure analyses of all adverse events (AEs) reported to the U.S. Vaccine Adverse Event Reporting System (VAERS) during the 2003–04 and the 2004–05 influenza seasons[97] and for the seasons from 2005 to 2013[98,99] have been reported for trivalent LAIV. In the first two postlicensure seasons, approximately 2,500,000 persons received trivalent LAIV, and VAERS received 460 AE reports.[97] No fatalities were reported. There were seven reports of possible anaphylaxis, two reports of Guillain-Barré syndrome (GBS), one report of Bell palsy, and eight reports of asthma exacerbation among persons with a prior history of asthma. Events in persons for whom the vaccine was not indicated accounted for 73 reports (16%). Between 2005 and 2013, approximately 50 million doses of trivalent LAIV were distributed for use in adults and children.[98] Separate reviews of reports to VAERS were conducted for adults[99] and children.[98] Available safety data were limited, particularly for adults. Over eight influenza seasons, 1207 events were reported to VAERS for adults of the approved age range of 18 to 49 years, of which 8.9% were serious. Nine deaths were reported. Deaths and nonfatal SAEs were only reported in the U.S. Department of Defense population, which includes military personnel and Department of Defense beneficiaries treated at military clinics or hospitals. The most common categories of reported serious nonfatal events were neurological (39%), cardiovascular (14%), or other noninfectious conditions (19%). A higher rate of GBS and cardiovascular events were reported in the Department of Defense versus the civilian population. This finding is the subject of further investigation. The most commonly reported AEs were not serious in nature: headache, fever, and nausea. There were 207 reports of the administration of expired vaccine, 155 reports of administration of vaccine to adults 50 years of age and older, and some reports of inadvertent administration to pregnant women, none of which resulted in a SAE. Over the seven influenza seasons between 2005 and 2012, there were 2619 AE reports to VAERS associated with administration of trivalent LAIV in children, of which 7.5% were serious, and there were five deaths.[98] Four of the five deaths were children with chronic underlying conditions. An increase in reported events was observed from 2005 to 2011, mainly attributable to an increase in reports of nonserious AEs. SAE reports for 2- to 4-year-old children peaked in the 2007–08 influenza season at 25% (15 of 60 vaccinees); this was the first influenza season following approval for use of LAIV in this age group. The two most frequently reported categories of nonfatal SAEs were neurologic (29.2%) and respiratory events (22.4%). The most frequent neurologic diagnoses were seizures and GBS, and the most frequent respiratory diagnoses were pneumonia and asthma or reactive airway disease. The most commonly reported vaccine administration error was administration of expired vaccine, which was not associated with AEs. For both pediatric and adult populations, the reviews of reports

to VAERS for trivalent LAIV were reassuringly consistent with prelicensure safety analyses and postlicensure safety data from the first two influenza seasons after FDA approval. Because VAERS is a passive surveillance system it is not possible to assess the reports for causal associations between vaccines and AEs.[98,99]

A similar analysis was conducted for Q/LAIV for the first influenza season following licensure, 2013–14,[100] when approximately 12.7 million doses of Q/LAIV were distributed in the United States. VAERS received 779 AE reports for individuals in the approved age range of 2 to 49 years, 95% of which were nonserious. In children, the most commonly reported AE was administration of expired vaccine (42%), followed by fever (13%) and cough (8%). In general, the safety profile of Q/LAIV was similar to that of trivalent LAIV. The most commonly reported AEs in adults were headache (18%), administration of expired vaccine (15%), and exposure during pregnancy (12%). One death, resulting from complications of brain tumors, was reported in children, and no deaths were reported for adults. Neurologic conditions were the most commonly reported nonfatal serious events in both children and adults. Two cases of GBS were reported in children, and none in adults; disproportional reporting of this AE compared to other vaccines was not observed. There were 100 reports of Q/LAIV being administered to individuals outside of the approved age range; 28 children younger than the age of 2 years and 72 adults older than the age of 49 years received Q/LAIV. Seventy-five percent of these children and 87.5% of the adults did not experience AEs. There were 49 reports where the age of the vaccinee was unknown. As was the case with the postlicensure VAERS reporting for the trivalent LAIV, the most commonly reported vaccine administration error was administration of expired vaccine. Administration of expired vaccine may be related to the change in LAIV formulation from frozen to refrigerator-stable in 2007 that resulted in a shorter shelf life of the vaccine, or to the relatively shorter shelf life for LAIV compared to IIV.[101] This error would be expected to result in lack of protection afforded by the vaccine, rather than adverse reactions to it. The administration of expired vaccine, administration to individuals outside the approved age group, and administration to individuals in whom LAIV is contraindicated (e.g., pregnant women) underscore the need for continued education and training of healthcare workers. In summary, none of the postlicensure reviews of events reported to VAERS for trivalent or quadrivalent LAIV identified any unexpected serious risks when the vaccine was used as approved.

TRANSMISSION OF LIVE ATTENUATED INFLUENZA VACCINE

LAIV strains replicate in the nasopharynx of the recipient and are shed in the respiratory secretions. Data in the published literature failed to detect transmission of vaccine viruses derived from the passaged cold-adapted master donor viruses in multiple age groups and settings.[63,83,85,102–109] In an evaluation of direct transmissibility in a daycare setting in children 8 to 36 months of age, 80% of the 98 FluMist recipients shed one or more vaccine strains, with a mean shedding duration of 7.6 days. One possible transmission event was observed among the 99 placebo recipients and was confirmed by phenotype and genotype analysis. The safety profile for this child after transmission was similar to that for the other children in the study who received vaccine or placebo; the child was not ill and did not experience an SAE.[85] An open-label, single-arm, multicenter Phase II study reported by Mallory and colleagues[110] demonstrated that at least one component of seasonal LAIV was shed by 79% of children between 6 and 59 months of age. In this study, virus shedding was most frequently seen in children 6 to 23 months of age, and it occurred mostly on postvaccination days 1 through 11, with shedding beyond day 11 detected almost exclusively in the younger age group. Peak titers of vaccine virus were detected on postvaccination day 2, and mean peak titers were generally less than 10^3 $TCID_{50}$ (median tissue culture infective dose)/mL.

In the year after initial FDA approval, the clinical use of LAIV was restricted in some institutions because of a theoretical concern that live attenuated virus might transmit from vaccinated hospital employees to unvaccinated patient contacts. After several years of experience, this concern proved to be unfounded, and person-to-person transmission of LAIV has not been a problem. Izurieta and colleagues[97] summarized vaccine AE reporting regarding LAIV, and no instances of recognized transmission were identified. In the one case in which transmission was suspected, and in which viral isolates were obtained, it turned out that a wt influenza virus, not vaccine virus, was involved in the transmission. The Advisory Committee on Immunization Practices (ACIP) to the CDC modified its initial recommendation to reflect the practicalities of vaccine usage in hospitals. The only concern that remains now is a theoretical one. Employees who are vaccinated should not have contact within 7 days with immunosuppressed patients who are in reverse isolation (such as bone marrow transplant recipients). All other situations are considered acceptable for using LAIV.

Immunogenicity of Live Attenuated Influenza Vaccine

Replication of LAIV in the upper respiratory tract induces both antibody and cellular immune responses. This section addresses the induction of these immune responses in healthy adults and children.

Antibody Responses

Serum antibody titers are the most common immune response measured in vaccine studies, in part because antibody alone is sufficient to protect from influenza virus infection. Serum HAI antibody titers induced by IIV are generally accepted as a correlate of protection.[111] Influenza-specific antibody titers measured by HAI assays serve as a surrogate for virus-neutralization assays. The enzyme-linked immunosorbent assay (ELISA) detects binding antibodies that may be broadly cross-reactive[112] while HAI and neutralization assays detect more strain-specific and functional antibody.

Numerous studies have found that seroconversion following vaccination with LAIV occurs more frequently in immunologically naïve individuals. Because adults are immunologically primed by repeated prior exposure to influenza strains that have circulated in recent years, seroconversion rates in adults vaccinated with LAIV are often not significantly higher than in placebo recipients.[87,113,114] This observation correlates with reduced shedding of vaccine virus after vaccination with LAIV in adults compared with young children.[113] In influenza-naïve children, LAIV elicits a robust serum antibody response in both HAI and neutralization assays.[89,92,115–118] Lee and colleagues[116] and Belshe and associates[89] compared serum antibody responses after LAIV in 1- to 5-year-old seronegative children by neutralization assay or HAI, and they reported seroconversion rates of 58% and 16% against H1N1, 100% and 92% against H3N2, and 100% and 88% against influenza B, respectively, after a single dose of the trivalent LAIV vaccine. Administration of a second dose of LAIV 28 to 60 days later improved the seroconversion rate against the H1N1 virus in these studies to 77% and 61%, respectively.

The influenza virus HA is a homotrimer with a globular head, bearing the receptor binding pocket and five antibody binding sites, and a relatively conserved stem region. The majority of antibody produced against the HA is directed at the globular head, where antigenic changes in the protein occur. There is renewed interest in conserved epitopes in the HA stem region to induce broadly cross-reactive antibodies that neutralize influenza A viruses from different subtypes.[119–126] These cross-reactive HA stem antibodies have been detected using sensitive new technologies including phage display libraries and immortalization of memory B cells from humans after natural influenza virus infection or after receipt of TIV. It is likely that LAIV also induces stem antibodies, but the proportion of the serum anti-HA antibody response that is directed to the stem is not known. The presentation of a stem epitope in a vaccine may have potential as a universal influenza virus vaccine.[127,128]

Serum antibody responses in healthy adults after LAIV vaccination are known to be low in comparison to those elicited by TIV, but production of mucosal antibodies and circulating antibody-secreting cells have been described in a higher percentage of LAIV recipients.[129–131] Clements and colleagues[132] found that the titer of influenza-specific immunoglobulin (Ig) A in the nasal wash of adults vaccinated with monovalent LAIV against H1N1 or H3N2 viruses was higher than in those vaccinated with TIV (83% for LAIV vs 38% for TIV). Interestingly, however, the reverse was true for influenza-specific IgG titers in nasal wash specimens: 94% of persons vaccinated with TIV had an increase in IgG titer, compared with 59% of LAIV recipients. Although it is still unclear how the presence of mucosal antibodies mediates protection, levels of IgA in nasal wash specimens correlated with protection on challenge with wt influenza virus.[132] In contrast to the case in children, mucosal IgA in adults vaccinated with LAIV declined 6 months after vaccination.[129] In a study of 79 adults who received LAIV, twofold or greater rises in IgA antibody titer in nasal wash specimens were seen in 33% of subjects, compared to 9% with fourfold rises in serum antibody titer.[133] It has been demonstrated that adults vaccinated with LAIV had a more prominent vaccine-specific IgA plasmablast response compared to subjects who received IIV.[134] In a small study in children who received LAIV or TIV followed by LAIV, levels of mucosal IgA measured by kinetic ELISA or Luminex were marginally higher in children following the first dose of LAIV, but this was not statistically significant.[135] Ambrose and colleagues reported significantly higher vaccine-specific nasal IgA antibody in a subset of children who received LAIV compared to placebo in three prospective, 2-year randomized clinical trials.[136] Although mucosal IgA responses are elicited by LAIV, their precise role in vaccine efficacy remains to be elucidated. Newer studies suggest that circulating memory B cells are also an important measure of vaccine-induced immunity in adults[130,131,134] and children.[137,138] B-cell and antibody responses, particularly plasmablast responses, to LAIV and TIV were compared in young adults.[134] Six to 8 days postvaccination, the titers of plasmablast-derived polyclonal antibodies were significantly higher in TIV recipients, but higher levels of cross-reactive plasmablast-derived polyclonal antibodies that bound heterologous strains from the previous year's influenza vaccine were observed in LAIV recipients. In children, LAIV resulted in a significant increase in naïve, memory, and transitional B cells in peripheral blood 30 days after vaccination,[137] whereas TIV elicited an increase in plasmablasts 7 days after vaccination.

The majority of the antibodies measured in studies of LAIV were against the influenza virus HA protein. There is evidence that anti-NA antibodies may be important in limiting the severity of influenza in humans.[139] The immune response elicited against the NA after vaccination with LAIV has not been extensively studied. In recent years, there has been a renewed interest in studying the contribution of antibody to the NA in influenza vaccine–mediated protection. Improved assays to measure NA inhibiting (NAI) antibodies in sera are being employed in this effort.[140,141] The levels of serum NAI antibodies in a recent study comparing the relative efficacy of TIV and LAIV were correlated with protection in individuals who had measurable HAI responses.[142] Responses were observed by HAI and NAI assays in 77%, and 37% of recipients of IIV, respectively, and in 21%, and 6% of LAIV recipients. Higher NAI titers in recipients of IIV were associated with a lower frequency of vaccine failure.

Cellular Responses

In contrast to the antibody response that is directed at the surface glycoproteins of the virus, the main targets of the cellular immune response to influenza are the conserved internal proteins of the virus. Although cell-mediated immunity plays an important role in clearance of influenza virus during natural infection, it is unclear to what extent vaccine-induced, cell-mediated immunity contributes to protection against disease, because the rate of influenza virus replication is very rapid, and the recall of memory T cells likely occurs after the peak of viral replication.[143] However, it is anticipated that a robust T-cell response induced by vaccination would protect against complications or severe disease. Because LAIV replicates in host cells, it induces $CD4^+$ and $CD8^+$ T-cell immunity. However, assays for the assessment of cellular immune responses to LAIV in clinical trials have lagged behind antibody assays and have primarily focused on the induction of T-helper cell type 1 (Th1)-mediated immunity by enumeration of interferon gamma (IFNγ)-producing T cells and natural killer cells by flow cytometry, ELISA, and enzyme-linked immunosorbent spot (ELISPOT) assay. Significant increases in $IFN\gamma^+$, $CD4^+$, and $CD8^+$ T cells after LAIV have been observed in both children and adults.[137,138,144–147]

In summary, immune responses to LAIV are more frequently detected in influenza-naïve subjects, making the responses in children more robust than in adults. It is important to note that although humoral and cellular immune responses to LAIV can be measured, an immune correlate of protection for LAIV has not yet been identified (see "Immune Correlates of Protection for Live Attenuated Influenza Vaccine" later).

Innate Immune Responses

As the pathways by which the innate immune system senses and responds to pathogens are being unraveled, it is becoming clear that the innate immune response plays a very important role in determining the adaptive immune response to pathogens and vaccines. The application of systems biology to the field of vaccinology promises to bring a new understanding of this interplay.[148–150] One study that compared the whole genome transcriptional profile of peripheral blood mononuclear cells collected from children vaccinated with TIV or LAIV found differences in the early immune responses elicited by the two vaccines.[151] Increased expression of IFN response genes was observed in mucosal RNA samples from adults vaccinated with LAIV.[133] In children, both LAIV and TIV induced overexpression of IFN response genes, but this occurred early (day 1 postvaccination) in all age groups for TIV, and only on day 7 postvaccination in children younger than 5 years of age for LAIV recipients.[137] These data suggest that early induction of IFN response genes may be important for the development of antibody responses.

Efficacy and Effectiveness of Live Attenuated Influenza Vaccine

Vaccine efficacy data were initially obtained from double-blind, placebo-controlled clinical trials in adults that measured the ability of the vaccine to protect persons from infection with a homologous wt virus. To prove that influenza vaccines are efficacious against natural infection, it is necessary to vaccinate a population before an influenza epidemic and compare the infection rates in vaccinated subjects with a group given a placebo. Case definitions of influenza vary according to study and may include culture-positive infection, fourfold rise in antibody titer during the influenza epidemic, or clinical observations of influenza-like illness (ILI). Specific point estimates of vaccine efficacy are ideally obtained by RT-PCR–positive or culture-positive identification of cases, thereby eliminating clinical syndromes caused by other viruses.[152] Efficacy field trials with culture-positive end points are often conducted in young children[89,153] because attack rates of influenza in children are high and they shed high quantities of virus for several days, making virus detection a sensitive and specific case-finding method. Efficacy trials in adults are more commonly conducted using clinical end points such as febrile respiratory illness. Influenza is the most common cause of febrile respiratory illness in adults, and virus isolation to identify influenza infection is difficult because adults may shed virus in low quantity and for a brief duration. Serologic diagnosis of a fourfold antibody rise in paired sera obtained before and after an epidemic is also often used in adults, with case definition being any subject with a fourfold antibody rise in response to the epidemic virus. However, subjects with vaccine-induced antibody may not produce sufficient antibody during the subsequent natural infection to result in a detectable fourfold antibody increase. Therefore, this definition has inherent bias, unlike the clinical end point for adult studies or the virus isolation end point for pediatric efficacy trials. Also, it is difficult to directly compare efficacy rates obtained from challenge studies with rates from field trials because challenge studies are typically conducted in small numbers of subjects who are seronegative for the challenge virus, whereas field trials are typically much larger and include subjects with a variable history of previous exposure to influenza.

Vaccine effectiveness and vaccine efficacy can be confused. In this chapter, *vaccine efficacy* means the specific reduction in attack rates of laboratory-confirmed influenza, and it is represented mathematically as $(1\text{-Rv/Rp}) \times 100\%$, where Rv is attack rate in vaccinees, and Rp is attack rate in placebo recipients.

Vaccine effectiveness refers to the reduction in clinical and socioeconomic events that can be expected to be associated with influenza but could also be caused by other agents. End points such as ILI, medically attended acute respiratory illness (MAARI), acute respiratory illness with cough, febrile acute respiratory illness, days of work missed by caretakers, and days absent from daycare have been used to determine vaccine effectiveness.

In this section, we describe the efficacy of LAIV against culture-confirmed influenza in healthy adults and children as determined from double-blind, placebo-controlled challenge studies and field trials. Studies with end points other than laboratory-confirmed diagnosis were used to determine vaccine effectiveness.

Efficacy in Adults

Several challenge studies, summarized in Table 32.2,[103,114,154–157] have been conducted in adults. Many studies of monovalent LAIV vaccines found that LAIV significantly reduced the number of persons presenting with clinical signs of respiratory illness after challenge. However, vaccination did not dramatically reduce the frequency of persons shedding challenge virus (Table 32.2).[114,154–157] Reduction in mean titer and duration of shedding varied by influenza type and subtype,[103,114,156] with a statistically significant effect on H3N2 virus shedding. A challenge study that compared the efficacy of trivalent LAIV to placebo in adults found that LAIV was 85% efficacious against culture-confirmed infection and respiratory illness after experimental challenge with wild-type influenza virus.[114]

Two randomized, double-blind, placebo-controlled, multiyear studies assessed the efficacy of LAIV and TIV in protection against naturally occurring influenza (see "Efficacy of Live Attenuated Influenza Vaccine Compared with Trivalent Inactivated Influenza Vaccine," later); both studies used culture-confirmed influenza as the primary end point.[87,158–160] In years when the circulating strain was well matched to the vaccine strain, the efficacy of LAIV was 90% (95% confidence interval [CI], 72–96%) for H1N1 in 1988–89, 56% (95% CI, 1–81%) for H3N2 in 1989–90, 43% (95% CI, 15–70%) for H3N2 in 2005–06, and 36% (95% CI, 0–59%) for H3N2 and B in 2007–08.[87,158,159] It is important to note that the study done during the seasons from 1988 to 1990 included persons 1 to 65 years of age, whereas the later studies included only adults 18 to 49 years of age.

Efficacy in Children

Given the risk of severe illness associated with influenza infection in young children, challenge studies with wt virus cannot be conducted in this population. Therefore, efficacy in children is assessed either by evaluating replication of an attenuated vaccine virus as a surrogate for challenge with a wt virus (see Table 32.3),[154] or by following vaccinated children through natural exposure to influenza virus over the course of an influenza season. Efficacy was assessed by challenge with an attenuated H1N1 vaccine virus in a placebo-controlled study in healthy children. More placebo recipients shed virus than LAIV-vaccinated children, demonstrating a vaccine efficacy of 83% (95% CI, 60–93%).[154]

Several studies measured the efficacy of LAIV in children against naturally acquired culture-confirmed illness as the primary end point; children younger than 2 years of age were included in most of these studies.[88,89,91,93,94,161,162] Table 32.3 summarizes the efficacy rates of LAIV from comparable studies in large cohorts of children and those that have undergone post hoc analysis to include data only from children 2 years of age or older. These studies found efficacy rates between 70% and 94% against antigenically similar strains.[163,164] Even more striking are the Kaplan-Meier curves for the time to the first culture-confirmed influenza illness in children given one or two doses of LAIV (Fig. 32.3).[91,164] The data from these studies demonstrate that LAIV is efficacious in young children and that even a single dose can protect seronegative children from disease.

Efficacy Against Antigenic Drift Variants

Because antigenic drift is a constant process that can occur after vaccines are manufactured, efficacy of influenza vaccines against antigenic drift variants is a desirable property. This is usually assessed in large, community-based, randomized, controlled studies that span a period during which an antigenic variant virus emerges. Interestingly, many of the efficacy studies extended over 2 or more years and included a season in which the predominant circulating influenza virus was antigenically distinct from the vaccine strain (Table 32.4). The efficacy of LAIV against drifted strains was higher in children than in adults, just as it is against antigenically similar

TABLE 32.2 Efficacy of LAIV in Protection From Experimental Challenge With Wild-Type Influenza Virus

Study	Population[a]	Vaccine Virus, Dose	Time to Challenge	Challenge Virus, Dose	No. of LAIV Recipients			No. of Placebo Recipients		
					Total	Infection[b]	Illness[c]	Total	Infection[b]	Illness[c]
Clements et al (1984)[155]	College-age students	Monovalent H3N2, $10^{7.5}$ $TCID_{50}$	5–8 wk	H3N2, $10^{6.0}$ $TCID_{50}$	16	3	0	24	23	11
		Monovalent H3N2, $10^{6.5}$ $TCID_{50}$			12	11	5			
		Monovalent H3N2, $10^{5.5}$ $TCID_{50}$			13	6	3			
Clements et al (1986)[156]	18–35 y	Monovalent H1N1, $10^{7.8}$ $TCID_{50}$	7 mo	H1N1, $10^{4.0}$ $TCID_{50}$	16	11	2	27	25	12
		Monovalent H3N2, $10^{7.5}$ $TCID_{50}$		H3N2, $10^{6.0}$ $TCID_{50}$	14	7	4	15	11	6
Sears et al (1988)[157]	18–40 y	Monovalent H1N1, $10^{7.5}$ $TCID_{50}$	4–7 wk	H1N1, $10^{6.4}$ $TCID_{50}$	20	6	1	28	23	12
		Monovalent H3N2, $10^{7.5}$ $TCID_{50}$		H3N2, $10^{7.0}$ $TCID_{50}$	11	5	0	10	10	3
Clements et al (1990)[103]	18–40 y	Monovalent B, $10^{7.5}$ $TCID_{50}$	6 wk	B, $10^{7.0}$ $TCID_{50}$	13	9	0	12	10	5
Treanor et al (1999)[114]	18–45 y	Trivalent, $10^{7.0}$ $TCID_{50}$ of each strain	4 wk	H1N1, $10^{7.0}$ $TCID_{50}$	10	3	1	12	6	6
				H3N2, $10^{7.0}$ $TCID_{50}$	9	3	1	10	2	4
				B, $10^{7.0}$ $TCID_{50}$	10	1	0	11	2	4
Belshe et al (2000)[154]	34–91 mo	Trivalent, $10^{6.7}$ $TCID_{50}$ year 1; Trivalent, $10^{7.0}$ $TCID_{50}$ year 2	6–8 mo after year 2 vaccination	caH1N1[d], $10^{7.0}$ $TCID_{50}$	144	6	ND[e]	78	19	ND[e]

[a]All subjects were screened prior to vaccination to have serum antibody HAI titer ≤1:8.
[b]Determined by isolation of influenza virus from nasal wash.
[c]Any signs of respiratory illness, see text for description of clinical signs.
[d]Challenge with cold-adapted (ca) vaccine virus, A/Shenzhen/227/95-like H1N1, the H1N1 component of 1997–98 season which was year 2 of the study.
[e]Not determined in this study.
HAI, hemagglutination-inhibiting; LAIV, live attenuated influenza vaccine; $TCID_{50}$, median tissue culture infective dose.

TABLE 32.3 Efficacy in Children of Two Doses of LAIV vs Placebo Against Natural Infection by Antigenically Similar Viruses

Study Population (Age)	Location	Post Hoc Analysis Results				Reference
		Study Year[a]	% Efficacy[b]	95% CI	n	
2–6 y	United States	1996–97	94.6	88.6–97.5	713 LAIV, 335 placebo	89
2–3 y	South Africa, Brazil, Argentina	2000–01	81.8	66.8–90.8	344 LAIV, 332 placebo	91
1–3 y[c]	Southeast Asia	2000–01	72.9	62.8–80.5	1653 LAIV, 1111 placebo	93
0.5–3 y[c]	Europe	2000–01	83.5	72.6–90.6	1059 LAIV, 725 placebo	94

[a]Data from year 1 of each study, when the circulating strains of virus were antigenically similar to the vaccine viruses.
[b]Primary efficacy end point was the incidence of the first episode of culture-confirmed influenza illness caused by a virus antigenically similar to that in the vaccine.
[c]Includes data from children younger than 2 years of age.
CI, confidence interval; LAIV, live attenuated influenza vaccine.
Modified from Block SL, Toback SL, Yi T, et al. Efficacy of a single dose of live attenuated influenza vaccine in previously unvaccinated children: a post hoc analysis of three studies of children aged 2 to 6 years. Clin Ther. *2009;31:2140–2147.*

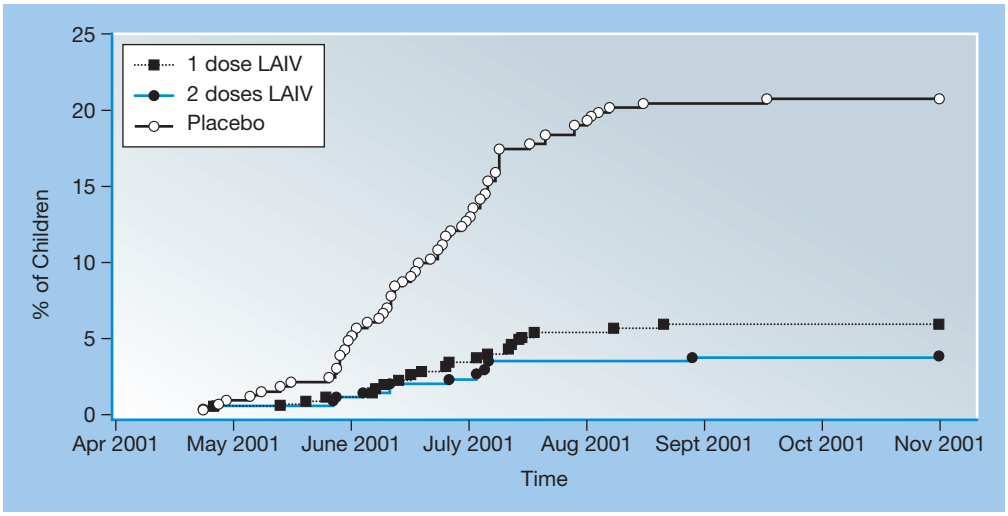


Figure 32.3. Kaplan-Meier curves from the time of vaccination with one or two doses of live attenuated influenza vaccine (LAIV) in year 1 to the first episode of culture-confirmed influenza illness. *(From Bracco Neto H, Farhat CK, Tregnaghi MW, et al; D153-P504 LAIV Study Group: Efficacy and safety of 1 and 2 doses of live attenuated influenza vaccine in vaccine-naive children.* Pediatr Infect Dis J. *2009;28:365–371; and Block SL, Toback SL, Yi T, et al. Efficacy of a single dose of live attenuated influenza vaccine in previously unvaccinated children: a post hoc analysis of three studies of children aged 2 to 6 years.* Clin Ther. *2009;31:2140–2147.)*

TABLE 32.4 Efficacy of LAIV Against Influenza Caused by Antigenic Drift Variants

Study Population (Age)	Year of Study	Antigenic Drift Strain(s)	Efficacy (%)		Comment	Reference
			Point Estimate	95% CI		
26–85 mo	1997–98	H3N2	86	75–92		153
6–36 mo	2001–02	B	33.3	–5.7 to 57.6	U.S./single-dose LAIV each year	91
6–36 mo	2001–02	B	24.1	–28.5 to 55.7	U.S./two doses LAIV in year 1	
12–36 mo	2001–02	B	56.7[a]	30.3–73.8	Asia/single-dose LAIV	93
12–36 mo	2001–02	B	64.2[a]	44.2–77.3	Asia/repeated LAIV	
6–36 mo	2001–02	B	70.2	42.7–85.3	Europe/2-dose LAIV	94
1–65 y	1986–87	H1N1	78	49–91		158
1–65 y	1987–88	H3N2	59	21–79		
Adults	2004–05	H3N2 and B	57	–3 to 82	Too few B cases to determine efficacy	160

[a]Efficacy data against any strain in year 2 of the study. The circulating influenza B viruses were not antigenically similar to the influenza B vaccine component.
CI, confidence interval; LAIV, live attenuated influenza vaccine.

strains. The cross-protection conferred by LAIV against antigenic drift variants may result from the breadth of the immune response elicited by LAIV vaccination (see "Immunogenicity of Live Attenuated Influenza Vaccine," earlier), which includes immunity to conserved viral proteins such as the M and NP proteins. In young adults who received LAIV, a higher level of NP-specific plasmablasts were observed compared to recipients of TIV, and the cross-reactive plasmablast response to heterologous influenza strains from the previous year's vaccine was also of greater magnitude in LAIV vaccinees.[134] However, LAIV was not effective against an H3N2 drift-variant in the 2014–15 influenza season, suggesting that the breadth of cross-protection conferred by LAIV is influenced by the extent of antigenic change.[165–167]

Effectiveness of Live Attenuated Influenza Vaccine

Table 32.5 summarizes key effectiveness results following a single dose of LAIV or placebo in healthy working adults. Vaccine significantly reduced the number of severe febrile illnesses and days of febrile upper respiratory tract illnesses among healthy, working adults. Vaccine also led to lower rates of work absenteeism, healthcare provider visits, and the use of prescription antibiotics and nonprescription medications. These benefits were observed during a season in which the predominant circulating influenza virus strain, A/Sydney/5/97 (H3N2), was not well matched to the A/Wuhan/359/95 strain contained in the vaccine.[78] In contrast, a similar effectiveness study of the TIV in similar age adults conducted in the same influenza season demonstrated no protection compared to placebo recipients.[168]

The frequencies of influenza-associated otitis media (OM) and MAARI have been used to measure the effectiveness of LAIV vaccination in children. Both were reduced in each year in three multiyear multicenter trials.[94,169,170] Belshe and colleagues concluded that LAIV was 98% effective in reducing OM during the 1996–97 season, based on one case of OM in the vaccine group and 20 cases in the placebo group, and

TABLE 32.5 Effectiveness of LAIV in Working Adults During the 1997–98 and 1998–99 Seasons

	LAIV[a]: 1997–98 Season[c]			TIV[b]					
				1997–98 Season[c]			1998–99 Season		
	Vaccine Group (*n* = 2833)	Placebo Group (*n* = 1420)	*P* Value[d]	Vaccine Group (*n* = 576)	Placebo Group (*n* = 554)	*P* Value[d]	Vaccine Group (*n* = 582)	Placebo Group (*n* = 596)	*P* Value[d]
Influenza-like illness/febrile illness	406	225	.1	161	132	.25	82	128	**<.001**
Physician visits	118	69	.07	64	48	.19	29	51	**<.001**
Taking any prescribed medication[e]	525	459	**<.001**	47	45	.6	26	40	.005
Hospitalization	NA	NA	NA	1	0	.5	0	0	NA
Lost workdays	465	267	**<.001**	167	111	.04	48	72	.002

[a]Data from Nichol et al.[78]
[b]Data from Bridges et al.[168]
[c]The predominant circulating strain in the 1997–98 influenza season was an H3N2 virus that was antigenically distinct from the vaccine strain.
[d]*P* values in bold are statistically significant.
[e]Nichol et al. only stated the number of individuals taking antibiotics, whereas Bridges et al. reported the number taking any prescribed drug.
LAIV, live attenuated influenza vaccine; NA, not available; TIV, trivalent inactivated influenza vaccine.

94% effective during the 1997–98 season during which an antigenic drift variant circulated, based on two cases in the vaccine group and 17 cases in the placebo group.[169] Gaglani and colleagues demonstrated a more modest impact (20% direct effectiveness [95% CI, 14–25%]) of LAIV on MAARI in children 1.5 to 18 years of age during the 2000–01 season.[170] Reduction in influenza-associated OM and MAARI associated with influenza also led to an increase in LAIV effectiveness as measured in time a caretaker takes off work, days missed from daycare, and incidence of outpatient or emergency department visits. Vesikari and colleagues measured the effectiveness of LAIV on socioeconomic end points during two influenza seasons.[94] LAIV was effective during a mild influenza epidemic in the first season, but a significant impact was also seen during the second season that was associated with a high attack rate in placebo recipients, with reductions in the need for a guardian to take time off work, the number of days of work lost, and days of daycare missed.[94] During the second season (2001–02), there was an unmatched influenza B component in the vaccine, demonstrating that LAIV was not only efficacious but also effective during a drift season in children.

The relative effectiveness of LAIV versus IIV against MAARI has been evaluated since 2010 in studies conducted by the U.S. Influenza Vaccine Effectiveness Network, using a test-negative study design, where influenza cases are defined as patients with MAARI confirmed to be influenza by RT-PCR. Patients with similar illnesses who do not have RT-PCR–confirmed influenza infection serve as control subjects. The test-negative design controls for factors associated with illness severity and propensity to seek care when ill.[171] In the 2011–12 influenza season the point estimates of vaccine effectiveness for children 9 to 17 years of age were similar for LAIV and IIV (adjusted vaccine efficacy [VE] of 60% [95% CI, −15 to 86] for LAIV vs 61% [95% CI, 28–79] for IIV), whereas LAIV was more effective than IIV in children 2 to 8 years of age (adjusted VE 61% [95% CI, 16–82] vs 40% [95% CI, 6–62]).[172] In the 2012–13 influenza season, LAIV was not significantly more effective than IIV in children 2 to 17 years of age against influenza A H3N2 (adjusted VE for LAIV 46% [95% CI, 13–66] vs IIV 36% [95% CI, 15–51]) or influenza B/Yamagata (adjusted VE for LAIV 53% [95% CI, 20–73] vs IIV 68% [95% CI, 54–77]).[173] In the 2013–14 influenza season—the first in which the Q/LAIV formulation was used—the predominant circulating strain was H1N1pdm09. Significant VE was demonstrated for IIV but not for Q/LAIV.[174] In fully vaccinated children 2 to 17 years of age, Q/LAIV was not effective against any influenza (adjusted VE 17% [95% CI, −39 to 15]), whereas IIV was effective in this age group (adjusted VE 60% [95% CI, 36–74]). Vaccine effectiveness was not demonstrated for Q/LAIV against H1N1pdm09 in 2- to 17-year-olds (adjusted VE 18% [95% CI, −38 to 51], or in any age-stratified model, but was demonstrated for IIV (adjusted VE 58% [95% CI, 50–65]). Similar effectiveness estimates were reported for the 2013–14 influenza season in postmarketing studies conducted by the manufacturer[175]: Q/LAIV did not show significant effectiveness against H1N1pdm09 (adjusted VE 13% [95% CI, −5 to 51]), but was effective against the influenza B/Yamagata virus circulating that season (adjusted VE 82% [95% CI, 12–96]). In this study, IIV was effective against both H1N1pdm09 (adjusted VE 74% [95% CI, 50–86]) and the B/Yamagata strain (adjusted VE 70% [95% CI, 18–89]). The postmarketing studies conducted by the manufacturer employ a test-negative study design similar to that of the U.S. Influenza Vaccine Effectiveness Network studies. In the 2014–15 influenza season, an H3N2 antigenic drift variant emerged shortly after the selection of the Northern Hemisphere vaccine strains, resulting in a mismatched vaccine. Estimates for vaccine effectiveness for influenza vaccines for the United States for 2014–15 were 19%.[165,166] An interim analysis of effectiveness indicated that neither Q/LAIV nor IIV provided significant protection in children 2 through 17 years of age; LAIV did not offer greater protection than IIV during this season.[167,176] Further analysis of data from the U.S. Influenza Vaccine Effectiveness Network from the 2010–11 to 2013–14 influenza seasons[177] found that the odds of influenza were similar for LAIV and IIV recipients during the first three seasons studied. In 2010–11 and 2013–14, the odds of influenza illness caused by H1N1pdm09 were significantly greater among LAIV recipients compared to IIV recipients 2 to 8 years of age.

Interim VE estimates reported by the CDC from the U.S. Influenza Vaccine Effectiveness Network for the 2015–16 influenza season have heightened concerns that Q/LAIV is not effective against H1N1pdm09,[178,179] although data from the manufacturer are not in agreement with these findings.[180] The effectiveness of LAIV against H1N1pdm09 strains since the emergence of the swine-origin pandemic H1N1 influenza virus in 2009 is discussed in detail below under "LAIV Against the 2009 Pandemic H1N1 Virus."

Relative effectiveness of LAIV and IIV in adults has not been reported in the U.S. Influenza Vaccine Effectiveness Network studies as data on LAIV in adults are sparse.

Retrospective effectiveness studies comparing LAIV to TIV were conducted in the U.S. military, in new recruits undergoing training as well as other military personnel (nonrecruits)[181,182] who receive annual influenza vaccination. The analyses relied on administrative records, and in most cases, effectiveness against culture-confirmed influenza illness could not be determined. A retrospective cohort analysis among military service members over the 2005–06 and 2006–07 influenza seasons demonstrated that TIV was slightly more effective than LAIV against ILI for nonrecruits over both seasons. However, in Army and Air Force recruits, significantly greater effectiveness against ILI was observed with LAIV.[181] A separate retrospective cohort analysis of effectiveness of LAIV and TIV[182] examined the incidence of healthcare encounters among U.S. military active-duty nonrecruits over three influenza seasons. Data from more than 1 million persons per year were analyzed and a statistically significant reduction in the incidence of healthcare encounters resulting in a primary diagnostic code consistent with pneumonia or influenza in recipients of TIV compared with no vaccine was reported. A consistent effect of LAIV vaccination was observed in only one season, and this effect was less than TIV in both total and propensity-matched cohorts. Both vaccines were comparable in effectiveness among vaccine-naïve service members. The relative effectiveness of LAIV and TIV in military personnel for the influenza seasons between 2006 and 2009 was similar: no differences in the rates of ILI, influenza, or pneumonia were observed.[183] In the 2010–11 influenza season, overall vaccine effectiveness was found to be low against clinically apparent, laboratory-confirmed influenza, although protection was marginally better with TIV.[184] Vaccine effectiveness was not observed against H1N1pdm09 virus infection. In another study of military personnel this was attributed to antigenic drift in the circulating H1N1pdm09 strain and decreased serologic response to H1N1pdm09 in vaccinees.[185] Although it is difficult to determine the role of antibody in effectiveness of LAIV in adults as serum antibody responses do not correlate with protection, these findings are consistent with the other reports of low effectiveness of LAIV against H1N1pdm09 in the 2010–11 season.

In summary, the relative effectiveness of LAIV and TIV vary in specific populations, and vaccination with LAIV appears to be more effective in children and military recruits than in adults.

Noninferiority Studies to Support Licensure of Quadrivalent Live Attenuated Influenza Vaccine. The strategy to obtain marketing approval for Q/LAIV from the FDA was based on the following similarities between the trivalent and proposed quadrivalent formulations: the vaccine components are based on the same attenuated ca master donor strains, manufactured using an identical process, all excipients in the vaccine are the same, they are given at the same dose in the same volume by the same delivery method, and are in the same refrigerator-stable formulation. Therefore, the body of clinical data that supported licensure of trivalent LAIV was relevant to Q/LAIV.[186] A bridging strategy was used, where two large clinical studies were designed to demonstrate that Q/LAIV was noninferior to trivalent LAIV in safety and immunogenicity in adults[77] and children.[76]

A randomized, double-blind, active-controlled clinical trial was conducted in 1800 healthy adults 18 to 49 years of age.[77] Subjects were randomized 4:1:1 to receive Q/LAIV ($n = 1200$), trivalent LAIV with the influenza B/Yamagata strain ($n = 300$), or trivalent LAIV with the B/Victoria strain ($n = 300$). The primary end point for the study was the comparison of postvaccination strain-specific geometric mean HAI titers, where the criterion for noninferiority was met if the upper bound of the two-sided 95% CI for the ratio of the geometric mean of the HAI titer for trivalent LAIV to Q/LAIV was 1.5 or less for all strains included in the vaccine. Although HAI antibody responses are not frequently observed in adults following administration of LAIV, and do not correlate with efficacy, they have served as an end point for other bridging studies including the transition from frozen to refrigerator stable formulation, assessment of consistency between lots of vaccine, and studies that examined the effect of concomitant administration of other live virus vaccines with LAIV. In addition to the primary immunogenicity end point, solicited symptoms and AEs were compared between groups. Q/LAIV met the criterion for immunologic noninferiority in adults, and solicited symptoms and AEs were similar for trivalent LAIV and Q/LAIV, an observation that was confirmed in analyses of postmarketing surveillance AE reporting data.[100]

A similar study was conducted in children 2 to 17 years of age.[76] Subjects were randomized 3:1:1 to receive Q/LAIV ($n = 1385$), trivalent LAIV with the influenza B/Yamagata strain ($n = 300$), or trivalent LAIV with the influenza B/Victoria strain. Children 2 to 8 years of age received two doses, and those 9 to 17 years of age received a single dose of LAIV. The immunologic noninferiority criterion was the same as for the adult study. Q/LAIV met the immunologic noninferiority criterion, although it was noted that the immunogenicity of the H3N2 and H1N1 vaccine components were lower than expected. The safety of Q/LAIV in children was comparable to that of trivalent LAIV, although a small but statistically significant increase in fever was noted in 2 to 8 year olds following the first dose of Q/LAIV. Q/LAIV was approved by the FDA in 2012 and was used for the first time in the 2013–14 influenza season.

Duration of Immunity Induced by Live Attenuated Influenza Vaccine

Longer-term persistence of immunity may emerge as a characteristic of the host response induced by LAIV. When the persistence of serum and nasal antibodies induced by TIV or LAIV in seronegative children were compared, LAIV recipients had significantly higher and more sustained levels of both serum and nasal antibodies measured by HAI and ELISA.[187,188] Prolonged humoral immunity may provide protection against antigenic drift variants that emerge. B-cell and T-cell immune responses in children vaccinated with LAIV were sustained for at least 1 year after vaccination.[138] The decline in HAI and NAI antibody responses over an 18-month period following vaccination with either IIV or LAIV was measured in the sera of adults who had participated in a placebo-controlled efficacy trial in Michigan between 2004 and 2007.[87] Although a greater proportion of IIV recipients than LAIV recipients had an HAI titer of 1:32 or greater and an NAI titer of 1:40 or greater 1 month after vaccination, the decline in antibody titers in both groups was slow; HAI and NAI antibody titers decreased twofold in approximately 600 days.[189] Community-based studies have found protection from illness up to 12 months after vaccination.[190] The durability of direct effectiveness against MAARI was assessed in children who were vaccinated with a single dose of LAIV during the 1999–2000 season and then were given placebo in the 2000–01 season; the direct effectiveness against MAARI of a single dose of LAIV administered a year prior to surveillance compared to unvaccinated children for both seasons was 22% (95% CI, 11%–32%).[170] Recent studies with investigational pandemic LAIV (pLAIV) have demonstrated long-term immunological

memory: individuals who received H5N1 or H7N7 pLAIV had a rapid, robust antibody response to a corresponding IIV administered years later[191,192] (see "Live Attenuated Influenza Vaccine Against Other Influenza Viruses of Pandemic Potential" below).

As with IIV, there is some concern that repeated annual vaccination may result in decreased effectiveness of LAIV. Data from systematic clinical trials conducted over several seasons that are specifically designed to address this question are not available. In a 5-year study comparing the efficacy of IIV and LAIV against influenza A,[158] a consistent decrease in efficacy was not observed. However, the authors noted that seroconversion rates were higher in the first year of the study compared to subsequent years. The same observation was made in another study in which HAI and NAI antibody titers were compared over 18 months following vaccination with either IIV or LAIV in adults[189]: recipients of both types of vaccine had significantly lower HAI titers in the second year of the study, although rates of antibody persistence did not differ between the two groups. Analysis of antibody and B-cell responses to influenza vaccination with either IIV or LAIV suggest that the type of vaccine received in the previous season can influence immune responses[193]; receipt of IIV in the previous season was associated with lower HAI antibody responses to IIV or LAIV in the subsequent season, and lower B-cell effector responses to IIV but not LAIV. In a study conducted by the U.S. Influenza Vaccine Effectiveness Network overall influenza vaccine effectiveness in the 2011–12 season appeared to be negatively affected by vaccination in the prior influenza season,[172] but this analysis did not take vaccine type into account, so it is not possible to determine whether the effect was similar for IIV and LAIV. Vaccination in the previous and current influenza seasons was reported to result in lower influenza vaccine effectiveness in households[194,195] and the community,[194] but, again, analyses were not stratified by vaccine type. Further studies to determine the effect of prior season vaccination on the effectiveness of LAIV are warranted, but relatively few adults receive LAIV compared to IIV.

IMMUNE CORRELATES OF PROTECTION FOR LIVE ATTENUATED INFLUENZA VACCINE

A serum HAI antibody titer of 1:40 (or 1:32) was established as the 50% protective titer against influenza infection in healthy adults who were experimentally challenged with an influenza virus.[111] It is widely accepted that this correlates with protective immunity induced by inactivated influenza vaccines[196] and this benchmark forms the basis of the licensing criteria for inactivated influenza vaccines in the United States and in Europe.[197] In addition, Ohmit and colleagues found that postvaccination HAI titers elicited by TIV or LAIV were not fully predictive of protection against influenza infection,[198] and the applicability of this correlate of immunity in children has been questioned.[199]

Intranasally administered LAIV elicits mucosal and cell-mediated immunity in addition to serum antibody.[146] The contribution of the different arms of the immune system to LAIV-induced protection has been evaluated in mice and ferrets.[200–202] In mice, both cellular and humoral immunity contribute to LAIV-mediated protection and their relative contribution to viral clearance depends on the location and replication of the vaccine virus. Optimal protection against wild-type virus challenge in mice requires the development of neutralizing antibody; the magnitude of the humoral response and access of antibodies to the respiratory tract are equally important determinants of protection.[202] However, the relevance of the findings from the mouse model for the human experience are not clear because LAIVs replicate in the lower respiratory tract of mice but not in the lower respiratory tract of humans. In ferrets, $CD4^+$ and $CD8^+$ T-cell responses correlated with protection from challenge virus replication in the upper respiratory tract.[200]

In humans, the protective antibody response to influenza viruses is primarily directed at the HA and NA glycoproteins. Both serum IgG and mucosal IgG and IgA antibodies independently contribute to this resistance,[102,132,142] with serum IgG antibodies providing protection primarily to the lower respiratory tract and IgA antibodies providing protection primarily to the upper respiratory tract (reviewed in reference 82). The contribution of T cells to protection from influenza virus infection in humans was evaluated in a challenge study and two recent prospective studies in the United Kingdom. In the challenge study with an H1N1 virus, the presence of preexisting cross-reactive influenza-specific $CD4^+$ T cells correlated with protection from severe illness and decreased shedding.[203] In a cohort study, Sridhar and colleagues found that in the absence of cross-reactive neutralizing antibodies, $CD8^+$ T cells specific to conserved viral epitopes correlated with cross-protection against symptomatic influenza illness during the 2009 pandemic.[204] Individuals who developed less-severe illness had higher frequencies of preexisting T cells to conserved CD8 epitopes.[204] In a separate study of a population cohort of 1414 unvaccinated individuals from 2006 to 2010, Hayward and colleagues found that preexisting cross-reactive T-cell responses to NP were independently associated with decreased odds of nasal viral shedding in those infected with seasonal or pandemic influenza. The presence of baseline influenza A NP-specific T cells correlated with less symptomatic, polymerase chain reaction (PCR)-positive influenza A infection (overall adjusted odds ratio, 0.27; 95% CI, 0.11–0.68; $P = .005$, during pandemic [$P = .047$] and seasonal [$P = .049$] periods). Protection was independent of baseline antibodies. Taken together, the data from these studies indicate that preexisting T-cell immunity independent of baseline antibodies protects against symptoms and viral shedding associated with influenza.

IIVs induce higher titers of serum HAI and NAI antibodies than LAIV in primed individuals, but LAIV is more efficient in inducing mucosal IgA antibody responses.[132,142] In human challenge studies and in field studies, LAIV was highly efficacious even in persons with modest serum HAI responses[73,114,158] and either serum antibody or nasal wash IgA was a predictor of protection.[154] The induction of influenza-specific T cells by LAIVs has been demonstrated in children.[132,145,146] In a large field study of seasonal LAIV administered to young children, more than 100 spot-forming cells per 10^6 peripheral blood mononuclear cells in an IFNγ ELISPOT assay was associated with protection against culture-confirmed influenza illness.[162]

In addition, it has been shown that LAIVs alter the expression of IFN-related genes, whereas IIVs do not, suggesting that the innate immune response plays an important role in protection mediated by LAIVs.[149] A robust Type I (IFNγ) interferon response was observed when peripheral blood mononuclear cells from LAIV recipients were cultured with LAIV.[147] A comparison of genome-wide transcript profiles in whole blood from young children 7 days following vaccination with LAIV or TIV revealed that LAIV induced higher expression of Type I IFN and interferon-stimulated genes than TIV.[151] In summary, our understanding of LAIV-induced immunity will be enhanced as assays that permit evaluation of cellular and innate immune responses are incorporated into clinical studies designed to explore immune correlates of protection for LAIV and biomarkers of early responses to LAIV.

EFFICACY OF LIVE ATTENUATED INFLUENZA VACCINE COMPARED WITH TRIVALENT INACTIVATED INFLUENZA VACCINE

Comparison of the efficacy of LAIV relative to TIV may provide useful data to inform public health policy decisions on the recommendations for influenza vaccination in different populations. Although LAIV and TIV were compared in several clinical trials,[205] definitive conclusions regarding relative efficacy cannot be drawn from studies that were not randomized controlled studies or when efficacy was not measured as reduction in culture-confirmed ILI.

Relative Efficacies of Live Attenuated Influenza Vaccine and Trivalent Inactivated Influenza Vaccine in Children

Between 1985 and 1990, randomized controlled clinical trials of a single dose of bivalent influenza A LAIV compared with inactivated vaccine or placebo were conducted at Vanderbilt University.[158] In 2001, Neuzil and colleagues reanalyzed the data from subjects who were younger than 16 years of age at the time of the study.[206] Efficacy was based on protection from culture-positive or serologically confirmed influenza illness. In general, the frequency of culture-positive influenza illness was too low to assess the rates of disease or VE by age group, and the attack rates for the different subtypes differed.

During the seasons when H1N1 influenza viruses circulated, the inactivated vaccine and LAIV had very similar efficacies against culture-positive ILI. Although the efficacies of inactivated vaccine and LAIV were also similar for the H3N2 years, at 77% and 68%, respectively, the CIs were extremely wide. There was a low rate of influenza illness and a high rate of seroconversion in placebo recipients; the latter affected the assessment of VE by serology.

LAIV was reported to be more efficacious than TIV in infants and young children in a large international multicenter study.[88] A statistically significant reduction in the number of cases of culture-confirmed influenza illness was observed for both H1N1 and H3N2 viruses compared with TIV. A significant reduction in the number of cases of influenza B was not observed with either vaccine. Statistically significant reductions in culture-confirmed lower respiratory tract illness and influenza-associated OM were also observed in recipients of LAIV. However, the absolute efficacy of either vaccine could not be determined from this study because a placebo group was not included.

The efficacy of LAIV versus TIV has also been studied in children at high risk for severe influenza disease. Fleming and colleagues compared the efficacy of LAIV and TIV in adolescents and children with asthma.[207] Children between the ages of 6 and 17 years with a clinical diagnosis of asthma received a single dose of either TIV or the refrigerator-stable formulation of LAIV. The primary end point of the study was efficacy as determined by the incidence of culture-confirmed influenza illness caused by a community-acquired virus that was antigenically similar to the vaccine and only relative efficacies were reported. Overall, LAIV was found to be 34.7% more efficacious than TIV. There was a trend toward greater efficacy of LAIV against influenza A H1N1 and influenza B, but there was no difference between the two vaccines against influenza A H3N2. Influenza B was the predominant strain during the influenza season of this study, and LAIV had an overall 31.9% greater efficacy than TIV against all influenza viruses. There was no difference between the two vaccines for the pharmacoeconomic end points such as use of prescribed medications or antibiotics, incidence of unscheduled healthcare provider visits, incidence of hospitalizations, and number of days of absence from school or work.

The efficacy of two doses of LAIV or TIV in young children with a history of recurrent respiratory tract infection was reported by Ashkenazi and colleagues.[208] In this randomized, open-label trial, there were 52.7% fewer cases of culture-confirmed influenza caused by influenza viruses that antigenically matched the vaccine in children who received LAIV. Greater relative efficacy was observed for LAIV against influenza A H1N1 and influenza B, but not against influenza A H3N2. LAIV recipients also had a slight reduction in the number of visits to healthcare providers for respiratory tract illness and in absences from school, kindergarten, or daycare.

In summary, although some studies had design limitations, LAIV has been reported to be more efficacious than TIV in children, and several metaanalyses have reached the same general conclusion.[205,209,210] Post hoc analyses of the studies just described have also been conducted to address the relative efficacy of LAIV compared with TIV in children by age group,[161] as a function of length of time after vaccination,[211] and against influenza-associated OM.[212] The relative efficacy of LAIV compared to TIV in three studies conducted in children did not vary by age group,[161] suggesting that prior influenza infection or vaccination may not result in reduction of efficacy of LAIV. Relative efficacy of LAIV versus TIV against matched strains was greater at more than 4 to 8 months postvaccination compared to 0 to 4 months postvaccination (range, 49–89% vs 25–60%) in three large randomized clinical trials in children,[211] and greater protection against influenza-associated OM was observed in LAIV recipients in two TIV-controlled clinical trials,[212] an effect that was most evident in children 2 years of age and older. The interpretation of these studies is limited because most of the studies did not include a placebo arm.

Relative Efficacies of Live Attenuated Influenza Vaccine and Trivalent Inactivated Influenza Vaccine in Adults

Few comparative studies of LAIV and TIV have been reported in adults, and most of these studies measured vaccine effectiveness against symptomatic influenza. Table 32.6 summarizes the estimated relative efficacies of LAIV and TIV in these studies. In the multiyear placebo-controlled study conducted at Vanderbilt University,[158] efficacies of LAIV and TIV in adults against culture-confirmed influenza A did not significantly differ among study years. Both vaccines were efficacious in preventing seroconversion, and TIV was more efficacious than LAIV in the years when H3N2 viruses circulated.

The most comprehensive comparative efficacy studies of LAIV and TIV in adults were part of a multiyear study conducted at the University of Michigan comparing the efficacies of TIV and LAIV relative to placebo.[87,159,160] The primary end point was laboratory-confirmed symptomatic influenza, determined by virus isolation or serology. In the first year of the study, 2004–05, an influenza A H3N2 antigenic drift variant and two lineages of influenza B were the predominant circulating viruses, permitting determination of VE against antigenically mismatched strains.[160] Although the data suggested greater efficacy with TIV, a significant advantage of TIV over LAIV in terms of relative efficacy could not be demonstrated because there were too few laboratory-confirmed illnesses. LAIV prevented influenza illness, but the absolute efficacy was not significant. These findings contrast with previous studies in adults and children, where LAIV was highly efficacious against an H3N2 antigenic drift variant.[78,153] In the subsequent year (2005–06), influenza activity was relatively low. Efficacy

TABLE 32.6 Relative Efficacy of LAIV Versus TIV in Adults

Study Design	End Point	Estimated Efficacy Against Indicated End Point		Reference
		LAIV	TIV	
Multiyear, double-blind, randomized, placebo-controlled	Culture-confirmed influenza A H1N1, any study year	85% (95% CI, 70–92%)	76% (95% CI, 58–87%)	158
	Culture-confirmed influenza A H3N2, any study year	74% (95% CI, 52–86%)	58% (95% CI, 29–75%)	
	Culture-confirmed influenza A for years when H3N2 was circulating	32% (95% CI, 17–44%)	73% (95% CI, 65–79%)	
Double-blind, randomized, placebo-controlled	Culture-confirmed influenza, any virus	57% (95% CI, –3–82%)	77% (95% CI, 37–92%)	160
	Culture-confirmed influenza A	74% (95% CI, –12 to 95%)	74% (95% CI, –11 to 95%)	
	Culture-confirmed influenza B	40% (95% CI, –103 to 87%)	80% (95% CI, 8–97%)	
	Symptomatic influenza confirmed by culture or RT-PCR	48% (95% CI, –7 to 74%)	75% (95% CI, 42–90%)	
	Symptomatic influenza confirmed by culture or serology	30% (95% CI, –57 to 67%)	67% (95% CI, 16–87%)	
Double-blind, randomized, placebo-controlled	Symptomatic influenza confirmed by culture or serology	43% (95% CI, –15 to 71%)	54% (95% CI, 4–77%)	87
Double-blind, randomized, placebo-controlled	Culture-confirmed influenza, any virus	36% (95% CI, 0 to 59%)	68% (95% CI, 46–81%)	159
	Culture-confirmed influenza A H3N2	29% (95% CI, –14 to 55%)	72% (95% CI, 49–84%)	

CI, confidence interval; LAIV, live attenuated influenza vaccine; RT-PCR, reverse-transcription polymerase chain reaction; TIV, trivalent inactivated influenza vaccine.

of TIV was demonstrated when serologic evidence of influenza infection was included, but efficacy of LAIV was not statistically greater than placebo.[87] Laboratory-confirmed influenza rates in the third year of the study were higher, so a more comprehensive analysis of absolute and relative efficacy was possible.[159] The data demonstrated greater efficacy of TIV than LAIV against culture-confirmed influenza A (see Table 32.6). Efficacy of either vaccine against influenza B could not be determined because there were too few isolates of influenza B. The Michigan study underscores the difficulties in conclusively demonstrating the efficacy of influenza vaccines in well-controlled clinical trials. The unpredictable attack rate, the types of influenza virus circulating from season to season, and the antigenic variability of influenza viruses are all factors that must be considered.

A challenge study designed to demonstrate efficacy of LAIV and TIV in humans was reported by Treanor and colleagues.[114] Subjects were randomized to receive LAIV, TIV, or placebo, and 28 days later they were challenged with antigenically matched wild-type influenza virus. No significant difference was observed between LAIV and TIV in efficacy against laboratory- confirmed influenza illness, demonstrated by isolation of virus or seroconversion. However, vaccination with TIV resulted in a statistically significant reduction in shedding of challenge virus and infection rate. Interpretation of this study was limited by the small numbers of subjects, and lower-than-expected infectivity of the challenge virus in the placebo group.

Live Attenuated Influenza Vaccine in High-Risk Populations

Populations at highest risk for severe disease associated with influenza infection include very young children, persons with asthma or cystic fibrosis (CF), those suffering from an immunodeficiency, and adults older than 65 years of age. LAIV is currently licensed in children 2 years of age or older, based on several studies in large cohorts of children. Although the vaccine was well tolerated in most children, there was a significant increase in the risk of reactive airways disease in children 18 to 35 months of age.[90] Another large cohort study comparing LAIV to TIV found a significant increase in the rates of hospitalization for any cause in 6- to 11-month-olds in the first 180 days after vaccination.[88] However, LAIV was more effective than TIV in the young children in this study; the LAIV vaccine group had 54.9% fewer culture-confirmed influenza infections than TIV ($P < .001$). Although LAIV may be effective in children younger than 2 years of age it is not recommended by the ACIP or by the American Academy of Pediatrics because of concerns associated with reactive airways disease and hospitalization.

LAIV is not currently licensed for use in persons with asthma in the United States. Because children with asthma are at particularly high risk for severe influenza illness, further studies have been undertaken to examine the safety of LAIV in adolescents with asthma. In a study comparing placebo with LAIV in 9- to 17-year-old children with moderate to severe asthma,[213] mild exacerbations of asthma were recorded in two of 24 LAIV recipients and zero of 24 placebo recipients. A more recent open-label study compared TIV with LAIV in more than 2000 asthmatic children 6 to 17 years of age.[207] Participants with severe disease and recipients of immunosuppressive therapy were excluded. Although runny nose and nasal congestion were more common after LAIV, there were no significant differences between the two groups with regard to wheezing, peak expiratory flow rate, asthma exacerbations, asthma symptom scores, or nighttime awakening scores, indicating that LAIV is safe and effective in asthmatic children.[207,214] The efficacy of LAIV in preventing influenza in asthmatic children was 34.7% (95% CI, 3.9–56.0%) higher than TIV. Additionally, the rate of emergency department visits or hospitalizations within 42 days was equivalent after vaccination with LAIV or TIV in children 2 to 5 years of age with asthma or recurrent wheezing.[215]

Severe morbidity in association with influenza infection has been found in patients with CF,[216] and vaccination with TIV is recommended for all patients with CF and their families. In a 3-year study in patients with CF and their families, both LAIV and TIV were effective.[217]

Concerns about the inadvertent exposure of immunodeficient patients to a live vaccine have prompted small studies of the safety and immunogenicity of LAIV in persons infected with HIV and those with cancer. In one study of noninfected adults and HIV-infected adults with $CD4^+$ counts greater than

200 cells/mm^3, LAIV resulted in similar rates of runny nose and other minor symptoms in HIV-infected and noninfected persons. Among HIV-infected persons, no difference in HIV viral load and CD4$^+$ levels were seen up to 6 months after vaccination between the vaccinated group and placebo recipients.[218] Additional studies to assess the immunogenicity of LAIV and shedding of vaccine virus have been conducted in a small number of children who were immunocompromised because of HIV infection or cancer.[219–222] None of these studies reported any SAEs or ILI. Additionally, HIV-infected children produced anti-influenza neutralizing antibodies and increased salivary IgG antibodies following receipt of LAIV.[222] These studies suggest that exposure to LAIV does not pose a risk to HIV-infected persons and that LAIV vaccine may be useful to protect HIV-infected persons against influenza infection.

Persons 65 years of age and older are one of the highest-risk groups for serious illness and death from epidemic influenza.[223] Although LAIV is approved only for healthy persons between 2 and 49 years of age, these vaccines have been evaluated in healthy adults 50 years of age and older, and in those with chronic cardiopulmonary conditions or diabetes mellitus. Table 32.7 summarizes these studies.[224–231] In most of these studies, LAIV was administered simultaneously with TIV and LAIV was safe and well tolerated. Coadministration of TIV and LAIV was reported to enhance local HA-specific IgA antibody responses.[225,229,230,232] However, the efficacy of the combination of the two vaccines compared with TIV alone remains equivocal. A recent study by Forrest and colleagues designed to determine the relative efficacy of LAIV versus TIV in persons 60 years of age and older[233] was inconclusive because of the low incidence of influenza in both vaccine groups.

TABLE 32.7 Safety and Efficacy of Live Attenuated Cold Adapted Influenza Vaccines in Individuals Aged ≥50 Years

Vaccine	Study Population and Age Range	Safety	Efficacy/Effectiveness		Reference
			Measure	% Efficacy (95% CI)	
Monovalent A/H1N1 LAIV alone or with TIV	Ambulatory elderly, 65–83 y	Very rare reports of rhinitis (LAIV) and pharyngitis (LAIV + TIV)	N/A	ND	230
Bivalent A LAIV (H1N1 and H3N2) alone or with TIV	Healthy elderly, 60–90 y	Very rare reports of low-grade fever with rhinitis and local reactions (LAIV + TIV)	N/A	ND	229
Trivalent LAIV with TIV	Elderly nursing home residents, mean age 84.1 y	No significant symptoms reported	Lab-documented influenza A Outbreak-associated respiratory illness Outbreak-associated ILI	60.6% (18–82%) 56.8% (23–76%) 65% (17–86%)	231
Bivalent A LAIV (H1N1 and H3N2) with TIV	Chronically ill elderly, ≥60 y	Vaccines well tolerated	N/A	ND	227
Trivalent LAIV with TIV	Chronically ill elderly with cardiovascular or pulmonary conditions, or diabetes mellitus, 65–75 y	Sore throat, runny nose more frequent with LAIV	N/A	ND	228
Trivalent LAIV with TIV	Adults with chronic obstructive pulmonary disease, ≥50 y	Significant increase in runny nose, shortness of breath, chills, headache and itchiness at injection site with LAIV + TIV	Lab-documented influenza-caused illness: • Any influenza virus • H3N2 influenza A • Influenza B • Possible advantage in reducing respiratory consequences of influenza infection as measured by pulmonary function and symptoms at the end of the study	Not statistically significant: 16% (–22 to 43%) 26% (–17 to 53%) 5% (–113 to 48%)	225, 226
Trivalent LAIV alone	Community-dwelling ambulatory adults, ≥60 y	Cough, sore throat, runny nose/nasal congestion, headache, myalgia, tiredness, loss of appetite more frequent with LAIV. Single report of bronchopneumonia and single report of asthma with LAIV	Culture-confirmed influenza, any vaccine strain Culture-confirmed influenza, any strain Culture-confirmed influenza A H3N2 Efficacy in subjects ≥70 y against influenza A H3N2 Efficacy in subjects 60–69 y against influenza A H3N2 Efficacy against influenza B	42.3% (21.6–57.8%) 41.6% (20.9–57.1%) 52.5% (32.1–67.2%) 65.7% (37.3–82.2%) 41.8% (8.3–63.7%) Not efficacious	224

CI, confidence interval; ILI, influenza-like illness; LAIV, live attenuated influenza vaccine; N/A, not applicable; ND, not determined; TIV, trivalent inactivated influenza vaccine.

Community Protection Afforded by Live Attenuated Influenza Vaccine

Indirect protection of unvaccinated individuals within a community that is afforded by the immunity of immunized members of the community has been demonstrated for infectious diseases of viral and bacterial etiology, and it is widely accepted that this phenomenon occurs with influenza.[234] Although influenza vaccine policy in the United States has been focused on the immunization of persons 65 years of age and older and vaccination rates increased from 31% to 67% between 1989 and 1997, influenza epidemics still cause widespread morbidity and mortality in this age group.[234–236]

Several lines of evidence suggest that widespread immunization of otherwise healthy populations may interrupt transmission of influenza and thereby indirectly protect those in high-risk groups. For example, vaccination of healthcare workers was associated with reduced morbidity and mortality from influenza among nursing home residents,[237–241] and healthcare workers now constitute a priority group for influenza vaccination in the United States.[223]

Widespread vaccination of schoolchildren has also been proposed as a measure to reduce the burden of influenza in the community, because children are important vectors for the spread of influenza. The ability to modify the course of an influenza outbreak by vaccination of schoolchildren has been demonstrated in several studies. A vaccination rate of 86% with a monovalent inactivated vaccine in schoolchildren in Tecumseh, Michigan, resulted in a threefold reduction in the excess attack rate for the community from influenza compared with a neighboring community in which schoolchildren were not vaccinated.[242] A clear difference was observed in the rates of school absenteeism between the communities, and there was evidence that protection was not limited to children of school age. In Japan, vaccination of schoolchildren was mandatory between 1977 and 1987. In 1987, the laws were relaxed, and parents could decide whether or not their children received vaccine. In 1994, vaccination rates fell to low levels amid doubts about the effectiveness of the vaccine. In an analysis of all-cause mortality and death attributed to influenza and of vaccination rates from Japan and from the United States between 1949 and 1998, Reichert and colleagues found that excess mortality rates, predominantly in older persons, dropped significantly in Japan with initiation of the program to vaccinate schoolchildren, from rates three to four times those in the United States to rates similar to those in the United States.[243] Excess mortality rates in Japan increased with discontinuation of the vaccination program for schoolchildren.

From a practical standpoint, LAIV could be an extremely effective method to achieve community protection if high vaccination rates with an efficacious vaccine are achieved in schoolchildren and influenza transmission to other segments of the community is interrupted. Large numbers of children could be vaccinated in a short period, and intranasal administration is preferred over injection of inactivated vaccine. There is evidence from several studies that this is an effective approach. A study conducted in the 1990s demonstrated that vaccination of schoolchildren with either inactivated or Russian LAIV resulted in significant protection. In schools where children received LAIV, vaccination rates and illness among staff and unvaccinated children were inversely correlated, suggesting reduction of transmission as a result of vaccination. Such a correlation was not seen in the schools where children received inactivated vaccine or in schools where children received placebo.[244]

There are several studies in a community in central Texas that report both direct and indirect protection against ILI afforded by vaccination of children. In these studies, age-specific rates of MAARI during the influenza season in intervention communities, where children received LAIV, were compared with rates in comparison communities, where children did not receive vaccine. Vaccination of approximately 20% to 25% of children, 1.5 to 18 years of age, in intervention communities resulted in indirect protection of 8% to 18% against MAARI in adults older than 35 years of age. This small effect may translate into a substantial effect at the population level. Moreover, the size of this effect may be diluted from use of clinical rather than laboratory end points.[245] In another study, significant protection against laboratory-confirmed influenza illness and pneumonia and influenza events was observed in the children who received LAIV, but not in those who received TIV. Indirect effectiveness against MAARI was observed in 5- to 11-year-old children and in 35- to 44-year-old adults.[246] In a third study,[247] when schoolchildren were vaccinated with LAIV against antigenically mismatched influenza viruses, significant indirect protection from influenza was observed in all age groups, except those 12 to 17 years of age. Combined virologic surveillance and MAARI visit data suggested that a single dose of LAIV provided better protection against influenza than TIV.

King and colleagues reported a small pilot study followed by a larger, multistate, school-based immunization intervention study using LAIV.[248,249] In the pilot study,[248] significant (45% to 70%) relative reductions in fever or respiratory illness–related outcomes including physician visits by adults, physician visits by children, prescription or other medicines purchased by household members, and family schooldays and workdays missed, were observed for intervention ("target") school households compared with control school households. In the larger trial, intervention school households reported significantly fewer ILI-related doctor or clinic visits for children; fewer episodes of fever plus cough or sore throat in children and adults; lower ILI-related prescription, over-the-counter, and herbal medication use for ILI; lower absenteeism rates for elementary and high-school students; and fewer missed workdays for adults caring for their own ILI or for others during the peak influenza week. Relative reductions across these outcomes ranged from 25% to 40%, again confirming indirect as well as direct benefit. Limitations of this study included the lack of placebo groups and the use of questionnaires for reporting of ILI.

A cluster, randomized, blinded trial was undertaken over three influenza seasons from 2012–15 in 52 Hutterite communities in Canada to determine whether vaccinating children and adolescents with trivalent LAIV would provide better community protection than IIV.[250] Influenza virus infection occurred at a rate of 5.3% (295 of 5560 person-years) in the LAIV group compared to 5.2% (304 of 5810 person-years) in the IIV group. The hazard ratio comparing LAIV with IIV for influenza A or B virus was 1.03 (95% CI, 0.85–1.24). The authors concluded that immunizing children with LAIV does not provide better community protection against influenza than IIV.

In summary, a large body of data demonstrates the effectiveness of vaccination of schoolchildren for the control of influenza in communities, from both direct and indirect effects of immunization. Both LAIV and TIV are highly efficacious against influenza in children, and a mechanism to explain how LAIV could afford better indirect protection is not clear. The data from these studies support the widespread vaccination of schoolchildren as a means of reducing morbidity and mortality in other high-risk members of communities.

Heterosubtypic Immunity

Immunity elicited by infection with an influenza A virus that provides protection against infection by a virus from another HA or NA subtype is referred to as heterosubtypic immunity. This phenomenon was first proposed after the 1957 pandemic, when adults may have had some protection against the pandemic H2N2 strain as a result of previous influenza infection with an H1N1 virus.[251,252] Heterosubtypic immunity has been reported in animal models,[253–256] but evidence of the phenomenon occurring in humans is weak. Given that vaccination with LAIV is known to elicit a broad range of immune responses against conserved influenza virus proteins (see "Immunogenicity of Live Attenuated Influenza Vaccine" earlier), it is possible that LAIV may confer heterosubtypic immunity. One study was designed to address the impact of heterosubtypic immunity induced by LAIV in children.[257] Children with preexisting HAI titers against either H1N1 or H3N2 viruses were challenged with a monovalent LAIV of the other subtype, and the ability of prior immunity to restrict the replication of the vaccine virus was sought as evidence of heterosubtypic immunity. Regardless of prior seropositivity, infection rates were equivalent, suggesting that LAIV did not induce heterosubtypic immunity. Larger population-based studies are underway to determine whether robust heterosubtypic immunity exists in humans.

Interference Between Strains

Infection with closely related viruses can lead to a predominance of one strain over another if replication of one virus interferes with the replication of the other strain. This phenomenon is of concern in the context of a multivalent live virus vaccine, because it can lead to variable efficacy of different components. Only a few studies have examined whether interference exists between influenza strains in the trivalent LAIV. Interference is most accurately assessed in subjects in whom a robust serum antibody response occurs; therefore, young seronegative children are the ideal subjects for such studies (see "Antibody Responses" earlier). Although there is insufficient evidence to conclude that interference between LAIV vaccine strains exists, the seroconversion rate in children against the H1N1 strain after the first dose was lower than for H3N2 and B strains, and this was rescued by administration of $10^{7.0}$ $TCID_{50}$ of each vaccine strain, which is approximately the dose in the licensed vaccine, or by administration of a second dose of vaccine.[89,116]

The question of interference between the component strains of LAIV came to the fore again when the Q/LAIV was being developed. A review of nonclinical studies in animal models and clinical trials in which interference between strains had been examined concluded that there is no clinically significant evidence of interference between component strains of LAIV.[258] However, it was recently proposed that interference between the component strains of LAIV may be a contributing factor to the observed reduced effectiveness of LAIV in recent seasons, particularly against the 2009 pandemic H1N1-like viruses.[178] It is possible that the change to the Q/LAIV formulation is associated with lower effectiveness of the H1N1 strain. It is important to note that in the immunogenicity bridging studies in humans to support licensure of Q/LAIV,[76,77] as well as the animal studies on interference reviewed by Bandell and colleagues,[258] the H1N1 components of LAIV were pre-2009 seasonal H1N1 viruses. Interference could compound other factors that were proposed to account for reduced vaccine effectiveness, such as thermostability of the HA[259] or increasing population immunity by repeated annual vaccination.

LIVE ATTENUATED VACCINES AGAINST PANDEMIC INFLUENZA

Experience with LAIV against seasonal influenza suggests that live vaccines may be an effective vaccination strategy for pandemic influenza. Potential advantages of LAIV include induction of humoral and cellular immunity, protection after a single dose, broad cross-protection against antigenic drift variants, high yield, and efficient manufacturing using infrastructure that is already in place for seasonal LAIV. To minimize the risk of the introduction of novel HA and NA genes into the environment, LAIV would not be used for vaccination against a novel pandemic influenza virus until the virus was known to be spreading in the human population.

Live Attenuated Influenza Vaccine Against the 2009 Pandemic H1N1 Virus

In response to the emergence of the H1N1pdm09 in 2009, a monovalent LAIV was rapidly produced by MedImmune.[70,260] The HA and NA genes of the vaccine virus were derived from the A/California/7/2009 (H1N1) influenza virus.[70] In randomized, double-blind, placebo-controlled clinical trials in healthy children (2 to 17 years old) and adults (18 to 49 years old), the monovalent H1N1pdm09 LAIV was found to have a safety profile similar to that of seasonal LAIV. As has been observed with seasonal LAIV, seroconversion rates (defined as a fourfold or greater rise in serum HAI titer) were modest. In children, seroconversion was observed in 11.1% and 32.0% of vaccine recipients by study day 29 (post–dose 1) and study day 57 (post–dose 2), respectively, compared with 6.3% and 14.5% in placebo recipients, regardless of baseline serostatus. In adults, seroconversion rates were 6.1% and 14.9% by study days 29 and 57, respectively, compared with 0% and 5.6% in the placebo group.[260]

In a study of H1N1pdm09 influenza vaccine effectiveness from four communities in the United States, it was reported that 21% of 1057 vaccine recipients received the live vaccine.[171] Effectiveness of the live vaccine, for those between 2 and 49 years old, was demonstrated only if vaccination more than 7 days prior to illness onset was considered, and the adjusted effectiveness estimate was 61% (95% CI, 12–82%). As recommended by the World Health Organization, the ca A/California/07/2009 (H1N1) was included in the subsequent seasonal trivalent LAIVs, and in the Q/LAIV for the 2013–14 season.

Table 32.8 summarizes vaccine effectiveness data for LAIV against A/California/07/2009-like H1N1pdm09 influenza. Low or no significant VE against H1N1pdm09 influenza was reported in the United States for the 2013–14 influenza season.[174,175,261] This was thought to be related to the thermal instability of the HA, resulting from the presence of the particular amino acid residue, E47, in the stalk region of A/California/07/2009-like H1N1pdm09 viruses.[259] Accordingly, the A/California/07/2009-like H1N1pdm09 component of Q/LAIV was replaced with a virus that did not have this residue in the HA stalk, A/Bolivia/559/2013 (H1N1). Despite this change in strain, the U.S. Influenza Vaccine Effectiveness Network reported no effectiveness of Q/LAIV against H1N1pdm09 in children and adolescents in an interim analysis of the 2015–16 influenza season, when H1N1pdm09 was the predominant circulating strain,[179] whereas IIV was found to be effective. However, data from a postmarketing study (ICICLE [Influenza Clinical Investigation for Children]) conducted by the manufacturer reported a point estimate for adjusted VE of LAIV of 50%,[180] which contrasted with a VE of −21% reported by the U.S. Vaccine Influenza Vaccine Effectiveness Network.[179] It should be noted that the adjusted VE point

TABLE 32.8 Effectiveness of LAIV Against H1N1 Influenza Since 2009

Season	LAIV	Study Description	Age Group (Years)	No. of Subjects Who Received LAIV Among		No. of Subjects Who Received IIV Among		Adjusted VE Estimate (95% CI)		Reference
				Flu+ Cases	Flu– Controls	Flu+ Cases	Flu– Controls	LAIV	IIV	
2009–10	Monovalent	4 U.S. communities	2–49 2–9	8/860 2/330	230/2931 140/977	14/999 7/373	875/5504 390/1647	61% (12–82)[a] 82% (14–96)[a]	62% (25–81)[b] 89% (15–99)[c]	171
2009–10	Monovalent	School-age children in Maine	5–14	4/73	135/693	19/87	458/865	81% (–37 to 97)	58% (–39 to 87)	281
2010–11	Trivalent	U.S. Influenza Vaccine Effectiveness Network	2–17	7/61	95/640	9/63	27/182	15% (–110 to 65)	75% (49–88)	177[d]
2013–14	Quadrivalent	U.S. Influenza Vaccine Effectiveness Network	2–17	24/172	132/773	27/182	433/1113	17% (–39 to 51)	60% (36–74)	174
2013–14	Quadrivalent	Postmarketing observational study (ICICLE)	2–17	26/94	145/389	14/82	272/516	13% (–5 to 51)	74% (50–86)	175
2013–14	Trivalent	Sentinel Physician Surveillance Network, Canada	2–19 2–8	1/75 1/41	15/74 11/84	3/77 2/42	26/185 7/80	86% (–11 to 98)[e] 83% (–33 to 98)[e]	75% (16–93)[e] 48% (–163 to 90)[e]	282
2013–14	Quadrivalent	Department of Defense (U.S. Air Force School of Aerospace Medicine) mid-season analysis[f]	Not stated	Not stated	Not stated	Not stated	Not stated	40% (–5 to 66)	74% (60–83)	261
2015–16	Quadrivalent	U.S. Influenza Vaccine Effectiveness Network	2–17	23/156	Not stated	41/174	Not stated	–21% (not stated but includes 0)	65% (not stated but does not include 0)	179
2015–16	Quadrivalent	Postmarketing observational study (ICICLE)	2–17	Not stated	Not stated	Not stated	Not stated	50% (not stated but includes 0)	71% (not stated but does not include 0)	180
2015–16	Quadrivalent	Department of Defense Laboratory-Based Influenza Surveillance	2–17	23/156	Not stated	18/156	Not stated	15% (not stated but includes 0)	68% (not stated but does not include 0)	179

[a]Not significant in primary analysis: only if vaccination >7 d, rather than >14 d, prior to illness onset.
[b]Overall (ages 0.5–65 y).
[c]10–49 y.
[d]Chung et al.[177] also discuss the 2013–14 data reported by Gaglani et al.[174]
[e]Unadjusted VE estimates. Adjusted VE was not estimated because of small sample size.
[f]Mid-season analysis. Analysis by subtype not performed.
CI, confidence interval; ICICLE, Influenza Clinical Investigation for Children postmarketing study sponsored by Astra-Zeneca; IIV, inactivated influenza vaccine; LAIV, live attenuated influenza vaccine; VE, vaccine efficacy.

estimates for IIV against H1N1pdm09 were similar and the Q/LAIV was less effective than IIV in both analyses. The point estimates for VE against any influenza for LAIV also differed greatly between the two studies (46% [95% CI, 7–69] in the ICICLE study versus 3% in the U.S. Influenza Vaccine Effectiveness Network study). Although it is widely acknowledged that observational studies have several limitations, the reported studies used a test-negative study design and were conducted in similar numbers of subjects. As of the time of writing, there is no clear explanation for the discordance in the findings with respect to effectiveness of Q/LAIV from the various studies conducted to date. This question notwithstanding, findings from the U.S. Influenza Vaccine Effectiveness Network interim analysis for the 2015–16 influenza season further fueled concerns that the H1N1pdm09 component of LAIV is particularly problematic, which led to the recommendation from the ACIP that Q/LAIV not be used for the 2016–17 influenza season (see "Indications for the Use of Live Attenuated Influenza Vaccine to Prevent Influenza" below). Notably, in a report published after the ACIP recommendation, trivalent LAIV and IIV were shown to be similarly effective in preventing RT-PCR–confirmed H1N1 influenza in the 2013–14 season in a cluster randomized trial in Hutterite communities in Canada, with a hazard ratio of 1.09 (95% CI, 0.34–3.49), although vaccine effectiveness was not assessed because the study did not include a placebo group.[250]

Live Attenuated Influenza Vaccine Against Other Influenza Viruses of Pandemic Potential

Although pLAIVs against other influenza viruses with pandemic potential have not been licensed, potential pLAIVs on the A/AA ca and Len/17 ca virus backbones are being evaluated.[71,262] Reassortant A/AA ca vaccine viruses against H9N2, H5N1, H7N3, H7N7, H6N1, and H2N2 subtypes of influenza were generated and evaluated in preclinical studies and in clinical trials.[191,263–267] In Phase I clinical trials in healthy adults, the A/AA ca pLAIV viruses were found to be safe, but were highly restricted in replication and were variably immunogenic.[191,263–267] Despite the absence of robust detectable antibody responses to the primary vaccination with pLAIV, subjects who received either H5N1 or H7N7 pLAIV exhibited rapid and robust antibody responses when they received a single dose of inactivated subvirion vaccine (ISV) of the same subtype years after they had received pLAIV.[191,192] Table 32.9 summarizes these findings. In the case of H5N1, subjects received ISV almost 5 years after receipt of H5N1 pLAIV, and the antibody responses to ISV in pLAIV-primed individuals occurred at higher frequency, were of higher titer, and higher affinity than in unprimed subjects.[192] In addition, the antibody in H5N1 pLAIV-primed subjects cross-reacted with several antigenically divergent viruses of other H5N1 clades. Similar observations were seen in subjects primed with H7N7 pLAIV who received an H7N7 ISV approximately 2 years later[191,268]: sera from primed subjects was of higher titer and cross-reacted with antigenically distinct H7 influenza viruses, including the recently emerged H7N9 influenza virus.[191,269] These findings suggest that receipt of pLAIV results in a long-lasting B-cell memory response. The observations have significant implications for pandemic preparedness, and studies evaluating the optimal interval between priming and boosting, as well as the order in which the LAIV and ISV are administered are underway. Clinical studies with pLAIV will be highly informative for determining the correlates of protection for LAIV.

In response to the emergence of H7N9 avian influenza in China in 2013, a candidate H7N9 LAIV was generated, bearing the HA and NA of the A/Anhui/1/2013 (H7N9) virus. This candidate vaccine was immunogenic and efficacious in ferrets[270] and is currently being evaluated in Phase I clinical trials. Pandemic LAIV based on the Russian Len/17 ca donor strain are also in development.[262] Several other approaches to the development of live attenuated vaccines for pandemic influenza are being pursued. An H5N1 vaccine based on an NS1 deletion mutant[271,272] was tested in a Phase I clinical trial in Europe. Shedding of the vaccine virus was not observed in any of the subjects, and it was found to be safe and immunogenic.[273] Cytoplasmic tail mutants of M2[52,274] and Newcastle disease virus–vectored vaccines[275] are also being explored, but they are still in the preclinical stage of evaluation.

INDICATIONS FOR USE OF LIVE ATTENUATED INFLUENZA VACCINE TO PREVENT INFLUENZA

LAIV is approved for use by the FDA for active immunization to prevent disease caused by influenza A and B viruses in healthy children, adolescents, and adults, between 2 and 49 years of age. In 2014, the ACIP issued a recommendation that when immediately available, LAIV should be used rather than IIV for healthy children 2 to 8 years of age who have no contraindications or precautions.[276] The guidelines also stated that IIV should be used if LAIV was not available, and vaccination should not be delayed if LAIV was not available.

TABLE 32.9 Immunologic Priming by H5 and H7 Pandemic LAIV

pLAIV				Serum HAI Antibody Titer at Indicated Time Point				
							Responders (Day 28 Post-ISV)[a]	
Vaccine	No. of Doses	ISV, Dose	Interval Between pLAIV and ISV	N	Pre-ISV GMT (Range); All Subjects	Post-ISV Day 28 GMT (Range); All Subjects	%	GMT
H5N1 VN04[b]	2	H5N1 VN04, 45 μg	56 mo	11	5[b]	87[c] (5–1280)	73	222
H5N1 HK03[b]	2	H5N1 VN04, 45 μg	54 mo	10	5[d]	29[c] (5–480)	50	146
H7N7 NL03[e]	2	H7N7 NL03, 45 μg	19–24 mo[f]	13	2[d]	181[g] (2–512)	69	234

[a]Response defined as ≥4-fold rise in titer after receipt of ISV.
[b]Talaat et al.[192]
[c]HAI titer vs H5N1 VN04 wild-type virus.
[d]Undetectable HAI titer was assigned a value of 5 for the H5N1 study and 2 for the H7N7 study.
[e]Babu et al.[191]
[f]Two separate cohorts.
[g]HAI titer vs H7N7 NL03 ca vaccine virus.
GMT, geometric mean titer; HAI, hemagglutination-inhibiting; ISV, inactivated subvirion vaccine; LAIV, live attenuated influenza vaccine; pLAIV, pandemic live attenuated influenza vaccine.

This preferential recommendation was based on the available efficacy data comparing LAIV and IIV in children. The age range was based on the existing upper age limit for receipt of two doses of LAIV in naïve children. However, this recommendation was reversed in March 2015 when low effectiveness of LAIV against H1N1 influenza in some previous seasons was reported.[100,176]

In 2016, based on estimates from the U.S. Influenza Vaccine Effectiveness Network of low effectiveness of LAIV against H1N1pdm09 in 2015–16 and data from prior influenza seasons since 2010 that contrast with higher and statistically significant effectiveness of IIV in the same studies, the ACIP recommended against the use of LAIV in the United States for the 2016–17 influenza season. The FDA, however, issued a position statement that the agency continues to find that the benefits of Q/LAIV outweigh potential risks and that no specific regulatory action is warranted at this time.[277] The FDA statement cited the inherent limitations of the observational studies upon which the ACIP recommendation was based, as well as the variability of influenza vaccine effectiveness across seasons. In the United Kingdom, where Q/LAIV is the cornerstone of the childhood influenza vaccination program, Public Health England found no reason to change the current recommendations regarding the use of Q/LAIV.[278,279] Effectiveness data from studies conducted in the United Kingdom and Finland, which demonstrated overall effectiveness of Q/LAIV in 2015–16,[180] informed their decision.

Prior to the ACIP recommendation for 2016–17, the ACIP guidelines for use of LAIV recommended that children 2 to 8 years of age should receive two doses at least 4 weeks apart in their first year of influenza vaccination; children in this age group who received at least two prior doses of influenza vaccine in the past, require only one dose. For persons 9 to 49 years of age, it was recommended that a single dose annually prior to exposure to influenza should be administered. In contrast to IIV, which has been used largely in populations at high risk for death or other complications of influenza, LAIV is currently indicated to prevent influenza in persons without underlying high-risk conditions. Table 32.10 compares and contrasts LAIV and IIV.

The age range up to 49 years was selected for initial clinical use in healthy persons because of the adequacy of safety and efficacy data in that age range. The effectiveness trial in healthy working adults was specifically designed to examine the clinical benefits of LAIV compared with placebo in all persons 18 to 64 years old, but there were limited effectiveness data in the subgroup of 50- to 64-year-olds, a population universally recommended to receive trivalent inactivated vaccine.[78] Post hoc analysis among persons 50 to 64 years of age (641 [14%] of the 4561 adults) did not reveal statistically significant reductions in illness measures (proportion, episodes, or days of illness), but there were significant reductions in illness-associated missed work days and visits to healthcare providers. In a similarly designed effectiveness study in more than 1000 adults 18 to 64 years of age conducted by the CDC, TIV was compared with placebo in the same season; there were no reductions in any illness measures in any age group.[168] In Canada, the upper age limit for LAIV is 59 years.

SPECIAL CONSIDERATIONS AND CONTRAINDICATIONS

The following precautions should be considered before vaccinating with Q/LAIV in the United States:

- Q/LAIV is not indicated for persons younger than 2 years of age.
- Q/LAIV is not indicated for persons 50 years of age and older.
- Q/LAIV is contraindicated for persons with a history of hypersensitivity, especially anaphylaxis, to any component in LAIV, including eggs.
- Q/LAIV is contraindicated for children or adolescents receiving aspirin or other salicylates because of the association of Reye syndrome with wild-type influenza infection.
- Persons with a history of GBS should not receive Q/LAIV.

TABLE 32.10 Comparison of Live Attenuated Influenza Vaccine (LAIV) and Inactivated Influenza Virus Vaccine (IIV)

	LAIV	IIV
Route of administration	Intranasal spray	Intramuscular injection
Type of vaccine	Live virus	Killed virus
Number of included virus strains	4 (2 influenza A, 2 influenza B)	4 (2 influenza A, 2 influenza B) Trivalent formulation is also available (2 influenza A, 1 influenza B)
Vaccine virus strains updated	Annually	Annually
Frequency of administration	Annual	Annual
Can be administered to children and adults at high risk[a] from complications arising from influenza	No	Yes
Can be simultaneously administered with other vaccines	Yes[b]	Yes[c]
If not simultaneously administered, can be administered within 4 weeks of another live vaccine	Yes, but prudent to wait 4 weeks	Yes
If not simultaneously administered, can be administered within weeks of an inactivated vaccine	Yes	Yes

[a]Populations at high risk from complications of influenza infection include persons older than 65 years of age; residents of nursing homes and other chronic-care facilities that house persons with chronic medical conditions; adults and children with chronic disorders of the pulmonary or cardiovascular systems; adults and children with chronic metabolic diseases (including diabetes mellitus), renal dysfunction, hemoglobinopathies, or immunosuppression; children and adolescents receiving long-term aspirin therapy (at risk for developing Reye syndrome after wild-type influenza infection); and women who will be in the second or third trimester of pregnancy during influenza season.

[b]No data exist regarding effect on efficacy; LAIV coadministration has been evaluated systematically only in children with measles-mumps-rubella (MMR) II and Varivax vaccines.

[c]Trivalent inactivated influenza vaccine coadministration has been evaluated systematically only in adults with pneumococcal polysaccharide vaccine.

- Pregnant women should not receive Q/LAIV unless clearly needed. This recommendation is based on a theoretical concern: there has been no indication of fetal harm from LAIV in animals studies, and it is not known whether LAIV can cause fetal harm when administered to a pregnant woman, or can affect reproductive capacity.
- Persons with asthma, reactive airways disease, or other chronic disorders of the pulmonary or cardiovascular systems; persons with other underlying medical conditions, including metabolic diseases such as diabetes, renal dysfunction, and hemoglobinopathies; and persons with known or suspected immune deficiency diseases or receiving immunosuppressive therapies should not receive Q/LAIV.

If close contacts of severely immunosuppressed persons (e.g., bone marrow transplant patients while they are in a protected environment) are given Q/LAIV, they should avoid close contact with the immunosuppressed patient for 7 days.

For vaccination of healthy persons 2 to 49 years of age in close contact with members of all other immunocompetent high-risk groups (e.g., persons with heart disease, lung disease, or diabetes), either IIV or Q/LAIV is an acceptable vaccination option.

TIMING OF LIVE ATTENUATED INFLUENZA VACCINE ADMINISTRATION

Vaccination with LAIV can begin as soon as vaccine is available for the season. Children younger than 9 years old receiving LAIV for the first time should begin in October or earlier in the Northern Hemisphere, because they will need a second dose a minimum of 4 weeks after the initial dose. It was observed that different two-dose regimens of TIV alone, LAIV alone, LAIV followed by TIV, or TIV followed by LAIV administered to children 6 to 35 months of age at an interval of 1 month were well tolerated and induced similar levels of HAI antibody.[146] The level of replication of LAIV was compared in children who were vaccinated with LAIV or TIV a month earlier. Children who were previously vaccinated with LAIV had less replication of the subsequent dose of LAIV than children who were vaccinated with TIV a month earlier.[135] Both studies were conducted with a small number of children, but the findings suggest that flexibility in immunization regimens may be an effective approach to vaccination of young children against influenza. The safety and immunogenicity of concurrent administration of LAIV with measles-mumps-rubella II and Varivax vaccines have been evaluated in children 12 to 15 months of age[280]; they were safe and well tolerated, and the immune responses to the relevant viral antigens were similar whether the vaccines were given concurrently or separately. In the absence of specific data indicating lack of interference with other vaccines, it is prudent to follow the ACIP General Recommendations on Immunization. Inactivated vaccines do not interfere with the immune response to other inactivated vaccines or to live vaccines. An inactivated vaccine can be administered either simultaneously or at any time before or after LAIV. A live vaccine not administered on the same day should be administered after a 4-week (or longer) interval when possible.

Administration of Live Attenuated Influenza Vaccine and Influenza Antiviral Use

It is not known whether administering influenza antiviral medications affects the safety or efficacy of LAIV; LAIV should not be administered until 48 hours after cessation of influenza antiviral therapy, and influenza antiviral medications should not be administered for 2 weeks after receipt of LAIV.

CONCLUSIONS

LAIV has the potential to significantly contribute to the control of influenza and influenza-associated illnesses. LAIV has significant advantages in convenience of administration. The observation of high efficacy of LAIV compared with TIV against matched strains and, in some seasons, drifted strains, are compelling reasons to use the vaccine in children. Effectiveness of LAIV in adults has also been demonstrated. However, low effectiveness of LAIV reported in some studies with the quadrivalent vaccine in recent influenza seasons, particularly in the face of significant effectiveness on IIV, is of concern. Efforts to further investigate these findings and identify contributing factors should be undertaken to ensure the continued availability of this important public health measure to protect against influenza infection and illness.

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33 Japanese Encephalitis Vaccines

Scott B. Halstead, Susan L. Hills, and Katrin Dubischar

Japanese encephalitis (JE), a mosquito-borne flavivirus infection, is the leading recognized cause of childhood encephalitis in Asia. Despite JE being vaccine preventable, thousands of cases and deaths are reported annually.[1] However, in many locations, the disease is not under systematic surveillance, and official reports undoubtedly underestimate the true number of cases.[2–4] JE virus (JEV) is transmitted throughout most of Asia and parts of the western Pacific, in a region supporting 3.2 billion people—almost 50% of the world's population (Fig. 33.1). Consequently, regional JE-associated morbidity probably exceeds worldwide morbidity from herpes encephalitis.[3,5,6] Because of the near-eradication of poliomyelitis, JE is the leading cause of childhood viral neurologic infection and disability in Asia.[7] The integration of JE vaccine into routine immunization programs in some of the higher-income Asian countries—including Japan, South Korea, Taiwan, and Thailand—has led to its near elimination, with the greatest disease burden now in many of the least-developed countries in Asia (Fig. 33.2).[7] By any standard, JE is a major public health problem. Fortunately, it is controllable by proven effective vaccines.[8]

HISTORY OF DISEASE

Summer-fall encephalitis outbreaks consistent with JE were recorded in Japan as early as 1871, the largest of which, in 1924, led to more than 6000 cases, with more than 60% of them fatal.[9] A filterable agent from human brain tissue was isolated in rabbits that year, and in 1934, Hayashi transmitted the disease experimentally to monkeys.[10] Soon after, the isolation of JE and the related St. Louis encephalitis (SLE) viruses made possible serologic confirmation of encephalitis cases occurring elsewhere in the region, including two cases in Americans acquired in China and Japan in 1934 and 1935.[11] The virus initially was called Japanese B encephalitis (the modifying "B" has since fallen into disuse) to distinguish the disease from von Economo type A encephalitis, which had different clinical and epidemiologic characteristics. The mosquito-borne mode of JE transmission was confirmed with the isolation of JEV from *Culex tritaeniorhynchus* mosquitoes in 1938. Subsequent field studies established the role of aquatic birds and pigs in the viral enzootic cycle. Viruses isolated from human cases in Japan in 1935 and Beijing in 1948 provided the prototype Nakayama, and Beijing and P3 strains, respectively. These strains were widely used in vaccine production for many years.

During the first half of the 20th century, JE was recognized principally in temperate areas of Asia with perennial outbreaks in Japan, Korea, and China.[12] Annual outbreaks of several thousand cases recurred in Japan until 1966. In Korea, 5616 cases and 2729 deaths were recorded in 1949 when JE became a notifiable disease, and epidemics continued every 2 or 3 years, culminating in an unprecedented 6897 cases in 1958.[13] As recently as 1982, 1197 cases were reported in South Korea.[14] JE also had a substantial impact on U.S. servicemen in Korea, including approximately 300 cases recorded in 1950.[15] In China, reporting of JE cases began in 1951, and many provinces recorded outbreaks in the early 1950s.[16,17] Between 1965 and 1975, more than 1 million cases were reported, almost 175,000 in 1971 alone. Until the 1990s, 20,000 to 40,000 cases were recorded annually from China (Fig. 33.3).[16,17] Since widespread use of JE vaccine in Japan from 1967, Taiwan from 1968, and Republic of Korea from 1983, JE epidemics have not occurred in these countries (see Fig. 33.2). While the combination of vaccination with occupational and environmental changes has led to the near elimination of the disease in humans, there have not been corresponding changes in zoonotic circulation of virus (Fig. 33.4). JE vaccine has also played a major role in reducing national JE incidence in China since its wider use in the late 1980s. From 2001 through 2007, fewer than 10,000 cases were reported annually, and since 2008, when JE vaccine was included in the national Expanded Program on Immunization (EPI) and the central government assumed the full cost of JE vaccination in all endemic provinces, fewer than 5000 cases have been reported each year.[17,18]

In Southeast Asia, although sporadic viral encephalitis cases were noted from early in the 20th century, JE was not recognized as a major public health problem until 1969, when an epidemic was reported from the Chiang Mai Valley in northern Thailand.[19] Yearly outbreaks producing thousands of cases and hundreds of deaths followed in the northern region, and JE became recognized as a leading cause of childhood mortality and disability.[20,21] This led to a successful effort to introduce JE vaccine as a routine childhood immunization. Subsequently, in 1974, the first of several epidemics was recorded in an area of Myanmar (Burma) adjacent to the Chiang Mai Valley.[22,23] In Vietnam, epidemics occurred in the late 1960s and during the 1970s, with incidence rates exceeding 18 per 100,000 population in some years.[12,24] After reinstatement of notification in the late 1970s, several thousand JE cases were reported annually. During the 1970s, JE was also recognized as an important cause of encephalitis in the Philippines, Malaysia, and Singapore.[25,26] Subsequently, there has been increased recognition of disease burden from JE throughout Southeast Asia and several countries have introduced JE vaccination programs.

In South Asia, serosurveys in 1952 and serologically confirmed clinical cases in 1955 in India marked the first recognition of disease in the region.[27,28] Outbreaks in India were documented only in southern areas until 1973 when a large outbreak occurred in the Burdwan and Bankura districts of West Bengal.[29] Large JE outbreaks, often involving adults, subsequently were reported from various Indian states. In 1978, Nepal experienced its first JE epidemic in the southern Terai region along the Indian border, with 422 cases and 119 deaths, and large outbreaks continued to regularly occur.[24,30] Although virus was isolated in Sri Lanka in 1968 and endemic transmission had occurred for many decades, the first major outbreak occurred in 1985.[31,32] Elsewhere in South Asia, Bangladesh reported a small outbreak in 1977, but following that the disease was not recognized and no systematic assessments occurred until 2003.[33,34] In the west, JEV has spread as far as the Indus valley in Pakistan.[35] Despite large-scale vaccination programs, JE remains a problem in India, while cases have markedly declined in Nepal and Sri Lanka (Fig. 33.5).

In the western Pacific region, self-limited outbreaks occurred on the islands of Guam in 1947 and Saipan in

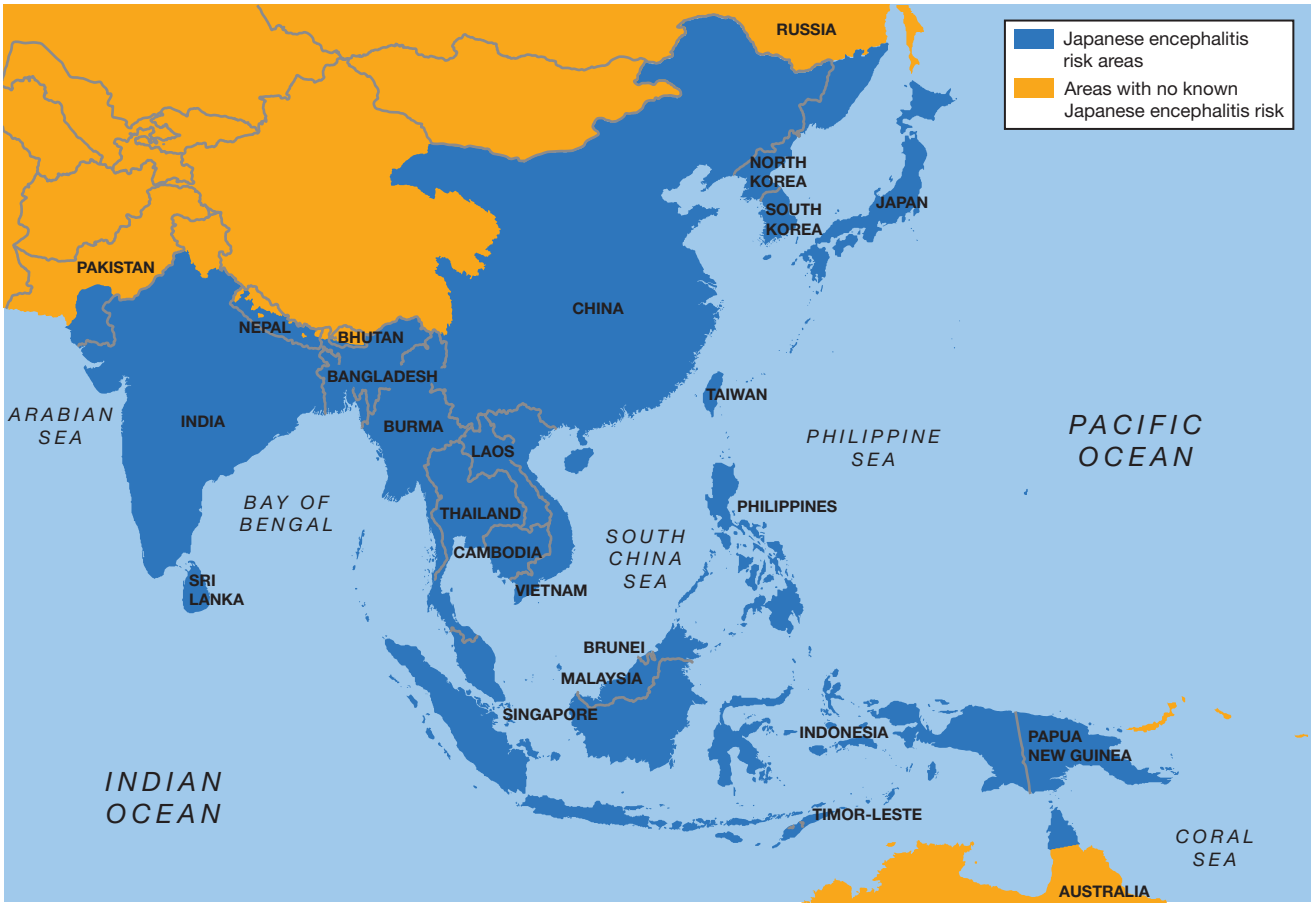


Figure 33.1. Areas with proven or suspected enzootic Japanese encephalitis viral transmission. *(From Centers for Disease Control and Prevention.* CDC Health Information for International Travel 2016. *New York, NY: Oxford University Press; 2016.)*

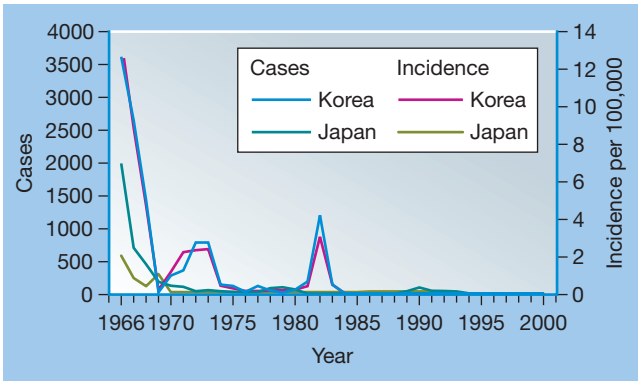


Figure 33.2. Reported Japanese encephalitis cases and incidence, Japan and Korea, 1966–2000. *(Data from World Health Organization reports.)*

1990.[36–38] During the 1990s, southward extension of the geographical range of JEV transmission was noted when cases occurred in northern Australia. JE cases were first reported in the outer Torres Strait Islands in 1995, and a case was detected on Cape York Peninsula on the northern mainland of Australia in 1998.[39,40]

WHY THE DISEASE IS IMPORTANT

JEV is the most common vaccine-preventable cause of encephalitis in Asia. Each year, an estimated 67,900 JE cases occur.[2] Approximately 75% of these cases occur in children younger than 15 years of age, with an annual incidence of 5.4 cases per 100,000 children.[2] There is no antiviral therapy or other therapy available to mitigate the effects of JEV infection, and disease outcome is often severe. The case fatality rate is 20% to 30%, and 30% to 50% of survivors have long-term physical or cognitive sequelae.[41,42] Given its incidence and severity, JE is a major public health problem. JE vaccination has gained wide support from public health authorities with growing recognition of the scope of the problem. However, in many Asian countries, introduction or expansion of vaccination programs is still needed. Additionally, as enzootic transmission of JEV continues even in locations that have achieved good control, high-quality vaccination and surveillance programs must be maintained long term.

BACKGROUND

Clinical Description

The great majority of infections are inapparent, with only one in approximately 250 infections resulting in symptomatic illness in susceptible Asians with higher overt infection rates in non-Asian populations.[43,44] The principal recognized clinical manifestation of illness is encephalitis. Milder clinical manifestations, such as aseptic meningitis and simple febrile illness with headache, can sometimes occur but often escape detection.[15,45–53] Patients occasionally also present with poliomyelitis-like acute flaccid paralysis.[54]

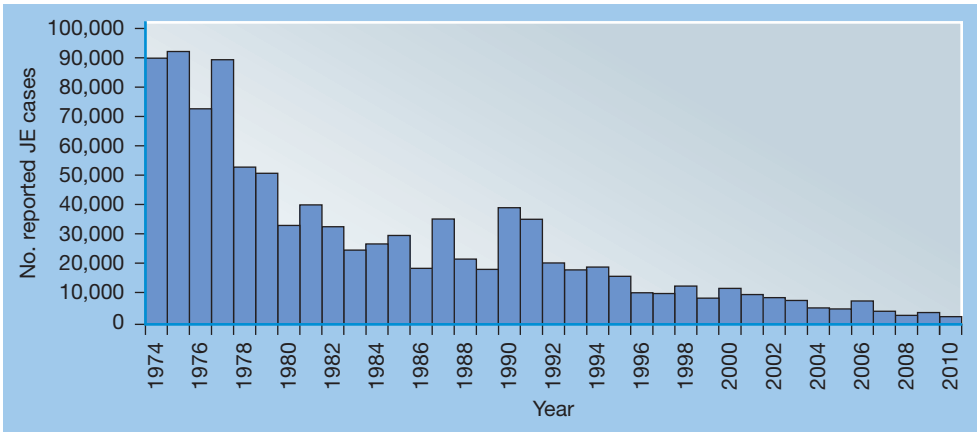


Figure 33.3. Reported cases of Japanese encephalitis (JE) for China, 1974–2010. *(Data from Ministry of Health Beijing, China.)*

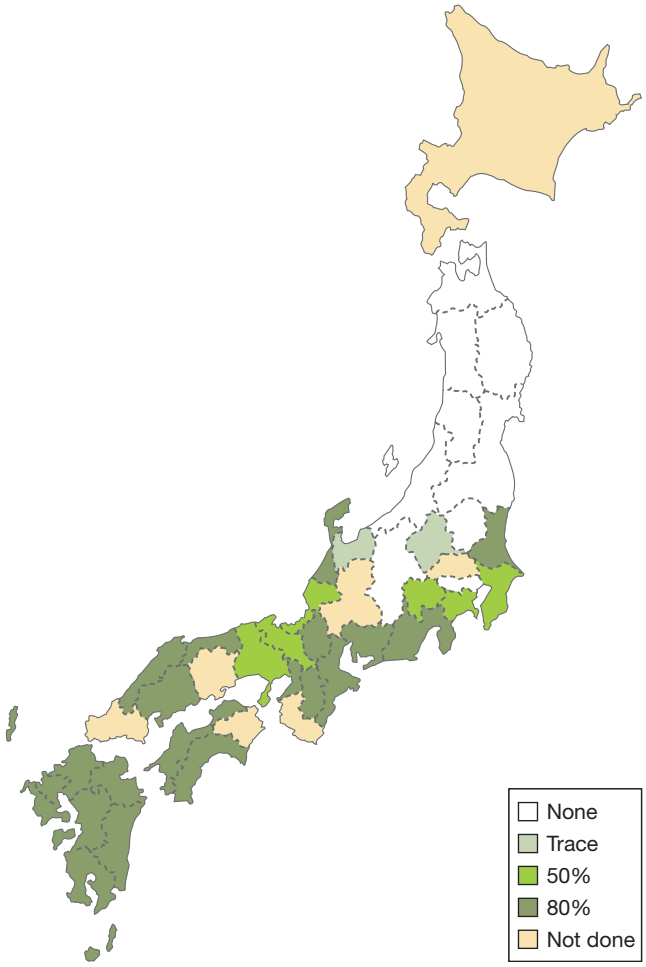


Figure 33.4. Japanese encephalitis seroprevalence in swine by district, Japan, October 1996. The absence of reported human cases belies continued transmission of the virus in its enzootic cycle, as demonstrated by high rates of pig infections. *(From Taniguchi K, Matsunaga Y. National epidemiologic surveillance of vaccine-preventable diseases, Japan, October 1996, with permission.)*

The incubation period is 5 to 15 days. Illness usually begins with abrupt onset of high fever, change in mental status, gastrointestinal symptoms, and headache, followed gradually by disturbances in speech or gait or other motor dysfunction. Irritability, vomiting, and diarrhea or an acute seizure may be the earliest signs of illness in an infant or child. Seizures occur in 55% to 85% of pediatric patients but are less frequent in adults.[53,55–58] A progressive decline in alertness eventually leads to stupor and coma. Patients may become completely unresponsive and require ventilatory assistance (Fig. 33.6). Generalized weakness and changes in muscle tone, especially hypertonia and hyperreflexia, are common, but focal motor deficits indicative of direct brain tissue damage, including paresis, hemiplegia, or tetraplegia; cranial nerve palsies (especially central facial palsy); and abnormal reflexes also may be present (Fig. 33.7). Sensory disturbances are seen less frequently. Central hyperpnea, hypertension, pulmonary edema, and urinary retention may complicate the illness. Although symptoms suggest elevated intracranial pressure in many cases, papilledema and other signs of increased intracranial pressure are rare. Signs of extrapyramidal involvement, including tremor, mask-like facies, rigidity, and choreoathetoid movements, are characteristic of JE, but these signs may be obscured initially by generalized weakness.

The typical clinical course in patients whose main presentation is poliomyelitis-like acute flaccid paralysis is a brief febrile illness followed by rapid onset of flaccid paralysis in one or more limbs, despite a normal level of consciousness.[59] Weakness occurs more frequently in the legs than in the arms and is usually asymmetric. Approximately 30% subsequently develop encephalitis, with a reduced level of consciousness and upper motor neuron signs. At 1 to 2 years later, persistent weakness and marked wasting in the affected limbs can be found. Flaccid paralysis has also been reported in patients with a more characteristic encephalitic presentation of JE.[15,58,60] In patients with flaccid paralysis, electrophysiological studies have confirmed anterior horn cell damage, and magnetic resonance imaging of the spinal cord has shown abnormal signal intensity on T2-weighted images.[59,61]

During an outbreak in Uttar Pradesh, India, a biphasic illness was described in which 2.7% of patients had recurrent symptoms after apparent recovery.[51] This was further described in a retrospective study in India among 62 patients, six of whom had early relapse characterized by progressive neural involvement with anterior horn cell damage.[62] Recurrent neurologic signs and symptoms have been observed as late as 8 to 9 months after recovery.[63] Although JEV has been isolated from peripheral blood mononuclear cells from patients with

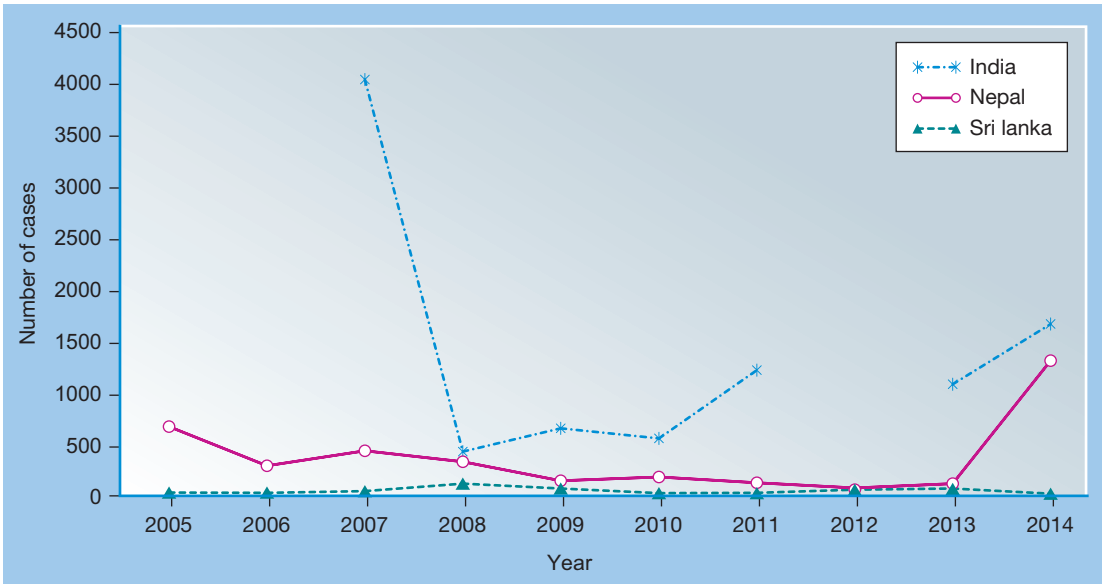


Figure 33.5. Reported Japanese encephalitis cases from India, Nepal, and Sri Lanka, 2005–2014. *(Data from reports to the World Health Organization; no data reported from India in 2005, 2006, and 2012.)*



Figure 33.6. A patient with Japanese encephalitis receiving respiratory support. Note hand ventilation. Hanoi, Vietnam, 2003.



Figure 33.7. Child with Japanese encephalitis carried to the hospital, Gorakhpur, India, 2005. During the epidemic peak, 40 patients were admitted to the hospital every day, with up to 20 deaths daily.

recurrent neurologic disease, a rise in antibody titers has not been observed.[63] Recurrent neurologic disease does not necessarily result in poorer outcome.[62,63]

Complications

Reports of case-fatality and disability rates vary greatly. Of the cases, 5% to 30% are fatal.[37,50,52,55,56,64–73] Some deaths occur after a brief prodrome and fulminant course lasting a few days, whereas others occur after a more protracted course with persistent coma. Severity may vary by age. Young children (younger than 10 years of age) are reported to be more likely than adults to die, and, if they survive, they are more likely to have residual or more severe neurologic deficits.[37,55,65] Disability rates vary widely, with study findings showing sequelae in 45% to 65% of patients within the first year and in 30% to 80% of patients up to 10 years later.[37,50,52,55,56,64–73,75] However, differences in rates partly reflect the intensity of the follow-up examination, including type of neurologic sequelae examined and use of formal cognitive and neurologic examinations, completeness of the follow-up examination, and whether calculations were based on the total patient cohort or survivors (Fig. 33.8). The reported rate of long-term, severe neurological disability among survivors reaches as high as 45%.[52,56,65,66]



Figure 33.8. A patient recovering from Japanese encephalitis (JE) in a private clinic in Adoni, India, 2002. Although JE is reportable in India, private clinics do not report into the system, resulting in underreporting.

Principal sequelae fall into three main categories: psychosocial, intellectual, and physical. Deficits can include memory loss, impaired cognition, behavioral disturbances, seizures, motor weakness or paralysis, and abnormalities of tone and coordination. Over time, abnormalities can improve or eventually resolve. However, some sequelae can worsen and new abnormalities can develop. In China, some patients developed additional neurologic signs and symptoms 3 to 17 years after acute infection, including optic nerve degeneration and seizures.[76]

A poor prognosis is associated with a short prodromal interval, deep obtundation at hospital admission, irregular and central hyperpneic respiratory patterns, focal deficits, status epilepticus, the presence of extrapyramidal signs, flexor and extensor posturing, and pathologic reflexes.[52–54,68,77–79] In some locations, more than 25% of JE cases have concurrent neurocysticercosis, which increases the risk for JEV neuroinvasive disease and contributes to higher JE mortality rates.[80–82] There is evidence that preexisting dengue immunity is associated with better outcome of JE, but this is not true of yellow fever (YF) vaccination and is unknown for other cocirculating flaviviruses like West Nile virus.[83–85]

Virology

The JEV is one of more than 70 viruses in the *Flavivirus* genus of the *Flaviviridae* family.[86,87] Morphologically, flaviviruses are spherical, positive, single-stranded RNA, approximately 40 to 50 nm in diameter, with a lipid membrane enclosing an isometric 30-nm–diameter nucleocapsid core.[88] Membrane surface projections are composed of a glycosylated envelope (E) and membrane (M) protein, a mature form of the premembrane (prM) protein. The JE viral RNA, 10,976 bases long, encodes an uninterrupted open reading frame, flanked by 95- and 585-base untranslated regions at the 5′ and 3′ ends, respectively.[89,90] Protein translation at the first encountered AUG codon near the 5′ end yields a polyprotein precursor of 3432 amino acids that is cotranslationally or posttranslationally processed by a virus-specific nonstructural (NS) protease complex, NS2B-NS3, host cell signal peptidase, or an unidentified host cell–specific protease into at least 10 mature viral proteins. The order of proteins encoded in the JEV open reading frame, as with other flaviviruses, is 5′-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3′.

Flaviviruses replicate in a variety of cultured cells of vertebrate and arthropod origin. Viral entry occurs by receptor-mediated endocytosis, with the formation of coated vesicles, or by direct fusion with endosomal membranes.[91–94] The nucleocapsid is uncoated by acid-dependent fusion of viral and endosomal membranes, releasing genomic RNA into the cytoplasm, where viral replication continues with immediate translation of the uncoated viral genome.[94] The translated polyprotein then is processed and assembled into a virus-specific replication complex.

The hydrophobic carboxyl terminus of the E protein provides a membrane-associated anchor, while an extensive ectodomain, stabilized by disulfide bridging, is folded into three structural and antigenic domains (I, II, and III) that are variably related to determinants representing flaviviral group, subgroup, and virus-specific epitopes and biologic functions.[95] Binding of JE virions to certain cells of the central nervous system (CNS) lineage may be associated with the presence of specific neurotransmitter receptors.[96] Dengue-2 virus selectively binds to cellular heparan sulfates of the glycosaminoglycan family via E protein glycosaminoglycan-binding motifs within the carboxyl terminal and externally accessible regions of domains I and III.[97,98] Similar mechanisms may apply to JEV and other flaviviruses.[99]

Virus-specific and cross-reactive neutralizing epitopes have been mapped to specific regions of the flavivirus E glycoprotein.[100,101] Comprehensive cross-neutralization studies indicate the close antigenic relationship of JEV to SLE, West Nile, Koutango, and Usutu viruses and several flaviviruses found in Australia (e.g., Murray Valley encephalitis, Alfuy, Stratford, and Kokobera viruses) with their classification into a single antigenic complex.[102,103] No serologic cross-reactivity with hepatitis C virus has been observed.[104]

The biochemical, antigenic, and genetic relationships of JEVs isolated from different geographic regions and at various times have been compared by using polyclonal and monoclonal antibodies, two-dimensional gel electrophoresis of T1 ribonuclease-digested virion RNA, and genomic sequencing.[105–107] The molecular phylogeny of JEVs, based on the 240-base nucleotide sequence of viral prM, divides JE isolates into five distinct genotypes (Gs), with a maximum divergence of 21% among the isolates (Table 33.1).[108–114] GIII has been the source of numerous JE epidemics throughout history and was the most frequently isolated genotype throughout most of Asia from 1935 until the 1990s. Three genotypes (I, III, and V) were confirmed to be cocirculating in China in both high- and low-prevalence areas.[115] GIII consisted of two clades (mainland clade and Taiwan clade). Two recent evolutionary studies have examined the genotype displacement of GIII by GI in China.[116,117] Compared to the mainland GIII clade, GI and the Taiwan clade were newly introduced and evolved more rapidly. In recent years, multiple reports have indicated that GI has displaced GIII as the most frequently isolated virus genotype in a number of Asian countries,[118] including Thailand,[119] Korea,[120] Japan,[111] Malaysia,[121] Vietnam,[122] India,[123] and Taiwan.[124] A recent study has reconstructed the spatiotemporal chronology of the emergence of GI throughout Asia and identified genetic determinants underlying the genotype displacement as it unfolded across Asia. A possible explanation of this displacement was the observed increase in vitro multiplicative ability of GI viruses compared to GIII viruses in avian and/or mosquito cells, suggesting an increase in the efficacy of mosquito-avian mosquito transmission cycles in nature.[125]

TABLE 33.1 Japanese Encephalitis Viruses by Genotype and Countries With Early Isolations of Specific Genotypes

Genotype	Country or Region (Year[s] of Isolation)
I	Japan (1935, 1955, 1957, 1959, 1979, 1982) China (1949, 1960) Korea (1982, 1987, 1991, 1994) Okinawa (1968–1992) Taiwan (1972, 1987) Philippines (1977, 1984) Vietnam (1964–1988) Nepal (1985) India (1963, 1970, 1972, 1975, 1978–1980, 1982, 1985) Sri Lanka (1969, 1987)
II	Thailand (1979, 1982–1985, 1992, 1993) Cambodia (1969)
III	Indonesia (1970, 1978, 1979, 1981) Thailand (1983) Malaysia (1970) Sarawak (1968) Australia (1995)
IV	Indonesia (1981)
V	Singapore (1994)

Data from references 108–114, 117, and 125–128.

An antigenic analysis using five virus-specific monoclonal antibodies classified strains into four antigenic types, without correspondence to the aforementioned genotypes.[129]

The JEV isolates from the same region but from different years show a high degree of nucleotide similarity. The 16 Vietnam and 23 Okinawa strains of JEV isolated between 1964 and 1988 and between 1968 and 1992 differed by only 3.2% and 4%, respectively.[108,111] However, viruses could be distinguished chronologically before and after 1986 in Okinawa and before and after 1975 in Vietnam. RNA copying errors and bottlenecking (e.g., survival of a single strain during harsh ecological conditions) seem to be the main mechanisms by which JEV continuously evolves, although novel viral introductions have been documented, indicating the potential for genotypic displacement.[38,112] However, when 92 genomes with complete envelope sequences in the GenBank were analyzed, the level of intergenotypic diversity was less than observed across poliovirus or dengue serotypes. This genetic analysis supports the contention that all known JEV isolates comprise a single serotype from the standpoint of protection.[130] This information is important to the design of JE vaccines.

Pathogenesis as It Relates to Prevention

After an infectious mosquito bite, viral replication occurs locally and in regional lymph nodes. Virions disseminate to secondary sites, where further replication contributes to low-level viremia. Invasion of the CNS probably occurs from the blood by antipodal transport of virions through vascular endothelial cells.[131,132] Infection in the CNS spreads by viral dissemination through the extracellular space or by direct intercellular spread.[133] Sensitized T-helper (Th) cells stimulate an inflammatory response by recruiting macrophages and lymphocytes to the perivascular space, parenchyma, and CNS, where the inflammatory response clears infected neurons, with subsequent formation of glial nodules.[131,134–136] The predominant cell type in the cerebrospinal fluid (CSF) and in the parenchyma is the helper-inducer (CD4) T cell, with B lymphocytes confined chiefly to the perivascular space.[135,137]

Pathologic abnormalities are found chiefly in the CNS; however, inflammatory changes in the myocardium and lung and hyperplasia of reticuloendothelial cells in the spleen, liver, and lymph nodes have been described.[134] Cerebral edema and congested leptomeninges are visible on gross examination of the brain, and "punched-out" necrolytic lesions in the gray matter may be seen.[134,136,138,139] Histopathologic examination discloses a pattern of diffuse microglial proliferation with nodular formation around dead or degenerating neurons, in which viral antigen can be demonstrated by immunohistochemical staining.[131,136] Viral antigen is distributed principally in the thalamus, midbrain, hippocampus, and temporal cortex, but also in Purkinje and granular cells of the cerebellum and in the brainstem reticular formation. Viral antigen also has been demonstrated in well-preserved neurons independent of glial reaction, well after the acute phase of illness, suggesting intracellular viral persistence.[140] Gliomesenchymal nodules are seen in a parallel distribution within the brain and anterior horn of the spinal cord. In patients dying with residual neurologic impairment several years after resolution of the acute illness, scarred rarefied foci are found in a characteristic distribution in the thalamus, substantia nigra, and hippocampus.[140] Virus has also been isolated from peripheral blood mononuclear cells 8 months after initial infection in three (38%) of eight asymptomatic children after cocultivation with primary mouse embryo fibroblasts.[63]

Why only one in several hundred infections in nonimmune residents of endemic areas develops into symptomatic neuroinvasive disease is unclear. It must be concluded that circulating JEV delivered by mosquito subcutaneously has poor capability of reaching the human brain even in genetically susceptible whites.[43] Some rare event or host factor may control the invasion of the CNS. Factors that contribute to neuroinvasion include a somewhat increased risk of encephalitis in children compared with adults, as observed in Guam, and, based on work in laboratory animals, genetic host factors.[36,141–144] Selective pressure for genetic resistance to infection in populations with long exposure to a fatal viral infection may explain the observed higher case-infection ratios in foreigners visiting endemic areas than in resident Asian populations. Innate immunity, such as levels of interferon-α and some genetic factors that affect cellular proteins, may be related to whether clinical illness develops in individual people. Viral replication is dependent on normal cellular proteins, while some host cells have allelic forms of these proteins that may be less supportive of or interfere with viral replication.[145,146] This is an example of the kind of cellular mechanism that may operate to reduce morbidity in Asian compared with non-Asian populations.

Macrophages are important in the nonspecific clearance of virus, and their depletion leads to extended viremia, CNS invasion, and death in experimentally infected mice.[147] However, circulating antibody fulfills a critical role in modulating infection by limiting viremia in the preneuroinvasive phase. Both JEV-specific and heterologous (e.g., dengue) antibodies contribute to protection, and low levels of neutralizing antibody may be sufficient to prevent viremia.[83,148–151] Guinea pigs or mice previously immunized with attenuated JE vaccine but without detectable neutralizing antibodies (<1:4) were protected against intraperitoneal challenge infection, and, their sera passively protected mice.[152] Experimentally infected monkeys immunosuppressed with cyclophosphamide had no measurable antibody response and exhibited increased susceptibility to paralytic encephalitis and a diminished CNS inflammatory reaction.[153] Conversely, passive transfer of JE

monoclonal antibodies after active infection enhanced neurovirulence in intracerebrally challenged mice.[154]

Clinically, high CSF interferon-α levels and low CSF virus-specific immunoglobulin (Ig) M and IgG antibodies have been associated with a fatal outcome, suggesting that delayed or poor local antibody response and uninhibited CNS virus proliferation determine outcome.[155,156] The reactivity of intrathecal and serum antibodies differs in Western blot analyses, but the relationship of these response patterns to outcome is unknown. Recovered patients develop antibodies to structural and NS proteins and exhibit CD4 and CD8 T-cell proliferative responses to JE viral lysate, rather than structural E and prM/M proteins only.[157] In a large clinical study, only 24% of patients developed a T-lymphocyte response to whole virus or viral E protein (structural proteins), and response was not correlated with survival.[158]

Other studies suggest an important role for immunopathology in the pathogenesis of JE. Antibodies to neural antigens (neurofilament and myelin basic protein) were associated with death, suggesting that neural damage resulted in a destructive autoimmune response.[139,159–161] CSF viral immune complex formation also was associated with mortality, further implicating the possibility of autoimmune injury.

Cell-mediated immune mechanisms have been described in athymic nude mice, which, in contrast with normal adult mice, die or develop an extended illness with secondary viremias after peripheral inoculation.[162,163] Antibodies did not appear in nude mice, indicating the functional importance of Th cells. Interestingly, although virus replicated to high titers in brain tissue, histopathologic evidence of encephalitis was absent, indicating that T cells are required in mediating pathologic changes. Mice immunosuppressed with cyclophosphamide and then immunized with live attenuated vaccine, but not inactivated vaccine, are able to resist experimental challenge. Specific JE viral immunity can be transferred passively with spleen cells from immune mice, including animals immunized with live attenuated JE vaccine, but not from animals immunized with inactivated vaccine.[152,164,165] Both Lyt2.2 and L3T4 cells are needed to protect adult mice against intracerebral challenge, and direct intracerebral introduction of the effector cells is required, suggesting the importance of local T-cell enhancement of antibody production in the CNS.

Conditions that compromise the integrity of the blood-brain barrier have been suspected to increase the risk for neuroinvasion and neurodissemination. Several observations suggest that dual infection with another infectious agent, especially *Taenia solium* in neurocysticercosis, is a risk factor.[80,138,166–168] The incidental finding of cysts in a disproportionate number of JE cases at autopsy suggested their potential contribution to a fatal outcome. There are anecdotal observations of dual herpes and JE viral infections in autopsied human JE cases.[168] The mechanisms by which dual infections apparently augment the risk of symptomatic illness are unclear, but increased CNS dissemination of JEV was shown experimentally in mice infected with both JE and herpes.[168] Other physiologic or structural conditions that compromise the integrity of the cerebral vasculature or the blood–brain barrier also may contribute to risk. Atherosclerotic and hypertensive cerebrovascular diseases are suspected risk factors for SLE, and foreign bodies (e.g., ventricular shunts) have predisposed patients to poliovirus neuroinvasion.[169] Experimental disruption of the blood–brain barrier by microwave irradiation was shown to predispose mice to JE viral neuroinvasion.[170,171]

The impact of HIV infection and AIDS on the outcome of JE has not been reported; however, HIV infection seemed to increase the risk for developing overt SLE or West Nile encephalitis after infection.[170,172]

Diagnosis

Although a history of exposure to mosquitoes in a JE endemic area and certain clinical features may suggest JE, there are no pathognomonic features, and laboratory confirmation, usually by serologic tests, is necessary.[173] The JEV occasionally can be recovered from blood in the preneuroinvasive phase (up to 3 to 7 days after infection), but most patients have low or undetectable levels of viremia by the time of clinical presentation with encephalitis.[45,156,174,175] Virus or viral RNA has been recovered from the CSF in 68% of patients when the highly sensitive system of isolation in *Toxorhynchites splendens* mosquitoes was used, and less frequently with other isolation methods or nucleic acid amplification tests.[176–178] However, neither viral isolation nor detection of viral RNA by nucleic acid amplification test is sufficiently sensitive for routine diagnostic purposes. Viral antigen or viral RNA often can be demonstrated in brain tissue when no virus can be isolated from the same specimen and when viral antibodies are undetectable. The JEV produces cytopathic effects in Vero, $LLCMK_2$ (monkey kidney epithelial cells), and PS (porcine kidney cell line) cells, and kills suckling mice inoculated intracerebrally. C6/36 and AP61 mosquito cell lines and *T. splendens*, inoculated intrathoracically, also are sensitive systems for viral isolation. Infection produces no cytopathic effect in C6/36 cells, so inoculated cultures must be examined for viral antigen by immunofluorescent antibody or other techniques. Viral isolates are readily identified by genetic tests or by immunofluorescent techniques using virus-specific monoclonal antibodies or by neutralization.

The most widely used serologic test is the enzyme-linked immunosorbent assay (ELISA) for detection of IgM antibodies.[156,179–184] JEV-specific IgM antibodies are detectable in CSF of most patients by 4 days after symptom onset and in serum by 7 days after onset.[77,156,177,185] For cases diagnosed during well-established outbreaks, a positive JE IgM ELISA result in an acute phase serum sample should be considered diagnostic. However, for an isolated case of encephalitis or for cases in countries where multiple dengue viruses are endemic, laboratory-confirmed JE requires the detection of JE IgM antibodies in the CSF. If acute samples collected within 10 days of illness onset lack detectable IgM, a convalescent sample from the CSF should be collected and tested.[186]

When plaque-reduction neutralization tests (PRNTs) are available, they can be used to measure virus-specific neutralizing antibodies and to discriminate between cross-reacting antibodies in primary flavivirus infections. In most countries in Asia, routine access to PRNTs is likely to be limited except through the World Health Organization (WHO) JE laboratory network.[8] Evidence of a recent JEV infection can be confirmed by a fourfold or greater rise in virus-specific neutralizing antibodies between acute-phase and convalescent-phase serum specimens collected 2 to 3 weeks apart. Hemagglutination inhibition assays can also be used to measure a fourfold or greater rise in antibodies and confirm acute JEV infection. Vaccination history, date of symptom onset, and information regarding other flaviviruses known to circulate in the geographic area that might cross-react in the assay should be considered when interpreting results. In patients who have been infected with another flavivirus in the past (i.e., who have secondary flavivirus infections) or have been immunized with a flaviviral vaccine (e.g., YF or tickborne encephalitis vaccines), cross-reactive antibodies may make it difficult to identify which flavivirus is causing the patient's illness.

The WHO defines a case of acute encephalitis syndrome (AES) as a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures) after the acute onset of fever (Box 33.1).[186,187] A suspected case

BOX 33.1 World Health Organization–Recommended Case Definition and Laboratory Criteria for Confirmation of JE: Field Tested Version

CLINICAL CASE DEFINITION	A case of acute encephalitis syndrome (AES) is defined as the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures[a]) in a person of any age, at any time of year. Other early clinical findings may include an increase in irritability, somnolence, or abnormal behavior greater than seen with usual febrile illness.
CASE CLASSIFICATION	
Suspected Case	A case that meets the clinical case definition for AES. Suspected cases should be classified in one of the following four ways:
Laboratory-Confirmed JE	A suspected case that has been laboratory-confirmed as JE.
Probable JE	A suspected case that occurs in close geographic and temporal relationship to a laboratory-confirmed case of JE, in the context of an outbreak.
AES, Other Agent	A suspected case in which diagnostic testing is done and a causative agent other than JE virus is identified.
AES, Unknown	A suspected case in which no diagnostic testing is done, in which testing identified no causative agent, or in which the test results were indeterminate.
LABORATORY CRITERIA FOR CONFIRMATION	Clinical signs of JE are indistinguishable from other causes of AES. Laboratory confirmation is, therefore, essential for accurate diagnosis of JE. Laboratory confirmation of a JE virus infection includes the following: 1. Presence of immunoglobulin (Ig) M antibodies specific to the JE virus in a single sample of cerebrospinal fluid (CSF) or serum,[b] as detected by an IgM-capture enzyme-linked immunosorbent assay (ELISA) specifically for the JE virus.[c] 2. Detection of JE virus antigens in tissue by immunohistochemical studies. 3. Detection of JE virus genome in serum, plasma, blood, CSF,[d] or tissue by reverse transcriptase–polymerase chain reaction or an equally sensitive and specific nucleic acid amplification test. 4. Isolation of the JE virus in serum, plasma, blood, CSF,[d] or tissue. 5. Detection of a fourfold or greater rise in antibodies specific to the JE virus as measured by hemagglutination inhibition or plaque reduction neutralization test in serum samples obtained during the acute and convalescent phases of illness. The two specimens for IgG should be obtained at least 14 days apart. The IgG test should be done in parallel with other confirmatory tests to eliminate the possibility of cross-reactivity, as indicated.[c]

Most JE infections are asymptomatic. Therefore, in areas that are highly endemic for JE, it is possible to have AES resulting from a cause other than JE virus and have JE virus–specific IgM antibody present in serum. To avoid implicating asymptomatic JE as the cause of other AES illnesses, sterile collection and testing of a CSF sample from all persons with AES are recommended when feasible.

Only the first 5 to 10 cases of an outbreak need be confirmed through laboratory testing. During periods of epidemic transmission of JE virus, laboratory confirmation of every case may not be necessary.

[a]A simple febrile seizure is one in a child 6 months to younger than 6 years; the only findings are fever and a single generalized seizure lasting less than 15 minutes, and the child recovers consciousness within 60 minutes of the seizure.

[b]A serum sample should be obtained at admission. Because it may not yet be positive in a JE-infected person, a second serum sample should be obtained at discharge, on the 10th day of illness, or at the time of death and tested for presence of JE virus–specific IgM.

[c]Further confirmatory tests (e.g., for cross-reactivity with other flaviviruses circulating in the geographic area) should be done when there is an ongoing dengue or other Flavivirus outbreak; when vaccination coverage is very high; or in cases in areas where there are no epidemiologic and entomological data supportive of JE transmission.

[d]Detection of virus genome or virus isolation in serum, plasma, or blood is specific for JE diagnosis; however, it is not sensitive because virus levels are usually undetectable in a clinically ill person with JE. Therefore, a negative result by these methods should not be used to rule out JE in a suspected case. Similarly, detection of virus genome or virus isolation in CSF is usually found only in fatal cases and, therefore, is not very sensitive and should not be used for ruling out a diagnosis of JE.

JE, Japanese encephalitis.

Modified from Hills S, Dabbagh A, Jacobson J, et al. Evidence and rationale for the World Health Organization recommended standards for Japanese encephalitis surveillance. BMC Infect Dis. *2009;9:214; and Solomon T, Thao TT, Lewthwaite P, et al. A cohort study to assess the new WHO Japanese encephalitis surveillance standards.* Bull World Health Organ. *2008;86:178–186.*

of JE is one that meets the clinical case definition for AES. Suspected cases are classified into four categories: (a) laboratory-confirmed JE; (b) probable JE; (c) AES, other agent; or (d) AES, unknown (see Box 33.1). A laboratory-confirmed case must meet the WHO laboratory criteria for confirmation of JE as follows: (a) presence of JEV-specific IgM antibody in a single sample of CSF or a serum sample obtained 7 or more days after onset of fever; (b) detection of JEV antigens in tissue by immunohistochemical analysis; (c) detection of the JEV genome in serum, plasma, blood, CSF, or tissue by reverse transcriptase–polymerase chain reaction or an equally sensitive and specific nucleic acid amplification test; (d) isolation of JEV in serum, plasma, blood, CSF, or tissue; or (e) detection of a fourfold or greater rise in JE antibodies as measured by hemagglutination inhibition or PRNT in serum obtained during the acute and convalescent phases of illness. These tests should be performed in parallel with other confirmatory tests to eliminate the possibility of cross-reactivity.[186,187]

Recommended Types of Surveillance

Surveillance for JE should be conducted year-round. Where feasible, surveillance for and reporting of JE should be performed within the context of integrated disease surveillance

and linked synergistically with similar surveillance activities, such as those for acute flaccid paralysis or meningitis.[186,187]

In All Asian Countries

Comprehensive syndromic surveillance for AES with aggregate reporting is recommended. In sentinel hospitals, surveillance should be case-based with specimens obtained for laboratory confirmation. The number of sentinel hospitals can be gradually increased when feasible logistically.

In Asian Countries Where a High Level of Japanese Encephalitis Control Has Been Achieved

Surveillance should be case-based throughout the country and include laboratory confirmation of all suspected cases. Regardless of the type of surveillance, reporting should be weekly or monthly and include "zero reporting" (i.e., no blanks should be left in the reporting forms; a zero should be used when no cases are detected). Outbreak investigations should be initiated if there is a sudden increase in cases or if cases reported are different from historical information in terms of season, geographic area, age group, or case fatality.

Treatment

No specific therapy is available, but supportive treatment can substantially reduce morbidity and mortality. Good nursing care and close attention to managing ventilation, fluids, and nutritional status, and prevention of bed sores and secondary infections, can improve outcome. Mannitol and other modalities to control intracranial pressure often are needed and seizure control is important. Aspiration pneumonia is a particular concern in any patient with a reduced gag reflex. Trihexyphenidyl hydrochloride and central dopamine agonists have been used to treat acute extrapyramidal symptoms.[64] Experimental studies in mice and monkeys suggested the potentially beneficial effect of interferon.[188] In an uncontrolled series of 14 patients treated with recombinant interferon-α, 13 survived.[189,190] However, in a double-blind, placebo-controlled trial with interferon-α2a in 87 Vietnamese children with confirmed JE, it showed no impact on the overall outcome.[191] For many years cortisone has been used to treat JE, but a randomized, placebo-controlled trial of dexamethasone in 40 patients showed no benefit of this treatment.[192] In an Indian trial, 70 patients received oral ribavirin, and 83 received placebo. Early mortality rates did not differ between the groups, and there were no statistically significant differences in secondary outcome measures.[193]

Use of JE antibodies or immunoglobulin preparations is discussed under "Passive Immunization and Cross-Protection" below.

EPIDEMIOLOGY

Incidence and Prevalence Data

In 2010, it was estimated that 67,900 JE cases occur annually, but changes in the intensity of JEV transmission cause fluctuations from year to year.[2] In developed Asian countries (e.g., Japan, Taiwan, and Korea), the incidence of JE has decreased over several decades and currently fewer than 10 to 20 cases are typically reported annually (see Fig. 33.2). Other countries, including Thailand, Nepal, and Sri Lanka, also have reported a substantial decline in cases during the past 1 to 2 decades. The major contributing factor to the decrease in cases has been immunization. However, a number of other factors probably have contributed to the decline, including secular trends toward a higher standard of living, a reduction in land under cultivation, widespread use of agricultural pesticides, and centralized pig production. The impact of economic development and secular factors has been demonstrated most clearly in Singapore, which has no national immunization program. Although JE previously was endemic on the island, only sporadic cases have been detected since 1992 and a serosurvey performed in 1993 demonstrated no antibodies in children younger than 15 years of age, indicating the near-elimination of viral transmission through the indirect effects of modern urban infrastructure, mosquito control, and complete prohibition of pig rearing on the island.[194–196] Banning the rearing of pigs, although possibly effective, is not reproducible in other constituencies.

Although JE incidence is now low in several of the 25 JE-endemic countries, the disease burden is still high in many areas. Almost 34,000 cases are still estimated to occur annually in China, and high incidence rates of 11.1 cases per 100,000 children 15 years of age and younger and 8.2 cases per 100,000 children younger than 10 years of age have recently been documented in Cambodia and Indonesia, respectively.[2,197,198] Surveillance activities also are improving the understanding of the extent of disease burden in countries with previously limited data, such as Bangladesh and the Philippines.[26,34]

In countries where childhood cases have been prevented by immunization with inactivated vaccine, the age distribution of cases has shifted toward adults, particularly to elderly people. In Japan, the previous bimodal age distribution of cases, with peaks in young children and elderly people, shifted toward a predominance of cases in adults (Fig. 33.9).[199–201] This shift does not necessarily mean an increase in the number of cases in older populations, but immunity of the younger populations has allowed the epidemiology of the elderly population to become more predominant. A similar pattern is seen in Korea and Taiwan and has emerged in some municipalities of China.[14,199,202] An analysis of age-specific incidence by decade in an area of Shanghai showed that, since 1961, case rates declined most dramatically in children, reflecting the impact of immunization; however, rates also declined in other age groups, reflecting a similar decrease in risk (Fig. 33.10). The result is that incidence rates are similar in adults and children.

In Taiwan, the loss of inactivated mouse brain vaccine-derived immunity has been inferred from declining JE antibody prevalence rates, from 49% in primary school to 38% in junior high school, 34% in junior college, and 29% in university students.[203] A similar trend also has been seen in Japan, where the induction and maintenance of immunity, presumably by immunization, through the first decade of life is followed by a subsequent decline, reflecting loss of vaccine-induced antibodies. Waning vaccine-induced immunity, age-related host factors, and reduced opportunities for natural exposure and "boosting" infections in an increasingly urban population may contribute to increased risk of acquiring JE in adults, even adults who might have been infected naturally earlier in life.[204] Surveillance to measure the incidence of JE in adults and to detect cases of secondary vaccine failure should be undertaken to determine the need for booster doses after childhood.

JEV has increased in geographic range across Asia in recent decades, and transmission in new areas continues to be documented.[205] Some studies correlate the apparent spread to or amplification of JE in new areas with agricultural development and intensive rice cultivation supported by irrigation schemes.[206] In Sri Lanka, the first recognized large outbreak in 1985 occurred after rerouting a river to a previously arid district for agriculture. Subsequent attempts at vector control failed to prevent an outbreak the following year. Similarly in

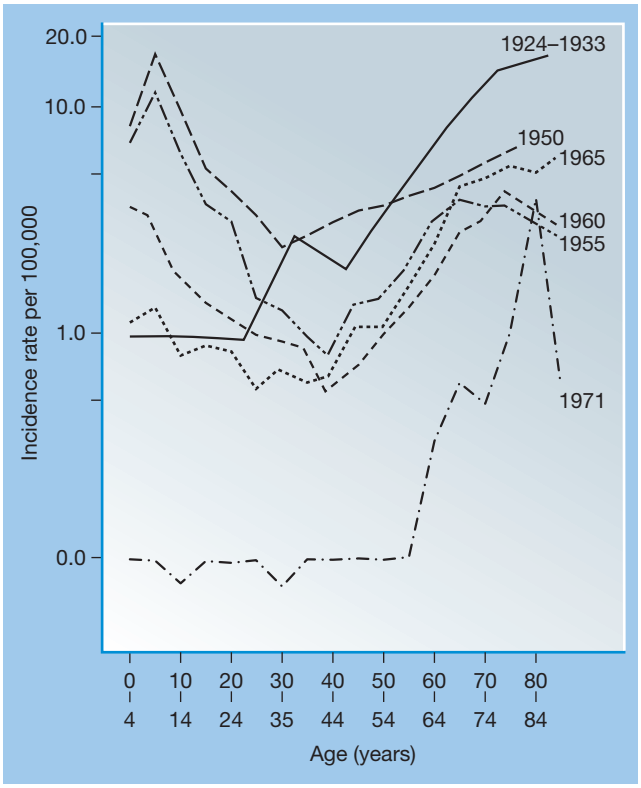


Figure 33.9. Age-specific incidence of Japanese encephalitis (JE) in Japan, 1924–1971. Although JE was most visible as a childhood disease, the age-specific incidence was bimodal, with elevated risk in children and elderly persons. As a result of universal childhood immunization, sporadic cases now occur almost exclusively in elderly persons. The causes of increased risk with advanced age are unknown. *(Modified from Oya A. Epidemiology of Japanese encephalitis, Rinsho to Biseibutsu.* Clin Diagn Microbiol. *1989;16:5–9.)*

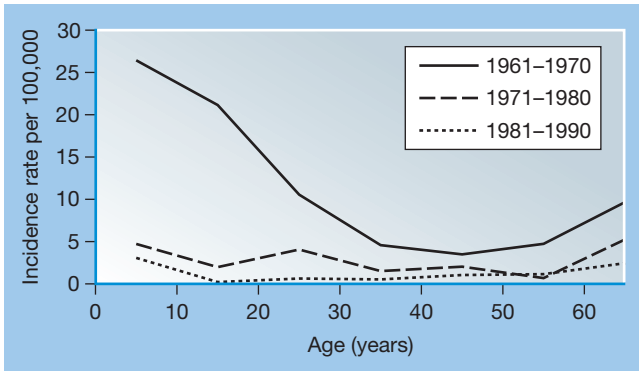


Figure 33.10. Age-specific incidence of Japanese encephalitis, Nanshi District, Shanghai, by decade. *(Data from Z.Y. Xu, personal communication.)*

southern Nepal, hyperendemic transmission of malaria and JE were documented to follow deforestation and development in the Terai. Viremic migratory birds or windblown mosquitoes are other possible mechanisms of JE viral importation over significant distances. Such factors are hypothesized to have led to outbreaks on Saipan and the Torres Strait Islands between New Guinea and northern mainland Australia.[38,40]

JE incidence may fluctuate yearly or regionally, but sustained childhood immunization programs are essential



Figure 33.11. Free pigs being herded out of city streets during the 2005 outbreak in Uttar Pradesh, India.

because JEV is maintained in an enzootic cycle and viral eradication from nature is not possible (Fig. 33.11). This is well illustrated in Japan, where viral surveillance using sentinel pigs and horses continues to document yearly high levels of transmission of JEV (see Fig. 33.4).[207] When the recommendation for JE vaccination was temporarily suspended in 2005 in Japan, vaccination rates in young children declined and two unvaccinated children with JE were reported in 2006 and 2009, before the recommendation for routine childhood vaccination was reinstated.[208]

Modes of Transmission and Reservoirs of Infection

Enzootic Areas in Asia

JE occurs in epidemics or in an endemic pattern or both in virtually every country of Asia (see Fig. 33.1; see also Table 33.14). Officially reported cases significantly underestimate the geographic extent or magnitude of risk because of underreporting (Fig. 33.12). Transmission is seasonal, occurring approximately from May to September in most temperate areas of Asia. Farther south, the transmission season is somewhat longer, extending from March through October (Fig. 33.13). In tropical areas and subtropical areas of Asia transmission can occur year-round and often intensifies following monsoon rains with the possibility of two transmission intervals in a calendar year.[26,197,198]

JE is principally a disease of rural areas in which vector mosquitoes proliferate in close association with birds and pigs, which serve as vertebrate amplifying hosts, with contribution of other species to sustained transmission less well studied (Figs. 33.14 through 33.16).[1,2,45,209,210] Humans and horses may become ill after infection, but such infections contribute minimally to the transmission cycle.[211,212] Experimental observations and field studies indicate that the virus overwinters in infected adult mosquitoes.[210] Vertical infection of mosquitoes may contribute to virus survival.[209] Long-term persistence in tissues and blood of JEV-infected vertebrate hosts, such as bats and reptiles, has been demonstrated. Introduction of the virus by viremic migratory birds or by windblown mosquitoes also may occur.[209,213] Self-limited outbreaks on western Pacific islands—Guam in 1947, Saipan in 1990, and the Australian Torres Strait islands in 1995 and

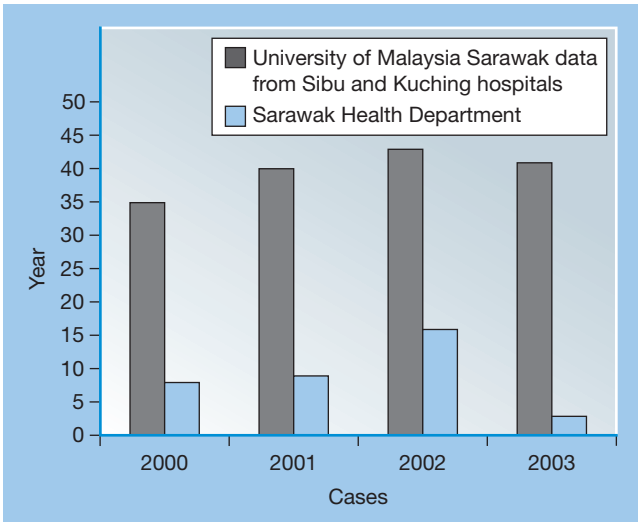


Figure 33.12. Underreporting of Japanese encephalitis (JE) happens for many reasons, including lack of recognition, no diagnostics, political pressure, and poor record keeping. In this example from Malaysia, JE cases are reported to the local ministry office from the hospitals, but the information is not passed to the central level to be recorded. *(Jane Cardosa, PhD, University of Malaysia Sarawak, personal communication.)*

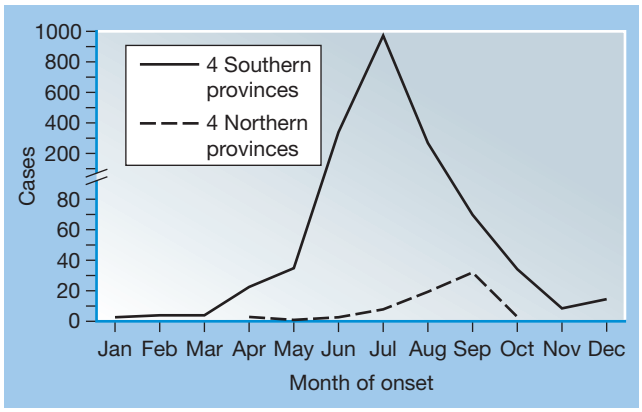


Figure 33.13. Seasonal distribution of Japanese encephalitis cases in four southern and four northern provinces, China, 1993. *(From Yu YX. Japanese encephalitis in China.* Southeast Asian J Trop Med Public Health. *1995;26[Suppl 3]:17–21.)*

1998—were examples of viral introductions possibly by migratory birds or, in the last case, by windblown mosquitoes.[36,38,40,214]

C. tritaeniorhynchus is the principal JEV vector in most areas of Asia, but various other ground pool– and rice paddy–breeding species, including *Culex vishnui*, *Culex pseudovishnui*, *Culex gelidus*, *Culex fuscocephala*, *Culex bitaeniorhynchus*, *Culex infula*, *Culex whitmorei*, and *Culex annulus*, are also important locally.[209,210] The JEV has been identified in 16 different mosquitoes in India and 19 in Indonesia,[45] and JEV has been recovered from *Culex pipiens pallens* and *Culex quinquefasciatus* in urban locations. In addition, *Culex annulirostris* has been identified as a vector in the western Pacific, *Aedes togoi* in sylvatic locations in Siberia, and members of the *Anopheles hyrcanus* group in northeastern India.[112,209,215] Although vector abundance and risk for human infection are associated with rainfall, with the introduction of wet rice cultivation,

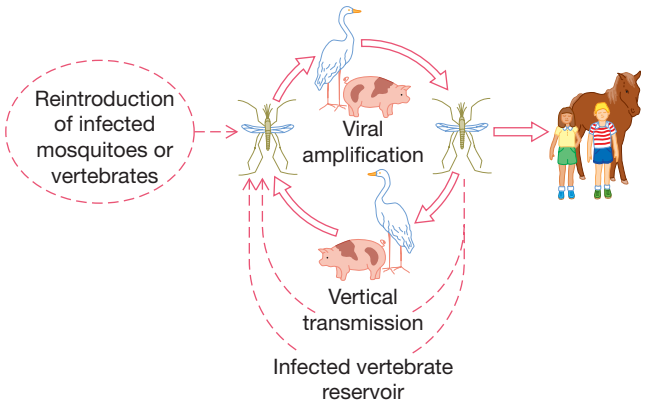


Figure 33.14. Transmission cycle of Japanese encephalitis (JE) virus. The open arrows indicate known portions of the cycle, and the dashed arrows indicate speculative portions. Infections and illnesses in humans and horses are incidental to the transmission cycle. The overwintering mechanism for JE virus is undefined, but experimental field observations suggest a role for vertical transmission in vector mosquitoes.



Figure 33.15. In Indonesia, flooded rice paddies are the principal breeding sites for larval stages of Japanese encephalitis virus mosquito vectors. The disease is transmitted chiefly in rural areas, especially in areas using irrigation schemes, where vector mosquitoes and pigs, the principal vertebrate amplifying host, are abundant near human residences.



Figure 33.16. Rice cultivation in Kurnool District, India, which alone is responsible for almost 46% of the Japanese encephalitis cases in Andhra Pradesh State.

paddy-flooding schedules have come to influence vector bionomics. Single paddies can produce more than 30,000 adult mosquitoes in a day. Collectively, these human-made breeding sites overpower the impact of natural sources. In these circumstances, mosquito abundance fluctuates with periodic rice field flooding and can peak at any time of the year, including the dry season (see Figs. 33.15 and 33.16).[216,217]

In temperate regions, vector mosquitoes emerge in May, and, after several initial rounds of viral amplification, high rates of pig seroconversion are detected. This is followed almost immediately by the onset of human cases, typically in July and August. By virtue of high levels and lengthy periods of viremia after infection and their prevalence as domestic animals, pigs are the key hosts for viral amplification (see Fig. 33.11). Infections in adult pigs are asymptomatic, but gestational infection frequently results in abortions and stillbirths, with significant economic losses. In some locations, enzootic transmission of the virus among aquatic birds has been well established, and, in outbreaks in which pigs are absent birds may serve as amplifying hosts.[218] Other domesticated animals, such as cattle, dogs, sheep, cows, and chickens, and peridomestic rodents may become infected, but these animals fail to develop sufficient viremia to support further viral amplification.[219] JEV mosquito vectors are zoophilic; consequently, cows and certain other animals can reduce risk to humans by diverting vector mosquitoes (zooprophylaxis).[220] Immunization of pigs prevents abortion and stillbirths and also may reduce viral transmission by nullifying the role of pigs as viral amplifiers. Experimental immunization of nearly the entire pig population on one island led to a significant reduction in human cases.[220–222] Unfortunately, this model is expensive and not easily reproducible with the high birth rates in the swine population and impossible in settings where pigs forage freely, a common practice in low-resource areas. Therefore, in rural parts of Asia, immunization of pigs has not been found to be an effective public health measure.[223] In humans, neither the use of permethrin-impregnated or untreated mosquito nets provide statistically significant protection.[224–227]

Other modes of transmission have been documented on rare occasions. In India, JEV was isolated from an aborted fetus, suggesting that intrauterine transmission of JEV can occur.[228,229] Based on experience with similar flaviviruses, blood transfusion and organ transplantation also are considered potential modes of JEV transmission.[230,231] At least 22 laboratory-acquired JEV infections have been reported in the literature.[232] Transmission in the laboratory setting could occur through needle sticks, and theoretically, through mucosal or inhalational accidental exposures.

RISK GROUPS

Residents in Endemic Area

Behavioral and other factors associated with the risk of acquiring JE vary regionally. Rural location, household crowding, religion, ethnicity, exposure to domestic animals, outdoor evening activities, and lack of air conditioning were detected as risk factors in some studies.[9,38,233–236] Risk of JE is highest in children younger than age 15 years, as most adults have acquired natural immunity after childhood infection (Fig. 33.17).[81,237,238] Although risk for acquiring JE is greatest in rural areas, conditions that permit enzootic viral transmission exist within or at the periphery of many Asian cities. JE cases in persons residing in urban area are commonly reported, including from suburban areas of major cities such as Bangkok, Beijing, Shanghai, and Delhi.[238–243] Sporadic reports of cases from Hong Kong and Singapore attest to the possibility of enzootic viral transmission near highly developed urban areas.[196,238] Reports of JE antibodies in feral pigs on a Singapore offshore island and JE reported in horses in Hong Kong strengthen these arguments.[195,244]

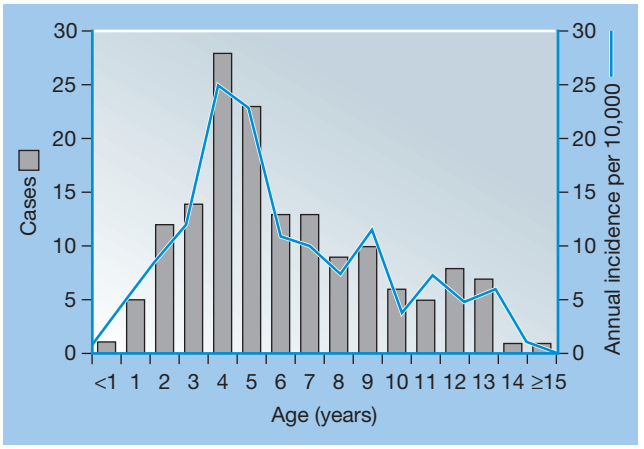


Figure 33.17. Reported Japanese encephalitis cases and age-specific incidence, Nallur Primary Health Centre, South Arcot District, Tamil Nadu, India, 1986–1990. *(Modified from Gajanana A, Thenmozi V, Samuel P, et al. A community-based study of subclinical Flavivirus infections in children in an area of Tamil Nadu, India, where JE is endemic.* Bull World Health Organ. *1995;73:237–244.)*

Previously, areas with high Muslim populations were believed not to be at risk because pig rearing is rare compared with Hindu and Christian populations.[34,245,246] In Indonesia, despite substantial data demonstrating JEV transmission in animal and mosquito populations since the 1970s, the perception that populations in predominantly Muslim areas were not at risk of JE led to a lack of human surveillance and an underappreciation of JE disease. However, a study of patients hospitalized with AES found serologically confirmed cases in all areas of the country, including areas with high Muslim populations.[198,237,245,247] Bangladesh, another predominantly Muslim country, also has demonstrated that JEV is a cause of encephalitis there.[34]

Travelers and Expatriates

Although JE vaccine is used principally in Asia to protect local populations, vaccines also are marketed in developed countries for travelers to Asia, expatriates, and, especially, military personnel (see Table 33.14). For the purposes of this discussion, expatriates are defined as residents through a transmission season. Sporadic cases have been reported in travelers from North America, Europe, Russia, Israel, Australia, and New Zealand, and, paradoxically, in Japanese and Taiwanese tourists to other endemic areas of Asia.[248–252] Data on JE cases among travelers from nonenzootic countries have been published.[251–256] Between 1973 and 2008, there were 55 cases of JE that occurred in citizens of 17 countries, an average of 1.5 published cases per year. The age range of patients was 1 to 91 years with a median of 34 years.[252] Of the 55 patients, 10 (18%) died and 24 (44%) had mild to severe sequelae. Of 37 persons with travel histories, 24 (65%) had spent 1 month or more in JE-endemic areas, and most had factors identified that may have increased infection risk. It is estimated that for every published case there may be an additional case of JE among travelers.[256] U.S. Department of Transportation statistics indicate that 4 million U.S. citizens traveled by air to Asia in 2004; however, this is not the population at risk. Most

TABLE 33.2 Japanese Encephalitis in Nonimmunized Americans and Other Western Military Personnel, 1945–1991

Location	Year	No. of Cases	Approximate Population at Risk	Rate/10,000	Reference
Okinawa	1991	3	19,000	0.1[a]	263
Philippines	1986	2		?	264
Thailand	1972	9	2,500	2.1[b]	47
Vietnam[c]	1966–1967	2	2,000	0.9[d]	258
Korea	1958	3	860	1.6[d]	43
Korea[e]	1950	103	114,813	0.4[d]	262
Korea	1946	3	1,500	0.9[d]	261
Okinawa	1945	11	77,000[f]	0.05[a]	265

[a]Rate based on exposure during a 6-month transmission season.
[b]Rate based on exposure during 4 months.
[c]Australian personnel.
[d]Rate based on exposure during a 5-month transmission season.
[e]American and British personnel.
[f]Partially immunized population.

travelers have brief itineraries during nonenzootic season, without outdoor or nighttime exposure in at-risk areas. Some may have been immunized.[257] The annual incidence in American travelers can be estimated roughly at less than 1 per 1 million population.

Risk also can be extrapolated from attack rates in unimmunized American, Australian, and British military personnel exposed in Asia. Rates have ranged from 0.05 to 2.1 per 10,000 population per week in soldiers who were exposed intensely under field conditions, in some cases during epidemics (Table 33.2).[43,47,252,256,258–262] These rates are similar to rates among children residing in hyperendemic areas, where annual reported incidence rates typically are in the range of 0.1 to 2 per 10,000 population. By accepting the high estimate and recognizing that transmission is limited to about a 5-month period in most areas, the monthly risk can be estimated as 1 per 50,000 population, or 1 per 200,000 population per week.

The risk for acquiring JE after a single mosquito bite can be estimated by considering the following probabilities of infection and illness:

1. Only bites of vector species are potentially infectious.
2. Even in epidemic circumstances, viral infection rates in vector mosquitoes rarely exceed 3%.
3. If a nonindigenous person is infected, symptomatic neuroinvasive illness may occur at a rate of 1 per 30 to 50 infections.[43]

Further reducing the risk of an infectious bite, *C. tritaeniorhynchus* and other JE vectors are chiefly zoophilic and prefer animal rather than human hosts. They feed chiefly in the evening and during the crepuscular (twilight) periods at dawn and dusk, and, although they may enter houses to feed, they principally are exophilic and seek hosts outdoors. Travelers can reduce risk by wearing mosquito repellent and long-sleeved shirts and trousers, by avoiding outdoor activities in the evening, and staying in screened or air-conditioned rooms.[224,266,267]

Risk for acquiring JE during travel is highly variable and depends on the destination and season of travel and the activities of the traveler (Table 33.3; also see Table 33.14). Travelers who remain in rural areas for extended periods are at greatest risk. Cases have been reported in travelers with brief itineraries staying in resorts or urban locations, but most have had factors identified that put them at risk for mosquito bites and JEV infection.[249–251] For example, in 2010, two JE cases were reported in U.S. citizens who had traveled to Asia for 21 and 10 days, respectively.[268,269] One of them, a child who visited the Philippines, stayed in a screened house in Metro Manila but on four occasions visited coastal and rural destinations, including evening walks on the beach.[268] The second, an adult, stayed in Shanghai, China, but made three trips, each of about 2 days' duration to three outlying areas. During at least one of his side trips, he participated in outdoor activities, including swimming and hiking.

TABLE 33.3 Risk and Protective Factors for Acquiring Japanese Encephalitis During Travel to Asia

RISK FACTORS
Travel to developing country
Travel during transmission season
Travel to rural areas
Extended period of travel or residence
Outdoor activities, especially in twilight period and evenings
Advanced age
Pregnancy (risk to developing fetus)
PROTECTIVE FACTORS
Repellents
Protective clothing
Residence in air-conditioned or well-screened areas
Permethrin-treated mosquito nets

PASSIVE IMMUNIZATION AND CROSS-PROTECTION

JE immune plasma and immunoglobulin are not commercially available. Experimental data in mice, goats, and rhesus monkeys indicate a prophylactic and therapeutic potential for polyclonal antibodies or a mixture of JE monoclonal antibodies, although combined use with peripheral and intraspinal or subdural administration was required for maximum effect.[270–273] A small controlled trial in humans suggested a

potential therapeutic benefit of passive immunization with antibodies.[270] More recently, intravenous injection of human immunoglobulin prepared from donors resident in JE-endemic areas was administered in an exploratory double-blinded clinical trial to Nepalese children with JE. The product was administered according to protocol and well-tolerated. The sponsors recommend larger clinical trials.[274] However, experience with prophylaxis of tickborne encephalitis (TBE) using human TBE-specific immunoglobulin indicates that antibody must be administered within a short period after tick exposure to be effective and that late administration (i.e., after 4 days) may worsen outcome.[275] This enhancement phenomenon has been replicated in animal models using JE and Murray Valley encephalitis.[276] Although there are few data, early treatment with interferon-α, perhaps in combination with immune plasma, may prevent illness after a known exposure, such as a laboratory accident, although treatment with interferon-α alone has not been found effective.[45,189–191]

All flaviviruses share many common antigens. Immunity, presumably from antibodies, against some flaviviruses offers low-level protection against disease from different species of flaviviruses. For example, prior dengue infection with one or more types has been found to reduce the incidence and the severity of sequelae from subsequent infections by encephalitis viruses, including SLE and JE. Original observations were made during a virgin soil epidemic of JE on Guam in 1947 to 1948, where much of the population was immune to dengue 1 virus from World War II epidemics.[36,277] Similar observations were made on the incidence of SLE during an outbreak in Florida, where some persons had been infected previously by dengue 2 virus.[278] Further observations on the possible protective effect of prior dengue infection on the incidence of clinical JE have been suggested in prospective studies in Thailand.[55,83,279–281]

ACTIVE IMMUNIZATION

Worldwide, at least eight different types of JE vaccines are produced and used, some in several countries (Table 33.4). For many years, only inactivated JE vaccine produced in mouse brain was available in developed countries.[283] Biken, a Japanese manufacturer, distributed approximately 2 million doses of this product abroad through Sanofi Pasteur for many decades; however, production of this vaccine was discontinued in December 2005, and all remaining stocks expired in early 2011.[284,285] The two vaccines currently most widely available internationally are the live attenuated SA 14-14-2 JE vaccine produced by Chengdu Institute of Biological Products (CDIBP), China and the inactivated Vero cell-derived JE vaccine manufactured by Valneva Scotland Ltd and licensed by Valneva Austria GmbH, Vienna, Austria (formerly Intercell AG). Live attenuated SA 14-14-2 vaccine is in use or licensed in at least 10 countries and was prequalified by the WHO for persons 8 months of age and older in 2013. In the decade through 2011, 145 million doses had been exported to JE-endemic countries and 245 million doses had been used in the domestic Chinese market.[286] Producers of the Vero-based inactivated JE vaccine produced by Valneva Scotland Ltd are listed below. Following technology transfer from Valneva, Biological E produced a similar JE vaccine in India which is distributed under the trade name JEEV. JEEV was WHO prequalified in 2013 for adults 18 to 49 years of age, and in 2014 for children 12 to 36 months of age.[287] A live YF 17D-JE chimeric vaccine (trade name, IMOJEV, IMOJEVMD, and THAIJEV) is manufactured by Sanofi Pasteur and by Government Pharmaceutical Organization–Merieux Biological Products Co. (GPO-MBP), a joint venture between GPO, Thailand and Sanofi Pasteur (Fig. 33.18). It is used or licensed in Australia, Thailand, Brunei Darussalam, Malaysia, Myanmar, and the Philippines.[286,288] It was prequalified for individuals 9 months of age and older in 2014.[287]

Inactivated Mouse Brain–Derived Japanese Encephalitis Vaccine

History of Vaccine Development

Initial Approaches That Were Abandoned. Inactivated mouse brain–derived JE vaccines were produced in Russia and Japan in the 1930s.[289] During World War II, a simple uncentrifuged 10% suspension of infected mouse brain, inactivated with formalin, was produced in the United States as a vaccine for the military. The vaccine was variably immunogenic, and efficacy field trials could not be completed.[265,290–292]

A more stable inactivated chick embryo–derived vaccine, also developed by the U.S. military, had an 80% efficacy in Japanese children when given as a combination of mouse brain and chick embryo–derived vaccines.[293–297] However, the latter vaccine was less immunogenic in adults, and its efficacy in soldiers was never evaluated. Although this vaccine was given to all U.S. soldiers assigned to Asia from 1948 to 1951,

TABLE 33.4 Licensed Japanese Encephalitis Vaccines

Vaccine Type	Substrate	Viral Strains	Manufacturers
Inactivated	Mouse brain	Nakayama, Beijing-1	Korea: Green Cross; Boryung Biopharma; Taiwan: Adimmune Corp; Thailand: Government Pharmaceutical Organization; Vietnam: Vabiotech
	Vero	Beijing-1	Japan: Biken (Research Foundation for Microbial Diseases of Osaka University), Kaketsuken
	Vero	Beijing P-3	China: Beijing Tiantan Biological Products Co; Liaoning Chengda Biotechnology Co
	Vero	SA 14-14-2	United Kingdom: Valneva Scotland Limited; India: Biological E
	Vero	Kolar strain (JEV 821564XY)	India: Bharat Biotech
	Hamster kidney cells	Beijing-1	China: Liaoning Yisheng Biopharma Co.
Live attenuated	Hamster kidney cells	SA 14-14-2	China: Chengdu Institute of Biological Products, Wuhan Institute of Biological Products, Lanzhou Institute of Biological Products
Chimera	Vero	JE SA 14-14-2/yellow fever 17D	Thailand: Government Pharmaceutical Organization-Merieux Biological Products Co.; Sanofi Pasteur

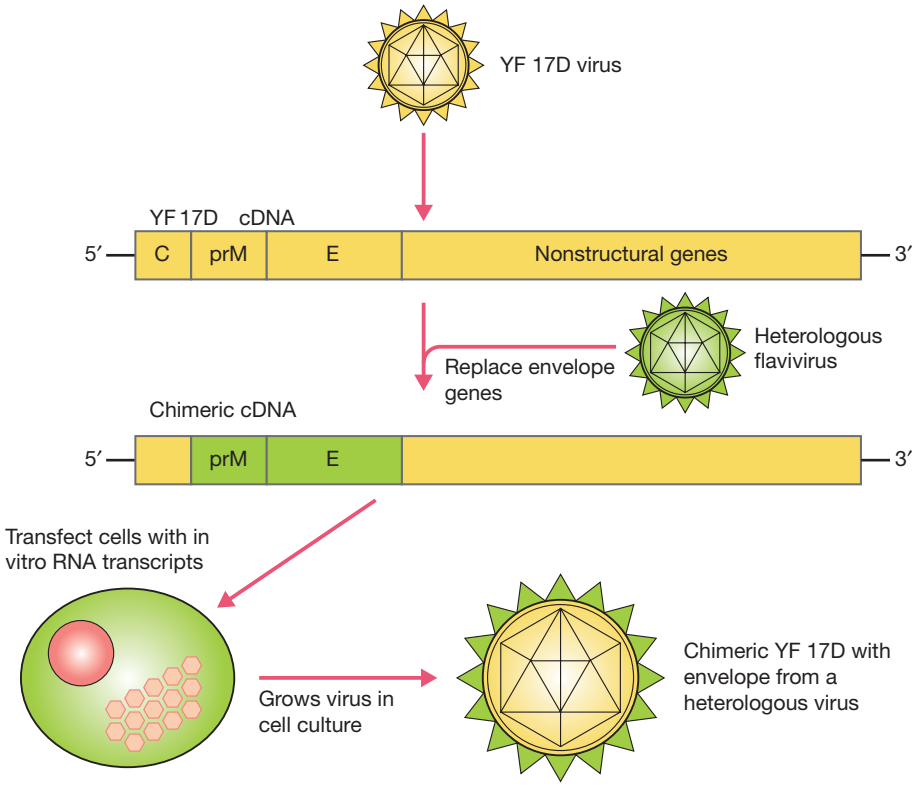


Figure 33.18. Chimeric Japanese encephalitis (JE) vaccine concept. The chimeric JE vaccine results from the insertion of the prM and E structural genes from the SA 14-14-2 virus into the nonstructural genes of yellow fever vaccine 17D (YF 17D). cDNA, complementary DNA. *(Data from Acambis, Inc.)*

its use was discontinued in 1952 after review of available data failed to produce convincing evidence of immunogenicity and efficacy.[258–262]

Description of Current Vaccine and History of Development. Successive refinements of the mouse brain vaccine were introduced by research institutes in Japan, resulting in a highly purified vaccine marketed worldwide but no longer produced by Biken.[298,299] It is not clear whether inactivated mouse brain vaccines currently manufactured in developing countries are subject to the same purification procedures as used for the Biken vaccine. Information regarding constituents and methods of production of the Biken vaccine will be of continuing interest as persons in some countries continue to be immunized with similar vaccines. The use and timing of booster doses of inactivated JE vaccines must be addressed for many decades to come.

Constituents, Including Antibiotics, Preservatives, and Adjuvants

The Nakayama strain of JEV, isolated from the CSF of a patient in 1935 and maintained by continuous mouse brain passage, has been the principal strain used in mouse brain–derived vaccines produced throughout Asia.[283] The strain was chosen because of good propagation characteristics and because it provided protection against several JE viral strains in mice. Cross-immunization studies in mice with strains from diverse areas of Asia indicated that strains of the JaGAr01/Beijing type (e.g., Beijing-1, known as P1 in China and the equivalent P3 strain; see later discussion) confer a broader neutralizing antibody response against various JE viral isolates than does the Nakayama strain.[106,300,301] The Beijing-1 strain grows to higher titer, and the vaccine produces higher heterologous antibody titers in immunized mice than does the Nakayama strain vaccine. Although vaccine produced from either strain meets standardized mouse protection tests for potency, the Beijing-1 vaccine is formulated in half the volume. Biken, the principal Japanese manufacturer of JE vaccine, used the Beijing-1 strain since 1989 in vaccine produced for domestic consumption, whereas the Nakayama strain was used in vaccines distributed internationally.

Manufacture of Vaccine

Mouse brain–derived vaccines are currently produced in Thailand, Vietnam, Taiwan, and South Korea by inoculating 3-week-old mice intracerebrally. When mice show signs of illness, brains are harvested, pooled, and suspended. Vaccine is made using similar sequences of centrifugation, ultrafiltration, protamine sulfate precipitation, and formalin inactivation in the cold, followed by further purification by ultrafiltration, ammonium sulfate precipitation, and continuous zonal centrifugation on sucrose density gradients. National standards in Japan specify minimal immunogenicity and potency in mice (compared with a vaccine standard) and maximal total protein (80 g/mL) and myelin basic protein (MBP) content (2 ng/mL), among other specifications. Bulk vaccine is diluted with medium 199 and phosphate buffer to meet a potency standard.[302,303] Although the quantity of JE E protein is not controlled, in one study, a dose was estimated to contain approximately 50 g. The vaccine is stabilized with gelatin and sodium glutamate and preserved with thimerosal.

Producers

Inactivated JE vaccines are produced by national manufacturers in several Asian countries, as detailed in Table 33.4.

Preparations Available, Including Combinations

Vaccines produced in Vietnam, Taiwan, and Thailand are in liquid form. No combination inactivated vaccines are available. However, in several countries, JE vaccine is frequently coadministered with other vaccines on the immunization schedule. In a study of children 15 months old, simultaneous administration of inactivated JE vaccine with measles-mumps-rubella vaccine did not result in reduced immunogenicity or increased side effects.[304] Under the Thailand EPI, JE vaccine is given concurrently with the fourth doses of diphtheria, tetanus toxoid, and pertussis (DTP) and oral poliovirus vaccines at 18 months of age.[305]

Dosage and Route of Administration

In most areas of Asia, vaccine produced from the Nakayama strain is given subcutaneously in two 0.5-mL doses 1 to 4 weeks apart (1.0 mL for people >3 years), usually beginning at the age of 12 months or older, with a dose at 1 year and one or more additional booster doses as outlined below.[283] In practice, immunization schedules are variable. The Biken package insert recommended an interval of 1 to 2 weeks for primary immunization; however, many immunogenicity studies have used a 4-week interval. Primary vaccination is recommended at 18 months of age in Thailand, at 15 months in Taiwan, and at 12 to 23 months in Korea. With no evidence from efficacy studies, boosters are recommended to be given 1 year later. In Korea, subsequent booster doses were given annually until 15 years of age, but now a revised schedule of giving boosters at 6 and 12 years of age is recommended. In Taiwan, an additional booster is given at school entry (age 5 or 6 years). Beijing-1 strain–derived vaccine is formulated with a higher antigen concentration, and the recommended dose is 0.5 mL (0.25 mL for children <3 years).

The primary series has been administered to infants along with DTP vaccine as early as 2 months of age in clinical trials, but, because JE uncommonly occurs in children younger than 1 year, vaccination is typically recommended starting in children 1 year of age and older.[305] A comparison of administration routes in adults showed that a 0.1-mL intradermal dose given at two sites may be as immunogenic as the standard administration of 1.0 mL subcutaneously, at least when given as a booster.[306]

Vaccine Stability

Lyophilized Biken vaccine was stable at 4°C for at least 1 year and retained more than 90% of its potency after 28 weeks at 22°C. At 37°C, lyophilized vaccine retained 95% of its original potency after 4 weeks.[299]

Inactivated Primary Hamster Kidney Cell–Derived JE Vaccine

Because inactivated primary hamster kidney (PHK) vaccine has been produced exclusively in China and newer vaccines are now in use there, details on history of vaccine development, constituents, production, and administration have been omitted. Readers are referred to earlier versions of this chapter for details.

Inactivated Vero Cell-Derived Japanese Encephalitis Vaccine (SA 14-14-2 Virus Strain)

Description of Current Vaccine and History of Development

The wild-type parental virus, SA-14, was isolated from a pool of *C. pipiens* larvae from Xian, China, following 11 passages in mouse brain. The derivation of the strain SA 14-14-2 was performed through an empirical process of serial passage performed in Beijing, China, in PHK cells and was given the strain designation SA14 clone 14-2 (also designated SA 14-14-2).[307] At the Walter Reed Army Institute of Research (WRAIR), the SA14 clone 14-2 was first adapted to primary dog kidney (PDK) cells and then to Vero cells. Attenuated SA 14-14-2 vaccine strain was initially passaged eight times in certified PDK cells. The PDK cell–passaged virus was then passaged in certified Vero cells and used as the master seed for making the inactivated vaccine.[308] Phase I and Phase II clinical trials were carried out at WRAIR, and the vaccine was then licensed to the Austria-based company Intercell AG (now Valneva Austria GmbH) for further development.[308,309] The vaccine was tested under the name IC51 and in 2009, was licensed for adults in the United States and Europe as IXIARO and in Australia as JESPECT. The vaccine was licensed for pediatric use in 2013.

Constituents, Including Antibiotics, Preservatives, and Adjuvants

In contrast with the mouse brain–derived inactivated vaccines, inactivated Vero cell–derived SA 14-14-2 vaccine contains no preservatives, antibiotics or gelatin stabilizers. The vaccine contains aluminum hydroxide at a concentration of 0.1% as an adjuvant. Other excipients are phosphate-buffered saline (PBS) consisting of sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, and water for injection. From the manufacturing process, inactivated Vero cell–derived SA 14-14-2 vaccine also contains formaldehyde (≤200 ppm), bovine serum albumin (≤100 ng/mL), host cell DNA (≤200 pg/mL), sodium metabisulfite (≤200 ppm), host cell proteins (≤300 ng/6 μg of protein), and protamine sulfate (≤1 μg/mL).

Manufacture of Vaccine

Working viral seed is produced at Valneva Scotland Ltd (Livingston, UK). The JE vaccine production seed is prepared by inoculating Vero cells grown in roller bottles. After virus inoculation and propagation, virus harvests are filtered, pooled, and concentrated. The concentrated virus pool is treated with protamine sulfate to remove Vero cell DNA. The virus pool is then purified by batch centrifugation in a sucrose gradient. The purified material, containing hemagglutinating virus, is inactivated with formaldehyde, which is then neutralized with sodium metabisulfite. Following neutralization, the inactivated virus is sterile filtered through 0.2 μm filters and diluted to a specified antigen content of 12 μg/mL. Finally the preparation is adsorbed to 1.5 mg/mL aluminum hydroxide and dispensed as a 0.5-mL dose per syringe.

Producers

The vaccine is licensed and distributed under the trade name IXIARO in Europe, North America, Hong Kong, Singapore and Israel, while in Australia and New Zealand, the vaccine is licensed and distributed by bioCSL under the trade name JESPECT. Following transfer of the manufacturing technology for IC51, the Indian company Biological E produces

a similar vaccine under the trade name JEEV at its facility in Hyderabad.

Preparations Available, Including Combinations

Inactivated Vero cell–derived SA 14-14-2 vaccine is provided as prefilled syringes containing 0.5 mL of vaccine, corresponding to a single dose for adults and children 3 years of age and older. For children 2 months through 2 years of age, each dose is 0.25 mL, and a marked syringe has been developed to allow administration of the 0.25-mL dose. No combination vaccines are available, but the vaccine can be coadministered with inactivated hepatitis A, rabies, or meningococcal vaccines without detrimental effects on immunogenicity.[310–312] There are no data on coadministration with measles, mumps, rubella (MMR) or other childhood vaccines.

Dosage and Route of Administration

For adults and children 3 years of age and older, the dose is 0.5 mL. Each 0.5-mL dose contains 6 µg of JEV strain SA 14-14-2 viral protein adsorbed on 0.25 mg Al^{3+} (hydrated aluminum hydroxide), corresponding to a potency of 460 ng ED_{50} (median effective dose) or less. For children 2 months through 2 years of age, each dose is 0.25 mL (3 µg of viral protein) (Table 33.5).[313,314] The vaccine is administered intramuscularly. The primary series consists of two doses administered 28 days apart. An accelerated schedule with doses administered 7 days apart provides noninferior seroprotection rates to a standard schedule.[312] If administration of the second dose is delayed, a second dose given up to 11 months after the first dose also provides similar seroprotection rates to the standard 0 and 28 day schedule.[315] Seroconversion rates after a single dose were only 21% to 29% at day 10 and 40% to 43% at day 28, but reach 97% by 7 days after administration of a second dose.[316] Protection before administration of the second dose is therefore uncertain and the full schedule should be completed 7 days before departure to a JEV endemic area. For adults, booster doses of vaccine should be administered before a potential reexposure if 12 months or more have elapsed since initiation of the primary series.[315,317] Six years after a booster dose, 96% of subjects remain seroprotected.[318]

TABLE 33.5 Recommended Immunization Schedule for Vero Cell–Derived Inactivated Japanese Encephalitis Vaccine

Vaccines	Primary[a]	Booster[a]
Adults and children ≥3 y	0.5 mL given on days 0 and 28[b] (conventional schedule) Complete series 1 wk before travel Adults 18–65 y can be vaccinated in a rapid schedule of 0.5 mL given on days 0 and 7[c]	Age ≥18 y: 1–2 y after primary series given in a conventional or rapid schedule, administer 0.5 mL prior to travel to JE endemic countries Age 3–17 y: Need for and timing of a booster dose not determined yet
Children 2 mo to <3 y	0.25 mL given on days 0 and 28	Need for and timing of booster doses not determined yet

[a]All doses given intramuscularly.
[b]IXIARO Summary of Product Characteristics, http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000963/WC500037287.pdf
[c]A rapid schedule is not approved in some countries, including the United States.

Existing recommendations for repeated booster suggest that a second booster should be given 10 years after the first booster dose, prior to potential exposure to JEV.[395] Similarly in children, a booster should be administered before potential reexposure if 12 months or more have elapsed since the primary series. Two years after a booster dose, 100% of children remain seroprotected.[395]

Vaccine Stability

The vaccine has an approved shelf life of 18 to 24 months, depending on the local regulatory approval. Immunogenicity of the vaccine is slightly lower toward the end of the shelf life but still induces seroconversion in more than 93% of vaccinees.[319]

Live Attenuated Japanese Encephalitis Vaccine

History of Vaccine Development

Initial Approaches That Were Abandoned. Attenuated JE viral strains have been sought by passaging wild strains serially in various cell culture systems, including PHK, chick embryo, and mouse embryo skin cells.[320–323] Loss of neurovirulence in mice, hamsters, pigs, or any combination of the three initially suggested the possibility of safe use in humans. Attenuation may be correlated with decreased binding to mouse brain cell receptors.[324] In China, the vaccine parent strain, SA 14, was isolated in 1954 from *C. pipiens* larvae collected in Xian (Table 33.6). After isolation and 11 serial passages in weanling mice, the virus was attenuated through 100 passages in PHK cells at 36°C to 37°C. Neurovirulence in monkeys had been lost at this passage level. Further plaque selection and cloning in chick embryo cells and subpassages in mice and hamsters by peripheral and oral infection were performed resulting in a stable nonneurovirulent virus. The resulting SA 14-5-3 strain no longer reverted to an established criterion of neurovirulence after intracerebral passage in suckling mice while remaining potent in mouse immunization-challenge studies.[325–334] The SA 14-5-3 virus did not kill 3-week-old mice by subcutaneous or direct intracerebral inoculation. Direct intrathalamic and intraspinal inoculation of the virus in monkeys resulted in no mortality or morbidity and only minimal CNS inflammation, limited to areas around the injection sites. Histopathologic

TABLE 33.6 Attenuation History of Japanese Encephalitis SA 14-14-2 Virus[307]

	Name
Parental Japanese encephalitis SA14 virus isolated from pool of *Culex pipiens* larvae by 11 passages in mouse brain	SA14
100 serial passages in primary hamster kidney (PHK) cells followed by three plaque purifications	SA 14-12-1-7
Two plaque purifications	SA 14-17-4
One mouse spleen passage (intraperitoneal inoculation) followed by four plaque purifications	SA 14-9
One mouse skin passage (subcutaneous inoculation) followed by one plaque purification	SA 14-9-7
Six hamster spleen passages (oral inoculation) followed by two plaque purifications	SA 14-5-3
Five suckling mice skin passages (subcutaneous inoculation) followed by two plaque purifications	SA 14-14-2
Amplification in PHK or primary dog kidney cells[334]	

changes were characterized by perivascular lymphocytic cuffs and focal mononuclear cell infiltration, with rare direct neuronal degeneration or necrosis.

The SA 14-5-3 vaccine was shown to be safe in humans, and field trials in endemic areas demonstrated seroconversion rates greater than 85%. However, rates of only 61% were obtained in subjects from nonendemic areas.[325] Expanded field trials in southern China involving more than 200,000 immunized children confirmed the vaccine's safety and yielded efficacies ranging from 88% to 96% over 5 years. However, the vaccine's poor immunogenicity in *Flavivirus*-naïve subjects from nonendemic areas suggested that the SA 14-5-3 virus, like previous live JEV candidate vaccines, was overattenuated and did not replicate uniformly in humans. To increase immunogenicity, the SA 14-5-3 virus was serially passaged five times by subcutaneous inoculation of suckling mice, using skin, subcutaneous tissue, and local peripheral lymph nodes as the passage material.[307,326] After plaque selection and cloning twice in PHK cells, the SA 14-14-2 strain was obtained. The SA 14-14-2 virus was equally attenuated but more immunogenic in mice, pigs, and humans, producing seroconversion rates greater than 90% in nonimmune subjects.[329,330] The response to intracerebral inoculation of live attenuated JE vaccines in infant mice is a reliable surrogate for more expensive use of monkeys for this purpose.[307,335]

Description of Current Vaccine and History of Development. The history of vaccine development and the genotypic and phenotypic attributes of SA 14-14-2 vaccine have been described.[307] Workers at the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) in Beijing managed the attenuation of PHK-passaged JE strain SA 14-14-2 and found it to be safe and immunogenic in animals and humans (see Table 33.6). The vaccine's efficacy was demonstrated in field trials, and the vaccine was licensed in the China in 1988 (for details see the 6th edition of this text). Attenuation of the SA 14-14-2 virus was produced empirically by serial passage in nonneural tissues, and the underlying molecular basis of its neuroattenuation is partially known. The nucleotide sequence of the neurovirulent parent SA 14 virus differs from that of SA 14-14-2 and two other attenuated SA 14-2–derived vaccine viruses in seven amino acid substitutions found in all three attenuated strains. Four were in the envelope protein (E138, E176, E315, and E439), one was in NS protein 2B (NS2B63), one was in NS3 (NS3105), and one was in NS4B (NS4B106).[336–338] These mutations have been shown to be very stable. An amino acid change at E138 in SA 14-14-2 virus was shown to be sufficient for mouse neuroattenuation when introduced into a wild-type JE complementary DNA infectious clone. Nucleotide sequences of structural protein genes revealed that the attenuated and parental viruses differed by eight amino acid mutations in the E protein. Attenuated JEVs also have been obtained by selecting neutralization escape variants. Attenuation was associated with single base changes resulting in single E protein amino acid changes and was linked with altered early virus–cell interactions but not with replication.[339,340]

The reduced neurovirulence of the SA 14-14-2 strain was confirmed in 3-week-old mice and monkeys.[307] Compared with the parent SA-14 strain, which killed weanling mice by subcutaneous or intracerebral inoculation with median lethal doses (LD_{50}) of $10^{5.5}$ and $10^{8.3}$ LD_{50}/mL, respectively, the SA 14-14-2 virus produced no mortality and only minor clinical signs in a few intracerebrally inoculated mice. Combined intrathalamic and intraspinal inoculation of rhesus monkeys produced no clinical illness and only minor inflammatory reactions in the substantia nigra and cervical spinal cord. Mice were more sensitive than monkeys to intracerebral infection, with some animals showing mild neuronal lesions in the cerebral cortex, hippocampus, or basal ganglia.[307] Compared with histopathologic lesions produced by the parent SA-14 virus, the inflammatory reaction and neuronal necrosis seen after SA 14-14-2 virus were significantly less. In 5-week-old mice inoculated intracerebrally with the virus pair, ultrastructural studies showed that the parent virus produced cytopathologic changes in the majority of neurons, particularly in the rough endoplasmic reticulum and Golgi apparatus of the neuronal secretory system, although it could not be confirmed that the vaccine strain replicated at all and neurons appeared normal.[332]

No deaths or histopathologic abnormalities were observed in athymic nude mice after intraperitoneal or subcutaneous inoculation of a viral dose greater than 10^7 median tissue culture infective doses ($TCID_{50}$), and virus could not be recovered from brain tissue.[164] Although cyclophosphamide increases susceptibility of mice (and also of monkeys) to virulent JEV, immunosuppression with cyclophosphamide did not lead to encephalitis in mice inoculated peripherally with the SA 14-14-2 virus.[165] The strain also did not kill intracerebrally inoculated weanling hamsters. Phenotypic characteristics of the vaccine strain (PHK8), such as small plaque, reduced mouse neurovirulence, and genetic properties, were stable through at least 10 additional PHK cell culture passages.[307,333]

No evidence of viremia up to 14 days following SA 14-14-2 JE vaccine administration was found in a study among 19 adults in India previously unexposed to JEV.[341] The SA 14-2-8 strain, closely related to SA 14-14-2, propagated well in *C. tritaeniorhynchus* mosquitoes after intrathoracic inoculation but could not be transmitted. In oral infection trials, only 11% of *C. tritaeniorhynchus* mosquitoes ingesting vaccine became infected compared with 100% with wild-type JE.[342] The virus did not revert to a neurovirulent phenotype after mosquito passage.[342] Intrathoracic inoculation of the SA 14-14-2 strain into vector mosquitoes replicated to titers similar to parental SA-14, but the authors did not test transmissibility of vaccine virus.[343]

Constituents

Several hundred ampules of seed virus, prepared from the sixth passage level of SA 14-14-2 virus, are maintained at the NICPBP, Beijing. Lyophilized seed virus (PHK5) is provided to production institutes, where it is passaged once for the production seed (PHK6). The PHK cells are obtained from 10- to 12-day-old golden Syrian hamsters maintained in colonies at the Chengdu and Wuhan production institutes. Current Chinese pharmacopeia and WHO guidelines specify the use of specific pathogen-free hamsters for the primary cell cultures. A specific pathogen-free hamster colony has been established at the Chengdu facility and is used for all vaccine production.[344] Monolayers are inoculated with diluted virus, and cells are fed with minimal essential medium containing human albumin, gentamicin, and kanamycin. Infected cell culture fluid with an infectious titer of approximately $10^{7.2}$ plaque-forming units (PFU)/mL is harvested at 78 to 96 hours and coarsely filtered, and the resulting liquid vaccine is lyophilized. Gelatin (1%) and sucrose (5%) are added as stabilizers. The major components of the final vaccine are human serum albumin, gelatin, sucrose, lactose, and carbamide. Lyophilized vaccine is reconstituted and diluted with sterile water for injection (single-dose vial) or PBS (five-dose vial).[287,345]

The reconstituted vaccine should be a transparent orange-red or light pink liquid. The PFUs of vaccine is not to be lower than $10^{5.7}$ PFU/mL in the reconstituted vaccine.

Manufacture of Vaccine

Vaccine must meet standards according to the WHO guidelines and Chinese pharmacopeia for absence of neurovirulence in adult mice, stability for reversion to neurovirulence after intracerebral passage in suckling mice, and freedom from adventitious agents, including retroviruses.[346] A highly sensitive product-enhanced reverse-transcriptase assay could not detect any reverse transcriptase activity in finished or bulk vaccine. A cocultivation assay on production cell culture using human cell lines followed by product-enhanced reverse-transcriptase assay allowed an expanded opportunity of detecting cross-species infection of retroviruses into human cells and did not show any reverse transcriptase activity in PHK cells. No evidence was found in SA 14-14-2 vaccine of murine leukemia virus-related viruses.[347]

Producers

Vaccine is produced by three manufacturers in China: CDIBP, Lanzhou Institute of Biological Products, and Wuhan Institute of Biological Products (see Table 33.4). In 1998, 200,000 doses of vaccine from CDIBP were donated to Nepal. In 2001, CDIBP became the first and only manufacturer to officially export vaccine outside of China when it successfully licensed and began exporting vaccine to Nepal. Since that time, CDIBP has additionally licensed vaccine in South Korea (2002), Sri Lanka (2002), India (2006), Thailand (2007), Laos (2008), Democratic People's Republic of Korea (2009), Myanmar (2010), Vietnam (2013), and Malaysia (2013). With local authority approval, children in Cambodia have also been vaccinated, commencing in 2009. CDIBP's vaccine is distributed under the trade names CD.JEVAX or RS.JEV. As of 2015, CDIBP is the only facility to fully comply with all the WHO production standards for the live attenuated vaccine.[287]

Preparations Available, Including Combinations

No combination vaccine is available. However, live attenuated SA 14-14-2 vaccine is commonly coadministered with measles-containing vaccines because their schedules coincide. In a randomized trial in the Philippines in which 8- to 10-month-old children were administered measles and SA 14-14-2 JE vaccines concomitantly or separated by 1 month, measles and JE seroprotection rates were high and comparable in all groups and reactogenicity profiles were similar.[348,349] Safety and immunogenicity of coadministration of measles and SA 14-14-2 JE vaccines were also assessed in a single arm study in Sri Lankan infants 9 months of age, which demonstrated good seroprotection rates and no safety concerns.[350]

Dosage and Route of Administration

Live attenuated SA 14-14-2 vaccine is prequalified by WHO as a single 0.5-mL dose for children 8 months of age and older. The vaccine is frequently administered to children at 8, 9, or 12 months of age to coincide with other routine immunizations, and to provide protection as early as possible while avoiding potential interference from passively acquired maternal antibodies.[349,350] In endemic settings, the need for a booster dose has not been established. In China and India, a two-dose schedule provides a second opportunity for vaccination and aims to ensure high coverage with at least one dose. In other countries, two doses are routinely given (e.g., South Korea, Thailand).

Vaccine Stability

The infectious titer of lyophilized vaccine is not appreciably changed after storage at 37°C for 7 to 10 days, at room temperature for 4 months, or at 2°C to 8°C for at least 1.5 years. After reconstitution with sterile saline or distilled water and storage at 23°C, the vaccine's infectious titer is stable for 2 to 4 hours or 2 hours, respectively.[345] The vaccine should be stored and shipped at 2°C to 8°C, protected from sunlight, and used within 18 months after the titration test is completed. Multidose vials once reconstituted should be used during the immunization session or within 6 hours, whichever comes first.[287]

Phenotypic and genotypic attributes of the SA 14-14-2 vaccine have remained stable over several decades.[307]

Chimeric Vaccines

History of Vaccine Development

The chimeric JE vaccine is constructed from the YF 17D genome that contributes NS genes and the SA 14-14-2 genome that contributes prM and E genes (see Fig. 33.18). The product was invented at St. Louis University; developed by Acambis, Cambridge, Massachusetts; and licensed for global distribution to Sanofi Pasteur, Lyon, France.[351] The chimera is constructed from DNA, transcribed to RNA and electroporated into Vero cells. The resultant infectious clone has the neurovirulence properties of SA 14-14-2 but Vero cell growth characteristics of 17D.[352,353] Attenuation of the chimeric virus was shown to depend on clusters of at least three of the six amino acid changes in the JE E protein.[353] JE-CV has proved to be immunogenic in rhesus monkeys and protects against intracerebral and intranasal challenge with the wild virus strain.[354,355] The chimera has not been shown to be transmitted by mosquito vectors of JE or YF viruses.[356]

A number of preclinical studies have shown that the JE-CV vaccine is safe, immunogenic, and protective in mouse and nonhuman primate models.[335,352–354,357] A clinical trial in adults (18 years of age and older) found that a single dose of IMOJEV generates comparable seroconversion rates (~99%) to those seen after three doses of JE-VAX (~95%), with approximately 94% of the participants seroconverting within 14 days.[358] A low-level, transient viremia was present in the majority of subjects (10/12 naïve and 11/12 YF-immune JE-CV recipients) and was similar in magnitude and duration to the viremia induced by YF 17D. In 100% of JE-CV subjects, including all YF-immune subjects, neutralizing antibodies developed against JE (seroconversion) by 30 days following vaccination; YF-immune subjects responded with higher JE neutralizing antibody titers than did naïve subjects.[359] A 5-year follow-up study found approximately 87% of vaccinees receiving one dose had detectable neutralizing antibodies at both month 6 and 5 years; this percentage increased to approximately 96% with a booster at month 6.[360] This result has been modeled to achieve a predicted 10 years of "protection."[361] In Thailand, the Philippines, and Taiwan, clinical trials in children (12 to 18 years of age or 24 months of age) have shown that single-dose administration elicits seroconversion in approximately 95% of naïve toddlers. In the Thai trial, among 182 flavivirus-naïve children, 96% developed neutralizing antibody titers to 1:10 or greater at 28 days, but 15% of primary responders had titers less than 1:10 at 1 year after single-dose immunization[362–364] There have been no serious safety concerns following administration of this vaccine.[358,360,363] Furthermore, the JE-CV virus is restricted in its ability to infect and replicate in six potential vector mosquito

species following oral feeding of artificial blood meals containing high titers of virus.[356,365] In a multicenter trial in Taiwan, 550 children 12 to 18 months of age were alternately given JE-CV or live-attenuated licensed MMR vaccine at 6-week intervals, or they were administered concomitantly.[366] The immune responses were higher than 95% in all groups and remained high for 12 months after vaccinations in the groups that had sequential administration of vaccines, but decreased slightly in the coadministration group.

Constituents. The vaccine contains Vero cell proteins, mannitol, lactose, glutamic acid, potassium hydroxide, histidine, human serum albumin, sodium chloride, and water for injection. No adjuvant or antimicrobial preservatives are added.

Manufacture of Vaccine. The vaccine is grown in cultured Vero cells.

Producers. The chimeric JE vaccine is manufactured by Sanofi Pasteur and licensed in Australia as IMOJEV. The vaccine is manufactured from furnished seed by the GPO-MBP in Thailand where it is licensed and marketed as THAIJEV.

Preparations Available, Including Combinations. There are no combination vaccines.

Dosage and Route of Administration. One dose (0.5 mL) containing $10^{4.0–5.8}$ PFUs, is administered subcutaneously. It is licensed in Australia for individuals older than the age of 12 months and WHO prequalified for children 9 months of age and older.

Vaccine Stability. Vaccine is stored at 2°C to 8°C and has a shelf life of 2 years.

IMMUNOGENICITY OF JAPANESE ENCEPHALITIS VACCINES

The JE viral strains isolated in different geographic locales or at different times exhibit minor antigenic differences and biologic characteristics such as growth in cell culture and neuroinvasiveness in experimentally infected mice. However, there is no evidence of corresponding differences in human pathogenicity and no evidence that immune responses to one strain, whether as a live viral infection or as a vaccine, does not protect against disease caused by another.[130,367–370] In the modern era, antibodies raised by administration of JE vaccines have been measured by IgM and IgG ELISA, hemagglutination inhibition, complement-fixation, and tissue culture–based neutralization tests of varying formats. A neutralizing antibody titer of 1:10 or greater is accepted as evidence of protection and postvaccination seroconversion.[371] Passively immunized mice that acquire this level of neutralizing antibody are protected against challenge from 10^5 LD_{50} of JEV, a typical dose transmitted by an infectious mosquito bite. Indirect observations from human trials have associated efficacy with this criterion.[372] PRNTs are used most frequently, and procedural differences, such as choice of challenge virus strain, cell systems, addition of exogenous complement, and choice of end points (ranging from 50% to 90% plaque reduction in serum dilution tests), affect test sensitivity.[373] In one assessment, neutralizing antibody titers in three laboratories (the U.S. Centers for Disease Control and Prevention [CDC], Japan's National Institutes of Health, and the Yale Arbovirus Research Unit) were shown to be highly correlated (R. DeFraites, unpublished data, 1998). This same result was not replicated when several laboratories evaluated standard serum samples for a test serum bank being developed by the WHO.[373] An international standard of protective antibody units is in the process of being established.[371]

Inactivated Mouse Brain Vaccine

Antibodies

Among Asian children immunized with two doses of Nakayama strain– or Beijing-1 strain–derived vaccines, neutralizing antibody responses to the respective homologous vaccine strains are in the range of 94% to 99%; responses to strains representing a heterologous antigenic group are lower.[374–377] The proportion of vaccinees retaining detectable neutralizing antibodies and their geometric mean antibody titers (GMTs) declined rapidly in the year after the primary two-dose series, so that only 78% to 89% of Nakayama vaccine recipients and 88% to 100% of Beijing-1 vaccine recipients still had detectable levels before the scheduled 1-year booster. Antibody persistence was greater among Beijing-1 vaccine recipients. After booster immunization (or third vaccine dose), antibody response rates were uniformly high (100%). During the history of the administration of JE vaccines in Asia, additional booster doses have been given empirically, usually with the goal of restoring neutralizing antibody titers to the detectable range and according to the philosophy that more is better, as illustrated in a U.S. study of mouse brain vaccine (Fig. 33.19). However, there are no studies of the efficacy of such booster doses in Asian countries. In fact, the long-term efficacy of two or three doses of killed vaccine has not been measured. There is evidence of sustained protection in adults who do not receive booster doses of JE vaccines in Korea, Japan, and Taiwan. This evidence is compatible with solid protection being provided to immunized persons with very low titers of neutralizing antibodies.

Immunogenicity studies in Asian subjects should be interpreted in the light of the immunologic background of vaccinees. Although some studies have been done in nonendemic areas and in subjects without JE viral antibodies, in other studies, undetected exposures to JE, dengue, and other flaviviruses prevalent in Asia may have resulted in an augmented antibody response after immunization.

Where the influence of previous flaviviral infections was unlikely, vaccinees receiving two doses had lower seroconversion rates and lower GMTs than vaccinees receiving three doses.[378–382] After administration of two doses of inactivated vaccine, neutralizing antibody titers declined to less than 1:8 in 90% of vaccinees at 6 to 12 months following vaccination. A three-dose primary schedule was more immunogenic, resulting in seroconversion rates exceeding 90% and significantly higher neutralizing antibody titers.[378–382] Administration of vaccines at long (days 0, 7, and 30) versus short intervals (days 0, 7, and 14) using three doses resulted in uniform seroconversions in all subjects but significantly higher neutralizing antibody titers in vaccinees immunized over the longer period (30 days). Vaccine prepared from the Beijing-1 strain seems to be more immunogenic, despite its smaller delivered volume, yielding higher seroconversion rates and higher antibody titers to heterologous Nakayama virus.[383–385] Similar but more marked differences were seen in comparative neutralization of field viral strains from Taiwan, paralleling those in experimentally immunized mice.[386] The clinical importance of these differences in strain reactivity in the neutralization test is uncertain. Results of an efficacy trial comparing a monovalent Nakayama strain vaccine with a bivalent vaccine also containing Beijing-1 antigen showed that the two were equally efficacious.[387] Neutralizing activity below the threshold of detection may be protective. B-cell

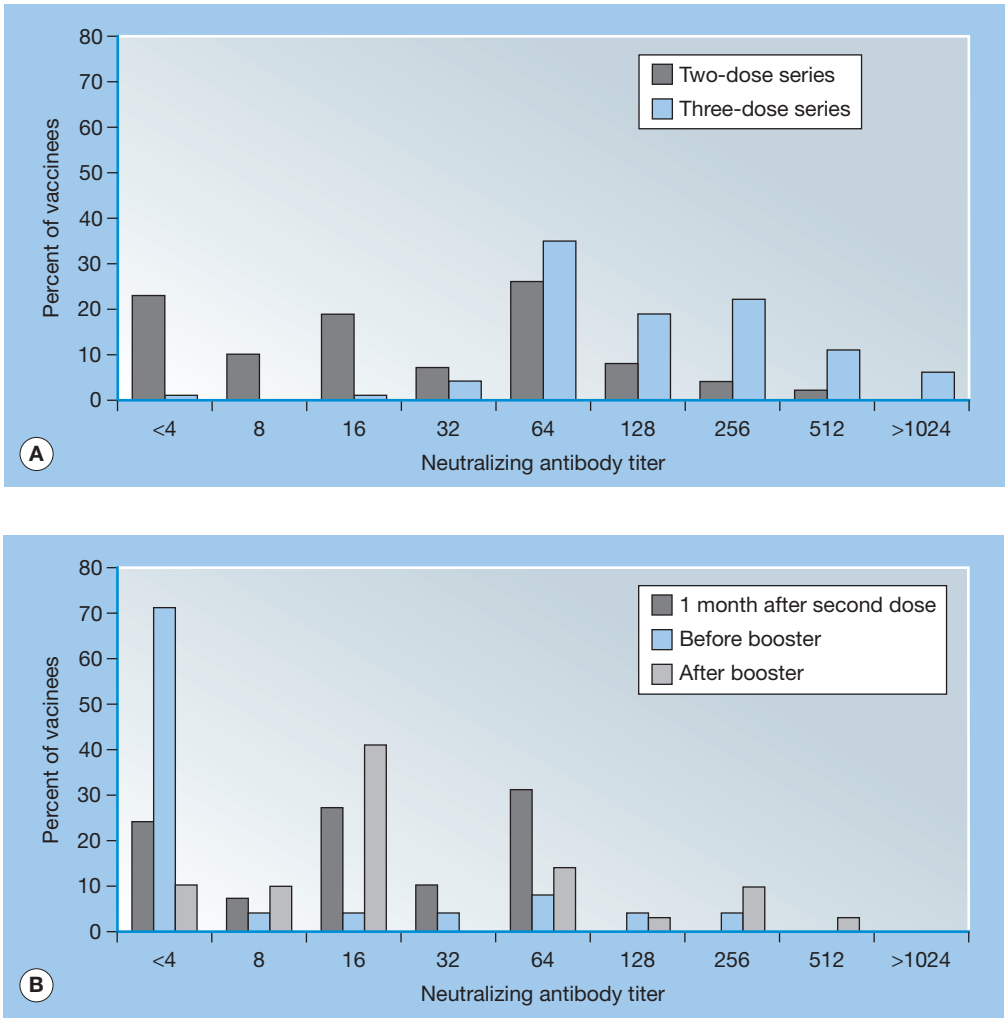


Figure 33.19. A, Antibody response to inactivated mouse brain–derived Japanese encephalitis vaccine in a trial among U.S. citizens. Only 77% of vaccinees who received two doses seroconverted, compared with 99% of vaccinees who received three doses. Geometric mean antibody titers also were higher in the latter group (28 vs 141). **B,** At 6 to 12 months after primary immunization, only 10% of vaccinees who were given two doses retained protective levels of neutralizing antibody. Booster immunization led to a greater than 90% response. *(Modified from Poland JD, Cropp CB, Craven RB, et al. Evaluation of the potency and safety of inactivated Japanese encephalitis vaccine in US inhabitants.* J Infect Dis. *1990;161:878–882.)*

memory, anamnestic antibody responses, and T-cell memory may contribute to protect seronegative vaccinees, but nevertheless provide immunologic help to clear infections on reexposure. Previous exposures to dengue and certain other flaviviruses probably enhance the immune response to JE vaccine.

Cellular Responses

Persons given inactivated vaccine are exposed only to viral structural proteins, and, in contrast to convalescent patients, they do not produce radioprecipitating antibodies to viral NS proteins.[387] Their memory T-cell proliferative responses to a viral-like particle containing only structural proteins also differ from those of recovered patients, with CD4 and CD8 cell responses to viral NS proteins.[157] The implications of these immune response differences are uncertain. While cellular responses have been studied following infection with wild-type JE or immunization with JE vaccines, these have not been correlated with protection or disease outcome.

Correlates of Protection

Neutralizing antibodies measured in a mouse or tissue culture neutralization test have been generally accepted as a correlate for protection. A WHO expert panel determined that a 50% PRNT ($PRNT_{50}$) titer of 10 or greater was acceptable as an immunologic correlate of protection from JE in humans.[371]

In Special Groups, Such as Immunosuppressed Persons

Impaired responses to vaccination were observed in infants with vertically acquired HIV infection when compared with HIV-uninfected infants born to HIV-infected women: five (36%) of 14 HIV-infected children and 18 (67%) of 27 control children developed JE antibodies after immunization (odds ratio [OR], 0.3; $P = .06$); among children with positive titers, the GMT of HIV-infected children also was lower (15.1 vs 23.8; $P = .17$).[388] The response to additional doses beyond the primary two doses was not studied. Immune response in other immunocompromised states has not been studied systematically.[389]

Inactivated Vero Cell Vaccine

Correlates of Protection

Passive immune transfer of human sera with high PRNT titers after immunization with inactivated Vero cell–derived SA 14-14-2 vaccine that resulted in a titer equal to or greater than 1 : 10 in mice protected them from lethal challenge with SA-14 and the non–genotype 3 JE strain KE-093.[390] Groups of 10 female ICR mice (6–7 weeks old) received human antisera in 0.5-mL amounts (intraperitoneal injection) and were then challenged intraperitoneally 17 to 18 hours later with a lethal dose of SA-14 or KE-093 strain. Challenged mice were observed for 20 days, and blood samples were obtained just before challenge and at day 21. Mice with JE neutralizing antibody titers equal to or greater than 1 : 10 before challenge had survival rates of 86% (6/7) and 100% (10/10) after challenge with the KE-093 and SA-14 strains, respectively.[390] Human immune serum samples from clinical trial participants were tested for neutralization of other JEV strains and showed broad neutralizing capacity against a variety of strains (SA-14, Nakayama, Beijing, P-20778).[41,391,392]

Antibodies

Immunogenicity of inactivated Vero cell–derived SA 14-14-2 vaccine served as an indicator of vaccine efficacy in the clinical development of the vaccine. A validated PRNT was applied to determine neutralizing antibody response to vaccinations and the WHO-recommended immunological correlate of protection ($PRNT_{50}$ titer of ≥10) was used. Licensure of inactivated Vero cell–derived SA 14-14-2 vaccine was based on noninferior immunogenicity of the vaccine compared with field-effective, mouse brain–derived JE vaccine (JE-VAX, Biken; distributed by Sanofi Pasteur). Seroconversion rates and GMTs were comparable for both vaccines (Table 33.7).[316,391,393] Inactivated Vero cell–derived SA 14-14-2 vaccine led to 96.4% seroconversion rate (all subjects were seronegative at baseline) at day 56, compared with 93.8% seroconversion rate for JE-VAX. GMTs were 243.6 and 102.0, respectively; however, the $PRNT_{50}$ assay used SA 14-14-2 JEV strain as the challenge virus and use of the homologous strain to the inactivated Vero cell–derived SA 14-14-2 vaccine likely accounted for the higher titer for the Vero cell–derived vaccine.

A study investigating the kinetics of the neutralizing antibody response after vaccination detected a 97.3% seroconversion rate by 7 days after the second dose, suggesting rapid boosting of immunity following the second dose (Fig. 33.20).[316] One study has investigated the response to an accelerated schedule of vaccine. JE vaccine doses were administered on days 0 and 7, and rabies vaccine was also administered on days 0, 3, and 7. The JE seroprotection rate in the accelerated schedule group was noninferior to the rate in the group who received the standard schedule of two doses of JE vaccine on days 0 and 28 (99% vs 100%). The accelerated schedule led to higher GMTs at both 7 and 28 days after the last active immunization and up to 12 months later (Fig. 33.21).[394,395]

The pivotal pediatric clinical trial was conducted in children 2 months to 17 years of age in the Philippines.[393,396] Among children who received two age-appropriate doses, 384 (100%) of 385 were seroprotected at 28 days after the second dose (95% confidence interval [CI], 96–100%). At 6 months, 134 (88%; 95% CI, 82–92%) of 152 children 2 months to 2 years of age and 224 (95%; 95% CI, 91–97%) of 237 children 3 to 17 years of age were seroprotected. Immunogenicity was also investigated in a small cohort of children from nonendemic countries who were traveling to endemic regions. Among 54 children 10 months to 17 years of age (median, 15 years of age), the seroprotection rate was 100% at day 56.[393] In a small trial in India, 22 (96%; 95% CI, 87–100%) of 23 children 1 and 2 years of age were seroprotected at 28 days after receiving two doses.[313] No statistically significant differences were detected in the seroprotection rates between this group receiving two doses and children who received three doses of the inactivated mouse brain–derived JE vaccine (manufactured by Korean Green Cross).

In Special Groups

Immunogenicity of inactivated Vero cell–derived SA 14-14-2 vaccine has not been studied in immunocompromised persons. In 200 elderly persons (65 years of age and older) the

TABLE 33.7 Seroconversion Rates and Geometric Mean Titers After Inactivated Vero Cell–Derived SA 14-14-2 Vaccine or Mouse Brain–Derived Inactivated Japanese Encephalitis Vaccine, Per Protocol Population

SEROCONVERSION RATES[a]			
Time point	Vero cell–derived vaccine (N = 365) SCR (n/N) [95% CI]	Mouse brain–derived vaccine (N = 370) SCR (n/N) [95% CI]	Rate difference [95% CI]
Prevaccination screen	0	0	
Day 56[b]	96.4% (352/365) [94.0–97.9]	93.8% (347/370) [90.9–95.8]	2.6% [– 0.5 to 6.0][a]
GEOMETRIC MEAN TITERS			
	GMT [95% CI]	GMT [95% CI]	GMT ratio [95% CI]
Prevaccination screen	5.0[c] (n = 365)	5.0[c] (n = 370)	
Day 56[b]	243.6 [216.4–274.1] (n = 361)	102.0 [90.3–115.2] (n = 364)	2.33 [1.97–2.75][d]

[a]Seroconversion rates (SCRs): Noninferiority of inactivated Vero cell–derived SA 14-14-2 vaccine compared with mouse brain–derived inactivated Japanese encephalitis vaccine for SCRs was demonstrated if the lower bound of the two-sided 95% confidence interval (CI) for the SCR difference (Vero cell minus mouse brain) was more than –10% at day 56.
[b]28 days after dose 2 for Vero cell–derived vaccine and after dose 3 for mouse brain–derived vaccine.
[c]Prevaccination titers were negative by definition in the per protocol population and have been imputed to 5.
[d]Geometric mean titers (GMTs): Noninferiority of Vero cell compared with mouse brain for GMTs was demonstrated if the lower bound of the two-sided 95% CI for the GMT ratio (Vero cell:mouse brain) was >1 : 1.5 at day 56.
JE, Japanese encephalitis.
Data from U.S. Food and Drug Administration (FDA) Approved Prescribing Information for IXIARO. Revised August 2015. Available at: http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM142569.pdf and Tauber E, Kollaritsch H, Korinek M, et al. Safety and immunogenicity of a Vero-cell-derived, inactivated Japanese encephalitis vaccine: a non-inferiority, phase III, randomised controlled trial. Lancet. *2007;370:1847–1853.*

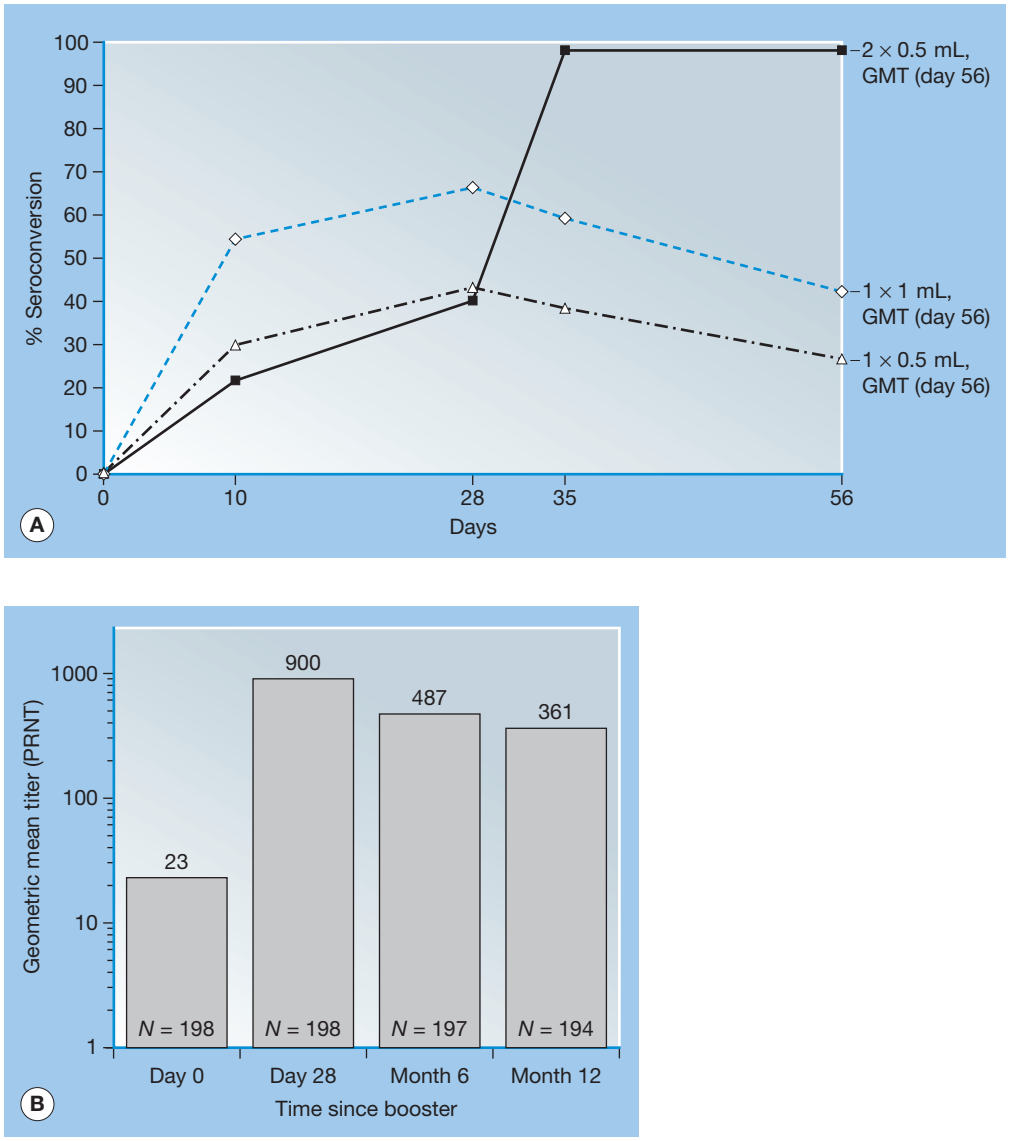


Figure 33.20. A, Kinetics of neutralizing antibody response to vaccination with Vero cell–derived, inactivated Japanese encephalitis vaccine (IXIARO). Seroconversion rates at days 10, 28, 35, and 56 after vaccination with a single high dose (1 × 1.0 mL), the standard two-dose schedule (2 × 0.5 mL), or a single dose (1 × 0.5 mL) of IXIARO. **B,** Persistence of neutralizing antibodies following a booster dose of IXIARO. GMT for neutralizing antibodies measured before (day 0) and 1, 6, and 12 months after a single booster dose of Vero cell inactivated JE vaccine, administered 15 months after the first dose of the primary series. The seroprotection rate ($PRNT_{50}$ titer, ≥1 : 10) was 69% before and 100% at 1 month after the booster and remained at 98.5% at 6 and 12 months later. GMT, geometric mean titer; JE, Japanese encephalitis; PRNT, plaque-reduction neutralization test; $PRNT_{50}$, 50% plaque-reduction neutralization test. (***A,*** *Modified from Schuller E, Klade CS, Wölfl G, et al. Comparison of a single, high-dose vaccination regimen to the standard regimen for the investigational Japanese encephalitis vaccine, IC51: a randomized, observer-blind, controlled phase 3 study.* Vaccine. *2009;27:2188–2193.* ***B,*** *Modified from Eder S, Dubischar-Kastner K, Firbas C, et al. Long term immunity following a booster dose of the inactivated Japanese encephalitis vaccine IXIARO, IC51.* Vaccine. *2011;29:2607–2612.)*

vaccine was less immunogenic compared to younger populations, with a seroconversion rate of 65% and a GMT of 37 at 70 days after the first dose.[393]

Live Attenuated Vaccine, SA 14-14-2

Antibodies

In early studies in China, after a single dose, antibody responses were produced in 85% to 100% of nonimmune 1- to 12-year-old children, with a response gradient that paralleled the viral titers.[307,329,330,397] Lower seroconversion rates were obtained with vaccine dilutions that had infectious titers less than $10^{5.7}$ PFU/mL, which has been established as the minimal standard of vaccine infectivity.

More recent clinical trials with live attenuated SA 14-14-2 vaccine have been conducted in the Philippines, Bangladesh, Thailand, and South Korea. In the Philippines trial, immunogenicity was assessed among children 8 to 10 months of age who were randomly assigned to receive one dose of live attenuated SA 14-14-2 JE vaccine 1 month before, after, or coadministered with measles vaccine.[348,349] JE seroprotection rates in the three groups 28 days postvaccination were 92.1% (81/88), 90.6% (163/180), and 90.5% (201/222), respectively. GMTs were 203, 155, and 139. In a study among Bangladeshi infants 10 to 12 months of age (N = 727 in the

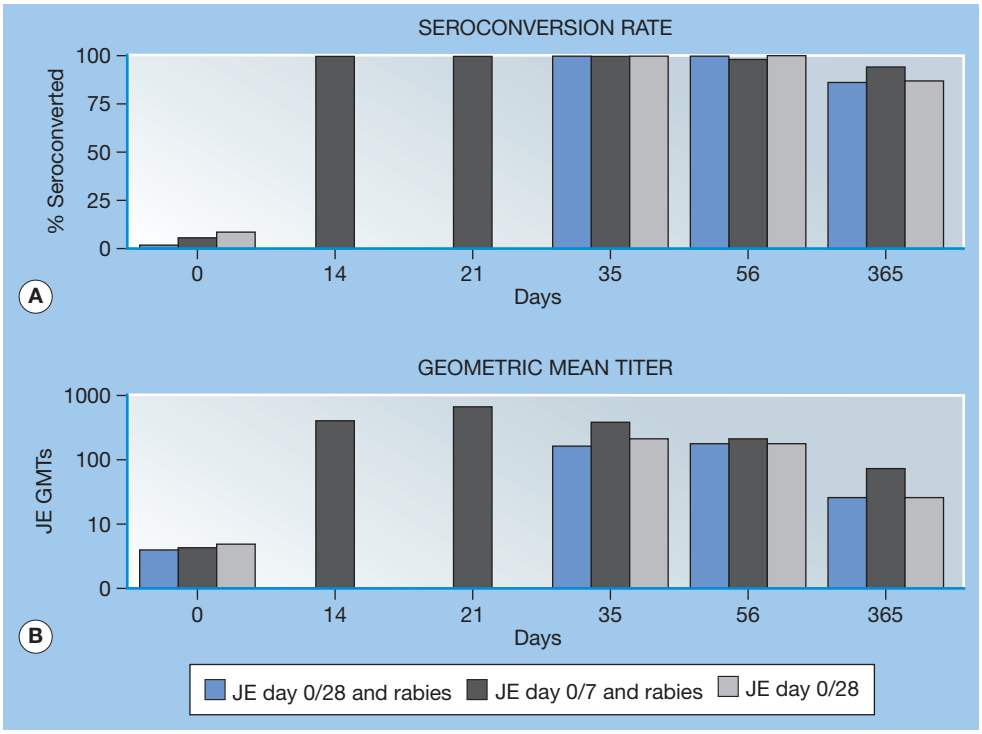


Figure 33.21. Japanese encephalitis (JE) seroconversion rates **(A)** and geometric mean titers **(B)** at intervals up to 365 days following administration of inactivated Vero cell–derived JE vaccine (IXIARO). The Vero cell–derived vaccine was administered in an accelerated days 0, 7 schedule (together with purified chick embryo cell [PCEC] rabies vaccine administered on days 0, 3, and 7) or in one of two comparison regimens of a standard days 0, 28 schedule (together with PCEC rabies vaccine on days 0, 7, and 28) or a days 0, 28 schedule alone. Seroconversion was defined as a $PRNT_{50}$ titer ≥1 : 10. $PRNT_{50}$, 50% plaque-reduction neutralization test. *(Modified from Jelinek T, Burchard GD, Dieckmann S, et al. Short-term immunogenicity and safety of an accelerated pre-exposure prophylaxis regimen with Japanese encephalitis vaccine in combination with a rabies vaccine: a phase III, multicenter, observer-blind study.* J Travel Med. *2015;22:225–231; and IXIARO Summary of Product Characteristics, available at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000963/WC500037287.pdf.)*

per-protocol analysis) randomized to receive one of four different lots of vaccine, seroprotection rates at 28 days ranged from 80.2% to 86.3%, and GMTs ranged from 52.8 to 77.3.[398] When live attenuated SA 14-14-2 JE vaccine was used in the control arm in a JE vaccine study in Thailand among children 9 to 18 months of age, 97.6% (122/125) of participants who received live attenuated SA 14-14-2 JE vaccine had seroconverted by day 28.[364] GMT was 171 when SA 14-14-2 JEV was used in the PRNT assay. In a similar study in South Korea among children 12 to 24 months of age, 99.1% (116/117) seroconverted by day 28 with a GMT of 579 (chimeric virus in the PRNT assay).[399]

Cellular Responses. Induction of cellular immunity was shown by higher levels of protection when cyclophosphamide-suppressed immunized mice were vaccinated with live attenuated SA 14-14-2 vaccine compared to inactivated PHK cell vaccine (see earlier discussion).[328,400]

Correlates of Protection

Although correlates of protection were not well established when earlier studies with the SA 14-14-2 vaccine were conducted, recent studies have used the WHO-recommended $PRNT_{50}$ titer of equal to or greater than 10 as the immunological correlate of protection. This recommendation may not apply to immune responses to attenuated viruses, as measured efficacy rates are higher than measured neutralizing antibody responses. Attenuated vaccine was found to be protective in a challenge model using JE strains from various Asian countries collected over a span of 50 years.[328,370,400]

In Special Groups

There are few data on use of live attenuated SA 14-14-2 vaccine in immunosuppressed patients. A trial in Thailand evaluated patients who had undergone allogenic hematopoietic stem cell transplantation 2 or more years earlier and were off immunosuppressive medications for 6 months or longer.[401] Among 18 patients who were not seroprotected at baseline, nine (50%) had seroconverted by 3 months after one dose, but only six (33%) were still seroprotected at 12 months, seven (39%) seroconverted by 3 months following two doses (all remained seroprotected 12 months later), one (6%) seroconverted by 12 months following two doses, and one (6%) did not develop a seroprotective titer. Although this is a small study, these results suggest a poorer immunologic response is likely in immunosuppressed children than in healthy children. There are no data on the use of vaccine in pregnant or breastfeeding women.

Chimeric Vaccine

Antibodies

A single dose of chimeric JE vaccine given to children 16 to 18 months old raised similar levels of neutralizing antibodies against seven strains of JEV, genotypes I and III, within the margin of measurement error.[402]

Correlates of Protection

One Phase III study enrolled 820 participants across 10 centers (five each in the United States and Australia). Half were randomized to receive chimeric JE vaccine and the other half to receive licensed mouse brain vaccine. Ninety-four percent of participants completed the trial. Incidence of adverse events was acceptable in the chimeric JE vaccine arm and JE neutralizing antibody responses were equivalent or better than to standard licensed mouse brain vaccine through day 60.[358]

Duration of Immunity/Protection

In a long-term follow-up assessment in a randomized control Phase II trial, 97.6% (95% CI, 93.3–98.8) of adults showed seroprotective levels 6 months after a single administration of IMOJEV. At 60 months after vaccination 86% had seroprotective levels, but no data exist for seroprotection after 5 years.

EFFICACY AND EFFECTIVENESS OF VACCINE

Inactivated Mouse Brain Vaccine

Efficacy of the Nakayama vaccine was initially evaluated in two masked, randomized, placebo-(tetanus toxoid–) controlled field trials (Table 33.8). In the first one in 1965 in Taiwan, two doses of the inactivated mouse brain–derived vaccine yielded an 80% efficacy in the first year after immunization.[403–405] A subsequent masked, randomized, placebo-controlled field trial in Thailand compared the efficacies of the monovalent Nakayama vaccine with a specially formulated bivalent vaccine also containing Beijing-1 antigen.[282] Two doses of vaccine or placebo were given 1 week apart to children 1 year of age or older. After 2 years, efficacies of the monovalent and bivalent vaccines were identical at 91%. Reduced attack rates of dengue and dengue hemorrhagic fever were also observed in JE-vaccinated groups, although the differences were not significant.

Two recent case control studies evaluated the effectiveness of the mouse brain–derived inactivated vaccines produced in Thailand and Vietnam. Vaccine effectiveness in Thailand among children 18 months to 5 years of age who had received at least 1 vaccine dose was 97.5%.[390,406] In Vietnamese children, vaccine effectiveness among children aged younger than 15 years of age who were vaccinated with at least three doses was 92.9%.[407]

Experimental studies in monkeys and neutralizing antibody studies in humans suggest that immunization against JE might provide cross-protection against West Nile virus, leading some workers to recommend JE vaccine to protect laboratory workers against West Nile virus.[151,408] A small number of workers at the U.S. CDC laboratory in Fort Collins, Colorado, who had been immunized against JE, nevertheless had mild overt West Nile infections.[409]

Inactivated Vero Cell Vaccine

Direct tests of efficacy and effectiveness of inactivated Vero cell–derived SA 14-14-2 vaccines were not undertaken as there was consensus in a WHO consultation that efficacy studies comparing new with existing JE vaccines would be infeasible owing to prohibitive sample sizes.[371] However, immunogenicity as an indicator of efficacy was demonstrated with noninferior immune responses to Vero cell vaccine when compared to mouse brain–derived vaccine (see Table 33.7).[391]

Live Attenuated Vaccine, SA 14-14-2

Five major efficacy trials of SA 14-14-2 vaccine, completed in China from 1988 to 1999 in 1- to 10-year-old children, consistently demonstrated protection rates of greater than 98%.[410] (See the 6th edition of this text for more details.) Inactivated vaccine was already widely used at the time, so placebo-controlled trials were not ethical. A noninferiority trial was conducted with the inactivated vaccine using immunogenicity end points (unpublished data).

A case-control study performed in 1993 in Sichuan Province, China, in children younger than age 15 years, measured effectiveness of routinely delivered SA 14-14-2 vaccine at 80% (95% CI, 44–93%) for a single dose and 97.5% (95% CI, 86–99.6%) for two doses given at a 1-year interval.[411] The PFU per dose was not available for this trial; after 1996, the method of determining PFU dose was refined and standardized (Y.F. Yao, personal communication, 2004; L.J. Yang, personal communication, 2005). In addition, the study authors noted that program quality was unknown, including maintenance of cold chain and vaccine administration techniques, and these factors could have affected the results. In Nepal in 1999, more than 160,000 children 1 to 15 years of age received a single dose of SA 14-14-2 vaccine ($10^{5.8}$ PFU/0.5 mL) just a few days before the onset of a large JE outbreak and vaccine effectiveness was 99.3%. In a case-control study, no JE cases had a history of vaccination compared with 59% of age- and sex-matched village control subjects.[412] The effectiveness findings for a single dose in preventing JE cases in the next year and 5 years later were 98% and 96%, respectively.[413,414] This study provided evidence that SA 14-14-2 was useful in combating epidemics. Although the vaccine lot used in this field trial was made for export and the vaccine packaged in individual 1-mL syringes, this study clearly demonstrates that under supervised administration, SA 14-14-2 vaccine can be highly protective after just one dose.

TABLE 33.8 Efficacy of Inactivated Mouse Brain–Derived Japanese Encephalitis Vaccine[a]

Country	Study Group	No. at Risk	Case Rate/100,000	Efficacy (95% CI) (%)
Taiwan, 1965[348,350]	Total vaccinated	133,943	4.48	76 (63–90)
	1 Dose	22,194	9.01	50 (26–88)
	2 Doses	111,749	3.58	80 (71–93)
	Placebo	131,865	18.20	—
	Nonvaccinated	140,514	24.91	—
Thailand, 1984–1985[332]	Total vaccinated	43,708	4.60	91 (70–97)
	Monovalent	21,628	4.60	91 (54–98)
	Bivalent	22,080	4.50	91 (54–98)
	Placebo	21,516	51.10	—

[a]Two doses of Nakayama or bivalent Nakayama/Beijing mouse brain–derived vaccines.
CI, confidence interval.

Following the widespread use of SA 14-14-2 vaccine in India, two case-control studies measured vaccine effectiveness. In one study among children 1 to 15 years of age, effectiveness was 94.5% at 6 months following a mass immunization campaign.[415] In another study, vaccine effectiveness was measured following administration of a single dose of vaccine as part of the routine immunization program. Among children 24 to 54 months of age, vaccine effectiveness was 84% up to 3 years after vaccination.[416] These and other data from China attest to the effectiveness of the SA 14-14-2 vaccine used in a single-dose preventive campaign complemented by routine EPI administration to infants in reducing the public health burden of JE.

Chimeric Vaccine

Efficacy

Because several effective JE vaccines are available, a randomized, controlled efficacy trial would have been logistically very difficult. Consequently, the chimeric vaccine was licensed on the basis of its ability to induce neutralizing antibodies, which is thought to be a reliable correlate of efficacy.

DURATION OF IMMUNITY AND PROTECTION

Inactivated Mouse Brain Vaccine

Studies in Asia to determine the persistence of vaccine-derived immunity are complicated by boosting through cocirculating related flaviviruses such as dengue and West Nile viruses or reexposure to JEV itself, all of which may reinforce and broaden vaccine-derived immunity to JEV.[102,103,148–150] Even with the potential for these reinforcing infections, several studies in Asian and Western subjects (see previous discussion) indicate a progressive decline in antibody levels in the first year after primary immunization with two doses of mouse brain vaccine.[298,380] Cross-sectional serosurveys in Japan and Taiwan (see earlier discussion) demonstrate a rapid decline of antibody levels in childhood. Observations of vaccine efficacy in the Taiwan field trial parallel these results: in the second year after immunization, protective efficacy declined from 80% to 55% (95% CI, 39–75%).[404] In the Thailand field trial of the Nakayama vaccine, efficacy was shown through 2 years of observation. Further follow-up data are not available. A retrospective study on Taiwan estimated the efficacy of a single dose of Nakayama strain inactivated JE vaccine at 86.7%.[417] However, efficacy data on control subjects were simply extrapolated from historical estimates of JE vaccine delivery in Taiwan and not individual vaccination records. Vaccine efficacy for a 30-year period was calculated using the JE attack rate in a single year, 1967, and it is likely that as the population has become more urban and rice cultivation has declined, JE infection rates in human beings would have fallen significantly.

Booster doses after a two-dose primary immunization series in endemic settings are universally recommended. Indeed, JE cases have been observed in children with a history of adequate primary vaccination.[418] Booster doses are followed by significant increases in neutralizing antibody titer and uniform anamnestic responses in subjects who had reverted to seronegative. A small study of vaccinees receiving an Indian-manufactured JE vaccine found that 34 (97%) of 35 retained neutralizing antibodies 3 years after a primary series of three doses and 31 (91%) of 34 retained antibodies at 4.5 years, with GMTs of 71 and 32, respectively. However, the boosting effect of naturally acquired flaviviral infections in these subjects cannot be ruled out.[419] During the history of the administration of JE vaccines in Asia, additional booster doses have been given empirically, usually with the goal of restoring neutralizing antibody titers to the detectable range with the philosophy that more is better (see Fig. 33.19).

Flavivirus-naïve U.S. Army soldiers who received a three-dose primary immunization series retained protective neutralizing antibody titers for at least 1 year (GMT: 76), with antibody titers at 12 months unchanged from those observed at 3 months after immunization (GMT: 78). A booster dose given at 12 months was followed by a significant anamnestic response (GMT: 1117). In a limited number of subjects studied 3 years after the primary series, 16 (94%) of 17 who had neither traveled to Asia nor received a booster dose retained neutralizing antibody titers of greater than 1 : 10, with their GMTs at 6 months and 3 years remaining unchanged.[381] A large study of 293 laboratory workers revealed that more than 50% lost neutralizing antibody titers to below 1 : 10 just over 2 years after immunization. The authors also noted significant lot-to-lot variation in antibody response to vaccination.[420] Although these observations suggest that the first booster immunization is needed no sooner than 2 to 3 years after primary immunization, the interval for subsequent boosters has not been established. Although studies suggest that antibodies quickly wane no matter how many doses of inactivated vaccine are given, if a person with an undetectable level of antibodies is bitten by an infected mosquito, a B-cell memory response likely occurs, raising antibodies to protective levels before JEV invades the CNS.

Inactivated Vero Cell Vaccine

The persistence of antibodies following immunization with inactivated Vero cell–derived SA 14-14-2 vaccine was investigated in three studies among adults from nonendemic areas with varying results. Findings showed that between 58% and 83% of subjects still had neutralizing antibody titers of 1 : 10 or more at 12 to 15 months after the primary immunization (Table 33.9).[315,316,392] Reasons for the differing results are not completely understood, but the studies were carried out in different geographic areas, and a high prevalence of prior TBE vaccination in the trial showing higher antibody persistence might have contributed to this difference.[317]

Booster doses of inactivated Vero cell–derived SA 14-14-2 vaccine are needed in adults to sustain long-lasting titers of neutralizing antibodies 1 : 10 or greater. Based on data on the persistence of antibodies and immunogenicity of booster doses, the CDC's Advisory Committee on Immunization Practices (ACIP) recommends that adults should be given a booster dose if it is more than 12 months after the primary series and there is the potential for JEV exposure.[421] In a booster trial in 198 subjects 18 years of age and older, inactivated Vero cell–derived SA 14-14-2 vaccine was administered at 15 months after primary immunization. The booster dose was highly immunogenic and yielded a GMT of 900 (a 40-fold increase relative to prebooster titer), with 100% of subjects achieving a $PRNT_{50}$ titer of 1 : 10 or more by 28 days after the booster dose. At 1 year postbooster, the GMT was 361 and 98.5% of subjects still had a $PRNT_{50}$ of 1 : 10 or more (see Fig. 33.20B).[317] A follow-up study included 67 (34%) of the original 198 study participants. At a mean of 75.5 months following a booster dose, 64 of the subjects (96%) still had a neutralizing antibody titer equal to or greater than 10 and the GMT was 148.[318] In six subjects 50 years of age and older, the GMT of 52 (95% CI, 10–269) was lower than the GMT of 165 (95% CI, 118–230) in those younger than age 50 years. GMTs were not significantly different in subjects with and without a history of TBE or YF vaccination. Among the 67 participants, 17 (25%) had traveled to a JE-endemic country since the booster dose and the impact on the immune response of possible exposure

TABLE 33.9 Persistence of Neutralizing Antibodies Against Inactivated Vero Cell–Derived SA 14-14-2 Vaccine Following Two-Dose Primary Immunization Schedule

Study Code	Day 56	Month 6	Month 12 or 15	Month 24	Month 36
SEROPROTECTION RATE % (NO. SEROCONVERTED)					
IC51–303[392,393] (n = 181)	99 (179)	95 (172)	83 (151)	82 (148)	85 (129/152)
Mouse brain–derived vaccine group[392,393] (n = 82)	98 (80)	73 (61)	Not done	Not done	Not done
IC51–305[315] (n = 116)	97 (113)	83 (96)	58 (67)	48 (56)	Not done
IC51–311[317] (n = 198)	97 (192)	Not done	69 (137) (month 15)	Not done	Not done
GEOMETRIC MEAN TITER (95% CONFIDENCE INTERVAL)					
IC51–303[392,393] (n = 181)	311 (269– 359)	84 (71–98)	41 (34–49)	44 (37–53)	44 (n = 152) (37–53)
Mouse brain–derived vaccine, group[392,393] (n = 82)	100 (77–129)	35 (25–46)	Not done	Not done	Not done
IC51–305[315] (n = 116)	219 (180–267)	47 (37–59)	18 (14–23)	16 (13–21)	Not done
IC51–311[317] (n = 198)	171 (146–200)	Not done	23 (19–27) (month 15)	Not done	Not done

Both seroconversion rate and seroprotection rate are defined as the rate of subjects with $PRNT_{50}$ ≥1 : 10.
PRNT, plaque reduction neutralization test.

to wild JEV is unknown. Modeling of antibody decline following a booster dose suggested an average duration of protection of 14 years (range: 2–25 years) and that an estimated 75% of subjects would be protected for at least 10 years. In another study, vaccine recipients whose titers had dropped to less than 1 : 10 were administered booster doses at 11 months (n = 16) or 23 months (n = 24) after primary immunization. All subjects seroconverted following the booster dose, regardless of the time of booster dose administration.[315] No data are available on immunogenicity of booster doses administered longer than 23 months after primary immunization.

There are limited data on duration of protection in children and in individuals living in endemic areas. In a study in the Philippines, the seroprotection rate in children 2 months of age and older to younger than age 17 years given two doses of vaccine was 90% 3 years after the primary series.[396] Among 23 traveling children from JE-nonendemic countries (mean age: 14.3 years; one child younger than 3 years of age at priming) protection rate was was 91.3% (21/23 subjects) 2 years after the primary series.[422]

The response to a booster dose in children was studied in 150 children in the Philippines 9 months of age and older to younger than 17 years. A booster administered 12 months after the primary series resulted in an approximately 40-fold increase in GMT, with highest GMTs in the youngest children. Two years after the booster, the seroprotection rate was maintained at 100%. Currently existing booster recommendations for children are similiar to adults; that is, a booster should be administered before potential reexposure if 12 months or more have elapsed since the primary series.[395]

Live Attenuated Vaccine, SA 14-14-2

In a case-control study in Nepal, persons given a single dose of SA 14-14-2 vaccine in 1999 were followed up in 2000 and again in 2004. The protective efficacy of JE vaccine 12 to 15 months after administration was 98.5% (95% CI, 90.1–99.2%) and at 5 years was maintained at 96.2% (95% CI, 73.1–99.9%).[413,414] As suggested by long-term experience of up to 11 years of sustained reduction in JE cases in areas where many children received only a single dose of SA 14-14-2 vaccine, efficacy of this and possibly other JE vaccines greatly exceeds the ability to detect circulating neutralizing antibodies.

An immunogenicity study conducted in the Philippines followed children for 3 years after a single dose of live attenuated SA 14-14-2 vaccine.[396,423] In infants vaccinated at 8 months of age, seroprotection rates 1, 2, and 3 years later were 90%, 81%, and 79%, respectively. Among infants vaccinated at 10 months of age, corresponding seroprotection rates were 86%, 81%, and 82%. The 1-year results were consistent with findings from a JE vaccine immunogenicity study conducted in Thailand in which SA 14-14-2 JE vaccine was administered to 125 children 9 to 18 months of age in the study's control arm. At 1 year following vaccination, 87.5% of infants who received a single dose of SA 14-14-2 vaccine still had a seroprotective titer.[364] GMT decreased between 1 and 6 months and then remained stable until month 12.

Chimeric Vaccine

Duration of Immunity/Protection

In a long-term follow-up assessment of a randomized control Phase II trial, 97.6% (95% CI, 93.3–98.8) of individuals showed seroprotective levels 6 months after a single administration of IMOJEV, with projected seroprotection rates estimated to be 86.8% 60 months after vaccination.[360] There are no data for seroprotection after 5 years.

ADVERSE EVENTS

Inactivated Mouse Brain–Derived Japanese Encephalitis Vaccine

Common Adverse Events

Localized Reaction. Local tenderness, redness, or swelling at the injection site occurs in approximately 20% of persons immunized with inactivated mouse brain–derived vaccines. Mild systemic symptoms, chiefly headache, low-grade fever, myalgias, malaise, and gastrointestinal symptoms, are reported by 10% to 30% of vaccinees (Table 33.10).[376,378,379,424–426]

Systemic Hypersensitivity Reactions. Vaccine-related allergic adverse events, not reported previously from Asia, were recognized after 1989 in Australia and several European and

TABLE 33.10 Reported Side Effects of Inactivated Mouse Brain–Derived Japanese Encephalitis Vaccine

Country	No. of Subjects	Local Side Effects (%)[a]	Systemic Side Effects (%)[b]	Reference
Thailand	490	<1	1.7–2.9	282
United States	59	18	9	380
United States	1328	12	2	380
	526			382
	Dose 1	20	5	
	Dose 2	12	2	
	Dose 3	11	1	
United States	3573	23	10–13	379
United States, Europe	435			391
	Dose 1	46	29	
	Dose 2	42	12	
Thailand	448	2	1.3–1.8	282

[a]Local tenderness, redness, swelling, itching, and numbness.
[b]Chiefly fever, headache, malaise, rash; also chills, dizziness, myalgia, nausea, vomiting, abdominal pain, diarrhea, sore throat, blurred vision, increased salivation and taste, difficulty concentrating, and emotional instability.

North American countries as the vaccine became used widely for travelers.[263,424–435] Hypersensitivity reactions have consisted principally of generalized urticaria, angioedema, or both, which in a few patients were potentially life threatening. These reactions generally have responded to oral antihistamines or corticosteroids, but recalcitrant cases have required hospitalization and parenteral steroid therapy. A temporally related death was reported in a man with multiple hypersensitivities who also had received plague vaccine.[263] Numerous lots and different manufacturers have been implicated in the hypersensitivity reactions.[429] In retrospect, allergic side effects, including urticaria, angioedema, and moderate dyspnea, were also observed in recipients of the crude mouse brain vaccine administered on Okinawa in 1945.[265]

An important feature of the hypersensitivity reactions is the potential for delayed onset, particularly after a second dose. In a prospective study of 14,249 U.S. Marines, the median interval between immunization and onset was 18 to 24 hours after the first dose, with 74% of reactions occurring within 48 hours.[263,428] Among those experiencing a reaction after the second dose, there was a greater delay, with a median interval of 96 hours and a range of 20 to 336 hours. Reactions have also developed after a second or third dose when earlier doses were given uneventfully. A nested case-control study found an elevated risk with history of various allergic disorders (e.g., urticaria: OR, 11.4 [95% CI, 2.4–62.1]; allergic rhinitis: OR, 9.2 [95% CI, 2.8–23.1]; asthma, rhinitis, or both: OR, 6.5 [95% CI, 2.1–20.8); and any allergy: OR, 5.7 [95% CI, 1.8–18.1]).[263] Another small study also implicated alcohol consumption and receipt of another vaccine 1 to 9 days previously, as opposed to simultaneous administration, as risk factors.[433]

Reported rates of hypersensitivity have varied according to the approach to ascertainment. Prospective and retrospective studies have found risk of an allergic adverse event, usually defined as objective urticaria or angioedema, in the range of 18 to 64 per 10,000 vaccinees.[427,432,433,435,436] A cluster of two deaths resulting from anaphylactic shock in children receiving JE vaccine was reported in Korea in 1994. In a follow-up study to measure the incidence of JE vaccine–related adverse events, one case of anaphylactic shock with syncope and collapse, three cases of generalized urticaria, and three cases of severe erythema were found in 15,487 Korean children immunized between May 15 and June 30, 1995. The rate of 0.03% was lower than that observed in adult travelers, which could reflect biologic differences in reactivity or the sensitivity of surveillance (Y.M. Sohn, unpublished data, 1996).

Reported rates of hypersensitivity reactions during postmarketing surveillance in the United States and Japan in the 1990s were 6.3 and 0.8 events per 100,000 doses, respectively.[437] In a subsequent analysis of reports submitted between 1999 and 2009 to the U.S. Vaccine Adverse Event Reporting System (VAERS), 106 (35.3%) of 300 total events were classified as hypersensitivity reactions for a rate of 8.4 per 100,000 doses distributed.[438]

Although the pathogenesis of the hypersensitivity reactions is not proven, in three Japanese children experiencing systemic reactions, IgE antibodies to gelatin were demonstrated, suggesting that gelatin, which is added as a vaccine stabilizer, may be a provoking antigen.[439] Further analysis of reactions showed two patterns: One was a combination of urticarial rash and wheezing, which was associated with the presence of antigelatin IgE in the serum, and the second was a cardiovascular collapse syndrome, in which antigelatin IgE was absent and was apparently caused by another mechanism.[439] Immunologic studies demonstrated IgE antibodies to human albumin, which is added to the vaccine as a stabilizer and chemically altered by the inactivating agent β-propiolactone.[440] Allergic reactions in recipients of crude mouse brain vaccine in Okinawa were attributed to formalin-altered proteins.

Rare Adverse Events

From the beginning, the vaccine's neural tissue substrate raised concern about the possibility of postvaccination neurologic side effects.[441] The manufacturing process purifies the infected mouse brain suspension extensively, and MBP content is controlled below 2 ng/mL, well below the dose considered to have an encephalitogenic effect in a guinea pig test system. However, measurements of other acute disseminated encephalomyelitis (ADEM)-associated neural proteins (e.g., proteolipid protein, myelin-oligodendrocyte glycoprotein) have not been reported. Experimental immunization of guinea pigs and cynomolgus monkeys with adjuvant and 50 times the normal dose of vaccine did not result in clinical or histopathologic evidence of encephalomyelitis.[442–445]

One case of Guillain-Barré syndrome, temporally related to JE immunization, was reported among approximately 20,000 American soldiers immunized with the vaccine before U.S. licensure. An early prospective study to detect vaccine-associated adverse events in Japan found no neurologic complications occurring within 1 month after vaccination in 38,384 subjects receiving crude or purified vaccine.[441] A countrywide study to detect neurologic complications found 26 temporally related cases (meningitis, seizures, demyelinating disease, polyneuritis) between 1957 and 1966, but rates and comparisons with nonimmunized control subjects were not available.

In 1992, two anecdotally reported cases of temporally related, vaccine-associated ADEM in Japan prompted a survey of 162 Japanese medical institutions to solicit additional cases.[446] Five more cases spanning 22 years were reported, including two with elevated CSF MBP levels.[447] Neither the numerator of cases nor the denominator of vaccinees was defined rigorously, but the authors estimated that ADEM occurred in fewer than 1 in 1 million vaccinees. An unrelated

report described two deaths from anaphylactic shock, and four ADEM cases (two fatal) temporally related to vaccination in Korea in 1994; one, also fatal, was reported in 1996. An additional fatal case of acute encephalopathy occurred in a 15-year-old girl who received her ninth dose of JE vaccine and her third dose of a hantaviral vaccine (also made in mouse brain) at 4 and 2 weeks, respectively, before onset of stupor and seizures (Y.M. Sohn, unpublished data, 2002). In Denmark, after a vaccinee developed ADEM in 1995, a review of the national database disclosed two similar temporally related cases in 1983 and 1989, all in adults.[448] A high rate of temporally related ADEM, one in 50,000 to 75,000 vaccinees, was determined.

In the United States, postmarketing surveillance between 1993 and 1998 detected one case of Guillain-Barré syndrome in a 55-year-old male 17 days after vaccination. He had also received rabies vaccine 5 weeks prior and had gastroenteritis 1 week prior to onset. One febrile seizure was also reported for a reporting rate of 0.2 events per 100,000 doses for all neurological adverse events.[437] Between 1999 and 2009, four neurologic events were reported in temporal association with vaccination for a reporting rate of 0.3 per 100,000 doses distributed; the events included one case each of Bell palsy, generalized weakness, ataxia, and seizure.[438] In Japan, 17 vaccine-related neurologic disorders were reported from April 1996 to October 1998.[437] In 2005, the government of Japan withdrew its recommendation for routine immunization using inactivated mouse brain–derived JE vaccine.[285] Routine immunization was recommended again once Vero cell–derived vaccines were licensed.

Adverse Events in Immunosuppressed Persons

There are few data on the safety and immunogenicity of inactivated JE vaccines in immunocompromised persons. A small study of children with various chronic diseases, including some oncology patients, disclosed no difference in immunogenicity or reactogenicity compared to responses in healthy children.[389] Infants vertically infected with HIV responded less well to the vaccine (see earlier discussion), but no unusual adverse events were recorded.[388]

Adverse Events During Pregnancy

No adverse outcomes of pregnancy have been associated directly with JE vaccine. Currently, the ACIP notes that the safety of JE vaccine in pregnancy is unknown, and because vaccination poses a hypothetical risk to the fetus, routine vaccination during pregnancy is not recommended. However, the ACIP also advises that pregnant women traveling to a JE-endemic area should be vaccinated if the risk of infection is greater than the theoretical risks of immunization.

Spread to Contacts

Spread to contacts is not a risk because the vaccine is inactivated.

Inactivated Vero Cell Vaccine

Common Adverse Events

The adverse event profile of inactivated Vero cell–derived SA 14-14-2 vaccine was characterized in a series of prelicensure clinical trials, comparing the local and systemic safety profiles with alum-containing PBS and with inactivated mouse brain–derived JE vaccine.[391,449]

Localized Reactions. The localized reactions seen after immunization with inactivated Vero cell–derived SA 14-14-2 vaccine are similar to local reactions after other intramuscularly administered, alum-adjuvanted vaccines. In several trials, the major local reactions have been injection site pain and tenderness, which have been reported in 20% to 30% of recipients of vaccine and placebo (Table 33.11). Local redness, swelling, itching, and hardening were less commonly observed.

TABLE 33.11 Injection Site–Solicited Adverse Reactions[a] After Inactivated Vero Cell–Derived SA 14-14-2 Vaccine or Placebo Control[b] in Subjects in the Safety Population With Evaluable Diary Cards

	Incidence					
	After Dose 1 (% of Subjects[c])		After Dose 2 (% of Subjects[c])		After Dose 1 or 2 (% of Subjects[c])	
Adverse reaction	Vero cell JE vaccine (n = 1963[d])	Control (n = 645[d])	Vero cell JE vaccine (n = 1951[d])	Control (n = 638[d])	Vero cell JE vaccine (n = 1963[d])	Control (n = 645[d])
Any Reaction	48.5	47.7	32.6	32.2	55.4	56.2
Pain	27.7	28.2	17.7	18.2	33.0	35.8
Tenderness	28.8	26.9	22.5	18.1	35.9	32.6
Erythema	6.8	5.4	4.6	4.1	9.6	7.4
Induration	4.8	5.3	4.0	3.0	7.5	7.4
Edema	2.4	3.3	2.3	1.6	4.2	4.6
Pruritus	2.6	3.3	1.6	1.9	3.8	4.5

[a]Injection-site reactions were assessed for 7 days after each dose.
[b]The control was phosphate-buffered saline and aluminum hydroxide.
[c]Denominators used to calculate percentages are based on the number of evaluable diary card entries (defined as documented presence on any day [i.e., entry of "yes"] or absence on all days [i.e., entry of "no"]) for each individual symptom and observation period.
[d]Number of subjects who returned diary cards after each dose.
JE, Japanese encephalitis.
Data from U.S. Food and Drug Administration (FDA) Approved Prescribing Information for IXIARO. Revised August 2015. Available at: http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM142569.pdf; and Tauber E, Kollaritsch H, von Sonnenburg F, et al. Randomized, double-blind, placebo-controlled phase 3 trial of the safety and tolerability of IC51, an inactivated Japanese encephalitis vaccine. J Infect Dis. *2008;198:493–499.*

Most local reactions after immunization with inactivated Vero cell–derived SA 14-14-2 vaccine are transient and mild. The local reactogenicity profile seemed to be more favorable than the mouse brain–derived JE vaccine in terms frequency of severe reactions, with any severe symptom reported in nine (2.1%) of 421 subjects versus 59 (13.8%) of 427 subjects ($P < .0001$), respectively.[391]

Common Systemic Reactions. Headache and myalgia were the most common systemic reactions reported in vaccinated subjects and placebo recipients in clinical trials of inactivated Vero cell–derived SA 14-14-2 vaccine (Table 33.12). Other mostly mild systemic reactions observed in vaccinees were fever, rash, nausea, vomiting, diarrhea, and excessive fatigue. All reactions were seen at similar frequencies in recipients of placebo.

Systemic Hypersensitivity Reactions. Adverse events that generally might be associated with systemic hypersensitivity reactions were analyzed in a metaanalysis of clinical trials that included 3558 subjects receiving inactivated Vero cell–derived SA 14-14-2 vaccine; 657 subjects receiving placebo control (PBS + alum); and 435 subjects receiving a mouse brain–derived inactivated JE vaccine.[450] Total potential hypersensitivity-related adverse events were reported at similar frequencies in recipients of inactivated Vero cell–derived SA 14-14-2 vaccine (3.5%) and a PBS + alum control vaccine (3.7%), but the incidence was significantly higher in the group receiving the mouse brain–derived JE vaccine (5.5%; for difference between inactivated Vero cell–derived SA 14-14-2 vaccine and mouse brain–derived inactivated JE vaccine, $P = .04$). Urticaria was reported in four of 3558 subjects receiving inactivated Vero cell–derived SA 14-14-2 vaccine (two generalized and two localized reactions of mild to moderate severity) and one of 657 subjects receiving PBS + alum vaccination. All cases occurred between 6 and 28 days after administration of the vaccine. All recovered successfully; three subjects received no treatment, one was treated with antihistamines plus a corticosteroid, and one with antihistamines alone (both in the inactivated Vero cell–derived SA 14-14-2 vaccine group). Only the reaction following the PBS + alum vaccination was judged as possibly related to the vaccination by the investigator. Two subjects had no reaction to a second dose of vaccine. Angioedema was not reported in any of the cases.[450]

In an open-label pediatric trial in the Philippines, 195 infants 2 to 11 months of age were randomly assigned to receive the inactivated Vero cell–derived vaccine (N = 131) or

TABLE 33.12 Common Systemic Adverse Events[a] After Inactivated Vero Cell–Derived SA 14-14-2 Vaccine or Placebo Control[b] in the Safety Population

Adverse Event	Incidence (% of Subjects)					
	First Vaccination Period (Day 0–28)		Second Vaccination Period (Day 28–56)		Total Vaccination Period (Day 0–56)	
	Vero Cell JE Vaccine (n = 1993[c])	Control (n = 657[c])	Vero Cell JE Vaccine (n = 1968[c])	Control (n = 645[c])	Vero Cell JE Vaccine (n = 1993[c])	Control (n = 657[c])
Headache[d]	21.6	20.2	13.4	13.0	27.9	26.2
Myalgia[d]	13.3	12.9	5.6	5.3	15.6	15.5
Fatigue[d]	8.6	8.7	5.2	5.9	11.3	11.7
Influenza-like illness[d]	8.2	8.5	5.8	4.3	12.3	11.7
Nausea[d]	4.7	5.3	2.6	3.7	6.6	7.5
Nasopharyngitis	2.3	1.8	2.6	2.3	4.7	4.0
Pyrexia[d]	1.9	2.1	1.5	1.7	3.2	3.0
Rhinitis	1.0	0.8	0.5	0.6	1.4	1.4
Upper respiratory tract infection	0.9	0.9	0.8	0.9	1.7	2.0
Back pain	0.8	0.9	0.6	0.2	1.3	1.1
Pharyngeal pain	0.8	0.9	1.0	0.5	1.6	1.4
Rash[d]	0.8	0.9	0.7	0.8	1.3	1.5
Diarrhea	0.8	0.8	0.7	0.3	1.5	1.1
Cough	0.8	0.8	0.6	0.6	1.2	1.2
Vomiting[d]	0.6	0.8	0.8	0.9	1.4	1.7

[a]Adverse events are those observed at an incidence of ≥1% in the IXIARO or control group.
[b]Placebo control was phosphate-buffered saline and aluminum hydroxide.
[c]Number of subjects in the safety population (subjects treated with at least one dose) who received the respective dose.
[d]These symptoms were solicited in a subject diary card. Percentages include unsolicited events that occurred after the 7-day period covered by the diary card.
JE, Japanese encephalitis.
Data from U.S. Food and Drug Administration (FDA) Approved Prescribing Information for IXIARO. Revised August 2015. Available at: http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM142569.pdf; and Tauber E, Kollaritsch H, von Sonnenburg F, et al. Randomized, double-blind, placebo-controlled phase 3 trial of the safety and tolerability of IC51, an inactivated Japanese encephalitis vaccine. J Infect Dis. *2008;198:493–499.*

seven-valent pneumococcal conjugate vaccine (N = 64). Another 1674 children 1 to 17 years of age received inactivated Vero cell–derived vaccine (n = 1280) or hepatitis A vaccine (n = 394). The incidences of local, systemic, medically attended, and serious adverse events were similar between children who received JE or the relevant comparison vaccines. The main local reactions were redness in children younger than age 1 year and pain and tenderness in older children. The predominant systemic reaction was fever.[341]

Rare Adverse Events

One evaluation of postmarketing surveillance data reviewed adverse event reports from the first 12 months following licensure of inactivated Vero cell–derived SA 14-14-2 vaccine in Europe, Australia, and the United States. Data analyzed included consumer reports, regulatory authority reports, European pharmacovigilance data, and reports from the VAERS database. Overall, there were 25 adverse drug reactions (ADRs) reported, including 13 from Europe, 6 from the U.S., and 6 from Australia, translating into a reporting rate of 10.1 per 100,000 doses distributed. Four of these ADRs (16%) were serious (reporting rate for serious ADRs: 1.6 per 100,000 doses distributed). The serious ADRs were distinctive plexus neuritis of all extremities, meningism (later downgraded by the local regulatory authority to be a nonserious case), oropharyngeal spasm, and iritis. No cases of anaphylaxis or angioedema were reported.[451]

The second review analyzed adverse events reported to VAERS in the first 3 years postvaccine licensure in the United States from May 2009 to April 2012.[452] Although events occurred in temporal association with vaccination, causality cannot generally be assessed for VAERS reports. All reports were for adults 17 years of age or older as the review period was prior to pediatric licensure, and some reports overlapped those reviewed in the multicountry analysis described above. There were a total of 42 adverse events reported during the 3-year period, equating to a reporting rate of 15.2 adverse events per 100,000 doses distributed. Among these 42 reports, there were 12 hypersensitivity adverse events for a reporting rate of 4.4 per 100,000 doses distributed, and six neurologic adverse events for a reporting rate of 2.2 per 100,000 doses distributed. Among the hypersensitivity events, four occurred within 2 hours (immediate hypersensitivity events) and eight occurred between 2 hours and 10 days (delayed hypersensitivity events) after vaccination. Among the six neurologic events, three were classified as central and three as peripheral neurologic events. Overall, the most commonly reported events were the 12 hypersensitivity events and 10 reports of fever, with three or fewer reports each of musculoskeletal pain, local reactions, nonspecific somatosensory complaints, rash, and syncope. In total, 27 (64%) events occurred after the first dose and 10 (24%) followed a second dose. The inactivated Vero cell–derived vaccine was the only vaccine administered in 17 (40%) of the reports. Five events (12%) were classified as serious as they resulted in hospitalization (n = 4) or "observation for a life-threatening condition," for a reporting rate of 1.8 per 100,000 doses distributed. The five serious adverse events included one case each of immediate hypersensitivity, delayed hypersensitivity, appendicitis, encephalomyelitis, and myopericarditis. Other vaccines, including smallpox and anthrax vaccines were administered at the same time for three of these patients (Table 33.13). Overall, most events were nonserious, no unexpectedly high reporting rates for specific events were identified, and no deaths were reported. Therefore, these postmarketing surveillance data from the first 3 years postlicensure supported the good safety profile documented in prelicensure clinical trials.

Adverse Events in Immunosuppressed Persons

Safety of inactivated Vero cell–derived SA 14-14-2 vaccine has not been studied in immunocompromised persons. Postmarketing data through the first 5 years of marketing did not contain reports on use in immunocompromised persons. Naturally, concerns that the vaccine could cause JE in immunocompromised persons are not applicable to an inactivated vaccine.

Adverse Events During Pregnancy

Inactivated Vero cell–derived SA 14-14-2 vaccine is pregnancy category B (United States). There are limited data from the use of the vaccine in pregnant or breastfeeding women. In a reproductive and prenatal/postnatal toxicity study in animals, no vaccine-related effects were observed on reproduction, fetal weight, survival, and development of the offspring. However, incomplete ossification of parts of the skeleton was observed in the group receiving two doses, but not in the group receiving three doses. As a precautionary measure, use of the vaccine during pregnancy or lactation should be avoided as per European labeling. After reviewing data from 28 women who became pregnant during clinical trials with inactivated Vero cell–derived SA 14-14-2 vaccine, the European regulatory authority concluded that there was currently no evidence for a causal relationship of abnormal pregnancy outcomes with the vaccine (Valneva, on file). Cases in which women are inadvertently vaccinated during pregnancy should be reported to the manufacturer for follow-up of the pregnancy and infant health status. Further data will be generated as part of a vaccine safety surveillance program.

TABLE 33.13 Serious Adverse Events Reported to the U.S. Vaccine Adverse Event Reporting System Following Receipt of IXIARO, May 2009 to April 2012

Event Type	Days Postvaccination	Age (y)	Sex	Primary Symptoms or Condition	Concurrent Vaccines
Immediate hypersensitivity	0	45	M	Dyspnea, sensation of throat closure	MMR, HepA
Delayed hypersensitivity	2	56	M	Dyspnea, cough	None
Appendicitis	5	40	M	Abdominal pain	None
Myopericarditis	11	22	M	Chest pain	Smallpox, Anthrax, Typh
Central neurological	39	20	M	Encephalomyelitis	Smallpox, Typh, HepA/HepB

HepA, hepatitis A vaccine; HepB, hepatitis B vaccine; MMR, measles, mumps, rubella vaccine; Typh, typhoid vaccine.
From Rabe IB, Miller ER, Fischer M, et al. Adverse events following vaccination with an inactivated, Vero cell culture-derived Japanese encephalitis vaccine in the United States, 2009–2012. Vaccine. *2015;33:708–712.*

Spread to Contacts

Spread to contacts is not a risk because the vaccine is inactivated.

Live Attenuated Japanese Encephalitis Vaccine

Common Adverse Events

Safety data are available from clinical trials as well as from postmarketing surveillance following the use of live attenuated SA 14-14-2 JE vaccine in more than 400 million individuals in Asia. In an early safety study in China, of 867 children in whom fever was closely monitored during a 21-day period after immunization, temperatures of 37.6°C or higher were recorded in fewer than 2% of vaccinees, and fever-onset days were distributed throughout the observation interval, mitigating against a vaccine-related febrile illness after a specific incubation period.[453] In the same study, symptoms were recorded from 588,512 other vaccinees; fever was reported in 0.05% of subjects, rash in 0.01%, dizziness in 0.003%, and nausea in 0.003%, but these rates are difficult to interpret in the absence of similar observations in control subjects.

A block-randomized cohort study investigated vaccine safety in 13,266 vaccinated and 12,951 nonvaccinated 1- to 2-year-old children who were followed up prospectively for 30 days. No cases of encephalitis or meningitis were detected in either group, and rates of hospitalization, new onset of seizures, fever lasting more than 3 days, and allergic, respiratory, and gastrointestinal symptoms were similar in the two groups. Based on study findings, a vaccination-related encephalitis risk above 1 in 3400 could be excluded.[454]

In a randomized trial conducted in the Philippines from 2005 to 2006, local reactions were reported in 27 (27%) of 100 children 8 months of age and 27 (12%) of 224 children 10 months of age.[348] The majority of local reactions were mild and transitory. Systemic reactions were reported in 58% and 39% of children, respectively. The most common early systemic reactions were irritability and drowsiness. Fever within 7 days was reported in 24% and 25% of infants in both groups, but no fever was 40°C or higher. No serious adverse events were reported. As there was no placebo group in the trial, the proportion of systemic events attributable to live attenuated SA 14-14-2 JE vaccine could not be determined. In four other recent studies, the proportion of children with local reactions ranged from 1% to 44%.[350,364,398,399] In only one of these studies were any serious adverse events thought to be vaccine related reported; two children in a study in Korea had fevers that were classified as serious as they resulted in hospitalization of the children.[399] In the same study, one child had urticaria considered by investigators to be vaccine-related.

Rare Adverse Events

In national postmarketing surveillance in China during 2009–2012, 6024 adverse events temporally associated with immunization were reported, for a rate of 9.7 events per 100,000 doses distributed. There were 70 events classified as serious including febrile convulsions, thrombocytopenic purpura, and myelitis. Among nine encephalitis cases, one was considered possibly vaccine-related while the others were classified as coincidental illnesses. No other safety signals were identified.[341,455] Following use of the vaccine among more than 9.3 million children 1 to 15 years of age in mass vaccination campaigns in India, 65 serious adverse events were reported. Most were considered to be unrelated to the vaccine. Two clusters of encephalitis-like syndromes were identified. WHO's Global Advisory Committee on Vaccine Safety (GACVS) reviewed available information and considered the events were unlikely related to vaccine. On other occasions, the GACVS has reviewed data on the live attenuated SA 14-14-2 JE vaccine and noted its excellent safety profile.[341,456]

Adverse Events in Immunosuppressed Persons

Although experimental data suggest that JE SA 14-14-2 virus may not be neurotropic in immunosuppressed animals, there are no data on the vaccine's safety in immunocompromised persons, specifically HIV-infected patients. It should not be administered to persons known to be immunosuppressed; instead, inactivated JE vaccine should be used.

Adverse Events During Pregnancy

No observations on the vaccine's safety in pregnant women have been reported. Live attenuated JE vaccine has a theoretical risk in pregnant women. Usage advisories are that pregnant women should not be vaccinated. When JE vaccine must be given to pregnant women or to immunocompromised persons, available inactivated JE vaccine should be used rather than live vaccine.

Spread to Contacts

No spread to contacts has been reported. Transmissibility of vaccine virus by vector mosquitoes is not well studied.

Chimeric Vaccine

Adverse Events

Common postvaccination symptoms include tiredness, malaise, injection-site pain, headache, and myalgia. Less-common postvaccination events include itching at injection site, joint pain, gastrointestinal symptoms, upper respiratory symptoms, rash, and fever.

Rare Adverse Events

A small number of serious adverse events were reported during clinical development of the vaccine, including high-grade fever.[358] No serious hypersensitivity reactions or neurologic adverse events were identified in clinical trials. However, because the vaccine has been used in limited numbers of people, the possibility of rare serious adverse events cannot be excluded. Additional postmarketing safety data collected from larger populations are needed to fully evaluate this issue.

Adverse Events During Pregnancy

Animal studies did not indicate direct or indirect harmful effects with respect to pregnancy, embryo-fetal development, parturition, or postnatal development. However, as with all live attenuated vaccines, pregnancy constitutes a contraindication.

Contraindications

The chimeric JE vaccine should not be administered to anyone with HIV, congenital or acquired immune deficiency, or a history of severe allergic reaction to any component of the vaccine. Vaccination must be postponed in case of febrile or acute disease. Vaccine should not be given to patients on immunosuppressive therapies such as chemotherapy or high doses of systemic corticosteroids given generally for 14 days

or more. IMOJEV must not be administered to pregnant or lactating women.

Adverse Events in Immunosuppressed Persons

None have been reported, but the vaccine should not be administered to this group.

Spread to Contacts

No reports.

INDICATIONS FOR VACCINE (WHO AND WHY)

Endemic Areas

In rural areas of Asia, intense JEV transmission in the enzootic cycle leads to a high risk of exposure at an early age. The risk of infection increases sharply with the waning of the protective effects of maternal immunity and as increasing outdoor activity places young children at risk of bites from infected mosquitoes. In hyperenzootic settings, there may be almost universal seroconversion by the age of 15 years, indicating that by that age everyone has been exposed and had an asymptomatic, mild illness, or clinical disease. The WHO recommends that JE vaccination should be integrated into the national immunization program in all areas where JE is recognized as a public health priority, and notes that even if the number of JE-confirmed cases is low, vaccination should be considered where there is a suitable environment for JEV transmission, such as the presence of animal reservoirs, ecological conditions supportive of transmission, and proximity to other areas with known JEV transmission.[42] For the most part, stepwise implementation of national JE vaccination programs, initially in epidemic foci and in areas with hyperenzootic transmission, has been necessary because of economic considerations.[457,458] The current recommendation is to mount a one-time catch-up campaign to cover all at-risk age groups (typically children <15 years) followed by the introduction of routine immunization through the EPI. Although most JE immunization programs were initiated in limited areas, as surveillance systems improved, programs have expanded to the entire country.[459,460] It is important to note that immunization schedules and boosting may be different in endemic than in nonendemic settings.

Although incidence may vary from year to year and within countries, universal childhood immunization is desirable because, even in economically advanced countries, viral transmission cannot be eliminated and the cumulative risk of acquiring the illness over a lifetime of exposure justifies universal protection. This ongoing risk was confirmed in a Japanese cross-sectional serologic study measuring NS1 antibodies that showed average annual infection rates of 1.8% in west Japan from 2004 to 2008 and 1.3% in Tokyo from 2004 to 2006.[285] When only unvaccinated persons were included, average annual infection rates were 2.6% in both areas.[207,285,461] Indeed, when Japan temporarily withdrew its recommendation for routine childhood immunization in 2005, two reports of JE in unvaccinated children followed in 2006 and 2009.[208] Conditions leading to epidemic transmission are unpredictable, and, at intervals, outbreaks may lead to large numbers of cases, even in urban areas.

Expatriates

With safe and effective new JE vaccines available, vaccination is recommended for all expatriates whose principal residence is in an area where JE is enzootic.[41,253,426,462] Risk of acquiring JE among expatriates is variable and depends principally on the specific location of residence, housing conditions, nature of activities, and the possibility of unanticipated exposure to high-risk areas (see the next section, "Travelers"). Risk varies regionally and within specific countries, and viral transmission can fluctuate from year to year in a given location. Fig. 33.1 and Table 33.14 summarize and extrapolate available data on locations and seasonality of risk by country and

TABLE 33.14 Risk of Japanese Encephalitis by Country, Region, and Season[a]

Country	Affected Areas/ Jurisdictions	Transmission Season	Vaccinating	Comments
Australia	Outer Torres Strait islands	December–May	Targeted populations (outer Torres Strait Islands)	1 Human case reported from north Queensland mainland
Bangladesh	Probably widespread	Cases throughout the year; peak, May–October	No	Sentinel surveillance identified cases in Chittagong, Dhaka, Khulna, Rajshahi, Ranjpur, and Sylhet Divisions
Bhutan	Rare reports	No data	No	Virus transmission likely
Brunei	Rare reports	Presumed year-round transmission	Private market	Virus transmission likely
Cambodia	Enzootic throughout country	Year-round with peak season May–October	Included in the Expanded Programme on Immunization (EPI) (subnational)	Sentinel surveillance identified cases in 15 of 23 provinces
China	Cases in all provinces except Xizang (Tibet), Xinjiang, and Qinghai; rare cases in Hong Kong	Northern China: May–September; southern China: April–October	Included in the EPI in all affected provinces	Vaccine not routinely recommended for travelers to major cities; highest JE rates in Guizhou, Shaanxi, Sichuan, and Yunnan Provinces, and Chongqing City

Continued on following page

TABLE 33.14 Risk of Japanese Encephalitis by Country, Region, and Season[a] *(Continued)*

Country	Affected Areas/ Jurisdictions	Transmission Season	Vaccinating	Comments
India	Cases in all states except Dadra, Daman, Diu, Gujarat, Himachal, Jammu, Kashmir, Lakshadweep, Meghalaya, Nagar Haveli, Orissa, Punjab, Rajastan, and Sikkim	Northern India: July–December; southern India: Goa, May–October; Tamil Nadu: October–January; Karnataka: April–December; Andhra Pradesh: September–December	Included in the EPI in all affected districts	Highest rates reported from West Bengal, Bihar, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Assam, Uttar Pradesh, Haryana, and Goa
Indonesia	Presumed to be endemic nationwide	Year-round; peak period with rainy season, November–March	No	Sentinel surveillance has identified cases in Bali, Kalimantan, Java, Nusa Tenggara, Papua, and Sumatra
Japan	Enzootic in all islands, except Hokkaido	June–October, except Ryukyu Islands (Okinawa), April–October	Yes	Vaccine not routinely recommended for travelers to major cities
Korea, North	Presumed to be endemic nationwide	July–October	Vaccine campaigns	
Korea, South	Enzootic throughout country	May–October	Yes	Vaccine not routinely recommended for travelers to major cities
Laos	Enzootic throughout country	June–September	Included in the EPI (subnational)	
Malaysia	Enzootic in peninsular Malaya and Borneo	Year-round transmission	Subnational program	Most human cases reported from Sarawak
Myanmar	Enzootic throughout country	May–October	No	
Nepal	Enzootic in Terai; some cases reported from Kathmandu Valley and other hill and mountain districts	June–October	Included in the EPI (subnational)	Highest rates of disease reported from western Terai districts; vaccine not routinely recommended for those trekking in high-altitude areas
Pakistan	Presumed enzootic in central Pakistan	June–January	No	
Papua New Guinea	Low-level transmission	Year-round	No	
Philippines	Enzootic throughout country	Year-round with peak season July–September	No	
Russia	Enzootic in Far Eastern provinces	July–September	No	
Singapore	Little transmission	Year-round	No	Vaccine not routinely recommended
Sri Lanka	Enzootic throughout country	Scattered cases year-round; peak season follows monsoon rains	Included in the EPI	Highest rates of human disease reported from Anuradhapura, Gampaha, Kurunegala, Plonnaruwa, and Puttalam districts
Taiwan	Enzootic throughout country	May–October	Yes	Vaccine not routinely recommended for travelers to major cities
Thailand	Enzootic throughout country	Cases occur year-round in south; May–October in north	Included in the EPI	
Vietnam	Enzootic throughout country	Year round with peak season May–October, especially in the north	Included in the EPI	Highest disease rates in the northern provinces
Western Pacific	Sporadic introductions	Insufficient data	No	Vaccine not recommended

[a]Assessments based on published reports, reports to the World Health Organization, and personal correspondence. Because virus is transmitted in a zoonotic cycle, reports of human cases in countries with vigorous vaccination programs are not a reliable indicator of risk. For an update, refer to the U.S. Centers for Disease Control and Prevention, Travelers' Health (http://wwwnc.cdc.gov/travel).

region. Patterns of viral transmission may change, and physicians and travelers are cautioned to consult public health officials for current data and trends. The risk may be difficult to assess because JE is poorly reported, accurate data may not be available, and local immunization prevents disease, giving a false sense of security.

Travelers

Administration of a licensed JE vaccine is recommended for select travelers to Asia, but is not recommended as a routine immunization. In the United States, the vaccine is recommended for persons spending 1 month or more in enzootic settings during the transmission season. This includes recurrent travelers or expatriates who will be based in urban areas but who are likely to visit rural areas during a high-risk period for JEV transmission. Vaccine should be considered for short-term (<1 month) travelers if they have an increased risk for JEV exposure, such as spending a substantial amount of time outdoors in rural areas, participating in extensive outdoor activities (e.g., camping, hiking, fishing), or staying in accommodations without air conditioning.[41,462]

JE vaccine is not recommended for short-term travelers whose visit will be restricted to urban areas or time outside of a well-defined transmission season.[463] European recommendations vary by country with some (e.g., Germany and France) having broad recommendations for immunization for travelers with higher risk itineraries regardless of duration of travel, and some having more restrictive recommendations focusing on prolonged travel.[465,466] In many Asian countries, the use of vaccines in endemic areas masks viral activity, making it difficult to determine risk accurately. Travelers and their physicians should weigh individual risk factors and disease risk in the area and season of anticipated travel (see Figs. 33.1 and Tables 33.3 and 33.14). Advanced age and pregnancy may affect risk and outcome of JE. Repellents and other protective measures are recommended regardless, because other vector-borne diseases may be transmitted in the same areas. General precautions are especially important for travelers in whom vaccine is contraindicated, who are unable to complete immunization because of departures on short notice, or who do not choose to be immunized because their visits to high-risk areas are brief or carry an equivocal risk. The recommendations for vaccination may change as data on cost and risk profiles become available for vaccines licensed for use in travelers.

Research Laboratory Workers

There have been at least 22 cases of laboratory-acquired JEV, principally in research settings where infectious JEV was used.[232,467] Infection can be transmitted by percutaneous or mucous membrane exposures and potentially by aerosols, especially from preparations containing high viral concentrations, which occur during viral purification. Immunization presumably protects against percutaneous exposures; however, it is unknown whether vaccine-derived immunity, especially from inactivated vaccine, protects against aerosol infection. Immunization is advised for all research laboratory personnel who potentially may be exposed to field or virulent strains of the virus. Based on a review of data firmly establishing the attenuation of vaccine SA 14-14-2 strain, the U.S. Subcommittee on Arbovirus Laboratory Safety recognizes that immunized workers should be permitted to handle SA 14-14-2 strain under biosafety level 2 conditions, paralleling recommendations for the attenuated vaccine strains of YF, Junin, Rift Valley fever, chikungunya, and Venezuelan equine encephalitis viruses.[468]

CONTRAINDICATIONS AND PRECAUTIONS TO IMMUNIZATION

Mouse brain–derived JE vaccine is contraindicated in people who have had an allergic reaction to the vaccine, to gelatin, or to other rodent-derived products, including previous doses of JE vaccine. As previously noted, hypersensitivity reactions to mouse brain–derived JE vaccine are more common in persons with allergic conditions (e.g., asthma; allergic rhinitis; drug or Hymenoptera venom sensitivity; and food allergy, especially to gelatin-containing foods). If these persons are offered JE vaccine, they should be advised of the potential for vaccine-related angioedema and generalized urticaria. Hypersensitivity to a protein found in mouse urine is common in animal caretakers and certain laboratory workers. It is unknown whether this sensitivity carries a specific risk in recipients of JE vaccine. Anecdotal reports of ADEM occurring in temporal relationship to vaccination suggest that the mouse brain–derived vaccine should not be used in persons who have recovered from ADEM or Guillain-Barré syndrome or who have multiple sclerosis or other demyelinating disorders. Contraindications for inactivated Vero cell–derived vaccine are hypersensitivity to one of the components, for example, protamine sulfate, or allergic reaction to a previous dose of the vaccine or another JE vaccine. Live JE vaccines pose a theoretical risk to the developing fetus but no adverse outcomes of pregnancy have been associated directly with JE vaccine. Travelers and their physicians must balance the theoretical risks of JE vaccine in pregnancy against the potential risks of acquiring JE and the adverse outcome of the disease. The live attenuated SA 14-14-2 and chimeric JE vaccines are not recommended for administration to pregnant women, and are also contraindicated in immunosuppressed persons, those known to have HIV, and individuals with a severe allergy to any of the vaccine components.[287]

A theoretical risk, explored by passive transfer of antibody in animal models, is the antibody-mediated enhancement of JE infection and disease.[276] A similar phenomenon has occurred in humans who contracted respiratory syncytial virus (RSV) infection or measles after their antibody levels had waned below protective thresholds following immunization with inactivated RSV or measles vaccines. A similar phenomenon has been demonstrated in laboratory animals using inactivated versus live attenuated vaccines for JE or Murray Valley encephalitis.[469,470] Antibody-dependent enhancement is also postulated as a pathogenic mechanism in severe dengue infections. Fundamental differences in cellular pathogenesis may preclude this phenomenon from occurring in JE where the brain and not macrophages or pulmonary epithelial cells are major targets of viral infection.[471]

PUBLIC HEALTH CONSIDERATIONS

Epidemiologic Effects of Vaccination

JE has a devastating effect on health resources and the affected communities. Epidemics frequently result in pressure from the community to take action to control the disease and provide a safe living environment. Vaccination for JE has been the single most effective and reliable means to control JE in endemic settings. Although a secular trend toward declining JE incidence has been observed with widespread use of JE vaccine, coincident socioeconomic changes also may have contributed to falling disease incidence. It was this same change in socioeconomic status that allowed developed Asian countries to afford vaccine and introduce it. In general, the higher the socioeconomic status of each country, the earlier that universal JE vaccine coverage was introduced. Less-affluent countries

are catching up. For example, in Nepal, a JE immunization program was launched in 2006, and by 2009, mass campaigns had been implemented in 23 districts. Analysis of surveillance data from 2004 to 2009 on laboratory-confirmed JE and clinical AES cases demonstrated that postcampaign, the JE incidence rate of 1.3 per 100,000 population was 72% lower than expected had no campaigns occurred, and an estimated 891 JE cases were prevented. In addition, AES incidence was 58% lower, with an estimated 2787 AES cases prevented, suggesting that three times as many disease cases may have been prevented than indicated by the laboratory-confirmed JE cases alone.[458] Owing to interventions managed by PATH, a not-for-profit organization in Seattle, Washington, with funding from the Bill and Melinda Gates Foundation, SA 14-14-2 JE vaccine is now routinely administered in Sri Lanka and large quantities were imported to combat epidemics in India.[350] In Thailand, a vertical control program based on vector control was implemented from 1970; the program demonstrated no impact on disease. The program was then integrated with an increased focus on outbreak response, again without impact on disease. In 1986, vaccine began to be integrated into the routine immunization system in the highest-risk areas of the country; this had a dramatic impact on disease incidence as immunization was expanded to additional provinces (Fig. 33.22).[21,472]

Observations from China, also reinforce the impact of immunization. The JE incidence rates in Beijing and other areas of China where high immunization rates are maintained have declined dramatically and remained low (Fig. 33.23).[2,16,473] Until recently, vaccine coverage, while high in prosperous regions in China, remained low in many poorer high-risk rural locations. The principal barrier to immunization had been the cost of vaccine to the family because JE vaccine was not subsidized by the government as a routine childhood vaccine. However, in 2008, JE vaccine was expanded into the EPI system in endemic provinces.

Disease Control Strategies

As a zoonotic disease with natural viral reservoirs, JE never can be eradicated. Although its transmission can be modulated by factors mentioned earlier, these approaches alone or in combination cannot be relied on to reduce disease incidence. The only effective strategy is vaccination. Successful control of JE by universal immunization in many Asian countries provides ample proof that expansion of vaccine coverage throughout the continent will lead to the near-elimination of the disease.[474]

Cost-to-Benefit Information

Several studies have demonstrated the economic benefits of JE vaccination with live attenuated SA 14-14-2 and mouse brain-derived inactivated vaccines.[475–478] Factors including vaccine price, disease incidence, number of doses used in the vaccine schedule, and vaccination strategy selected (campaign and/or routine immunization) impact the calculations. In a recent evaluation conducted in Guizhou province, China, investigators modeled use of live attenuated SA 14-14-2 JE vaccine in a two-dose schedule in the routine EPI in a theoretical cohort of 100,000 persons followed from birth to 65 years. The selected strategy would cost about US$435,000 each year and prevent 406 JE cases, 102 deaths, and 122 chronic disabilities. If future cost savings were ignored and only EPI program costs used, the program would cost US$95.50 per disability-adjusted life-year (DALY), less than China's Gross Domestic Product per capita in 2009 of US$3741, making it a cost-effective investment. Taking into account future savings, use of JE vaccine in the EPI for a 100,000-person cohort would lead to savings of US$1,591,975 for the health system and US$11,570,989 from the societal perspective.[18] Other assessments in Cambodia, Indonesia, and India similarly have found that vaccination programs with SA 14-14-2 JE vaccine would be cost-effective, if not cost saving.[477–479]

A partnership was developed between CDIBP and PATH to increase the supply of live attenuated vaccine with pricing well below US$1 per dose for the poorest countries of Asia (Gross National Product [GNP] <US$1000). The availability of this vaccine at a lower price should result in increased access to JE vaccine throughout Asia.

Unlike populations in JE-endemic countries, universal JE vaccination for all travelers is not considered to be cost-effective. Although evaluations have not been done, given the large numbers of travelers to Asia (135.5 million travelers to JE enzootic countries of Asia in 2015), the very low risk for JE for most travelers to Asia (<1 case/million travelers overall), and the high cost of JE vaccine (US$400 to US$500 per two-dose primary series for the inactivated Vero cell–derived

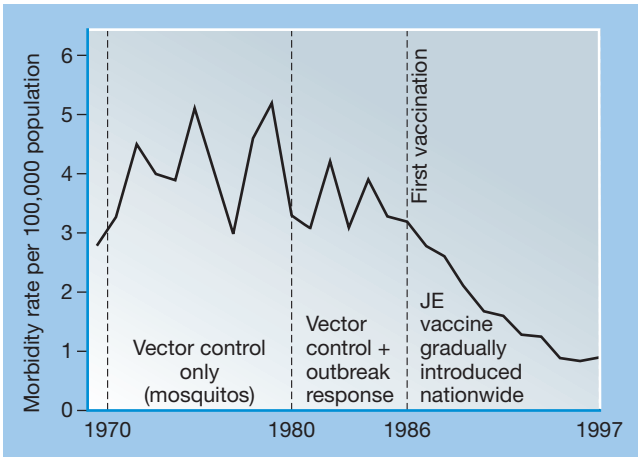


Figure 33.22. In Thailand, Japanese encephalitis (JE) cases failed to respond to control measures directed against vector mosquitoes; attack rates declined only after introduction of JE vaccine.

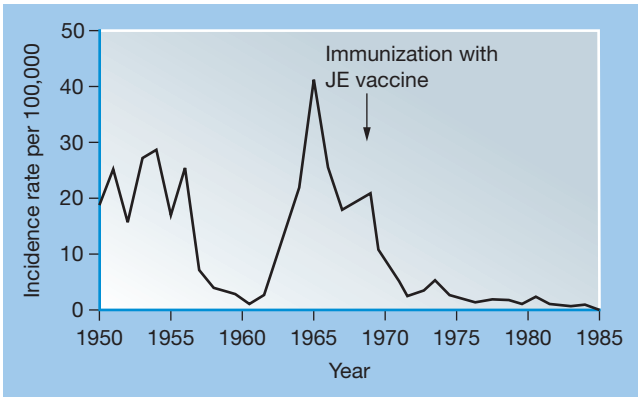


Figure 33.23. Declining incidence of Japanese encephalitis (JE) in Beijing and association with mass immunization, 1950 to 1985. *(Modified from Gu PW, Ding ZF. Inactivated Japanese encephalitis (JE) vaccine made from hamster cell culture (a review).* JE HFRS Bull. *1987;2:15–26.)*

vaccine), universal vaccination of travelers is not cost effective. However, for selected travelers at high risk and for resident expatriates, the equations change significantly.

Eradication or Elimination, If Feasible

Eradication of JEV from nature is not feasible. Elimination of clinical JE in humans is possible through universal vaccine coverage of the at-risk population. Disease control relies on individual protection because there is no herd immunity with JE as there is no human-to-human transmission.

NEW AND FUTURE VACCINES

A number of JE vaccines are in development, some have been licensed, and others are approaching licensure. Vero-based inactivated JE vaccines have been licensed by Biken and Kaketsuken for the Japanese domestic market. As the postlicensure experiences with these vaccines are somewhat limited, they are described in summary form here.

Inactivated Vaccines

Several groups have produced experimental inactivated whole-virion vaccines from infected Vero cell cultures.[480–482] Cell culture medium with high viral infectious titers, harvested continuously from microcarrier cultures, inactivated with formalin, and further concentrated has yielded candidate vaccines meeting mouse protection potency standards established for the inactivated mouse brain vaccine. The vaccine produced by Intercell has been licensed widely in the world. Its properties were described earlier in the chapter.

The Research Foundation for Microbiological Disease of Osaka University, Biken, has produced JEBIK V, a Vero-based vaccine formulated in alum and using the Beijing-1 JE strain. By using a bellows system for feeding Vero cells and batch harvesting, high yields of virus have been obtained.[482] Virus has been grown in serum-free medium using glycine and sorbitol stabilizers in the inactivation process.[483] A 2001 Phase I study of a two-dose regimen showed the vaccine to be well tolerated, with only mild elevations of liver function test results, and immunogenic, with JE-neutralizing antibody seroconversions observed in all seronegative subjects. A two-dose Phase III trial in 110 Flavivirus-susceptible children produced neutralizing antibody in 100% of subjects with no adverse events. A second Phase III open-label trial resulted in high levels of neutralizing antibodies in all children previously immunized with mouse brain vaccine and given one dose of JEBIK V. The vaccine was licensed in Japan in 2009.[484,485] Administration of this vaccine is as follows: for persons 3 years of age or older, 0.5 mL is given intramuscularly; 0.25 mL is given to children younger than 3 years of age. Two doses are given, 1 to 4 weeks apart. A booster dose is given approximately 1 year after the two-dose series. A fourth dose is given to 9- to 13-year-old children (I. Kurane, personal communication, 2010).

The Chemo-Sero-Therapeutic Research Institute, Kaketsuken, one of the Japanese producers of mouse brain–derived JE vaccines has produced a purified, unadjuvanted, Vero cell–derived vaccine (Encevac) formulated without gelatin or thimerosal. Vero cell DNA and protein concentrations are several picograms per dose in the final product. In a Phase I trial, 30 JE-susceptible adults were given three doses of vaccine at 0 and 4 weeks and 6 months with no serious adverse events observed and all subjects developing JE-neutralizing antibodies. Phase II studies involved 200 children 6 to 90 months of age who were given three doses with 100% rate of seroconversion. Mild adverse events were noted in as many as 45% of subjects following administration of the first dose. In 2011, the company obtained a license to market this vaccine in Japan.[485] Similarly, Vero cell–inactivated vaccines are now being produced in China by Beijing Tiantan Biologial Products Co, Ltd. and Liaoning Chengda Biotechnology Co, Ltd., and in India by Bharat Biotech.[486]

A collaborative group in Australia and Japan has grown the Beijing-1 strain of JEV in American Type Culture Collection (ATCC)-derived Vero cells grown in microcarrier cultures without serum.[482,483] When this virus was inactivated with formalin and stabilized with 0.5% glycerin, two doses given 1 week apart raised neutralizing antibodies 1 week later to several genotype I and genotype III JEVs in vaccinated mice. PRNTs were comparable to those titers obtained following two doses of standard mouse brain JE-VAX.[483] This early result was improved by adding delta inulin, a polysaccharide-based adjuvant (ADVAX). Two doses of unadjuvanted and cell culture–based adjuvanted vaccine (JE-ADVAX) were given at 1-week intervals to mice, and neutralizing antibody responses were compared with those obtained following immunization with two doses of comparison vaccines. Neutralizing antibody titers in mice given Vero-based vaccine incorporated with delta inulin were 100-fold higher than in mice given cell culture JE vaccine. Mice given JE-ADVAX were protected against lethal intranasal challenge with JE Nakayama strain, whereas mice vaccinated with cell culture–based or mouse brain–based vaccines were not. JE-neutralizing antibody responses in mice vaccinated with the chimeric JE vaccine and with licensed IXIARO (Jespect) vaccines were comparable to responses observed to JE-ADVAX.[481] A similar immunization regimen in mice provided protection against lethal challenge with West Nile virus with protection attributed to antibodies and could be adoptively transferred by memory B lymphocytes but not T cells.[487]

Vaccinia-Vectored Vaccines

Replication-deficient canary pox (ALVAC) and highly attenuated vaccinia viruses (NYVAC) have been used as vectors for delivering PrM-E or PrM-E-NS1 genes.[488] Several candidate JE vaccines are in various stages of clinical and preclinical development or research.[374,375] Recombinant vaccine candidates engineered by inserting four JE viral genes—prM, E, NS1, and NS2a—into vaccinia (NYVAC) or canarypox (ALVAC) viruses have been extensively evaluated. Both recombinants expressed the encoded JE structural and NS gene products and stimulated JE protective antibodies in mice; two doses of the former also protected rhesus monkeys against lethal viral challenge. In a Phase I human trial, the vaccines were somewhat more reactogenic than mouse brain vaccine but were otherwise safe. Two doses of the NYVAC-JE recombinant were nearly as immunogenic as three doses of inactivated mouse brain JE vaccine, but only in vaccinia-naïve volunteers. JE-neutralizing antibodies were elicited in all five non–vaccinia-immune recipients but in none of the five vaccinia-immune volunteers. Antibody responses were observed in only one of 10 ALVAC-JE vaccinees.[489] Although the NYVAC-JE recombinant virus proved safe and potent in vaccinia-nonimmune subjects, the apparent failure to replicate in vaccinia-immune subjects places some limits on its utility.

Other Genetically Engineered Constructs

Neuroattenuated JEV was produced by introducing defined nucleotide substitutions into the E gene. Virions and virion subunits derived from complementary DNA protected mice from death following challenge.[490,491] The JE viral subunit proteins produced in various expression systems, including

Spodoptera frugiperda, *Escherichia coli*, *Saccharomyces cerevisiae*, and *Drosophila* Schneider 2 cells, have had variable success in eliciting mouse immunogenicity and protective potency, depending on the expressed epitopes and their conformation.[491–495] The identification of peptides mimicking the conformation of viral epitopes may be expedited by screening pentapeptide libraries against monoclonal antibodies with previously defined specificity.[496] A vaccinia–JEV recombinant that releases extracellular particles composed of JE, prM, and E proteins resulted in an apparently more authentic configuration than when presented as simple peptides.[497] When given without adjuvant, extracellular particles induced long-lasting antibody and memory T cells in immunized mice. Immunogenic subviral particles containing JEV E protein also have been produced in other systems, including an alphaviral recombinant virus.[498,499] Novel delivery systems and adjuvants have been explored as a means to improve immunogenicity, to direct the Th1 or Th2 response, or to improve the convenience of immunization (e.g., the inactivated mouse brain vaccine has been microencapsulated in glycolide and lactide polymer microspheres designed to degrade at specific intervals).[500]

A pseudoinfectious JEV (named RepliVAX) has been made by deleting the capsid gene (C). The virus can be propagated in C protein–expressing cells, permitting them to form infectious particles structurally identical to live virus and secreted forms of NS1, but, in normal cells, RepliVAX JE cannot produce infectious particles. This vaccine was shown to protect mice and hamsters from lethal challenge.[501]

Subunit Vaccines

Vaccines expressing JE envelope protein or domain III have been presented to mice in an adenovirus construct or after expression in a baculovirus, J12#26 cells, *E. coli* or *Drosophila* cells, respectively.[502–507]

DNA Vaccines

Another approach being explored is the intramuscular or intracutaneous injection of naked DNA plasmids encoding viral prM and E genes under the control of a cytomegalovirus immediate early promoter. Using JE plasmids, immunized mice were protected against challenge with wild-type viruses.[508–511] In swine, two intramuscular doses produced high antibody titers and high anamnestic responses to challenge with live attenuated virus.[512] Another vaccine in development includes the secretory signal sequence derived from tissue plasminogen activator fused to the full-length or partial JEV envelope protein gene. Cells transfected with the latter construct secreted E protein and produced better protection against intracerebral challenge in mice.[513]

References for this chapter are available at ExpertConsult.com.

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34 Lyme Disease Vaccines

Allen C. Steere

Lyme disease was described as a separate entity in 1976 because of the geographic clustering of children in Lyme, Connecticut, who had been thought to have juvenile rheumatoid arthritis.[1] The rural setting of the case clusters and the identification of an expanding skin lesion, erythema migrans, as a feature of the illness suggested that the disorder was transmitted by an arthropod. It soon became apparent that Lyme disease was a multisystem illness that affected primarily the skin, nervous system, heart, and joints.[2] Epidemiologic studies of patients with erythema migrans implicated certain ixodid ticks as vectors of the disease.[3]

In addition to providing clues about the cause of the illness, the initial expanding skin lesion linked Lyme disease in the United States with certain syndromes in Europe that were described in the early to mid-20th century.[4–6] Lyme disease and these various syndromes were brought together conclusively in 1982, when Burgdorfer and Barbour isolated a previously unrecognized spirochete, now called *Borrelia burgdorferi*, from *Ixodes scapularis* ticks.[7] The spirochete then was recovered from patients with Lyme disease in the United States[8,9] and in patients with erythema migrans, meningopolyneuritis, or acrodermatitis in Europe[10–12]; additionally, the patients' immune responses were linked conclusively with this organism.[8]

Later, *B. burgdorferi sensu lato* (*B. burgdorferi* in the general sense) was divided into three pathogenic groups.[13] To date, all North American strains have belonged to the first group, *B. burgdorferi sensu stricto* (*B. burgdorferi* in the strict sense). Although all three groups have been found in Europe, most isolates there have been group 2 *(Borrelia garinii)* or group 3 *(Borrelia afzelii)* strains; only groups 2 and 3 have been found in Asia.

Lyme disease (or Lyme borreliosis) is now the most common vector-borne disease in the United States and Europe.[14] As efforts to prevent tick bites have been largely ineffective, vaccination is an attractive strategy for prevention of the illness.

CLINICAL DESCRIPTION

Lyme disease occurs in stages, with different manifestations at each stage. In 70% to 80% of patients in the United States, the infection begins with a characteristic expanding skin lesion, erythema migrans (stage 1), which occurs at the site of the tick bite.[15–17] After an incubation period of 3 to 32 days, redness forms around the site and expands slowly (Fig. 34.1).[18] Within days to weeks, *B. burgdorferi* in the United States often disseminates hematogenously (stage 2). During this period, patients often have influenza-like symptoms such as malaise and fatigue, headache, neck pain, arthralgias, myalgias, fever, or regional lymphadenopathy. In addition, secondary annular skin lesions may develop that are similar to initial erythema migrans lesions.

Within weeks, during or shortly after the period of early, disseminated infection, objective signs of acute neuroborreliosis develop in approximately 15% of untreated patients.[19,20] Possible manifestations include lymphocytic meningitis, subtle encephalitis, cranial neuropathy (particularly unilateral or bilateral facial palsy), or motor or sensory radiculoneuritis. During this same period, approximately 5% of untreated patients have acute cardiac involvement, most commonly fluctuating degrees of atrioventricular block.[21,22] Even without treatment, these manifestations of the illness usually resolve within weeks to months. Months after the onset of illness (stage 3), approximately 60% of untreated patients in the northeastern United States begin to have intermittent attacks of oligoarticular arthritis, usually in one or a few large joints at a time, especially the knee.[23] By the time arthritis is present, the infection often seems localized to affected joints, and systemic symptoms are minimal. Synovial tissue in joints shows synovial hypertrophy, vascular proliferation, and a marked infiltration of mononuclear cells.[24,25] However, even in untreated patients, intermittent or persistent Lyme arthritis usually resolves completely within several years.

Months to several years after disease onset, usually after the period of oligoarticular arthritis, up to 5% of untreated patients in the United States experience chronic neurologic manifestations of Lyme disease,[26] primarily a mild encephalopathy[26,27] or an axonal polyneuropathy.[28,29] In patients with encephalopathy, intrathecal antibody production to the spirochete can often be demonstrated by antibody-capture enzyme immunoassay,[30] and in those with polyneuropathy, electromyograms typically show diffuse involvement of both proximal and distal nerve segments.[28,29] The basic outlines of Lyme borreliosis are similar worldwide, but there are regional variations, primarily between the illness found in America and that found in Europe.[31]

A small percentage of patients develop chronic pain, neurocognitive symptoms, or fatigue symptoms in association with or soon after Lyme disease.[31] Compared with Lyme disease, these pain and fatigue syndromes tend to produce more generalized and disabling symptoms, including marked fatigue, headache, diffuse musculoskeletal pain and stiffness in many joints, diffuse dysesthesias, difficulty with concentration, and sleep disturbances. These syndromes, which are sometimes called chronic Lyme disease, are not specific for that infection, and they may be triggered by other infectious or noninfectious conditions. In fact, the majority of patients now diagnosed with chronic Lyme disease lack evidence of past or present *B. burgdorferi* infection.[32]

BACTERIOLOGY

The structure of *Borrelia* species, including *B. burgdorferi*, is similar to that of all spirochetes: a protoplasmic cylinder surrounded by periplasm containing the flagella, which is surrounded, in turn, by an outer membrane (Fig. 34.2).[33] The genome of *B. burgdorferi* is quite small (approximately 1.5 Mb) and consists of a highly unusual linear chromosome of 950 kb as well as nine circular and 12 linear plasmids.[34,35] The remarkable aspect of the *B. burgdorferi* genome is its large number of sequences for predicted or known lipoproteins, including plasmid-encoded outer surface proteins (Osps) A through F. In addition, during early, disseminated infection, a surface-exposed lipoprotein, called VlsE, undergoes extensive antigenic variation.[36] The only known virulence factors of *B. burgdorferi* are surface proteins, which allow the spirochete to attach to a variety of mammalian cells.



Figure 34.1. Classic erythema migrans skin lesion (9 cm in diameter) near the axilla. The lesion has partial central clearing, a bright red outer border, and a target center. *(Courtesy Dr. Vijay K. Sikand, East Lyme, CT.)*



Figure 34.2. Scanning electron micrograph of *Borrelia burgdorferi*, the causative agent of Lyme disease. Of the *Borrelia* species, *B. burgdorferi* is the longest (20 to 30 μm) and narrowest (0.2 to 0.3 μm), and has the fewest flagella (7 to 11). *(From Johnson RC, Hyde RW, Rumpel CM. Taxonomy of the Lyme disease spirochetes.* Yale J Biol Med. *1984;57:529–537.)*

PATHOGENESIS

To maintain its complex enzootic cycle, *B. burgdorferi* must adapt to two markedly different environments, the tick and the mammalian host.[37] In the midgut of the tick, the spirochete expresses OspA and OspB.[38] When a blood meal is taken, these two proteins are downregulated and OspC is upregulated as the spirochete traverses to the tick salivary gland and then to the mammalian host. In the salivary gland, OspC binds a tick protein (Salp15 [saliva protein of 15 kDa]), which protects the spirochete from antibody-mediated killing in the host.[39] In addition, some strains of *B. burgdorferi* have complement regulator–acquiring surface proteins that bind complement factor H and factor H–like protein 1, which protect the organism from complement-mediated killing.[40]

After injection into the skin, *B. burgdorferi* usually first multiplies locally in the skin at the site of the tick bite. Several days later, the spirochete begins to spread in the skin, and, within days to weeks, it may disseminate to many sites. A number of mechanisms may aid in dissemination. For example, the sequences of OspC vary considerably among strains, and certain types, particularly OspC type A followed by OspC type I, have a greater inflammatory potential[41] and can more often be detected in blood.[42,43] Spreading through the skin and other tissue matrices may be facilitated by the binding of human plasminogen and its activators to the surface of the spirochete.[44] During dissemination and homing to specific sites, the organism attaches to certain host integrins,[45] matrix glycosaminoglycans,[46] and extracellular matrix proteins.[47] *Borrelia* decorin-binding proteins (Dbps) A and B bind decorin,[48] a glycosaminoglycan on collagen fibrils, which may explain why the organism is commonly aligned with collagen fibrils in the extracellular matrix in the heart, nervous system, or joints.

Both innate and adaptive immune responses, especially macrophage- and antibody-mediated killing, are required for optimal control of the infection and spirochetal eradication.[49–52] The primary role of *B. burgdorferi*–specific T-helper 1 cells is to prime T-cell–dependent B-cell responses to *B. burgdorferi* proteins.[53] Furthermore, *B. burgdorferi* has two immunogenic glycolipids,[54] which induce immunoglobulin (Ig) G antibody responses in some patients with early infection and in almost all patients with Lyme arthritis,[55] a late disease manifestation. The combination of these responses leads to the production of antibodies against many components of the organism, which promote spirochetal killing by opsonization and complement fixation.[49] Despite innate and adaptive immune responses, the organism may sometimes survive in selected niches for several years. In histologic sections, *B. burgdorferi* has been seen only extracellularly[56]; it has not been shown to survive intracellularly for prolonged periods.

DIAGNOSIS

B. burgdorferi can be cultured in a complex liquid medium called Barbour-Stoenner-Kelly medium.[8,9] However, except for acrodermatitis in Europe, a late disease manifestation, positive cultures have been obtained only early in the illness, primarily from skin biopsy specimens of erythema migrans lesions,[57] less often from plasma samples,[58] and only occasionally from cerebrospinal fluid samples in patients with meningitis.[59] Later in the infection, polymerase chain reaction (PCR) testing has been used for the detection of *B. burgdorferi* DNA in joint fluid.[60,61] However, because *B. burgdorferi* DNA at this site is derived from moribund or dead spirochetes, PCR testing of joint fluid is not an accurate indicator of active spirochetal infection.[62] Moreover, PCR testing is not often positive with samples from other sites later in the illness.[63] Thus, the diagnosis of Lyme disease is usually based on recognition of a characteristic clinical picture, exposure in an endemic area, and, except in patients with erythema migrans, a positive antibody response to *B. burgdorferi* by enzyme-linked immunosorbent assay (ELISA) and Western blot,[64] interpreted according to the criteria of the Centers for Disease Control and Prevention (CDC).[65] In Europe, where there is less expansion of the antibody response, no single set of criteria for the interpretation of immunoblots results in high levels of sensitivity and specificity in all countries.[66]

During the first several weeks of infection, when most patients have erythema migrans, serodiagnostic tests are insensitive, and they depend primarily on the detection of an IgM response to the spirochete.[67] However, after 4 to 8 weeks of infection, when most patients in the United States have disseminated infection, the sensitivity and specificity of the IgG response to the spirochete are both very high, in the range of 95% to 99% as determined by the two-test approach of ELISA and Western blot.[67] A positive IgG blot requires reactivity with at least five of the following 10 bands (18, 23, 28, 30, 39, 41, 45, 58, 66, and 93 kDa).[65] In persons who have been ill for longer than 4 to 8 weeks, a positive IgM test alone is likely to represent a false-positive result; therefore, such a response should not be used to support the diagnosis of Lyme disease after the first 2 months of infection. A new serologic test, an IgG ELISA that uses a peptide in the sixth invariant region of the VlsE lipoprotein of *B. burgdorferi*, may be valuable, as this test typically becomes positive before five IgG bands are present on Western blots.[67,68] However, the VlsE test as a standalone test is not quite as specific as standard two-tiered testing.[68a] Another approach is to use two EIAs, such as the whole sonicate EIA followed by the VlsE C6 peptide EIA, which is not quite as sensitive as the VlsE test by itself, but it maintains the specificity of standard two-tiered testing.[68b]

Antibody titers fall slowly after antibiotic treatment, but IgG and even IgM responses may persist at low levels for many years after treatment.[69] Moreover, Western blots, a nonquantitative test, do not change much after treatment. Thus, current serologic tests demonstrate exposure to the spirochete, but they cannot distinguish accurately between past infection and active infection. In addition, *B. burgdorferi* may cause asymptomatic infection. In the SmithKline Beecham Lyme disease vaccine trial in the United States, asymptomatic IgG seroconversion was demonstrated by Western blotting in approximately 10% of persons with Lyme disease.[70] Under these circumstances, positive serologic results for Lyme disease may cause diagnostic confusion.

Alternate approaches for diagnosis, such as determination of a metabolic biosignature, are being researched.[71]

TREATMENT AND PREVENTION WITH ANTIMICROBIALS

Evidence-based treatment recommendations for Lyme disease have been published by the Infectious Diseases Society of America (Box 34.1).[72] For early localized or disseminated infection, doxycycline (100 mg twice daily for 14 to 21 days) is recommended in persons 8 years of age or older, except for pregnant women.[73,74] Amoxicillin (500 mg three times daily), the second-choice alternative, should be used in children or pregnant women.[73,74] In case of allergy or other contraindication to doxycycline or amoxicillin, cefuroxime axetil (500 mg twice daily) is a third-choice alternative.[75] Erythromycin (250 mg four times daily) or its congeners, which are fourth-choice alternatives, are recommended only for patients who are unable to take doxycycline, amoxicillin, or cefuroxime axetil.[74]

For patients with objective neurologic abnormalities, 2- to 4-week courses of IV ceftriaxone (2 g once per day) are given most commonly.[26,76,77] Parenteral therapy with cefotaxime or penicillin G may be a satisfactory alternative. Objective evidence of relapse is rare after a 4-week course of therapy. In patients with high-degree atrioventricular nodal block, IV therapy for at least part of the course and cardiac monitoring are recommended, but insertion of a permanent pacemaker is not necessary.

Either oral or IV regimens are usually effective for the treatment of Lyme arthritis, although some patients require IV therapy for successful treatment of their arthritis.[78,79] However, despite treatment with either oral or IV antibiotic therapy, a small percentage of patients in the United States have persistent proliferative synovitis for months or even several years after 2 or more months of oral antibiotics or 1 or more months of IV antibiotics, or both. This outcome is thought to involve

BOX 34.1 Antibiotic Treatment Regimens for Lyme Disease[a]

EARLY INFECTION (LOCAL OR DISSEMINATED)

Adults

- Doxycycline, 100 mg orally twice daily for 14 to 21 days
- Amoxicillin, 500 mg orally 3 times daily for 14 to 21 days
- Alternatives in case of doxycycline or amoxicillin allergy:
  - Cefuroxime axetil, 500 mg orally twice daily for 14 to 21 days
  - Erythromycin, 250 mg orally 4 times a day for 14 to 21 days

Children (Age ≤8 Years)

- Amoxicillin, 250 mg orally 3 times a day or 50 mg/kg per day in 3 divided doses for 14 to 21 days
- Alternatives in case of penicillin allergy:
  - Cefuroxime axetil, 125 mg orally twice daily or 30 mg/kg per day in 2 divided doses for 14 to 21 days
  - Erythromycin, 250 mg orally 3 times a day or 30 mg/kg per day in 3 divided doses for 14 to 21 days

NEUROLOGIC ABNORMALITIES (EARLY OR LATE)

Adults

- Ceftriaxone 2 g IV once a day for 14 to 28 days
- Cefotaxime, 2 g IV every 8 hours for 14 to 28 days
- Na penicillin G, 20 million U IV in 6 divided doses every 4 hours for 14 to 28 days
- Alternative in case of ceftriaxone or penicillin allergy:
  - Doxycycline, 100 mg orally 3 times a day for 30 days; this regimen may be ineffective for late neuroborreliosis
  - Facial palsy alone: oral regimens may be adequate

Children (Age ≤8 Years)

- Ceftriaxone, 75 to 100 mg/kg per day (maximum: 2 g) IV once a day for 14 to 28 days
- Cefotaxime, 150 mg/kg per day in 3 or 4 divided doses (maximum: 6 g) for 14 to 28 days
- Na penicillin G, 200,000 to 400,000 U/kg per day in 6 divided doses for 14 to 28 days

ARTHRITIS (INTERMITTENT OR CHRONIC)

- Oral regimens listed above for 30 to 60 days or
- IV regimens listed above for 14 to 28 days

CARDIAC ABNORMALITIES

- First-degree atrioventricular block: oral regimens, as for early infection
- High-degree atrioventricular block (P-R interval >0.3 s): IV regimens and cardiac monitoring; once the patient has stabilized, the course may be completed with oral therapy

PREGNANT WOMEN

- Standard therapy for manifestation of the illness; avoid doxycycline

[a]Antibiotic recommendations are based on the guidelines from Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Disease Society of America. *Clin Infect Dis*. 2006;43:1089–1134.

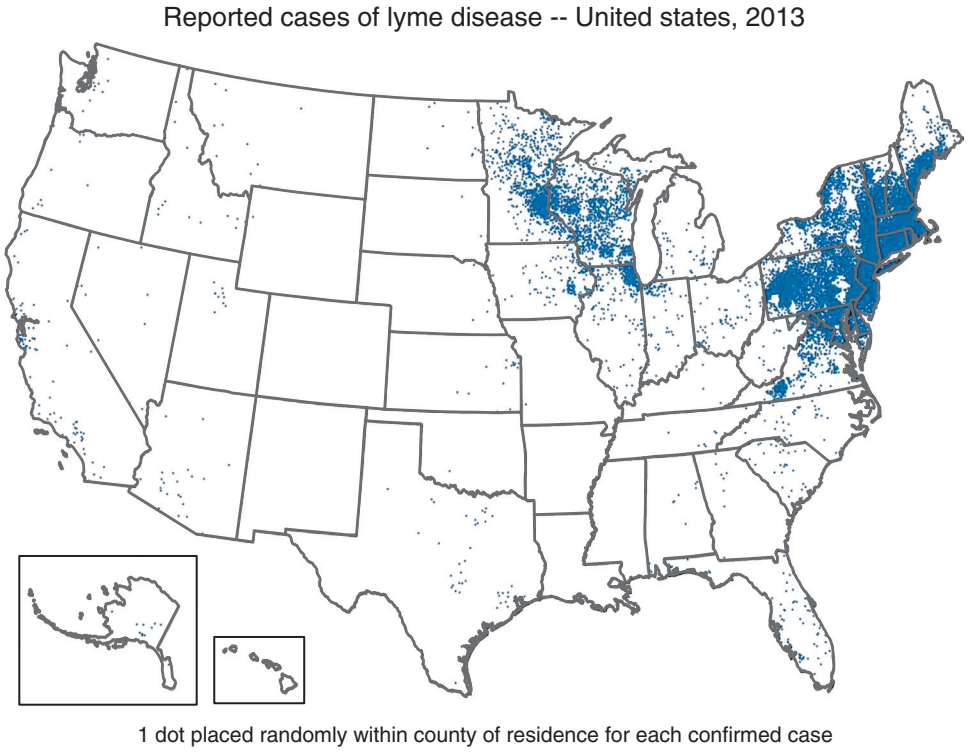


Figure 34.3. Number of cases of Lyme disease in the United States, by county, reported to the Centers for Disease Control and Prevention in 2013. Most of the cases occurred in the northeast and upper midwest. *(From the Centers for Disease Control and Prevention. http://www.cdc.gov/lyme/stats/graphs.html.)*

excessive inflammation,[80] immune dysregulation,[81] and infection-induced autoimmunity.[82,83] Patients who have persistent arthritis despite this treatment may be treated with antiinflammatory agents, disease modifying antirheumatic drugs, or arthroscopic synovectomy.

Should tick bites be treated with antibiotic prophylaxis? In studies, the frequency of Lyme disease after a recognized tick bite has been only approximately 1%,[84] perhaps because at least 24 hours of tick attachment seems to be necessary for transmission to occur.[85] Thus persons should remove an attached tick as soon as possible, and no other treatment is usually necessary. However, if the tick is engorged, indicating a longer duration of attachment, a single 200-mg dose of doxycycline usually prevents Lyme disease when given within 72 hours after the tick bite.[86]

Five double-blind, placebo-controlled trials of additional antibiotic therapy have been conducted in patients who have pain, neurocognitive symptoms, or fatigue symptoms after standard courses of antibiotic therapy for Lyme disease. None of the trials showed sustained benefit from additional oral or IV antibiotic therapy, but they documented the potential for severe adverse reactions, including IV catheter–related infections and antibiotic-associated colitis.[87–89a]

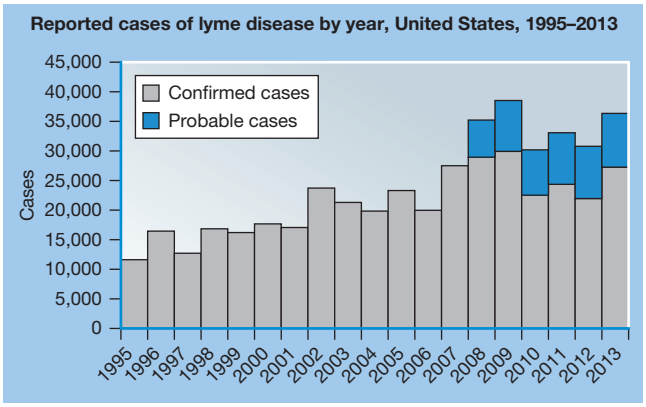


Figure 34.4. Number of cases of Lyme disease in the United States, by year, reported to the Centers for Disease Control and Prevention (CDC) from 1995 through 2013. For the past several years, more than 30,000 cases have been reported annually, but the CDC estimates that the actual number is 10 times higher, or 300,000 cases annually. *(From the Centers for Disease Control and Prevention. http://www.cdc.gov/lyme/stats/graphs.html.)*

EPIDEMIOLOGY

Incidence and Prevalence Data

Lyme disease occurs primarily in three distinct foci in the United States: in the Northeast from Maine to North Carolina, in the Midwest in Wisconsin, Minnesota, and Michigan, and to a lesser degree in the West, primarily in northern California (Fig. 34.3).[14,90] In Europe, the highest reported frequencies of the disease are in middle Europe and Scandinavia, particularly in Germany, Austria, Slovenia, and Sweden.[91] The infection is also found in Russia, China, and Japan.

Since surveillance for Lyme disease was begun in the United States in 1982, the number of cases reported to the CDC has increased dramatically (Fig. 34.4). In recent years, more than 30,000 cases have been reported yearly, accounting for more than 95% of all reported cases of vector-borne illness in the United States.[14] However, the CDC estimates that the actual number of cases is at least 10 times that number, or 300,000 or more cases per year.[92,93] The proliferation of deer, which are

the preferred host of the adult *I. scapularis,* was a major factor in the emergence of epidemic Lyme disease in the northeastern United States during the late 20th century.[49] Moreover, OspC type A and type B strains, which cause 30% to 50% of the infections in the northeastern United States,[42,94,95] have a high transmission frequency among ticks[96]; they may be increasing in frequency in nature, and this may also be an important factor in the intense emergence of Lyme disease in this region.[97,98]

Lyme disease has evolved differently in different geographic locations. In the northeastern and north-central United States, *I. scapularis* ticks are abundant, and a highly efficient cycle of *B. burgdorferi* transmission occurs between immature larval and nymphal *I. scapularis* and white-footed mice.[99] This cycle results in high infection rates in nymphal ticks and a high frequency of human Lyme disease during the late spring and summer months.[14] In the SmithKline Beecham Phase III Lyme disease vaccine trial, the yearly incidence of the disease in participants from hyperendemic areas was greater than 1%, and in some regions the seroprevalence of antibody to *B. burgdorferi* was as high as 5%.[100]

The vector ecology of *B. burgdorferi* is quite different on the West Coast in northern California, where the incidence of Lyme disease is low.[14] There, two intersecting cycles are necessary for disease transmission, one involving the dusky-footed wood rat and *Ixodes spinipalpis* ticks, which do not bite humans, and the other involving *Ixodes pacificus* ticks, which do.[101] Because nymphal *I. pacificus* prefer to feed on lizards, which are not susceptible to *B. burgdorferi* infection, only the relatively few *I. pacificus* nymphs that previously fed on infected wood rats in the larval stage are responsible for spirochetal transmission to humans.

Significance as a Public Health Problem

Even though the occurrence of Lyme disease is highly focal, certain locations, primarily in the northeastern United States, have been greatly affected.[14] Such areas have included suburban locations near Boston, New York, Philadelphia, Baltimore, and Washington, DC, which are some of the most heavily populated parts of the country.[102] In addition, *I. scapularis* ticks continue to spread, and one can anticipate that epidemics of Lyme disease will continue to occur in new locations in the United States. Currently, the major preventive measures are use of protective clothing, tick repellants, and personal inspection after tick exposure, but their efficacy is limited.[103, 104] Thus, vaccination would be an attractive strategy for prevention of the infection.

PASSIVE IMMUNIZATION

Although passively transferred sera from Lyme borreliosis patients to mice protected them from the infection,[105] passive immunization is not an important factor in protective immunity in Lyme disease. The spirochete is transmitted only by tick bite; person-to-person transmission has not been noted. Most patients have symptoms of the infection for months before protective immunity develops.

ACTIVE IMMUNIZATION

History of Vaccine Development

In 1986, Johnson and colleagues reported that Syrian hamsters could be immunized successfully against homologous strains of *B. burgdorferi* using a formalin-inactivated, whole-cell lysate of the spirochete.[106,107] These seminal studies showed that vaccination against Lyme disease was feasible. However, cross-protection among strains was limited. Immunization with European strains did not provide protection against U.S. strains, and immunization with U.S. strains did not provide protection against European strains. Moreover, hamsters inoculated with a Northeast coast strain were not protected from infection with a West coast strain. Subsequently, an inactivated whole-cell vaccine with a proprietary polymer-based adjuvant, called *B. burgdorferi* bacterin (Fort Dodge Laboratories, Fort Dodge, IA) and a bivalent whole-cell killed vaccine (Solway Animal Health, Mendota Heights, MN) were licensed in the United States for use in dogs.[108,109]

In 1990 in the United States, Fikrig and colleagues reported that immunization with a recombinant OspA protein of *B. burgdorferi* protected mice from challenge with several strains of the spirochete.[110] At the same time in Germany, Schaible and colleagues showed that injection of a monoclonal antibody to OspA provided protection in a murine model of the infection.[111] Protection was afforded by the development of high-titered antibody to a conformational antibody epitope, called LA-2, in the C-terminal portion of the protein.[112] Because *B. burgdorferi* expresses OspA primarily in the midgut of the tick, spirochetal killing was accomplished mainly in the tick before transmission to the human host.[113] Immunization with the lipid component of the protein was important in the induction of the primary immune response.[114] In addition to mice, protective immunity to OspA was demonstrated in hamsters,[115] dogs,[116] and monkeys.[117] As with whole-cell lysates, immunization with OspA was highly protective when the challenge isolate was either identical or closely related to the one from which the protein was derived.[118,119] However, protection from more diverse isolates was more limited.[118,120]

At the same time, other approaches to vaccination were studied. In one study, a recombinant bacille Calmette-Guérin vaccine expressing an OspA lipoprotein protected mice against intradermal challenge with *B. burgdorferi*.[121] In addition, antibody responses to other Osps of *B. burgdorferi,* including OspB,[122] OspC,[123] OspF,[124] and DbpA,[125] afforded some degree of protective immunity in animal model systems, but not the complete protection observed with OspA (Table 34.1). In contrast, antibody reactivity with OspE or the flagellar protein of the spirochete did not provide protective immunity.[122]

TABLE 34.1 Protection of Experimental Animals From *Borrelia burgdorferi* Infection[a]

Immunization	Challenge	Protection[b]
B. burgdorferi	*B. burgdorferi*	+++++
OspA	*B. burgdorferi*	+++++
OspB	*B. burgdorferi*	++++
OspC	*B. burgdorferi*	+++
OspE	*B. burgdorferi*	–
OspF	*B. burgdorferi*	+
P35 and P37	*B. burgdorferi*	++++
Flagellin	*B. burgdorferi*	–
P39	*B. burgdorferi*	+
P66	*B. burgdorferi*	+
DbpA	*B. burgdorferi*	++

[a]The data reflect the authors' interpretation of published studies.
[b]The degree of protection is graded on a scale from – to +++++, with – representing no protection and +++++ representing substantial (nearly complete) protection. Intermediate grades reflect mild to moderate protection.
DbpA, decorin-binding protein A; Osp, outer surface protein.

In the early 1990s, two companies, Pasteur Mérieux Connaught (now Sanofi Pasteur) and SmithKline Beecham (now GlaxoSmithKline) began the development of recombinant OspA vaccines for human use.[126,127] The vaccine made by GlaxoSmithKline was a recombinant OspA lipoprotein with adjuvant,[127] whereas the Sanofi Pasteur vaccine was a recombinant OspA lipoprotein without adjuvant.[126] In December 1998, the vaccine made by GlaxoSmithKline, called LYMErix, was licensed and sold commercially until February 2002. Several review articles were written about Lyme disease vaccination.[128–135]

LYMErix Vaccine

LYMErix, produced by GlaxoSmithKline, contained lipidated OspA adsorbed onto aluminum hydroxide adjuvant in phosphate-buffered saline with phenoxyethanol as a preservative (pH 6.5 to 7.1).[100,128] It was produced using recombinant DNA technology. The OspA gene from the ZS7 strain of *B. burgdorferi sensu stricto* was placed in the pOA15 plasmid vector and grown in *Escherichia coli* strain AR58.[100,128] The OspA lipoprotein produced was a single polypeptide chain of 257 amino acids; the lipid moiety was covalently bonded to the N-terminus after translation. The LYMErix preparation was not formulated in combination with other vaccines.

Dosage and Route of Administration

Each 0.5-mL dose of LYMErix contained 30 µg of lipidated OspA with adjuvant, which was administered intramuscularly, usually in the deltoid muscle.[100] For primary immunization, it was recommended that three injections be given: one at the first visit, the second 1 month later, and the third at 12 months. However, equivalent antibody levels could be achieved by giving the third injection at either 2 or 6 months rather than at 12 months.[136,137] The important point was that the third injection should be in April to provide maximum levels of protection during May through July, the questing period for nymphal *I. scapularis* ticks.

Vaccine Stability

The recommendation was to refrigerate LYMErix at 2°C to 8°C. With refrigeration, the vaccine had an expiration date that was 24 months after the date of manufacture. The vaccine was stable at room temperature for up to 4 days. If freezing occurred, the vaccine was to be discarded.

Results of Vaccination

Immunogenicity of the Vaccine

Antibody Responses. Lyme disease vaccine induced an IgG antibody response to OspA, including reactivity with the protective conformational epitope, LA-2, in the C-terminal portion of the protein.[112] Because the mechanism of the vaccine was antibody-mediated killing of the spirochete in the tick,[113] OspA antibody titers were the critical factor in vaccine efficacy. The levels of LA-2–equivalent antibodies were determined in a competitive-inhibition enzyme immunoassay in which a murine monoclonal antibody (also called LA-2) competed for binding with the patient's LA-2–equivalent anti-OspA antibodies.[111,128] An alternative assay was the determination of IgG antibody titers to full-length OspA by indirect ELISA.[138] This assay measured the amount of antibody not only to the protective antibody epitope of OspA but also to other OspA epitopes. For this reason, full-length OspA levels were not as accurate in predicting protection as were LA-2–equivalent antibody levels.

In the immunogenicity portion of the SmithKline Beecham Phase III trial, 95% of the 938 vaccine recipients tested from one site had LA-2–equivalent OspA antibody levels of 100 ng/mL or greater at month 2, 1 month after the second injection (Fig. 34.5).[100] Ten months later, the mean titer had declined markedly. At month 13, 1 month after the third injection, a marked anamnestic response was seen, and 99% had levels of 100 ng/mL or greater. At month 20, the mean titer had again declined. Thus, after three injections, most vaccine recipients developed high levels of LA-2–equivalent OspA antibody, but the antibody levels waned rather rapidly.

In the vaccine study, seven individuals were identified with very low antibody titers after vaccination.[139] The macrophages of these individuals produced less tumor necrosis factor-α and interleukin-6 after OspA stimulation, and had lower expression of Toll-like receptor (TLR)-1 than normal cells. TLR1 and TLR2 are required for lipoprotein recognition, and defects in the TLR1/2 signaling pathway may account in part for hyporesponsiveness to OspA vaccination.

Cellular Immune Responses. In the SmithKline Beecham Phase III trial, T-cell proliferative responses were determined to full-length OspA and to 20 overlapping OspA peptides in 41 vaccine and 44 placebo recipients from one site. Although the strength of the responses was often quite low, reactivity in

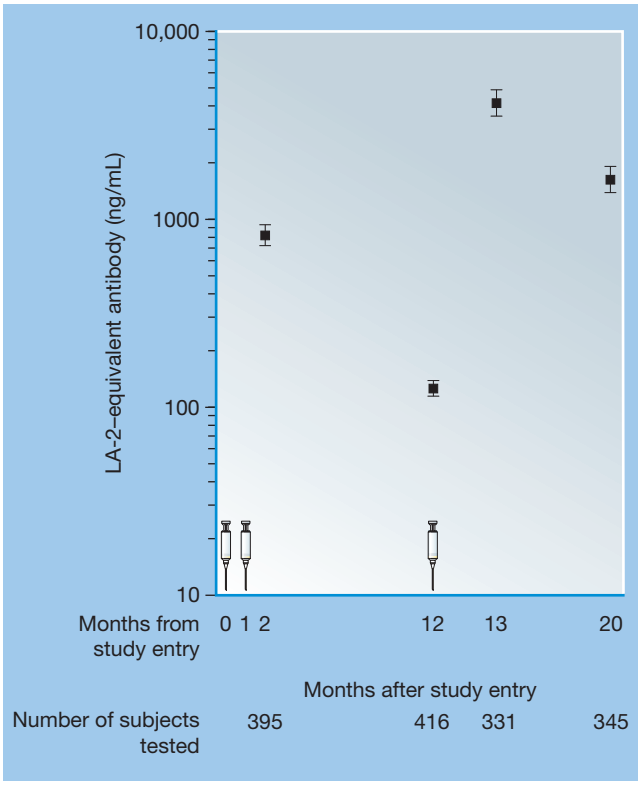


Figure 34.5. Levels of antibody to the protective epitope of OspA (LA-2–equivalent antibody) in vaccine recipients at one study site. At month 2, 1 month after the second injection, the geometric mean antibody titer was 816 ng/mL. Ten months later, the mean titer had declined. At month 13, 1 month after the third injection, a marked anamnestic response was seen, and the mean value was 4127 ng/mL. At month 20, the mean response had again declined, but it was still twice as high as at month 2. The vertical bars indicate 95% confidence intervals. *(From Steere AC, Sikand VK, Meurice F, et al. Vaccination against Lyme disease with recombinant* Borrelia burgdorferi *outer-surface lipoprotein A with adjuvant.* N Engl J Med. *1998;339:209–215. Copyright Massachusetts Medical Society.)*

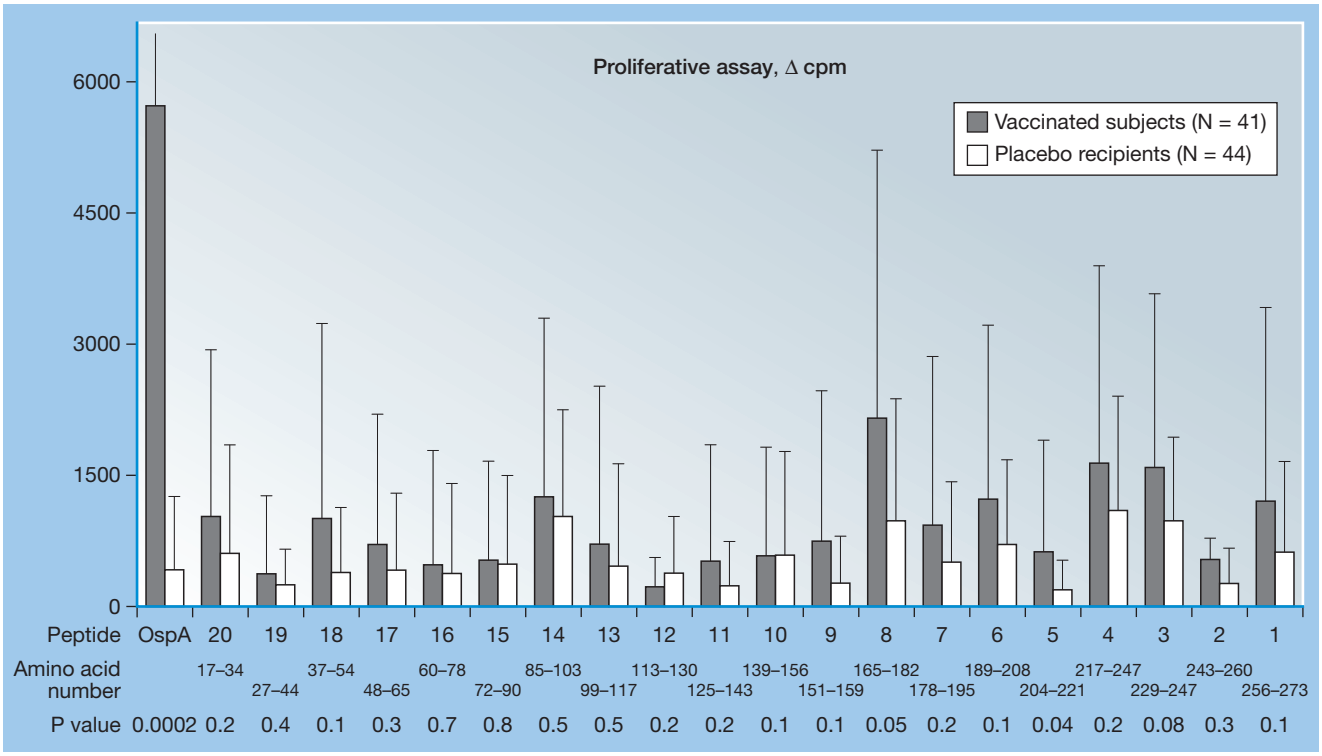


Figure 34.6. T-cell proliferative responses to full-length OspA and to 20 overlapping OspA peptides in 41 vaccine and 44 placebo recipients at one study site. Compared with that in placebo recipients, the responses in vaccine recipients were greater to full-length OspA and to epitopes contained in peptides 18, 14, 8, 4, 3, and 1. Significant differences between vaccine and placebo recipients were in the responses to full-length OspA ($P = .0002$) and peptide 8 ($P = .05$) as determined by *t*-test.

the 41 vaccine recipients was significantly greater to full-length OspA and to peptide 8 than in placebo recipients (Fig. 34.6). This peptide contains the immunodominant T-cell epitope of OspA recognized by individuals with the human leukocyte antigen (HLA)-DRB1*0401 or other related alleles. Although a T-helper–cell response may prime B cells to produce antibody to OspA, at least in some individuals, vaccine-induced cellular immunity was not thought to play a direct role in spirochetal killing.

Correlates of Protection

In animal models, the levels of LA-2–equivalent antibodies, which were directed against the protective antibody epitope of OspA, accurately predicted the probability of becoming infected with *B. burgdorferi*.[116] In the SmithKline Beecham Phase III vaccine trial, the antibody titers were significantly lower in vaccinated subjects with breakthrough cases of Lyme disease than in subjects in two comparison groups ($P < .01$ for each comparison) (Fig. 34.7).[100] Later, these serum samples were used to determine the IgG antibody titers to full-length OspA by indirect ELISA. Titers of 700 to 1400 ELISA units (EL.U.)/mL provided 70% to 95% sensitivity in discriminating between vaccine success and failure.[138] After three injections, 90% of the adults tested and 100% of children attained levels of 1400 EL.U./mL.[140]

Herd Immunity. *B. burgdorferi* survives in a horizontal cycle of transmission between ticks and mice[49]; human infection is a dead end in the spirochetal life cycle. Therefore, herd immunity in human populations does not influence the frequency of infected ticks or the enzootic cycle of infection.

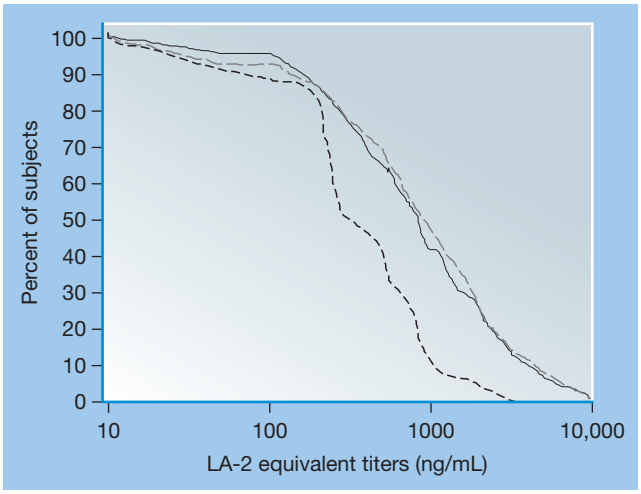


Figure 34.7. Reverse cumulative curve of LA-2–equivalent OspA antibody levels at month 2 in 20 vaccinated subjects with breakthrough cases of definite Lyme disease, in 512 vaccinated subjects evaluated for Lyme disease during year 1 whose diagnosis was not confirmed, and in 395 vaccinated subjects from one study site. At month 2, 1 month after the second injection, the titers were significantly lower in vaccinated subjects with breakthrough cases of Lyme disease than in the other two comparison groups ($P = .01$ for each comparison). *(From Steere AC, Sikand VK, Meurice F, et al. Vaccination against Lyme disease with recombinant* Borrelia burgdorferi *outer-surface lipoprotein A with adjuvant.* N Engl J Med. *1998;339:209–215, Copyright Massachusetts Medical Society.)*

Vaccine Efficacy

The efficacy of recombinant OspA vaccines was determined in two Phase III trials, one sponsored by SmithKline Beecham[100] and the other sponsored by Pasteur Mérieux Connaught.[141] The SmithKline Beecham study involved 10,936 subjects, ages 15 to 70 years, who lived in 10 states endemic for Lyme disease. Participants received an injection of either recombinant *B. burgdorferi* OspA lipoprotein with adjuvant, or placebo, at enrollment and 1 and 12 months later. In cases of suspected Lyme disease, culture of skin lesions, PCR testing, or serologic testing was done, and serologic testing was performed in all study participants 12 and 20 months after study entry to detect asymptomatic infection.

In the first year, after two injections, 22 subjects in the vaccine group and 43 in the placebo group contracted definite Lyme disease ($P = .009$); the point estimate of vaccine efficacy was 49% (95% confidence interval [CI], 15% to 69%).[100] In the second year, after the third injection, 16 vaccine recipients and 66 placebo recipients contracted definite Lyme disease ($P < .001$); the point estimate of vaccine efficacy was 76% (95% CI, 58% to 86%). Except for one participant each year who presented with Lyme arthritis, all definite cases in vaccine recipients had erythema migrans. Moreover, the arthritis case in year 1 most likely acquired the infection in the year before enrollment. During the first year, two subjects in the vaccine group and 13 in the placebo group had asymptomatic IgG seroconversion to *B. burgdorferi* ($P = .004$). In the second year, all 15 subjects with asymptomatic seroconversion were in the placebo group ($P = .001$). Thus, the efficacy of the vaccine in preventing asymptomatic infection was 83% in the first year and 100% in the second year.

In the SmithKline Beecham vaccine trial, the clinical picture of nonspecific systemic symptoms, without erythema migrans, accompanied by IgM or IgG seroconversion to *B. burgdorferi* sonicate was defined as possible Lyme disease.[100] Vaccine efficacy was not demonstrated among the participants who had this clinical picture.[100] However, in a post hoc analysis, in which this clinical picture and IgG seroconversion with the more sensitive and specific VlsE C6 peptide ELISA was redefined as definite Lyme disease, 17% of the 201 patients with definite Lyme disease presented with nonspecific systemic symptoms, without erythema migrans.[16] Among these patients, vaccine efficacy was similar to those with erythema migrans.[142]

In the trial sponsored by Pasteur Mérieux Connaught,[141] 10,305 subjects 18 years of age or older were recruited. The first two injections were administered 1 month apart, and some patients received a booster dose at 12 months. The primary end point was the number of new clinically and serologically confirmed cases of Lyme disease. The efficacy of the vaccine was 68% in the first year and 92% in the second year among the 3475 subjects who received the third injection. Thus, both studies showed that three injections of a recombinant OspA vaccine were quite effective in the prevention of Lyme disease for at least one tick transmission season.

Duration of Immunity and Protection. Although vaccinated subjects usually developed high levels of antibody to the protective epitope of OspA after three injections, the response declined rather rapidly.[100] If the correlate of protection was defined as 1400 EL.U./mL, a level with 95% sensitivity to discriminate between vaccine success and failure,[138] a fourth injection probably would have been needed in most patients the following year. Although long-term immunogenicity studies were not completed, it was anticipated that additional booster injections would have been needed in adults approximately every other year to maintain antibody titers at this level.

Postexposure Prophylaxis and Therapeutic Vaccination

There was no role for postexposure prophylaxis or therapeutic vaccination with recombinant OspA vaccines. Because *B. burgdorferi* downregulates OspA expression during transmission to the human host,[38] an antibody response to this protein does not afford protection once the spirochete is transmitted to the human host. However, in patients with Lyme arthritis in the northeastern United States, OspA seems to be upregulated again, and OspA antibody responses are common.[136a]

Safety

Common Side Effects

During both Phase III clinical trials, pain and tenderness at the vaccination site were the most commonly reported adverse events.[100,141] In the SmithKline Beecham Phase III trial, approximately 24% of vaccine recipients noted pain at the injection site, compared with 7% of placebo recipients ($P < .001$) (Table 34.2).[100] In addition, approximately 3% of vaccine recipients reported systemic symptoms of myalgias, achiness, fever, and chills, a significantly higher percentage than seen in placebo

TABLE 34.2 Percentage of Subjects With Symptoms (Overall Incidence, ≥1%) Classified as Related, Possibly Related, or Unrelated to Vaccination[a]

Symptoms	Vaccine (%)	Placebo (%)	*P* Value
RELATED OR POSSIBLY RELATED TO VACCINATION			
Local (Injection Site)			
Soreness	24.1	7.6	<.001
Redness	1.8	0.5	<.001
Swelling	0.9	0.2	<.001
Systemic			
Early (≤30 Days)			
Arthralgia	3.9	3.5	.34
Headache	3.0	2.5	.14
Myalgias	3.2	1.8	<.001
Fatigue	2.3	2.0	.37
Achiness	2.0	1.4	.01
Flu-like symptoms	2.0	1.1	<.001
Fever	2.0	0.8	<.001
Chills	1.8	0.5	<.001
Upper respiratory tract infection	1.0	1.1	.69
Totals	19.4	15.1	<.001
Late (>30 Days)			
Arthralgia	1.3	1.2	.54
Totals	4.1	3.4	.06
UNRELATED TO VACCINATION			
Early	27.1	27.9	.37
Late	53.3	52.6	.48

[a]Totals include all early or late, related or possibly related systemic events, not just those with a frequency of 1% or greater.

From Steere AC, Sikand VK, Meurice F, et al. Vaccination against Lyme disease with recombinant Borrelia burgdorferi *outer-surface lipoprotein A with adjuvant.* N Engl J Med. *1998;339:209–215, copyright Massachusetts Medical Society.*

recipients. These reactions usually occurred within 48 hours after vaccination and lasted for a median duration of 3 days. They were usually mild or moderate in severity, and the severity usually did not increase with subsequent injections. At one site, where information was solicited about fever, no patient reported a temperature above 39°C. No hypersensitivity reactions were noted. Eleven percent of participants had a past history of Lyme disease, and 2.3% were seropositive for *B. burgdorferi* at study entry.[100] These individuals were not different from the rest of the subjects in the safety profile of the vaccine.[100]

Similarly, in a study of safety and immunogenicity of LYMErix in 4087 children ages 4 to 18 years, children generally achieved higher LA-2–equivalent antibody titers than adults, and the responses persisted longer, suggesting that the vaccine may be more effective in children than adults.[140] In the vaccine group, 20.4% had local injection-site reactions or systemic symptoms compared with 5.9% of the placebo recipients (P = .001). However, most reactions had a mean duration of only 2 to 3 days and were mild or moderate in severity.

Rare Reactions

In the natural infection, a small percentage of patients with Lyme arthritis have persistent synovitis for months or even several years after 2 to 3 months of treatment with antibiotic therapy, termed antibiotic-refractory Lyme arthritis.[143] Although PCR results for *B. burgdorferi* DNA are usually positive before antibiotic therapy, they are frequently negative after such courses of antibiotics,[60] and both PCR and culture results have been uniformly negative in synovial tissue obtained at synovectomies 12 months or more after the start of antibiotics.[62] These observations suggested that Lyme arthritis may continue after the near or total eradication of *B. burgdorferi* from the joint, perhaps sustained by an infection-induced autoimmune response.[144] This outcome is associated with HLA-DR molecules that bind the $OspA_{163–175}$ epitope of *B. burgdorferi* (DRB1*0101, DRB1*0102, DRB1*0401, DRB1*0402, DRB1*0404, DRB1*0405, and DRB5*0101),[145] and with T-cell recognition of this epitope.[146–149] Human lymphocyte function–associated antigen (LFA)-1$\alpha_{L\ 332–340}$, which has partial sequence homology with $OspA_{163–175}$, was originally proposed as a candidate autoantigen in antibiotic-refractory Lyme arthritis.[147] Subsequently, it was shown that the LFA-1 peptide was not a relevant autoantigen in this illness.[150,151]

Nevertheless, the question arose whether vaccination with recombinant OspA might exacerbate Lyme arthritis or induce autoimmune arthritis in genetically susceptible individuals. In the Phase III vaccine trials, however, vaccination with OspA was not associated with arthritis, neurologic abnormalities, or any other late syndrome 30 days or more after vaccination.[100,141] Although several subjects in the SmithKline Beecham Phase III trial developed inflammatory polyarthritis during the study and three subjects developed Lyme arthritis, these persons were found in both the vaccine and the placebo groups. In the Pasteur Mérieux Connaught trial, subjects with prior Lyme arthritis did not develop arthritis after vaccination.[141] Moreover, in a separate study of 30 patients with previous Lyme disease, those with a previous history of Lyme arthritis did not have a recurrence of arthritis after vaccination.[152] In the pediatric safety and immunogenicity study, one child had transient ankle swelling after dose 2, which resolved and did not recur after dose 3.[140] Nevertheless, because of lingering concerns about the theoretical potential for arthritogenicity, caution was urged with Lyme disease vaccination, particularly in people with arthritis.

As with any vaccine trial, rare side effects may not be recognized in comparisons of vaccine and placebo groups. Thus, the question remained: Might vaccination with OspA induce arthritis but at a frequency below the level of detectability in vaccine trials? In 2001, four patients in a case series were reported to have developed arthritis after OspA vaccination.[153] In one of the patients, *B. burgdorferi* infection was probably acquired before vaccination, but joint swelling became manifest months after vaccination. The arthritis resolved with antibiotic therapy. This phenomenon was observed in three patients in the SmithKline Beecham Phase III trial, two in the vaccine group and one in the placebo group.[100] Three months after the third injection of vaccine, the second patient developed a moderate effusion of a knee, which lasted for several months. The third patient developed swelling of the finger and toe joints within 24 hours after the second injection, and his symptoms improved over the next 5 days. The final patient developed, 24 hours after the second vaccination, synovitis of multiple proximal interphalangeal joints, which lasted approximately 7 weeks.

In 2002, the CDC and the U.S. Food and Drug Administration (FDA) published the reports relating to Lyme disease vaccination from their Vaccine Adverse Event Reporting System (VAERS). From December 1996 through August 2000, during the first 19 months after licensure, approximately 1.4 million doses of LYMErix were distributed, and, during this period, 905 adverse events (0.06%) were reported to the VAERS.[154] The most common reports were of arthralgias (250 reports) or myalgias (195 reports) occurring after the first dose. These frequencies were consistent with the events observed in the clinical trials. In addition, arthritis was reported to have developed in 59 patients, arthrosis in 34 patients, and rheumatoid arthritis in nine patients, usually after the second or third dose.[154] This frequency of arthritis was thought to be below the expected background incidence of arthritis occurring during this time period in an unvaccinated population. An analysis of HLA-DR profiles and OspA immunity in a subgroup of these patients was not suggestive of a vaccine-induced process.[155]

As detailed in a recent review,[156] recent work in a mouse model suggests that OspA in genetically susceptible persons stimulates a particularly strong T-helper cell type 1 response,[157] which may be one of several factors that can set the stage for a putative autoimmune response in affected joints. However, vaccination with OspA did not induce arthritis in this mouse model, and case and control comparisons in human vaccine trials did not show an increased frequency of arthritis among OspA-vaccinated individuals. Thus, a vaccine-induced immune response to OspA does not replicate the sequence of events needed in the natural infection to induce antibiotic-refractory Lyme arthritis.

Immunosuppressed Individuals

Lyme disease vaccines were not tested in immunosuppressed patients, and thus no information is available about the effectiveness of vaccination in such persons.

Spread to Contacts

Because Lyme disease vaccines consisted of only a recombinant OspA protein, there was no risk of spread to contacts.

Indications for Vaccine

Most *B. burgdorferi* infections result from periresidential exposure to infected ticks during property maintenance, recreation, or leisure activities.[103] Thus, persons who live or work in residential areas surrounded by woods or overgrown brush infested with vector ticks are at risk for acquiring Lyme disease. However, cost-effectiveness analyses concluded that

vaccination for Lyme disease was economically attractive only for those who have a seasonal probability of *B. burgdorferi* infection of greater than 1%.[158–160] Although these analyses were performed to inform a public health decision, an individual patient in a lower-risk area who is willing to pay out of pocket could reasonably decide in favor of vaccination.

The CDC's Advisory Committee on Immunization Practices advised that vaccination should be considered by persons 15 to 70 years old who live in or visit high-risk areas and have frequent and prolonged exposure to *I. scapularis* ticks.[161] Vaccination was not recommended for persons with minimal or no exposure to such ticks. However, as vaccination is not currently available, this preventive approach to Lyme disease is not possible at this time.

Contraindications and Precautions

Recombinant OspA vaccines were tested only in normal individuals. Therefore, safety information was not available about vaccine use by patients with other diseases or by pregnant women. Although studies in children were underway, these vaccines were not licensed for use in children at the time of withdrawal of the vaccine.

Nonimmunologic Prevention

Protective measures for the prevention of Lyme disease include the avoidance of tick-infected areas, the use of protective clothing, the use of repellants and acaricides, tick checks, and modifications of landscapes in or near residential areas.[103] However, these methods of prevention have not been shown to lower the incidence of Lyme disease,[133] presumably because people have trouble maintaining these tick prevention efforts throughout the entire tick-transmission season.

Withdrawal of LYMErix

Although the FDA approved LYMErix, the first new recombinant Lyme disease vaccine, in late 1998, the manufacturer voluntarily withdrew its product from the market a little more than 3 years later, in 2002, amid media coverage, fears of vaccine side effects, and declining sales. Several articles have reviewed the factors involved.[162–166] Some of the reasons included the low risk of Lyme disease in most parts of the country, the manufacturer's direct-to-consumer advertising that overstated the risk in these areas, the need for booster injections every or every other year, the relatively high cost of this preventive approach compared with antibiotic treatment of early infection, and the theoretical (although never proven) concern that in rare cases, vaccination might trigger autoimmune arthritis. For all these reasons, the vaccine received a tepid endorsement by the public health community. Most importantly, the Lyme disease advocacy community, which initially supported the vaccine, became critical opponents.[166] For the most part, advocacy groups consisted of patients with other illnesses, particularly pain and fatigue syndromes,[32] but they said that vaccination made their "Lyme disease" worse. Paradoxically, the vaccine's success in scientific studies was seen as threatening their central position that Lyme disease is chronic, protean, and difficult to treat.[166] An additional problem is that the vaccine was not covered by the Vaccine Injury Compensation Program, which made it a target for personal injury lawyers. One law firm filed a class action suit in December 1999, and individual suits began to flood in.[163] In July 2003, GlaxoSmithKline, now the maker of LYMErix, settled the class action suits.[163] The final agreement included over $1 million in legal fees for prosecuting attorneys, but it provided no financial compensation to the vaccine "victims."

FUTURE CONSIDERATIONS

OspA Vaccination for Global Use

The OspA Lyme disease vaccines developed in the 1990s should be considered first-generation vaccines. A goal of future vaccines would be to provide longer-term protection and broader protection against variant strains of *B. burgdorferi*. The development of a Lyme disease vaccine for use in Europe has been more difficult than in the United States because all three pathogenic species of *B. burgdorferi sensu lato* cause the infection there.

Recently, Baxter Healthcare in Vienna, Austria, developed a multivalent OspA Lyme borreliosis vaccine (mv rOspA LB vaccine) containing three recombinant OspA antigens, which was designed to provide protection against almost all *B. burgdorferi sensu lato* strains associated with human disease worldwide. For this development, knowledge of protective OspA epitopes was used to design recombinant OspA molecules combining the protective properties of two OspA antigens in a single molecule. In addition, the sequence of the *B. burgdorferi* epitope ($OspA_{163–175}$) that has partial sequence homology with human LFA-1 was replaced with a corresponding (non-hLFA-1) sequence from a serotype-2 OspA molecule (Fig. 34.8).

In a proof-of-principle studies, a single recombinant OspA containing protective elements from two different OspA serotypes (1 and 2) induced antibody responses that protected mice against infection with either *B. burgdorferi sensu stricto* (serotype-1) or *B. afzelii* (OspA serotype-2),[167,168] as well as *B. garinii, Borrelia spielmanii, Borrelia valaisiana, Borrelia lusitaniae,* and *Borrelia japonica* strains,[169] further indicating that the vaccine had the potential to prevent Lyme disease worldwide.

In Phase I/II clinical trials to evaluate the safety and immunogenicity of the mv rOspA Lyme borreliosis vaccine, with and without adjuvant, the 30-μg adjuvanted formulation showed an excellent tolerability profile and induced the highest antibody titers following a booster dose, 9 to 12 months after the initial immunization.[170] Despite these promising results, Baxter Bioscience terminated their Lyme disease vaccine research program in 2014.

Valneva Austria GmbH has also developed a lipidated OspA vaccine. The vaccine includes three proteins, each containing the C-terminal half of two OspA serotypes linked to form a heterodimer along with a N-terminal lipid moiety.[171] Three immunizations protected mice from spirochetes expressing OspA serotypes 1, 2, and 5.

Other Altered OspA Proteins

Other altered OspA proteins have been developed as vaccine candidates. In one study, the entire 255-amino-acid ectodomain of OspA was inserted into the major B-cell epitope of the hepatitis B virus capsid protein.[172] This fusion protein induced an antibody response to the LA-2–protective epitope similar to that of recombinant lipidated OspA. In another study, a C-terminal fragment of OspA was generated that lacked approximately 45% of the residues from the N-terminus.[173]

Human OspA-Specific Monoclonal Antibody

Another experimental approach is injection of a humanized OspA-specific, borreliacidal monoclonal antibody, rather than injection of an OspA protein, to induce an OspA antibody response.[173a] When passively administered to mice prior to challenge with *B. burgdorferi*–infected ticks, these humanized monoclonal antibodies were capable of preventing

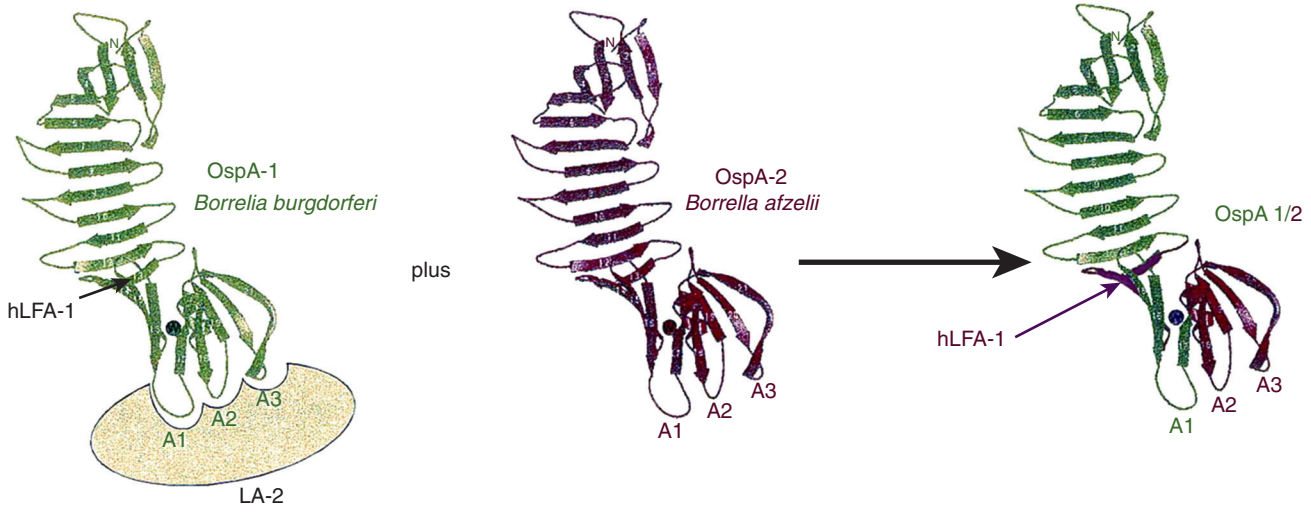


Figure 34.8. Proximal portion of a serotype-1 OspA sequence is fused to the distal portion of a serotype-2 OspA sequence. The resultant composite molecule contains the first of the three surface-exposed loops recognized by protective monoclonal antibody LA-2. The second and third loops recognized LA-2 are replaced by equivalent sequences from the serotype-2 molecule. The hLFA-1 epitope in β-sheet 13 from the serotype-1 sequence was replaced with a 25-amino-acid sequence derived from the serotype-2 sequence.

transmission completely. This approach has not yet been tested in human subjects, but if successful, it would presumably require infusions of OspA antibody each year prior to the tick transmission season.

OspC Vaccine Candidates

In an effort to provide protection against the range of European strains, Baxter Healthcare in Vienna initially researched a multivalent OspC vaccine. A potential advantage of this approach was that *B. burgdorferi sensu lato* strains express OspC on transmission and entry into mammalian hosts.[174] Therefore, anamnestic immune responses in the host could provide protection, rather than depending solely on the maintenance of high levels of circulating OspA antibodies, which kill spirochetes in the tick while it feeds. However, a major disadvantage was the greater variability of OspC compared with OspA. The company developed a 14-valent OspC vaccine, which was given in three 100-µg or 140-µg doses. In Phase I trials, the major problem was vaccine-induced erythema and swelling at the injection site, particularly after the third injection, which occurred in up to half of persons. Consequently, this approach was discontinued. Subsequently, one research group found that combinations of as few as two OspC proteins identified all patients who had OspC antibodies.[175] Another research group developed a tetravalent OspC-based, recombinant, chimeric vaccinogen, with linear epitopes presented by OspC types A, B, K, and D.[176] When mice were vaccinated with this construct, they developed complement-dependent bactericidal antibodies against strains expressing each of the OspC types.

OspA Immunization of Rodent Reservoirs

Another novel approach is being researched in which mouse reservoirs of *B. burgdorferi* are immunized with OspA. In one study, wild white-footed mice *(Peromyscus leucopus)*, the preferred host for nymphal *I. scapularis* ticks, were immunized by subcutaneous injection with either recombinant OspA or a negative control antigen in experimental or control grids.[177] In two large-scale experiments, the authors estimated that approximately 69% of the mouse population was immunized more than once, approximately 55% of all mice in each experimental group seroconverted to OspA, and the nymphal tick infection prevalence declined by approximately 16%. In a 5-year-prospective field trial, oral vaccination of wild white-footed mice with OspA resulted in a reduction of 23% in nymphal infection prevalence at 2 years and a 76% reduction at 5 years.[178] Similarly, another study predicted that a mouse-targeted vaccine would reduce infection prevalence among ticks by 56%.[179] However, the authors warned that other factors, particularly contributions to pathogen maintenance from non-mouse hosts, would preclude elimination of *B. burgdorferi* through mouse vaccination alone. They proposed increasing the impact of wildlife vaccination by the use of concombinant acaricide applications.

Immunization Using Multiple *B. burgdorferi* Proteins

In mice, antibody to OspA gives the best protective response of any single, known *B. burgdorferi* protein.[122–125] However, in these models, other responses, including responses to OspB, OspC, and DbpA, also provide protection against cultured *B. burgdorferi*, and reactivity with OspF, DbpB, and BBK32 provides partial protection.[135] Although reinfection may occur in patients with early infection who are treated with antibiotic therapy, it has not been noted in patients with Lyme arthritis, a late manifestation of the illness, suggesting that long-term immunity in the natural infection is generated from reactivity with a combination of spirochetal proteins.

To identify uncharacterized outer-surface proteins, one group performed a comprehensive gene expression profiling analysis of temperature-shifted and mammalian-host-adapted *B. burgdorferi*.[180] They identified seven uncharacterized *B. burgdorferi* outer-surface proteins, all of which elicited bactericidal antibody responses. In addition, two glycolipid antigens of *B. burgdorferi* are known to be immunogenic,[55,181] and antigenic epitopes of ACG have been identified.[182] However, it is not known whether glycolipid antigens might have a role as an adjuvant or in protective immunity in Lyme disease.

Reverse Vaccinology

Reverse vaccinology makes use of the known sequence of genes of *B. burgdorferi* to determine vaccine candidate targets using in silico analyses. Selected vaccine candidates are formulated to generate prototype immunogens, which are then used to immunize naive mice prior to challenge with live, infectious *B. burgdorferi*.[182a]

Immunization With Tick Proteins

I. scapularis ticks, which transmit the Lyme disease agent, have been implicated in the transmission of five other infectious agents. Thus, host immune responses that interfere with tick feeding could potentially help in preventing not only Lyme borreliosis, but also other *I. scapularis*–associated diseases. In the last decade, several tick molecules with the potential to serve as vaccines have been identified.[183–185] The *Ixodes* salivary protein Salp15 specifically binds to *B. burgdorferi* OspC and thereby protects spirochetes against borreliacidal antibodies.[39] By using Salp15 as a vaccine candidate, specific antibodies not only neutralized the immunosuppressive effects of this protein but also bound *Borrelia* surface-coated Salp15, enhancing clearance by phagocytic immune cells.[186] As a next step, an adenovirus expression system was used to deliver four salivary proteins (Salp15, Salp25A, Salp25D, and Isac) derived from *Ixodes scapularis* ticks.[187] In a mouse model, vaccination with these tick proteins modulated a Th1 response and partially controlled spirochete load. Using a phage display library, another protein of *I. scapularis*, a metalloprotease (MP1), was shown to be immunogenic.[188] In another study, vaccination with vaccinia virus expressing subolesin, a conserved tick protein, inhibited tick infestation by 52% compared with control vaccination, and reduced uptake of *B. burgdorferi* among surviving ticks by 34%.[189] Moreover, vaccination targeting tick or spirochetal proteins are not mutually exclusive. In a mouse model, immunization with Salp15 and OspA provided better protection against *B. burgdorferi* infection than either alone.[186]

SUMMARY

An effective human vaccine could be very beneficial in preventing Lyme disease,[190] and experience gained with OspA vaccines in the 1990s has shown the feasibility of this approach. However, given the fiasco associated with the introduction of these OspA vaccines in the late 1990s,[165] a new vaccine would need to meet high standards of safety, efficacy, cost, and public acceptance.[190] Nevertheless, an effective vaccine has already been developed for the United States market for this potentially serious infection, but the Lyme disease advocacy community has prevailed in keeping it from the public. This represents a failure of disease prevention efforts in the United States.[165]

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35 Malaria Vaccines

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Human malaria is caused by five species of the protozoan *Plasmodium*: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. The life cycle and parasite-host interaction of each species determines the severity and pathogenesis of clinical disease.[1,2] *P. falciparum* has the highest case fatality rate, especially in young children. The ability of *P. vivax* to develop dormant intrahepatocytic hypnozoites results in clinical relapses and contributes to the greatest geographic distribution.[3] Malaria occurs where the environment supports competent *Anopheles* mosquito vectors with access to malaria-infected hosts.[3,4] Until the early 1900s, malaria was endemic across every continent except Antarctica. By the 1950s, malaria was controlled in the United States, Europe, and Australia through the use of insecticides and environmental strategies.[1] Recently, a multifaceted strategy including environmental control, insecticides, bed nets, and chemotherapy has allowed some countries to report a dramatic fall in malaria incidence.[5] Nevertheless, a safe and effective vaccine remains an essential missing tool in the fight against malaria.

BACKGROUND

All species of malaria have a complex life cycle that begins in humans when female *Anopheles* mosquitos deposit sporozoites subcutaneously during a blood meal. Sporozoites enter the bloodstream and lymphatics and rapidly migrate to the liver, invading hepatocytes to form schizonts, each of which eventually yields tens of thousands of merozoites. *P. vivax* hypnozoites may remain quiescent in the liver for months to years before emerging into the circulation. Once merozoites erupt and invade circulating red blood cells (RBCs), symptoms of clinical disease become apparent. Over 24 to 48 hours, each merozoite develops into 8 to 10 new merozoites that rupture through the erythrocyte membrane and infect other RBCs. Some parasites develop into sexual-stage gametocytes, which, if taken up by an *Anopheles* during a blood meal, develop into male or female gametes, undergo fertilization to form ookinetes, and penetrate the gut epithelium to form oocysts. Sporozoites develop during the next 2 to 3 weeks, emerge from oocysts, and localize to the salivary glands, where they become infectious for humans.

Characteristic signs and symptoms of malaria include fever, malaise, headache, photophobia, muscle aches, anorexia, nausea, and vomiting.[6] Symptoms appear to coincide with the synchronized lysis of RBCs by schizonts releasing malarial toxins along with merozoites. Severe disease occurs most frequently with *P. falciparum* because of its ability to multiply infect erythrocytes, leading to massive destruction of RBCs, and because mature schizont-infected RBCs cytoadhere and obstruct capillary walls.[2,6] Severe clinical sequelae (such as acute renal failure, severe anemia, hypoglycemia, cerebral malaria, and pulmonary edema) occur most commonly in malaria-naïve populations, such as children and travelers.[7] Pregnant women and the developing fetus are at special risk for severe disease, indicating unique mechanisms of pathogenesis.[8] Coinfection with HIV predisposes to more severe disease, especially in children and malaria-naïve adults.[9]

EPIDEMIOLOGY AND BURDEN OF DISEASE

Quantification of the clinical burden of malaria is imprecise and is impacted by variable access to diagnosis and treatment, species prevalence, case definition, and, in many malaria-endemic settings, low specificity of diagnosis.[10,11] Approximately 170 million to 300 million clinical cases occur per year globally, 25% of which are caused by *P. vivax*. Estimates of annual malaria-attributable deaths in 2013 ranged from 367,000 to 755,000,[12] with the majority of deaths occurring in children in sub-Saharan Africa who were infected with *P. falciparum*. *P. knowlesi* was recognized in 2008 by the World Health Organization (WHO) as the fifth human *Plasmodium* species potentially leading to fatal outcome.[13] Tourism and economic development in southern and Southeast Asia will likely lead to a further increase in cases among locals and travelers.

The number of malaria cases diagnosed within the United States has been stable at approximately 1000 to 1500 per year for the past decade, with most cases occurring in travelers and military personnel. The United States has a very small number of congenital, blood-transfusion, and locally acquired cases.[14] Cases of imported malaria in Europe have increased steadily for several decades, while in Central Asia, local malaria transmission has been largely interrupted, with Tajikistan and Turkey making significant progress toward becoming malaria-free.[15]

VACCINOLOGY

Multiple lines of evidence suggest that a malaria vaccine is feasible. Immunization of humans with radiation-attenuated sporozoites confers sterile protection in roughly 50% to 90% of naïve volunteers who are challenged within 12 months.[16–18,18a] Because this model requires exposure to approximately 1000 irradiated, malaria-infected mosquitoes over several months, it is impractical.[19] Naturally acquired clinical immunity develops during the first 2 decades of life in people living in malaria-endemic countries.[20,21] In general, immunity is imperfect, wanes rapidly, is linked to intensity of exposure, and primarily impacts the severity of clinical disease.[22–25] An immunologic correlate of naturally acquired protection has yet to be identified.

The approximately 5000 open reading frames of the *P. falciparum* genome are carried on 14 chromosomes.[26–29] Throughout its life cycle, the malaria parasite is susceptible to vector and host immunity and passes through several "biological bottlenecks" where the number of parasites in the vector or host is limited. These bottlenecks are logical targets for vaccine development. Most candidate vaccines currently in development focus on a single stage of the life cycle. Although some target antigens are expressed in multiple stages,[30–32] a highly efficacious vaccine might require distinct antigens from different stages.

Vaccines Targeting the Preerythrocytic Stages

Vaccine strategies targeting sporozoites aim to generate immune responses that neutralize its ability to invade or develop within hepatocytes (Fig. 35.1). Studies in rodent and simian models confirm that humoral and cell-mediated

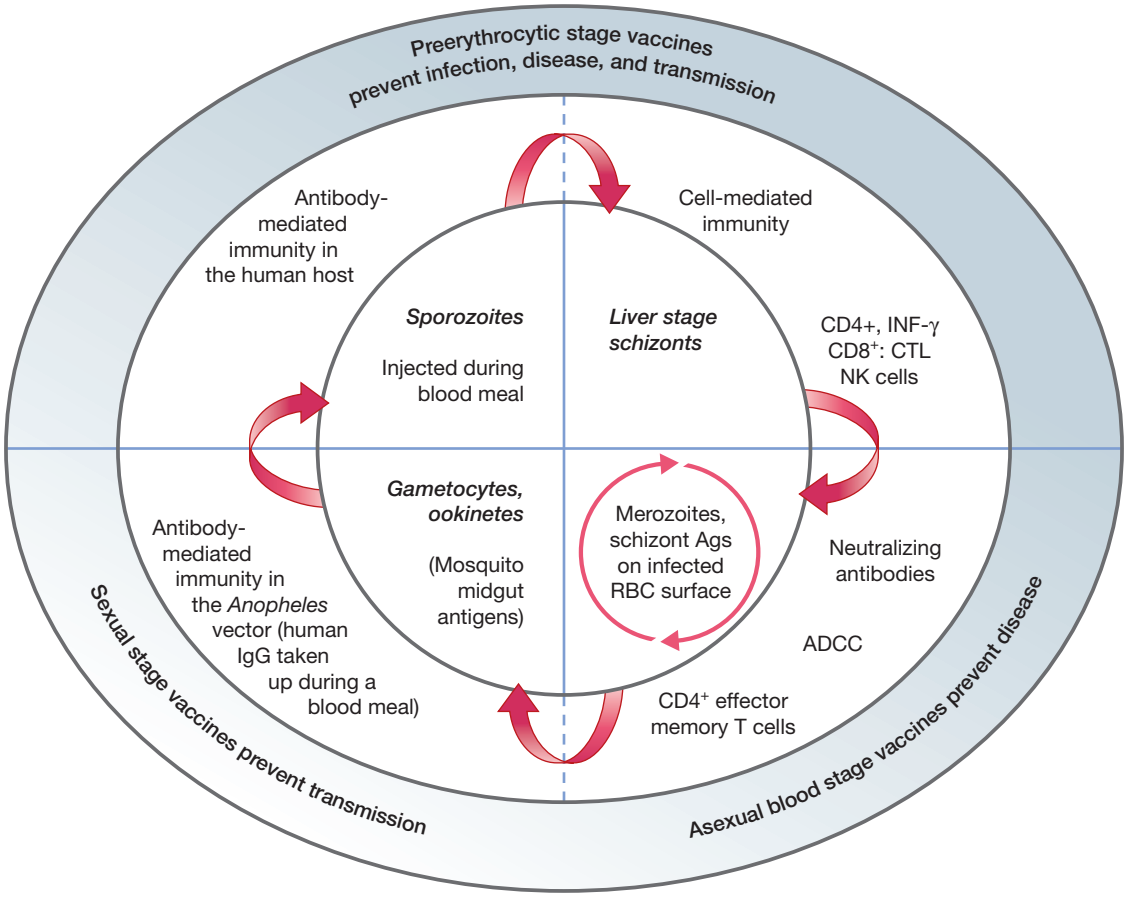


Figure 35.1. The inner circle highlights key stages of the parasite life cycle targeted by malaria vaccines under development. The middle circle shows the immune responses thought to be most important in protective immunity against these stages. The outer circle shows the major classes of malaria vaccines under development and the potential effect that a highly effective vaccine of that class might have on malaria. Stages in italics represent biological bottlenecks where relatively few parasites (<100) must be targeted to break the life cycle. For comparison, the total number of merozoites/schizonts in the human host can exceed 10^{12}. ADCC, antibody-dependent cellular cytotoxicity; Ags, antigens; CTL, cytotoxic T lymphocyte; Ig, immunoglobulin; INF, interferon; NK, natural killer; RBC, red blood cell.

immunity contribute to protection.[33–37] Experiments involving irradiated sporozoite challenge models have implicated $CD4^+$ and $CD8^+$ T cells, natural killer T cells, and γδ T cells in the inhibition of intrahepatic schizogony.[38–40] A highly effective preerythrocytic stage vaccine that confers sterilizing immunity, delivered widely within a malaria endemic region, should logically reduce malaria transmission.[41] Partially protective preerythrocytic vaccines that significantly decrease the load of merozoites emerging from the liver[42] could have a large public health impact if, like other partially effective malaria interventions (insecticide-treated bed nets, intermittent drug therapy), they reduced the risk of clinical malaria.[43–45]

Vaccines Targeting the Asexual Blood Stages

Merozoites enter the bloodstream after erupting from hepatocytes and within seconds invade RBCs. Neutralizing antibodies can inactivate merozoites before invasion or engage antibody-dependent cytotoxic effector mechanisms against target malarial antigens expressed on schizont-infected RBC membranes, but as these antigens are generally subject to significant antigenic variation, are large, and/or are structurally complex, most candidate vaccines based on these stages target only a portion of the native protein.[46–48] Rodent models have implicated CD4 T cells, nitric oxide, and γδ T-cell activity against blood-stage parasites,[49] and CMI likely has a role in naturally acquired immunity in humans.[50,51] Vaccines against these stages ideally would prevent disease by limiting the number of infected RBCs, but generating immune responses sufficiently broad to cover the many antigenic variants that circulate in communities will be challenging.[52–54]

Vaccines Targeting the Sexual Stages

Transmission-blocking vaccines rely on human antibody and complement taken up by mosquitoes during a blood meal. Within the mosquito, sexual-stage parasite surface antigens or mosquito mid-gut binding sites can be targeted to prevent successful development of infectious sporozoites.[55–57] This type of vaccine would not prevent disease in a vaccinated person, but would prevent the further spread of malaria.[58,59] If effective, such a vaccine could be particularly useful in areas of low-to-moderate transmission intensity or as an important component of a multiantigen vaccine.

VACCINES IN LATE-STAGE DEVELOPMENT

Currently, there are no registered malaria vaccines. A large number of malaria vaccine candidates have been tested preclinically or in Phase I/II clinical trials,[60–63] but only one is in late-stage development. The RTS,S Clinical Trials Partnership has recently completed a large Phase III trial of the RTS,S

vaccine candidate conducted in 11 sites across seven sub-Saharan African countries. An application for a Committee for Medicinal Products for Human Use (CHMP) scientific opinion through the European Medicines Agency (EMA) Article 58 procedure was placed.[64] A positive opinion was received from EMA in July 2015, and the WHO recommended pilot introduction of the vaccine in January 2016.[64a,64b] The program was designed in consultation with the WHO to demonstrate that RTS,S can provide significant durable protection against clinical malaria, including severe malaria, in young children and infants when administered via the Expanded Programme on Immunization (EPI).

RTS,S was developed and tested by GlaxoSmithKline Biologicals (GlaxoSmithKline), the Walter Reed Army Institute of Research (WRAIR), Programs for Appropriate Technology in Health (PATH) Malaria Vaccine Initiative, and several other academic partners in a collaboration that spanned many years.[65] The vaccine is based on the circumsporozoite (CS) protein of *P. falciparum*, a major sporozoite surface protein that contains B- and T-cell epitopes.[66] The CS protein undergoes important conformational modification as it transits from the mosquito to the mammalian host, permitting motility and invasion of hepatocytes.[67] RTS,S consists of a chimeric virus-like particle protein construct expressing 19 NANP repeats of the central region and the entire C-terminal flanking region (amino acids 207 to 395) fused to the hepatitis B virus surface antigen (HBsAg).[68] The vaccine is produced using recombinant DNA technology that uses a *Saccharomyces cerevisiae* fermentation process. The overall manufacturing process is similar to that used for production of commercial HBsAg vaccine. The fusion protein is coexpressed with HBsAg, yielding a product that is composed of CS-HBsAg fusion protein and HBsAg in a ratio of approximately 1 : 3.

A series of pivotal clinical trials conducted at WRAIR that incorporated an experimental malaria sporozoite challenge demonstrated an absolute requirement for adjuvantation of RTS,S with a formulation containing the Toll-like receptor (TLR)-4 agonist monophosphoryl lipid A and a purified saponin derivative QS21 (*Quillaja sapanaria* Molina, fraction 21; licensed by GSK from Antigenics Inc, a wholly owned subsidiary of Agenus Inc).[68–72] Two related adjuvant formulations were studied subsequently during Phase II, AS02 in which the immunostimulants are combined with an oil-in-water emulsion, and AS01, in which the immunostimulants are combined with liposomes.[73] Based on superior immunogenicity, an equivalent safety profile and a trend toward better efficacy, the AS01 formulation was ultimately selected for Phase III.[71,74–76] RTS,S induces immune responses in humans that are believed to protect against infection with malaria sporozoites. These include high levels of antibodies against CS capable of inhibiting sporozoite infection of liver cells in vitro. It also induces CMI responses against CS that have been shown in numerous animal models to associated with protection.[77–80] The vaccine also induces very high levels of antibodies against HBsAg.

RTS,S was studied in more than 4000 subjects before Phase III, including malaria-naïve and malaria-experienced adults, as well as young children and infants, in malaria-endemic settings. The vaccine is generally well tolerated and immunogenic in all age groups and has an acceptable safety profile.[68–71,74–76,81–91] Because of the complexity of falciparum malaria as a disease target and the use of novel adjuvant technology in RTS,S, the Phase II/III program in Africa was complex and included more than a dozen clinical trials designed to select the proper vaccine dose, formulation, and schedule, while evaluating vaccine safety and immunogenicity in progressively younger age groups down to neonates. The program also included specific studies to demonstrate that RTS,S could be coadministered with routine EPI vaccines, including diphtheria, tetanus toxoid, whole-cell pertussis (DTPw), *Haemophilus influenzae* type b (Hib), hepatitis B (HepB), oral polio, measles, rotavirus, pneumococcal conjugate, and yellow fever, and to HIV-infected children. Interim and final results of the Phase III trial have now been published.[64,92–94] Overall, 8922 children and 6537 young infants were enrolled, randomized to receive either three doses of RTS,S plus a booster (R3R), three doses of RTS,S without a booster (R3C), or three doses of control vaccine plus a booster control vaccine (3C3). Follow-up in the older children and younger infants continued for approximately 48 and 38 months respectively. The trial was conducted in the context of high coverage of bednets (approximately 80%) and different transmission settings. Malaria incidence in the infant group that received the control vaccines ranged from 0.03 to 4.27 episodes per infant during the first 12 months of the study. As a consequence of intense monitoring and ready access to medical care, all-cause mortality in the trial was low (1.8% in children, 2.3% in infants). Tables 35.1 and 35.2 summarize the data on vaccine efficacy.

TABLE 35.1 Vaccine Efficacy in Infants Age 6–12 Weeks at First Dose

	Vaccine Efficacy Against All Episodes of Clinical Malaria (95% CI)	Vaccine Efficacy Against Severe Malaria (95% CI)	Vaccine Efficacy Against Hospitalization Caused by Malaria (95% CI)
>12 months follow-up from dose 3 (ATP[a] cohort, n = 6003)	33% (26–39)	37% (5–58)	32% (7–50)
>18 months follow-up from dose 3 (ATP[a] cohort, n = 6003)	27% (20–32)	15% (–20 to 39)	17% (–7 to 36)
PRIMARY VACCINATION ONLY (ATP[a] COHORT, n = 5997)			
>30 months follow-up from dose 3	20% (13–27)	11% (–22 to 35)	10% (–15 to 30)
36 months follow-up[b] from dose 3	18% (11–25)	13% (–17 to 35)	13% (–9 to 31)
PRIMARY AND BOOSTER VACCINATION (ATP[a] COHORT, n = 5997)			
>30 months follow-up from dose 3	28% (22–34)	17% (–14 to 40)	25% (3 to 42)
36 months follow-up[b] from dose 3	27% (21–32)	21% (–7 to 42)	27% (7 to 43)

[a]According-to-protocol (ATP) cohort, all children were immunized according to schedule.
[b]Median time to follow-up after dose 3.
Mosquirix 2015 product information available at http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/document_listing/document_listing_000395.jsp&mid=.

TABLE 35.2 Vaccine Efficacy in Children Age 5–17 Months at First Dose

	Vaccine Efficacy Against All Episodes of Clinical Malaria (95% CI)	Vaccine Efficacy Against Severe Malaria (95% CI)	Vaccine Efficacy Against Hospitalization Caused by Malaria (95% CI)
>12 months follow-up from dose 3 (ATP[a] cohort, n = 6880)	51% (47–55)	45% (22–60)	48% (35–59)
>18 months follow-up from dose 3 (ATP[a] cohort, n = 6885)	46% (42–49)	36% (15–51)	42% (29–52)
PRIMARY VACCINATION ONLY (ATP[a] COHORT, n = 6918)			
>30 months follow-up from dose 3	34% (29–39)	2% (–28–25)	18% (1–32)
46 months follow-up[b] from dose 3	26% (21–31)	–6% (–35 to 17)	12% (–5 to 26)
PRIMARY and BOOSTER VACCINATION (ATP[a] COHORT, n = 6918)			
>30 months follow-up from dose 3	46% (42–50)	32% (10–50)	40% (26–52)
46 months follow-up[b] from dose 3	39% (34–43)	29% (6–46)	37% (24–49)

[a]According-to-protocol (ATP) cohort, all children were immunized according to schedule.
[b]Median time to follow-up after dose 3.
Mosquirix 2015 product information available at http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/document_listing/document_listing_000395.jsp&mid=.

Vaccination with RTS,S reduced the number of cases by half in children and by a quarter in infants over the first 18 months.[94] Vaccine efficacy waned over time. Over the 4 years of follow-up, vaccine efficacy against clinical malaria in children was 39% (95% confidence interval [CI], 34–43) for the R3R group and 26% (95% CI, 21–31) for the R3C group, suggesting that the booster dose helped to maintain vaccine efficacy. In children, protection against severe malaria was likewise higher over the 4-year study period in the R3R as compared to the R3C group (29%, 95% CI, 6–46 vs. −6%, 95% CI, −35.0 to 17). Similar patterns of vaccine efficacy were detected against secondary end points such as severe malaria anemia, hospitalization for malaria, and requirement for blood transfusion during the trial, reflecting the overall impact of vaccine efficacy against clinical malaria provided by RTS,S. The number of cases averted varied according to exposure, age category, and whether a booster was administered. Depending on study site, 205 to 6565 cases of clinical malaria were averted per 1000 children (older age category) vaccinated with the R3R vaccine regimen. The R3R booster dose was more reactogenic than R3C and C3C boosters for both age groups, although grade 3 reactions were infrequent. The incidence of serious adverse events was similar in groups, but postvaccination febrile seizures were more frequent in children receiving RTS,S as compared with control vaccines. Compared with the control groups, rates of meningitis reported as an serious adverse event were higher in children, but not in infants, who received RTS,S. This imbalance could be a chance finding as events were caused by various pathogens, were clustered to a few sites, and occurred without temporal association with vaccination. This signal would be followed up during postregistration studies.

VACCINES IN EARLY-STAGE DEVELOPMENT

Candidate vaccines targeting each of the distinct stages of the parasite in humans are currently in early development, and some are progressing to field trials (Fig. 35.2). Asexual-stage candidate vaccines based on adjuvanted recombinant *P. falciparum* MSP-1, AMA-1, and alum-adjuvanted GLURP+MSP-3 (GMZ2) vaccine have failed to demonstrate compelling vaccine efficacy in Phase II field trials.[95–98a] PfRH5 is a reticulocyte binding-like homologue protein for which compelling preclinical data are accumulating, including protection in Aotus monkeys.[99] A thorough discussion of the many candidate malaria vaccines in early development is beyond the scope of this chapter, but the WHO maintains a regularly updated website that follows their progress and is useful for monitoring their progress.[100] The desire to induce strong cell-mediated responses has led investigators to explore the use of live attenuated recombinant bacterial and viral vectors.[62,101] Recently, a number of prime-boost regimens have been explored,[102–105] the most advanced of which uses a recombinant chimpanzee adenovirus 63 vector expressing multiple preerythrocytic-stage antigens (ME-TRAP) followed by boosts with modified vaccinia Ankara (MVA) vector expressing the same antigens.[105] Experimental challenge studies indicate that this vaccine can induce partial protection against infection and a Phase Ib study in Kenyan adults has shown evidence of protection from infection over 8 weeks of follow up.[106] It is currently in exploratory field trials in African children.

Vaccines against *P. vivax* are also entering clinical development.[107,108] These have targeted antigens for which homologous protection has been seen in human or animal models of falciparum malaria, including the vivax forms of MSP-1, CSP, TRAP, AMA-1, and the Pvs 25 and Pvs 28 ookinete antigens. In addition, several unique vivax asexual-stage antigens, including the Duffy binding protein, have received considerable focus.[109] As the *P. vivax* life cycle cannot currently be maintained in the laboratory, developing a vivax sporozoite challenge model for use in vaccine challenge trials is much more complex. Progress is being made, but clinical vivax relapses constitute an unresolved complexity for this model.[110,111]

Another innovative strategy relies on the large-scale manual dissection and purification of radiation-attenuated sporozoites from mosquitoes and delivering them by intramuscular, subcutaneous or intravenous injection.[112] The basis of this vaccine strategy is the high level of protection seen in human volunteers immunized by the bites of large numbers of malaria-infected irradiated mosquitoes, but the translation of this approach to a practical delivery method has been challenging.[113] A Phase IIa study has reported that five intravenous doses of 135,000 sporozoites fully protected six of six volunteers against experimental infection. These six protected volunteers subsequently underwent repeat CHMI 21 weeks after the initial challenge, but vaccine efficacy had declined

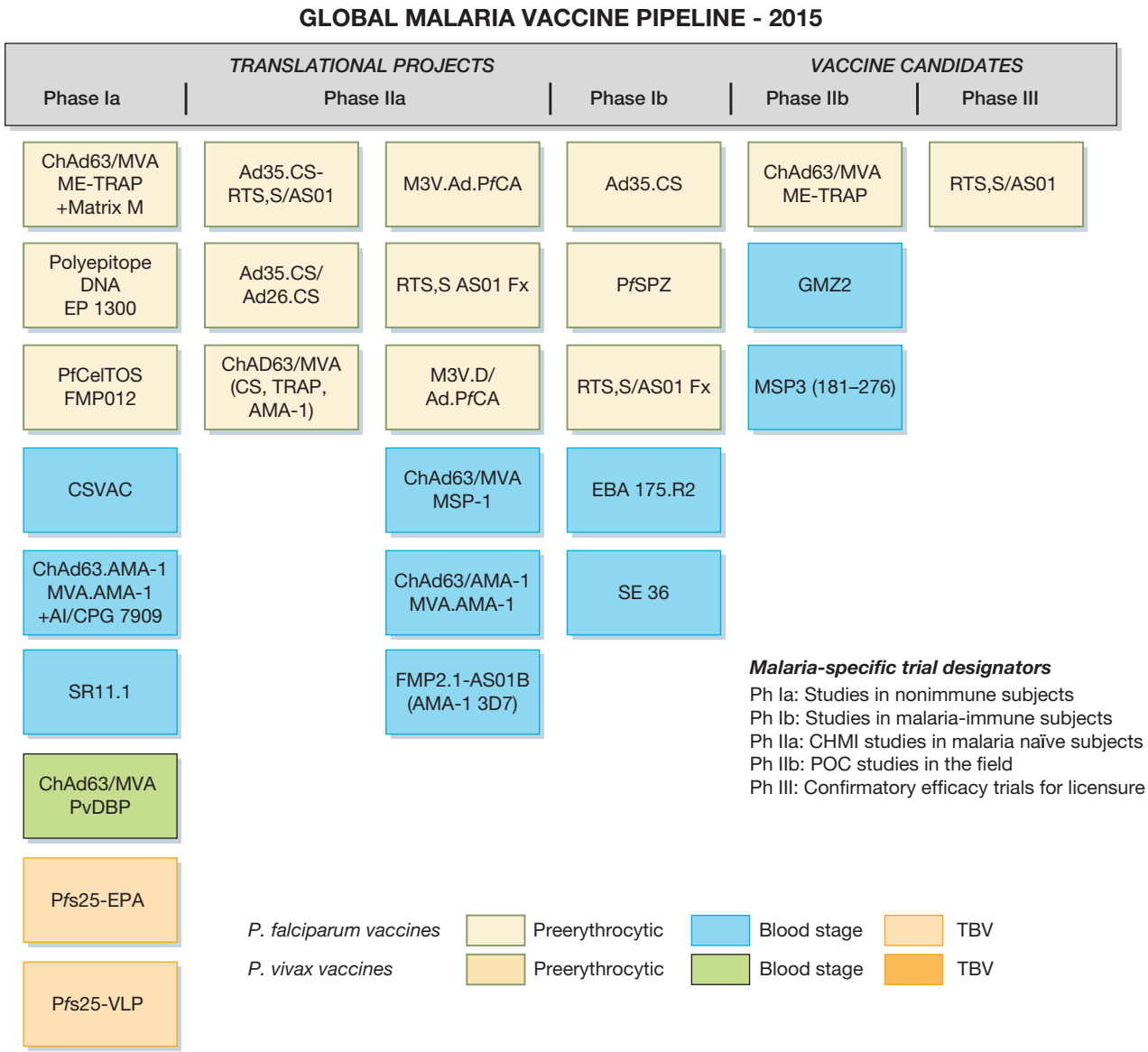


Figure 35.2. Overview of the global malaria vaccine pipeline currently in clinical development. The figure is organized according to stage of clinical development and color coded to identify the target species and life-cycle stage being targeted. Note that trial designations (Phases Ia, Ib, IIa, IIb) in common use within the malaria vaccine community may not be identical to those used for other vaccine development programs. CHMI, controlled human malarial infection; POC, proof of concept; TBV, transmission-blocking vaccine. *(Modified from World Health Organization.* Tables of Malaria Vaccine Projects Globally: Rainbow Table. *Available at: http://www.who.int/vaccine_research/links/Rainbow/en/index.html.)*

to 25%. In addition, 8 of 8 previously immunized subjects who were not protected at the first CHMI were similarly not protected after the second CHMI, indicating that exposure to malaria parasites was insufficient to boost immunity to protecctive levels.[114]

The impact of higher intravenous doses of irradiated sporozoites and the durability of protection was explored in a subsequent trial in which subject were randomized to receive from twofold to 3.3-fold the number of sporozoites administered in the previous trial, and were challenged at 3 weeks; those who remained protected were rechallenged at approximate or 12 months.[114a] Cumulative vaccine efficacy was approximately 55% and protection was correlated with Pf-specific antibodies up to 6 months, but levels waned thereafter. No correlation was seen between protection and numbers of circulating $CD4^+$ or $CD8^+$ T cells. A substudy in nonhuman primates that underwent a similar immunization procedure suggested that the long-term protection seen at 12 months may have been due to liver-resident $CD8^+$ T cells not detectable in the peripheral circulation.[114a]

Protection was associated with the development of both antibodies and cellular responses against sporozoite antigens. To eliminate the requirement for irradiation attenuation, genetically attenuated sporozoites are being developed, and whole-parasite blood-stage vaccines are also being explored,[62,115,116] but these strategies face additional challenges in establishing a balance between safety from malaria breakthroughs and induction of adequate immunogenicity. These strategies remain highly experimental and face enormous hurdles for large-scale manufacturing, formulation, and delivery.[117]

In the midterm (5–10 years), improvements on RTS,S may be feasible through repeated dosing, the use of more immunogenic regimen, or combination with other vaccines, targeting the same or other antigens.[118] Chimpanzee adenovirus type 63

(ChAd63) and Modified Vaccinia Ankara (MVA) expressing the synthetic pre-erythrocytic antigen ME-TRAP have been administered in a prime boost regimen along with RTS,S/AS01B and studied for safety, immunogenicity, and vaccine efficacy following CHMI.[118a] Both regimens were well tolerated. The combination regimen (RTS,S/AS01B ×3 plus Chad63/MVA ME-TRAP) protected 14 of 20 volunteers while the RTS,S/AS01B protected 12 of 16. Rechallenge of protected volunteers at 6 months revealed sustained high levels of protection (83–88%). Protection correlated with antibodies against CS on the day of challenge, but not with T-cell responses against either CS or TRAP or antibodies against TRAP. The recent finding that postpriming avidity increase is associated with a lower risk of clinical malaria suggests that this may be a useful surrogate for schedule optimization.[119] Priming with a single dose of attenuated human adenovirus 35 expressing the CS protein and boosting with two doses of RTS,S/AS01B (ARR) was compared with three doses of RTS,S/AS01B (RRR).[119a] Both regimens were well tolerated and immunogenic, but while vaccine efficacy at CHMI was similar (ARR, 44%; RRR, 52%), protection appeared to be mediated by different mechanisms, with the ARR schedule inducing great polyfunctional $CD4^+$ T-cell responses than did RRR, while the RRR schedule induced higher levels of antibodies to CS.

An alternative dosing regimen of RTS,S in which the third vaccination was delayed and reduced from 0.5 mL to 0.1 mL (delayed fractional third dose schedule [Fx]) was compared to a standard 0, 1, 2 months full dose (STD) schedule for safety, immunogenicity, and efficacy following CHMI.[119b] The Fx schedule recapitulated that used in the landmark RTS,S/AS02A CHMI trial[70] that first demonstrated high level efficacy against sporozoite challenge (7 of 8; 86% of the volunteers were protected). This unusual schedule resulted from a concern about reactogenicity (severe headache and malaise) observed in one volunteer after dose 2 and resulted in a pause in the trial and an 80% reduction in the volume of the third dose. Subsequent studies using the STD schedule were reassuring in their safety profile, and the high level of vaccine efficacy for Fx attributed to small numbers. To confirm that this was not the case, 30 subjects received RTS,S/AS01B on the Fx schedule and underwent CHMI, 26 (87%) were protected compared to 10 of 16 (63%) who received the STD schedule ($P = .04$, log rank). Antibody titers to CS were marginally lower in the Fx group than in the STD group, but avidity against CS on the day of challenge was higher in protected than in nonprotected individuals. The Fx schedule also significantly increased antibody somatic hypermutation. Fx dose boosting at 6 months was highly effective at maintaining or restoring protection upon rechallenge.

Finally, a global scientific effort is underway to understand what will be necessary to eliminate and eventually eradicate malaria. This has led to the concept of "vaccines that interrupt malaria transmission" (VIMT), which may include components from one or more of the parasite stages described in this chapter.[120–122] It is likely that a significant proportion of future funding for malaria vaccine development will focus on the long-term elimination/eradication goal and that such vaccines are developed within a framework that will position their use in context with other new tools being developed to support the malaria eradication agenda.

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36 Maternal Immunization

Saad B. Omer and Denise J. Jamieson

WHY MATERNAL VACCINATION?

The term *maternal immunization* has been used to describe vaccination of women prior to, during, and after pregnancy. We use *maternal immunization* to refer to vaccination of women during pregnancy and/or in the immediate postpartum period. Maternal vaccination can be used for the benefit of the mother, for the benefit of the infant, or for the benefit of both.

Several infections disproportionately impact pregnant women. Although infections such as influenza, herpes simplex virus, and hepatitis E virus are not associated with increased susceptibility during pregnancy, they result in more adverse outcomes among pregnant women compared to nonpregnant women. On the other hand pregnancy makes women more susceptible to infections such as malaria, listeriosis, and (possibly) HIV. In the case of malaria, but not HIV, pregnancy is associated with more-severe outcomes after infection.

In addition to maternal benefits, maternal immunization has the potential to impact early childhood morbidity, and in some cases, mortality. Infections such as respiratory syncytial virus (RSV), influenza, and pertussis are associated with adverse outcomes in young infants. Influenza vaccines are generally not recommended for children younger than 6 months of age and pertussis vaccines are not started during an infant's most vulnerable period, the first month of life. Similarly, vaccines against Group B streptococcus and influenza could have a protective effect on birth outcomes such as low-birth-weight, preterm, and small-for-gestational-age births.

Gains in reducing global childhood mortality mostly have been outside the neonatal period. Approximately 40% of global childhood deaths occur in the neonatal period. Many of these deaths are caused by infections that can be prevented through existing maternal vaccines and those vaccines in development.

IMMUNOLOGY OF PREGNANCY

The increased susceptibility to and/or severity of several infections in pregnancy may be explained by immunological changes in pregnancy. Moreover, understanding these immunological changes might provide insights into vaccine responses in pregnancy. While there are several human studies on the immunological changes in pregnancy, a substantial portion of the evidence base comes from animal models.

Conventionally, pregnancy has been perceived as a generalized state of immune suppression with the biological goal of preserving the embryo/fetus by avoiding its potential allogeneic effects.[1] However, emerging evidence suggests that this conventional model may be too simplistic. For example, fetus-specific cytotoxic T cells with broad functional capacity can be detected in approximately half of all normal pregnancies—often as early as the first trimester. These cells are not deleted through the course of pregnancy. These data demonstrate that a fetus-specific adaptive cellular immune response is a part of normal human pregnancy.[2] In fact, a lower number of decidual cytotoxic $CD8^+$ lymphocytes is associated with development of preeclampsia.[3]

It is more appropriate to consider pregnancy as a physiologically dynamic state in which the immune response is progressively altered and not across-the-board suppressed.[4] There is an overall increase in the levels of sex hormones during the course of pregnancy (Fig. 36.1). As most of the changes in the immune responses during pregnancy are mediated by sex hormones, the responses during pregnancy vary by trimester. Low estradiol levels are associated with T-helper (Th)-1 type responses and higher cell-mediated immunity.[4,5] Conversely, high estradiol levels generally result in enhanced Th2 type responses. Increased progesterone levels are associated with overall suppression of maternal immune responses. During pregnancy, the number of T lymphocytes as well as B lymphocytes decline, with a resulting decline in Th1 and Th2 responses. By contrast, phagocytic activity, α-defensin expression, and the numbers of neutrophils, monocytes, and dendritic cells are maintained and may even increase during the second and third trimesters, representing some enhancement of innate immunity. The altered cell-mediated immunity may be the reason for severe disease in pregnancy after infection with organisms such as influenza virus in which cell-mediated immune responses are important to suppression of pathogen replication.[6–8] Enhancement of other aspects of the immune system may help explain why pregnancy does not result in overall immune suppression and how pregnant women are able to mount adequate immunologic responses when vaccinated.

Infections that result in more severe clinical outcomes among pregnant women compared to nonpregnant women often vary in severity by stage of pregnancy. For example, compared to early pregnancy, influenza infection results in more severe outcomes in later stages of pregnancy.[9–12] In contrast, *Plasmodium falciparum*–associated malaria is more severe in the first half of pregnancy compared to the second half.[13,14] These pregnancy-stage–dependent differences in postinfection outcomes may be, in part, a result of the types of immune responses involved in the control of various pathogens and the progressive alteration of the immune system during the course of the pregnancy.[15] For example, during implantation and placentation in the first trimester, extensive tissue remodeling is associated with a local inflammatory reaction. Later in pregnancy when the tissue remodeling substantially diminishes and when there is rapid fetal growth, there is a switch to an antiinflammatory *milieu*—ostensibly to ensure fetal survival.[15] As the pregnancy approaches the final phase, fetal development is complete and there is a resurgence of inflammatory processes in the uterus to initiate parturition-related events such as smooth muscle contraction. It has been hypothesized that the increased severe outcomes after parasitic infections (e.g., *P. falciparum* malaria) may be a result of predominantly local inflammatory Th1 and Th17 responses associated with enhancement of local tissue damage.[15–19] In contrast, the higher severity of influenza virus infections during late pregnancy may reflect the reduced Th1 type response.[19]

While there is an overall decrease in maternal $CD4^+$ and $CD8^+$ T cells and in natural killer cells with increasing gestational age, components of the innate immune system are either maintained or enhanced with the progression of pregnancy. It has been suggested that the reduced clearance of pathogens resulting from the decrease in $CD4^+$ and $CD8^+$ T cells is balanced by increases in the cells of the innate immune

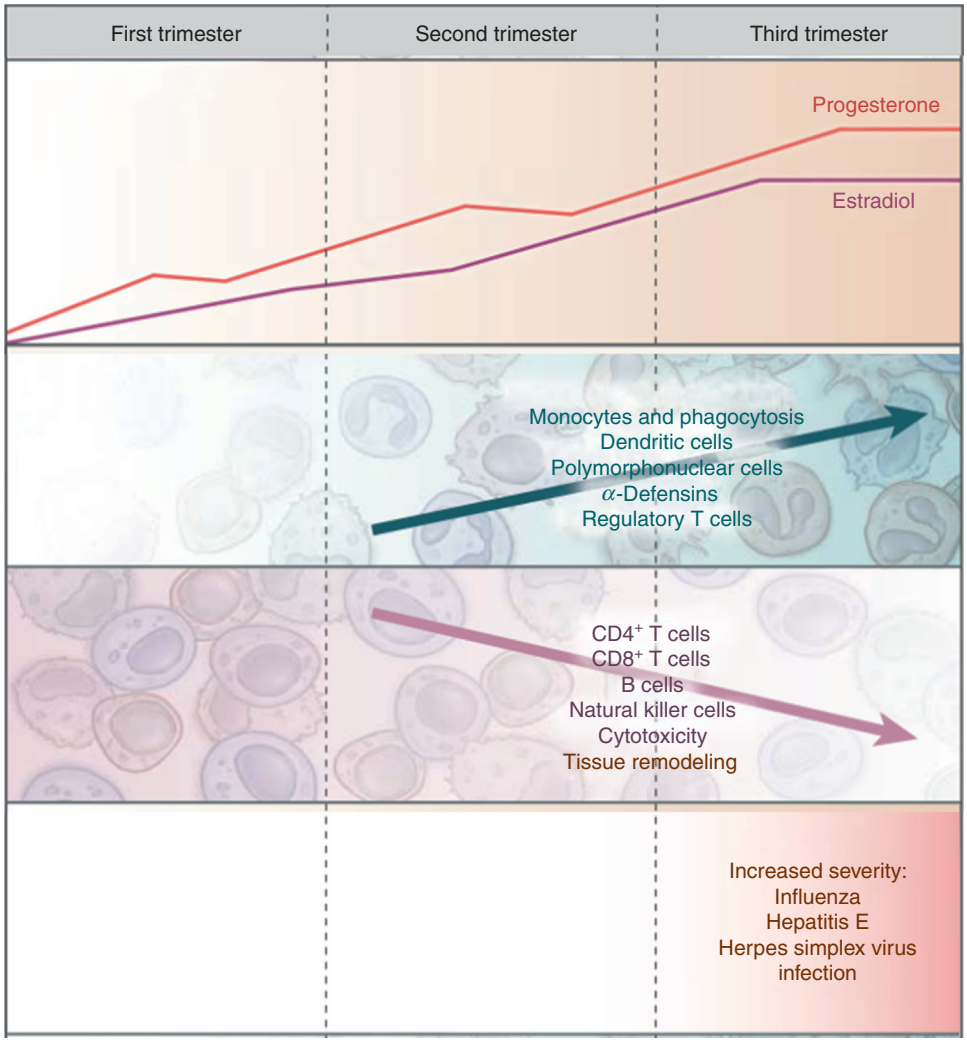


Figure 36.1. Physiologic and immunological changes in pregnancy and their association with infection severity. *(Modified from Kourtis AP, Read JS, Jamieson DJ. Pregnancy and infection.* N Engl J Med. *2014;371[11]:1077.)*

system, resulting in no change in susceptibility to many infections.[4]

RESPONSE TO VACCINES IN PREGNANCY

The evidence regarding the quality and magnitude of response to vaccines in pregnancy, most frequently measured as immunogenicity, is scant but developing. Most of the evidence base is composed of studies of inactivated influenza vaccine. These studies paint a mixed picture: studies involving 1962–63 seasonal vaccine,[20] the monovalent A/New Jersey/8/76 influenza vaccine,[21] and the 2010–12 seasonal vaccines documented equivalent responses among pregnant versus nonpregnant women.[22] However, other studies reported lower immunogenicity of influenza vaccine among pregnant women compared to nonpregnant women.[23–25] Similarly with yellow fever, some evidence suggests that the vaccine is potentially less effective because of the decreased immunogenicity in pregnancy[26]; however, other studies report no difference in response between pregnant and nonpregnant women.[27] Recently, Munoz and colleagues reported lower titers of anti–pertussis toxin, anti–filamentous hemagglutinin, antipertactin, and anti–fimbriae-2/3 antibodies in pregnant women who received tetanus, diphtheria, and pertussis (Tdap) vaccine compared to nonpregnant women.[28]

Timing of vaccination during pregnancy may have implications for quality of vaccine responses. In a study evaluating quality of cord blood immunoglobulin (Ig) G after Tdap vaccination in pregnancy, there was higher avidity of cord blood anti–pertussis toxin antibodies with pertussis immunization at 27 to 30 weeks of gestation compared to the group that received Tdap after 31 weeks of gestation.[29] These findings, may reflect the effect of additional time available for affinity maturation in mothers vaccinated relatively early in pregnancy, rather than differences in immune response later in pregnancy.[29] On the other hand, two studies conducted in Belgium and Vietnam, respectively, did not find an association between gestational age at pertussis vaccination and avidity of antipertussis antibody in cord blood samples.[30] The variable findings in studies evaluating the association between gestational age at maternal vaccination and avidity may be a result of their small sample size, prior disease and/or vaccine exposure, composition of any prior pertussis vaccine received (acellular vs whole cell), and underlying differences in study populations and study designs.[30] Moreover, in another relatively small study, women immunized with Tdap during pregnancy had a substantial decline in antipertussis IgG levels 9 to 15 months postpartum.[31]

Coinfections such as HIV and malaria could also potentially impact responses to vaccines in pregnancy. Lower

antibody responses to seasonal inactivated influenza vaccine have been observed among HIV-positive pregnant women compared to HIV-infected nonpregnant women in the United States[32] and South Africa.[33] Interestingly in the South African study, compared with HIV-unexposed infants, HIV-exposed infants had lower antibody levels at birth but similar antibody levels after 8 weeks of life.[33] Moreover, HIV-infected South African pregnant women had similar influenza vaccine efficacy as nonpregnant women,[34] which highlights the limitations of using antibody levels alone as predictors of influenza vaccine efficacy, particularly, in HIV-infected pregnant women.

PLACENTAL TRANSFER

The immune system in newborns and young infants is functionally immature at birth. Notably, this functional immaturity is not universal, as some functions are mature even in preterm infants.[35] Antibody responses in neonates have an overall delayed onset, reach lower peak levels, and have a shorter duration.[35] Moreover, neonates have low levels of several components of the complement system.[36] Neonatal $CD4^+$ T cells have diminished Th1 effector capacity and a limited ability to generate memory cells.[37]

Placental transfer of antibodies provides a mechanism to compensate for functional deficiencies in some aspects of the immune responses among newborns and young infants. Maternal IgG is efficiently transferred across the placenta using active transport through syncytiotrophoblasts. This transfer is mediated by neonatal Fc receptors (FcRns).[35] Two cell layers are encountered by substances that are transferred from maternal to fetal blood. The first layer consists of syncytiotrophoblasts, the multinucleated cells of placental villi. The second layer consists of endothelial cells of fetal capillaries. Moreover, the villous stroma is inhabited by placental macrophages (Hofbauer cells) and fibroblasts that may play a role in binding and trapping immune complexes.[38]

There are multiple mechanisms for transfer of substances from maternal to fetal blood. Substances smaller than approximately 500 Da are transferred through diffusion across the placental tissue. Very large molecules such as IgG (which is approximately 160 kDa) are transferred through active transport.[35] IgG from maternal blood is taken up by syncytiotrophoblasts through fluid-phase endocytosis followed by binding with FcRns in the acidic endosomal environment.[39] The endosomes with bound IgG may move across the syncytiotrophoblast and release the bound IgG when it reaches the basolateral side of the cell upon exposure to neutral pH (i.e., pH 7.4).[40–42] This pH-dependent IgG transport enables movement of antibodies against the concentration gradient and often results in a higher concentration of maternal antibodies in the fetus compared to maternal circulation. After being transcytosed through syncytiotrophoblasts, the IgG passes through the fetal capillary epithelium, either directly or after passing through the villous stroma, and enters fetal circulation.

The mother-to-fetus transfer of IgG may begin as early as 13 weeks of gestation; however, the largest amount of antibodies is transferred in the third trimester.[43] The IgG_1 subclass is more effectively transferred from the mother to the fetus compared to other IgG subclasses. The quantity of IgG transferred is dependent on maternal IgG levels, gestational age, coinfection, and (possibly) nutritional factors. IgG titers are generally lower in preterm infants compared to term infants.[44] Similarly, low birth weight is associated with reduced transfer of maternal IgG to the infant.[45] However, this transfer is not influenced by parity, maternal age, weight, height, and type of delivery.[46,47]

There may be differences in transfer for antibodies against polysaccharide versus protein antigens. In a study of paired maternal delivery and cord blood samples, antibodies specific for *Neisseria meningitidis* serogroup C polysaccharide, *Haemophilus influenzae* type B (Hib) polysaccharide, diphtheria toxin, tetanus toxin, and multiple antigens from *Bordetella pertussis* were examined.[48] In this study, antibody concentrations directed toward polysaccharides were equal in maternal and cord blood, whereas antibody concentrations to proteins were 1.6 times higher in cord blood than in maternal blood.[48]

Infection with "unrelated" pathogens (i.e., pathogens not specifically targeted by a given vaccine) can potentially impact mother-to-fetus antibody transfer. Studies of the effect of malaria infection during pregnancy on placental antibody transfer, mainly focusing on antimeasles and anti–tetanus toxin antibodies, have produced contrasting results.[49–59] In mothers immunized against tetanus, malaria infection during pregnancy is associated with reduced infant antibodies against tetanus antigens.[60,61] Among women with documented placental malaria, there was a 48% reduction in neonatal tetanus antibody titers when compared with neonates born without evidence of placenta malaria infection.[60] However, malaria during pregnancy was not associated with reduced measles antibodies in neonates using the same set of specimens.[57] A study in Papua New Guinea clearly showed interference with placental antibody transfer of tetanus antibodies associated with severe malaria in the mother.[52] Other studies in Malawi and the Gambia found no association between placental malaria and tetanus antibody levels in the newborn.[56] It is possible that the differing results relate to variations in prevalence of severe malaria. Nonetheless, these considerations need to be assessed with regard to maternal immunization.

HIV infection may also reduce transplacental antibody transfer of measles and tetanus antibodies.[60,63] However, there is a need for further evaluation of the impact of infection on maternal antibody transfer, particularly in the context of nonparasitic infection.

Kinetics and decay of transferred maternal antibodies in infants may vary by source of antibody. For example, after the mother's vaccination, RSV antibodies have a relatively shorter half-life in infants compared to maternally derived RSV antibodies after natural exposure.[64–69] Moreover, at least for influenza, the half-life of maternal antibody depends on the specific viral antigen (e.g., H3N2 vs H1N1 vs influenza B) included in an (inactivated) vaccine.[70] However, limited evidence suggests that there may be no difference in antibody decay by study population/location, but this evidence primarily comes from evaluation of measles antibody transfer and may not be generalizable to other antigens.[71]

Maternal antibodies, particularly after vaccination in pregnancy, can potentially interfere with vaccine responses in infants—particularly humoral responses. The impact of maternal antibodies on infant vaccine responses is not only variable by vaccine but could also vary by population specific factors.[72] Most of the evidence for mechanisms of inhibition have been generated in contexts other than vaccination (e.g., passive intravenous antigens).[15] The mechanisms for inhibition of infant vaccine responses by maternal antibodies that have supporting evidence include F(ab')2-dependent mechanisms such as inhibition of B-cell recognition of vaccine antigens through masking; neutralization of (live viral) epitopes; and opsonization, phagocytosis, and clearance of vaccine antigens mediated by maternal IgG.[73–76] Moreover, hypotheses focusing on Fc-dependent inhibition have found supporting evidence. These potential Fc-dependent mechanisms include inhibition of infant B-cell activation and antibody production, FcγR-mediated postopsonization phagocytosis resulting in vaccine antigen clearance, maternal IgG saturation of infant

endothelial or myeloid FcRn and acceleration of catabolism of vaccine-induced infant IgG, inhibition of infant dendritic cells by ingested and absorbed maternal IgA via FcαRI and FcαμR.[15,76–84]

CLINICAL ISSUES REGARDING VACCINATIONS DURING PREGNANCY

Whereas inactivated vaccines are fairly safe in pregnancy, live vaccines are generally avoided during pregnancy and during the 4 weeks preceding a pregnancy. For many live vaccines, immunization poses a theoretical rather than a documented risk. For example, measles, mumps, and rubella vaccine (MMR) has not been linked to fetal infection, congenital anomalies, or other adverse pregnancy outcomes.[85–88] By contrast, vaccination with live smallpox vaccine has resulted in fetal infection with vaccinia, the virus contained in the vaccine. However, this risk is very low; a recent systematic review of the worldwide literature identified only 21 presumed cases from 1809 to 2014.[89]

Some vaccines contain adjuvants which are added to boost the immune response and potentially provide better protection against antigenically drifted viruses. For example, aluminum salts have used in a variety of vaccines such as hepatitis A, hepatitis B, and tetanus vaccines. Data from aluminum-based adjuvanted vaccines in pregnancy are reassuring with no increased risk of adverse pregnancy outcomes. Newer adjuvants such as oil-in-water emulsions (e.g., AS03, MF59) were used in some H1N1 influenza vaccine preparations. These adjuvanted vaccines were administered to pregnant women in several European countries and no increased risk of adverse outcomes was found.[90] Thimerosal is a mercury preservative used in some vaccines (thimerosal has been removed from most vaccines in the United States). Despite speculation about a link between childhood vaccine exposure and autism, extensive epidemiologic investigation does not support such an association.[91] Furthermore, there is no evidence that thimerosal-containing vaccines administered in pregnancy are harmful to offspring.[92] Consequently, the Advisory Committee on Immunization Practices (ACIP) does not recommend that pregnant women avoid thimerosal-containing vaccines. However, thimerosal-free formulations of most vaccines, such as seasonal influenza, are available.

Women are vaccinated during pregnancy for variety of reasons. Table 36.1 summarizes the recommendations and rationale for use of vaccinations in pregnancy.

Currently, there are only two vaccines routinely recommended in pregnancy in the United States and many other high-income and middle-income countries: the seasonal influenza vaccine and Tdap (several low-income and middle-income countries include maternal tetanus toxin or diphtheria and tetanus toxoids in their immunization programs). For these recommended vaccines, rates of vaccination use in pregnancy remain disappointingly low in the United States. Although there has been a sustained increase in vaccination coverage rates for seasonal influenza among pregnant women since the 2009 H1N1 influenza pandemic, coverage rates in the United States remain at approximately 50% among pregnant women.[104] When healthcare providers specifically recommend to their pregnant patients that they receive the influenza vaccine, vaccination rates are higher. Rates of Tdap vaccination during pregnancy are even lower.[119]

Maternal Immunizations for Maternal Benefit

Vaccination during pregnancy may be particularly important for infectious diseases to which pregnant women are more susceptible or more likely to develop severe disease. For influenza, there is evidence that infection is more severe in pregnancy, that vaccination reduces the risk of maternal influenza illness, and that the vaccine is safe in pregnancy.[120–128] Because of the maternal as well as fetal and infant benefits, seasonal influenza vaccine is routinely recommended in pregnancy. The ACIP recommends that women who are or will be pregnant during influenza season receive the vaccine as early as possible in the season, regardless of trimester.[129]

For other vaccine-preventable illnesses, the calculation of risks and benefits may be less straightforward, particularly with live vaccines. For example, yellow fever is a mosquito-borne illness that can cause severe disease and death. Therefore, the ACIP recommends that people be vaccinated with the live vaccine before traveling to endemic areas. Because live vaccines are generally not recommended in pregnancy, pregnant women should be advised to avoid travel to areas with endemic yellow fever. However, if travel is unavoidable, then vaccination is not contraindicated and should be considered.[118]

Both smallpox and anthrax are potential bioterrorism agents that could be intentionally introduced. With both pathogens, pregnant women are at risk for severe disease. However, due to benefit versus risk considerations, vaccination is not recommended in a preevent setting. In postevent settings, the recommendations for vaccine are the same for pregnant women as they are for nonpregnant adults.[89,93]

Maternal Immunizations for Neonatal Benefit

Maternal antibodies that are transferred across the placenta may provide passive immunization of the fetus and infant. These antibodies may protect the fetus from maternal in utero or intrapartum transmission. In addition, because young infants fail to mount an adequate immune response to vaccination, persistent maternal antibodies may provide immunologic protection until the child is old enough to be vaccinated.

Worldwide, neonatal tetanus has been a leading cause of neonatal death in resource-limited countries where aseptic obstetric and safe umbilical cord practices are not commonly practiced. Immunization of pregnant women with tetanus toxoid was first recommended by the World Health Organization (WHO) in 1974 to prevent neonatal tetanus. Despite this recommendation, only about one-quarter of eligible pregnant women were receiving the recommended two doses of tetanus toxoid in the 1980s and the burden of neonatal tetanus remained high. In 1990, WHO launched its neonatal tetanus elimination program which emphasized the importance of maternal tetanus immunization. In 1999, WHO renamed this initiative the Maternal and Neonatal Tetanus Elimination Program to emphasize the role of maternal tetanus and its contribution to maternal mortality. Although great strides have been made, neonatal tetanus continues to cause a substantial number of neonatal deaths worldwide. Continued efforts to promote maternal immunization, in addition to clean delivery practices, are critical.[113,130]

In the United States, Tdap is recommended for pregnant women close to delivery, ideally at 27 to 36 weeks of gestation. Tdap was recommended as a strategy to protect infants from pertussis as young infants are at the highest risk for morbidity and mortality from pertussis. This ideal gestational age was chosen to maximize passive immunity as antibody levels wane fairly rapidly. The ACIP has recommended Tdap in pregnancy since 2011 and in 2012 the ACIP updated the guidance to include reimmunization with each subsequent pregnancy.[112] Although this is one of the two vaccines specifically recommended in pregnancy, coverage rates for Tdap during pregnancy remain low in the United States.[119,131]

TABLE 36.1 Recommendations and Rationale for Use of Vaccinations in Pregnancy

Infection/Vaccine	Recommendations	Type of Vaccine	Rationale and Clarification
ANTHRAX			
Anthrax Vaccine Adsorbed (AVA)[93]	Per the CDC, AVA is not recommended for pregnant women in a preevent setting. Postexposure vaccination is recommended for pregnant women at high risk for inhalational anthrax, regardless of pregnancy trimester.	Inactivated	Although it is not clear whether pregnant women are more susceptible to anthrax or more likely to develop severe disease, anthrax in pregnancy has resulted in maternal and neonatal deaths. Given the severity of anthrax, pregnant women should receive the same postexposure prophylaxis as the general population, which includes AVA vaccination. From reports of vaccination during pregnancy, largely from inadvertent predeployment vaccination of women in the military, no concerning maternal or infant safety patterns were reported.
GROUP B STREPTOCOCCUS DISEASE			
Group B Streptococcus (GBS) vaccine[94–96]	Not available.	Inactivated	In development. GBS infection remains a source of infant morbidity and mortality. Although intrapartum antimicrobial prophylaxis reduces early onset disease, it does not prevent later onset disease (beyond 7 days of life). Maternal vaccination with a GBS vaccine could potentially reduce the burden of GBS disease in both infants as well as women (e.g., postpartum endometritis).
HAEMOPHILUS INFLUENZAE			
Haemophilis influenzae type b (Hib) vaccine[97]	In developing country settings where Hib remains an important cause of meningitis in children, maternal vaccination may provide early protection.	Inactivated	The ACIP does not have recommendations for Hib as childhood vaccination programs in the U.S. have dramatically reduced morbidity and mortality from Hib. However, in settings where Hib is still a common cause of bacterial meningitis in children, maternal Hib vaccination may provide some protection for children <18 months of age who have not yet completed their vaccination series. There are reassuring safety and immunogenicity data from vaccination in the third trimester of pregnancy.
HEPATITIS			
Hepatitis A Vaccine (HAV)[98,99]	Per the ACIP, high-risk susceptible pregnant women may be vaccinated after weighing the potential risks and benefits.	Inactivated	Risk factors for hepatitis A include, but are not limited to, chronic liver disease, HIV infection, and travel to countries with high prevalence of disease. If a pregnant woman is exposed to hepatitis A, she should receive HAV and immunoglobulin. In the VAERS, no concerning patterns of adverse events in pregnant women vaccinated with HAV or their infants have been observed.
Hepatitis B Vaccine (HBV)[100]	High-risk susceptible pregnant women may be vaccinated.	Recombinant	Risk factors for hepatitis B include, but are not limited to, chronic liver disease, HIV infection, multiple sex partners, injection drug use, working in healthcare, chronic hemodialysis, and travel to countries with high prevalence of disease. The recommended schedule is 0, 1 month, and 6 months but an accelerated schedule is possible in pregnancy. Safety data indicate no concerning patterns of adverse events in vaccinated pregnant women or their infants.
Combined HAV and HBV[98–100]	High-risk susceptible women may be vaccinated.	Inactivated, recombinant	In VAERS, no concerning patterns of adverse events in vaccinated pregnant women or their infants.
HUMAN PAPILLOMAVIRUS			
Human Papillomavirus (HPV) vaccine[101,102]	Not recommended for use in pregnancy.	Inactivated	Although harmful effects from vaccination in pregnancy have not been found, HPV vaccine is not recommended in pregnancy. If a woman who started the vaccine series is found to be pregnant, she should delay completion of the vaccine series until completion of the pregnancy. Trial data, registry data, and VAERS found no unexpected patterns in maternal or fetal outcomes among pregnant women inadvertently vaccinated with the bivalent or quadrivalent vaccine.
INFLUENZA			
Inactivated influenza vaccine[103]	Per the ACIP, women who are or will be pregnant during influenza season should receive as early as possible in pregnancy regardless of trimester. In some non–U.S. countries, the vaccination is recommended after the first trimester.	Inactivated	Pregnant women with influenza are more likely to develop severe disease and die compared with nonpregnant women. Multiple studies including randomized controlled trials in Bangladesh and South Africa have demonstrated the benefits of influenza vaccine for pregnant women and their infants. In the U.S., influenza vaccination was first recommended in pregnancy in 1960 and has a long record of safety and effectiveness data.

Continued on following page

TABLE 36.1 Recommendations and Rationale for Use of Vaccinations in Pregnancy *(Continued)*

Infection/Vaccine	Recommendations	Type of Vaccine	Rationale and Clarification
Live-attenuated influenza vaccine (LAIV)[104]	Per the ACIP, LAIV is not recommended for use in pregnancy.	Live	Live vaccines have the potential for fetal infection and in general should be avoided in pregnancy. From VAERS, there are no concerning patterns of birth outcomes following inadvertent vaccination with LAIV.
JAPANESE ENCEPHALITIS			
Japanese encephalitis (JE) vaccine[105]	Per the ACIP, JE vaccination should generally be deferred. However, pregnant women who must travel to an area where JE risk is high should be vaccinated if the benefits outweigh the risks.	Inactivated	Although little is known about Japanese encephalitis virus infection in pregnancy, intrauterine transmission likely occurs. However, even in endemic areas, Japanese encephalitis virus infection is rare.
MEASLES, MUMPS, AND RUBELLA			
Measles-mumps-rubella (MMR) vaccine[85,106]	Per the ACIP, should not be used in pregnancy.	Live	Because of theoretical risks of this live vaccine, pregnant women or those planning to become pregnant should not receive this vaccine. Women should be counseled to avoid pregnancy for 28 days following receipt of the vaccine.
MENINGOCOCCAL DISEASE			
Meningococcal polysaccharide vaccine (MPSV4)[107]	Per the ACIP, pregnancy should not preclude vaccination if indicated.	Inactivated	Quadrivalent (serogroups A, C, W, Y) meningococcal polysaccharide vaccine was licensed in the United States in 1981. Generally used when person has a contraindication to the quadrivalent vaccine *and in adults aged ≥55 years*. No reports of concerning patterns of adverse effects in vaccinated pregnant women or their infants.
Meningococcal (MenACWY)[107]	Per the ACIP, pregnancy should not preclude vaccination if indicated.	Inactivated	Conjugated quadrivalent (serogroups A, C, W, Y) vaccine. In VAERS, no concerning patterns of adverse effects in vaccinated pregnant women or their infants.
Meningococcal B (Men B)	Per the ACIP, no recommendation for pregnant women.	Inactivated	Serogroup B vaccine. No randomized controlled clinical trials have been conducted to evaluate use of MenB vaccines in pregnant or lactating women. Vaccination should be deferred in pregnant and lactating women unless the woman is at increased risk, and, after consultation with her healthcare provider, the benefits of vaccination are considered.
PNEUMOCOCCAL DISEASE			
Pneumococcal polysaccharide vaccine (PPSV23)[108]	Vaccination is not routinely recommended in pregnancy but can be given to those at increased risk of disease.	Inactivated	Although the ACIP states that there is inadequate information to make a recommendation, it has been used in the second and third trimester without an apparent increased risk of adverse outcome. There is less information about use in the first trimester. A Cochrane review summarizing 6 randomized trials concluded that there was insufficient evidence to assess whether vaccination during pregnancy reduces infant infections. Women with indications for vaccination (e.g., those with asplenia, cochlear implants, emphysema, chronic heart disease, chronic liver disease) should be vaccinated prior to pregnancy.
Pneumococcal conjugate vaccine (PCV13)[108]	If vaccination against pneumococcal disease is indicated in pregnancy, it may be reasonable to preferentially vaccinate with PPSV23.	Inactivated	Conjugated vaccine. The ACIP has not published pregnancy recommendations owing to lack of information about use in pregnancy. Although there are no data from pregnant humans, animal studies have not shown adverse effects of the vaccine in pregnancy. Use of PCV13 is limited among women of childbearing age.
POLIOVIRUS			
Inactivated polio vaccine (IPV)[109]	Vaccination should be avoided in pregnancy unless the pregnant woman is at increased risk for polio infection and requires immediate protection.	Inactivated	Polio has been eliminated from many countries including the United States, where routine vaccination is no longer recommended. Pregnant women should avoid travel to polio endemic countries if possible. If they do travel to these areas, they should be vaccinated. IPV is the only vaccine available in the United States; live-attenuated oral polio vaccine is available outside of the United States. No reports of concerning patterns of adverse effects in vaccinated pregnant women or their infants.

TABLE 36.1 Recommendations and Rationale for Use of Vaccinations in Pregnancy *(Continued)*

Infection/Vaccine	Recommendations	Type of Vaccine	Rationale and Clarification
RABIES			
Rabies vaccine[110]	Pregnancy is not a contraindication to postexposure vaccination. If a woman is at high risk for exposure, preexposure vaccination may also be considered.	Inactivated	No increased risk of adverse events among women vaccinated in pregnancy or their infants. For postexposure prophylaxis, immunoglobulin may also be given.
RESPIRATORY SYNCYTIAL VIRUS			
Respiratory syncytial virus (RSV) vaccine[97]	Not available	Inactivated (products being considered for maternal use)	In development. RSV is an important cause of morbidity and mortality among young infants. Because of poor immunogenicity of vaccines given to young infants, maternal vaccination might be an effective strategy for prevention of RSV in infants. A recombinant RSV fusion protein nanoparticle vaccine (RSV F vaccine) candidate was immunogenic and safe in a Phase II trial in women of childbearing age.[111]
SMALLPOX			
Smallpox vaccine[89]	Per the ACIP, pregnant women should not be vaccinated in a preevent setting. Per the CDC, pregnant women should be vaccinated in a postevent setting if they are exposed to or at high risk for developing disease.	Live	Although smallpox has been eradicated, there is concern that it could be intentionally reintroduced. Pregnant women are at increased risk for severe disease and death from smallpox. Fetal infection with vaccinia (live virus in the vaccine) is a possible but rare outcome from vaccination in pregnancy. The overall risk of adverse outcomes from vaccination in pregnancy is low, but there may be a small increased risk of congenital defects from vaccination during the first trimester of pregnancy.[110–112]
TETANUS, DIPHTHERIA, AND PERTUSSIS			
Tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccine[112]	Per the ACIP, pregnant women should receive a dose of Tdap during each pregnancy, ideally between 27 and 36 weeks of gestation.	Toxoid, inactivated	As a consequence of recent increases in the number of cases of neonatal pertussis in the United States, the ACIP recommends Tdap vaccination during pregnancy to protect young infants who are most susceptible to severe illness and are unable to be vaccinated. This vaccine is recommended in each pregnancy close to the time of delivery because of waning antibody levels. Maternal vaccination close to delivery provides passive immunity to infants.
Tetanus and diphtheria (Td) vaccine[112]	Per the ACIP, pregnant women without 3 prior doses of tetanus and diphtheria vaccination should receive 3 vaccinations in pregnancy including 1 dose of Tdap, ideally at 27–36 weeks of gestation.	Toxoid	The recommended schedule is 0 and 4 weeks, and 6–12 months. Tdap should replace 1 dose of Td.
Tetanus toxoid[113]	Per the WHO, in areas with maternal and neonatal tetanus cases, pregnant women without adequate tetanus vaccination should receive 2 doses of tetanus toxoid during pregnancy and 1 dose in each subsequent pregnancy up to a total of 5 doses.	Toxoid	In some developing countries, mainly in Asia and Africa, tetanus contributes substantially to maternal and neonatal mortality, largely because of unclean delivery practices such as the care of the umbilical cord. To reduce mortality, the WHO implemented the Maternal and Neonatal Tetanus Elimination initiative to promote vaccination of women of childbearing age, including those who are pregnant.
TUBERCULOSIS			
Bacille Calmette-Guérin (BCG) vaccine[114]	Per the ACIP, not recommended in pregnancy.	Live	Although harmful effects have not been shown, BCG is not recommended for use in pregnancy.
TYPHOID			
Typhoid vaccine[115]	If indicated, the inactivated vaccine should be used rather than the live vaccine in pregnancy.	Inactivated	Although the ACIP has not published guidance, the inactivated vaccine should be used rather than the live vaccine in pregnancy. If possible, pregnant women should avoid travel to typhoid affected areas.
Typhoid oral vaccine[115]	Avoid the live vaccine in pregnancy.	Live	Although the ACIP has not published guidance, the inactivated vaccine should be used rather than the live vaccine in pregnancy.

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TABLE 36.1 Recommendations and Rationale for Use of Vaccinations in Pregnancy *(Continued)*

Infection/Vaccine	Recommendations	Type of Vaccine	Rationale and Clarification
VARICELLA			
Varicella vaccine[116]	Per the ACIP, pregnant women should not be vaccinated.	Live	Varicella vaccine should not be given during pregnancy because it is a live vaccine. If a nonimmune pregnant woman is exposed to varicella, immunoglobulin should be given.
Zoster vaccine[117]	Per the ACIP, zoster vaccine is not recommended for use in pregnancy.	Live	Because this vaccine is generally used in older adults (>60 years), pregnant women are unlikely to require vaccination. Also, because it is a live vaccine, herpes zoster vaccine should not be used in pregnancy. There is no safety information available in pregnancy.
YELLOW FEVER			
Yellow fever (YF) vaccine[118]	Per the ACIP, pregnancy is a precaution but not a contraindication for vaccination; in cases where the risks of exposure are felt to outweigh the vaccination risks, a pregnant woman should be vaccinated.	Live	In cases where a pregnant woman is traveling to an endemic country and she is not vaccinated because the vaccination risks seem to outweigh her risk of exposure, she should be issued a medical waiver to fulfill travel requirements.

ACIP, Advisory Committee on Immunization Practices; CDC, Centers for Disease Control and Prevention; VAERS, Vaccine Adverse Events Reporting System; WHO, World Health Organization.

Modified from Centers for Disease Control and Prevention. Pregnancy and Vaccination: Guidelines for Vaccinating Pregnant Women: ACIP: Guidance for Vaccine Recommendations in Pregnant and Breastfeeding Women. *2014. Available at http://www.cdc.gov/vaccines/pubs/preg-guide.htm; and Rasmussen SA, Watson AK, Kennedy ED, et al. Vaccines and pregnancy: past, present, and future.* Semin Fetal Neonatal Med. *2014;19:161–169.*

Group B streptococcus (GBS) is a leading cause of infection-related neonatal deaths. Although intrapartum antibiotic prophylaxis has resulted in a dramatic drop in the incidence of early-onset neonatal sepsis caused by GBS, the rates of early-onset disease have plateaued and late-onset GBS disease is not reduced by intrapartum prophylaxis. Therefore, efforts have been focused on development of a safe and effective GBS vaccine that could be administered to pregnant women to protect their infants. Since the 1990s, the safety and immunogenicity of several candidate GBS polysaccharide–protein conjugate vaccines have been under investigation. Currently, a trivalent GBS polysaccharide–protein conjugate vaccine is in Phase II trials among pregnant women in Europe, North America, and Africa.[94,95,132]

Globally, RSV is the most significant cause of viral acute lower respiratory tract illness.[133,134] RSV has a particularly high burden among young infants, making this disease a potential target for maternal immunization.[135] There are ongoing efforts to develop a maternal RSV vaccine. Phase II trials of an RSV vaccine were recently completed. A recombinant RSV fusion protein nanoparticle vaccine (RSV F vaccine) candidate was shown to be immunogenic and safe in a Phase II trial in women of childbearing age.[111]

Maternal Immunization and Impact on Birth Outcomes

Pathogens such as influenza and GBS have been associated with adverse birth outcomes including fetal loss, preterm birth, low birth weight, and small for gestational age.[120,136–139] The evidence for influenza's impact on birth outcomes is strongest for pandemics, although such evidence also exists for seasonal influenza.[120,136,138,140–145] A significant proportion of this evidence comes from studies conducted prior to the development of modern methods for assessing quality of epidemiological evidence, making it challenging to apply modern quality criteria to these data. However the consistency of evidence, particularly in the context of pandemics, strongly suggests that influenza infection in pregnancy is associated with adverse birth outcomes.[120,136,140]

Potential biological mechanisms through which pathogens can result in adverse birth outcomes include local/placental infection and systemic inflammation.[146] Inflammation, irrespective of the cause, is thought to play a substantial role in adverse birth outcomes. For example, inflammation may be a contributory factor in as much as 40% of preterm births.[146] Increase in proinflammatory (and decrease in antiinflammatory) cytokines is associated with physiological cascades that eventually result in increased levels of mediators of parturition such as prostaglandins and matrix metalloproteinases.[147] Many viral and bacterial pathogens have been associated with systemic increases in proinflammatory cytokines and, in some cases, preterm birth.

Because (with only a few exceptions) influenza does not cause direct placental or fetal infection, the most probable mechanism for adverse birth outcomes after influenza infection of pregnant women is through inflammation. On the other hand, GBS infection can result in bacteremia, chorioamnionitis, or endometritis. These infections can result in spontaneous abortion, preterm delivery, and stillbirth.

Adverse birth outcomes, such as preterm birth and low birth weight, are associated with significant morbidity and mortality worldwide. Given the role of maternal infection in adverse outcomes, maternal vaccination can potentially act as a useful tool for countering the burden and consequences of adverse birth outcomes.

There is compelling, but as yet inconclusive, evidence that maternal influenza immunization protects against outcomes such as preterm birth,[138,148–153] small for gestational age,[138,145,148] and stillbirth.[120,154] Most of this evidence comes from observational studies, although data are also publically available from three trials of maternal influenza immunization. Generally, larger observational studies and studies that account for influenza virus circulation tend to show a protective effect of maternal influenza vaccination on outcomes such as preterm birth and stillbirth, often restricted to the influenza virus

circulation period.[138,148] In a randomized, controlled trial in Bangladesh, maternal immunization during pregnancy was associated with a lower proportion of infants who were small for gestational age and an increase in mean birth weight during the period when influenza virus was circulating.[145] In contrast, trials in South Africa and Mali reported no impact of maternal influenza immunization on birth outcomes,[34,154a] although data stratified by the time of influenza circulation have not been published for these trials. A large randomized, controlled trial in Nepal demonstrated a 15% reduction in low birth weight among newborns of mothers who received influenza vaccination in pregnancy, compared to newborns of unvaccinated women.[155]

A few considerations should be kept in mind when interpreting the trial results in regard to bird outcomes.[155a] For example, the Nepal trial is unique as, in addition to lab-confirmed influenza, it was designed and powered to evaluate the impact against low birth weight—the only trial with a birth outcome as a (co-) primary end point.[156] This translated into a lower liklihood of type II error and a more robust assessment of birth outcomes, such as measuring birth weight in grams rather than kilograms (hence, having a higher ability to detect a difference in birth weight). Moreover, the women in the Nepal trial were vaccinated at an earlier gestational age,[156] providing an opportunity to protect the fetus for a longer period. Maternal influenza immunization may be particularly useful in reducing adverse birth outcomes at locations where influenza tends to circulate for more months than most other regions, therefore potentially exposing a higher proportion of pregnancies to influenza. Similarly, assessment of gestations age is suboptimal if it is conducted for the first time in late pregnancy (e.g., for women enrolled in the third trimester) or at delivery. In the otherwise high-quality Mali trial, the main approach to assess gestational age was the New Ballard score. However, ultrasound information was available for approximately 13% of the women. In the subgroup of women with ultrasound data, the correlation between ultrasound and Ballard core-based gestational age was only 0.4.[154a]

Importantly, newborns in areas where the Nepal and Bangladesh trials were conducted had lower birth weight compared with populations where the South Africa and Mali trials were conducted.[156] A potential implication of lower baseline birth weights in trials showing a protective effect of maternal influenza vaccination on birth outcomes is that maternal influenza vaccine may be more useful for vulnerable populations.

Inadvertent Vaccination During Pregnancy

A common clinical question that arises is what to do when a pregnant woman inadvertently receives a vaccine not recommended for use in pregnancy. These inadvertent exposures often occur during the first trimester, before pregnancy is recognized and during the period of organogenesis. For many vaccines, there is less known about safety in the first trimester compared with other trimesters, even for well-studied vaccines such as influenza. As pregnant women are often excluded from prelicensure clinical trials of vaccines, postmarketing surveillance is important and several systems and registries are in place to monitor safety in pregnancy. There are also some efforts underway in the United States to include pregnant women in future vaccine trials.[157]

Safety of Maternal Vaccines

Safety of vaccines administered in pregnancy should be conceptualized in the context of a developing embryo/fetus (Table 36.2). Embryonic and fetal development is often categorized into three stages relevant to teratogenesis: preimplantation, embryonic, and fetal stages. Each stage is associated with a different profile of potential adverse effects. For example, one would be concerned about spontaneous abortions in the preimplantation period (gestational age 2–4 weeks); organ malformations during the embryonic period

TABLE 36.2 Gestational Time Periods Relevant to Vaccination in Pregnancy

Period	Gestational Age (Weeks Since LMP)[a]	Events	Potential Teratogenic Effects
Preimplantation	2–4 wk	Fertilization, cell division, blastocyst formation, implantation	Injury to large number of cells → spontaneous abortion *or* Injury to small number of cells → survival without abnormalities
Embryonic	4–11 wk	Organogenesis	Malformations, altered function Onset for major defects in organs: • CNS: 5 weeks • Heart: 5 weeks • Sensory organs (eyes, ears): 6 weeks • Limbs: 6 weeks • Palate: 8 weeks • Teeth: 8 weeks • Genitalia: 9 weeks
Fetal	11 wk to term	Growth, differentiation, maturation	SGA/IUGR, fetal death, minor malformations, and altered function Continued susceptibility period for minor defects in organs: • CNS: to end of period • Sensory organs: to end of period • Palate: through 11 weeks • Teeth: to end of period • Genitalia: to end of period

[a]Note: Gestational age values, as presented, specifically reference time since LMP. Embryonic age is considered to be 2 weeks less than gestational age.

CNS, central nervous system; IUGR, intrauterine growth restriction; LMP, last menstrual period; SGA, small for gestational age.

Modified from Bednarczyk RA, Adjaye-Gbewonyo D, Omer SB. Safety of influenza immunization during pregnancy for the fetus and the neonate. Am J Obstet Gynecol. *2012;207:S38–S46; Buhimschi CS, Weiner CP. Medications in pregnancy and lactation: part 1. Teratology.* Obstet Gynecol. *2009;113:166–188; and Hill MA. Early human development.* Clin Obstet Gynecol. *2007;50:2–9.*

(i.e., 4–11 weeks of gestation); and outcomes such as preterm birth, stillbirth, small for gestational age, and functional malformations during the fetal (11 weeks through term) period (Table 36.2).[158,159,160]

MATERNAL VACCINES IN CLINICAL USE

Influenza Vaccine

Pregnant women with influenza are more likely to develop severe disease and die compared with nonpregnant women, particularly during pandemics.[97]

The live attenuated influenza vaccine is not recommended for use in pregnancy based on theoretical risks of adverse events potentially associated with live vaccines. In the United States, inactivated influenza vaccine was first recommended in pregnancy in 1960 and has substantial safety and effectiveness data.[103,158,161] According to the ACIP, women who are or will be pregnant during influenza season should receive the inactivated influenza vaccine as early as possible in pregnancy regardless of trimester.[103] In some non-U.S. countries, the vaccination is recommended after the first trimester.

Multiple studies, including randomized, controlled trials, have demonstrated the benefits of influenza vaccine for pregnant women and their infants. In a randomized, controlled trial in Bangladesh, the vaccine reduced the risk of influenza like illness among women vaccinated in the third trimester of pregnancy by approximately one-third.[162] The efficacy for lab-confirmed influenza among infants younger than 24 weeks of age in the Bangladesh trial was 63%.[162] Similarly, in a randomized, controlled trial in South Africa, the efficacy of inactivated influenza vaccine administered in second and third trimesters of pregnancy was approximately 50% among HIV-uninfected women and their infants younger than 24 weeks of age.[34] In a parallel cohort including HIV-infected women, the vaccine efficacy was 58% among mothers.[34] However, there was no statistically significant impact among infants of HIV-infected women, although with a study cohort of 194, there was very limited statistical power to detect a difference. The South Africa study also documented substantially higher attack rates among HIV-infected women compared to noninfected women.[34] In a trial in Mali, where pregnant women were randomized to either receive influenza vaccine or meningococcal vaccine, the efficacy for protection against influenza in infants younger than 6 months was 33%. In another randomized trial conducted in Nepal, the infants of women who received influenza vaccine between 17 and 34 weeks' gestation were 30% less likely to have influenza compared with infants of women who received placebo.[155]

An important consideration for clinical and public health maternal immunization recommendations is the duration of protection for infants. In the Bangladesh trial, the maternally derived antibodies in infants had a half-life of approximately 6 to 7 weeks.[70] In the South Africa trial the vaccine was only protective through the first 8 weeks of life.[162a] On the other hand, in the Mali trial vaccine efficacy was 68% through 4 months of age.[156] Although the efficacy diminished to 57% in the fifth month, the vaccine continued to provide protection through the first half of infancy.[156] Further detailed results are awaited from the randomized controlled trials of maternal influenza immunization in Nepal, Mali, and South Africa aimed at evaluating the impact of inactivated influenza vaccine on pregnant women and their infants.[156]

Randomized controlled trials have been very useful in establishing the efficacy of maternal influenza vaccine for pregnant women and their infants. However, the trial sample sizes ranged from approximately 300 to approximately 4000. Therefore these trials are limited in their ability to detect an impact on severe but relatively rare outcomes such as hospitalization. Fortunately, there are several recent large observational studies that have evaluated the effectiveness of maternal influenza immunization against severe outcomes. In a study of approximately 250,000 women in the United States, maternal immunization was associated with an 83% reduction in hospitalizations with laboratory-confirmed influenza. Similarly, in a series of studies in Western Australia, maternal influenza immunization was associated with a 25% reduction in infant hospitalization with acute respiratory illness, an 81% reduction in maternal emergency department visits, and a 65% reduction in hospitalizations during pregnancy.[162b,162c]

Overall, (inactivated) influenza vaccine administered in pregnancy has a good safety record.[103,122] However, most of the safety data come from the late second and third trimester and from studies evaluating the 2009 pandemic H1N1 vaccine and the data from the first trimester are limited. In a small retrospective cohort study with 439 first trimester births, there was no increase in major malformations, stillbirths, and neonatal pneumonia; however, there was increased risk of neonatal intensive care unit admissions among newborns of women vaccinated in the first trimester.[125] One of the larger retrospective studies that included approximately 8000 first trimester vaccinees did not find increased risk of stillbirth, early neonatal death, preterm birth, low birth weight, or small for gestational age.[163]

Data to support safety of maternal immunization in the second and third trimesters are substantial and robust.[103,122] However given the limited first trimester data and hitherto conflicting findings in studies evaluating the safety of first trimester influenza vaccination, there is a need to conduct further studies with first trimester vaccination. Further retrospective studies will continue to have limitations associated with retrospective studies (e.g., confounding associated with differential health seeking among vaccine recipients vs nonrecipients). Therefore, future efforts should focus on prospective studies. To capture early first trimester events, these studies may have to include reproductive-age women with a declared intention to get pregnant (e.g., presenting for prepregnancy consultation).

Vaccination Against Pertussis

Maternal immunization in pregnancy is a potential strategy to protect young infants against pertussis in two ways: first, passively transferred antipertussis antibodies can directly protect the infant; and second, prevention of pertussis in the mother can indirectly protect the child through decreasing exposure. Since 2012, both the United Kingdom's Department of Health and the United States' Centers for Disease Control and Prevention (CDC) recommended that all pregnant women receive a single dose of tetanus and diphtheria toxoids and acellular pertussis vaccine (Tdap) in the late second or third trimester with every pregnancy irrespective of previous Tdap history.[164,165] Although Tdap is already recommended for pregnant women in the United Kingdom and the United States, as well as in a few other countries such as Argentina and Brazil, it is not part of routine antenatal care in developing countries where the burden of pertussis is much higher.

There have been no Phase III trials of maternal Tdap administration. Therefore, data on efficacy of Tdap administration in pregnancy are not available. However, estimates of vaccine effectiveness from the United Kingdom suggest that, in a high-income-country context, pertussis vaccine in pregnancy has high effectiveness in preventing pertussis in early infancy. In the United Kingdom, pertussis vaccine administered in pregnancy was estimated to be 91% effective

(95% confidence interval [CI], 84 to 95) using the screening method, and 93% (95% CI, 81–97%) using the case-control method.[166,167] Similarly, data from a Phase I/II trial of Tdap in pregnancy, conducted in the United States, documented high concentrations of antibodies against pertussis in the first 2 months of life.[168] Another small trial in Vietnam reported higher geometric mean concentrations for all three measured pertussis antigens (i.e., pertussis toxin, filamentous hemagglutinin, and pertactin) in the infants of the Tdap group compared to the tetanus toxoid group, up to 2 months of age.[169]

Overall, the safety data for pertussis vaccination in pregnancy is reassuring. The data from United Kingdom's maternal pertussis program did not find any safety signals among mothers or infants. In a large study evaluating safety of Tdap administration in pregnancy using the Vaccine Safety Datalink (VSD) project, vaccination was not associated with increased risk of small-for-gestational-age, preterm birth, and hypertensive disorders of pregnancy.[170] However, compared to nonvaccinated women, approximately 20% more vaccinated women were diagnosed with chorioamnionitis (5.5% vs 6.1%). This finding needs to be interpreted with some context. In the United States, as a consequence of litigation-related concerns, many fevers in the third trimester are thought to be labeled as chorioamnionitis without further investigation. In fact, in the VSD study, chart reviews revealed that only half of the chorioamnionitis cases had a clinical presentation consistent with chorioamnionitis.[170] Moreover, in this study, the lack of increased risk of preterm birth, the most significant clinical consequence of chorioamnionitis, was reassuring.[170]

With the expansion of the maternal immunization platform, a theoretical concern is a potential for an increase in adverse events with concomitant administration of multiple vaccines. Reassuringly, a retrospective cohort study comparing adverse events among pregnant women receiving concomitant Tdap and influenza vaccination and women receiving these vaccines sequentially did not find a difference in adverse events between the two groups.[171] In another study, there was no difference in rates of acute postvaccination events (fever, allergy, local reactions) and adverse birth outcomes (small for gestational age, preterm delivery, low birth weight) in women vaccinated with Tdap vaccine in pregnancy, regardless of the time since the previous dose of a tetanus-containing vaccine.[172] Similarly, there was no elevated risk of structural birth defects, including microcephaly, among the offspring of women who received Tdap in pregnancy compared with offspring of women who did not.[172a] However, like influenza vaccine, most of the safety data for pertussis vaccination comes from the late second and the third trimesters.

Theoretically, maternal pertussis vaccination during pregnancy could result in attenuation of the infant immune response to childhood doses of pertussis vaccine. However, the evidence regarding attenuation of infant immune response to pertussis vaccines in the presence of maternal antibodies as a result of maternal vaccination in pregnancy or infection during or prior to pregnancy is inconsistent. Multiple studies published in the 1990s[173–175] evaluated differences in infant response to pertussis vaccines by presence of maternal antibodies prior to receipt of infant doses of (whole-cell or acellular) pertussis vaccine. As these studies were conducted prior to the recommendation of pertussis vaccination in pregnancy, the maternal antibodies were a result of infection or childhood immunization. Although there was considerable variability in findings from these studies, generally maternal antibodies were associated with greater attenuation in infant response to diphtheria and tetanus toxoids combined with whole-cell pertussis (DTwP) than that observed for diphtheria, tetanus, and acellular pertussis (DTaP).[173–175]

There are limited data on attenuation of infant DTaP responses after pertussis vaccination in pregnancy and there are no published studies assessing attenuation of DTwP response. A U.S. study found 0.7- to 0.8-fold lower concentrations of antipertussis antibodies after three doses of DTaP at 2, 4, and 6 months.[176] However, both prior to and following the recommended 12- to 18-month pertussis vaccine booster, antipertussis antibody levels in children of both vaccinated and control mothers were similar.[176] In a Phase I/II trial of maternal pertussis vaccination in the United States, the infant post-DTaP primary series responses were lower among the maternal vaccination group compared to infants of mothers who did not receive pertussis vaccine in pregnancy.[28] However, these differences did not persist after the 12-month booster dose. Another study from the United Kingdom documented substantially (0.51- to 0.67-fold) lower antipertussis antibodies after completion of the 2–3–4-month primary series with DTaP among infants of mothers who received pertussis vaccine in pregnancy compared to infants of women who were not vaccinated.[177] In a small trial in Vietnam of Tdap vaccination in pregnancy, potential attenuation was evaluated 1 month after completion of the 2–3–4-month primary infant vaccination with the acellular vaccine. In this trial, antipertactin antibodies (but not anti–pertussis toxin and anti–filamentous hemagglutinin antibodies) were lower in the infants born to mothers in the Tdap group.[169] The findings from the United Kingdom and Vietnam are particularly important because, like most countries, no pertussis booster is given the second year of life in the United Kingdom and Vietnam.

As there is no immunological correlate of protection for pertussis, the clinical significance of attenuation of infant DTaP responses is uncertain. Moreover, there is a need to evaluate attenuation of infant responses in countries that use DTwP for infant vaccination and countries with the 6–10–14-week schedule.

Tetanus Toxoid

Tetanus, caused by an exotoxin produced by *Clostridium tetani*, is an often fatal disease characterized by muscular rigidity and spasm.[113] Maternal and neonatal tetanus, which are associated with unhygienic birth practices such as unclean deliveries and care of the umbilical cord, are common causes of maternal and neonatal mortality in some resource-limited settings.[130] The Neonatal Tetanus Elimination Program, launched by the World Health Assembly in 1989, was later expanded to include maternal tetanus and renamed the Maternal and Neonatal Tetanus Elimination Program.[180] The aim of the initiative was to eliminate maternal and neonatal tetanus through promotion of clean birth and neonatal care practices, as well as maternal immunization with tetanus toxoid. Since these programs were launched, there has been a dramatic reduction in neonatal deaths worldwide and many more countries are now deemed free from neonatal tetanus (defined as less than 1 case of neonatal tetanus per 1000 live births). In addition to a commitment to safe delivery and neonatal care practices, this was accomplished by maternal immunization with tetanus toxoid. Because tetanus spores are widespread in the soil, it is not possible to eliminate them completely from the environment. Therefore, it is important to continue to immunize populations and in many settings pregnant women are routinely immunized.

The tetanus toxoid vaccine is considered very safe and effective in pregnancy. Neonates born to women immunized against tetanus are protected by passively acquired maternal antibody.[181] Furthermore, through widespread vaccination programs many pregnant women have been vaccinated with tetanus toxoid without any resulting safety concerns.

The ACIP recently began recommending routine Tdap vaccination during pregnancy because of recent neonatal and young infant pertussis cases.[112] Although pertussis is of current concern, protection against tetanus is a minor issue in the United States where tetanus is exceedingly rare.

PRECONCEPTION AND POSTPARTUM VACCINES

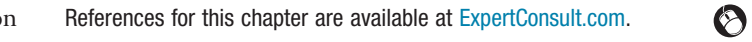
The postpartum period during the delivery hospitalization may represent an ideal time for vaccination given that pregnant women are accessible and known not be pregnant. For example, rubella immunity is routinely tested for during prenatal care and susceptible women are generally vaccinated prior to discharge from the hospital. Vaccinated women are advised to avoid pregnancy for 28 days following vaccination (the manufacturer recommends 3 months) based on a theoretical risk of embryopathy. Postpartum women who have not previously received Tdap are recommended to receive Tdap in the United States immediately after delivery.[112] A recent outbreak of measles also highlights the importance of ensuring that all women are up to date on measles-containing vaccine.[106] It is critical that healthcare providers review vaccination history in preconception care, during pregnancy, and postpartum. Furthermore, they need to actively recommend vaccines and facilitate vaccination (e.g., vaccinate in their office).

RESEARCH CONSIDERATIONS

Maternal immunization is an emerging and active area of research. There are several issues that deserve specific attention in the context of research on maternal immunization. For example, a review of adverse event definitions reported that there was substantial heterogeneity in how outcomes such as stillbirth were defined by various studies of maternal immunization[183] making it very challenging to compare rates of adverse outcomes across studies and populations. Therefore, there is a need for standardized outcome definitions. Moreover, assessing impact of maternal vaccines on birth outcomes could be one of the most important public health contributions of maternal vaccination. However, few studies are specifically designed and adequately powered to evaluate birth outcomes, resulting in an evidence base comprised mostly of retrospective studies and post hoc analyses of trials. Given that potential protective birth effects of maternal vaccines are likely to work through reduction of infections, it is essential to account for infection at the individual or community level when conducting such analyses. In fact, it is inappropriate to interpret data on protective effects of vaccines on birth outcomes without accounting for infection. Moreover, as mentioned in a previous section of this chapter, there is a need to conduct additional studies evaluating safety of first trimester vaccination.

In summary, maternal immunization has the potential for preventing substantial morbidity and mortality among mothers and infants.[155a,184] However, immunological changes specific to pregnancy should be kept in mind when considering clinical and research implications of vaccines in pregnancy.

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37 Measles Vaccines

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The written history of measles is classically traced to the writings of the Persian physician Rhazes, also known as Abu Becr, who lived during the 10th century.[1–3] However, the disease was apparently recognized as early as the 7th century by such persons as the Hebrew physician Al Yehudi.[2] Rhazes referred to measles as hasbah, which means "eruption" in Arabic.[2] *Rubeola* and *morbilli* are descriptive Latin words first used in the Middle Ages. The latter is a diminutive of *morbus*, meaning "disease," which was reserved to refer to the bubonic plague; *morbilli* referred to a minor disease. *Measles* is probably derived from *mesels,* the anglicized form of *misellus,* which, in turn, is a diminutive of the Latin word *miser,* meaning miserable and referring to the sufferer of various eruptions or sores. The presence of nonspecific leprous sores was incorrectly identified with the disease called morbilli in Latin. Thus, mesels came to be equated with the disease and not the sufferer of ill-defined skin lesions.[3]

Rhazes appears to have been the first to make the distinction between measles and smallpox.[2,3] He considered measles to be a severe disease, "more to be dreaded than smallpox." Although Rhazes did distinguish between the two diseases, he and others still probably considered them to be closely related.[3] Furthermore, although he was aware of the seasonal nature of measles, he did not think the disease was infectious.[2]

The distinction between measles and smallpox was becoming clearer by the beginning of the 17th century, when annual bills of mortality in London in 1629 listed the two diseases separately.[3] Thomas Sydenham clearly described the clinical characteristics of measles during this period and believed the disease to be infectious.[2–4] It was, however, Francis Home, a Scottish physician who worked in Edinburgh in the mid-18th century, who truly recognized the infectious nature of the illness in his attempts to prevent it.[5] Understanding of the epidemiology of measles was greatly enhanced by the classic investigation of a measles epidemic on the Faroe Islands in 1846 by the young Danish physician Peter Panum.[6] He not only confirmed that measles was contagious but also defined the 14-day interval between exposure and appearance of exanthem, recognized the higher mortality at the extremes of age, and observed that infection provided lifelong immunity.

In 1911, using infected material from acute cases, Goldberger and Anderson[7] transmitted human measles infection to monkeys, clearly demonstrating the existence of an infectious agent or substance responsible for measles. This finding antedated the technology to isolate and culture the measles virus. In 1954, John F. Enders and Thomas C. Peebles[8] successfully isolated the measles virus in human and monkey kidney tissue cultures. Adaptation of the virus to chicken embryos[9] and cultivation in chicken embryo tissue culture[10] paved the road to vaccine development and licensure in 1963.[11–21]

Widespread vaccination of children in the United States[22–27] and others[28–41] has had a dramatic effect on the incidence of measles and its associated complications. Reductions in morbidity and mortality have been so great that global eradication has been proposed[42–44] and judged feasible.[45–47] This would be a fitting end to a disease once confused with smallpox, the first infectious disease eradicated from the world.

BACKGROUND

Clinical Description

Usual Clinical Course

The first symptoms of measles occur after a 10- to 14-day incubation period that follows wild measles virus exposure from a measles-infected person. A systematic review estimated the median incubation period to be 12.5 days (95% confidence interval [CI], 11.8–13.2) based on 55 observations from eight observational studies.[48] Measles incubation periods vary by setting depending on population dynamics, measles virus transmission patterns during outbreaks, and the nature of infection events.[49] For example, if infection occurs after parenteral exposure, the incubation period is shortened by 2 to 4 days. Individual case reports have suggested potential exposure events occurring at least 21 days prior to rash onset[2,11,49–53]; therefore, to ensure high sensitivity for disease surveillance, an incubation period of 7 to 21 days is often used for the purposes of conducting epidemiological investigations, and identifying contacts and potential transmission events to guide control activities.[54] Immunosuppressed persons may have a prolonged incubation period because host immune responses, which are responsible for the signs and symptoms of measles, may be absent or delayed in persons with cellular immune deficiencies.[55,56] The prodromal stage is heralded by the onset of fever, malaise, conjunctivitis, coryza, and tracheobronchitis (manifesting as cough) and lasts 2 to 4 days. This symptom complex is similar to that seen with any upper respiratory infection. The temperature rises during the ensuing 4 days and may reach as high as 40.6°C. Koplik spots, the enanthema believed to be pathognomonic of measles, appear as multiple 2- to 4-mm bluish-white plaques on the buccal mucosa, usually on the inside of the cheek opposite the first or second molar, 1 to 2 days before the onset of a rash and may be noted for an additional 1 to 2 days after rash onset.[57–59] Koplik spots are believed to occur in greater than 70% of measles patients and because they usually first appear prior to rash onset, their early finding by a clinician during physical examination can prompt laboratory testing for rapid confirmation of measles, early treatment, implementation of infection-control practices, and postexposure vaccination of close contacts. Measles rash is an erythematous maculopapular eruption that usually appears 14 days after exposure and spreads from the head (face, forehead, hairline, ears, and upper neck) over the trunk to the extremities during a 3- to 4-day period. The exanthem is usually most confluent on the face and upper body and initially blanches on pressure. During the next 3 to 4 days, the rash fades in the order of its appearance and assumes a nonblanching brownish appearance.

Virus can be isolated from subcrevicular oral fluid, the nasopharynx, and blood during the latter part of the incubation period and during the early stages of rash development.[8,60] Although virus has been isolated from the urine

as late as 4 to 7 days after rash onset,[61] viremia generally clears 2 to 3 days after rash onset in parallel with the appearance of antibody.[62] Persons with measles are generally considered to be infectious 4 days before through 4 days after rash onset.

Measles virus infection causes simultaneous activation and suppression of the immune system.[63–67] Measurement of cytokines released during measles suggests activation of $CD8^+$ T cells, which are important for viral clearance, and type 2 $CD4^+$ T cells, which provide optimal antibody production. Measles virus infection leads to a decrease of $CD4^+$ lymphocyte counts that begins before the onset of rash and lasts for up to 1 month.[68] A study of measles patients in The Gambia found marked suppression of the production of interleukin-12, a known regulator of cellular immunity, and suggests a mechanism for the immune suppression associated with measles infection.[67] Recovery from infection is associated with the production of serum and secretory antibodies[62,69–77] as well as the establishment of cellular immunity.[65,66,78–84] Although subclinical infection with boosting of antibody may occur with subsequent exposure,[70,72] immunity after natural infection is believed to be lifelong.[6,85]

Complications

The complications associated with measles infection have been the subject of much description and review.[50,52,86–111] Measles illness severity, complication rates, and clinical outcomes vary by the person's age, preexisting health and nutritional status, comorbidities, access to treatment, and quality of care. In industrialized countries and settings with access to optimal care, the most commonly cited complications associated with measles infection are otitis media (7–9%), pneumonia (1–6%), diarrhea (8%), postinfectious encephalitis (1–4 per 1000–2000 cases of measles), subacute sclerosing panencephalitis (SSPE) (1 per 2500–10,000 measles cases),[112] and death (1.0–3.0 per 1000 cases).[113] Complications are likely to be present if the fever has not lysed within 1 to 2 days of rash onset. The risk of serious complications and death is increased in children younger than 5 years and adults older than 20 years.[2,97,98,102,105] Pneumonia, which is responsible for approximately 60% of deaths, is more common in young patients, whereas acute encephalitis occurs more frequently in adults.[97,105] Pneumonia may occur as a primary viral pneumonia (Hecht pneumonia) or as a bacterial superinfection, most commonly with staphylococcus, pneumococcus, or typeable (encapsulated) *Haemophilus influenzae*. Other described complications include thrombocytopenia, laryngotracheobronchitis, stomatitis, hepatitis, appendicitis and ileocolitis, pericarditis and myocarditis, glomerulonephritis, hypocalcemia, and Stevens-Johnson syndrome.[50,52] Based on clinical observations, it has long been assumed that measles infection exacerbates active tuberculosis and activates latent tuberculosis because of the theoretical risk from the measles virus–induced immunosuppression[114]; however, results from available published studies attempting to determine the occurrence and frequency of this potential additional measles complication are inconclusive.[115–117]

Measles infection runs a devastating course in children in developing countries and settings with minimal care, where measles mortality rates can be as high as 2% to 15%.[2,50,52,110,118–133] The rash is intense and may be hemorrhagic (black measles), and it resolves after marked desquamation. Pneumonia is the most common severe complication from measles and is associated with the greatest number of measles-associated deaths.[134,135] Inflammation of the mucosa leads to stomatitis and diarrhea. Diarrhea is a frequent cause of death because it may persist long after the acute insult and further aggravate a preexisting malnourished state.[106,136,137] Mediastinal and subcutaneous emphysema, keratitis, corneal ulceration, and gangrene of the extremities are not uncommon. The combination of vitamin A deficiency and keratitis results in a high incidence of blindness.[138–140] Secondary bacterial infections, often with staphylococci, produce pustules, furuncles, pneumonia, osteomyelitis, and other pyogenic complications. Neurological complications, including acute viral or bacterial encephalitis, which often result in permanent brain damage, occur in approximately 1 to 4 per 1000 cases.[3,56,113,141]

Following acute measles virus infection, a persistent infection in the central nervous system tissue with a defective measles virus may occur causing SSPE, a degenerative central nervous system disease that is characterized by behavioral changes, intellectual deterioration, mental and motor deterioration, seizures, and death.[141–145] SSPE signs and symptoms begin an average of 7 years after the primary measles infection. Once symptoms start, the person with SSPE develops progressive personality changes, myoclonic seizures and motor disability, and eventually lapses into a coma and dies.[146] Because SSPE symptoms are nonspecific and have very late onset after the initial measles infection,[147] SSPE cases largely go undetected, particularly in resource-limited settings. The global burden of SSPE is unknown; and the previous estimate of 1 SSPE case per 100,000 measles cases is now thought to have been an underestimation. During the resurgence of measles in the United States during 1989–91, the occurrence of SSPE was estimated to be 22 per 100,000 reported measles cases.[144] In Germany, among reported measles cases during 1994–2001, the risk of developing SSPE for children younger than 5 years of age contracting measles infection was estimated to be 1:1700 to 1:3300.[112,144] SSPE incidence depends on the measles epidemiology of a particular setting, in part because the risk for SSPE is higher when the primary measles infection occurs at a younger age,[141,148] and among reported SSPE cases, males outnumbered females 2:1 to 4:1.[4,100,104,149] The pathogenesis of measles in SSPE cases is not fully understood; it is thought that measles virus enters the brain, likely during the acute primary measles infection, then persists in the nervous system. Patients with SSPE have high titers of measles-specific antibodies in their sera and cerebrospinal fluid, and measles virus can be detected in affected brain tissue by electron microscopy and polymerase chain reaction (PCR).[150] Measles viruses isolated from affected brain tissue of SSPE cases have been found to have critical mutations in *M*, *H*, or *F* genes that prevent normal virus replication and budding from the host membrane. Measles viruses from SSPE cases do not play a role in virus transmission.[142,151]

Measles infection during pregnancy is associated with an increased risk of maternal, fetal, and newborn complications including miscarriage, preterm birth, neonatal low birth weight, and maternal death.[152–156] Because physiological adaptations in the immune system during pregnancy can increase a woman's susceptibility, pregnant women infected with measles are more likely to be hospitalized, develop pneumonia, and die than nonpregnant women.[25,26] Clinical illness in the newborn after intrauterine exposure follows a shortened incubation period and may vary from mild to severe, and fatal.[157,158] There is no convincing evidence that maternal infection with measles is associated with congenital malformations.[157,159]

Exploratory studies of other potential measles-related complications have been conducted. For example, a team of investigators in the United Kingdom has suggested that infection with measles virus or measles vaccine virus may result in a persistent infection of the gastrointestinal tract, leading to Crohn disease or ulcerative colitis later in life.[160–163] They proceeded to study a subset of children with developmental

disorders and ileocolonic lymphonodular hyperplasia by using reverse transcriptase–PCR (RT-PCR) techniques, and reported finding evidence of measles virus in biopsies from the terminal ileum of these children.[164] The findings of this group of investigators have led to speculation that combined vaccination with measles-mumps-rubella (MMR) vaccine may be responsible not only for some forms of inflammatory bowel disease but also the increasing reported incidence of childhood autism. Other researchers, however, have been unable to replicate earlier laboratory findings by using similar or more sensitive and specific methods.[165,166] Several subsequent well-conducted epidemiologic studies have found no association between measles vaccination and development of inflammatory bowel disease[167–169] or autism.[169–171] In fact, the available laboratory and epidemiologic evidence does not support hypotheses that measles or measles-containing vaccines might contribute to or induce inflammatory bowel disease or autism[172] (see "Vaccine Safety"). Furthermore, in 2010, the editors of *The Lancet* retracted the original article that led to the controversy,[173] and in January 2011, the *British Medical Journal* published an investigative review that found the medical records had been incorrectly abstracted to falsely implicate the vaccine.[174]

Measles has been proposed to be a causal factor for several other diseases. Studies from different laboratories have revealed conflicting evidence for persistence of measles virus in affected tissues from patients with otosclerosis[175] and Paget disease.[176] Also, current evidence is inconclusive regarding a possible role of measles infections in the pathogenesis of multiple sclerosis.[177]

Clinical Variants

The typical course of measles described in the preceding section can be modified by the presence of previously acquired cell-mediated immunity or antibodies.[2,50,52] This situation usually arises in the infant with residual maternal transplacental antibody, in a person given immunoglobulin (Ig) or vaccination after exposure, in an attempt to prevent or attenuate disease, or in previously vaccinated persons exhibiting an anamnestic immune response following measles virus exposure.[72,178–181] In these persons, subclinical infection[182] or a mild abbreviated illness may occur that confers lasting immunity.[181,183] However, if immunity is incomplete,[184] a second clinical measles infection may occur.[52,185] Typical or modified measles illness also may (rarely) follow reinfection after either natural infection[186,187] or vaccination.[187–196] Schaffner and colleagues[186] reported a case of typical, albeit mild, measles in a 16-year-old girl who reportedly had measles 8 years earlier. She had a hemagglutination inhibition (HI) antibody titer of 1:200 on the second day of rash and titers of 1:1600 and 1:320 at 23 days and 6 months, respectively, after rash onset. The rapidity of antibody appearance, the high titer achieved, and the absence of IgM antibody in all the specimens suggested a secondary immune response. Although reports examining immunity after infection rely on antibody determination, immunity relies heavily on T-lymphocyte memory and function.

Measles infection in the immunocompromised host (e.g., persons with malignancies or HIV infection) can be prolonged, severe, and frequently fatal.[197–202] Infection in these persons may occur in the absence of rash.[197,200–203] The severity of illness is believed to be primarily a result of impaired cell-mediated immunity.[204–207] Two especially severe complications are an acute progressive encephalitis (measles inclusion body encephalitis)[103,208,209] and a characteristic giant-cell pneumonia (Hecht pneumonia).[197–199,210] Measles has been found to be more severe in persons with HIV infection. In the United States, the case-fatality rate has been reported to be as high as 50% in HIV-infected children.[211]

An atypical variant of measles occurred in some recipients of killed measles vaccine who were subsequently exposed to wild-type virus.[50,52,185,187,212–224] Early studies found that patients with atypical measles lacked antibody to the measles virus fusion (F) protein[141,224,225] and had exaggerated cellular responses to measles antigen.[80,226] Affected patients had extremely high levels of measles-specific circulating antibody.[52,80,81,213,224] Studies in monkeys show that this illness is caused by antigen–antibody immune complexes.[227] The formalin-inactivated vaccine induced complement-fixing antibodies that failed to undergo affinity maturation; after exposure to measles, an anamnestic production of nonprotective, complement-fixing antibodies resulted in immune complex deposition and atypical measles.[228] After an incubation period of 1 to 2 weeks, a prodrome consisting of high fever, headache, abdominal pain, myalgia, and cough ensued. In the next 2 to 3 days, an unusual rash erupted on the extremities and spread centripetally. Whereas the exanthem could be erythematous and maculopapular, it was frequently petechial or vesicular and accompanied by edema; occasionally it was pruritic. Hepatocellular enzymes were sometimes strikingly elevated. The illness was frequently mistaken for Rocky Mountain spotted fever and had to be differentiated from meningococcemia, Henoch-Schönlein purpura, and drug eruptions.[50,52] A nodular pneumonitis with pleural effusion was common.[221,229] Despite the potential for serious illness, there was only one report of a possible atypical measles-related fatality.[230] This syndrome also has been reported (rarely) after receipt of live measles vaccine exclusively.[231,232] Persons with atypical measles are believed to be noncontagious.[52,213,214] On the basis of humoral and cellular immunity studies, they also are thought to be protected from subsequent illness after exposure to measles.[80,81,224] The syndrome probably can be prevented by appropriate immunization with live vaccine.[80,81,233,234]

Virology

The measles virus is a pleomorphic, nonsegmented, single-stranded, negative-sense RNA virus with a diameter of 120 to 250 nm.[141,225,235] It is a member of the genus *Morbillivirus* in the family Paramyxoviridae. There are six known species in this genus and family: measles virus in humans, rinderpest virus in cattle, peste-des-petits-ruminants virus in goats and sheep, canine distemper virus in domestic dogs, phocine distemper virus in seals, and cetacean morbillivirus in cetaceans. These viruses are all lymphotropic, induce highly contagious and devastating diseases with marked immunosuppression in their host, and have high host specificity.[236,237] Phylogenetic analysis suggests divergence of measles virus from its closest relative, rinderpest virus, occurred as early as the 5th century, which is consistent with the earliest recorded clinical descriptions of measles disease.[238] The only natural host for the measles virus is humans. In susceptible human populations, measles is one of the most transmissible viruses known. The viral structure is composed of eight proteins encoded within a 16-kb genome, including replication factors (polymerase [L] and phosphoprotein [P]), structural proteins (hemagglutinin [H], fusion [F], nucleoprotein [N], and matrix [M]), and two accessory proteins of unknown function (C and V).[225] The H and F proteins are essential in viral pathogenesis. The measles H protein is involved in the attachment and entry of measles virus into host cells via interaction with cell surface receptors. The F protein is involved in the viral/host-cell membrane fusion and viral entry into the host cell.

The entire 15,894-nucleotide–long genome of the prototype Edmonston strain of measles and the genomes of the predominant measles vaccine strains[239–241] have been fully sequenced, as well as an increasing number of wild-type strains, including some isolated from SSPE cases.[242–244] Although measles virus is considered a monotypic virus, sequence analysis of the *N*, *H*, *P*, and *M* genes has shown that there are multiple, distinct lineages of wild-type viruses.[245–252] This sequence variability has made it possible to use molecular techniques to help monitor transmission pathways of measles virus.[253,254] Molecular epidemiologic data, when analyzed in conjunction with standard epidemiologic information, can confirm or suggest the source of outbreaks and, over time, can provide a measurement of the effectiveness of vaccination control programs and monitor elimination.[251] For example, genetic analysis of wild-type viruses detected in the United States from 1988 to 2000 helped document the interruption of endemic transmission in the United States.[255] Comparison of genetic sequences from wild-type strains isolated in the United States with those isolated elsewhere in the world has suggested international importations of measles virus as sources of outbreaks.[253] To facilitate the expansion of virologic surveillance activities, the World Health Organization (WHO) has recommended a standard nomenclature and analysis protocol, and established a global database of measles virus sequences.[256] The WHO currently recognizes 24 genotypes (Fig. 37.1) of measles virus based on phylogenetic analysis of the *N* gene and has established a set of reference sequences.[257] In 2000, the WHO established the Global Measles and Rubella Laboratory Network to provide standardized procedures for case confirmation and virus characterization. This network was built on the existing network for global polio eradication; it continues to expand and now includes more than 730 laboratories in all six WHO regions.[258] Although virologic surveillance is incomplete, increased laboratory capability and improved surveillance since 2000 has allowed for monitoring the global distribution of genotypes (Fig. 37.2).[259] Following elimination efforts globally, circulating genotypes of wild-type measles virus have decreased; of the 24 known measles virus genotypes, 13 were detected and reported during 2005–14.[260] Although the biologic significance of differences in the genetic sequence of wild-type strains is not known, there is no evidence that strains vary by transmissibility or virulence. The immune response generated through vaccination protects against all measles virus strains.

Measles virus can be cultured from clinical specimens and is best isolated in the Vero/hSLAM cell line comprised of Vero cells transfected with a plasmid encoding the gene for the human signaling lymphocyte activation molecule (SLAM).[261] The sensitivity of Vero/hSLAM cells for isolation of measles virus is equivalent to that of the previously used B95a cells, which were coinfected with Epstein-Barr virus. In addition, Vero/hSLAM cells are sensitive to laboratory-adapted measles strains, including vaccine viruses.[262] Measles virus can also be detected directly from clinical specimens, such as oral fluid or throat swabs, through molecular techniques that are being used to monitor measles transmission patterns without the need for virus culture. Measles virus is inactivated rapidly in the presence of sunlight, heat, and extremes of pH.[141] It can, however, be safely stored for long periods at −70°C.

In cell cultures, the virus causes two distinct cytopathic effects.[19,141,263,264] The first is formation of multinucleated syncytia (giant cells) containing numerous nuclei of fused cells. This corresponds to the predominant pathologic process observed in infected tissues, including skin and Koplik spots.[265] When observed in lymphoid tissue, the giant cells are referred to as Warthin-Finkeldey cells; otherwise they are known as epithelial giant cells.[141] Whereas this cytopathic effect is characteristic of wild-type virus isolates, the cytopathic effects of passaged virus may additionally include spindle-cell transformation. This difference in cytopathic effects as well as other factors, such as ability to grow in chick embryo fibroblasts, plaque morphology, interferon production, and optimal growth temperature, help differentiate wild-type virus from attenuated vaccine virus strains, as does sequencing.[141,266–268]

Pathogenesis as It Relates to Prevention

Until recently, standard theoretical models of measles virus pathogenesis suggested that in a person who has exposure to measles virus during contact with another person who is infectious with measles, transmission occurs with initial cell entry of the virus in the epithelium of the upper respiratory tract, nasopharynx, and possibly of the conjunctivae,[50,52,86–88,268–273] However, based on recent findings from molecular studies, our current understanding of measles virus pathogenesis suggests that the initial infection actually occurs in the lower respiratory tract, where measles virus likely first enters alveolar macrophages and subepithelial dendritic cells.[273a] Following this initial cell entry, measles virus is rapidly transported to regional lymph nodes, leading to extensive measles virus replication in the lymphatic system and an acute viremia and leukopenia. During viremia, measles virus spreads systemically via infected immune cells, primarily B cells, T cells, and monocytes, to organs throughout the body, as well as to the epithelial cells in the upper airway and nasopharynx.[274] Once the virus reaches epithelial cells systemically, the virus enters the basolateral side[275] of epithelial cells in the upper respiratory system. Following virus entry into upper respiratory epithelial cells, rapid spread of infected cytoplasm occurs laterally through intercellular pores to adjacent epithelial cells creating multinucleated epithelial giant cells that can be detected in nasal secretions and conjunctivae at the end of the incubation period, during the prodrome and the first days of the rash. This leads to sloughing of infectious tissue in the upper airway mucosa that induces coughing and sneezing of aerosolized respiratory droplets, marking the start of the infectious period and thereby, facilitating the survival of measles virus through continued transmission from person to person.[276] Measles transmission can occur during the infectious period, lasting a total of approximately 9 days, starting 4 days before, until 4 days after rash onset. The onset of rash marks the start of the adaptive immune response and virus clearance from the body.[277–279]

This current model of measles virus pathogenesis is based on the results of extensive research, including human and animal studies of the measles virus genome, structure, cellular receptors, and immunology. Measles virus is known to target dendritic, epithelial, endothelial, and blood cells, including activated B cells, T cells, and monocytes[280–282]; however, all specific measles virus cellular receptors have not yet been identified. Three known cellular receptors that interact with the measles virus H glycoprotein are (a) membrane cofactor protein or CD46, (b) SLAM or CD150, and (c) nectin-4, also known as poliovirus receptor-like 4 (PVRL4). CD46 is a human complement regulatory protein that is prolific in humans and expressed on all nucleated cells, including epithelial, endothelium and ependymal cells. CD46 functions as a cofactor for proteolytic inactivation and binds to measles virus transmembrane hemagglutinin (H),[283] and acts as a costimulatory molecule for CD4 T cells.[283,284] Generally, CD46 is an important cellular receptor used by measles vaccine strains but not by wild-type measles strains.[285–287] The two receptors that allow morbilliviruses to circulate in nature are SLAM, expressed in immune cells, and nectin-4, expressed in polarized epithelial cells.[288] The SLAM family is a group of

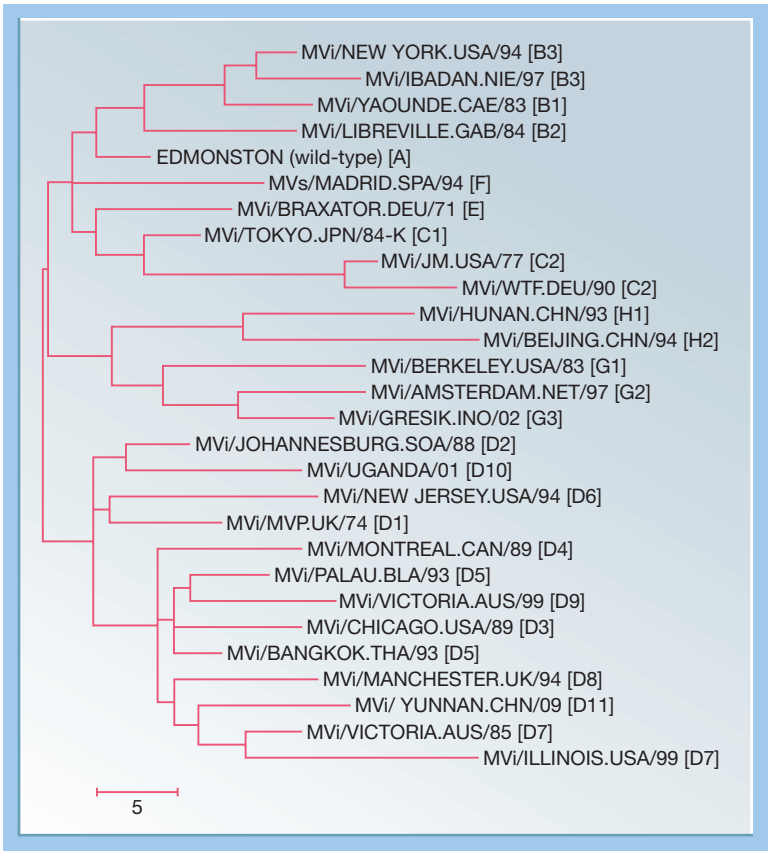


Figure 37.1. Genetic variation in wild-type measles viruses, 2015. The World Health Organization (WHO) recognizes 24 genotypes of wild-type measles virus (A, B1, B2, B3, C1, C2, D1, D2, D3, D4, D5, D6, D7, D8, D9, D10, D11, E, F, G1, G2, G3, H1, and H2). Figure shows phylogenetic tree based on the sequences of the 450 nucleotides that code for the 150 amino acids at the COOH terminal of the nucleoprotein gene of the WHO references strains for each genotype. Length of horizontal lines corresponds to genetic distance. Scale at lower left shows length for 5 nucleotide substitutions.

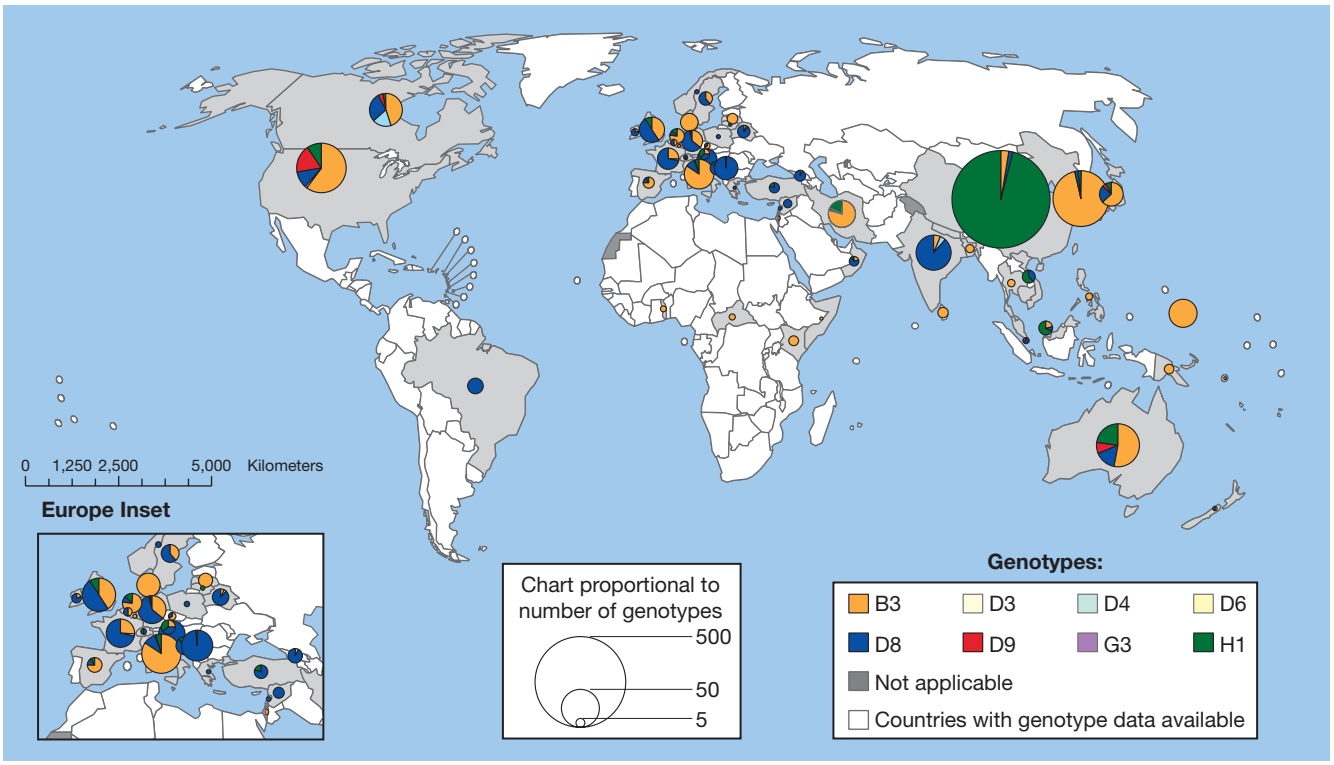


Figure 37.2. Worldwide distribution of measles virus genotypes, March 2014 to February 2015.

transmembrane receptors found in animals and humans, and were likely the major determinant[289] for the evolution of the host-virus specificity of morbilliviruses. In humans, SLAM/CD150 is a membrane glycoprotein expressed on cells of the immune system, including activated T and B lymphocytes, activated monocytes, immature thymocytes, and mature dendritic cells.[290] The initial cell entry of measles virus leading to primary measles infection occurs in alveolar macrophages or dendritic cells that express the immune cell–specific protein SLAM/CD150. After extensive replication in SLAM-expressing cells in lymphatic organs, the virus is likely delivered systemically by circulating SLAM-expressing immune cells to the basolateral side of epithelial cells. SLAM/CD150 is used by both measles vaccine strains and wild-type viruses.[291] Nectin-4 is a cell receptor on epithelial cells that has been found in animals and humans, and plays a critical role allowing measles virus in humans to rapidly spread laterally in the airway epithelium. In airway epithelial cells, measles virus uses the adherens junction protein nectin-4/afadin complex to initiate a viral membrane fusion apparatus opening intercellular membrane pores allowing infected cytoplasm transfer between columnar epithelial cells. This causes large infectious centers in the tracheae and upper airway that likely eventually detach, triggering coughing and sneezing of aerosolized[276] measles virus. Because the spectrum of measles disease affects multiple organs and tissues, additional measles virus cellular receptors that play a role in measles pathogenesis are known to exist, but have not yet been identified or have mechanisms that are not fully understood. For example, CD147 has been identified as potentially facilitating measles virus entry into epithelial and neuronal cells, a finding that could help explain the causes of central nervous system complications.[273] The development of new techniques in reverse genetics and recombinant virus strains that express enhanced fluorescent proteins have recently advanced tropism and pathogenesis studies, leading to important findings, including the identification of nectin-4 as the receptor that facilitates the virus passing through epithelial barriers to complete measles virus transmission from an infected person to a susceptible person. The pathogenesis of measles virus infection indicates that prevention of transmission from person to person could be accomplished by inhibiting replication at the initial site of infection, inhibiting the viremia that occurs during the incubation period, or preventing cell entry and invasion of the upper respiratory epithelial cells which allows shedding of virus for sustained transmission. The first approach requires the presence of local secretory IgA antibody or transudated IgG[292]; the second approach requires a sufficient titer of neutralizing antibody. Infectious measles can be prevented by an immune response with neutralizing antibodies.[178–182,268] Children with primary agammaglobulinemia do not have more-severe measles infections than do children with normal immune systems, and both develop long-lasting immunity after infection.[205,206] These observations indicate that measles antibodies prevent infection whereas cellular immunity acts to close off infection if it occurs.

Diagnosis

Measles should be suspected in persons who present with an acute erythematous rash and fever, preceded by a 2- to 4-day prodrome of cough, coryza, conjunctivitis, and photophobia. Recent experience suggests that clinical measles may be difficult to distinguish from other causes of febrile rash illness, particularly in areas where the incidence of measles has been low. Clinical features that support the diagnosis of measles include the presence of Koplik spots, the characteristic 2 to 4 days of intensifying prodromal symptoms, the progression of the rash from the head to the trunk and out to the extremities, and the lysis of fever shortly after the appearance of the rash. Healthcare providers working in measles-endemic areas may be more familiar with these clinical findings than are healthcare providers working in areas with a low incidence of measles. A clinical case definition for epidemiologic purposes is the presence of rash lasting 3 or more days; a fever (temperature ≥38.4°C, if measured); and cough, conjunctivitis, or coryza.[234] Its use for clinical diagnosis is limited, particularly because of the criterion requiring at least 3 days of rash before the diagnosis is made. In some parts of the world, a less-specific clinical definition not requiring rash duration of 3 days is being used for epidemiologic purposes. Laboratory tests are necessary to confirm the diagnosis, especially when measles is rare. Other illnesses, such as rubella, dengue, parvovirus B19, human herpesvirus type 6, and measles vaccine reactions, can meet these clinical definitions.[293–295] In the United States, it is recommended that clinicians obtain a blood or other suitable specimen for laboratory confirmation from all patients suspected of having measles.

Although virus isolation, direct cytologic examination of clinical material, or demonstration of virus antigen can be used to diagnose measles,[60,61,296–301] detection of measles-specific IgM antibody from a single blood specimen is the most commonly used method. An increase in measles antibodies between acute and convalescent serum specimens is also diagnostic but requires the collection of two blood specimens. RT-PCR can be used to identify measles virus RNA in urine, blood, oral fluid, and nasopharyngeal mucus.[302–305] Because measles is an RNA virus, RNA must be reverse transcribed to DNA before PCR analysis, resulting in a much reduced sensitivity for diagnosing measles compared with diagnosing DNA viruses.[306]

In primary acute infection, detectable antibodies generally appear in the serum within the first few days of rash onset, peak within approximately 4 weeks, and subsequently decline somewhat but persist for life (Fig. 37.3).[50,52,69–72,307–309] Both IgG and IgM antibodies are initially produced.[310–313] However, IgG antibodies are detectable long after infection, whereas IgM antibodies are rarely detected after 6 to 8 weeks. Serum and secretory IgA antibodies are also produced.[75–77,314,315] Reexposure usually induces a characteristic anamnestic response with a rapid boosting of IgG antibody; IgM may not be detected

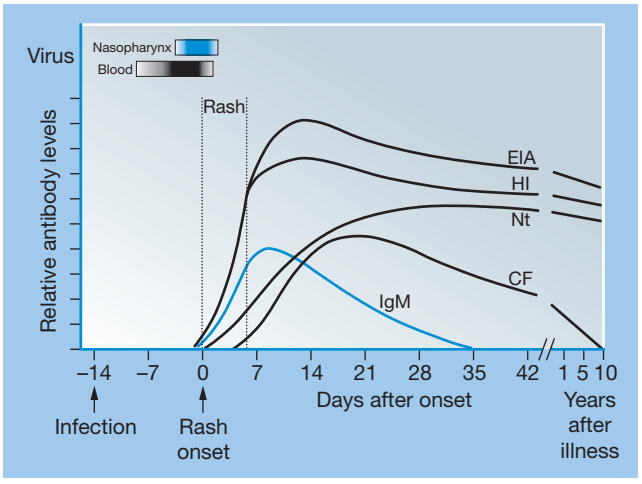


Figure 37.3. Schematic of the immune response in acute measles infection. CF, complement fixation; EIA, enzyme immunoassay; HI, hemagglutination; IgM, immunoglobulin M; Nt, neutralization.

after reexposure with use of some currently available serologic assays.[72,193,311,312] If IgM is detected after reexposure, it will be at a lower ratio to IgG than the IgM-to-IgG ratio in a previously unexposed person.[316]

The tests for complement fixation (CF), HI, and neutralization (Nt) have been the assays historically employed to detect fourfold rises in measles IgG antibody between acute and convalescent sera.[141] However, the CF test is less sensitive,[70–72,85,317] and the HI and Nt tests have undergone technical modifications over time.[318] The plaque-reduction neutralization test remains the gold standard to which other more rapid and simpler methods are compared, but, because it is time and personnel intensive, it is generally limited to specialized reference laboratories and research work.[318] Fluorescence tests are available,[319] as are enzyme immunoassays (EIAs), also referred to as enzyme-linked immunosorbent assays (ELISAs).[320–326] Currently EIA tests for measles IgG are the most widely used because they are generally sensitive and convenient. Good correlation has been shown between HI and Nt antibody in many studies,[327] as well as between EIA and other serologic methods for the diagnosis of acute measles.[321,322,328] The EIA for measles IgG is based on significant changes in optical density values and cannot be translated directly to antibody concentrations or titers.

Currently, the recommended laboratory method for the confirmation of clinically diagnosed measles is a serum-based IgM EIA collected at the time the patient is first seen for medical care.[329] A single specimen is adequate to detect the presence of IgM antibody.[315,325,326] A number of commercial kits using an indirect EIA method (with removal of the patient's IgG antibody before testing for IgM) or an antibody capture EIA technique (without removal of IgG) are now available and have been shown to have similar sensitivity (83–92%) and specificity (87–100%).[330] However, if measles disease prevalence is low (e.g., 1%), a modest reduction in the specificity of the assay from 99% to 95% will decrease the positive predictive value of the assay from 48% to 15% (i.e., only 15% of IgM-positive clinical cases will be true measles). Patients with parvovirus B19 or rubella infections may have false-positive reactions (rate of approximately 4%) when tested with measles IgM EIAs.[293,331]

Correct interpretation of serologic data depends on proper timing of specimens relative to rash onset. This is especially important in interpreting negative IgM results. For example, in one study, the sensitivity of an antibody capture IgM assay was approximately 80% within the first 72 hours after rash onset and rose to 100% between 3 and 14 days after rash onset.[332] Sensitivity of the assay within the first 3 days of rash onset is thought to be similar for other commercially available kits. If the validity of the initial measles IgM test is in doubt, a second convalescent specimen should be taken and tested for IgM and for a rise in IgG titer compared with the initial specimen. In some cases, interpretation may be difficult and requires precise information regarding dates of rash onset, prior measles vaccination, and specimen collection.

Two approaches provide alternatives to venipuncture for collection of diagnostic specimens: oral fluid and blood spots on filter paper. In the United Kingdom and elsewhere, commercial IgM EIA-based assays have been developed and used to test oral fluid specimens for measles. Compared with serum IgM results, reported sensitivities and specificities of oral fluid IgM assays ranged from 92% to 100%.[333–335] Use of oral fluid samples has appeal because the technique is noninvasive, can be used for rubella and mumps testing, does not require processing in the field, can be shipped without cold chain, and can be used to detect not only measles IgM and IgG but also the measles virus genome for molecular characterization. Although still considered an invasive technique, fingerstick as a method for sample collection may be more acceptable to parents than phlebotomy.[306] Blood spots collected onto filter paper do not require processing in the field, or a cold chain for transport to the laboratory, and can be used to test for measles and rubella as well as for molecular characterization of the measles virus genome. In addition, the eluted serum can be tested by using commercially available EIAs with minimal loss of sensitivity or specificity.[336–339]

Treatment

A number of preparations, such as interferon,[340,341] thymic humoral factor,[342] thymostimulin,[343] levamisole,[344] ribavirin,[345] and Ig,[178] have been used to treat measles. None of these is commonly used to treat uncomplicated measles, although limited studies with ribavirin have shown reduced duration of illness.[345] Ribavirin and interferon may be effective in treating severe measles in immunocompromised persons.[346] Recent research has identified anti-viral compounds that potentially could be developed as specific anti-paramyxovirus therapeutics.[347,348]

As antiviral therapies are not commonly used to treat measles, case management is focused on supportive care, as well as the prevention and treatment of measles complications and secondary infections to reduce morbidity and mortality. Clinical outcomes improve with early case detection and treatment. High doses of vitamin A have been shown to decrease mortality and morbidity in young children hospitalized with measles in developing countries.[349–353] The WHO currently recommends vitamin A for children with acute measles.[354] Vitamin A oral dosage is once daily for 2 days at 200,000 IU for children ages 12 months and older, 100,000 IU for infants 6 to 11 months of age, and 50,000 IU for infants younger than 6 months of age. In the United States, as well as in developing countries, children with measles have been found to have low levels of serum retinol, and those with more-severe illness have lower levels.[355–357] The American Academy of Pediatrics (AAP) recommends using two doses of vitamin A (200,000 IU) on consecutive days, which is associated with a reduction in the risk of mortality in children younger than 2 years of age (relative risk [RR], 0.18; 95% CI, 0.03–0.61) and the risk of pneumonia-specific mortality (RR, 0.33; 95% CI, 0.08–0.92). There is no evidence that vitamin A in a single dose is associated with a reduced risk of mortality among children with measles.[233,358] In addition, vitamin A therapy should be administered to children with measles who are immunosuppressed, have clinical evidence of vitamin A deficiency, or have recently immigrated from areas with a high mortality rate from measles.[233] Antibiotics, in the absence of pneumonia, sepsis, or other signs of a secondary bacterial complication, are generally not recommended.[359] However, a recent clinical trial in Guinea-Bissau found that case-patients who received prophylactic antibiotics (co-trimoxazole) had less pneumonia and conjunctivitis and higher weight gain, suggesting a beneficial role of prophylactic antibiotics in the treatment of measles in low-income countries.[360]

Various chemotherapeutic agents have also been used in patients with SSPE in an attempt to treat or at least alter the clinical course of the disease.[144,361–364] Of these, inosiplex (Isoprinosine) and interferon have been the most extensively studied[362–366]; despite anecdotal evidence of their effectiveness, controlled trials are lacking.

EPIDEMIOLOGY

General Epidemiology

In the absence of an immunization program, measles is a ubiquitous, highly contagious, seasonal disease affecting

nearly every person in a given population by adolescence.[2,367–369] Important exceptions are island and other isolated populations, which can remain free of infection for variable periods and then, after reintroduction of the virus, experience epidemic disease that involves all age groups not affected by the last wave of infection.[2,6,85,90,94,368] Thus, whereas peak transmission usually occurs among young children, outbreaks in isolated communities involve many older persons. This is exemplified by Panum's description of measles on the Faroe Islands,[6] in which the disease affected persons of all ages who were not affected by the last epidemic that had occurred 65 years earlier.

Measles is transmitted from person to person primarily by airborne aerosolized droplet nuclei.[2,370–373] The period of maximal contagion occurs during the prodrome.[7,50,52,95] Secondary attack rates in susceptible household (and institutional) contacts are high and can be on the order of 90% or greater.[51,70,307,374–376] Traditionally the incubation period for measles was thought to be 14 to 15 days.[375] Using a novel estimation method the mean incubation period and generation time for measles are estimated to be 11 to 12 days.[49] Almost all primary infections (except those modified by maternal antibody or parenteral Ig) are thought to be clinically overt.[2] Transmission from asymptomatic exposed immune persons has not been demonstrated.[377,378] Although susceptible monkeys may become infected with measles, there is no significant animal reservoir.[2,50,141]

Before the introduction of vaccines in most developed countries, school-age children had the highest risk of infection and accounted for the largest proportion of cases.[2,367] However, in dense urban areas, transmission among preschoolers took on greater importance.[367] Although serious complications did occur, they were relatively rare compared with the situation in developing countries. In the United States before the introduction of vaccine in 1963, major epidemics occurred approximately every 2 to 3 years.[2,379–381] Each year, disease peaked in late winter and early spring. The highest occurrence of disease was in children 5 to 9 years of age, who accounted for more than 50% of reported cases (Table 37.1). More than 95% of cases had occurred by age 15 years.[2,367] The highest risk of death was in children younger than 1 year of age and in adults.[97,98]

The measles virus is so contagious that it can be expected to circulate wherever a relatively large number of susceptible persons congregate, even in the face of a low population-susceptibility rate.[2] This explains the outbreaks that were typical among military recruits before the institution of routine measles vaccination.[382] Outbreaks among high school and college students, most of whom have been vaccinated, demonstrate the virus's capability to seek out the small number of remaining susceptible persons.[382–386]

Before widespread vaccination, in many developing countries, the average age at infection was much lower than that observed in developed countries.[2,118–125] In some areas of Africa, more than 50% of 2-year-old and 100% of 4-year-old children may be expected to have had measles. Poor nutrition and rapid loss of maternal antibody may explain why a greater proportion of these infants are susceptible at an earlier age than are those in developed areas,[327,387–391] and infection, in turn, results from the early age at which infants are exposed to the community at large.[118] Young age at infection contributes to the high risk of serious complications and death. Malnutrition, especially vitamin A deficiency, may also be an important factor leading to the marked severity of measles in the developing world because of defects in cellular (and possibly humoral) immunity.[126,392] However, there is some evidence that crowding, which leads to a potential increased dose of transmitted virus, may be a more significant determinant of the severity of infection than is protein-calorie malnutrition.[393–395]

Significance as a Public Health Problem

Although remarkable control of measles has been achieved in some areas of the world,[39,396] in 2000, measles was still the leading cause of vaccine-preventable deaths in children and the fifth leading cause of all deaths among children younger than 5 years of age.[397] Measles is also responsible for much diarrhea, respiratory disease, and blindness in the developing world.[130,135,136] Through mass vaccination campaigns and increased routine immunization coverage, the estimated number of measles deaths worldwide dropped 79% from 546,800 deaths in 2000 to 114,900 deaths in 2014 and measles vaccination was estimated to have prevented 17.1 million measles-related deaths during this period.[398,399] A comparison of the proportion of all-cause mortality resulting from measles among children less than 5 years of age found that measles accounted for approximately 7% of all child deaths in 1990 and 1% in 2008, contributing 23% to the overall reduction in mortality in this age group.[400]

In the United States in the prevaccine era, approximately 500,000 cases of measles were reported each year, but, in reality, an entire birth cohort of approximately 4 million persons was infected annually.[401] Associated with these cases were an estimated 500 deaths, 150,000 cases with respiratory complications, 100,000 cases of otitis media, 48,000 hospitalizations, 7000 seizure episodes, and 4000 cases of encephalitis, which left up to one quarter of patients permanently brain damaged or deaf. An economic analysis of the two-dose MMR vaccination program in the United States adjusted to 2001 dollars, found the direct and societal benefit-to-cost ratios to be 14.2 and 26.0, respectively.[403] Although endemic measles has been eliminated in the United States, importations continue to result in small but costly outbreaks. The total economic burden on public health institutions that dealt with 16 measles outbreaks involving 107 confirmed cases during

TABLE 37.1 Age Distribution and Mean Annual Incidence of Reported Measles Cases by Age Group

	1960–1964[a]		1981–1988[b]		1989–1991[b]		1992–2000[b]		2001–2014[b]	
Age (y)	Total (%)	Cases[c]	Total (%)	Cases[c]	Total (%)	Cases[c]	Total (%)	Cases[c]	Total (%)	Cases[c]
0–4	37.2	766.0	31.9	4.9	43.6	43.7	39.9	1.08	30.01	0.20
5–9	52.8	1236.9	11.7	1.8	10.4	10.4	9.6	0.26	9.7	0.06
10–14	6.5	169.1	19.7	3.3	9.2	9.8	9.6	0.26	10.2	0.06
≥15	3.4	10.0	35.6	0.6	36.9	3.5	41.0	0.10	50.0	0.03

[a]Data represent prevaccine years and are from four reporting areas: New York City, District of Columbia, Illinois, and Massachusetts.
[b]Data from the entire United States.
[c]Per 100.000.

2011 ranged from an estimated US$2.7 million to US$5.3 million.[404]

PASSIVE IMMUNIZATION

Passive immunity to measles can be acquired through physiologic transfer of maternal measles antibodies from mother to child or by administration of immunoglobulin preparations containing measles antibodies to persons at risk of measles.

Maternal antibodies provide time-limited protection against measles during the first months of life when infection can be most devastating.[70] Women who have antibodies to measles, either from measles infection or vaccination, transfer antibodies to their children during pregnancy via the placenta and after delivery by breastfeeding.[405,406] Among newborns, the duration of protection by transplacental measles antibody varies from some being unprotected at birth (i.e., their mother lacked immunity) to others being protected for up to 15 months of age. The factors that affect the duration of transplacental measles antibodies among infants and the effect of these antibodies on response to measles vaccination are presented in the section "Maternal Antibody and Age at Vaccination" below. Measles-specific antibodies, including secretory IgA, are excreted in the breast milk of immune mothers and have been shown to neutralize measles virus in vitro.[405,406] Many of the key factors related to breast milk antibodies against measles are not well determined. In two studies in Africa in the 1980s, one showed a significant reduction in the measles mortality rate of hospitalized children who were breastfed compared with those who had been weaned, while the other study showed no protective effect of breastfeeding.[407,408]

Administration of measles antibodies as immunoglobulin provides short-term protection from measles for susceptible persons. Preexposure prophylaxis against measles and other diseases is provided to persons with primary humoral immune deficiency diseases via frequent administration of immunoglobulin given intravenously (IgIV) or subcutaneously (IgSC). Postexposure prophylaxis with IgIM or IgIV is recommended for persons exposed to measles or at high risk of exposure for whom vaccine is either contraindicated or was not given within 3 days of exposure (see "Postexposure Prophylaxis" and "Use of Immunoglobulin" below).[409]

In 1926, Zingher[410] summarized early efforts to prevent measles by injection of whole blood, serum, or plasma from donors who had a previous measles infection. A significant reduction in the measles attack rate among persons who received convalescent blood products after exposure to measles was shown, and convalescent blood products were widely used as postexposure prophylaxis in the 1920s and 1930s. In the 1940s, Janeway demonstrated that administration of Ig within 6 days after intimate exposure prevented measles in about three of four persons, with mild measles occurring in the fourth.[179]

Multiple Ig preparations are available in the United States and include IgIM, IgIV, and IgSC. Ig products are prepared from plasma pools derived from thousands of donors. Measles disease results in higher antibody titers than does vaccination. Therefore, donor populations with predominantly vaccine-induced measles immunity yield lower measles antibody titers in Ig preparations. A Cochrane Review concluded that Ig was effective at reducing the risk of measles; however almost all the studies included in the review were conducted in the last century, when donor populations would have had higher titers as the result of predominantly disease-induced immunity.[411] A study in Japan showed lower measles antibody titers in IgIM may result in a reduced level of postexposure protection from measles.[412] Audet demonstrated a progressive decrease in measles virus neutralizing antibodies in IgIV lots in recent years in the United States, correlated with an increase in the proportion of plasma donors who were born in the vaccine era.[413] In the United States, the Food and Drug Administration (FDA) requires that all Ig preparations contain a measles-neutralizing antibody level that demonstrates adequate potency when compared with the U.S. standard.[414] In 2007, the FDA Blood Products Advisory Committee reduced the potency requirement from 0.60 to 0.48 of the standard lot titer for both IgIV and IgSC, which are given in much higher volumes than IgIM. The new potency requirement for IgIV and IgSC lots is calculated to provide a protective measles antibody titer given the recommended minimum doses and dose frequency.[409,415] The potency requirement for IgIM remains 0.60 of the standard lot titer.

Historically, IgIM has been the blood product of choice for short-term measles prophylaxis.[409] IgIV has been available since 1981 and is used primarily for the prevention of common infectious diseases for patients with primary immunodeficiency disorders. Although a larger dose can be administered with IgIV compared with IgIM, clinical use of IgIV has important disadvantages, including high cost and administration requiring extended observation in specialized settings by skilled professionals (e.g., hospital setting). IgSC has been available since 2006 with the same major indication as IgIV. However, administration requires a pump and advanced training. Also, multiple, consecutive weekly doses are needed to establish a steady-state with protective antibody levels.[409]

ACTIVE IMMUNIZATION

Vaccine Strains

Origin and Development

After the isolation and propagation of measles virus in tissue culture by Enders and Peebles[8] in 1954, vaccine development, testing, and licensure quickly followed.[11–21] The Edmonston strain, named after the youth from whom the virus was isolated, was used for many of the vaccines developed worldwide (Fig. 37.4).[416–433] To make the now-famous Edmonston B vaccine, Enders and colleagues[12,19,268] further passaged the Edmonston strain at 35°C to 36°C 24 times in primary kidney cells and 28 times in primary human amnion cells, adapted it to chicken embryos (six passages), and then passaged it in chicken embryo cells. This attenuated Edmonston B vaccine was licensed in the United States in March 1963 along with another Edmonston B virus strain that had been adapted to primary dog kidney cells.[434–436] Although the administration of the Edmonston B vaccine was associated with a high rate of fever (temperature of 39.4°C or higher in 20% to 40% of vaccinees) and rash (approximately 50%), the recipients remained remarkably well. However, simultaneous administration of a small dose of Ig with the vaccine (eventually set at 0.02 mL/kg) reduced the occurrence of high fever and rash by approximately 50% (Fig. 37.5).[307,419,422,423,434,435,437–442] Approximately 18.9 million doses of Edmonston B vaccine were administered in the United States between 1963 and 1975 (Table 37.2 and Fig. 37.6).

Killed Vaccine

A formalin-inactivated, alum-precipitated vaccine derived from the Edmonston strain was also licensed in the United States in 1963 and used until 1967 (see Table 37.2 and Fig. 37.6). This vaccine was also used in some provinces in Canada. Usually, three doses of killed

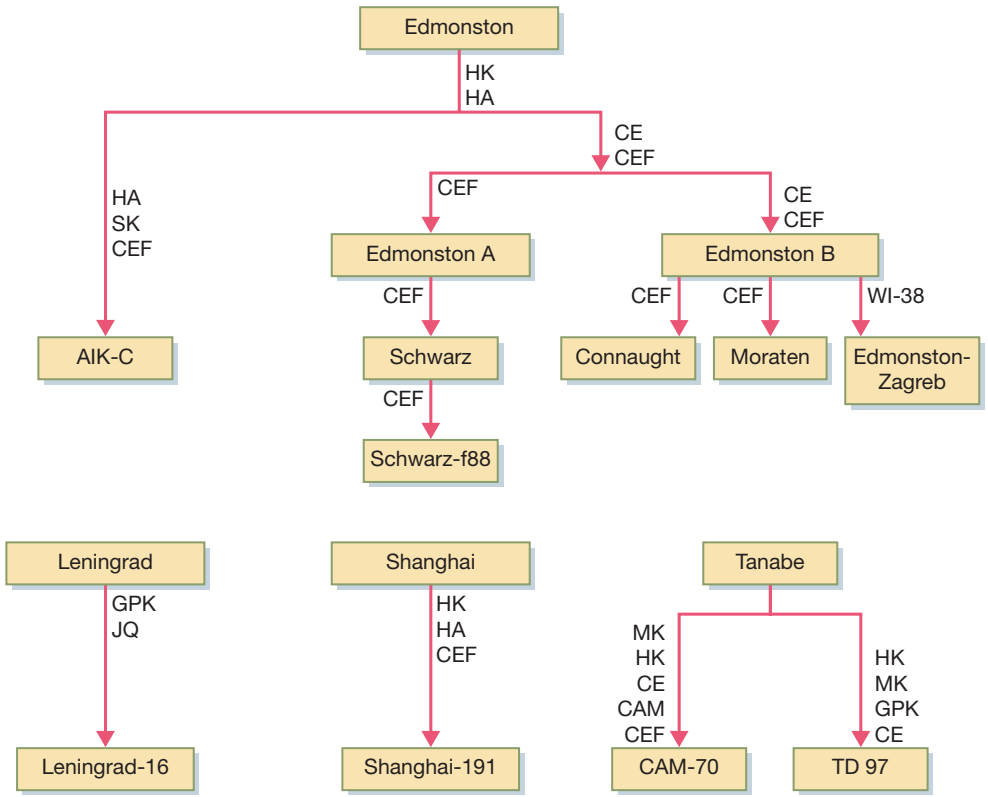


Figure 37.4. Attenuation history of selected measles vaccine strains. Cell cultures in which strains were passed during attenuation: CAM, chick chorioallantoic membrane; CE, chick embryo intraamniotic cavity; CEF, chick embryo fibroblast; GPK, guinea pig kidney; HA, human amnion; HK, human kidney; JQ, Japanese quail; MK, monkey kidney; SK, sheep kidney; WI-38, human diploid cell line.

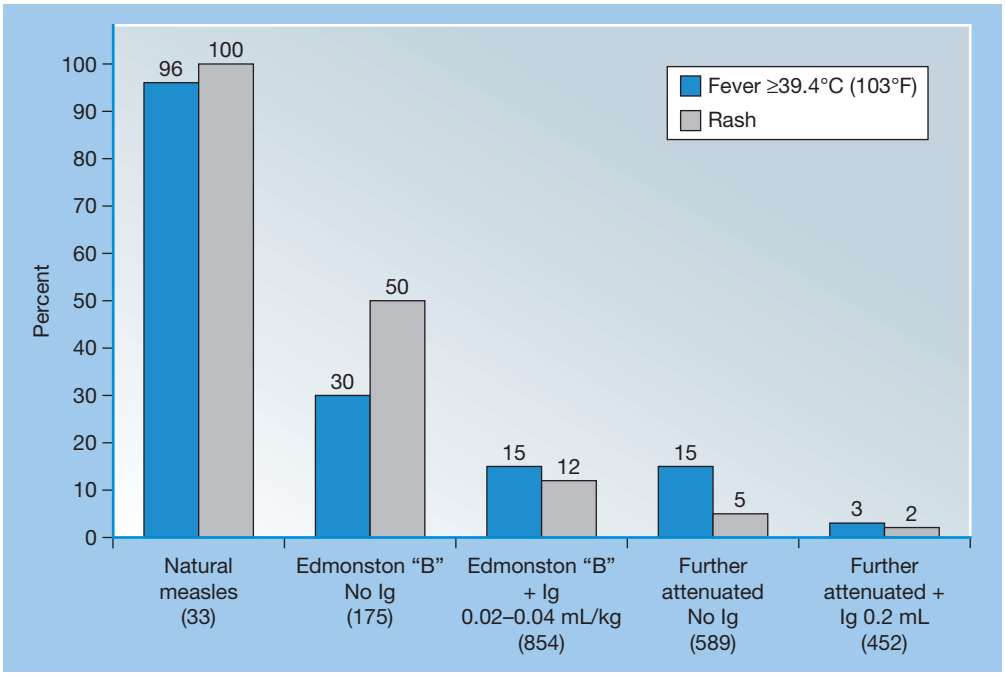


Figure 37.5. Incidence of fever and rash after natural measles infection and vaccination. The number of susceptibles is included in parentheses. Ig, immunoglobulin. *(Modified from Krugman S, Giles JP, Jacobs AM, et al. Studies with a further attenuated live measles-virus vaccine.* Pediatrics. *1963;31:919–928.)*

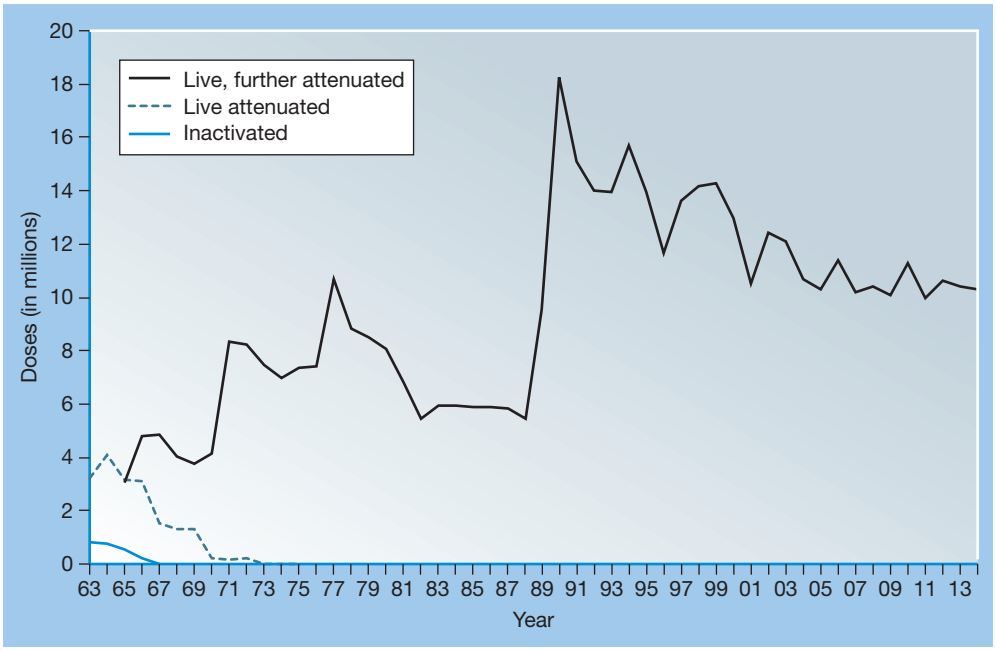


Figure 37.6. Doses of measles virus vaccines distributed by vaccine type, United States, 1963–2014. Live attenuated is the Edmonston B strain; live, further attenuated is the Schwarz and Moraten strains combined.

TABLE 37.2 Measles Vaccine Manufacture and Distribution in the United States, 1963 to 2016

Vaccine	Strain	Manufacturer	Brand	Years in Use	Doses Distributed
Inactivated	–	Lilly Pfizer	Generic Pfizer-Vax Measles-K	1963–1967	1.8 million
Live attenuated	Edmonston B	Lederle Lilly Merck Parke Davis Pfizer Philips Roxane	M-Vac Generic Rubeovax Generic Pfizer-Vax Measles-L Generic	1963–1975	18.9 million
Live, further attenuated	Schwarz Moraten	Pitman Moore- Dow Merck	Lirugen Attenuvax/MMR II	1965–1976 1968–2016	>450 million

Modified from Hayden GF. Measles vaccine failure: a survey of causes and means of prevention. Clin Pediatr. *1979;18:155–167.*

and one dose of live vaccine were administered at monthly intervals with few side effects.[72,307,419,422,443–450] Use of killed vaccine was eventually discontinued when it became apparent that this vaccine produced short-lived immunity and placed many recipients at risk for atypical measles infection.[451] It has been estimated that between 600,000 and 900,000 persons in the United States received the 1.8 million doses of killed measles vaccine that were administered (see Table 37.2 and Fig. 37.6).[452]

Further Attenuated Live Vaccines

Many further attenuated vaccines have been developed and are in active use worldwide (Table 37.3; see Fig. 37.4). Most were derived from the Edmonston strain. These further attenuated vaccines differ from each other in the viral isolate of origin, the number and temperature of cell culture passages, the type of cell culture used for passage and production, and whether plaquings were performed during the passages.[12,19,425,429,430,433,453] Although differences in plaque size,[425,454] subgenomic particles,[455] temperature sensitivity,[456] and pathogenicity in severe combined immune-deficient mice containing human thymic tissue implants[457] have been described among the further attenuated vaccine strains, their significance is uncertain.

Nucleotide sequence analysis of the *F*, *H*, *N*, and *M* genes showed no more than 0.6% variability among vaccine strains derived from the Edmonston strain.[250] Complete genomic sequence analysis of different measles vaccine strains have been compared with the Edmonston wild-type virus. AIK-C, Moraten, Schwarz, and Zagreb were all found to be of the Edmonston lineage, whereas CAM-70, Changchun-47, Leningrad-4, and Shanghai-191 were derived from four different wild-type isolates.[456,458] However, all the vaccine strains, whether derived from Edmonston or from other wild-type viruses, are members of the same genotype—genotype A.[250,259]

Studies have identified nucleotide sequence substitutions in the *H* gene that appear to mediate some of the biologic characteristics of the Moraten strain of vaccine.[459] Comparison of the noncoding regions of a low-passage Edmonston wild-type strain and five Edmonston vaccine viruses found 21 nucleotide positions at which the wild type and one or more of the vaccine types differed. Five nucleotide substitutions

TABLE 37.3 Measles Vaccine Manufacturers and Vaccine Strains Produced as of 2015

Manufacturer	Vaccine Strain
Merck (United States)	Moraten
SanofiPasteur (France)	Schwarz
GlaxoSmithKline (Belgium)	Schwarz
Government Pharmaceutical Organization (Thailand)	Schwarz
BioManguinhos (Brazil)	Schwarz
Takeda Phamarceutical Company (Japan)	Schwarz F88
Serum Institute of India	Edmonston-Zagreb
Institute of Immunology (Croatia)	Edmonston-Zagreb
Research Foundation for Microbial Diseases of Osaka University (Japan)	CAM-70
BioFarma (Indonesia)	CAM-70
Kitasato Pharmaceutical Industry (Japan)	AIK-C[a]
The Razi State Serum Institute (Iran)	AIK-HDC/Schwarz
Moscow Plant of Biological Preparations (Russia)	Leningrad-16
Beijing Tiantan Institute of Biological Products (China)	Shanghai-191
Beijing Minhai Biotechnology Co., Ltd. (China)	Chang-47
Lanzhou Institute of Biological Products (China)	Shanghai-191
Shanghai Institute of Biological Products (China)	Shanghai-191
Wuhan Institute of Biological Products (China)	Shanghai-191
Changchun Qijian Biological Products (China)	Chang-47

[a]AIK-C vaccine is produced in human diploid cells.

were conserved in all the vaccine strains. Comparison of protein-encoding nucleotide sequences of the *N*, *P*, *M*, *F*, *H*, and *L* genes of these vaccine strains with Edmonston wild-type virus identified amino acid substitutions in each of the genes; however, the overall level of heterogeneity did not exceed 0.3%.[240,241] The role that these sequence differences in both the coding and noncoding regions of the genome play in attenuation of measles viruses is being characterized by using specialized genetics systems.[460]

Two further attenuated live measles vaccines derived from the Edmonston strain that were licensed in the United States were the Schwarz strain in 1965 and the Moraten strain in 1968 (see Table 37.2 and Fig. 37.4). The Schwarz vaccine was derived from Edmonston virus passaged an additional 85 times at 32°C in chicken embryo cells.[461–464] The Moraten strain was also passaged at this lower temperature, but only an additional 40 times.[465] Compared with the Edmonston B vaccine, the frequency and severity of side effects attributed to these and other further attenuated vaccines were significantly lower (see Fig. 37.5).[307,419,424,461–466] A temperature of 39.4°C or higher occurred in only 5% to 15% and rash in only 3% to 5% of vaccinees. Simultaneous administration of the Schwarz strain and specially titered Ig in a low dose (0.2 mL) further reduced the incidence of high fever and rash to approximately 3% each (see Fig. 37.5).[307] These doses of Ig did not interfere with seroconversion, but the peak geometric mean antibody titer was lower than that observed without Ig administration. The further attenuated vaccines are used without Ig.[467]

The Moraten vaccine (Attenuvax contained in MMR II; Merck) is now the only measles vaccine used in the United States; the Schwarz vaccine is the predominant product in many other nations.[428] Several different further attenuated measles vaccines, including AIK-C, Schwarz F88, CAM-70, and TD97, have been developed and are being used in Japan.[429,430,433,453] The vaccine developed by Smorodintsev (Leningrad-16) was introduced in Russia in 1967 and was the principal vaccine virus strain in eastern Europe.[426] The CAM-70 and TD97 vaccines were derived from the Tanabe strain.[433,453] These vaccines, as well as those in use in China since 1965,[431] are the few not derived from the Edmonston virus.

Whereas most measles vaccines were attenuated and are produced in chick embryo fibroblasts, a few currently used vaccines were attenuated in human diploid cells. The Edmonston-Zagreb vaccine, used extensively in the former Yugoslavia since 1969, was derived from the Edmonston strain and underwent additional passage in WI-38 cells.[425] This vaccine is now produced by several other manufacturers and is the most widely used vaccine in developing countries (see Table 37.3). Other vaccine strains have been adapted to MRC-5 and R-17 human diploid cells in Iran[427,432] and in China.[431]

Dosage and Route of Administration

According to current regulations in the United States, measles vaccine must contain at least 1000 median tissue culture infective doses ($TCID_{50}$) at the end of the expiration date of the vaccine.[468] The vaccine is administered in a 0.5-mL dose. The minimum dose required to immunize a seronegative child has been found to be as low as 20 $TCID_{50}$ in some studies, but higher in others.[469–472] The regulated minimum dose in the commercial product is designed to compensate for some of the virus deterioration that may result either from improper storage or reconstitution or from exposure to light or heat before injection.

The recommended route of administration is subcutaneous injection. Although there are only limited data on the intramuscular route, it appears to be as effective as subcutaneous vaccination.[473] Studies with the Edmonston B and further attenuated vaccines have examined the effectiveness of other routes of administration, such as intranasal and conjunctival inoculation.[11,15,51,292,434,435,474–476] Most of the results were not favorable. In contrast, aerosol administration, which was evaluated during the early 1960s and 1970s, showed promising results.[476] During the 1980s, studies were undertaken to determine whether aerosol administration of measles vaccine could overcome maternal antibody and immunize younger infants.[476–481] Many of these studies found the Edmonston-Zagreb vaccine strain to be more immunogenic than the Schwarz strain when it is administered by aerosol.[482] However, whereas some investigators reported high seroconversion rates after administration by this route in young infants,[477–479,481] others found it inferior to subcutaneous administration (see "Future Vaccines" below).[480]

Aerosol administration of measles vaccine in South Africa[483] and of combined measles and rubella vaccines in Mexico[484] resulted in boosting of antibody responses among schoolchildren. These studies led to enthusiasm about the possible use of aerosol administration as a less-invasive alternative to needle-and-syringe administration during mass vaccination campaigns, especially among schoolchildren. Subsequently, aerosol administration of the Edmonston-Zagreb strain was shown to induce a primary immunization response at 9 months of age.[485] However, in a recent randomized clinical trial, aerosolized vaccine against measles was immunogenic but inferior to the subcutaneous vaccine with respect to the rate of seropositivity (see "Future Vaccines" below).[486]

Combination With Rubella, Mumps, and Varicella Vaccines

In the United States, vaccination against measles is accomplished with combined live vaccines that also contain attenuated rubella and mumps vaccine viruses. Combined vaccines were licensed in the United States in 1971. They contain at least 1000 $TCID_{50}$ of the measles Moraten strain, at least 5000 $TCID_{50}$ of the mumps Jeryl Lynn strain, and at least 1000 $TCID_{50}$ of the RA27/3 strain of rubella vaccine virus. The RA27/3 strain of rubella virus replaced the HPV-77:DE-5 strain as the rubella component in 1979. Currently, the only licensed MMR vaccine is produced by Merck (M-M-R II). In September 2005, a combined measles, mumps, rubella, and varicella (MMRV) vaccine produced by Merck was licensed for use in the United States.[487] The measles, mumps, and rubella vaccine viruses in this quadrivalent vaccine are identical and of equal titer to those in MMR vaccine. The titer of Oka/Merck varicella-zoster virus is higher in MMRV vaccine than in single-antigen varicella vaccine (see Chapter 38).

Combination products were developed as other countries began vaccinating children against rubella or mumps along with measles.[31–34,37,488] For example, GlaxoSmithKline (Research Triangle Park, NC) produces a vaccine that contains Schwarz measles vaccine, RIT4385 mumps vaccine strain (derived from the Jeryl Lynn strain), and the RA27/3 strain of rubella vaccine. As of 2015, Sanofi Pasteur (Swiftwater, PA) has discontinued production of a combined formulation with Schwarz measles vaccine, Urabe mumps vaccine, and RA27/3 rubella vaccine, and will continue single-dose measles vaccine production only through 2017. In Japan, several formulations of combined vaccines are available, including one containing the AIK-C measles virus, the Hoshino mumps virus, and the Takahashi rubella virus strains.[489] Two other combined vaccines are also licensed: one containing the CAM-70 measles strain and one containing the Schwarz F88 strain. The MMR vaccine containing the Urabe mumps strain is no longer being produced in Japan. A triple vaccine with the Edmonston-Zagreb strain of measles vaccine is being produced by the Institute of Immunology (Zagreb),[490] and the Serum Institute of India (Mumbai; Leningrad-Zagreb mumps strain and RA27/3 rubella strain). Serum Institute of India also manufactures combined measles-rubella vaccine (Edmonston-Zagreb measles strain and RA27/3 rubella strain) that is increasingly being used in developing countries as part of measles and rubella elimination efforts.

Safety and immunogenicity data indicate that combining the measles antigen with rubella and mumps antigens[488–505] in the MMR vaccine is both safe and efficacious. Initial results also showed similar safety and efficacy for MMRV vaccine compared with MMR given with varicella vaccine at the same time but at different injection sites.[506–509] However, when given as the first dose to young children, more recent studies show that the risk of febrile seizures[510,511] appears to be higher after MMRV compared with MMR and varicella vaccine administered simultaneously at separate sites (see "Vaccine Safety").[511] Based on the increased risk of febrile seizures when MMRV is used as the first dose in young children, the Advisory Committee on Immunization Practices (ACIP) has modified its recommendations for use of MMRV (see "General Immunization Guidelines" below).

Production and Constitution of Vaccine

Preparation methods for the Merck vaccine provide generally applicable information regarding the production and constitution of measles vaccines.[512] Although there are minor differences in dose, antibiotic content, and other details among manufacturers, there are no reports of significant differences in side effects or vaccine effectiveness.

The vaccine virus is cultured in primary chick embryo cells. After an initial cell growth phase, the cultures are inoculated with the further attenuated Moraten strain of measles virus. After several days' incubation at 32°C, the cells are washed to remove fetal bovine serum, and the medium is replaced with one containing 50 μg/mL of neomycin, sucrose, buffered salts, amino acids, and human albumin. Fluids containing virus can be removed from the cultures for a time as the cells are maintained at the same temperature. These fluids are frozen until determinations of the virus titer have been performed on retained aliquots. Harvested virus fluids having sufficient virus potency and satisfactorily passing tests are thawed, pooled, sampled for safety testing, clarified, dispensed, and refrozen.

When bulk vaccine has passed all quality control tests, portions of the vaccine are thawed, dispensed into vials, and lyophilized. At the time of use, the vaccine is reconstituted with fluid (sterile distilled water) provided by the manufacturer. A preservative-containing reconstitution fluid is not recommended for general use because it may inactivate the vaccine. Each vaccine dose contains approximately 25 μg of neomycin. Sorbitol and hydrolyzed gelatin are added as stabilizers. When reconstituted with the provided diluent, the vaccine is clear and yellow in color.

In 1996, the existence of reverse transcriptase in several vaccines that were grown in chick embryo fibroblast tissue culture was reported, including measles vaccine,[513] by use of a new and highly sensitive technique to detect the reverse transcriptase activity. With use of similar assays, other laboratories have confirmed the existence of the reverse transcriptase activity.[514] Subsequent studies showed that this reverse transcriptase activity is associated with the endogenous avian retrovirus EAV-0[515] and the endogenous avian leukosis virus ALV-E.[516] Extensive efforts to identify a transmissible virus have failed to link the reverse transcriptase activity with a transmissible virus. A study of 206 recipients of combined MMR vaccine found no evidence of infection with either avian leukosis virus or endogenous avian retrovirus.[517] These findings and the fact that transmission of retroviruses across disparate species may be restricted[518] indicate no apparent risk to vaccine recipients.

Stability of Vaccine

Measles vaccine is extremely stable between −70°C and −20°C.[519] Although measles vaccine is affected adversely by higher temperatures, the introduction of more heat-stable vaccines in 1979 led to increased stability under normal working conditions, which is especially important in the developing world.[520] In the United States, manufacturers must demonstrate that a minimum titer of 1000 $TCID_{50}$ is maintained through the labeled expiration date when the vaccine is stored at 2°C to 8°C. The WHO has a requirement that lyophilized measles vaccine, after exposure to 37°C for at least 1 week, cannot lose more than 1 $\log_{10}$ and must maintain a titer of at least 1000 $TCID_{50}$.[521]

For the currently available vaccine in the United States, when it is stored at 2°C to 8°C, a minimum titer of 1000 $TCID_{50}$ can be maintained in unreconstituted vaccine for 2 years or more. This potency can be maintained for 8 months at room temperature (20°C–25°C) and 4 weeks at 37°C. Reconstituted vaccine loses 50% of its potency in 1 hour at 20°C to 25°C and almost all its potency when it is held at 37°C for 1 hour. Vaccine is also sensitive to sunlight; however, colored glass vials further minimize loss of potency. Notwithstanding its improved thermostability, the vaccine still needs

to be handled with care according to the recommendations of the manufacturer.

As stated in the package insert, Merck recommends that its product be shipped at a temperature of 10°C or less and stored, before reconstitution, at 2°C to 8°C and protected from sunlight. Reconstituted measles vaccines (M, MR, and MMR) should be used immediately. If reconstituted vaccine is not used within 8 hours, it must be discarded. MMRV vaccine must be stored frozen at an average temperature of less than or equal to −15°C. Unlike other measles-containing vaccines, MMRV vaccine cannot be stored at refrigerator temperature, and once reconstituted, it should be used immediately to minimize loss of potency and discarded if not used within 30 minutes.[487]

RESULTS OF VACCINATION

Immune Response

The immune response after successful vaccination is similar in most respects to that noted after natural infection. Although the interval between vaccination and an immune response is a few days shorter than that observed after natural infection,[11] immunization induces both humoral[21,72,75,77,307] and cellular[81–83] immunity and the production of interferon.[522–524]

Laboratory evidence of immunity is most conveniently documented by use of antibody assays because tests for cell-mediated immunity are not standardized. However, even with antibody assays, results of studies on vaccine-induced immunity may vary, depending on the sensitivity of the antibody assay used.[70–72,85,317–323,525–533] Although the presence of antibodies detected by HI, ELISA, or CF correlates with immunity, Nt antibodies are probably most important in clinical protection.[2,70,85,327] After measles vaccination, IgG, IgM, and IgA antibodies can be detected in both serum and nasal secretions.[75,77,311,313,534] IgM antibody can be detected in the serum between 2 and 6 weeks (peak at 3 weeks) after vaccination and disappears soon thereafter.[311,313,535] Although only small amounts of IgA have been detected in serum, IgA is the predominant antibody found in nasal secretions.[75,77] Although detectable serum IgA and IgM antibodies are transient, IgG antibodies generally persist for many years. The antibody titers elicited by vaccination do decline over time (as do those induced by natural infection) and may become undetectable.[72,308,309,536–542] A study done in 2007, found that measles antibody persisted in all vaccinees available for follow-up 10 years after a second dose of vaccine, with no seronegative results detected. However, declining titers suggested the need for vigilance in ensuring disease protection for the vaccinated population.[542]

Vaccine-induced antibody titers are typically lower than those induced by natural infection. Vaccine-induced immunity is subject to boosting on challenge by either vaccine or wild-type virus; likewise, similar boosting can be observed after natural infection.[70,72,308,309,463,534,538,539] Thus, as discussed in more detail later, immunization usually provides immunity similar to that induced by natural infection.

Many investigators have described the initial antibody response elicited by live measles vaccination.[72,182,307–309] In studies by Krugman and colleagues, depending on the assay used (CF, HI, or Nt), antibodies first appear between 12 (HI and Nt) and 15 (CF) days after vaccination and peak 21 to 28 days after vaccination. Although antibodies were detected in 95% or more of susceptible vaccinees, regardless of the vaccine strain, the CF geometric mean titer did vary by strain of vaccine (Table 37.4). The geometric mean titers among young children 1 month after receipt of Edmonston B vaccine alone, Edmonston B vaccine plus Ig, Schwarz vaccine, and Schwarz vaccine with Ig were 1:208,[182] 1:96, 1:56, and 1:24 to 1:32, respectively.[307] The titer observed in 33 children 1 month after natural infection was 1:128.[307] Hilleman and colleagues[465] also noted a difference in the geometric mean titer by vaccine strain when using the HI assay (Table 37.5). Although 98% or more of vaccinees seroconverted, the HI geometric mean antibody titer associated with the Edmonston B vaccine was 1:25, whereas that noted after vaccination with either the Schwarz or Moraten further attenuated strains was 1:16. Despite these differences in geometric mean antibody titer, receipt of these vaccines was associated with a markedly reduced risk of infection that did not decrease over time.[72,308,309]

Studies of measles-specific cell-mediated immunity after live attenuated vaccines are more challenging because of the lack of a simple in vitro assay. With the importance that

TABLE 37.4 Antibody Response 21 to 28 Days After Measles Vaccination With or Without Immunoglobulin[a]

Vaccination Regimen	Total Number	Seroconversion (%)	GMT[b]
EDMONSTON B VACCINE			
Alone	171 27[c]	96 –	– 1:208
Immunoglobulin, 0.02 mL/kg	185	99	1:96
FURTHER ATTENUATED VACCINE[d]			
Alone	121	99	1:56
Immunoglobulin, 0.1 mL total	89	95	1:32
Immunoglobulin, 0.2 mL total	452	98	1:32
Immunoglobulin, 0.02 mL/kg	193	95	1:24

[a]Neutralizing titer of 1:400/0.1 mL.
[b]Among 27 children vaccinated with Edmonston vaccine B alone, the postvaccination geometric mean titer (GMT) was 1:208.
[c]Complement fixation assay geometric mean antibody titer.
[d]Schwarz strain.
Modified from Krugman S, Giles JP, Jacobs AM, et al. Studies with live attenuated measles-virus vaccine. Am J Dis Child. *1962;103:353–363; Krugman S, Giles JP, Jacobs AM, et al. Studies with a further attenuated live measles-virus vaccine.* Pediatrics. *1963;31:919–928; and Hilleman MR, Buynak EB, Weibel RE, et al. Development and evaluation of the Moraten measles virus vaccine.* JAMA. *1968;206:587–590.*

TABLE 37.5 Antibody Response 28 Days After Attenuated and Further Attenuated Measles Vaccine

Vaccine	Total Number	Seroconversion (%)	GMT[a]
Edmonston B[b]	258	99	1:25
Schwarz[c]	250	98	1:16
Moraten[c]	273	98	1:16

[a]Hemagglutination-inhibition assay.
[b]Attenuated vaccine.
[c]Further attenuated vaccine.
GMT, geometric mean antibody titer.
Modified from Hilleman MR, Buynak EB, Weibel RE, et al. Development and evaluation of the Moraten measles virus vaccine. JAMA. *1968;206:587–590.*

cell-mediated immunity plays in natural infection,[65,204–207] it would seem that successful vaccination would stimulate such immunity. Cell-mediated immune responses after live attenuated vaccine appear to be similar to but less pronounced than those after natural infection.[204–207,543] For example, Gallagher and colleagues[83] reported that a positive lymphocyte stimulation index was noted in nine of nine subjects with natural immunity and in 10 of 16 vaccinees. Pabst and colleagues[544] found good correlation between antibody titers and lymphoproliferative responses in 124 children receiving their first dose of MMR. However, a study of the immune response to measles vaccination at 9 months of age among 55 Peruvian children found that 93% developed a humoral response, and only 23% had lymphoproliferative responses.[545] Gans and colleagues[546] studied the ability of infants ages 6, 9, or 12 months to respond to measles vaccine and found that, unlike humoral responses, T-cell responses can be established despite the presence of passive antibodies. Outbreak investigations have documented vaccination before the first birthday as a risk factor for vaccine failure,[547–550] suggesting that vaccine-induced cell-mediated immune responses in young infants may not be protective. Ward and colleagues[551] found good lymphoproliferative responses after revaccination, even among those whose antibody titers dropped to low levels. It appears that both wild-type virus and vaccine infection result in a biphasic immune response, beginning with transient production of interleukin-2 and interferon-γ, followed by more sustained production of interleukin-4. First, $CD8^+$ T cells are activated, which is important for viral clearance. Later, beginning about the time of rash onset, $CD4^+$ T cells are activated and are involved in antibody production.[65] These responses by the cell-mediated immune system correspond to an initial T-helper 1 (Th1)-type response with a shift to a Th2-type response.[65]

Vaccination suppresses cell-mediated immune function (as does natural infection).[114,543,552–556] This manifests in vitro as suppression of lymphocyte stimulation or in vitro as suppression of cutaneous delayed hypersensitivity to various antigens. Fireman and colleagues[553] noted suppressed cellular immune function up to 4 weeks after administration of live vaccine. Suppression did not occur after receipt of killed vaccine. A study of Bangladeshi infants found that delayed-type hypersensitivity reactions to *Candida* antigen were significantly reduced at 6 weeks postvaccination.[557] Data suggest that this suppression is the result of downregulation of interleukin-12, which is needed for cell-mediated immunity.[558] Although there was concern initially that this temporary suppression of cellular immunity might be harmful (e.g., in patients with unrecognized tuberculosis[115]), no increased risk has been documented.[558,559]

One would assume that the presence of a rash after parenteral injection of vaccine would be associated with viremia. The generalized stimulation of T and B lymphocytes after vaccination also suggests viremia. Few studies documented viremia after vaccination. Early studies with the canine cell vaccine isolated vaccine virus from blood,[434–436] and, more recently, van Binnendijk and colleagues[560] have isolated Schwarz strain vaccine virus from monkeys 7 to 9 days after vaccination. There are no reports of isolation of vaccine virus from blood in normal humans.[11–13,18,51] Although Mitus and associates[197] did isolate vaccine virus from the throat and conjunctivae of a susceptible leukemic patient who died of giant cell pneumonia after administration of Edmonston B vaccine, they failed to isolate virus from the blood. The apparent difficulty of isolating vaccine virus may reflect the low level of viremia after vaccination.

Because wild-type virus is so highly transmissible, both virologic and clinical studies with susceptible contacts were conducted in early vaccine investigations.[11,13–18,51,434,436] These studies showed no evidence of virus excretion or transmission by vaccinees. Measles vaccine virus was isolated from a throat swab taken from a 3-year-old boy who presented with fever only 12 days after vaccination with MMR vaccine.[561] Measles vaccine virus was detected by PCR in a throat swab and urine sample from a 14-month-old infant with a postvaccination rash without fever.[562] These case reports indicate that subcutaneous vaccination with live attenuated measles virus can result in respiratory excretion of the vaccine virus. However, person-to-person transmission of vaccine virus has never been documented.

Response to Revaccination

The immune response after revaccination depends on the results of the initial vaccination. Persons who have no response to initial vaccination typically generate a primary immune response to revaccination, with a significant rise in antibody titer and the production of IgM antibody. After revaccination of a person with some level of immunity, a fourfold or greater rise in antibody may appear sooner than that seen after initial vaccination, but there are usually no clinical signs of infection.[72,308,309,311,313,532,533,540,563] IgG antibodies are first detected within 5 to 6 days and peak around 12 days. IgM antibodies are not usually detected. As is the case with immunity after natural infection, these are the characteristics of an anamnestic immune response.[310,312] Such a boost is more likely to occur in the presence of a low or undetectable preexisting antibody titer, whereas persons with a high level of circulating antibody may not boost.[72,308,309,526,538–540,564] Krugman and colleagues[72] reported that, after revaccination, a significant increase in HI titer occurred in only one of six children with HI titers of 1:16 or 1:32, but in 25 of 36 children with titers of 1:8 or less. Similar findings were noted in vaccinees exposed to wild-type virus.

Boosting of antibody titers appears to be transient, with several investigators finding decay of antibody levels to the prerevaccination level within months to years.[546,565–567] In one study, antibody titers against measles in children with low levels of antibody before revaccination decayed during a 6-month period after revaccination; however, cellular immunity, measured in a lymphocyte proliferation assay, appeared to persist.[551]

The booster phenomenon is not unique to vaccine-induced immunity. It can also be observed after exposure to measles or after vaccination in persons who have had measles, but it occurs less frequently because antibody titers after natural infection are usually higher than those after vaccination.[70,72] Stokes and colleagues[70] reported that, on reexposure to wild-type virus, 6 of 12 naturally immune persons with preexposure Nt titers ranging from 1:2 to 1:8 experienced a boost in titer. However, such boosts were not seen in any of 22 persons with titers between 1:16 and 1:128. These data indicate that subclinical reinfection may occur after both natural infection and successful vaccination.

Studies of the response to revaccination[546,568–570] show that a high proportion of vaccinated persons who lack detectable antibody to measles will respond to the second dose. Among persons initially vaccinated after 12 months of age, at least 95% will respond to the second dose.

Effectiveness of Protection

Correlates of Protection

The most direct method of measuring the personal immunity effect of measles vaccine would be to challenge vaccinees and controls with wild-type measles virus and document the

clinical outcome. Such studies are not done in humans because of the harmful effects of exposure to measles virus. In studies in animals challenged with wild-type measles virus, vaccinated animals have significant decreases in clinical symptoms and measles virus replication compared with unvaccinated animals.[571] These studies are performed to evaluate the response to new vaccines in terms of clinical protection, reduced viral load, and development of serologic and cellular immunity.[571–573]

Because tests of cell-mediated immunity to measles are less widely available, antibody titers are most often used as evidence of protection from measles (i.e., as a correlate of protection). Although any detectable level of measles antibody has been interpreted as evidence of protection after vaccination, the development of more sensitive antibody tests[318] has raised concerns that low levels of antibody[532] may not be protective.

Plaque reduction Nt antibody titers at the time of exposure to virus have been most closely correlated with immunity against measles. In one study, no one with a titer of 120 or greater at the start of an outbreak developed measles.[574]

Eight of nine students with low but detectable levels of measles antibody immediately before the outbreak (plaque reduction neutralization [PRN] titer ≤120) developed typical measles compared with none of 71 with preexposure PRN titers greater than 120. Seven of 11 students with preexposure PRN titers of 216 to 874 had a fourfold or greater rise in antibody titer compared with none of seven students with a preexposure PRN titer of 1052 or greater, indicating that high titers were associated with protection against both infection and disease. Although many persons with low antibody titers may not develop disease after exposure, available data suggest that these low levels of antibody may not be fully protective.

Protection also can be evaluated by examining the immune response of vaccinees challenged with vaccine virus through revaccination.[189,309,540,560,565,575,576] Such challenges most often result in an anamnestic immune response (see "Response to Revaccination" above).

Efficacy and Effectiveness

The most commonly used method to quantify the protective effect of measles vaccine is to identify persons with similar likelihood of exposure to measles virus and compare the attack rate of disease in unvaccinated and vaccinated persons. The decrease in attack rate among vaccinated compared with unvaccinated persons is called vaccine effectiveness or vaccine efficacy.[577,578] (Refer to Chapter 77 for a more detailed discussion of this topic, including formulas and methods for calculating vaccine effectiveness.) In outbreak settings in which a high proportion of the population is vaccinated, the proportion of cases that are vaccinated will also be high, and it may appear erroneously that the vaccine effectiveness is low.[579,580] However, approximately 50% of cases can be expected to be vaccinated, with vaccine effectiveness and vaccine coverage each 90%.[577] This proportion of cases will increase to 60% with a 95% coverage rate. The majority of available data indicate vaccine effectiveness after a single dose in the United States of 90% to 95% or greater,[581] consistent with seroconversion data.

Reduction in the occurrence of measles after the introduction of vaccine is a common measure of vaccine-induced protection at the population level. Both Krugman and colleagues[307] and Baba and colleagues[582] noted virtual elimination of measles from a population of institutionalized children after vaccination became routine, despite high levels of infection in the surrounding community. Similarly, nationwide surveillance in many countries with high levels of immunization has documented a significant reduction in reported measles cases after vaccine licensure, and many have eliminated endemic transmission of measles.[22–41] For more examples of the impact of measles vaccination on measles incidence, see "Experiences with Measles Control and Elimination in Various Countries" later in this chapter.

Monitoring the reproductive rate, or R value, is another method of assessing the impact of vaccination on a population. The basic reproductive rate (R_0) is the average number of cases that would be expected to spread from a single case of measles in a completely susceptible population. Measles is a highly infectious disease, and R_0 has been estimated to be 12 to 18.[583] The effective reproductive rate (R) incorporates the level of immunity in a population with the factors that determine R_0. In a completely susceptible population, $R = R_0$. In other populations, the difference between R and R_0 is the effect of population immunity. When R is greater than 1, the number of cases increases from one generation to the next and an epidemic begins. As the spread of disease increases immunity in the population, R falls below 1, the number of cases decreases from one generation to the next, and the epidemic ends. To achieve interruption of endemic transmission of measles, it is necessary to achieve a level of population immunity that maintains R less than 1 (see Chapter 77). In the United States, assessment of the reproductive rates through surveillance data documented R less than 1 for the period from 1992 to 1999.[584] In Canada, an analysis of reported measles cases between 2002 and 2013 enabled estimation of the effective reproductive number at 0.86 (95% CI, 0.81–0.92) which was consistent with maintenance of measles elimination.[585]

Population protection from measles can be evaluated without waiting for an outbreak to identify susceptible groups by using tools such as population surveys of measles antibody levels and by mathematical modeling of population immunity. Although antibody levels are a very useful measure of measles immunity, a proportion of persons who do not have detectable measles antibody may have a level of protection via cellular immunity. In a national serosurvey conducted from 1988 to 1994, Hutchins[586] reported that overall only 7% of persons older than 6 years of age in the United States lacked serum IgG antibodies to measles. However, 19% of those born between 1967 and 1976 were seronegative.[586] This time frame represents the early implementation phase of measles vaccination in the United States, when high coverage levels had not been achieved, yet the incidence of measles was significantly decreased; therefore, a proportion of children grew up without getting measles or vaccination. This pocket of seronegativity has not been a major focus of measles transmission; however, it may have a significant impact on the amount of antibodies mothers born in these years pass on to their infants.[586] In follow-up surveys conducted 1999–2004 and 2009–10 in the United States, the lowest levels of seropositivity were again found in persons born in early vaccine implementation years of 1967 to 1976.[587,588] Serosurveys in some other countries have demonstrated low levels of seropositivity in persons born in the early vaccine implementation years.[589,590] Serosurveys have also been used to plan vaccine strategies or assess their impact.[591–593] More recently, oral fluid surveys have assessed population IgG antibodies to measure the impact of mass campaigns on population immunity and susceptibility in Kenya and Ethiopia.[594–596]

Mathematical modeling can be used to compile and interpret the factors related to measures of vaccine protection, including patterns of transmission, vaccination coverage estimates, measles antibody surveys, and effects of primary and secondary vaccine failure.[596–600]

Host Factors Affecting Protection

The quality and durability of measles vaccine-induced immunity is dependent on a number of factors that relate both to the vaccine and to the host.[308,601] In considering factors affecting protection, it is important to distinguish between primary vaccine failure, that is, a failure to seroconvert after vaccination, and secondary vaccine failure, that is, loss of protection after demonstrated seroconversion.

Maternal Antibody and Age at Vaccination

A number of host factors may be responsible for primary vaccine failure. The most important and well described is maternal antibody. Passively acquired measles antibodies may neutralize vaccine virus before a complete immune response develops. The most common sources of these antibodies are maternal transfer, Ig, and other blood products. The presence of maternally derived transplacental antibody is particularly important in evaluating the immunogenicity of live measles vaccine in early childhood.[327,602] The effect of maternal antibodies in breast milk on response to measles vaccination is unknown.

As noted by Orenstein and colleagues,[602] recommendations for the age at vaccination must balance two factors: (a) the earliest age at which high rates of seroconversion can be obtained and (b) the age group with the greatest risk of severe infection. A balance must be met that optimizes vaccine-induced protection while minimizing the risk of morbidity and mortality that would occur by delaying vaccination.[327,379] The age at vaccination that achieves this balance is lower in developing countries than in developed nations because of the increased risk of measles exposure among infants and because of earlier loss of maternally derived antibody.[603,604] The reasons for the variation in duration of passive protection from maternal antibody include differences in (a) levels of measles antibody in mothers, (b) efficiency of transport of IgG across the placenta, and (c) rate of loss of passively acquired antibody by the infant.[327,605,606]

Early in the clinical investigations of live measles virus vaccine, it was recognized that maternal antibody interfered with seroconversion by in vitro Nt of vaccine virus before adequate replication had occurred.[601] On the basis of data available at the time, it appeared that maternal antibody rarely persisted beyond 7 months of age and that an adequate immune response could be achieved if vaccination was limited to infants 9 months of age and older.[70,72,528,530,607] Whereas only 60% to 70% of infants younger than 9 months seroconverted, 95% or more of older infants produced antibodies.[21,602] Accordingly, when vaccine was licensed in 1963 in the United States, the recommended age for routine vaccination was 9 months.[608]

In the first few years after licensure, it became apparent, however, that maternal antibodies actually persisted in many infants until 11 months of age.[72,527,528,531] On the basis of these data, vaccination before age 1 year would be expected to be associated with a high risk of primary vaccine failure and subsequent infection in exposed vaccinees.[185,189,530,609–612] Thus, in 1965, the recommended age for vaccination was raised to 12 months.[467] The importance of this change is illustrated by Krugman's[613] data showing that only 86% of 123 infants 9 months of age seroconverted after administration of Edmonston B vaccine and Ig, compared with 97% of 899 children vaccinated at 12 months of age or older.

In 1965, it was also recommended that children vaccinated before 1 year of age be revaccinated because a large proportion of these children were susceptible and were expected to respond well to revaccination. A number of studies confirmed these findings,[540,609,610,614] but there are also some data indicating that early vaccination may alter the immune response after revaccination. Wilkins and Wehrle[530] first raised concerns by reporting that 19 (51.4%) of 37 children who did not respond when they were initially vaccinated at 6 to 10 months of age did not have detectable HI antibodies 8 months after revaccination. All 37 did, however, have detectable Nt antibody. Similarly, Linnemann and colleagues[615] reported that 29 (40.3%) of 72 children vaccinated before 10 months of age were HI-negative at a mean of 4.8 years after revaccination. In contrast, Lampe and colleagues[576] did not find any difference in seroconversion rates in children vaccinated once at 15 months of age or later, compared with children revaccinated after first being vaccinated before 1 year of age. By using an EIA assay, Murphy and colleagues[616] observed high seroprevalence rates at a mean of 6 months after vaccination in 302 children revaccinated after early vaccination and in 300 vaccinated once at 15 months of age or older (98% seroprevalence rate for both groups). However, they did observe that the titers in the revaccinated group were lower than those in the children vaccinated once. These findings were confirmed by McGraw.[473]

One of the most complete descriptions of an altered immune response is provided by Stetler and colleagues.[533] These authors reported that children revaccinated after an initial vaccination before 1 year had no difficulty seroconverting; postrevaccination HI antibody was detected in 116 (95.9%) of 121 children lacking HI antibody before revaccination. Eight months later, HI antibodies were undetectable in 58 (47.9%) of 121 children, but, after retesting with a cytopathic effect neutralization (CPEN) test, only 5 (4.2%) of 120 sera were negative. Successful priming in some of the early vaccinees was also suggested by the finding that IgM was detected in the sera of only 14 (22.2%) of 63 revaccinated children lacking HI, EIA, and CPEN antibody, compared with 37 (74.0%) of 50 random control vaccinees. These findings were similar to those noted by Black and colleagues.[575]

Although there may be some alteration of the immune response, the most reliable indicator of the actual effectiveness of revaccination of these children is the evaluation of their risk of infection. Although revaccination may not provide 100% protection, available data indicate that revaccination is effective.[610,614] Shasby and colleagues[609] reported that, in an outbreak, the attack rate in 73 children vaccinated once before 12 months of age was 35.6% (26 of 73), whereas the attack rate in 55 children revaccinated after 12 months of age was only 1.8% (1 of 55). Davis and colleagues[614] noted that none of 80 students revaccinated after their first vaccination before 12 months of age became infected during an outbreak. For comparison, the attack rate in children vaccinated twice at 12 months of age or older was 1.4% (2 of 138), that in children vaccinated once at 12 months of age or older was 1.8% (21 of 1191), and that in unvaccinated children was 57.1% (4 of 7). Hutchins and colleagues[617] reported a vaccine effectiveness of 99.5% for children vaccinated with a first dose at age 6 to 11 months and a second dose after age 12 months. Thus, the available data indicate that revaccination of children first vaccinated before 1 year of age will result in good vaccine-induced protection, although it may be associated with an altered immune response, manifested as a lower antibody titer.[618,619] This conclusion is especially important because vaccination of U.S. children as young as 6 months, with subsequent revaccination, is recommended in certain outbreak situations and for children traveling internationally.[233,234]

The recommended age at vaccination was again changed in 1976 from 12 months to 15 months because newer data

indicated that children vaccinated at 15 months of age and older were even more likely to make and maintain antibodies and were less likely to be infected in outbreak situations than those children vaccinated earlier.[602] Although some studies provide contrary results, examination of data on seroconversion rates and prevalence of antibodies after vaccination indicates that, in general, 79% to 89% of children vaccinated at 12 months of age have detectable antibodies, compared with 87% to 99% of those vaccinated at 15 months or later.[527–529,531,602,609,620–622] Similarly, a number of studies measuring the risk of measles in vaccinated and unvaccinated children indicated that measles occurred in children vaccinated at 12 months of age approximately 1.5 to 5.0 times more frequently than in those vaccinated later than 12 months.[547–550,602,609,610]

Data on measles antibody titers in umbilical cord blood and in infants suggest that infants whose mothers have vaccine-induced immunity may be receiving less maternal antibody than infants whose mothers had natural measles.[544,622–627] Many mothers born in the vaccine era have antibodies induced by vaccination, which typically results in lower titers than does measles disease. Also, an increased proportion of mothers born since the introduction of measles vaccine may not have any measles antibodies because they have not had measles or been vaccinated. Serologic surveys show that up to 19% of women of childbearing age in the United States, born between 1967 and 1976, are measles seronegative.[586,587] Infants born to seronegative mothers would lack maternal antibody from birth onward. In 2009, greater than 99% of women giving birth in the United States were born after 1963, the year measles vaccine was licensed.[628]

Infants whose mothers were born in the vaccine era are susceptible to disease at a younger age and are more likely to respond to vaccination at an early age. In a 1992 cohort study in the United States, Papania and colleagues[629] documented a greater than 2.5 RR of measles among infants whose mothers were born in the vaccine era compared with infants whose mothers were born before 1963. Higher rates of seroconversion at 12 months also have been found in children born to women with vaccine-induced immunity.[623–625] Based on these findings, in 1994, the recommended age of vaccination in the United States was changed again to between 12 and 15 months.[234]

Factors that alter the efficiency of transport of IgG across the placenta also can affect the amount of antibody infants receive. Maternal measles antibody is transferred across the placenta from mother to infant via an active transport system, which results in a higher antibody titer in the infant than in the mother. Because the majority of the maternal measles antibody transfer occurs late in the third trimester, infants born prematurely often have low antibody titers.[630] Several diseases that affect the mother, most importantly HIV infection and possibly malaria, have been shown to decrease the placental transfer of maternal measles antibody and result in lower infant titers.[631,632]

Because infants in developing countries may lose maternal measles antibody at an early age and may be exposed to measles virus at an early age as a result of intense transmission, the WHO recommendations indicate that 9 months is the most appropriate age for delivery of the first dose of measles vaccine in most developing countries (see "Indications" below).[633]

Studies of early measles vaccination regimens have found that infants 6 months of age had lower seroconversion rates and geometric mean titers than older infants or toddlers. This effect was evident even among 6-month-old infants who lacked maternal measles antibody, suggesting an age-related delay in maturation of humoral immunity unrelated to passively transferred maternal antibody.[634] In contrast, markers of cell-mediated immunity (e.g., T-cell proliferation) were equivalent after vaccination at 6, 9, or 12 months, regardless of the presence of passive antibodies. In addition, infants initially vaccinated at 6 or 9 months experienced increases in both humoral and cell-mediated responses after administration of a second dose. Although the cell-mediated immunity resulting from a dose at 6 months alone may not be protective, early vaccination may prime the humoral response to the second dose, thereby providing clinical benefits.[635]

Intercurrent Illness and Malnutrition

There is a theoretic concern that interferon produced by an intercurrent infection may interfere with successful vaccination.[539,636] Studies addressing this question, which have been conducted in developing and in developed countries, have produced discordant results. Two studies in developing countries found no differences in seroconversion rates in ill and well children after receipt of measles vaccine.[390,637] A small study in the United States found that 80% of children with rhinorrhea seroconverted, compared with 98% of well children.[638] Subsequent studies conducted in the United States and Canada found equivalent seroconversion rates among children with upper respiratory infections or mild illness and well children.[639–641] In a study of 128 mildly ill and 258 well children given MMR, seroconversion to measles was 97% in well children compared with 99% in those with mild illnesses, mostly upper respiratory tract infection[641]; no differences were observed in seroconversion to mumps or rubella. A study conducted in Wisconsin found no association between vaccine failure and vaccination during the high-risk season for respiratory infections.[642] One study conducted in Thailand found lower geometric mean antibody titers for children vaccinated at 9 months of age among those who experienced an upper respiratory tract infection in the 2 weeks after vaccination.[643] However, the benefits of vaccinating mildly ill children outweigh any theoretic risk of lower seroconversion rates.[644] Several studies found seroconversion rates in malnourished children similar to those in children who are well nourished.[389,390,645,646]

Immunosuppression and HIV Infection

Measles vaccination is not recommended for most immunocompromised patients, and as a result, few data are available on the immune response in such patients. However, the increasing number of children with HIV infection, the high risk of severe measles in these children, and the limited side effects to measles vaccine found in HIV-infected children have resulted in recommendations for vaccination. Studies in the United States found that HIV-infected children have poor responses to vaccination, lose antibody more quickly after vaccination, and respond poorly to revaccination.[200,647–650] Retrospective studies found that only 3 (12.5%) of 24[200] and 22 (59.5%) of 37[648] vaccinated children with HIV infection had detectable measles antibody. Seroconversion rates in prospective studies range from 13 (33.3%) of 39 children to 15 (60%) of 25 children.[211,647] In the Democratic Republic of Congo, seroconversion rates after vaccination at 9 months were 77% in asymptomatic HIV-infected children and 36% in symptomatic HIV-infected children.[651] In two other studies in Africa, seroconversion rates after vaccination at 6 months were greater than 75% in HIV-infected children.[652,653] A study in Thailand found that by age 9 months, 100% of 30 infants of HIV-infected mothers had lost their maternally acquired antibodies

and that by 12 weeks postvaccination, HIV-positive infants had lower seroconversion and median antibody levels when compared with HIV-negative infants.[654] A recent study in Zambia found 88% of HIV-infected infants vaccinated at 9 months of age developed protective antibody levels; by 27 months after vaccination only 50% of children who survived had maintained protective antibody titers.[644] HIV-infected children may respond better when they are vaccinated at earlier ages, before they become severely immunocompromised.[655,656] In sub-Saharan Africa where the burden of pediatric HIV infection is greatest, antiretroviral treatment programs have scaled-up dramatically, increasing access to life-prolonging treatment for HIV-infected children. Revaccination with measles-containing vaccine (MCV) after immune reconstitution with highly active antiretroviral therapy (HAART) should be recommended to protect these children against measles, and ensure high levels of population immunity (see "Persons Infected with HIV" below).[657]

Adults who were vaccinated in childhood before becoming infected with HIV appear to retain protective levels of measles antibody. High seroprevalence of measles antibody in HIV-infected adults in the United States has been documented,[658] and antibody levels are maintained, even as immunosuppression progresses. In one study, measles seronegative HIV-infected adults who were vaccinated while on HAART (HAART) responded to measles vaccine but lost antibody rapidly after vaccination.[659]

Vitamin A Supplementation

Investigators in Indonesia found a lower rate of seroconversion among children vaccinated at 6 months of age who received supplementary vitamin A compared with children not receiving such a supplement.[660] Subsequent studies conducted in Guinea-Bissau, India, and West Java found similar rates of seroconversion among 9-month-old children receiving and not receiving vitamin A supplements.[661–663] Among malnourished children in the Indian study, the postvaccination geometric mean antibody titer was significantly higher in the group receiving vitamin A compared with the placebo group.[662] A large multicenter clinical trial confirmed the safety of vitamin A supplementation administered with routine immunizations at 6, 10, and 14 weeks of age and at 9 months with measles immunization.[664] The balance of evidence supports the WHO recommendation that, in vitamin A–deficient countries, infants should receive a vitamin A supplement at the time of measles immunization.[665]

Other Host Factors

Even after receipt of potent vaccine, approximately 2% to 5% of vaccinees will fail to respond for unknown reasons.[21,72,307] A group of investigators at the Mayo Clinic has explored the role of genetic factors and found associations with particular human lymphocyte antigen (HLA) types and nonresponse or hyperresponse to measles vaccination.[666] A study of monozygotic and dizygotic twins found a high degree of heritability of measles antibody level and subsequently showed associations between homozygosity for class I and class II HLA alleles and poor response to measles vaccination.[667] Of importance, two doses of MMR vaccine appear to induce sufficient antibody levels and lymphoproliferative responses against measles regardless of homozygosity status, suggesting genetically related nonresponse to the first dose is overcome by administration of a second dose.[668]

Although there may be some exceptions in the very young,[473,530,533,575] revaccination has been found in both epidemiologic and serologic studies to induce the same high rate of immune response that follows initial vaccination.[532,540,568–570,609,610,629]

Vaccine Factors Affecting Protection

Vaccine Antigen and Strain

Although most further attenuated measles vaccines are equally immunogenic in older children,[465,477] differences in the ability of vaccine strains to immunize young infants have been reported.[478,480,669–673] High measles morbidity and mortality in developing countries among infants younger than 9 months, the recommended age for vaccination,[674,675] has stimulated research on strategies for immunization of younger infants. In the early 1980s, Sabin and associates[477,478] investigated aerosol administration of two measles vaccines, the Edmonston-Zagreb and Schwarz strains. Their finding of higher seroconversion rates after Edmonston-Zagreb than after Schwarz vaccine focused attention on this vaccine strain. Several subsequent studies found that the Edmonston-Zagreb strain was more immunogenic than the Schwarz vaccine in this age group when given by injection (see "Vaccine Dose" below). The AIK-C strain of measles vaccine also has been found to be highly immunogenic in young infants and, in some studies, more immunogenic than either Schwarz or Edmonston-Zagreb vaccines.[676–678] The reasons for these differences are not known.

Vaccine Dose

Small doses of vaccine can effectively immunize older infants and children; however, the dose of vaccine administered is important in immunizing young infants.[669,670,672] Increasing the Edmonston-Zagreb vaccine dose from 10,000 to 40,000 plaque-forming units (PFU) in The Gambia resulted in an increase in response rate from 73% to 100% in 4- to 6-month-old infants.[669] In Mexico, serologic response to vaccination of 6-month-old infants increased for both Schwarz (66% to 91%) and Edmonston-Zagreb (92% to 98%) vaccines, with a 100-fold increase in dose[670]; the effect was greater for Schwarz than for Edmonston-Zagreb vaccine. Because of these data and interest in a vaccination strategy for children younger than 1 year of age in developing countries, in 1990 the WHO recommended that a higher dose (initially defined as >100,000 PFU and later changed to >50,000 PFU) of Edmonston-Zagreb vaccine be administered at 6 months of age in areas where measles mortality in young infants was a major health problem.[679] However, problems with vaccine availability resulted in little use of the vaccine on a large scale. Questions were raised regarding the safety of high-dose measles vaccine in several developing countries, with a higher all-cause mortality rate in girls (but not in boys) who received high-titer vaccines compared with those who received standard-titer vaccines.[678–682] Increased mortality rates after vaccination with a high-titer vaccine were not observed in developed countries or in countries with an infant mortality rate less than 100 per 1000 births.[683] High-titer measles vaccines are not currently recommended.[684]

Vaccine Handling

Vaccine failures with live vaccine have stemmed from improper handling. Loss of potency of live vaccines can result from poor shipping or storage practices.[685,686] Although administration of impotent vaccine had previously been implicated in outbreaks of measles in vaccinated persons, the likelihood of this occurring now is reduced because of the addition in 1979 of the stabilizers discussed previously.[471,519]

Persistence of Immunity

The majority of data suggest that a dose of live vaccine properly administered to an appropriate host that results in seroconversion will afford lifelong protection to nearly all vaccinees.[687] The duration of vaccine-induced immunity has been documented by studying the persistence of measurable antibody, the clinical characteristics and serologic response of measles cases in vaccinated persons, the effects of vaccine challenge, and the attack rate in vaccinees as a function of the time since vaccination.

Although vaccine-induced antibody titers are lower than those achieved after natural infection, this difference does not appear to result in decreased personal protection from measles. Serologic studies limited to children vaccinated at 12 months of age or older indicate that, although antibody titers do decline over time, detectable antibodies are present in most vaccinees.[72,308,309,525,526,529,532,534,536–538,542,688] Because titers can fall to levels undetectable with some assays, the test used is important in the assessment of immune status. Furthermore, many persons lacking detectable antibody manifest a secondary immune response on revaccination or exposure to wild-type virus, indicating persistence of some level of immunity.[316]

Krugman[72,308,309] followed the serologic status of a population of institutionalized children for 16 years. The HI geometric mean antibody titer in 70 persons who received further attenuated vaccine was 1:333 at 1 month but, in the absence of exposure, had fallen to 1:6 after 13 years (Fig. 37.7).[309] Thirteen percent had a HI titer of 1:4, 10% had a titer of 1:2, and 13% had no detectable antibody. In contrast, whereas 47 naturally infected children had a comparable 1-month HI geometric mean antibody titer of 1:410, 16 years after disease, the geometric mean titer was 1:22 (Fig. 37.7). Only 4% had a HI titer of 1:2, and none had undetectable antibodies. Sixteen sera with HI titers ranging from less than 1:2 to 1:4, obtained from persons vaccinated 6 to 15 years earlier, were retested with the more sensitive PRN assay[318]; the PRN titers ranged from 1:4 to 1:46.[309] Typical booster responses after revaccination of some of the HI-negative persons were also found.

Dine and associates[689] followed-up adults vaccinated in a vaccine trial as children in the 1970s. The 56 participants, who were all seropositive by HI tests after vaccination, all had detectable measles antibody by PRN at least 26 years after vaccination. PRN titers greater than 1:120 were found in 92% of the subjects, and the strongest predictor of lower titers was lower HI titers in the original study. None of the subjects had been revaccinated in the interim period or had had measles or known exposure to measles. Fewer than five cases were reported annually from Cincinnati, the study setting, over the 12 years before the follow-up study began. Therefore, boosting of antibody titers from exposure to wild-type measles virus was unlikely to play a major role in sustaining antibody titers in most of the study subjects.

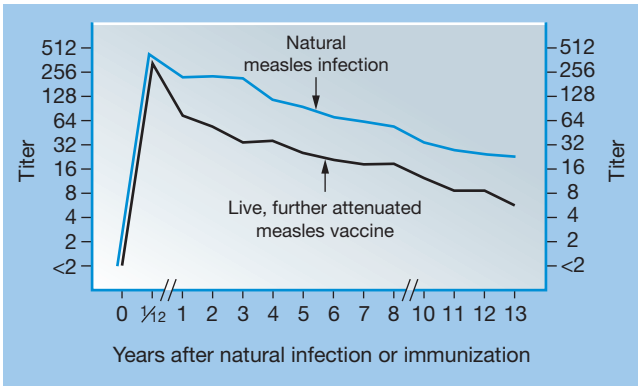


Figure 37.7. Measles geometric mean hemagglutination–inhibition antibody titers after natural infection and immunization with live, further attenuated vaccine. *(Courtesy S. Krugman.)*

Seroprevalence studies also provide useful information about duration of immunity, but, because information on seroconversion is lacking, one cannot be sure whether persons are seronegative because of primary or secondary vaccine failure. In addition, sensitivity of the assay procedure is important. These variables account for some discrepancies in reported findings. For example, Bass and colleagues[526] reported that HI antibodies were detectable in 29 (73%) of 40 children more than 8 years after vaccination; however, 39 (98%) of the 40 children had Nt antibody. Orenstein and colleagues[505] reported that the seroprevalence among 1871 high-school students (10th, 11th, and 12th graders), 1835 (98.1%) of whom had been vaccinated at 14 months of age or older and who had no history of measles, was 86.9% by an HI assay. However, 98.8% were positive with both the HI assay and the same PRN assay used in the Albrecht study.[318] Orenstein and colleagues[532] further documented the specificity of the PRN assay by vaccinating HI-negative children. IgM was detected in 14 (87.5%) of 16 students with a PRN titer less than 1:4, but in only 1 (1.5%) of 68 who were PRN positive.

Although live-virus vaccine-induced immunity typically provides lifelong protection, secondary vaccine failures occasionally result from waning immunity. Secondary vaccine failures are measles cases in persons who initially developed a protective immune response following vaccination which diminished over time to a nonprotective level. The most direct evidence of secondary vaccine failure comes from case reports of measles in persons whose post vaccination immunity was documented before the acute episode of measles.[192,194,690] In a study in Canada, 9 (5%) of 175 of persons who initially seroconverted after receipt of measles vaccine developed measles within 10 years of vaccination. In this study, persons who developed measles had lower postvaccination titers than those who did not.[192] Also, current laboratory analyses can be used to assess preexisting immunity at the time of acute measles onset. Absence of IgM antibody in cases confirmed virologically, IgG antibody at the time of rash onset, distinctively high levels of neutralizing antibodies and high IgG avidity all provide evidence of preexisting immunity. In vaccinated persons evidence of preexisting immunity indicates secondary vaccine failure[184,187–191,193,691,692] Some studies also suggest that the secondary vaccine failure cases can be mild and may be more likely to occur in persons vaccinated at younger than 12 months. In epidemiologic studies of persons vaccinated with live measles vaccine at 12 months of age or older, some studies show that persons with increased time since vaccination have slight increases in attack rates compared with those more recently vaccinated; however, none has yet documented a significant increase (Table 37.6).[548,609,610,612,626,693–695] During outbreaks, observed attack rates in persons vaccinated 15 years or more before infection have been on the order of 5% or less, and calculated vaccine effectiveness has been 90% to 95% or greater. These results are consistent with the expected frequency of primary vaccine failure that occurs in everyday practice. Data from the United Kingdom, the United States, and other countries[385,687,688,696,697] have not found significant increases in vaccine failures with time since vaccination. With overall incidence of measles in the United States at record low levels and no evidence of increasing incidence among

TABLE 37.6 Epidemiologic Studies of Duration of Measles Vaccine–Induced Immunity

	Attack Rate (%) by Years Since Vaccination[a]			
Study	**0-4 y**	**5-9 y**	**>10 y**	**≥15 y**
Shasby et al[609,b]	9.4 (3/32)[c]	6.9 (7/101)	5.4 (8/52)	–
Nkowane et al[610,d]	0 (0/18)	1.1 (1/21)	1.4 (10/158)	–
Davis et al[614]	1.1 (2/187)	1.7 (11/661)	2.6 (8/308)	0 (0/35)
Marks et al[612,e]	4.0/1000	4.2/1000	5.4/1000	11.7/1000
Hutchins et al[693]	0 (0/33)	0 (0/143)	2.2 (12/549)	3.1 (6/192)
Robertson et al[956]	0 (0/2)	1.4 (1/74)	3.4 (10/292)	0 (0/3)
Guris et al[695]	11.8 (2/17)	0 (0/7)		18.2 (2/11)
Lynn et al[694,f]	0 (0/40)	0 (0/46)	1.0 (18/1803)	0.3 (1/357)

[a]Single vaccination at age ≥15 months except for Shasby, Nkowane, and Davis (≥12 months).
[b]Years since vaccination: 5 to 8 and 9 or more.
[c]Number ill/total number.
[d]Projected from a 25% random sample.
[e]Cases per 1000 person-weeks at 0 to 3 (2 of 499), 4 to 6 (6 of 1420), 7 to 9 (5 of 929), and 10 to 12 (4 of 343) years since vaccination.
[f]Years since vaccination: grouped 0–4, 5–8, ≥9, >15 years.
Modified from Markowitz LE, Preblud SR, Fine PE, et al. Duration of live measles vaccine-induced immunity. Pediatr Infect Dis J. *1990;9:101–109.*

previously vaccinated persons, waning immunity does not appear to constitute a public health problem in the United States. Although secondary vaccine failures have been documented, taken collectively, the serologic and epidemiologic data during the past 50 years indicate that vaccine provides long-term immunity. However, increased measles transmission among adults has been reported in several countries, including China. Serologic studies show low seroprevalence in some adult age groups. It will be important to determine what portion of serosusceptibility, and of measles cases, is the result of failure to vaccinate, versus primary or secondary vaccine failure in populations that have different vaccine schedules with different measles vaccines.[698]

Combined Vaccines and Simultaneous Vaccination

Live measles vaccine has been administered successfully in combination or in conjunction with a variety of immunizing agents, such as yellow fever vaccine, poliovirus vaccine, diphtheria and tetanus toxoids and whole-cell pertussis vaccine (DTP), meningococcal vaccine, hepatitis B vaccine, and smallpox vaccine.[496,499,503,699–708] There is one report of interference with the measles antibody response after simultaneous administration of meningococcal A and C vaccines,[704] although a different team of investigators did not find interference with simultaneous administration of measles vaccine and meningococcal A vaccine.[703]

In developed countries, measles vaccine is administered today along with rubella and mumps vaccines as a combined vaccine (MMR) and as part of routine childhood immunization programs.[233,234] Use of MMR vaccine instead of single-antigen measles vaccine increases the benefit-to-cost ratio of the measles vaccination program in the United States from 17.2 : 1 to 21.3 : 1.[404] The varicella vaccine was included in the routine childhood schedule in 1996, licensure of a combined MMRV vaccine occurred in 2005, and the ACIP recommendation for a routine second dose of varicella vaccine occurred in June 2006.[709] Thus, in the United States, both recommended doses of measles vaccine could be administered as MMRV. However, given the increased incidence of febrile seizures with dose one of MMRV, most such doses are likely to be MMR.[510,511] MMR vaccine is also frequently administered to susceptible adults.

Studies consistently show that combinations of measles, mumps, and rubella antigens, regardless of the virus strain, elicit the same high rates of seroconversion seen with each component individually and that there is no increased risk of reactions in persons susceptible to all three antigens (Tables 37.7 and 37.8).[488–505] Furthermore, vaccination of persons already immune to one or more of the antigens, from either previous vaccination or infection, is not associated with any increased risk of vaccine-associated adverse events.[705,710–714] Although there are reports of directly mixing measles vaccine with DTP,[714] this should not be done routinely. Rather, the vaccines should be administered in separate syringes and at separate sites.[715]

Immunization against varicella along with measles, rubella, and mumps, either as two vaccines (varicella and MMR) or as a quadrivalent vaccine (MMRV), has been studied.[716,717] Seroconversion rates have been reported to be 95% or greater to all antigens, although titers against varicella were lower among persons receiving the quadrivalent vaccine. A second dose of MMRV administered 6 to 8 weeks after the first dose resulted in greater than or equal to 98% seropositivity for all four antigens.[506] Concomitant administration of MMRV, *H. influenzae* b/hepatitis B (Hib/HepB), and diphtheria-tetanus-acellular pertussis (DTaP) vaccines was found to be immunogenic and well tolerated.[507]

Postexposure Prophylaxis

Routine vaccination before exposure to measles provides the best protection against measles. However, persons without measles immunity who are exposed to measles can receive some protection through measles vaccination or Ig administration after exposure.

Use of Vaccine

Exposure to measles is not a contraindication to measles immunization. Available data suggest that live virus measles vaccine, if given within 72 hours of measles exposure, will prevent or modify disease.[447,559,718] Also, the vaccine should induce protection against subsequent measles exposures

TABLE 37.7 Antibody Response After Administration of Measles Vaccine Alone, in Combination With Mumps and Rubella Vaccines, and in Combination With Mumps, Rubella, and Varicella Vaccines

Vaccine	Total Number	Measles		Mumps		Rubella	
		%	GMT	%	GMT[a]	%	GMT
Measles[b]	23	100	82	–	–	–	–
Measles,[b] mumps,[c] rubella (RA27/3)	91	96	89	90	31	100	301[d]
Measles,[b] mumps,[c] rubella (HPV-77:DE-5)	85	99	77	89	15	99	144[d]

	Total Number	Measles		Mumps		Rubella		Varicella	
		%	GMT[e]	%	GMT[e]	%	GMT[e]	%	GMT[f]
Measles,[b] mumps,[c] rubella,[g] varicella[h]	2,331–2,532	97.1	2985.0	96.0	95.9	98.8	89.2	93.5	17.9

[a]Indirect immunofluorescent assay geometric mean antibody titer.
[b]Moraten strain.
[c]Jeryl Lynn strain.
[d]Hemagglutination–inhibition assay geometric mean antibody titer.
[e]Enzyme-linked immunosorbent assays (ELISA) measured 6 weeks after vaccination; mIU/mL for measles, ELISA antibody (Ab) units for mumps, and ELISA Ab units for rubella.
[f]Glycoprotein antigen-based ELISA (gp ELISA) units/mL measured 6 weeks after vaccination.
[g]RA27/3 strain.
[h]Oka/Merck strain.
GMT, Geometric mean titer.
Modified from Lerman SJ, Bollinger M, Brunken JM. Clinical and serologic evaluation of measles, mumps, and rubella (HPV-77:DE-5 and RA27/3) virus vaccines singly and in combination. Pediatrics. *1981;68:18–22 (top of table); and Lieberman JM, Williams WR, Miller JM, et al. The safety and immunogenicity of a quadrivalent measles, mumps, rubella and varicella vaccine in healthy children: a study of manufacturing consistency and persistence of antibody.* Pediatr Infect Dis J. *2006;25:615–622 (bottom of table).*

TABLE 37.8 Proportion of Children With Fever and Rash After Administration of Measles Vaccine Alone, in Combination With Mumps and Rubella Vaccines, and in Combination With Mumps, Rubella, and Varicella Vaccines[a]

Vaccine	Total Number	Fever ≥39.4°C (%)	Rash (%)
Measles[b]	43	5	12
Measles,[b] mumps,[c] rubella (RA27/3)	141	11	20
Measles,[b] mumps,[c] rubella (HPV-77:DE-5)	142	8	17
Placebo	42	0	9
		Fever ≥38.9°C [Total (%)]	**Rash [Total (%)]**
Measles,[b] mumps,[c] rubella,[d] and varicella[e] given separately		982 (33.1[f])	1000 (4.5)
Measles,[b] mumps,[c] rubella,[d] varicella[e]		2839 (39.1[f])	2877 (5.7)

[a]During the 6 weeks after vaccination.
[b]Moraten strain.
[c]Jeryl Lynn strain.
[d]RA27/3 strain.
[e]Oka/Merck strain.
[f]$P = .001$. For fever measured 5–12 days after vaccination: 15.6% for measles, mumps, rubella (MMR) and varicella vaccine recipients vs 22.8% for measles, mumps, rubella, and varicella combined vaccine recipients ($P < .001$).
Modified from Lerman SJ, Bollinger M, Brunken JM. Clinical and serologic evaluation of measles, mumps, and rubella (HPV-77:DE5 and RA27/3) virus vaccines singly and in combination. Pediatrics. *1981;68:18–22, 1981 (top of table); and Lieberman JM, Williams WR, Miller JM, et al. The safety and immunogenicity of a quadrivalent measles, mumps, rubella and varicella vaccine in healthy children: a study of manufacturing consistency and persistence of antibody.* Pediatr Infect Dis J. *2006;25:615–622 (bottom of table).*

should the initial exposure fail to result in measles and protection. Therefore, vaccine given within 72 hours of measles exposure is the intervention of choice for persons older than 12 months of age who are exposed to measles in most settings (e.g., healthcare facilities, schools, colleges, healthcare facilities), unless contraindicated (see "Precautions and Contraindications" below). Infants 6 to 11 months of age may receive measles vaccine instead of Ig if the initial exposure is detected within 72 hours. Infants vaccinated before age 12 months must be revaccinated on or after the first birthday with two doses of MMR vaccine separated by at least 28 days (see "Indications" below).

Use of Immunoglobulin

Guidelines for Ig dosage and use in measles postexposure prophylaxis should be driven by local measles epidemiology, population immunity, and Ig product antibody levels. Current U.S. recommendations are to give human IgIM or IgIV to susceptible persons exposed to measles and for whom vaccine either is contraindicated or was not given within 72 hours of initial exposure. The United Kingdom has similar guidelines.[719] Measles is infectious during the prodrome and often is not diagnosed until the rash onset. Therefore, many exposed persons are not identified until more than 72 hours after

initial exposure, which is too late for prophylaxis with measles vaccine. Human Ig provides measles antibodies to prevent or modify the disease when given up to 6 days after exposure.[179–181,410] However, any immunity conferred is temporary (lasting approximately 3 to 4 weeks) unless modified or typical measles occurs.[179,410] Ig should not be used to control measles outbreaks.

Postexposure prophylaxis with Ig is especially important for exposed persons who are at increased risk for complications from measles (i.e., infants younger than 12 months of age, pregnant women, and immunocompromised persons). Because donor pool concentrations of antibodies are lower in the United States than in the prevaccine era, the ACIP increased the recommended dose of IgIM to 0.5 mL/kg within 6 days of initial exposure. However, because the maximum dose by volume is 15 mL, persons who weigh more than 30 kg receive less than the recommended dose and will have lower titers than recommended.[409] The recommended dose of IgIV is 400 mg/kg. Any nonimmune person exposed to measles who received Ig should subsequently receive MMR vaccine, which should be administered no earlier than 6 months after IgIM administration or 8 months after IgIV administration, provided the person is then 12 months of age or older and the vaccine is not otherwise contraindicated. Most infants younger than 5 to 6 months of age have partial or complete immunity to measles because of passive maternal antibody, if their mothers are immune. However, in some countries up to 19% of women of childbearing age do not have measurable measles antibody titers.[600,720] Infants born to these women would be susceptible to measles at birth. Human Ig is not indicated for exposed persons who have received at least one dose of measles vaccine at 12 months of age or older unless they are immunocompromised.

The following patient groups are at risk for severe disease and complications from measles and, if exposed, should receive Ig: infants younger than 12 months of age, pregnant women without evidence of measles immunity, and severely immunocompromised persons. IgIM can be administered to other, exposed persons who do not have evidence of measles immunity, but priority should be given to persons exposed in settings with intense, prolonged, close contact (e.g., household, daycare, and classroom). For exposed persons without evidence of measles immunity, a rapid IgG antibody test can be used to inform immune status, provided that administration of Ig is not delayed.[409]

Because infants are at higher risk for severe measles and complications, and infants are susceptible to measles if mothers are nonimmune or their maternal antibodies to measles have waned,[721,722] IgIM should be administered to all infants younger than age 12 months who have been exposed to measles.[409] For infants 6 through 11 months of age, MMR vaccine can be administered in place of Ig if administered within 72 hours of exposure.

Because pregnant women might be at higher risk for severe measles and complications,[721,722] IgIV should be administered to pregnant women without evidence of measles immunity who have been exposed to measles. IgIV is recommended to be administered in doses high enough to achieve estimated protective levels of measles antibody titers (400 mg/kg).

Severely immunocompromised patients who are exposed to measles should receive IgIV prophylaxis regardless of immunologic or vaccination status because they might not be protected by the vaccine. Severely immunocompromised patients include patients with severe primary immunodeficiency; patients who have received a bone marrow transplant until at least 12 months after finishing all immunosuppressive treatment, or longer in patients who have developed graft-versus-host disease; patients on treatment for acute lymphocytic leukemia within and until at least 6 months after completion of immunosuppressive chemotherapy; and patients with a diagnosis of AIDS or HIV-infected persons with severe immunosuppression, which is defined as a CD4 percent of less than 15% (all ages) or a CD4 count of less than 200 lymphocytes/mm^3 (age >5 years) and those who have not received MMR vaccine since receiving effective antiretroviral therapy (ART). Some experts include HIV-infected persons who lack recent confirmation of immunologic status or measles immunity.[409] For persons already receiving IgIV therapy, administration of at least 400 mg/kg body weight within 3 weeks before measles exposure should be sufficient to prevent measles infection. For patients receiving IgSC therapy, administration of at least 200 mg/kg body weight for two consecutive weeks before measles exposure should be sufficient.[409]

Vaccine Safety

Adverse reactions after receipt of live, further attenuated measles vaccines (alone and in combination) are generally mild.[722] Except for hypersensitivity reactions, adverse reactions are limited to susceptible vaccinees and occur primarily during the 6 to 12 days after vaccination when the peak in replication of the live virus occurs.[307,419–424,461–464,488,489,492,495–503,705,715,723–733] Fever of 39.4°C or higher occurs in approximately 5% to 15% of vaccinees between the 7th and 12th days after vaccination and lasts approximately 1 to 2 days. In a study of twins, when one twin received MMR vaccine while the other received placebo, increased rates of fever occurred almost exclusively 9 or 10 days after vaccination.[488,733]

Rash occurs in approximately 5% of recipients, beginning 7 to 10 days after vaccination and lasting for 1 to 3 days. Determining the etiology of a rash illness after measles vaccination can be difficult because other infections, such as human herpesvirus type 6 and human papillomavirus type 19, can often occur early in life and can be confused with a vaccine reaction.[294] Systemic adverse events have been reported less frequently among infants receiving MMR at age 6 to 8 months when compared with those aged 9 to 11 months and 12 to 14 months, indicating that early MMR vaccination is well tolerated.[733a]

MMR vaccine should be given to eligible persons who present with mild illness or low-grade fever. For persons with moderate or severe illness, including untreated, active tuberculosis, vaccination with MMR should be postponed and given after they have recovered. Although, there are no reports of persons being treated for tuberculosis, having experienced an exacerbation of tuberculosis following MMR vaccination, or of any adverse effect of MMR or MMRV vaccines on persons with untreated tuberculosis, a theoretical concern exists that measles vaccine might exacerbate tuberculosis. Therefore, for persons with known untreated active tuberculosis, antituberculous therapy should be started prior to administering MMR vaccine. However, testing for latent tuberculosis infection should not be a prerequisite for MMR vaccination.

When the vaccine is administered to a person already incubating natural measles disease, it is not usually possible to determine if fever and rash are caused by the vaccine or if the child has a modified case of measles, unless wild-type virus is detected by laboratory testing. Measles-like illnesses developing less than 6 days after vaccination are usually the result of the wild-type virus or other concurrent illnesses because the measles-like rash associated with the vaccine is very unlikely to occur within this time period.[488] The frequency of fever, rash, and other side effects after second doses of MCVs is lower than after the first because most children are already immune from the initial vaccination, thus preventing significant viral replication.[724,725] One study found a slightly higher rate of

adverse effects after a second dose of MMR when that dose was given to 10- to 12-year-olds compared with 4- to 6-year-olds.[731] This difference was most likely the result of a higher rate of susceptibility among 10- to 12-year-olds and the recognized increased rate of arthralgia caused by rubella vaccine in older children.[731] Adverse events resulting in medical visits within 1 month after vaccination of 10- to 12-year-olds were still rare (attributable risk 1.7/1000) and consisted mainly of rashes and joint pain.

A study conducted in the United States showed that during the 5 to 12 days after vaccination, children receiving the first dose of MMRV vaccine had significantly higher rates of elevated temperatures than those who had been vaccinated with MMR and varicella vaccine administered separately (27.7% vs 18.7%, respectively).[732] Measles-like rash was also more common after the first dose of MMRV than after MMR and varicella vaccine administered separately (5.9% vs 1.9%, respectively).[507] A multicenter study found rates of fever between days 5 and 12 postvaccination to be higher in recipients of MMRV compared with recipients of MMR and varicella vaccine administered separately (22.8% vs 15.6%); no statistical differences were observed for fever greater than or equal to 40°C between the study groups.[508] Another study conducted in Germany also showed a higher incidence of fever greater than 37.5°C within 15 days after vaccination among children vaccinated with MMRV when compared with children vaccinated with MMR and varicella vaccine (67.7% vs 48.8%). However, the incidence of fever greater than 39°C was not statistically different between the two groups.[506,510,511] No increased fever or rash was noted when these vaccines were administered as the second dose of MCVs.[511,734–737]

Measles vaccine is associated with a mild decrease in platelet counts within a few days after the vaccine is given, and rare cases of idiopathic thrombocytopenic purpura (ITP) have been associated with MCVs.[738] A review of 12 studies revealed a median incidence of 2.6 cases of ITP per 100,000 children receiving the first dose of measles vaccine. In two studies in the United Kingdom, an increased risk of ITP was documented after measles vaccination, at a rate of 1 case in every 25,000 doses in the 6 weeks after immunization.[739] The Vaccine Safety Datalink Project found an attributable risk of 1 case of ITP per 40,000 doses of MMR.[740] The disease was generally mild and resolved in 93% of children within 6 months. Recurrences were uncommon. Therefore, although advisory committees recommend caution when administering MCVs to children with thrombocytopenia or a past history of ITP, data from several small studies of children who had ITP did not reveal recurrences after the administration of MMR.[233,234,739,740]

Immediate hypersensitivity reactions, including hives and anaphylaxis, occur rarely.[741] The rate of anaphylaxis is approximately 1 to 3.5 per million doses administered. The rate of other immediate hypersensitivity reactions is estimated to be approximately 10 per million doses.[742] Measles vaccine is produced in chick embryo tissue culture, and for many years, there were concerns about administering these vaccines to children with egg allergy. Although rare cases of anaphylaxis have been reported in children with egg hypersensitivity, the allergen in the vaccine responsible for these reactions is uncertain.[743] The majority of children with immediate hypersensitivity reactions to MCVs have evidence of allergy to the gelatin stabilizer or neomycin.[744,745] Children with immediate hypersensitivity to eggs can be safely immunized with measles vaccines, and no special precautions are necessary.[744]

Febrile seizures are the most commonly reported neurologic adverse event after measles vaccination.[268,726,729–733] Fever from any source lowers the threshold for seizures, and a febrile seizure is not a sign of central nervous system (CNS) infection or disease. Febrile seizures occur primarily in the second and third year of life and are not associated with long-term sequelae. The risk of febrile seizures is approximately one case for every 3000 to 4000 doses of MMR vaccine administered,[733,746] and the risk typically occurs 6 to 14 days after receipt of MMR.[510,511,726,733,747,] Among children 12 to 23 months of age who received the first dose of MMRV vaccine compared with children who received MMR and varicella vaccines separately, there is an approximate twofold increased risk for febrile seizures. In children 4 to 6 years of age,[748] no increased risk for febrile seizures was observed after vaccination with MMRV vaccine. Having a personal or family history of seizures of any etiology is a precaution for children for the first dose of MMRV, but not MMR, vaccination.[749,750] In the United States, the ACIP has recommended that children with a personal or family history of seizures of any etiology generally should be vaccinated with MMR for their first dose of measles vaccine, because the risks of using MMRV vaccine outweigh the benefits in this group.[722] MMRV vaccine has not been studied in persons with HIV infection and should not be substituted for MMR vaccine. The rates of fever and febrile seizures after administering measles vaccines are much lower than the rates after measles disease.

There has been no increased risk of adverse neurologic events observed in children who received MMR vaccines compared with measles vaccine administered alone.[733]

Postinfectious encephalitis is a recognized complication of measles, but there is uncertainty whether or not encephalitis is caused by further attenuated measles vaccines in normal hosts. In children who have died during the rash phase of measles, measles virus has been found in brain tissue, but measles virus rarely has been found anywhere in the CNS with postinfectious encephalitis, which is thought to be the result of an altered immune response to myelin proteins.[234] Encephalitis has been reported after measles immunization at a rate of approximately 1 case per 1 million vaccine recipients.[751] This rate is lower than the background rate of encephalitis of unknown etiology in unvaccinated children in the general population. There is temporal clustering of reports in the 5 to 15 days after immunization when other adverse effects from the vaccine occur.[714] There has been one report of a vaccine isolate recovered from the cerebrospinal fluid of a normal person who received Edmonston B strain vaccine.[752] There have been no cases of vaccine-strain virus identified as the cause of encephalitis in persons with intact immune systems confirmed by neuropathologic studies. Nine cases of encephalitis after administering MCVs were reported in the United States during 1979 to 1986, when 22.7 million doses of measles antigen–containing vaccine were distributed, for a rate of 1 per 2.5 million doses (0.4 per 1 million doses).[713] In Albania, two cases of encephalitis were identified after 867,000 doses of measles and rubella (MR) vaccine were administered during a mass vaccination campaign.[753] This observed rate is lower than that noted for severe neurologic disorders of unknown etiology in unimmunized children of the same age range, suggesting a possible chance temporal association.[754] In Finland and the United States, there was no evidence of an increased risk of encephalitis after measles vaccines.[755,756] In the United Kingdom, studies found an increased rate of serious neurologic disease in the 6 to 11 days after vaccination.[757,758] However, some of the increase was from complex febrile seizures, and other viruses known to cause encephalitis (herpes simplex virus, human herpesvirus [HHV]-6, and HHV-7) were identified in some affected patients. The Institute of Medicine concluded, in 1994, that there was inadequate evidence to accept or reject a causal relation between measles vaccine and encephalitis or encephalopathy.[738] However, in a 1998 study, done by Weibel and colleagues,[758] the authors found a clustering of 17 cases of encephalopathy on days 8

and 9 after MMR vaccination, but they stated that with the large denominator of children vaccinated throughout 23 years, encephalopathy would be an extremely rare complication (0.06 per 100,000 vaccinees). If measles vaccines do cause acute encephalitis, the rate is at least 1000 times less than the rate after natural infection.

There are case reports but no evidence of increased risk or causal associations of several other disorders after measles vaccines, including Reye syndrome, oculomotor palsy, optic neuritis, retinopathy, hearing loss, cerebellar ataxia, arthralgia, arthritis, and soft-tissue reactions.[268,713,759–768] There is no increased risk of Guillain-Barré syndrome after measles vaccine.[769,770] In a cohort study of 167,240 children, no significant association between timing of the receipt of measles vaccine and asthma was found.[771]

SSPE is caused by persistence of defective measles virus in the CNS through as-yet undefined mechanisms.[147] No specific immune deficiency has been identified in children with SSPE to explain the failure of the immune system to eliminate the virus.[772] There was an initial concern that measles vaccine virus might cause a persistent CNS infection because some patients with SSPE who have received vaccine had no history of disease.[99,104,149,772] However, when investigated carefully, most of these persons have histories of measles-like illnesses and/or known exposures to measles followed by administration of passive Ig.[739,773] Genetic sequencing of viruses obtained from the brains of patients with SSPE, including patients with no history of having had measles, has revealed only viruses of wild-type origin.[143,144,774,775] Case-control studies and the marked decline of SSPE in parallel with the decline in measles after the introduction and widespread use of measles vaccine demonstrate that the vaccine protects against SSPE (Fig. 37.8).[147] It is not known if measles virus persists in immunologically normal persons who do not develop SSPE.[772] Measles virus genomic RNA was detected frequently in persons who had been immunized or exposed to measles more than 2 months before testing in one study, and one group of investigators found evidence for persistence of measles virus nucleocapsid in a variety of tissues many years after measles.[776,777] An HIV-infected intravenous drug user developed progressive measles retinitis and subsequent progressive CNS disease diagnosed as SSPE at 30 years of age.[778] His illness resembled measles encephalitis in immunocompromised persons. He had a history of measles at 2 years of age, and HIV infection most likely occurred in later life, secondary to intravenous drug abuse or sexual contact.

Measles encephalitis in immunosuppressed children, including children with HIV infection or leukemia, is sometimes referred to as subacute or inclusion body measles encephalitis and is caused by progressive measles virus infection.[779,780] Onset is usually 5 weeks to 6 months after acute measles, and virus has been identified in brain tissue and cerebrospinal fluid specimens. Virus isolated from brain tissue specimens from several patients has been shown to be wild-type measles virus.[143,779] One patient with an undefined immune disorder who developed this disorder at 21 months of age was found to have measles vaccine virus in brain tissue.[781] The Institute of Medicine concluded in 2011 that there was adequate evidence to accept a causal relation between measles vaccine and measles inclusion body encephalitis in persons with demonstrated immunodeficiencies (causation is attributed to the measles component of the vaccine).[773]

In persons with severe immune suppression, there is an increased risk of progressive measles vaccine virus replication and severe complications, including fatal pneumonia.[782,783] HIV infection is not associated with increased risks of adverse events in the few weeks after measles vaccination (see Chapter 70 for additional information on immunization of HIV-infected persons).[784,785] Only one well-documented instance of a severe complication after measles vaccine in an HIV-infected person has been reported. Measles vaccine virus was identified in the lung of a 20-year-old man who died of progressive pneumonitis that was recognized approximately 9 months after receiving a second dose of measles vaccine.[783,786] One

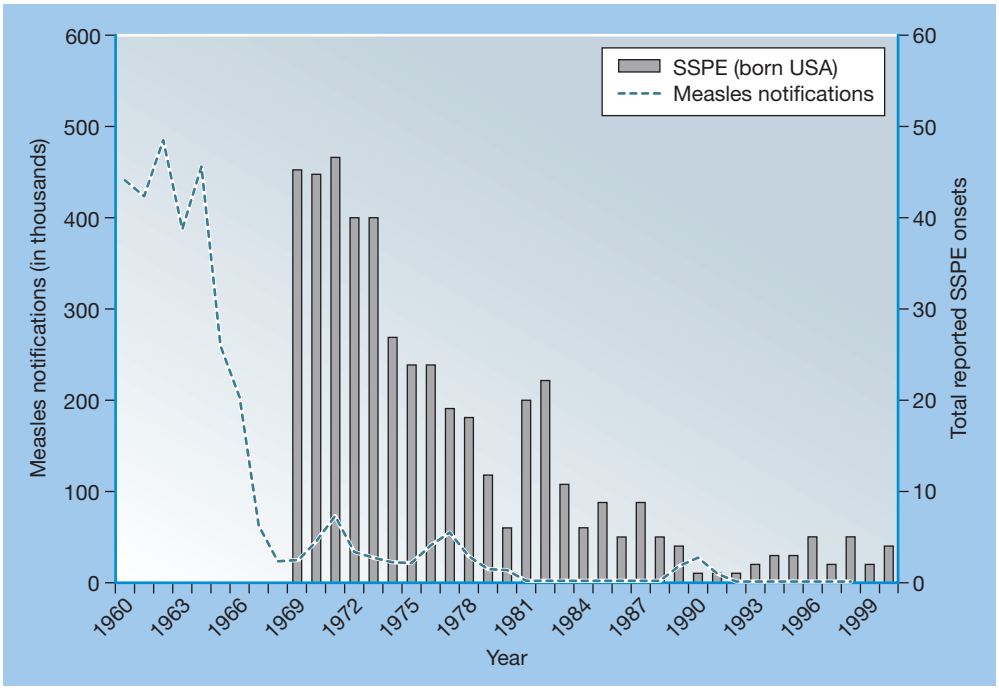


Figure 37.8. Subacute sclerosing panencephalitis (SSPE) cases and measles notifications in the United States, 1960–2000. *(Modified from Campbell H, Andrews N, Brown KE, et al. Review of the effect of measles vaccination on the epidemiology of SSPE.* Int J Epidemiol. *2007;36:1334–1348.)*

HIV-infected child who had received MMR vaccine at 15 months of age was found to have characteristic inclusion bodies on brain biopsy at 18 months of age, but no specific testing was performed to determine the source or identity of the virus.[779] Also, a 19-year-old HIV-infected man with hemophilia had paramyxovirus nucleocapsids in intranuclear inclusion bodies, and there was evidence of measles antigen on immunohistochemical staining, but the virus was not sequenced.[780] He had received measles vaccine at 10 years of age, and there was no history of measles exposure in the year preceding the biopsy. The ages at which he acquired HIV infection and possible subsequent exposures to measles were not reported. The WHO recommends measles vaccine for asymptomatic HIV-infected children and those with early signs of HIV-induced immunosuppression because of the high risk of serious complications from measles in HIV-infected persons.[622,785] In the United States, where the risk of measles exposure is low, the ACIP recommends withholding measles vaccine from persons with severe immunosuppression. The ACIP recommends two doses of MMR vaccine for HIV-infected persons who are 12 months of age or older and who do not have evidence of measles, rubella, and mumps immunity or evidence of severe immunosuppression.[722] Absence of severe immunosuppression is defined as CD4 percentages 15% or greater for 6 months or longer for persons 5 years of age or younger and CD4 percentages 15% or greater and CD4 count 200 lymphocytes/mm^3 or higher for 6 months or longer for persons older than 5 years of age. When only CD4 counts or CD4 percentages are available for those 5 years of age or older, the assessment of severe immunosuppression can be on the basis of the CD4 values (count or percentage) that are available. When CD4 percentages are not available for those 5 years of age or younger, the assessment of severe immunosuppression can be on the basis of age-specific CD4 counts at the time CD4 counts were measured (i.e., absence of severe immunosuppression is defined as ≥6 months above age-specific CD4 count criteria: CD4 count >750 lymphocytes/mm^3 while ≤12 months of age and CD4 count ≥500 lymphocytes/mm^3 while 1 through 5 years of age). The ACIP recommends two doses of MMR vaccine for older children and adults with newly diagnosed HIV infections and without evidence of measles, rubella, or mumps immunity, unless they have evidence of severe immunosuppression, which is defined as a CD4 percentage of less than 15% or, in persons older than 5 years of age, a CD4 count of less than 200 lymphocytes/mm^3.[722,787,788]

After bone marrow transplantations, advisory committees recommend waiting 2 years to administer measles vaccine to allow immune reconstitution.[234] However, in a study of 51 patients, vaccination 1 year after transplantation was not associated with any increased rate of adverse reactions.[789]

The possibility that measles virus persists in selected tissues after measles has been proposed by investigators who found evidence for measles antigen or genomic material in human tissues, including brain, lung, intestine, and bone, years after measles disease.[777] The investigators proposed that these viruses contribute to or cause inflammatory diseases in these organs, including Paget disease,[176,790] otosclerosis,[175] and inflammatory bowel disease.[791] However, other investigators have not found any evidence for measles viruses or measles genomic material in tissues from affected or normal patients.[792,793] The hypothesis that measles vaccine given in combination with mumps and rubella vaccines (MMR) caused inflammatory bowel disease and autism was promoted by one particular investigator who had unrevealed conflicts of interest. A later review of the clinical records from the original 12 patients found evidence of fraud in the data, and the article has been withdrawn.[791,794–797] Some of the other reports of persistent measles virus genomic material in children with autism were based upon PCR methods that amplified host genomic material, not measles virus.[793] In-depth reviews conducted by the Institute of Medicine[172,798–802] concluded that the evidence favors rejection of the MMR-autism hypothesis, and an expert review by the AAP[792] concluded that the evidence does not support either of these hypotheses. A systematic review of the literature by the Cochrane Collaboration reached similar conclusions.[803] Advisory committees in many countries recommend the use of MMR to protect against these three diseases.[233,234]

Indications

General

The goal of measles vaccination programs is to protect persons from the severe consequences of measles infection by providing lifelong individual immunity to vaccinees and by reducing susceptibility to measles in the population to prevent transmission of this disease. The major source of measles susceptibility in a population is the birth of children, who will all lose their maternal antibody and become susceptible in the first or second year of life. Maternal antibody interferes with the response to live measles vaccines. The most critical task of any vaccination program is the timely delivery of the first dose of measles vaccine to young children. The timing of the first dose must balance the proportion of infants who have lost maternal antibody and are able to respond to vaccination at a given age against the risk of measles infection before that age.[602] Therefore, recommendations for measles vaccination vary by country.

The WHO recommends measles vaccine be administered at 9 months of age in countries with ongoing transmission of measles in which the risk of measles mortality among infants is high.[804] In countries with low rates of measles transmission (i.e., those near elimination) the WHO recommends the first dose of measles vaccine be given at 12 months to take advantage of higher seroconversion rates achieved at this age.[622,804,805] In 2009, the WHO recommended that a two-dose measles immunization schedule should be the standard of care for all countries. The second dose may be offered either at a scheduled age through routine services or periodically through mass campaigns, depending on which strategy achieves higher coverage.[622,804]

All developed and many developing countries now have schedules for two routine doses of measles vaccine. In developed countries, the first dose is usually recommended early in the second year of life; the age for the second dose varies.[30–33,409,622] The following section provides detailed recommendations of measles vaccination for the United States.

General Immunization Guidelines

In general, use of licensed combination vaccines, such as MMR, is preferred over separate injections with equivalent component vaccines, to minimize the number of injections and reduce missed opportunities to protect through vaccination. ACIP, AAP, and American Academy of Family Physicians (AAFP) recommend that combination vaccines may be used whenever any components of the combination are indicated and its other components are not contraindicated.[806,807] Single-antigen measles, mumps, or rubella vaccines are no longer available in the United States.

MMR or MMRV vaccine may be administered simultaneously with other vaccines to persons at the recommended age to receive these vaccines. Neither theoretic considerations nor practical experience indicate that the simultaneous administration at separate anatomic sites of MMR or MMRV and other

live or inactivated vaccines will produce a diminished immune response or increase the incidence of adverse events among vaccinated persons.[409]

The combined live attenuated MMRV vaccine was licensed in 2005 in the United States and recommended in 2006 for use in children 12 months to 12 years of age.[487] In May 2010, ACIP updated its 2006 MMRV recommendations[709] based on study results showing a roughly twofold increased risk for febrile seizures after the first dose of MMRV compared with the first dose of MMR and varicella vaccine administered separately at the same visit.[510,511,808] The routinely recommended age for the first dose of MMRV vaccines is age 12 to 15 months; children not vaccinated according to the routine schedule may receive the first dose of MMRV vaccine up to age 12 years. For the first dose of MMRV vaccines at age 12 to 47 months, either MMR vaccine and varicella vaccine or MMRV vaccine may be used. Providers who are considering administering MMRV vaccine should discuss the benefits and risks of both vaccination options with the parents or caregivers. Unless the parent or caregiver expresses a preference for MMRV vaccine, MMR vaccine and varicella vaccine should be administered as separate vaccines for the first doses in this age group. Use of MMR vaccine and varicella vaccine avoids the increased risk for fever and febrile seizures after MMRV vaccine. The 47-month cutoff was selected on the basis of the epidemiology of febrile seizures. Approximately 97% of febrile seizures occur in children 47 months of age or younger.[510,511,808]

Although the routinely recommended age for the second dose of MMRV vaccines is 4 to 6 years, the second dose may be administered before age 4 years, provided it has been 3 months or longer since the first dose. MMRV vaccine is licensed for use among children from 12 months through 12 years of age. For the second dose of MMRV vaccines at any age (15 months to 12 years) and for the first dose at age 48 months or older, use of MMRV vaccine generally is preferred over separate injections of its equivalent component vaccines (i.e., MMR vaccine and varicella vaccine). Catch-up vaccination is recommended to ensure a second dose for all children, adolescents, and adults who previously had received one dose.[709]

At least 1 month should elapse between doses of any live virus vaccines. For children 12 months to 12 years of age at least 3 months should elapse between doses of varicella-containing vaccines. Simultaneous administration of the most widely used live and inactivated vaccines has produced seroconversion rates and rates of adverse reactions similar to those observed when the vaccines are administered separately.[806,809] Therefore, MMRV may be administered simultaneously with other vaccines recommended at ages 12 months to 12 years, although data are absent or limited for the concomitant use of MMRV vaccine with DTaP, inactivated polio, pneumococcal conjugate, influenza, and hepatitis A vaccines.[487]

Specific criteria for documentation of measles immunity have been established to identify the appropriate level of immunization for different groups. Persons can generally be presumed immune to measles if they have documentation of adequate vaccination, laboratory evidence of immunity to measles, laboratory confirmation of disease, or were born before 1957 (few persons in the United States born before 1957 escaped natural infection). Physician-diagnosed measles is no longer considered presumptive evidence of immunity.[409,810]

In general, one dose of MMR is considered adequate vaccination for preschool children and adults who are not at high risk of exposure to measles. Two doses of MMR are indicated for schoolchildren, students at post–high school educational institutions, healthcare personnel, persons in measles outbreak settings, and international travelers because these groups are at increased risk for exposure to measles. Criteria accepted as evidence of immunity for the purpose of meeting school or college entry requirements or other government regulations may vary among state and local jurisdictions.[233,409]

Proper documentation of immunization is critical to ensuring that every person receives the appropriate vaccines. Vaccination status and date of administration of all vaccinations should be documented in the patient's permanent medical record. Only doses of vaccine for which written documentation of the date of administration is presented should be considered valid. Healthcare professionals should provide a vaccination record for a patient only if they have administered the vaccine or have seen a record that documents vaccination.[233,409]

Because of reduced efficacy of measles vaccine when it is administered to young children, doses of measles vaccine administered to children younger than 12 months are not considered valid doses in the United States. Children who are vaccinated at ages younger than 12 months should be revaccinated at 12 to 15 months of age (provided at least 28 days have elapsed) to be considered to have received a single dose of measles vaccine.[233,409]

Routine Vaccination Schedule

Preschool Children. All children should receive the first dose of MMR or MMRV at 12 to 15 months of age (Table 37.9). Children exposed to measles or at high risk of exposure (e.g., during an outbreak or while on international travel) may be vaccinated with MMR as early as 6 months of age.

The second dose of MMRV or MMR vaccine is recommended when children are 4 to 6 years of age (i.e., before a child enters kindergarten or first grade). This recommended timing for the second dose has been adopted jointly by the ACIP, the AAP, and the AAFP.[233,409] Evidence now indicates that (a) the major benefit of administering the second dose for measles is a reduction in the proportion of persons who remain susceptible because of primary vaccine failure; (b) waning immunity is not a major cause of vaccine failure and has little influence on measles transmission; and (c) revaccination of children who have low levels of measles antibody produces only a transient rise in antibody levels.[192,566]

For children 12 months of age and older who are exposed to measles or at high risk of exposure (e.g., during an outbreak or for international travel), the second dose may be given as early as 28 days after the first dose based on the principle that live-virus vaccines not administered at the same time should be separated by at least 1 month.[233,409] For vaccines containing varicella antigens, a 3-month interval between doses is required.

Schoolchildren. All children in school from kindergarten through 12th grade should have documentation of two doses of measles vaccine, unless vaccination is contraindicated. Children who do not have documentation of adequate vaccination against measles or other acceptable evidence of immunity should be admitted to school only after administration of the first dose of MMR vaccine. If required, the second MMR dose should be administered as soon as possible, but no sooner than 28 days after the first dose. The ACIP, AAP, and AAFP recommend the health maintenance visit at age 11 to 12 years should serve as a special opportunity to evaluate vaccination status and to administer MMR vaccine to all persons who have not received two doses at the recommended ages.[233] As of the 2012–13 school year, all states require two doses for school entry.[811] The estimated national two-dose coverage rate in the 2013–14 school year at kindergarten entry was 94.7%[812]; state

TABLE 37.9 Summary of Measles Vaccination Recommendations in the United States[a]

Preschool children	
Routine childhood schedule	First dose: age 12–15 mo Second dose: age 4–6 y
Special circumstances	
Outbreak settings or before international travel	First dose: may be given as early as 6 mo of age Repeat first dose: 12 mo of age Second dose: may be given as early as 13 mo of age[b]
HIV infection	First dose: 12 mo of age Second dose: may be given as early as 13 mo of age[b,c]
School children and adolescents	All children in kindergarten through 12th grade should have documentation of two doses of MMR unless they have other evidence of immunity[d]
Adults	Adults without other evidence of immunity[d] should have documentation of receipt of at least one dose of MMR
Special circumstances	Students and staff in colleges and other post–high school educational institutions, persons working in healthcare facilities, and international travelers should have documentation of receipt of two doses of measles vaccine unless they have other evidence of immunity[d]

[a]All recommendations exclude persons for whom measles vaccination is contraindicated.
[b]Wait at least 28 days after any dose before giving a subsequent dose.
[c]Revaccination is recommended for persons with perinatal HIV infection who were vaccinated before establishment of effective antiretroviral therapy (ART) with two appropriately spaced doses of MMR vaccine once effective ART has been established.
[d]Persons born before 1957 or who have laboratory confirmation of disease or laboratory evidence of immunity.

estimates ranged from 81.7% to 99.7% or greater.[812] However, there are limitations to the kindergarten survey data, because the methodology for collection of the data differs among the states.

Vaccination of High-Risk Groups

Colleges and Other Post–High School Educational Institutions. Risks for transmission of measles at post–high school educational institutions can be high because these institutions may bring together large concentrations of persons who may be susceptible to measles. College entry requirements for measles immunity substantially reduce the risk for measles outbreaks on college campuses where they are implemented and enforced.[813] Therefore, colleges, universities, technical and vocational schools, and other institutions for post–high school education should require that all undergraduate and graduate students have received two doses of MMR vaccine or have other acceptable evidence of immunity before enrollment. Students without documentation of any measles immunization or immunity should receive a dose on entry, followed by a second dose 4 weeks later.[233,409]

Outbreak Settings

During outbreaks in schools and other institutions, all students and personnel born in 1957 or later, should have documentation of two doses of MCV on or after the first birthday.[233,409] All persons who do not have such documentation or other evidence of immunity should be vaccinated or excluded from the school until 21 days after the onset of rash in the last case of measles. Persons receiving their second dose and unimmunized persons receiving their first dose as part of the outbreak control program may be readmitted immediately to school. In outbreaks where there is increased risk of exposure for infants younger than 1 year of age, vaccination with MMR vaccine is recommended for infants as young as 6 months of age. Children who are vaccinated before their first birthday should be revaccinated when they are 12 months of age (provided at least 28 days have elapsed since the first dose given before age 12 months) and again at school entry. During outbreaks, Ig should be administered to immunocompromised persons, susceptible pregnant women, and unvaccinated children younger than 12 months of age who are exposed to measles. However, Ig should not be used to attempt to reduce measles transmission or control outbreaks. Likewise, serologic screening before vaccination generally is not recommended during an outbreak because waiting for serology results can impede the rapid vaccination needed to curb the outbreak.

Every suspected measles case should be reported immediately to the local health department, and every effort must be made to rapidly verify that the illness is measles, especially if this may be the first case in the community. Subsequent prevention of the spread of measles depends on prompt immunization of persons at risk of exposure or already exposed who cannot readily provide documentation of measles immunity, including the date of immunization.[233,409]

If an outbreak occurs in a healthcare setting, healthcare facilities should recommend two doses of MMR vaccine for unvaccinated healthcare personnel regardless of birth year. All employees who were born in 1957 or later who cannot provide documentation that they have received two doses of measles vaccine on or after their first birthday or other presumptive evidence of immunity to measles should be offered the first dose of MMR vaccine immediately and receive the second MMR vaccination at least 28 days later. Some healthcare personnel who were born before 1957 have acquired measles while at work. Therefore, during an outbreak of measles, healthcare facilities should recommend completion of two doses of MMR vaccine for unvaccinated or undervaccinated personnel born before 1957 who lack laboratory evidence of measles IgG in their serum or laboratory confirmation of infection.[810] Healthcare personnel without evidence of immunity who are not vaccinated after exposure should be removed from all patient contact and excluded from the facility from day 5 after their first exposure through day 21 after the last exposure, even if they have received postexposure immunoglobulin. Healthcare personnel who become ill should be relieved of patient contact until 4 days after rash develops. Those with documentation of one vaccine dose may remain at work and should receive the second dose.[233,409]

Healthcare Personnel

Persons who work in healthcare facilities are at greater risk for acquiring measles than the general population. During 1985 to 1989, physicians had an eightfold increased risk and nurses a twofold increased risk of measles compared with non–healthcare workers of the same age.[814] In the 120 measles outbreaks that occurred during 1993 through 2001, healthcare

facilities were the most commonly reported settings, with 24 outbreaks reported.[815] In the postelimination years 2001 to 2008, 27 measles cases were reported in U.S. healthcare facilities, accounting for 5% of all reported U.S. measles cases.[816]

Because persons working in medical settings have been infected with measles and have transmitted the virus to patients and other staff, the ACIP recommends that persons born in or after 1957 working in healthcare facilities be required to provide documented receipt of two doses of MCV, laboratory evidence of measles immunoglobulin (IgG) in serum (i.e., a single specimen test for measles IgG), or laboratory confirmation of acute prior measles infection (i.e., positive serologic test for measles IgM antibody or fourfold rise in measles IgG antibody level or documentation of seroconversion by any standard serologic assay, detection of measles RNA in a clinical specimen, or isolation of measles virus from a clinical specimen).[810,817]

During 2001 to 2008, 12.5% (1 of 8) of measles cases reported to the Centers for Disease Control and Prevention (CDC) among healthcare personnel occurred in persons born before 1957.[810] Thus, because measles has occurred in persons born before 1957, healthcare facilities should consider vaccinating personnel born before 1957 who lack documented receipt of two doses of MCV, laboratory evidence of measles IgG in their serum, or laboratory confirmation of infection, with two doses of MMR vaccine at the appropriate interval.[233,409] Vaccine recommendations during outbreaks differ from routine recommendations for this group (see "Outbreak Settings" above).[810,817]

Serologic screening need not be done before vaccinating for measles and rubella unless the medical facility considers it cost-effective.[817,818] For healthcare personnel who have two documented doses of MMR vaccine or other acceptable evidence of immunity to measles, serologic testing for immunity is not recommended.

International Travelers

Measles is endemic in many countries, including industrialized countries in Europe and Asia. Measles importations reflect both the incidence of measles in countries around the world and travel patterns.[816] Of the 222 measles cases reported in the United States in 2011, 200 (90%) were associated with importations. Almost half of the imported cases occurred in people who acquired measles in Europe.[819] Among the 288 measles cases reported in the United States between January and May in 2014, 280 (97%) were associated with importations, and more than a half of the importations were travelers returning from the Philippines.[820] Protection against measles is especially important for persons planning international travel. Before their departure from the United States, children 12 months of age or older should have received two doses of MMR vaccine separated by at least 28 days, with the first dose administered on or after the first birthday. Children ages 6 to 11 months should receive a dose of MMR vaccine before departure. For infants vaccinated before 12 months of age, revaccination should be at 12 to 15 months of age (12 months of age for those remaining in areas of endemic or epidemic measles). The second dose should be administered at least 28 days after the first dose.[233,409]

Because the risk of complications from measles is increased in adults, it is also important to protect susceptible adults. Most persons born in the United States before 1957 are likely to be immune. However, for persons born after 1956 who travel internationally, two doses of measles vaccine should be administered separated by at least 28 days, unless there is documentation of receipt of two doses, other evidence of immunity, or a contraindication.[233,409]

Persons Infected With Human Immunodeficiency Virus

HIV-infected persons are at increased risk for severe complications if infected with measles.[203,211] Among HIV-infected persons who did not have evidence of severe immunosuppression, no increase in serious or unusual adverse events have been reported after measles vaccination.[211,821] Therefore, MMR vaccination is recommended for all asymptomatic HIV-infected persons who do not have evidence of severe immunosuppression and for whom measles vaccination would otherwise be indicated. MMR vaccination also should be considered for all symptomatic HIV-infected persons who do not have evidence of severe immunosuppression.[822] Testing asymptomatic persons for HIV infection is not necessary before administering MMR or other MCVs.[233,409]

Because the immunologic response to vaccines may decrease as HIV disease progresses, vaccination early in the course of HIV infection may be more likely to induce an immune response.[655] Therefore, HIV-infected infants without severe immunosuppression (as defined in "Vaccine Safety") should routinely receive MMR vaccine as soon as possible on reaching the first birthday (i.e., at age 12 months).[822] Consideration should be given to administering the second dose of MMR vaccine as soon as 28 days (i.e., 1 month) after the first dose rather than waiting until the child is ready to enter kindergarten or first grade. In addition, if at risk for exposure to measles (e.g., in an outbreak setting or for international travel), HIV-infected infants who are not severely immunocompromised should be administered MMR vaccine at age 6 to 11 months.[233,409,622] These children should receive another dose as soon as possible on reaching their first birthday, provided at least 1 month has elapsed since the administration of the previous dose of MCV. An additional dose of MMR vaccine can be administered as early as 28 days after the second dose. Newly diagnosed HIV-infected adults and children ages 12 months or older without acceptable evidence of measles immunity should receive MMR vaccine as soon as possible after diagnosis, unless they have evidence of severe immunosuppression.

Revaccination of Persons With Perinatal HIV Infection Who Do Not Have Evidence of Severe Immunosuppression

Persons with perinatal HIV infection who were vaccinated with MMR before establishment of effective ART should be considered unvaccinated and should receive two appropriately spaced doses of MMR vaccine (i.e., one dose once effective ART has been established and a second dose at least 28 days later). Established effective ART is defined as receiving ART for 6 months or longer in combination with CD4 percentages 15% or greater for 6 months or longer for persons 5 years of age and younger and CD4 percentages of 15% or greater and a CD4 count of 200 lymphocytes/mm^3 or greater for 6 months or longer for persons 5 years of age and older.[233,409]

Close Contacts of Immunosuppressed Patients

To minimize the risk of exposure to measles among patients with immunosuppression, including HIV infection, all family and other close contacts of these patients should be vaccinated with two doses of MMR vaccine, unless they are immunosuppressed, have other contraindications, or have other acceptable evidence of measles immunity.[233,234] No transmission of measles vaccine virus from a vaccinee to a contact has ever been documented, which suggests that the risk of exposure to

vaccine virus among immunosuppressed contacts is very low. However, because of the extreme vulnerability of hematopoietic cell transplantation (HCT) recipients, vaccine recipients who develop a fever and/or rash postvaccination should be excluded from visiting the HCT center while symptomatic and should avoid close contact with HCT recipients in the home setting.[823]

Patients Treated With Chemotherapy, Organ Transplantation, or Bone Marrow Transplantation

When cancer chemotherapy or immunosuppressive treatment is being considered, measles vaccination ideally should precede the initiation of chemotherapy or immunosuppression by at least 2 weeks.[822] Persons who were not immune to measles when diagnosed with leukemia may receive MCV during remission. Measles vaccination is contraindicated during the period of immunosuppression, and at least 3 months should elapse after termination of chemotherapy before measles vaccination.[234] Patients exposed to measles or at high risk of exposure while immunosuppressed should be given Ig regardless of previous immunization status (see "Postexposure Prophylaxis" above). Routine vaccination with MMR is recommended for all children and seronegative adults who have received hematopoietic stem cell transplants. The first dose should be given 24 months after transplantation if the recipient is presumed to be immunocompetent, is not on immunosuppressive medications, and is not affected by graft-versus-host disease. A second dose should be given 6 to 12 months after the first dose.[822–826] Regular testing of long-term HCT survivors for maintenance of measles antibody levels is recommended approximately every 4 to 5 years. The need for revaccination has to be assessed on an individual basis.[823]

Revaccination of Persons Vaccinated According to Earlier Recommendations

Anyone vaccinated at any age with inactivated (killed) measles vaccine alone or killed vaccine followed by live vaccine within a 3-month period, and anyone vaccinated between 1963 and 1967 with a vaccine of unknown type, should be revaccinated with the current live vaccine to ensure protection and to prevent atypical measles illness. Revaccinating persons who received an unknown type of vaccine is recommended because Edmonston B, inactivated, and further attenuated live vaccines all were in use during this interval (see Fig. 37.6). As noted previously, vaccination of an immune person is not associated with any increased risk of adverse events. However, revaccination of recipients of killed vaccine may be associated with local reactions of pain, swelling, erythema, and regional lymphadenopathy lasting 1 to 2 days.[80,81,213,214,727,728,827] These reactions have been reported to occur in 4% to 55% of vaccinees. More-severe reactions have been noted, but only rarely.[81] Whereas revaccination has not been proved to totally eliminate the risk for atypical measles,[220] available data suggest that the risk is reduced considerably.[80,81,224] Thus, the risk of these reactions is outweighed by the risk associated with atypical measles.[234]

A dose of further attenuated live vaccine with Ig administered simultaneously should not be considered adequate vaccination. This recommendation is based on the theoretical consideration that passively derived antibody might interfere with seroconversion. Whereas low doses of Ig do not appear to interfere with seroconversion,[307] there is no information available about the dose used in general practice shortly after the introduction of further attenuated vaccines in the United States. Furthermore, at least one study suggested that there is an increased rate of seronegativity when further attenuated vaccine was administered with Ig.[609] Finally, and perhaps most importantly, revaccination is indicated if there is any uncertainty about the validity of doses in the vaccination record.[550]

Precautions and Contraindications

In general, measles vaccination is contraindicated for patients with concurrent moderate or severe illnesses, immunosuppression, pregnancy, or a personal history of an anaphylactic reaction to neomycin or severe allergic reaction to any component of the MCV. After administration of IgIM or IgIV or IgSC or other blood product, vaccination should be delayed for the recommended interval, unless the patient is exposed to measles or at high risk of exposure.[409] Clinical judgment must be used in deciding whether to vaccinate a patient with a history of thrombocytopenia, but recent studies suggest that the risk of stimulating a recurrence of ITP is very low.[740,828] Mild illness, personal or family history of seizures, steroid therapy that is not immunosuppressive, and allergic reactions to eggs, chicken, feathers, or penicillin are not contraindications to measles vaccination. Persons with contraindications to measles vaccination should receive Ig if they are exposed to measles or at high risk of exposure. Unfortunately, this only provides protection for a short time (see "Passive Immunization" above). To reduce the risk of exposure to measles in persons for whom the vaccine is contraindicated, their household contacts should be immune to measles or be vaccinated (unless contraindicated). To prevent nosocomial exposure to measles in patients with contraindications, all healthcare personnel should be immune to measles or be vaccinated (unless contraindicated) (see "Indications" above).

Moderate or Severe Illness With or Without Fever

MCVs should not be administered to patients with concurrent moderate or severe illnesses, including those with significant fever or evolving neurologic conditions. To avoid confusing the evolution of the illness with possible adverse events resulting from measles vaccine, vaccination should be deferred until the acute severe illness is resolved.

Withholding vaccination for false contraindications is a major cause of late vaccination and low vaccine coverage. Measles vaccination should not be deferred in the presence of mild illnesses, with or without low-grade fever, such as mild upper respiratory infections, otitis media, or diarrhea.[602] Multiple large studies in the United States and in other countries have documented that children with mild illness have similar serologic responses to measles vaccination compared with well children. These studies also showed that the ill children had no increased risk of adverse events after vaccination compared with well children.[409,639–641]

Tuberculosis

Because of a theoretic concern that measles vaccination might exacerbate tuberculosis, patients with untreated active tuberculosis should begin antituberculosis therapy before measles vaccination. This theoretic concern is based on the reputed role of measles disease as an aggravating factor in tuberculosis and the similar suppression of delayed hypersensitivity for up to 4 to 6 weeks seen after both measles disease and measles vaccination.[552,554] However, a 1976 review of studies examining the effect of measles disease on tuberculosis patients concluded that none of the studies provided conclusive evidence that measles disease has a deleterious effect on tuberculosis.[115] A review of the incidence of tuberculosis after a measles outbreak in Korea did not reveal an increased risk of tuberculosis.[116] In several small studies in the 1960s, measles vaccine did not exacerbate tuberculosis in children receiving

antituberculosis therapy.[559,829,830] However, there are no data available on the effect of measles vaccine on patients with untreated tuberculosis.

Tuberculin skin testing is not a prerequisite for measles immunization. In patients for whom tuberculin skin testing is indicated, such testing can be undertaken at the same time that vaccine is administered. If skin testing is not done at that time, it is advisable to wait 4 to 6 weeks after vaccination before administering a tuberculosis skin test, because measles immunization temporarily may suppress tuberculin skin test reactivity.[233,409]

Immunosuppression

Live virus measles vaccine should not be administered to persons who are immunosuppressed because of medication (e.g., high-dose steroids, alkylating agents, and antimetabolites), other therapy such as radiation, or underlying illness (e.g., congenital immunodeficiency, leukemia, lymphoma, generalized malignant disease) (see "Indications" above). Although patients with severe immunosuppression related to HIV infection should not receive measles vaccine, HIV-infected persons who are not severely immunosuppressed may be vaccinated. Immunosuppressed patients who are exposed to measles or at risk of exposure should receive Ig regardless of their history of measles disease, vaccination, or serology, because they are at high risk of complications and death from measles (see "Passive Immunization" above). Replication of live vaccine virus may be augmented and prolonged in patients who are immunocompromised, and adverse events after vaccination, including death, are more common in immunosuppressed patients (see "Vaccine Safety").[779,781,786,831]

Systemic corticosteroid treatment can result in immunosuppression. Because the minimum dose and duration of steroid therapy necessary to induce immunosuppression are not well defined, clinical judgment must be used to assess whether a patient on steroid therapy is immunosuppressed. Most experts would consider a steroid dose equivalent to or greater than a prednisone dose of 2 mg/kg of body weight per day (or ≥20 mg/day for persons who weigh >10 kg) administered for 14 days or more to be sufficiently immunosuppressive to contraindicate measles vaccination.[409] Patients receiving immunosuppressive doses of steroids should not receive measles vaccine until 1 month after the end of steroid therapy. Vaccination is not contraindicated in persons receiving topical, localized (e.g., intraarticular, bursal, or tendon injection); low- to moderate-dose steroids; or physiological steroid replacement. Patients who receive high-dose steroids for less than 14 days generally can be vaccinated immediately after cessation of steroid therapy. Measles vaccine is contraindicated in any patient on any dose of steroids with clinical or laboratory evidence of immunosuppression or an underlying disease that is immunosuppressive.[409,822]

MMR and other MCVs are not recommended for HIV-infected persons with evidence of severe immunosuppression (as defined above) because of the risk of adverse events, the impaired immune response to MMR vaccination, and the current low risk of exposure to measles in the United States.[233,409,788,] One case of fatal vaccine-associated pneumonitis has been documented in a severely immunocompromised patient with HIV infection (see "Vaccine Safety").[786] Available data suggest that persons with HIV infection who are not severely immunosuppressed may be vaccinated safely.[211,652,653,788] Because measles has been documented to be severe in HIV-infected persons in both the United States and developing countries,[832] measles vaccine is recommended routinely for asymptomatic HIV-infected children and adults without evidence of measles immunity and should be considered for those with symptomatic HIV infections who are not severely immunosuppressed. Asymptomatic patients at risk for HIV infection do not need to be screened for HIV before vaccination (see "Indications").[233,409,788]

Pregnancy

On theoretical grounds, live-virus vaccines, including measles vaccine, should not be administered to a pregnant woman. However, in contrast to rubella and mumps vaccines, measles vaccine virus has not been shown to cross the placenta and infect the fetus. ACIP recommends that susceptible women of childbearing age be asked if they are currently pregnant or attempting to become pregnant before administering MMR vaccine. Vaccination should be deferred for those who answer "yes." Those who answer "no" should be vaccinated and advised to avoid pregnancy for 28 days after vaccination because of the theoretical risks to the fetus.

Allergies

Severe immediate hypersensitivity reactions (urticaria, angioneurotic edema, wheezing, hypotension, and shock) are very rare serious complications after measles vaccination. From 1991 to 2000, the U.S. Vaccine Adverse Event Reporting System received reports of events that were classified as probable or possible anaphylaxis at an average of two reports per 1 million doses of MMR distributed.[745] A detailed review of patients enrolled in health maintenance organizations in the U.S. Vaccine Safety Datalink Project found three cases of anaphylaxis among 848,945 persons who received MMR vaccine (two had also received other vaccines at the same visit) for an estimated risk of 3.5 episodes of anaphylaxis per million doses of MMR administered.[742,745] In China, the rate was 6.5 per million doses administered.[833] No anaphylaxis deaths have been reported in association with measles vaccination in the United States, but anaphylaxis can be life threatening. Most patients with anaphylactic reactions after measles vaccination do not have clinically evident risk factors for anaphylaxis. Adequate treatment for hypersensitivity reactions, including epinephrine injection, should be available for immediate use whenever measles vaccines are administered, along with staff trained in treatment of anaphylactic reactions.[233]

Measles vaccines typically contain hydrolyzed gelatin as a stabilizer. Allergic reaction to gelatin has been recognized as a major cause of anaphylaxis after MMR vaccination.[834–836] Patients with severe immediate hypersensitivity reactions have been shown to have high levels of antigelatin IgE and IgG and increased gelatin-specific T-cell responses.[834,835] In a study in Japan, 24 of 26 children who had allergic reactions to vaccines had antigelatin IgE. Only two of the children had allergic reactions to gelatin (in food) before vaccination.[836] In contrast, substantially fewer children (6 of 22) with severe allergic reactions to MMR in the United States had antigelatin IgE, and none had a reported history of allergic reactions to gelatin in food.[745] Previous severe allergic reaction to any component of the vaccine, including gelatin, is a contraindication to vaccination.[409]

Because measles vaccines contain trace amounts of the antibiotic neomycin, persons who have experienced anaphylactic reactions to topically or systemically administered neomycin should not receive these vaccines. However, allergic reactions to topical or systemic neomycin are rare and typically consist of delayed or cell-mediated immune responses, such as contact dermatitis. A history of contact dermatitis to neomycin is not a contraindication to receiving MMR-containing vaccine.[409]

Measles vaccines do not contain other antibiotics, such as penicillin; consequently, penicillin allergy is not a contraindication to vaccination.

Measles (and mumps) vaccines licensed in the United States are grown in chick embryo fibroblast cultures. Fasano and colleagues[837] measured a 37 pg/dose of ovalbumin cross-reacting protein in the MMR vaccine currently licensed in the United States. Because some children with egg allergy have had anaphylactic reactions to measles vaccines,[838] previous recommendations included an algorithm of vaccine skin testing and desensitization for children who have had anaphylactic reactions after egg ingestion.[839] However, more than 1200 children with severe allergic reactions to eggs have been vaccinated safely in two studies,[840,841] and the rare serious allergic reactions after MMR vaccination are not believed to be caused by egg antigens but by other components of the vaccine, such as gelatin or neomycin.[409] Vaccine skin testing and desensitization are no longer recommended for persons with severe egg allergies.[233] These persons may be vaccinated by using the same precautions (treatment for anaphylaxis immediately available) as for persons without severe egg allergy. Also, persons with less-severe allergic reactions to eggs, or allergy to chicken or feathers, may be vaccinated as usual.[233,409]

Administration of Immunoglobulin and Other Blood Products

Because passively derived antibody may interfere with vaccine seroconversion, vaccination should be deferred for 3 to 11 months after receipt of IgIM or IgIV or IgSC or other blood products, depending on the dose of the blood product received.[233,409,842] In addition, vaccination should precede receipt of Ig by at least 2 weeks whenever possible,. However, unvaccinated persons may not be fully protected by the passive antibodies received during the entire interval recommended prior to vaccination after receipt of blood products. Therefore, additional doses of Ig or measles vaccination may be necessary if the patient is repeatedly exposed to measles or at high risk of exposure to measles. If measles vaccination is given before the recommended interval has elapsed, the dose should be repeated (at least 28 days after the earlier measles vaccination and after the recommended interval from the time of blood product administration has elapsed).

Thrombocytopenia

Oski and Naiman[843] reported significant depression of platelet counts without clinical symptoms after vaccination with Edmonston B vaccine, including one infant who had thrombocytopenia repeatedly after three doses. Previous immunity to measles did not prevent depression of the platelet count. ITP is associated with MCVs currently in use.[738] The increased risk of ITP after MMR vaccination is estimated at three to four cases for every 100,000 doses.[844,845] There are no reports in which ITP solely associated with measles vaccines has resulted in death.

Two cases of recurrent MMR-associated ITP have been reported in patients with ITP associated with a previous MMR dose,[846,847] and one case of MMR-associated exacerbation of chronic ITP was reported in a 19-year-old female whose onset of chronic ITP was associated with rubella vaccination in the second year of life.[848] In a study that included 21 children with ITP before their first dose of MMR, Miller and colleagues[844] reported that none had a recurrence of ITP after vaccination. A recent review identified 131 children with a history of ITP who received MMR without a recurrence, including 26 children whose ITP followed a first dose of MMR.[739] Advisory committees recommend caution when administering MCVs to children with thrombocytopenia or a past history of ITP, especially when the prior episode of ITP was associated with measles vaccination.[233,409] Serologic assessment of immunity may be useful in assessing the risk and benefits of immunization in such patients.

Family or Personal History of Seizures of Any Etiology

Measles vaccination, like other causes of fever, may cause febrile seizures in young children. Studies suggest that children who have a personal or family history of seizures of any etiology are at increased risk for febrile seizures after measles vaccination compared with children who do not have such histories. In one study in Denmark, children vaccinated with MMR at 15 to 17 months had an increased risk of febrile seizure during the following 14 days of 1.6 per 1000 compared to unvaccinated children of the same age.[749] For siblings of children with a history of febrile seizures the increased risk was 4.0 per 1000 and for children with a personal history of febrile seizures the increased risk was 19.5 per 1000. Febrile seizures after vaccination do not increase the risk of epilepsy or other neurologic disorders. The benefits of administering measles vaccine to children with personal or family history of seizures substantially outweigh the risks, and these children should be vaccinated following the standard recommendations. Their parents should be advised of the benefits of the vaccination and the minimal increased risk of febrile seizures. The risk of febrile seizures after the first dose among children 12 through 23 months of age is higher for MMRV compared to receiving MMR and varicella vaccines separately at the same visit,[233] thus, a personal or family history of seizures of any etiology is a precaution for the first dose of MMRV but not MMR.[233,409] Because the fever induced by measles vaccine occurs between 6 and 12 days after vaccination and seizures may occur with the onset of fever, it is difficult to use antipyretics to prevent febrile seizures after measles vaccination. Patients taking anticonvulsants should continue these medications after measles immunization.[233,409]

FUTURE VACCINES

Currently available measles vaccines are safe, highly effective, and inexpensive; however, they have limitations and alternative measles vaccines and novel delivery methods remain a global priority for research,[849] particularly for measles eradication. Despite high immunogenicity, the effectiveness of currently available measles vaccines are limited by the presence of passively acquired maternal antibodies.[850] Moreover, these vaccines are formulated as lyophilized powder requiring cold chain storage at 4 °C to 8 °C until use. Prior to administration, lyophilized vaccine must be reconstituted with sterile water diluent from the manufacturer and administered via needle and syringe by trained medical staff. Once reconstituted, the vaccine must be discarded after 6 hours because of the risk of bacterial contamination and the loss of potency from exposure to light and increased temperatures. Multidose (i.e., five-dose, 10-dose) vials are commonly used in developing countries. Vaccinators' concerns of potential inefficient use of vaccine supply and vaccine wastage impact decisions for when to open a multidose vial for vaccinating individual children, and lead to missed opportunities for vaccination. Measles vaccinations are administered by subcutaneous injection and injection pain caused by hypodermic needles can be a deterrent for vaccination acceptance. Reconstitution errors and lack of adherence to strict vaccine storage and handling requirements have caused avoidable adverse events and deaths. Safe use and handling of currently available measles vaccines

requires considerable resources, trained medical staff, and maintenance of cold chain. These requirements and complex logistics make house-to-house vaccination campaigns, a strategy that proved to be a key to success for the smallpox and current polio eradication efforts, extremely difficult. Vaccination efforts, particularly in remote locations, could be facilitated by the availability of alternative measles vaccines that are thermostable, and have simplified requirements for storage, handling, and administration.[482]

Intradermal delivery of measles vaccines has been studied for decades and is the likely optimal alternative to current subcutaneous injection. Skin vaccination using needle-free jet injectors[851,852] with single-dose disposable cartridges and with microneedle (also called microarray) patches is currently being evaluated for MCVs. Promising new dissolving measles and MR microneedle patches have proven to be thermostable and fully immunogenic in cotton rats and rhesus macaques.[853,854] The micron-scale (500–800 µm), water-soluble microneedle patches encapsulate a dry formulation of vaccine that rapidly dissolves in the skin upon application. Microneedles cause little or no pain, are easily administered and transported, require minimal storage and disposal capacity, and because they dissolve in the skin, do not require reconstitution with diluent, cannot be reused, and do not generate sharps waste. Human clinical trials using MR microneedle patches are needed to evaluate safety and immunogenicity.

Pulmonary delivery of measles vaccine research has been ongoing for more than 30 years, and is focused on dry powder and nebulized liquid aerosol vaccine formulations, as well as multiple delivery devices for these vaccines in humans and macaques. A measles vaccine dry powder (MVDP) for pulmonary delivery being developed since 2005,[855,856] has demonstrated robust immune responses in macaques, including serum antibody, T-cell responses, and protection from viral challenge.[857,858] This MVDP, which uses two delivery devices, was recently evaluated in a randomized, controlled Phase I human clinical study in adult males 18 to 45 years of age. No adverse events were reported, and the MVDP was well tolerated and produced serologic responses similar to subcutaneous vaccination.[858a] Further studies will be needed to evaluate the safety and immunogenicity of the MVDP in children. Liquid aerosol administration techniques with practical devices and immune response rates of measles vaccine strains have improved over time.[859] The aerosol administration of the Edmonston-Zagreb strain as a second dose to older children and adults induced a booster response at least equivalent to vaccination by subcutaneous injection; however, the use of the Schwarz strain via the aerosol route is no longer being pursued because of low response rates.[860] Recognizing that aerosol administration could potentially simplify administration of measles vaccine to large numbers of children in community-based campaigns, similar to those used in the polio eradication effort,[502,861,862] the WHO, the CDC, and the American Red Cross established a Measles Aerosol Project to complete the necessary research and regulatory requirements to license aerosol delivery of measles vaccine in the developing world.[863] Although previous studies suggested that aerosol measles vaccine administration in infants might be equally immunogenic as the same vaccine administered by injection, a recent randomized, controlled trial found it was immunogenic, but inferior to the currently available injectable measles vaccine.[482,861,863–865] The study was a randomized, open-label, controlled trial of a single 0.2-mL dose of reconstituted measles vaccine administered by liquid aerosol using a nebulizer for 30 seconds through a single-use nonvented face mask in unvaccinated healthy infants 9 to 11 months of age. The nebulizer generated aerosol with a median diameter of 5.1 µm. At day 91 postvaccination, 673 (85.4%) of 788 infants in the aerosol group and 754 (94.7%) of 796 children in the subcutaneous group were seropositive, a difference of −9.3 percentage points (95% CI, −12.3 to −6.4), not meeting the a priori study noninferiority threshold. In this study, no serious adverse events were attributable to measles vaccination and adverse-event profiles were similar in the two groups.[864,865]

To effectively immunize infants at younger ages, research led to the development of subunit DNA and vectored measles vaccines that might be potentially immunogenic in young infants in the presence of maternal antibody.[866–869] A DNA vaccine expressing measles virus hemagglutinin and fusion glycoproteins induced long-lasting, high-avidity neutralizing antibodies in neonatal mice born to measles-immune mothers despite the presence of high levels of maternal antibodies.[868,870] Studies in the monkey model for atypical measles reveal that a DNA vaccine expressing hemagglutinin did not prime for atypical measles.[228] However, some vaccine constructs did induce antibodies of poor avidity.[869] Oral administration methods for measles vaccination have been theorized and studied in animal models. Vaccination using an enteric-coated live, attenuated measles vaccine was not successful in monkeys.[871] In mice, oral administration of a measles peptide incorporated into a vesicle induced a cell-mediated immune response to measles[872] and orally administered adenovirus modified to express measles nucleocapsid antigen induced both measles antibody and cytotoxic T-cell responses.[873]

PUBLIC HEALTH CONSIDERATIONS

Epidemiologic Results of Vaccination

Measles is one of the most contagious diseases of humans and is a classic communicable disease of childhood. The goal of measles vaccination is to prevent illness and death caused by measles directly among vaccinated persons and indirectly among unvaccinated persons as a result of decreased transmission.[874] In the absence of vaccination, measles occurs in epidemic cycles. The magnitude and frequency of the epidemics depends on the population size, contact rates between persons, and the rate at which new susceptible persons are added to the population through births or migration.[875] In England and Wales in the 1940s, epidemics occurred every second year, starting in the large cities of London, Manchester, and Liverpool, and spreading outward to towns and rural villages.[39,876] In the large population centers, chains of transmission were sustained during the periods between epidemics, and these cities were the reservoirs of measles. In towns and villages, transmission died out after an epidemic and had to be reintroduced for each subsequent epidemic. Epidemic cycles of measles also can be described in terms of the effective reproduction number, R, defined as the average number of secondary cases produced by a typical case in a population (see Chapter 77).[583,877,878] When R is less than 1, the average case gives rise to less than one case, and the number of cases occurring subsequently begins to decrease. R for measles varies between and within populations based on factors important for virus transmission, including overall measles susceptibility, population density, contact rates, age structure, heterogeneity of population immunity, and migration patterns.

Introduction of measles vaccination leads to a reduction in the size of epidemics and an increase in the interval between measles epidemics.[874] The widened interepidemic interval has been referred to as the "honeymoon period."[879,880] Vaccination of successive cohorts of young children results in a decrease in incidence among vaccinated cohorts, an overall decline in measles incidence in all age groups (because of dampened transmission), and, when outbreaks occur, an increase in the

proportion of cases among older children.[385,880,881] Susceptibility to measles among older cohorts occurs most often because these persons missed natural disease as young children and either missed getting vaccinated (program failure) or failed to respond to vaccination (primary vaccine failure). Waning of vaccine-induced immunity (secondary vaccine failure) occurs in 0% to 5% of vaccinees but does not appear to play a major role in measles virus transmission or reducing overall population immunity to measles.[192,687,697]

Herd Immunity

As vaccination coverage increases among successive birth cohorts, measles transmission decreases, reducing the risk of measles even among unvaccinated persons. At some vaccine-induced immunity level lower than 100%, measles virus transmission is interrupted.[882–884] Mathematical models have estimated the herd immunity threshold for measles in the United States at 92% to 95%.[584] If this level of population immunity is maintained by a vaccination program, endemic transmission of measles will die out and measles elimination is achieved. Knowledge of the minimum level of vaccination capable of preventing measles transmission in an age group is helpful for establishing program targets for measles elimination. During the measles resurgence in the United States, 1989–91, epidemiological studies found that a minimum vaccine coverage of approximately 80% at the second birthday may be sufficient to prevent measles transmission among preschool-age children if population immunity is 93% among persons 6 years of age and older.[885] Similar differences in age-specific measles transmission rates have been observed in the WHO European Region, and mathematical modeling suggests a 6.4% susceptibility target for all age groups; however, achieving a lower level (5%) in age groups with the highest transmission rates, namely secondary school children and young adults, allows higher levels of susceptibility in preschool (15%) and primary school (10%) children.[583]

Elimination does not result in zero measles cases because importations of measles from endemic areas continue to occur. However, spread from these importations is short lived. Documenting the elimination of measles is challenging. De Serres and associates[584] have proposed using the proportion of cases imported and the distribution of outbreak sizes to monitor elimination. These surveillance parameters can be used to estimate R, which must be maintained at less than 1 to achieve elimination. Global eradication of measles will occur when the last chain of transmission of measles virus is interrupted and has been defined as the worldwide interruption of measles virus transmission in the presence of a surveillance system that has been verified to be performing well.[54]

In industrialized countries, a single dose of measles vaccine administered in the second year of life induces immunity in approximately 95% of vaccinees.[581] With a primary vaccine failure rate of 5%, 100% of the population would have to be vaccinated to reach a 95% immunity level with a single-dose strategy. Approximately 95% of persons who fail to respond to the first dose respond to a second dose,[568] and with high vaccine coverage, the herd immunity target can be reached if two doses are administered.

In developing countries, high morbidity and mortality resulting from measles among infants led to a recommendation to vaccinate infants at 9 months of age, a time when maternal antibody may interfere with seroconversion.[887] Studies have found the median seroconversion rate with vaccination at age 9 months is 85% (range: 70% to 98%).[888] This leaves three times more infants susceptible (15% of vaccinees) than does a rate of 95%. After a single dose with 90% coverage and 85% seroconversion, 77% of the population would be immune. To improve immunity, some have considered providing a second dose, usually through routine health services at an older age, when seroconversion is 95%. However, if only first-dose recipients were revaccinated through routine health services, given 90% coverage with both doses, immunity levels could rise to only 90%, leaving 10% susceptible. Alternatively, if vaccination activities were intensified both within and outside the routine health services, such that many children never vaccinated could receive a first dose while previously vaccinated children receive a second dose, the resulting population immunity level would be much higher. This is known as a second opportunity strategy.[805] For example, if the second opportunity reached 90% coverage of prior first-dose recipients and previously unvaccinated children, population immunity would increase to greater than 95%. In countries with poor access to preventive services, the second opportunity for measles vaccination is most often provided through nationwide supplementary immunization activities or mass campaigns, because these are more effective at reaching children who have never been vaccinated.

Nonspecific Effects

Wild measles infection is immunosuppressive and causes loss of memory responses to infections from other etiologies, which increases all-cause mortality. Observations in monkeys and humans, supported by immunological studies, suggest that measles vaccination reduces mortality beyond its protection against measles.[889–894] A recent study by Mina and colleagues suggests substantial nonspecific benefits of measles vaccination occur by preventing measles-induced adverse impact on the overall immune system, which, in turn, contributes to increased nonmeasles infectious disease mortality.[895] In April 2014, WHO's Strategic Advisory Group of Experts concluded that available evidence suggests possible beneficial effects of measles vaccination on all-cause mortality but found the evidence too weak to guide vaccination policy.[896]

Experiences With Measles Control and Elimination in Various Countries

Industrialized Countries

Experience in industrialized countries shows that a single dose of measles vaccine, widely administered, can reduce measles transmission, but a two-dose strategy is necessary for elimination of indigenous transmission.[28,897–899] Many countries introduced measles vaccine as a single-dose schedule and then added a second opportunity for vaccination because of the persistence of outbreaks despite high single-dose coverage (Table 37.10). Industrialized countries have found that the costs of patient care and outbreak control outweigh the cost of a second opportunity for immunization.[900] Some countries (e.g., Canada, United Kingdom, Australia, and New Zealand) have adopted a two-dose schedule and have conducted a one-time, nationwide vaccination campaign to reduce susceptibility among school-age children. Other countries (e.g., the United States, Finland, and Sweden) have relied on a two-dose schedule alone.

In the United States, the distribution and use of more than 450 million doses of live measles vaccine between the year of licensure (1963) and the end of 2014 (see Fig. 37.6) is associated with a marked reduction in measles (Fig. 37.9) and its associated complications, and estimated savings of billions of dollars.[26,404] In the years prior to vaccine introduction, approximately 4 million cases occurred annually, whereas on average 400,000 to 500,000 cases were reported. In 2010, only 64

TABLE 37.10 Evolution of Measles Vaccination Policy in Selected Countries and the Expanded Programme on Immunization, 1963–2014

First Opportunity			Second Opportunity			
First Dose in Schedule			Second Dose in Schedule		Supplementary Campaign	
Country	Year	Recommended Age	Year	Recommended Age	Year	Age Range
United States	1963	9 mo				
	1965	12 mo				
	1976	15 mo				
			1989	4–6 or 11–12 y		
	1994	12–15 mo				
			1999	4–6 y		
Canada	1963	9 mo				
	1968	12 mo				
			1996	18 mo or 4–6 y	1996	5–16 y
United Kingdom	1968	12–23 mo				
	1988	13–15 mo				
			1996	Before school entry	1994	5–16 y
					2013	10–16 y
	2012	12–13 mo	2012	3–5 y		
Brazil	1973	8 mo				
	1976	7 mo				
	1982	9 mo	1992	12–15 mo	1992	9 mo–14 y
					1995	1–3 y
					1997	6 mo–4 y
					2000	1–4 y
					2001–2002	12–29 yr women only
					2004	1–4 y
					2008	20–39 y
	2000	12 mo	2004	4–6 y		
China	1978	8 mo				
			1986	7 y		
			2006	18–24 mo		
					2010	8 mo–4 y or 14 y
WHO EPI	1983	9 mo				
	1989	6 mo				
	1991	9 mo				
			2001	Either routine second dose or regular supplementary campaigns		
			2009	15–18 mo (or school entry) and/or regular supplementary campaign		

EPI, Expanded Program on Immunization; WHO, World Health Organization.

confirmed cases were reported, with 56 (87.5%) of those cases linked to importations. This represented a reduction of more than 99.9% compared with the years preceding vaccine licensure. The reported occurrence of SSPE also declined greatly (see Fig. 37.8) and was virtually eliminated in the late 1980s and early 1990s. A small number of cases had been reported each year during the late 1990s as a result of the resurgence of measles during 1989 to 1991.

The United States has embarked on three elimination efforts (see Fig. 37.9).[22,23,883,901] The first, in 1966, was based on a single-dose strategy with vaccination at 12 months of age (see Table 37.10). The second, announced in 1978, had three components: (a) a high level of population immunity through vaccination with a single dose of measles vaccine, (b) disease surveillance, and (c) prompt response to outbreaks.[22,25] To reach high coverage, vaccination requirements for school entry were enacted and enforced in every state. These requirements, which mandated not only measles vaccination but also other childhood vaccinations, have become the major legacy of this elimination initiative. Although sustained interruption of transmission of measles was not achieved, measles was eliminated from most of the country; 54% of counties in the United States were measles free for the entire decade from 1980 to 1989, and only 17 (0.5%) counties reported measles every year during that period.[902] During this interval, outbreaks continued to occur in schools among school-age persons with histories of vaccination and among unvaccinated pre–school-age children.[383,550,610,614,693,903] In 1989, the United States introduced a routine second dose of measles vaccine at ages 4 to 6 years or 11 to 12 years to address the problem of school outbreaks (see Table 37.10).

After relatively low incidence during the 1980s, a 3-year epidemic of measles began in 1989 and resulted in more than 55,000 cases and 123 deaths.[904,905] The average annual incidence during 1989 to 1991 was 7.4 per 100,000 population compared with an average incidence for 1981 to 1988 of 1.8 per 100,000. Incidence was increased in all age groups; however, the greatest increases were in children younger than 1 year and 1 to 4 years, resulting in almost half of all cases occurring in children younger than 5 years (see Table 37.1). This epidemic was a consequence of low measles vaccination levels among pre–school-age children, particularly in inner cities,[906–909] and was part of a hemisphere-wide measles epidemic.

In 1993, the third elimination initiative was launched. In addition to the three components of the earlier strategy, this

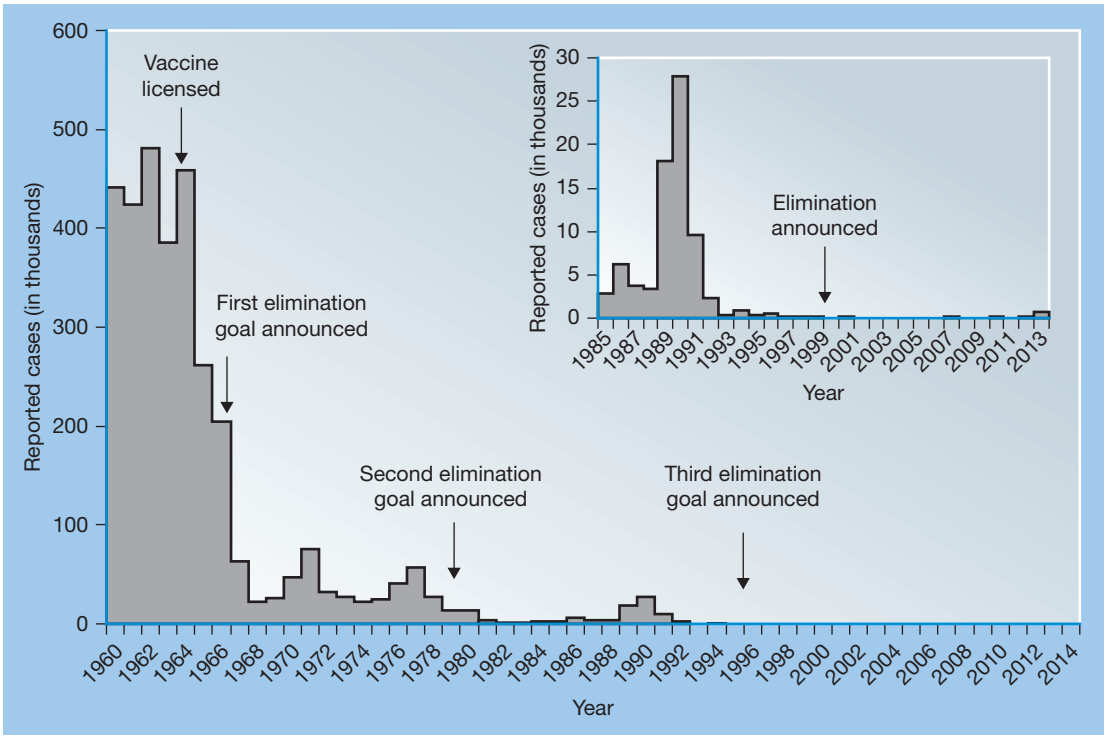


Figure 37.9. Reported measles cases, United States, 1960–2014.

initiative focused on increasing preschool immunization levels to greater than 90% and vaccination of all school children with a second dose.[901] To implement these vaccination strategies, between 10 and 16 million doses of measles vaccine were distributed annually between 1994 and 2005 (see Fig. 37.6). In March 2000, the CDC convened a panel of experts to review the pattern of measles transmission in the United States. Evidence presented included an annual measles incidence of less than 1 case per 1 million population since 1997,[910] a maximum outbreak size of 15 cases during 1999 to 2001,[815] an increase in the proportion of imported cases from 14% (1993 to 1996) to 35% (1997 to 2001),[911] an overall estimated population immunity against measles of 93%,[912] and the absence of an endemic measles virus genotype since 1994.[255] Based on this evidence, each participant concluded that measles was no longer an endemic disease in the United States.[26,913] In 2011 a second panel convened by the CDC concluded that elimination of endemic measles transmission had been sustained from 2001 to 2011. The data reviewed by the panel included continued measles incidence less than 1 case per million population, with 88% of measles cases imported or importation related and continued absence of an endemic genotype, all in the presence of an adequately functioning surveillance system and with documented high levels of population immunity. The four components of the current U.S. measles elimination strategy are to maximize population immunity through vaccination, to ensure adequate surveillance, to respond rapidly to outbreaks, and to work with other countries to achieve global measles control. The two key factors in the effort to maximize population immunity are to improve timely immunization of pre–school-age children and to deliver a second dose of measles vaccine to all school children.[901] National estimates of one-dose MMR coverage among children 19 to 35 months of age remained above 90% from 1996 through 2013.[914–917] Among the 49 states reporting coverage for children in kindergarten in 2014–15, the median vaccination coverage was 94.0% for two or more doses of MMR.[918] In 2014, a national survey estimated 91% of adolescents 13 to 17 years of age had received two or more doses of MMR.[919] The importance of maintaining very high vaccination coverage is illustrated by an analysis of age-specific measles vaccination data from the National Immunization Survey–Teen (2008–2013), which showed that 12.5% of U.S. children and adolescents are susceptible to measles.[919a]

Challenges to maintaining elimination in the United States include frequent international travel, large outbreaks of measles in highly traveled developed countries, and clusters of U.S. residents who remain unvaccinated because of personal belief exemptions.[920,921] As evidence of these ongoing challenges, from January 1 through May 23, 2014, a total of 288 confirmed measles cases were reported to CDC, surpassing the highest reported yearly total of measles cases since elimination.[820] By the end of 2014, the annual total had increased to 667 measles cases, the highest number reported since 1996. There were 63 imported measles cases, seven in foreign visitors and 56 in U.S. residents, including eight unvaccinated infants. The increase in cases following importations reflects recent increases in the incidence of measles in countries with high levels of travel to and from the United States. The source of almost half of the direct measles importations in 2014 was the Philippines. Most of the 288 measles cases reported between January and May 2014, were in persons who were unvaccinated (200 [69%]) or who had an unknown vaccination status (58 [20%]); 30 (10%) were in persons who were vaccinated. Among the 195 U.S. residents who had measles and were unvaccinated, 165 (85%) declined vaccination because of religious, philosophical, or personal objections; 11 (6%) were missed opportunities for vaccination; and 10 (5%) were too young to receive vaccination.[820] Similarly, from January 4 through April 2, 2015, 159 measles cases were reported to CDC, with one outbreak that originated in Disney theme parks in California accounting for 70% of cases.[921a] Among 110 California residents linked to this outbreak, the majority were either unvaccinated (49 [45%]) or had an unknown

vaccination status (47 [43%]); 14 (13%) had received one or more doses of a measles-containing vaccine or were immunoglobulin G seropositive.[921b] The outbreak spread to seven U.S. states, Mexico, and Canada, garnered considerable media attention, and led California legislators to pass laws eliminating nonmedical (philosophical and religious belief) exemptions to school immunization requirements in their state.[921c] Investigating and responding to measles outbreaks is time consuming and labor intensive, and imposes a significant economic burden on local and state health departments. Sixteen measles outbreaks occurred during 2011 and cost local and state health institutions an estimated US$2.7 to US$5.3 million.[922]

Canada began a measles immunization program in 1963 (see Table 37.10). Despite achieving coverage in excess of 95% with a single dose of measles vaccine throughout the 1980s, measles outbreaks continued to occur.[28] In 1995, 2362 measles cases were reported in Canada, more than one-third of the total number of cases reported in the Western Hemisphere. School outbreaks among children who had received a single dose of MCV accounted for most cases. Mathematical modeling predicted a sizable measles epidemic, and cost-to-benefit analyses showed that a national vaccination campaign to prevent the predicted epidemic would save $2.50 for every dollar spent.[900] On the basis of these analyses, 11 of the 12 provinces of Canada undertook mass vaccination campaigns targeting school-age children by using measles-rubella vaccine in the spring of 1996. A coverage level of more than 90% was achieved. In addition, a second dose was added to the routine schedule at age 18 months or 4 to 6 years of age (see Table 37.10). From 1998 through 2001, Canada interrupted endemic measles transmission as evidenced by the occurrence of only a few small outbreaks, many linked to importation.[28] In 2007, there was an outbreak of 94 cases characterized by transmission lasting 25 weeks in unvaccinated persons belonging to unrelated networks dispersed in the population.[923] Genotyping of measles virus in Canada supports epidemiologic information indicating importation as the source of current measles cases.[924] An analysis of the epidemiology of measles in Canada from 2002 through 2013 found a trend toward a greater annual number of importations over the period. While five chains of transmission had more than 30 cases, none of the importations resulted in sustained transmission for 12 months or longer, confirming that Canada has maintained elimination between 2002 and 2013.[585]

In 1968, the United Kingdom introduced measles vaccine for children between the ages of 1 and 2 years, but throughout the 1970s and early 1980s coverage remained below 80%. In 1988, combined MMR vaccine was introduced in place of measles vaccine at age 13 to 15 months (see Table 37.10) and coverage improved to greater than 90%. In November 1994, all children ages 5 to 16 years in England and Wales were offered combined measles-rubella vaccine, regardless of prior vaccination history, in an attempt to prevent a predicted epidemic of measles. During the campaign, 6.2 million doses were administered for a reported coverage of 92%.[898] Between 1995 and 2000, among 594 confirmed measles cases, 212 (36%) were sporadic and 382 (64%) were associated with 51 clusters. Forty-eight sporadic cases (23%) and 18 (35%) of 51 clusters were associated with an importation of infection from overseas. A wide variety of measles virus genotypes were identified during this period. The pattern of sporadic cases and small clusters associated with importations is consistent with elimination of sustained indigenous measles transmission.[898] However, unfounded concerns about vaccine safety, specifically the purported relation of MMR and autism, that began in 1998 (see "Vaccine Safety") contributed to a decline in MMR coverage that has resulted in a number of large outbreaks. In 2007, measles endemicity was reestablished with genotype D4 as the predominant genotype.[925] In 2013, based on an increase in the number of confirmed measles cases in children 10 to 16 years of age, the National Health System and Public Health England launched a national MMR vaccination campaign. This campaign increased MMR vaccination coverage to over 95% among 10- to 16-year-old children and resulted in a marked decline in the number of reported measles cases in the United Kingdom.[926]

Excellent control of measles has been achieved in other industrialized countries. In 1982, Finland initiated a measles elimination program based on a two-dose vaccination strategy. Efforts to improve vaccination coverage included mass media, a registry system to track defaulters, and an intensive outreach program. Measles elimination was achieved in Finland by 1996 and has been maintained as a result of consistently high (greater than 95%) two-dose vaccination coverage.[34,927] Sweden has reported similar success with a two-dose schedule.[32] Australia and South Korea have achieved elimination by using a two-dose routine schedule supplemented by a one-time catch-up campaign.[928,929] However, in other industrialized countries (e.g., Italy,[930] Germany,[931] France, and Switzerland[932]), measles remains endemic, with frequent large outbreaks, in part because of the perception that vaccination may be more risky than the disease itself.[933]

The European Region of the WHO, which includes the countries of the former Soviet Union, has updated its strategic plan for eliminating measles and rubella by 2015 and identified the accelerated actions needed to achieve the goal.[933] In addition to Finland and Sweden, a number of European countries are close to, or have already achieved, elimination (e.g., Hungary, Poland, the Netherlands, Slovenia, and the Russian Federation[934]). However, a review of the epidemiology of the 31,520 reported measles cases in Europe in 2013 found most cases occurred in nine countries: Georgia (7830), Germany (1773), Italy (2216), the Netherlands (2499), Romania (1074), the Russian Federation (2174), Turkey (7404), Ukraine (3308), and the United Kingdom (1900). The authors concluded that without sustained political commitment and accelerated action by Member States and partners, the elimination of measles in the WHO European Region may not be achieved on time.[935]

Less Industrialized Countries

Since the late 1970s, the Expanded Program on Immunization has played a major role in the development of immunization programs in less industrialized countries. Measles vaccination coverage in these countries rose from 18% in 1981 to 76% in 1990, and an estimated 1.7 million measles deaths were prevented in 1995 alone. During the 1990s, donor support for the Expanded Program on Immunization decreased substantially, and measles vaccination coverage levels plateaued or decreased, with the estimated annual number of measles deaths stabilizing at about 1 million.[396]

Worldwide in 2000, measles was estimated to cause between 535,300 and 777,000 deaths (i.e., 1.4% of 55.7 million total deaths).[397,398] Among children younger than 5 years of age, measles was the fifth leading cause of death, accounting for 5.4% of the 10.9 million total deaths in this age group. The vast majority of measles deaths (>95%) occur in less industrialized countries because of lower measles vaccination coverage (i.e., underdeveloped routine health services and lack of a second opportunity for measles vaccination) and a higher case-fatality ratio in these countries. The higher risk of death from measles in less industrialized countries compared with industrialized countries is associated with a younger median age of cases, increased intensity of exposure,

increased likelihood of secondary infections, and malnutrition, especially vitamin A deficiency. Poverty, crowded living conditions, and large family size are the underlying socioeconomic factors contributing to this epidemiologic pattern.[887] Global immunization programs have resulted in a decrease in worldwide measles mortality to an estimated 134,000 deaths in 2015 and prevented 20.3 million deaths since 2000.[936]

During the late 1980s and early 1990s in less industrialized countries, two different approaches were developed to address the problem of severe measles in children younger than 9 months, the age for vaccination recommended by the WHO (see Table 37.10). First, high-titer measles vaccines (e.g., Edmonston-Zagreb measles vaccine) were developed to overcome interference by maternal measles antibody when administered at 4 to 6 months of age. For a short period from 1989 to 1991, the WHO recommended use of high-titer Edmonston-Zagreb measles vaccines for 6-month-old infants in countries where measles was a significant cause of death for infants younger than 9 months.[679] Although high-titer vaccines were more immunogenic than standard-titer vaccines, they were unexpectedly associated with excess delayed mortality among females. For this reason, the WHO withdrew the recommendation for use of these vaccines in 1992.[684] The second approach, developed by the Pan American Health Organization (PAHO),[805] was based on the observation that older siblings were often the source of measles infection for infants. The concept of a one-time nationwide mass vaccination campaign for children ages 9 months to 14 years, regardless of prior disease or vaccination history, was pioneered in Cuba in 1987. The objective of this so-called catch-up campaign was to interrupt measles virus transmission by rapidly reducing susceptibility among older children and thereby prevent transmission to infants younger than 9 months of age. This approach proved highly effective and has been developed further into the PAHO strategy for measles elimination.

Pan American Health Organization Strategy

In 1994, ministers of health of countries of North and South America established the goal of eliminating measles from the Western Hemisphere by the end of 2000. To accomplish this goal, PAHO developed a strategy with three essential vaccination components: (a) catch-up—a one-time mass vaccination covering all children ages 1 to 14 years, regardless of prior disease or vaccination status; (b) keep-up—achievement of 90% or greater immunization coverage in each successive birth cohort; and (c) follow-up—subsequent mass campaigns conducted every 3 to 5 years, covering all children ages 1 to 5 years irrespective of prior disease or vaccination history.[805] Follow-up campaigns should be conducted before the estimated number of measles-susceptible pre–school-age children (determined by vaccination coverage and presumed vaccine effectiveness) reaches the size of the birth cohort. After the catch-up campaigns, most countries have increased the measles vaccination age to 12 months to maximize vaccine effectiveness. With the establishment of a rubella elimination goal in the Americas by the year 2010, countries in Latin America conducted nationwide vaccination campaigns targeting more than 450 million women and men up to age 39 years with MR vaccine. The addition of these "speed-up" campaigns to the PAHO Strategy resulted in the elimination of rubella in the Western Hemisphere by 2009 and reinforced efforts leading to measles elimination.[937] In addition to the vaccination strategy, case-based surveillance with laboratory confirmation of suspected measles cases has been established in all countries of the Americas.[938]

In Brazil, the National Immunization Program was created in 1973, and routine measles vaccination was recommended at 8 months of age.[939] National measles control was intensified in 1980–81 with campaigns focused in areas of low coverage. The minimum recommended age for measles vaccination was changed to 7 months in 1976, and then to 9 months in 1982. In 1992, with the adoption of a measles elimination goal by 2000, a second dose was recommended at age 12 to 15 months (see Table 37.10). Based on the success of statewide mass vaccination campaigns in Parana and Saõ Paulo in 1987, Brazil conducted a nationwide catch-up campaign in 1992 during which 48 million doses of measles vaccine were administered to children ages 9 months to 14 years. In 1995, the first national follow-up campaign for children ages 1 to 3 years was conducted; the state of Saõ Paulo did not participate in this campaign.

In 1997, after a 4-year period of low incidence, Brazil experienced a major resurgence of measles, with a total of 53,335 cases and 61 deaths.[939] Transmission was concentrated in the metropolitan area of Saõ Paulo State but subsequently spread to involve all states. The highest age-specific incidence rates were among children younger than 1 year (1577 per 100,000), young adults ages 20 to 29 years (539 per 100,000), and children ages 1 to 4 years (205 per 100,000). This age distribution was similar throughout Brazil, with 55% of cases occurring among adults 20 to 29 years of age, a cohort born between 1968 and 1977, when the vaccination program was being initiated. Characterization of measles viruses during the outbreak in 1997 identified the D6 genotype. This same genotype was identified in subsequent measles outbreaks in Argentina and Uruguay in 1998 and in Chile, Bolivia, and the Dominican Republic during 1999 to 2001.[940]

The resurgence of measles in Brazil in 1997 led to a nationwide follow-up campaign in 1997 for children ages 6 months to 4 years. A third follow-up campaign was conducted in 2000 for children ages 1 to 4 years. After two follow-up campaigns, measles transmission was interrupted. In 2008, as part of their rubella elimination strategy, Brazil conducted a national MR vaccination campaign for women and men ages 20 to 39 years, which both eliminated rubella and served to maintain measles elimination until 2013.[937]

Implementation of the PAHO strategy in the Western Hemisphere has resulted in a greater than 99% decline in reported measles cases, from a high of almost 250,000 cases in 1990 to 1824 cases in 2014 (Fig. 37.10). The pattern of measles importations into the United States confirms the success of the PAHO strategy.[911] In 1990, at the height of the regionwide resurgence in measles, 242 of 300 importations of measles were from Latin America; in contrast, of 27 importations in 2004, none was from Latin America.[27] During 2001, measles outbreaks in Haiti and the Dominican Republic were brought under control, ending known transmission of the D6 measles virus genotype in the Western Hemisphere. Following the interruption of transmission with the D9 genotype in Venezuela and Colombia in November 2002, the Western Hemisphere was free of endemic measles for more than 12 years. However, importation of D8 genotype in March 2013 into Pernambuco State in Brazil led to an outbreak of more than 1000 cases. This outbreak spread to the neighboring state of Ceara and lasted through May 2015, resulting in the reestablishment of endemic transmission of measles in the Americas. Intensive control efforts were successful in stopping transmission in Brazil and on 27 September 2016, PAHO announced that elimination of measles had been verified in all countries in the Americas.[940a]

The success of the PAHO strategy has led to expansion of its use outside the Western Hemisphere. In Africa, the PAHO strategy has been adopted in an effort to reduce measles mortality. Between 1996 and 1999, seven southern African countries (South Africa, Namibia, Zimbabwe, Swaziland, Malawi,

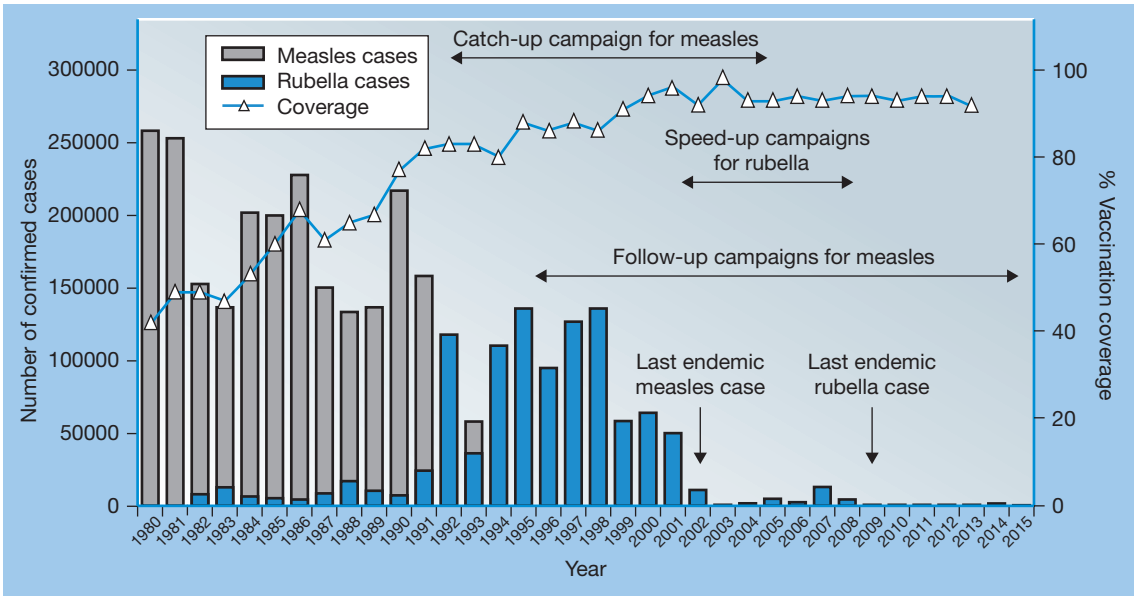


Figure 37.10. Reported measles cases, Latin America and the Caribbean, 1980 to June 2015. *(Data courtesy the Immunization Unit, Family and Community Health Area, Pan American Health Organization.)*

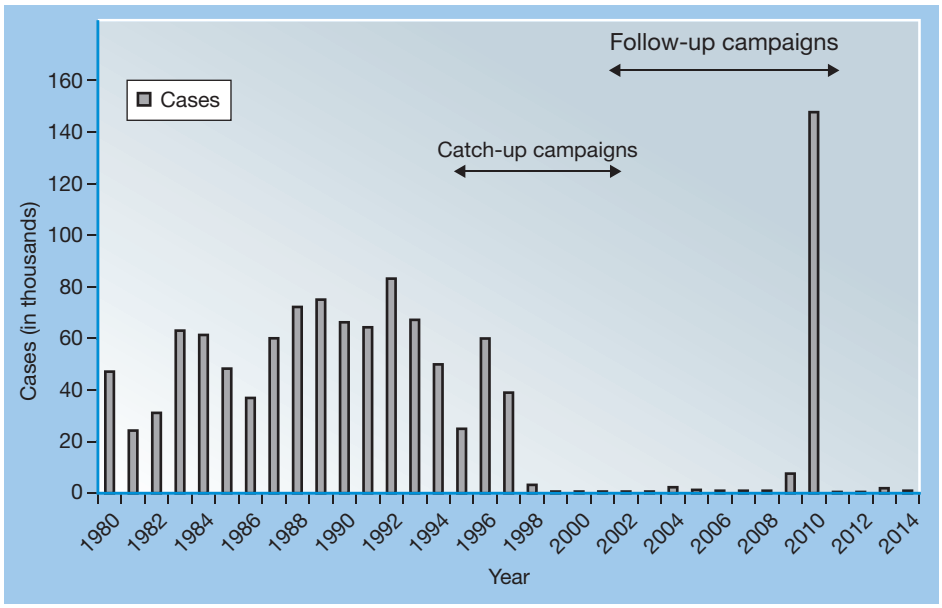


Figure 37.11. Reported measles cases, seven southern African countries, 1980–2014. *(Data courtesy the African Regional Office of World Health Organization.)*

Botswana, and Lesotho) conducted catch-up vaccination campaigns and introduced case-based measles surveillance.[941] Reported measles cases in these countries decreased from more than 50,000 in 1996 to between 20 and 2234 reported measles cases in the years 1998 to 2008 (Fig. 37.11). This period of low transmission was followed by a widespread resurgence across all countries in southern Africa that started in 2009 and peaked in 2010 with more than 140,000 reported measles cases and 764 measles deaths.[942,943] Possible reasons for the resurgence include build-up of susceptibles as a result of gaps in immunization coverage, both routine and during campaigns, and increased migration into South Africa following democratic elections in 1994. During 2011–14, reported measles incidence has remained low in southern Africa (Fig. 37.11).

Mortality Reduction Strategies

In February 2001, at a meeting hosted by the American Red Cross, a new partnership was formed to advocate for reduction of measles mortality in Africa.[944] Worldwide, during 2000 to the end of 2008, more than 650 million children received measles vaccine through supplementary immunization activities, and global routine immunization coverage with one dose of measles vaccine increased from 71% to 83%. These accelerated control efforts resulted in a 78% decrease in the estimated number of measles deaths worldwide by the end of 2008, thereby surpassing the 2005 global goal of a 50% reduction.[945] However, between 2009 and 2014 progress slowed and the estimated annual number of measles deaths remained above 100,000, with the result that the global goal set for 2015 to

reduce measles deaths by 95% compared with 2000 levels was not met on time.[936]

Based on the success of implementing the PAHO strategy in other regions of the world, a schedule that offers at least two doses of measles vaccine delivered either through routine services or mass campaigns has become the standard of care in all countries regardless of development status or routine vaccination coverage (see Table 37.10).[804]

Measles Eradication

The feasibility of measles eradication has been debated for many years.[42,44–46,946–948] Hopkins and colleagues[42] have proposed global measles eradication and pointed out both the similarities and differences between measles and smallpox. Measles will be substantially more difficult to eradicate than smallpox because of its higher contagiousness, younger median age at infection, and older age at which vaccine is effective.[949]

As of 2014, all six WHO regions have established target dates for measles elimination (the Americas by 2000, Europe and the Eastern Mediterranean initially by 2010 and revised to 2015, the Western Pacific by 2012, and Africa and Southeast Asia by 2020). The Region of the Americas stopped endemic measles transmission in November 2002 and remained free of endemic measles for over 12 years. At the global level, measles control targets have been set for 2015 to increase measles immunization coverage to 90% or greater at the national level and to 80% or greater in every district, to decrease the incidence of measles to less than five cases per million, and to reduce measles deaths by 95% compared with 2000 levels.[950]

In July 2010, the WHO convened a global technical consultation to review the feasibility of measles eradication.[47] There was a comprehensive review of the biologic, programmatic, vaccine supply, economic, and health systems aspects of measles eradication compared with mortality reduction targets. An epidemiologic and economic evaluation based on data collected in Bangladesh, Brazil, Colombia, Ethiopia, Tajikistan, and Uganda found that measles eradication by 2020 was the most cost-effective measles control option both in the six countries and globally, and it would cost an additional discounted $7.8 billion between 2010 and 2050.[951] After reviewing all the available information, the conclusion was that measles can and should be eradicated and that global eradication by 2020 is feasible. The report from the global technical consultation was reviewed and discussed by the Strategic Advisory Group of Experts in November 2010. They agreed that eradication of measles is technically and biologically feasible and recommended measurable progress toward existing regional elimination targets before establishing a target date for global eradication.[952] However, there has been little further progress since 2010, and the 2014 Annual Report of the Global Vaccine Action Plan concluded that regional elimination of measles by 2015 in the European, Eastern Mediterranean, and Western Pacific Regions was off-track.[953] In 2016, a midterm review of the Global Measles and Rubella Strategic Plan, 2012-2020,[953a] was conducted to determine the underlying causes for lack of progress toward regional elimination goals. The review concluded that the basic strategies articulated in the plan are sound; however, these require full implementation.[953b] The main impediments to full implementation have been inadequate country ownership and global political will, reflected in inadequate resources. The report recommends a shift in strategic focus toward greater reliance on ongoing services to administer two doses of MCV (rather than supplementary immunization activities) and toward use of disease incidence and epidemiology as a major indicator of progress (rather than vaccination coverage alone). The report emphasized that, while eradication is the ultimate goal, it is premature to set a date for its accomplishment, and existing regional elimination goals should be vigorously pursued to enable setting a global target by 2020.[953b]

Available evidence indicates measles meets the criteria for a disease that can be eradicated: (a) there is no animal or environmental reservoir, and humans are critical to maintaining transmission; (b) accurate diagnostic tests are available; (c) measles vaccine and existing vaccination strategies are effective and safe; and (d) measles transmission has been interrupted in a large geographic area (e.g., nationwide) for a prolonged period.[46] Major challenges to global measles eradication include (a) increasing urbanization and population density that will require very high vaccination coverage, (b) war and civil unrest, (c) the frequency of international travel and forced migrations that facilitate importations of measles, and (d) the HIV epidemic, which may reduce the effectiveness of measles vaccination and increase the transmissibility of measles.[832] Other potential impediments are lack of political will, risk of unsafe injections, and the possibility of sustained transmission among adults who are accounting for an increasing proportion of all cases. Measles in adults is emerging as a major challenge to elimination in countries with longstanding immunization programs (e.g., Western Europe[935] and China[954]) that experienced lower coverage in the early years of the programme. To address these challenges, research[849] is ongoing to improve diagnostic tests for measles by using filter paper blood spots[336,337] and oral fluid[334]; and to develop alternative routes for administration of existing measles vaccines (e.g., aerosol vaccination[482–485]), needle-free jet injectors, and microarray patches[853] to reduce the risk of unsafe injections; and new candidate vaccines that could be administered in the presence of maternal antibodies ("stealth vaccines").[955] In addition, strategies for disease surveillance, increasing vaccination coverage through routine immunization services and mass campaigns, and program monitoring are being strengthened to learn from recent field experience with measles mortality reduction and regional elimination activities. These efforts provide optimism that, in the future, the goal of global measles eradication may be achieved.

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38 Meningococcal Capsular Group A, C, W, and Y Conjugate Vaccines

Lee H. Harrison, Dan M. Granoff, and Andrew J. Pollard

Epidemics of meningococcal meningitis were first described in Geneva, Switzerland in 1805[1] and Medfield, Massachusetts in 1806.[2] Such epidemics occurred in the United States until 1944. In sub-Saharan Africa, meningococcal epidemics have been recognized for more than 100 years.[3] In the early 1900s, Flexner[4] demonstrated that intrathecal administration of antimeningococcal serum decreased mortality of meningococcal meningitis. In the past 15 years, huge advances have been made in new meningococcal vaccines, which are changing the face of meningococcal disease epidemiology globally.

Meningococcal disease can progress rapidly. The case fatality rate, which was 70% to 85% before the advent of antibiotics, is now 10% to 15%.[5] Epidemics historically occured every 5 to 10 years in sub-Saharan Africa with incidence rates as high as 1000 per 100,000 population,[3] but the epidemiology has changed dramatically in the past few years. Epidemics have not occurred in the United States and Europe since the 1940s, and during the past decade endemic disease incidence rates have fallen to rates not seen in the last 60 years. A group B epidemic in New Zealand with more than 5000 cases during a 15-year period beginning in the early 1990s demonstrated the potential of *Neisseria meningitidis* to cause epidemic disease in an industrialized country. Control measures were unsuccessful in New Zealand until development of an epidemic-specific outer membrane vesicle vaccine and mass immunization.

BACKGROUND

Clinical Description and Manifestations

The clinical features of meningococcal disease are highly variable. Classic clinical features of meningococcal disease are fever, rash, and meningitis, but initial presentation is often nonspecific and therefore may be indistinguishable from those of other bacterial, rickettsial, or viral infections.[6,7] In fact, many serious cases of meningococcal disease are initially misdiagnosed as a benign viral illness, which can have devastating consequences because rapid progression to life-threatening disease may follow within hours.[7]

The most common clinical manifestation is acute bacterial meningitis.[8] Older children and adults typically have an abrupt onset of fever, headache, photophobia, myalgias, and malaise. Seizures occur in approximately 20% of patients. Signs of altered consciousness, such as hyperactivity or lethargy, can be prominent. Nuchal rigidity is a common sign except in infants in whom a more gradual onset of fever, poor feeding, and lethargy are the typical initial complaints, and a bulging fontanel may be the major sign of central nervous system involvement. A rash is present in the majority of cases of meningococcal disease, which consists of typical petechiae or purpuric lesions that usually are most apparent on the chest, upper arms, and axillae.[9] Maculopapular rashes also are common and may occur early in the absence of petechiae.

Among patients with meningococcal disease, 10% to 20% have severe sepsis or meningococcemia. Meningitis may be absent, but the organism is widely disseminated in the bloodstream and in multiple organs. Spreading purpura heralds signs and symptoms of septicemia with rapid onset of hypotension and signs of multiple organ failure, although not all patients with shock or hypotension have purpura. White blood cell counts may be very high, normal, or low, and a prominence of immature band forms is often present. At the time of autopsy, acute adrenal hemorrhage may be present (Waterhouse-Friderichsen syndrome).

Up to 15% of patients with meningococcal disease have pneumonia, which is most commonly associated with capsular group Y strains.[10] In the absence of a characteristic rash and bacteremia, the cause of the pneumonia may go unrecognized because the clinical microbiology laboratory may not report isolation of *N. meningitidis* from oropharyngeal secretions as it is considered normal flora. Less-common manifestations of meningococcal disease include myocarditis, endocarditis, or pericarditis; arthritis; conjunctivitis; urethritis; pharyngitis; and cervicitis. Late in the course of treated disease, arthritis or pericarditis may occur as a result of immune complex formation. The case fatality rate from severe meningococcemia can be as high as 40%. More commonly, the course of meningococcal disease is less fulminant.

Complications of Meningococcal Disease

Approximately 10% to 20% of patients who survive meningococcal disease have permanent sequelae. In a multicenter study of 146 children surviving meningococcal infection, one required amputation of toes or all four extremities; 14 developed skin necrosis (four required skin grafting); 14 developed bilateral ($n = 8$) or unilateral ($n = 6$) deafness; four developed ataxia; and three developed hemiplegia.[6] Patients with certain genetic polymorphisms have an increased risk of dying or developing permanent sequelae (see subsequent text).[11] Patients with meningococcal meningitis who do not develop septic shock are less likely to die or have these sequelae (<10%), but they are at risk of developing neurosensory hearing loss, mild to moderate cognitive deficits, or seizure disorders.[12–17] In a study of UK children who survived group B meningococcal disease, approximately 9% had major disabling sequelae as compared with 2% of matched control children.[17] The risk of neurologic complications with meningococcal meningitis is generally lower than that of patients with meningitis caused by *Haemophilus influenzae* type b (Hib) or *Streptococcus pneumoniae*.[18–20]

Bacteriology and Molecular Epidemiology

Weichselbaum was the first to culture meningococcus from patients with meningitis in 1887.[21] The organism is a Gram-negative, encapsulated, oxidase-positive, aerobic diplococcus that naturally infects only humans.[22] Meningococci are usually grown on heated blood agar or Mueller-Hinton media in an

atmosphere containing 5% to 10% carbon dioxide. Catlin-6 medium[23] and Frantz medium[24] also have been used as defined and partially defined liquid culture media, respectively. Thirteen serologically distinct meningococcal capsular groups were reported on the basis of the immunochemistry of the polysaccharide.[25,26] However, what had been designated as group D was recently found to be an unencapsulated variant of group C; therefore, there are 12 known meningococcal capsular groups: A, B, C, E (formerly known as 29E), H, I, K, L, W (formerly known as W-135), X, Y, and Z.[27] Organisms with capsular groups A, B, C, W, X, or Y are responsible for almost all cases of disease.[28]

For epidemiologic purposes, meningococci have been further classified into serotypes and serosubtypes on the basis of the immunological reactivity of the PorB and PorA outer membrane proteins, respectively.[29–33] The accepted nomenclature lists the capsular group, PorB serotype, and PorA serosubtype, each separated by a colon (e.g., B:4:P1.7,4). The VR1 (loop 2) and VR2 (loop 4) encoded epitopes of PorA are given in order after the prefix P1.x, separated from one another by a comma.[34,35] Although phenotyping reagents for capsular group, type, and subtype are available,[36] these are not comprehensive. PorA subtypes are further classified into variants that cannot be classified serologically and can be defined by VR1 and VR2 gene sequence typing.[37] Another outer membrane protein, FetA, an enterobactin receptor, is also used for typing.[38–40] Sequencing of the meningococcal factor H (fH) binding protein gene has also been shown to provide additional evolutionary[41,42] and genomic epidemiologic information about meningococcal strains.[43] Meningococci also can be divided into immunotypes on the basis of variation in the lipooligosaccharide structure.[44]

The meningococcus is naturally competent for transformation with DNA, which provides a mechanism for horizontal exchange of DNA.[45,46] As a consequence, the organism has a highly panmictic population structure[47] and exchanges genes encoding key antigens that are used in the serologic characterization of isolates and as vaccine components.[40,48–50] The facility to exchange genes that encode for capsule and outer membrane proteins has implications for vaccine escape by virulent clones.[40,51,52]

For analysis of genetic lineage of isolates, multilocus sequence typing (MLST) is a commonly used method. MLST capitalizes on nucleotide sequence analysis to index variation based on sequence polymorphisms in housekeeping genes.[53,54] Despite the capacity of *N. meningitidis* to acquire DNA through horizontal gene transfer, a relatively small number of MLST-defined genetic lineages cause most invasive meningococcal disease (IMD).[28] This paradox may be explained by the presence of restriction modification systems that present a differential barrier to horizontal gene transfer and lead to the observed population structuring.[55]

Whole genome sequencing is replacing traditional molecular methods for meningococcal strain characterization. One of the most notable features of the meningococcal genome is the large number of repetitive elements, presumed to contribute to genome fluidity, and more than 100 putative phase-variable genes.[56] These features make an important contribution to the antigenic variability and phenotypic adaptability of the organism.

Risk Factors for Disease and Carriage

The meningococcus colonizes only the pharynx of humans and has no other known environmental niche. The organism is usually a harmless commensal, and there are major differences in carriage rates by age, geography, and time.[57–61] Carriage rates are usually highest among adolescents and young adults.[62–65] Aerosol droplets or direct contact leads to transmission of the meningococcus from person-to-person through respiratory secretions and requires close contact, such as kissing. Viral respiratory infections,[66–69] exposure to tobacco smoke[70–72] or indoor firewood stoves,[73] bar or discothèque patronage, and binge drinking[71,74–77] are all associated with increased rates of meningococcal carriage or disease. There are a variety of plausible mechanisms for the association between these environmental and behavioral factors and meningococcal infection. Respiratory viruses and tobacco smoking cause alterations in the mucosal surface that enhance bacterial binding or decrease the ability of the host to clear the organism from the nasopharynx. Bar patronage and binge drinking could increase transmission through crowding, coughing caused by exposure to tobacco smoke, and other close contact. In the United Kingdom, being a university student,[78] a freshman living in a university residence hall,[79] and intimate kissing with multiple partners were associated with an increased risk of acquiring meningococcal carriage or disease. These data suggest that behavior, not age or gender per se, is the main determinant of meningococcal carriage in this population. However, children with a history of preterm birth[78] and patients with HIV infection are at increased risk of developing meningococcal disease, which demonstrates that comorbidities can affect risk of disease as well.[80,81,81a]

Pathogenesis

As mentioned above, most IMD is caused by bacteria from a limited number of hypervirulent lineages.[28,51] Carriage isolates, in contrast, belong to a broader group of lineages, many of which have been rarely associated with disease.[60,82] These observations indicate that certain meningococci are genetically predisposed to cause disease. In addition, IMD is, with rare exception, caused by encapsulated strains, whereas colonizing strains often do not express capsule, which underscores the importance of the meningococcal capsule as a virulence factor.[83,84]

Following attachment to pharyngeal mucosal cells, the organism replicates and establishes a carrier state. Pili seem to be the most important adhesin, and two opacity-associated proteins (Opa and Opc), lipooligosaccharide, and interbacterial interactions (via PilX) facilitate subsequent tight attachment.[85–87] Attachment to epithelial cells is mediated by specific receptors such as CD46[87] and carcinoembryonic antigen receptors.[88] Attachment initiates loss of piliation by the bacteria as well as cytoskeletal rearrangements in the host cell that result in internalization of the bacteria within membrane-bound vesicles by endocytosis.[85,89] Once the organism has established the carrier state, the likelihood of acquiring IMD depends on the virulence of the particular isolate, host factors affecting innate susceptibility,[11] and the presence or absence of serum bactericidal antibodies.[90] Once the organism enters the bloodstream, the spleen is involved in bacterial clearance. Because of impaired clearance, patients with asplenia or hyposplenic function may be at increased risk of severe meningococcal disease.[91]

The complement cascade is activated by serum antibodies via the classical pathway, which results in opsonization and bacteriolysis of meningococci. In the absence of specific antimeningococcal antibodies, these complement-mediated functions also can be activated by the alternative pathway.[92] Given the central role of complement proteins in host defenses against invasive *N. meningitidis* disease, persons with underlying deficiencies of properdin, C3, or the late complement components (C5 through C9) are at greatly increased risk of acquiring meningococcal disease.[93–100]

fH is an important downregulatory molecule in the alternative complement pathway cascade. fH binds specifically to *N. meningitidis* and enhances resistance of the organism to serum bactericidal activity (SBA).[101,102] Binding of fH, therefore, is a mechanism by which the organism can evade complement-mediated killing. Polymorphisms within the complement fH and complement fH-related protein 3 have been found in genome-wide studies to be associated with a lower risk of meningococcal disease.[103] Two vaccines that were recently licensed for prevention of meningococcal group B disease target a ligand on meningococci that binds fH to the bacterial surface (referred to as fH binding protein).

Meningococcal disease causes rapidly progressive cutaneous hemorrhage and skin necrosis, disseminated intravascular coagulation, and shock. Many factors contribute to overwhelming meningococcal disease, but the most important are the rapid doubling time of the bacteria and their propensity to release endotoxin-rich outer membrane vesicles (blebbing), which results in very high levels of endotoxin in the circulation and porin proteins and DNA.[104] These trigger various pattern recognition receptors expressed on monocytes and macrophages (see subsequent discussion), which results in proinflammatory cytokine release and activation of fibrinolytic and complement pathways. Endotoxin (lipooligosaccharide) is the most potent toxic molecule,[105] and levels of lipooligosaccharide in the circulation correlate directly with severity of clinical manifestations and case fatality rate.[106]

Host Genetic Factors

Activation of the complement cascade and binding of complement components to the surface of *N. meningitidis* are critical host defenses against developing IMD. Deficiency of proteins in the complement cascade, particularly components of the membrane attack complex, such as C5, C6, or C7, has long been recognized as an important risk factor for IMD. Complement deficiency was recognized in 0.3% of 297 patients with meningococcal disease in the United Kingdom.[107] Other smaller studies have found no complement-deficient patients and in one population-based study, three (19%) of just 16 patients with meningococcal disease had a low complement 50 level (CH_{50}).[108–110] In another study, complement-deficient persons had a more than 1000-fold higher lifetime risk of developing IMD than the general population.[111] Complement deficiency is more common (30% or more) among individuals with disease caused by less-common capsular groups (X, Y, Z, W, 29E).[95,112] In addition, the complement-deficient patients frequently had recurrent episodes of IMD as teenagers or adults, and their disease was often caused by strains with capsular groups that rarely cause disease in the general population. Accordingly, complement deficiency should be considered in patients with recurrent meningococcal disease and patients with disease caused by strains with less-common capsular groups. Deficiencies in serum levels of C3 or C4, properdin, or alternative complement pathway activation from factor D deficiency are also associated with increased risk of IMD.[99,113] Mannose-binding lectin (MBL)[114–117] is a constituent of serum that binds to meningococci and activates the complement cascade.[111] Although there are conflicting data,[78] persons with low serum concentrations of MBL[113] or MBL polymorphisms associated with diminished function[115] are reported to be at increased risk of IMD.[118,119] Because the prevalence of polymorphisms of MBL is much greater in the general population than complement deficiencies, these structural variants of MBL may have an important role in overall susceptibility to meningococcal disease in the population, particularly in children and adolescents who have not yet acquired serum bactericidal antibodies.

Toll-like receptors (TLRs) are components of the innate immune system with specificity for different microbial molecules. TLR4 binds bacterial lipopolysaccharide and is responsible for innate host response to Gram-negative infections. Heterozygous missense mutations of TLR4 have been reported to be more frequent in patients with meningococcal disease than in the general population.[120]

A variety of other genetic polymorphisms are reported to increase the severity of meningococcal disease without affecting the risk of acquiring meningococcal disease.[11] These include a receptor on neutrophils for immunoglobulin (Ig) G_2 or IgG_3, termed Fcγ receptor IIA (FcγRIIA), which affects the ability of the phagocytic cell to clear opsonized bacteria from the bloodstream.[121–123] Several studies found an association between the FcγRIIA-R131 polymorphism, which binds IgG2 poorly, and an increased risk of severe forms of meningococcal disease.[121–125] Polymorphisms of the interleukin-1 gene family,[126] genotypes associated with high expression of interleukin-10[127] or with altered production of tumor necrosis factor-α,[127,128] and the presence of a specific allele of the angiotensin-converting enzyme are reported to increase the risk of severe meningococcal disease.[129] While most of these associations were identified in small sample sets and require independent confirmation, the accumulating data support a genetic component of altered host responses in otherwise healthy persons that underlies the disparities in clinical outcomes of persons exposed to meningococci. As mentioned above, mutations related to complement fH are associated with a lower risk of meningococcal disease.[103]

DIAGNOSIS

Confirmation of meningococcal disease is usually achieved through conventional cultures of blood, cerebrospinal fluid (CSF), or other infected sites. Gram staining to demonstrate the characteristic Gram-negative diplococcus also can be used for diagnosis. Latex agglutination testing for detection of meningococcal capsular polysaccharides in serum or CSF commonly leads to false-negative results.

A polymerase chain reaction (PCR) assay that detects meningococcal DNA in normally sterile body fluids can be used to establish the diagnosis and is particularly useful in the setting of negative cultures because of previous antibiotic use.[130] Capsular group-specific PCR assays are available to discriminate among capsular groups A, B, C, W, X, Y, Z, and 29E.[131–133]

In Europe, a substantial proportion of meningococcal cases are detected using PCR, as opposed to the United States where PCR is less commonly used. In the United Kingdom, for example, 55% of all cases were diagnosed based on positive PCR.[134,135] Using PCR for diagnosis of meningococcal disease has dual benefits: it provides a more accurate picture of disease burden and establishes the diagnosis in a greater proportion of cases.

TREATMENT AND PREVENTION

Antibiotics

Although many antimicrobial agents are effective for management of meningococcal disease, intravenous aqueous penicillin was traditionally considered the therapy of choice once meningococcal disease is confirmed. Although the clinical importance is unknown, some meningococcal isolates from the United States have intermediate susceptibility to penicillin (minimum inhibitory concentration: 0.1–1.0 μg/mL).[9,136]

In Belgium during 2011–2012, the frequency of clinical isolates with decreased susceptibility to penicillin increased; however, there was no resistance to expanded-spectrum cephalosporins, such as ceftriaxone.[137] Among 466 meningococcal isolates from the 10 Active Bacterial Core surveillance sites in the United States from 2004, 2008, 2010, and 2011, 10.3% had intermediate susceptibility to penicillin, 0.6% (three isolates) were resistant to penicillin, and all were susceptible to ceftriaxone.[138] The uncertainty about the clinical significance of intermediate susceptibility to penicillin, the more convenient dosing schedule, and the availability of generic ceftriaxone make it a reasonable option for therapy of meningococcal disease, even after the diagnosis has been established. In fact, pencillin is uncommonly used in the United States and ceftriaxone is the recommended antimicrobial agent for treatment of meningococcal disease in the United Kingdom.[138a] Early initiation of antibiotic therapy and attention to circulatory status in patients suspected of having meningococcal infection may help decrease morbidity and mortality.[139,140] Because the clinical features of meningococcal disease can overlap those of bacterial meningitis or sepsis caused by other bacterial pathogens, depending on the age of the patient, clinical manifestations, exposure history, the results of Gram stains of CSF or petechial hemorrhages, and the presence or absence of other epidemiologic risk factors, broader initial empirical therapy is standard before the diagnosis is confirmed.[9] Antibiotic treatment for 5 to 7 days is adequate therapy for most systemic meningococcal illnesses. In resource-poor settings, single-dose intramuscular chloramphenicol in oil or ceftriaxone has been effectively used.[141]

Other Therapies

Patients with purpura fulminans have the highest case fatality rates (as high as 50%). This syndrome is a result of a massive release of proinflammatory mediators[142] and development of shock and disseminated intravascular coagulation from a procoagulant state with local accumulation of thrombi in small and large arterial vessels.

One therapy for severe systemic meningococcal infection focused on activated protein C, an endogenous anticoagulant that suppresses excessive thrombosis and fibrin formation.[143,144] Although recombinant human activated protein C (drotrecogin alfa [activated]) was licensed based on a clinical trial, additional studies failed to show a survival benefit and the drug was withdrawn from the market.[144–147]

Dexamethasone therapy for 4 days, with the first dose given before the initiation of antibiotic therapy, is reported to decrease the rate of neurosensory deafness in patients with meningitis caused by Hib. In a randomized controlled trial in adults with bacterial meningitis, dexamethasone use also was associated with decreased mortality. A statistically significant benefit was seen only in patients with pneumococcal meningitis, but the study was not sufficiently powered to demonstrate efficacy for other pathogens.[148] High doses of corticosteroids have been demonstrated not to be beneficial in septic shock. Although low-dose corticosteroids are often administered,[149,150] a randomized, controlled study in patients with septic shock (Corticosteroid Therapy of Septic Shock [CORTICUS] trial) failed to demonstrate a benefit in overall 28-day mortality rate (33% for hydrocortisone recipients vs 31% for placebo recipients). Although the duration of shock was shorter in patients who received corticosteroids, adverse events, including new episodes of sepsis and septic shock, were more frequent in the hydrocortisone group. Sprung and colleagues concluded that for the majority of patients with septic shock, the potential advantages of corticosteroids do not outweigh the adverse risks.[151]

Chemoprophylaxis

Close contacts of a patient with meningococcal disease are at greatly increased risk of developing disease. The risk is greatest in household contacts and other close contacts exposed to oral secretions and in coattendees at child daycare. The risk of transmission to healthcare workers is low, but persons performing mouth-to-mouth resuscitation and unprotected healthcare workers exposed during management of endotracheal tubes are at increased risk.[152] Numerous cases of meningococcal disease among microbiology laboratory workers, primarily those who processed liquid cultures on an open bench, also have been reported.[153–155] In one report of 16 probable laboratory-acquired cases, 50% were fatal.[154] Rifampin, ceftriaxone, and ciprofloxacin eradicate nasopharyngeal *N. meningitidis* colonization, and these agents are recommended for chemoprophylaxis of close contacts.[5] Fluoroquinolone-resistant isolates were reported in 2008 in North Dakota and Minnesota, which prompted the Centers for Disease Control and Prevention (CDC) to recommend ceftriaxone, rifampin, or azithromycin for eradication of meningococcal carriage in areas where resistance has been identified.[156,157]

Chemoprophylaxis should be offered to household members and to other persons with a history of prolonged close contact. Prophylaxis of child daycare contacts and staff is recommended in the United States[5] but not in the United Kingdom.[158] Because the risk of disease in contacts is highest in the first week after onset of disease in the index patient, prophylaxis should be administered to contacts as soon as feasible, preferably within 24 hours of identification of the index patient.[267] Because penicillin therapy does not reliably eradicate nasopharyngeal colonization, additional antimicrobial treatment is recommended at the time of hospital discharge for eradication of colonization in patients treated exclusively with penicillin. Contacts who were previously immunized with meningococcal vaccines should still be considered for chemoprophylaxis because primary vaccine failure or lack of serum antibody persistence, or both, may render vaccinated persons susceptible to disease.

Chemoprophylaxis given to large populations in response to outbreaks of meningococcal disease may be of limited effectiveness in lowering the risk of disease, particularly as a standalone measure, and increases the likelihood of emergence of antimicrobial resistance. In small school-based outbreaks, chemoprophylaxis to all people within the population may be considered. Settings in which mass chemoprophylaxis may be of some benefit include relatively small populations that have limited mixing with outside populations and in which high antibiotic coverage can rapidly be achieved. If undertaken, the antbiotic should be administered to all persons at the same time.

EPIDEMIOLOGY

Incidence and Public Health Burden

Although meningococcal disease is a global problem that occurs in all countries,[28] the true burden of disease is unknown for many parts of the world because of inadequate surveillance.[159–161] The epidemiology is highly variable, dynamic, and fluid and is influenced by natural variation and immunization policy. In addition, the epidemiology varies substantially by capsular group. Group A meningococcus was historically remarkable for its ability to cause large epidemics.[3] Since World War II, epidemic meningococcal disease caused by group A organisms largely has been confined to developing countries, most often reported from the meningitis belt of sub-Saharan Africa. In this region, with recent widespread use

of a low-cost meningococcal A conjugate vaccine (see below), group A epidemics have largely been prevented.[162] Capsular group X and W strains now also cause substantial rates of disease in Africa. Group C disease, which in the past has rarely caused outbreaks in sub-Saharan Africa, has emerged in Nigeria and Niger.[163–166]

In the 1960s and 1970s, high rates of capsular group A disease were reported in China, Mongolia, and Nepal. As recently as 2005, group A epidemics were reported in India, and a meningococcal capsular group A outbreak occurred in the Philippines in 2004 and 2005, with 98 cases (33% mortality) reported to the World Health Organization (WHO).[167]

In Europe, most cases of meningococcal disease are sporadic and currently caused by capsular group B and C strains. However, some countries in Europe have experienced an increase in disease caused by group Y and W strains. During 2007–09, the proportion of invasive meningococcal cases caused by group Y strains in England and Wales increased from 3% to 6%, which was higher than in the previous three decades.[168] Nearly 40% of patients had underlying medical conditions. A high proportion of patients 65 years of age and older had pneumonia. Strains belonging to the ST-23 clonal complex were responsible for most of the increase. Other Eurpean countries have also reported increases in group Y disease.[169] The burden of endemic meningococcal disease varies substantially among countries and has declined dramatically in countries that introduced group C meningococcal conjugate vaccines. Using culture-confirmed cases, countries with the highest reported incidence rates per 100,000 population in 2012 were Lithuania (1.8), the United Kingdom (1.4 cases), and Ireland (1.3).[170] The capsular group distribution was group B (68% of cases), group C (17%), group Y (8%), and group W (4%).

Although a relatively small proportion of meningococcal disease in Europe is caused by group W, since 2009 the United Kingdom has experienced a rapid increase in group W disease, from 1.8% of cases in 2008–09 to 15% of cases 2013–14.[171,172] The increase was a result of clonal expansion of an ST-11 clonal complex strain. Analysis of whole-genome sequencing data indicated that the strain was not a descendent of the Hajj clone but more related to ST-11 group W strains that had been circulating pre-Hajj.[52,173] This observation is in contrast to a brief increase in group W disease that occurred in the early 2000s from the Hajj clone.[52] As a result of the increase and as discussed further below, a quadrivalent MenACWY immunization campaign for 14- to 18-year-olds was initiated in the United Kingdom in August 2015.[174]

Fig. 38.1 shows the annual incidence of meningococcal disease in the United States between 1984 and 2014.[175] The annual incidence of meningococcal disease since 2000 has been remarkably low, with an incidence in 2013 of 0.14 per 100,000.[176,177] The reasons for the decrease are not known but are likely multifactorial and include decreases in active and passive tobacco smoking, changing patterns of antibiotic use, increased use of influenza vaccines, declines in meningococcal carriage, and lack of novel antigenic variants among virulent strains to which the population does not have immunity.[178] A study from Maryland found that increases in capsular group C and Y disease incidence during the 1990s were associated with antigenic shifts involving key meningococcal outer membrane proteins.[40] Capsular switching from one meningococcal capsular group is also associated with emergence of meningococcal disease globally.[52] Meningococcal disease epidemiology is naturally cyclical; how the incidence will change in the future is unknown.

The age-specific incidence of meningococcal disease results from an interplay between the degree of immunity in the population and virulence of circulating strains. Disease

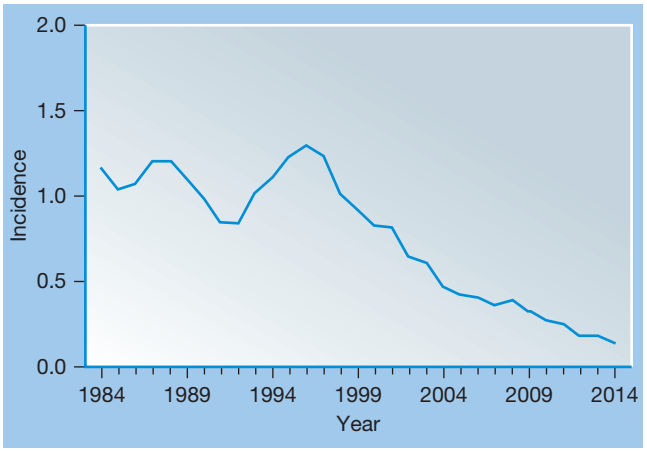


Figure 38.1. Incidence of meningococcal disease, 1982–2012. *(From Adams DA, Thomas KR, Jajosky RA, et al. Summary of notifiable diseases—United States, 2014.* MMWR Morb Mortal Wkly Rep. *2016;63:1–152.)*

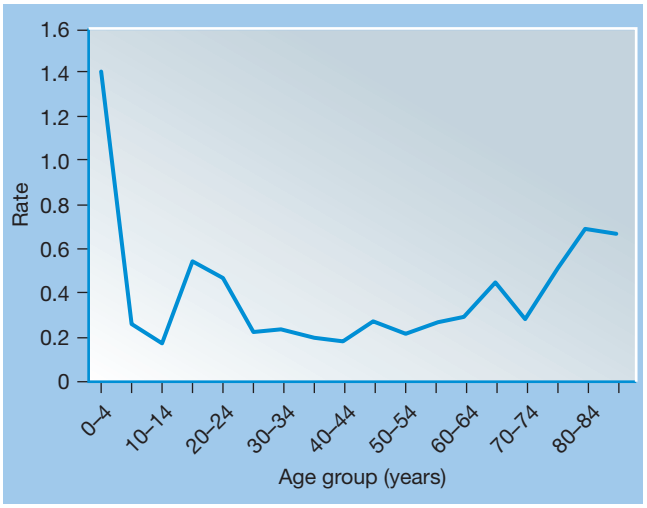


Figure 38.2. Rate per 100,000 of meningococcal disease, by age group, in the United States, 2002–2011. *(Cohn AC, MacNeil JR, Clark TA, et al, Centers for Disease Control and Prevention. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices [ACIP].* MMWR Recomm Rep. *2013;62:1–28.)*

incidence is highest among infants who have not yet acquired natural immunity. In the European Union, the reported rate of meningococcal disease (primarily group B) during 2012 among children younger than 1 year of age and between 1 and 4 years of age were 11.4 and 3.7 cases per 100,000 population, respectively.[170] During the same period, the incidence in the United States was much lower, 1.86, 0.41, and 0.20 among children younger than 1 year of age, children 1 year of age, and children 2 to 4 years of age per 100,000 population, respectively.[179] In the United States and Europe, there were peaks in incidence among adolescents and young adults, which have diminished over time (representative data for the United States is shown in Fig. 38.2).[5]

In temperate climates, rates of meningococcal infections are highest during the winter months. This association may be a result of closer personal contact during the winter months, lack of ventilation, or an increase in upper respiratory infections, all factors that facilitate transmission of or invasion by the organism, or both.

Meningococcal Capsular Group A Disease

Until recently, Group A strains of *N. meningitidis* were the major cause of epidemic and endemic meningococcal disease in the meningitis belt of sub-Saharan Africa, which extends from Senegal on the west coast to Eritrea on the east coast (Fig. 38.3).[28,180] These strains rarely cause disease in the United States[177,181] or in Europe.[170] A notable recent exception is Russia, where group A isolates were reported to account for the majority of isolates in Moscow as recently as 2010.[182–184]

The annual incidence of disease during group A epidemics in sub-Saharan Africa can exceed 1000 per 100,000 population, or 3000-fold higher than the current incidence of meningococcal disease in the United States. The case fatality rate during group A epidemics in Africa historically varied between 10% and 15%.[3] With the recent introduction of a monovalent group A conjugate vaccine, the incidence of group A disease in the meningitis belt has dramatically declined and substantial proportion of disease is now caused by groups W, X, and, more recently C.[163,164] Why disease caused by strains with these capsular groups has increased in the sub-Saharan region and why epidemics begin there with the dry season (generally November or December) and terminate abruptly with the onset of increased humidity and rain (generally June and July) is unknown. Most likely, environmental, host, and microbial factors in combination play a role.[185]

Meningococcal Capsular Group B Disease

See Chapter 39.

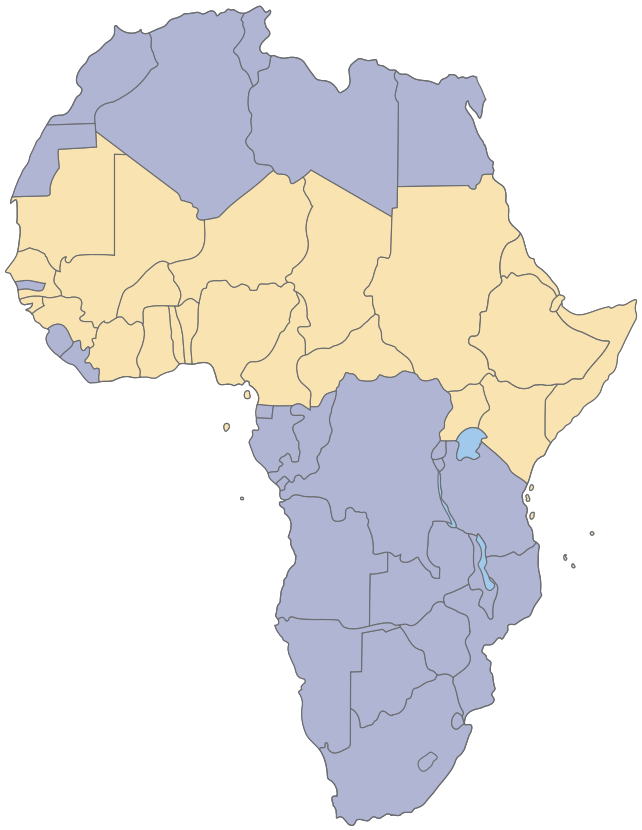


Figure 38.3. Sub-Saharan African meningitis belt. Countries shown in yellow experience explosive epidemics of meningococcal disease every 5 to 10 years that are usually caused by capsular group A strains. *(Courtesy the World Health Organization.)*

Meningococcal Capsular Group C Disease

Group C strains are a major cause of disease in many parts of the world, including North America, South America, Europe, and Australia, with the proportion of group C isolates varying by country. However, group C disease has declined substantially in countries that have introduced meningococcal conjugate vaccines into routine immunization schedules. For example, Canada, Australia, the United Kingdom, The Netherlands, and many other countries in Europe have experienced decreases in group C disease since the introduction of monovalent group C conjugate vaccines.[186–191] In 2012, 17% of disease in Europe was caused by group C strains, with 68% being caused by group B strains. More recently, group C conjugate vaccine was introduced into the routine pediatric immunization schedule in Brazil, with subsequent decreases in the incidence of group C disease.[192,193] In the United States, in part because of use of quadrivalent conjugate vaccines in adolescents, the incidence of disease in 2012–2013 caused by group C, in addition to groups W and Y, was only 0.05 per 100,000 population in persons 11 to 19 years old.[194] However, in 2013 group C strains still accounted for approximately 30% of all meningococcal disease in the United States.[195]

Group C meningococcus has periodically caused disease in the African meningitis belt but has not typically been a major cause of disease in this region. However, recent epidemics of group C disease have occurred in Niger and Nigeria.[163,164] Outbreaks of group C meningococcal disease have also been reported among men who have sex with men in the United States and Europe.[196–202a]

Meningococcal Capsular Group Y Disease

Group Y strains cause all the major meningococcal clinical syndromes, but patients with group Y disease are more likely to have pneumonia than patients with disease caused by strains with other capsular groups.[168,203,204] In the United States, the incidence of group Y disease increased substantially in the 1990s and by 1996 accounted for 33% of isolates.[205,206] In 2013, approximately 15% of IMD in the United States was caused by group Y strains.[195] Group Y strains have also been reported as a cause of a substantial proportion of disease in Colombia, parts of Canada,[207,208] South Africa,[209] Sweden,[210] and other European countries.[211] For reasons that remain unexplained, disease caused by group Y strains remains relatively rare in most other parts of the world despite observations that group Y strains are common causes of pharyngeal colonization.[59,64,212–214]

Meningococcal Capsular Group W Disease

In 2012, the nomenclature for what was previously referred to as group W135 was simplified to group W.[27] Until 2000, group W strains were a relatively uncommon cause of invasive disease worldwide and had not been known to cause outbreaks. That changed in 2000 and 2001, when outbreaks of group W disease occurred among Hajj pilgrims in Mecca, Saudia Arabia, where several million pilgrims from all the world gather each year in crowded conditions.[215–218] A group W clone belonging to the ST-11 complex caused the outbreak. ST-11 complex isolates typically expresss group C polysaccharide, which suggested that a group C to group W capsular switch had occurred at some point.[52,219] Group W strains belonging to the ST-11 complex were also isolated from patients with meningococcal disease in other African countries and globally.[220–223] In 2002, Burkina Faso reported more than 10,000 cases caused by group W isolates.[224,225] A study of a global collection of group W isolates dating from 1970 to 2013

using whole-genome sequencing suggested that the vast majority of ST-11 complex group W isolates arose from an ancestral strain that had undergone horizontal gene transfer events in the capsular operon.[219] By 2000, another series of horizontal gene transfer events involving, for example, fH binding protein, led to a strain that caused the Hajj outbreak and subsequent disease globally. W ST-11 strains unrelated to the Hajj outbreak contribute a significant proportion of W ST-11 cases globally.[52]

Group W strains now cause a substantial proportion of meningococcal disease in the African meningitis belt. For example, 81% of meningococcal cases in 2014 were caused by group W strains.[226] The recent emergence of non–group A strains in the meningitis belt comes in the face of the success of the monovalent group A meningococcal conjugate vaccine, which underscores the need for multivalent meningococcal vaccines for the meningitis belt.

An increase in disease caused by group W strains has also been reported from Argentina[227] and southern Brazil.[228] For example, the proportion of meningococcal cases caused by group W strains among children in Argentina increased from 7% in 2006 to 28% in 2008.[229] The proportion of cases caused by group W strains in the United Kingdom has increased, with 15% of cases being caused by group W during 2013–2014.[171]

Although an outbreak of 14 cases of group W meningococcal disease occurred in Florida between 2008 and 2009,[230] group W disease remains an infrequent cause of disease in the United States: for the period 2006–10, 3% of meningococcal infections in the United States were caused by group W strains.[181]

MENINGOCOCCAL IMMUNITY

Natural Acquisition of Humoral Immunity

Serum antibodies confer protection against meningococcal disease by activating complement-mediated bacteriolysis and, possibly, by enhancing phagocytosis (opsonic activity).[231] Naturally acquired serum antibodies are elicited by asymptomatic pharyngeal carriage of pathogenic and nonpathogenic meningococci,[232–234] or *Neisseria lactamica*.[62,235–237] Anticapsular antibodies stimulated by gastrointestinal colonization with *Escherichia coli* or *Bacillus pumilus* strains with cross-reacting polysaccharides also may contribute.[238–240]

Considerable data indicate that persons with SBA are protected from developing meningococcal disease (see below).[241]

Passive Immunization

Antibodies administered to infant rats passively conferred protection against meningococcal bacteremia.[242–252] However, studies of the ability of passive immunization to confer protection against bacteremia in infant rats likely overestimate protective activity because binding of certain complement downregulating proteins, such as fH, to *N. meningitidis* is specific for human fH (see below).

Immunity Following Active Immunization

Methods Used to Measure Vaccine Immunogenicity and Memory

Serum Antibody. Antigen-binding assays have been used to assess meningococcal vaccine immunogenicity.[253,254] Some investigations have suggested that an anticapsular antibody concentration of 2 µg/mL or greater is sufficient to confer protection against meningococcal disease.[255,256] The results, however, of antigen binding assays such as enzyme-linked immunosorbent assay do not consistently distinguish between bactericidal and nonbactericidal anticapsular antibodies.[243,244,254,257–259] Therefore, serologic assessments of meningococcal vaccine immunogenicity rely on measurements of complement-mediated SBA. In this assay, the test serum is heated to inactivate endogenous complement. Dilutions of the test serum are incubated for 60 minutes with the bacteria and a source of exogenous complement. The results are expressed as the dilution of serum giving 50% bacterial killing. The seminal study by Goldschneider and colleagues,[90] which demonstrated a correlation between naturally acquired SBA and protection against developing meningococcal disease, used endogenous complement (a fixed dilution of the human test serum that had been obtained to preserve endogenous complement and that had not been heated). For many years, infant rabbit serum was used as an exogenous source of complement for testing vaccine-induced bactericidal activity because of the difficulty of finding normal human serum without antibody that is suitable as a complement source for the assay.[258,260] Titers measured with rabbit complement are much higher than with human complement.[261] One reason is the presence of downregulatory complement proteins in human serum, such as fH, which demonstrate species specificity in binding to the bacteria.[101,102] In the absence of fH binding, the meningococcus is more susceptible to complement-mediated bactericidal activity.

Immunologic B-Cell Memory. Unconjugated polysaccharides elicit serum antibody responses largely without T-cell help (so-called T-cell–independent antigens).[262,263] Conjugation of a polysaccharide to a carrier protein confers T-cell–dependent properties to the polysaccharide. The immunologic hallmark of polysaccharide-protein conjugate vaccines is their ability to induce memory B cells capable of responding with a booster antibody response to a subsequent exposure to the polysaccharide. In practice, measuring serum antibody responses to an unconjugated polysaccharide vaccine "probe," following prior immunization with a conjugate, assesses induction of immunologic memory by the conjugate vaccine. Typically a reduced dose of the polysaccharide vaccine is used (as low as 1 µg),[264] based on the theory that a suboptimal immunogenic dose may be more useful for distinguishing memory antibody responses from antibody responses in subjects not primed by the conjugate vaccine.

The ability of a conjugate vaccine to prime for memory antibody responses on subsequently encountering meningococci was thought to be an important second mechanism of protection from developing disease in persons whose serum antibody concentrations had declined to subprotective levels.[265] However, persistence of functional antibody is now considered to be the most important determinant of MenC conjugate vaccine effectiveness, which casts doubt on the ability of memory antibody responses alone to protect against meningococcal group C disease.[266] The inability of memory antibody responses alone to confer protection is because it takes a minimum of 5 to 7 days to elicit a serum group C memory antibody response[264] and most IMD occurs within a few days after exposure to the organism.[90,267,268]

Induction of B-cell memory, therefore, has limited value as a potential mechanism of protection. In addition, the administration of test doses of unconjugated polysaccharide vaccine to assess memory antibody responses may be harmful by depleting antigen-specific memory B cells and impairing subsequent antibody responses.[269]

Induction of memory B cells is accompanied by avidity maturation, which reflects the effects of T cells on B-cell somatic mutation of antibody variable region genes. Memory

TABLE 38.1 Antibody Avidity in Relation to Age and Vaccine[a]

Vaccine	Age Group	Mean Avidity Constant (nM^{-1}) ± 2 SE		
		Pre	1 Mo Post	6 Mo Post
GROUP C ANTIBODY				
MPSV4	Adults	15 ± 3	26 ± 4	ND
MPSV4	3 y	ND	16 ± 2	17 ± 2
MCV4-DT	2 y	ND	22 ± 3	28 ± 3
GROUP W135 ANTIBODY				
MPSV4	3 y	ND	23 ± 2	21 ± 2
MCV4-DT	2 y	ND	27 ± 3	31 ± 4

[a]Vaccines: MPSV4, a quadrivalent meningococcal polysaccharide vaccine; MCV4-DT, a quadrivalent meningococcal conjugate vaccine with each of the four polysaccharides independently conjugated with diphtheria toxoid (see Table 38.3). At 1 and 6 months postvaccination, the mean antibody avidity is higher in the MCV4-DT group than in the MPSV4 group ($P = .002$ for capsular group C; $P < .02$ for capsular group W135). The increase in mean avidity between 1 and 6 months is statistically significant for the MCV4-DT group ($P < .001$ for group C, and $P < .05$ for W135) but not for the MPSV4 group.

ND, not done.

Data from Granoff DM, Morgan A, Welsch JA. Immunogenicity of an investigational quadrivalent Neisseria meningitidis*–diphtheria toxoid conjugate vaccine in 2-year-old children.* Vaccine. *2005;23:4307–4314.*

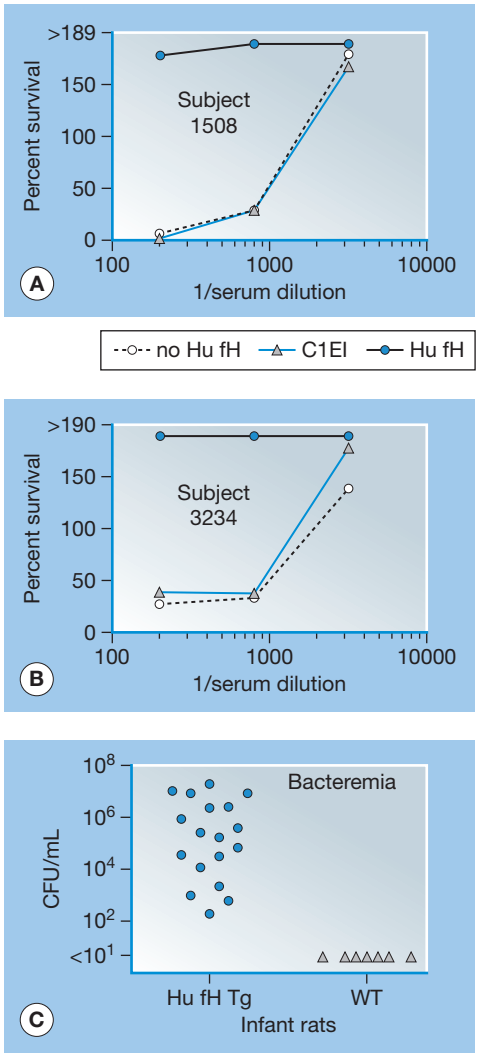


Figure 38.4. Effect of the addition of human factor H (fH) on survival of *Neisseria meningitidis*. A and **B,** Survival of group C strain 4242 when incubated with rabbit complement and dilutions of serum samples from children immunized with meningococcal polysaccharide vaccine. Open circles, no added human fH; triangles, addition of a negative control complement protein, C1 esterase inhibitor, 25 µg/mL; filled circles, addition of human fH (25 µg/mL).[277] **C,** Bacteremia 18 hours after intraperitoneal challenge of infant rats with group B strain H44/76 (≈1000 colony-forming units [CFU]/rat). Circles, human fH transgenic (Hu fH Tg) rats with serum human fH concentrations of 100–500 µg/mL; triangles, wild-type (WT) control littermates (human fH <12 µg/mL). *(Modified from Vu DM, Shaughnessy J, Lewis LA, et al. Enhanced bacteremia in human factor H transgenic rats infected by Neisseria meningitidis.* Infect Immun. *2012;80:643–650.)*

B-cell clones with mutated antibody combining sites have increased antibody avidity compared with antibodies elicited by primary immunization. Table 38.1 provides an example of avidity maturation of group C and W anticapsular antibodies in vaccinated children. During the 6 months of follow-up, a significant increase in avidity of the anticapsular antibodies was elicited by a dose of the meningococcal conjugate vaccine but not by a dose of the unconjugated polysaccharide vaccine. Thus, instead of administering a polysaccharide probe to assess induction of antigen-specific B-cell memory, it may be possible to use avidity as a correlate.[270–272] Other correlates include demonstration of booster antibody responses to a second injection of the conjugate vaccine (as demonstrated for groups C, Y, and W after quadrivalent meningococcal conjugate vaccination of adolescents; see later discussion)[273] and detection of antigen-specific peripheral blood memory B-cell responses after vaccination.[274,275] None of these approaches risks induction of antibody hyporesponsiveness or loss of immunologic memory that can accompany group C and, possibly, Y and W polysaccharide challenges.

Correlates of Protection. Since the first observations at the turn of the 20th century that some meningococci are serum sensitive while others are serum resistant, and that serum from some individuals has more bactericidal activity than serum from others,[276] it has become accepted that immunity to meningococci can be described by the titer of serum required to kill *N. meningitidis* in vitro. Epidemiological cohort studies[90] and vaccine evaluation studies[260] provide support for the routine use of a serum bactericidal assay as a correlate of protection. In the widely used assays, survival of meningococci following incubation in the presence of heat-inactivated test serum and an exogenous complement source (usually human or rabbit) is determined.

Fig. 38.4A and B show the percent survival of a group C strain incubated with rabbit complement and different dilutions of test serum obtained 1 month after immunization of two children with meningococcal polysaccharide vaccine. In the absence of added human fH, the bacteria were killed by rabbit complement and antibody at a serum dilution of approximately 1:800 (<50% survival after incubation for 1 hour compared with colony-forming units at time 0). The addition of 25 µg/mL of human fH eliminated the killing by binding to the bacteria and downregulating complement activation.[277] The addition of a negative control complement protein (C1 esterase inhibitor) had no effect on bacterial survival. Wild-type infant rats whose natural fH does not bind to

N. meningitidis are naturally relatively resistant to disease caused by many pathogenic group B *N. meningitidis* strains. After treatment with human fH, the rats became susceptible.[277] Human fH transgenic infant rats also are highly susceptible to meningococcal bacteremia, whereas control wild-type littermates whose rat fH does not bind to meningococci cleared the bacteremia (Fig. 38.4C). Collectively, these data illustrate one mechanism by which the meningococcus has uniquely adapted to cause disease in humans.

For meningococcal group B strains, there is consensus that only human complement bactericidal assays are reliable for predicting protection against disease.[241] As of 2012, rabbit complement assays were still used in Europe for licensure of new polysaccharide-protein conjugate vaccines against capsular group A, C, Y, or W strains[278,279] and were the basis of inferring efficacy for licensure in India of a new group A conjugate vaccine intended for Africa (see subsequent discussion). For group C strains, bactericidal titers as high as 1 : 128 using rabbit complement are needed to ensure that a titer of 1 : 4 is present if measured with human complement.[261] Using estimates of age-specific vaccine effectiveness from the United Kingdom, a titer of 1 : 8 or greater measured with rabbit complement has been suggested as the putative protective threshold following capsular group C conjugate vaccination.[265,280] A titer of 1 : 128 measured with rabbit complement was the basis of inferring efficacy for U.S. licensure of a quadrivalent meningococcal polysaccharide conjugate vaccine for adolescents (MCV4-DT; see subsequent discussion). Because meningococci cause disease only in humans and because of the exquisite human species specificity of binding complement–downregulating molecules by *N. meningitidis* and the corresponding uncertainty in inferring vaccine efficacy from bactericidal titers measured with rabbit complement, the U.S. Food and Drug Administration (FDA) requires bactericidal data from assays using human complement to support licensure of new meningococcal conjugate vaccines intended for infants and children.

History of Meningococcal Vaccine Development

Vaccines offering protection against meningococcal disease were first introduced into routine use in the military more than 40 years ago. Early attempts to develop meningococcal vaccines used killed whole bacterial cells.[281–283] Between 1900 and 1940, several trials were conducted, but the studies were poorly controlled, and, in most cases, it was impossible to tell whether the vaccines conferred protective immunity.[284] Pursuit of a whole-cell vaccine was curtailed by the excessive reactogenicity of such preparations. Following the successful development of tetanus and diphtheria toxoid vaccines in the 1930s, the protective potential of crude meningococcal culture filtrates containing inactivated exotoxin was explored.[285,286] These preparations were immunogenic, but they were certainly contaminated with capsular polysaccharide antigens, outer membranes, and endotoxin. Enthusiasm for development of meningococcal vaccines waned with the early successes with antibiotic treatment and prevention of secondary cases. By the early 1960s, sulfonamide-resistant isolates of *N. meningitidis* were widespread and represented an important problem among military recruits during the Vietnam War era, which promoted renewed interest in meningococcal vaccine development.[287,288]

During the early 1940s, Scherp and Rake demonstrated that serum from horses immunized with group-specific capsular polysaccharides protected mice against lethal challenge with *N. meningitidis*,[289] but purified preparations of capsular polysaccharide had failed to elicit antibody responses in humans.[290,291] The poor immunogenicity was subsequently attributed to the relatively low molecular size of the polysaccharide formulation tested, as studies showed that polysaccharide antigens of high molecular weight induced sufficient antibody responses in humans.[292,293] By the end of the 1960s, Gotschlich and colleagues had developed an alternative approach for the purification of high-molecular-weight meningococcal polysaccharides that was safe and immunogenic.[294,295] The principal limitation of polysaccharide vaccines is that they do not induce T-cell–dependent immunity. The lack of T-cell immunity has profound implications for the lack of immunogenicity of these vaccines in young children and the inability of polysaccharide vaccines to elicit long-term immunologic memory.[264,296,297] The chemical conjugation of polysaccharides to protein carrier molecules confers a T-cell–dependent immune response.[298] The success of this approach was first demonstrated in humans with Hib vaccines in the 1980s.[299–301] Subsequently, similar conjugate vaccines were investigated for the meningococcus for group A (*N*-acetyl mannosamine-1-phosphate) and group C (alpha 2–9 *N*-acetyl neuraminic acid [NANA]) polysaccharides,[302] group C polysaccharide,[303–306] and groups A, C, W (copolymer of NANA with galactose), and Y (copolymer of NANA with glucose).[246,259,307–310] These vaccines elicited high serum bactericidal antibody titers and boostable immune responses. As described, monovalent group C conjugate vaccines were introduced with great success in the United Kingdom in late 1999 and have been introduced into routine immunization shedules in Europe, Brazil, Canada, and Australia.

As of August 2015, two quadrivalent A, C, W, and Y meningococcal polysaccharide-protein conjugate vaccines were licensed in the United States and recommended for routine use in adolescents and young adults.[311] In addition, a meningococcal conjugate vaccine that contains capsular groups C and Y, as well as Hib, is licensed for use in infants and toddlers. Recently, two vaccines for prevention of capsular group B disease were licensed (see Chapter 39).

Polysaccharide Meningococcal Vaccines

Meningococcal polysaccharide vaccines are rapidly being replaced by conjugate vaccines because of the immunologic inferiority of the former. Although they have an acceptable safety profile and are immunogenic and effective in older children and adults, polysaccharide vaccines have substantial limitations relative to meningococcal polysaccharide-protein conjugate vaccines. They are poorly immunogenic in infants, do not provide a booster response, do not substantially decrease meningococcal pharyngeal colonization and therefore do not provide herd protection, and can induce the phenomenon of immunologic hyporesponsiveness. They are still used at times for outbreak control[312] and are still available in the United States and other countries. However, it is likely that their use will eventually be phased out. Table 38.2 shows currently licensed polysaccharide vaccines. For a comprensive review of this topic, refer to this chapter in the 6th edition.[313]

MENINGOCOCCAL POLYSACCHARIDE-PROTEIN CONJUGATE VACCINES

Meningococcal Capsular Group a Conjugate Vaccine for Africa

Until recently, the only meningococcal vaccines available in sub-Saharan Africa were a bivalent group A and C and a trivalent group A, C, and W polysaccharide vaccine (MPSV2 and MPSV3). These vaccines are poorly immunogenic in infants,

TABLE 38.2 Meningococcal Polysaccharide Vaccines[a]

Manufacturer	Vaccine	Active Constituents	Other Excipients	Diluent
Sanofi Pasteur	Mengivac	Group A polysaccharide Group C polysaccharide	Lactose (2 mg)	Phosphate-buffered saline solution
	Menomune	Group A polysaccharide Group C polysaccharide Group W135 polysaccharide Group Y polysaccharide	Lactose (2.5–5.0 mg) Sodium chloride (4.25–4.75 mg)	Pyrogen-free water
GlaxoSmithKline Biologicals	AC Vax	Group A polysaccharide Group C polysaccharide	Lactose (12.6 mg)	Saline solution
	ACWY Vax	Group A polysaccharide Group C polysaccharide Group W135 polysaccharide Group Y polysaccharide	Lactose (12.6 mg)	Saline solution

[a]Bivalent, trivalent, and quadrivalent meningococcal polysaccharide vaccines are referred to in the text as MPSV2, MPSV3, and MPSV4, respectively. All vaccines shown are licensed in European countries. Menomune is available in the United States and Canada and is sold in other countries under the trade name Menomune-A/C/Y/W135. All vaccines contain 50 μg of each of the capsular polysaccharides shown and are lyophilized, requiring reconstitution with preservative-free diluent. For multidose vials, the diluent used to reconstitute Menomune contains thimerosal (mercury derivative) 1:10,000 as a preservative. Note: although still licensed in Europe, the bivalent vaccines are generally no longer available.

the age group at greatest risk of acquiring disease, and have only a transient effect, if any, on colonization and spread of infection. For these reasons, meningococcal vaccination in Africa was largely done in response to epidemics, which was a logistically challenging and relatively ineffective strategy. Although an emergency response plan had been developed in collaboration with the WHO and the CDC,[314] implementation was often delayed because of limitations of surveillance or logistics. One study estimated that even under optimal circumstances, fewer than 60% of outbreak-related cases were prevented by implementing vaccination after an epidemic was recognized.[314] An improved meningococcal vaccine, therefore, was needed.

In sub-Saharan Africa, most meningococcal epidemics are caused by capsular group A strains. By the late 1990s, three vaccine manufacturers had developed capsular group C meningococcal conjugate vaccines for the United Kingdom, where there had been approximately 10,000 cases of disease caused by group C organisms and 1000 deaths during the previous decade.[315] There was little commercial interest in developing a group A meningococcal conjugate vaccine for sub-Saharan Africa, where there had been more than 700,000 cases and 100,000 deaths in the same decade.[3] The countries in this region remain some of the poorest in the world and could not afford to purchase the vaccine.

In 1999, the WHO funded a feasibility study to explore options to develop and manufacture a group A conjugate vaccine that could be sold in Africa for less than $0.50 a dose.[316,317] The results from this study formed the basis of a successful grant application to the Bill and Melinda Gates Foundation, which in 2001 awarded $70 million to the WHO and the Program for Appropriate Technology in Health to develop a meningococcal group A conjugate vaccine with the goal of eliminating group A epidemics in Africa.[316] Although there were concerns at the time about the potential for epidemics caused by capsular group W strains,[225,318–320] in the previous 100 years, more than 90% of epidemic meningococcal disease in sub-Saharan Africa had been caused by capsular group A strains. The dominance of group A disease and the greater technical challenges, higher cost, and extended timelines for developing a bivalent capsular group A and W conjugate vaccine made development of a monovalent group A vaccine more feasible as a first step.

A novel conjugation chemistry using reductive amination of group A polysaccharide aldehydes and tetanus toxoid hydrazides was developed by scientists at the FDA's Center for Biologics Evaluation and Research.[321] The process was transferred to the Serum Institute, a vaccine manufacturer in India.[322] Extensive testing of the vaccine in sub-Saharan Africa provided assurance of safety, immunogenicity, and manufacturing consistency.[323] In 2010, marketing authorization for MenAfriVac (MenA-TT conjugate vaccine) was granted by the Food and Drug Administration of India for the export of the vaccine to African countries. Mass vaccination of 1- to 29-year-olds was introduced in December 2010, as part of countrywide demonstration projects in Burkina Faso, Mali, and Niger.[324] More than 217 million people ages 1 through 29 years have received MenA-TT vaccine (containing 10 μg MenA polysaccharide) in 17 countries in the African meningitis belt by 2015.[325] It is hoped that high coverage in this age group will lead to herd protection of unvaccinated children younger than 1 year and elimination of epidemics of meningococcal A disease. In fact, MenA-TT led to a dramatic decline in group A carriage in African villages, suggesting that herd protection with this vaccine is substantial.[326,327] Once the mass vaccination campaigns are completed, the concern will be to protect subsequent birth cohorts throughout infancy. Two strategies are recommended: one for countries where MenA coverage is high and another where MenA coverage is low (<60%).[328] In high-coverage countries, vaccination should be administered as a single dose at 9 to 18 months of age. A lower-dose vaccine (5 μg of MenA polysaccharide) is available for children younger than 2 years of age. In low-coverage countries, periodic single-dose campaigns are recommended.

A major problem that has occurred since the widespread use of a capsular group A conjugate vaccine is the emergence of disease caused by strains with other capsular groups such as C, W, or X.[190,225,318,329–331] Currently there is no vaccine available for prevention of group X disease, which is extremely rare in North America and Europe.[332] For prevention of group W disease, quadrivalent (A, C, Y, and W) conjugate vaccines are available in industrialized countries (see earlier discussion), but these vaccines are expensive and not available in sub-Saharan Africa. A trivalent MPSV3 vaccine is available for Africa and kept in reserve by the WHO for response to capsular group C and W epidemics, but this vaccination strategy has

many limitations, as described. Work is currently underway to produce a low-cost pentavalent MenACWXY conjugate vaccine that could (potentially) replace the monovalent vaccine.

Monovalent Capsular Group C Conjugate Vaccines

Composition

Three group C conjugate vaccines (Table 38.3) are currently licensed in European countries, Australia, Brazil, and Canada; two are based on oligosaccharides derived from group C capsular polysaccharide that are conjugated to the nontoxic CRM_{197} derivative of diphtheria toxin; the third vaccine consists of de-O-acetylated group C polysaccharide conjugated to tetanus toxoid. Although these vaccines use different O-acetylated saccharides, all three vaccines are immunogenic in infants and elicit boostable serum meningococcal bactericidal antibody responses, whether or not the test organisms in the assay naturally express O-acetylated or de-O-acetylated group C capsules.

Meningitec (Pfizer, herein described as P-MenC-CRM) is prepared by the controlled treatment of group C polysaccharide with sodium periodate followed by the separation of the resulting oligosaccharides from high-molecular-weight saccharide and conjugation to CRM_{197} by reductive amination. Menjugate (GlaxoSmithKline Vaccines, recently acquired from Novartis Vaccines and Diagnostics, herein described as

TABLE 38.3 Meningococcal Conjugate Vaccines

Manufacturer	Vaccine	Other Designation	Active Constituents Per Dose	Adjuvant	Other Excipients	Presentation
Pfizer	Meningitec	P-MenC-CRM	10 μg *O*-acetylated group C oligosaccharide conjugated to 11–25 μg CRM_{197}	$AlPO_4$	Sodium chloride	Single-dose, liquid suspension vial
Pfizer (previously Baxter Bioscience)	NeisVac	MenC-TT	10 μg de-*O*-acetylated group C oligosaccharide conjugated to 10–20 μg tetanus toxoid	$Al(OH)_3$	Sodium chloride	Single-dose, prefilled syringe
GlaxoSmithKline vaccines (previously Novartis vaccines)	Menjugate	G-MenC-CRM	10 μg O-acetylated group C oligosaccharide conjugated to 11–25 μg CRM_{197}	$Al(OH)_3$	Mannitol, sodium phosphate buffer	Single-dose, freeze-dried, vial reconstituted with diluent
GlaxoSmithKline vaccines (previously Novartis vaccines)	Menveo	MCV4-CRM	5 μg each of groups C, Y, and W135 oligosaccharide and 10 μg of group A oligosaccharide conjugated to 33–64 μg CRM_{197}	None	Sucrose, potassium dihydrogen phosphate, sodium dihydrogen phosphate monohydrate, disodium phosphate dehydrate, sodium chloride	Single-dose vial with lyophilized group A component; single-dose vial with the three other components in liquid form
Sanofi Pasteur	Menactra	MCV4-DT	4 μg each of groups A, C, Y, and W135 polysaccharide conjugated to ≈48 μg of diphtheria toxoid	None	Sodium chloride	Single dose, prefilled syringe
Pfizer (previously GlaxoSmithKline)	Nimenrix	MCV4-TT	5 μg each of groups A, C, Y, and W polysaccharide conjugated to ≈44 μg of tetanus toxoid	None	Sucrose and trometamol	Liquid single dose
GlaxoSmithKline vaccines	Menitorix	Hib-MenC	5 μg Hib polysaccharide and 5 μg group C polysaccharide each conjugated to ≈17.5 μg of tetanus toxoid	None	Tris, sucrose, sodium chloride	Single-dose, freeze-dried vial reconstituted with prefilled syringe
GlaxoSmithKline vaccines	MenHibrix[a]	Hib-MenCY-TT	5 μg group C polysaccharide conjugated to 5 μg of tetanus toxoid, 5 μg group Y polysaccharide conjugated to 6.5 μg of tetanus toxoid, and 2.5 μg Hib polysaccharide conjugated to 6.25 μg of tetanus toxoid	None	Sucrose and trometamol	Single dose lyophilized with saline diluent

[a]GlaxoSmithKline announced that this vaccine would be discontinued as of February 28, 2017.

G-MenC-CRM) is manufactured from group C polysaccharide that has been partially hydrolyzed at low pH and size-fractionated before being conjugated to CRM_{197} through a bis *N*-hydroxysuccinimide ester of adipic acid.[302] In the manufacture of NeisVac-C (Pfizer, recently acquired from Baxter Bioscience, herein described as MenC-TT), the group C polysaccharide is first deacetylated with sodium hydroxide, but, because *N*-acetyl groups are part of the epitopes that elicit protective antibody, the amino groups are reacetylated with acetic anhydride.[333] Following limited depolymerization with sodium periodate and size fractionation, the de-O-acetylated oligosaccharide is conjugated directly to the tetanus toxoid by reductive amination. The single-dose formulations of all three vaccines do not contain preservatives.

Group C conjugate vaccines are stable at the recommended storage temperature (2°–8°C). Studies in which the conjugate vaccines have been stored at higher temperatures or subjected to repeated freeze–thaw cycles indicated that the structural stability of the oligosaccharide chains and of the protein carrier is related to the conjugation chemistry or formulation of the vaccine.[334] However, the immunogenicity of the vaccines in mice was affected only by incubation of the vaccines under more extreme conditions that resulted in the release of a substantial proportion of free saccharide.[335]

Immunogenicity

Prelicensure studies of monovalent meningococcal polysaccharide-protein group C conjugate vaccines demonstrated their safety and immunogenicity in all age groups. In infants and toddlers, the conjugate vaccines elicited higher antibody responses than did the polysaccharide vaccine, and the antibody elicited by the conjugate vaccine had greater bactericidal activity.[304,306,336,337]

Age-Related Immunogenicity

The hallmark of protection against meningococcal disease is SBA. Substantial evidence indicates that all three group C conjugate vaccines are highly immunogenic after two or three doses given in the first 6 months of life and elicit serum bactericidal antibody.[304,306,336,338] In most of these studies, the highest responses in infants or toddlers were with MenC-TT.[336,338,339] These early studies were performed with concomitant administration of combination vaccines containing diphtheria and tetanus toxoids and whole-cell pertussis (DTwP) vaccines. Since then, combination vaccines using acellular pertussis have largely replaced DTwP vaccines in most industrialized countries, and pneumococcal polysaccharide-protein conjugate vaccines have been introduced into the infant vaccination schedule. These changes have the potential to enhance or impair antibody responses to meningococcal conjugate vaccines.

A study in the United Kingdom compared serum antibody responses to the three MenC conjugate vaccines when administered at 2 and 3 months of age along with separate injections of a combination acellular pertussis vaccine ($DTaP_5$/IPV/Hib-TT, Pediacel, Sanofi Pasteur) and pneumococcal conjugate vaccine 7 (PCV7) (Prevnar, Pfizer).[340] At 2 months, a single dose of MenC-TT vaccine elicited higher serum bactericidal antibody responses than either of the MenC-CRM diphtheria toxoid vaccines (compare respective hatched bars in Fig. 38.5A). One month after the second dose (Fig. 38.5A, *bars without hatching*), the highest serum bactericidal geometric mean titer (GMT) was in the group given the G-MenC-CRM vaccine (1:682) followed by the MenC-TT (1:437) and P-MenC-CRM (1:229; $P < .02$) vaccines. In an earlier study with coadministration with $DTaP_5$/IPV/Hib-TT, the MenC-TT conjugate vaccine also elicited higher serum bactericidal antibody responses after one dose given at 2 months of age, but after two additional doses at 3 and 4 months of age, the responses were significantly higher to the G-MenC-CRM vaccine.[341]

The reasons for the higher group C antibody responses to one dose of the MenC-TT conjugate vaccine at 2 months and the higher responses to the G-MenC-CRM vaccine after a second or third dose are unknown. Epitopic or nonepitopic suppression to the tetanus toxoid carrier protein may have been induced by the first dose of the MenC-TT vaccine when coadministered with the DTaP vaccine, which dampened the anticapsular antibody responses to the second injection of the tetanus toxoid conjugate vaccine. This mechanism may explain the lower serum bactericidal antibody responses to the MenC-TT vaccine in preschoolers or adolescents, or both,

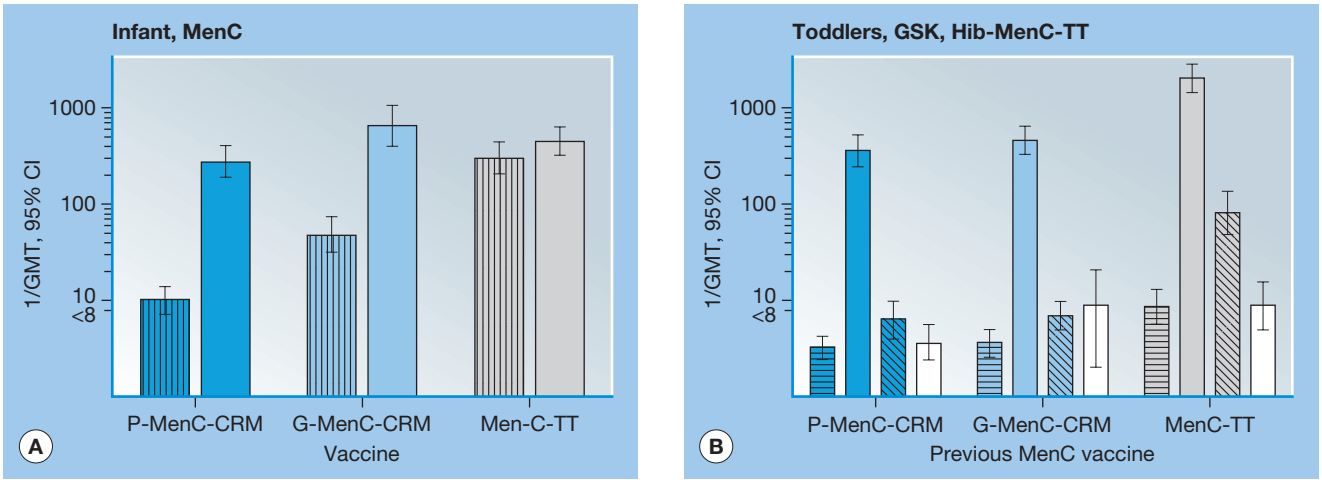


Figure 38.5. Serum bactericidal antibody responses of infants immunized with MenC conjugate vaccines (rabbit complement). A, Titers 1 month after a dose of vaccine given at 2 months *(hatched bars)* or after a second dose given at 3 months *(solid bars)*.[339] **B,** Serum bactericidal titers before *(horizontal hatched bars)* and after a booster dose of Hib-MenC-TT vaccine given at 12 to 15 months of age in relation to previous MenC vaccination at 2 to 4 months of age. Titers 1 month after the booster *(solid bars)*, at 24 months of age *(diagonal hatched bars)*, and at 36 months of age *(open bars)*.[352] CI, confidence interval; GMT, geometric mean titer; GSK, GlaxoSmithKline; Hib, *Haemophilus influenzae* type b.

given DT or Td at the same visit or at 1 month before the conjugate vaccine.[342–344] The clinical relevance of these lower responses is unclear because most subjects achieved putative protective titers (albeit measured with rabbit complement; see earlier discussion). Two studies also suggested interference might occur between coadministration of a combination of meningococcal and pneumococcal conjugate vaccines when CRM_{197} is the carrier protein of both vaccines.[345,346] In infants given P-MenC-CRM at 2, 3, and 4 months of age along with an investigational nine-valent pneumococcal conjugate vaccine that also used CRM_{197}, the meningococcal bactericidal GMT was 1:291 (95% confidence interval [CI], 208–407).[345] This response is substantially lower than the GMTs previously reported in children who received P-MenC-CRM alone. In another study, decreased meningococcal bactericidal antibody responses were observed in infants given an investigational combination pneumococcal-meningococcal CRM_{197}-based conjugate vaccine prepared by Pfizer, when compared with the responses of control infants given P-MenC-CRM (GMT, 1:179 vs 1:808, respectively).[346]

Anticapsular Antibody Kinetics and Persistence

Most adults immunized with group C conjugate vaccines develop protective serum bactericidal antibody levels within 7 to 10 days.[347] Serum bactericidal titers peak at approximately 2 to 4 weeks after immunization and persist for longer than a year.[348] Most teenagers immunized with the G-MenC-CRM conjugate vaccine had protective serum bactericidal titers at 3.7 years[268] or 5 years of follow-up.[349] Serum antibody persistence seems to be inversely correlated with age[262] as waning immunity was detected in the majority of 6- to 12-year-old children immunized an average of 6.8 years earlier as part of the United Kingdom national MenC catch-up campaign.[350] Similarly, only 44% of Australian children immunized at 2 to 8 years of age with a MenC vaccine had adequate serologic protection an average of 8.2 years following immunization.[351] As described subsequently, protective serum antibody levels decline even more rapidly after immunization of infants or toddlers.

At the end of the United Kingdom catch-up campaign, routine MenC conjugate vaccination was introduced for infants (three monthly doses beginning at 2 or 3 months of age). With this schedule, serum bactericidal titers declined to subprotective levels by 1 year (see Fig. 38.5B, *horizontal hatched bars*) and there was evidence of lower effectiveness in the second year of life.[266] To extend the duration of protective titers, a booster dose of a combined group C meningococcal-Hib tetanus toxoid conjugate vaccine (Menitorix, GlaxoSmith-Kline; see Table 38.2) was introduced in the United Kingdom for the 12- to 14-month age group. At 1 month after a booster dose, high serum bactericidal antibody titers were present in toddlers previously vaccinated with any of the three MenC conjugate vaccines (see Fig. 38.5B, *solid bars*).[352] By age 24 months *(diagonal hatched bars)* or 36 months *(open bars)*, however, the serum titers in the majority of the children had declined to less than the titers thought to be required for protection. In another study, infants were immunized at 2, 3, and 4 months of age with the G-MenC-CRM conjugate vaccine and boosted with MCV4-CRM at 12 months. At 40 to 60 months of age, 59% had protective titers of serum bactericidal antibody against capsular group C meningococci.[353]

Recent data assessing the impact of different conjugates (G-MenC-CRM or MenC-TT) or the numbers of doses (0, 1, or 2 doses) for primary immunization in the United Kingdom, on the persistence of protective titers after a Hib-MenC booster, again found that MenC-TT priming resulted in improved antibody persistence when compared with MenC-CRM priming. Of particular note, one priming dose of MenC-CRM resulted in superior booster responses to Hib-MenC when compared with two priming doses of MenC-CRM (Fig. 38.6).[354] Interestingly, antibody persistence to 2 years of age after the toddler Hib-MenC dose was the same with one or two priming doses as it was without any priming doses of MenC-CRM.[354]

Immunologic Memory

MenC conjugate vaccines prime for the ability to mount memory anticapsular antibody responses on subsequent exposure to a polysaccharide challenge (infants,[304,306,338] toddlers,[303] and adults[355]). In other studies, infants given lower doses of conjugated meningococcal A and C polysaccharide antigens[356,357] or fewer numbers of doses,[269,338] or both, mounted higher memory antibody responses to subsequent challenges with unconjugated polysaccharide vaccine than did infants who previously received higher doses or more injections of the conjugated antigen. Similarly, immunization with one dose of MenC-CRM resulted in higher memory responses than did two doses of MenC-CRM following a toddler dose of Hib-MenC-TT.[354] The immunization with the higher quantities of polysaccharide antigen per dose of conjugate vaccine or multiple injections, or both, resulted in higher serum bactericidal titers at 1 month after vaccination and greater serum antibody persistence, but lower memory responses.[356] Thus, the choice of the optimal dose of antigen and number of injections of a conjugate vaccine needs to consider the greater importance of having higher serum antibody concentrations for protection against meningococcal disease than the robustness of the memory antibody response. Underscoring this point are the UK data on decreasing vaccine effectiveness between 1 and 4 years after immunization of infants and toddlers,[266] at a time when the vaccinated children could mount robust memory antibody responses[271] but lacked protective levels of SBA.

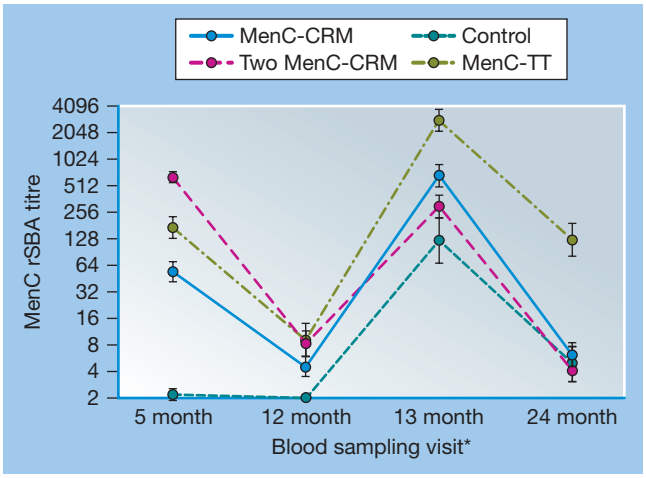


Figure 38.6. Geometric mean titers (and 95% confidence interval) for capsular group C meningococcal (MenC) rabbit serum bactericidal antibody (rSBA) at visits performed at ages 5, 12, 13, and 24 months according to type of MenC priming (G-MenC-CRM or MenC-TT) schedule and Hib-MenC boosting at 12 months of age (intention-to-treat population). Hib, *Haemophilus influenzae* type b. *(From Pace D, Khatami A, McKenna J, et al. Immunogenicity of reduced dose priming schedules of serogroup C meningococcal conjugate vaccine followed by booster at 12 months in infants: open label randomised controlled trial.* BMJ. *2015;350:h1554.)*

Effect of Prior Polysaccharide Vaccination Responses to MenC Conjugate Vaccine

Adults and toddlers previously vaccinated with one or more doses of meningococcal polysaccharide vaccine have lower serum antibody responses to a group C conjugate vaccine than do individuals immunized for the first time.[303,347,355,358] These lower responses to the conjugate vaccine probably are a result of depletion of memory B cells by exposure to the unconjugated polysaccharide. However, this phenomenon is not considered further here because conjugate vaccines have largely replaced unconjugated polysaccharides.

MenC Conjugate Vaccine Effectiveness

In England and Wales, the incidence of group C meningococcal disease rose substantially from 1994 to 1998.[359] Enhanced meningococcal surveillance was initiated, and the total number of group C cases for the 1998–1999 epidemiologic year (July 1 to June 30) was estimated at 1500 (Fig. 38.7, *left panel*), with at least 150 deaths. From November 1999, group C conjugate vaccination was introduced in the United Kingdom for all individuals younger than 19 years of age (later extended to younger than 24 years of age) in a catch-up program that took 12 months to complete. Individuals 1 to 18 years of age were given one dose, infants were given three doses at 2, 3, and 4 months of age along with their routine primary immunizations, with two doses for those 5 to 11 months of age.

Within 12 to 18 months after introduction of group C vaccination, a marked decline in the number of cases and deaths caused by group C disease occurred in the age groups targeted for immunization.[266,315,360,361] Ten years after vaccine introduction, group C meningococcal disease had been virtually eliminated in the UK population, including in the groups older than 24 years of age who were not vaccinated (see Fig. 38.7, *right panel*).

Formal estimates of age-specific vaccine effectiveness during the first year after vaccination were approximately 90% or more for all vaccinated age groups. Between 1 and 4 years after vaccination, however, effectiveness declined in all age groups.[266] The decline was most marked in infants vaccinated at 2, 3, and 4 months of age for whom there was no demonstrable effectiveness, –81% (95% CI, –7430% to 71%), and least in adolescents given one dose of vaccine, 90% (95% CI, 77% to 96%). In toddlers given one dose of vaccine, effectiveness between 1 and 4 years declined to 61% (95% CI, –327% to 94%).[266] In response to the decline in effectiveness in vaccinated infants, in September 2006, the ages of the routine infant UK MenC vaccination schedule were changed to 3, 4, and 12 months, with the final dose administered as a combination Hib-MenC vaccine.[266] Based on data inclusive from 2000 through the middle of 2009, vaccine effectiveness within 1 year of infant immunization was estimated to be 97% (95% CI, 91% to 99%) and was 68% (95% CI, –63% to 90%) at 12 or more months of follow-up.[188]

Between 2000 and 2003, 53 reported cases of group C conjugate vaccine failure occurred in persons from England or Wales.[361] There was no evidence of immunodeficiency in these immunized persons and the case fatality rate was 7.5%, which was similar to that of cases in unvaccinated persons in the same birth cohorts: 10.4%. Serum bactericidal titers in convalescent serum and avidity indices in acute serum were significantly higher in vaccine failures than in unvaccinated cases (5.7-fold higher for SBA titers, $P = .03$; 2.9-fold higher for avidity indices, $P = .01$), which are consistent with an anamnestic antibody response in the vaccine failures. These observations suggest that group C disease had occurred in vaccinated persons despite induction of immunologic priming (memory) by the group C conjugate.[361]

Additional effectiveness data are available from other countries that have introduced meningococcal group C conjugate vaccines using a catch-up campaign at the time of introduction, including Spain, The Netherlands, and Canada. These countries reported high vaccine effectiveness using various different schedules, but with some waning of effectiveness over time.[362–364] The decline in vaccine effectiveness in immunized infants and young children was consistent with the rapid decline in serum bactericidal antibody titers after vaccination (see Fig. 38.5). Booster doses for toddlers were widely introduced with the expectation that antibody, and thus protection, would persist better during early childhood after the 12-month dose. However, accumulating evidence indicates that persistence of serum antibody after this booster is poor,[354] more so with the MenC-CRM vaccines than the MenC-TT, and the observed protection against disease in the population was

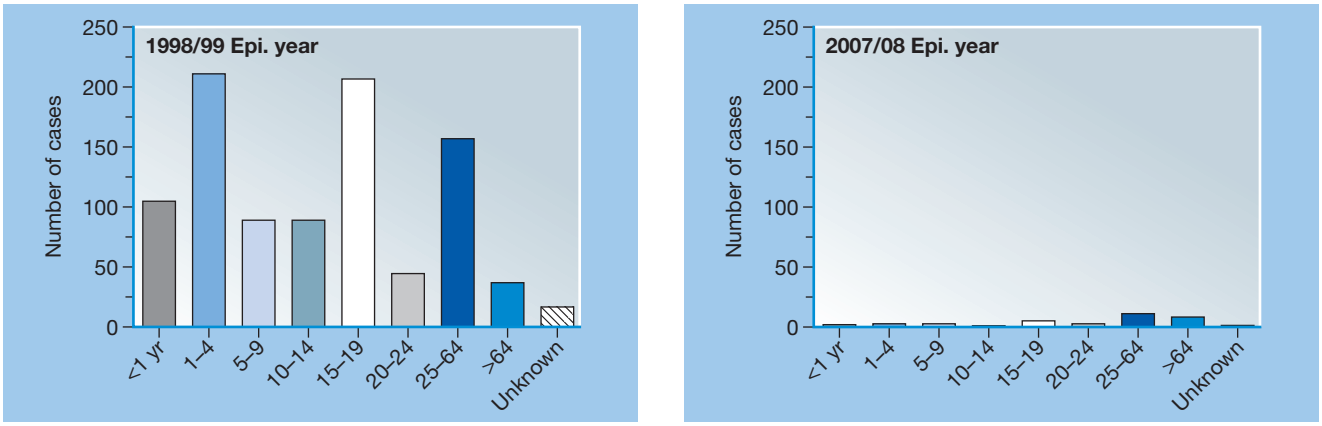


Figure 38.7. Group C meningococcal disease in the United Kingdom by age group, as determined by enhanced surveillance. *Left*, Cases for July 1998 to June 1999 before introduction of group C meningococcal conjugate vaccine. *Right*, Cases for July 2007 to June 2008 after vaccine had been used in the United Kingdom for 10 years. Epi. year, epidemiologic year. *(Data from Helen Campell, UK Health Protection Agency, Centre for Infections. From Campbell H, Andrews N, Borrow R, et al. Updated postlicensure surveillance of the meningococcal C conjugate vaccine in England and Wales: effectiveness, validation of serological correlates of protection, and modeling predictions of the duration of herd immunity.* Clin Vaccine Immunol. *2010;17:840–847.)*

likely maintained chiefly through herd protection from decreased group C meningococcal carriage. Thus, the focus of the UK program has now switched to maintaining herd protection rather than optimizing antibody levels in the first few years of life. Accodingly, in 2013 the UK infant schedule was changed to 3 and 12 months, with a second MenC booster offered in early adolescence.[365] In 2015 the teenage MenC dose was replaced with MCV4 in response to an increase in MenW cases. In 2016 the 3-month dose of MenC was dropped once the majority of teenagers had been immunized and herd protection had been secured.[366] Similarly, many other countries and regions with established infant/toddler immunization programs have either introduced an adolescent booster dose of MenC or MenACWY vaccine or plan to do so.

Effect of MenC Conjugate Vaccine on Carriage and Herd Protection

To measure the effectiveness of group C conjugate vaccines on decreasing carriage of meningococci,[367] oropharyngeal swabs were obtained in 1999 from 15,106 UK students 15 to 19 years of age before introduction of group C conjugate vaccines. After vaccine introduction (single dose for this age group), swabs were obtained from 18,095 students in 2000, and 19,710 students in 2001. After 1 year of vaccine use, there was a 66% decrease in carriage of encapsulated group C meningococci, which reached 75% by 2 years.[59] In contrast, there was no evidence of decreases or increases in carriage by isolates with other capsular groups.[59] Consistent with these data, there also was a 66% decrease in the attack rate of group C meningococcal disease in unvaccinated persons.[368] Thus, the near eradication of group C meningococcal disease from the United Kingdom during the past 15 years (see Fig. 38.7) occurred despite relatively limited vaccine efficacy beyond 1 year after vaccination in the youngest age groups.[188] It is likely that direct protection of the vaccinated indivduals was important in the rapid control of disease but, as antibody levels waned rapidly in the younger age groups, sustained control has been driven by decreased colonization and thus decreased circulation of hypervirulent group C strains as a result of vaccine-induced herd protection. Recent data show that the current carriage rates of capsular group C meningococci remain very low, even 15 years after introduction of the vaccine.[369,370] Herd protection with group C conjugate vaccines has been documented in other countries as well.[371,372]

Although the incidence of group C disease in the United Kingdom and the rates of group B carriage remain very low, serum group C anticapsular antibody levels in the childhood population have waned.[262,373] Thus, there is a large susceptible population potentially at risk of disease should a virulent group C clone be introduced. However, as discussed above, the addition of MenC adolescent boosters from 2013 and a MenACWY catch-up campaign among 14- to 18-year-olds from 2015, should boost immunity among teenagers and maintain herd protection, as sustained persistence of antbody has been observed in this age group.

Before introduction of group C conjugate vaccination in the United Kingdom, concerns were raised that immune pressure induced by vaccination might select for capsular switching from group C to group B strains.[374] Capsular switching between group B and group C strains had been observed in the United States.[51,181,375] Switching from group C ST-11 complex isolates to group B ST-11 complex isolates also has been reported in France, together with conservation of virulence.[376] To date, there is no evidence of increased rates of group B disease in the United Kingdom by strains that have undergone capsular switching; indeed rates of capsular group B disease have actually declined over the past decade.[377] There also is no evidence of capsular group switching or replacement observed among UK carriage isolates.[59] In contrast, since introduction of group C conjugate vaccine immunization in Spain, an increase in ST-11 capsular group B disease has occurred in Navarra, which suggests that group C–to–group B capsular switching and subsequent clonal expansion of the group B clone had occurred, but there is no clear evidence that this was driven by vaccination.[378] Similarly, an ST-11 W clone emerged in the United Kingdom in 2009, which appears to have originated in Latin America and is apparently unrelated to the MenC vaccine program.[174]

Safety of MenC Conjugate Vaccines

From prelicensure safety studies in the United Kingdom, transient headache of mild to moderate severity was the most commonly reported adverse event, with the highest rate (12%) in the first 3 days after vaccination. Headache was reported more frequently in secondary school than in primary school children, and more frequently in girls than in boys. Local reactions at the injection site consisted mostly of pain, tenderness, and occasional redness. These reactions tended to be of mild to moderate severity, were maximal on the third postvaccination day, and typically resolved within 1 day. Local reactions were more common after diphtheria toxoid or diphtheria toxoid–tetanus toxoid booster vaccination than after group C conjugate vaccination. Preexisting allergies did not seem to affect reactogenicity.[379] Postlicensure passive surveillance through reports of adverse vaccine events from health professionals to the UK Medicines Control Agency/Committee on Safety of Medicines indicated a rate of one adverse event per 2875 doses of meningococcal group C vaccine distributed in the first 10 months of the UK campaign.[380] Nearly all these adverse events consisted of transient headache, pyrexia, or dizziness, or local reactions. Anaphylaxis was reported at a rate of one per 500,000 doses distributed. Although some new reactions to group C conjugate vaccine were identified (e.g., headache, nausea, vomiting, abdominal pain, and malaise in all age groups), these were generally not serious, and the balance of risks and benefits for group C conjugate vaccines was considered overwhelmingly in favor of immunization.

Five cases of Guillain-Barré syndrome (GBS) were reported in persons immunized with monovalent group C conjugate vaccines during the UK catch-up campaign when millions of doses were administered. This number of cases was considered by the UK Health Protection Agency to be lower than the expected background rate.

MULTIVALENT MENINGOCOCCAL CONJUGATE VACCINES

Composition

As of September 2015, three meningococcal conjugate vaccines are licensed in the United States (see Table 38.3). One is a combination Hib-meningococcal C and Y conjugate (MenHibrix, GlaxoSmithKline, referred to herein as Hib-MenCY),[381–383] and the other two are quadrivalent conjugate vaccines: Menactra (Sanofi Pasteur; referred to as MCV4-DT), and Menveo (GlaxoSmithKline Vaccines, referred to as MCV4-CRM).[384–386] However, GlaxoSmithKline recently announced that Hib-MenCY will no longer be available. One or both of the quadrivalent vaccines also are licensed in Canada, Europe, and some countries in Latin America, the Middle East, and Asia. A third quadrivalent conjugate vaccine called Nimenrix (recently acquired by Pfizer Vaccines from GlaxoSmithKline, referred to herein as MCV4-TT) is licensed in Europe, Canada, and Australia and is under review or licensed in various other countries.

A combination Hib conjugate vaccine and group C meningococcal conjugate vaccine (without Y) (Menitorix, GlaxoSmithKline, referred to herein as Hib-MenC) is available in Europe and Australia. None of these vaccines contains an adjuvant or preservative.

MCV4-DT is prepared by performing controlled, limited depolymerization of capsular groups A, C, Y, and W polysaccharides, which are then derivatized with a dihydrazide-containing linker molecule. The derivatized polysaccharides are independently conjugated to the carboxylic acid groups on diphtheria toxoid. One dose of the quadrivalent vaccine contains 4 μg of each of the four polysaccharides and a total dose of approximately 48 μg of diphtheria toxoid in 0.5 mL of phosphate-buffered physiologic saline (see Table 38.2).[309] The vaccine is currently available in 0.5-mL single-dose vials and single-dose, prefilled syringes; the single-dose vials contain dry natural latex rubber stoppers.

For manufacturing MCV4-CRM, the oligosaccharide from each polysaccharide is prepared by the processes of hydrolysis, sizing, and reductive amination, and linked covalently to CRM_{197}. One dose contains 5 μg of oligosaccharide for each capsular group C, Y, and W, 10 μg of capsular group A oligosaccharide, and a total dose of 33 to 64 μg of CRM_{197}. The vaccine is presented as a single-dose vial containing the group A conjugate component in lyophilized form and another single-dose vial containing the three other components in liquid form. The group A component is reconstituted with the groups C, Y, and W liquid conjugate vaccine component immediately before administration.

For manufacturing MCV4-TT, the conjugate is made by sizing, cyanate ester formation, and derivatisation of the oligosaccharide with a spacer prior to conjugation to TT by carboxylic group activation (MenA and MenC) or sizing, and conjugation via a cyanate ester group (MenY and MenW). One dose contains 5 μg of each oligosaccharide conjugated to a total dose of 44 μg of tetanus toxoid.[387] MCV4-TT is presented as a freeze-dried powder in a single-dose vial for constitution with saline.

For manufacturing Hib-MenC or Hib-MenCY, the Hib polysaccharide is derivatized with a dihydrazide-containing linker molecule and conjugated to the tetanus toxoid carrier protein via carbodiimide condensation. The meningococcal polysaccharide(s) is activated by cyanylation and directly linked to tetanus toxoid through its amino group. One dose of the Hib-MenC vaccine contains 5 μg of Hib polysaccharide, 5 μg of meningococcal C polysaccharide, and a total dose of approximately 17.5 μg of tetanus toxoid.[388] The presentation is a single-dose vial containing the vaccine as a powder, which is reconstituted with 0.5 mL of saline solution that is provided in a prefilled syringe. One dose of the Hib-MenCY contains 2.5 μg of Hib polysaccharide, 5 μg each of C and Y polysaccharides, and a total dose of approximately 18 μg of tetanus toxoid, and is presented as a lyopholised product for reconstitution with saline.[389]

Immunogenicity

MCV4-DT

In adolescents, MCV4-DT elicited noninferior serum bactericidal antibody responses as compared with a U.S.-licensed meningococcal quadrivalent polysaccharide vaccine (MPSV4).[310] In children 2- to 11-years-old, the conjugate vaccine elicited superior serum bactericidal antibody responses.[309] In infants given three doses of MCV4-DT at 2, 4, and 6 months of age, serum bactericidal antibody responses 1 month after the third dose were approximately 50-fold lower than those obtained after three doses of monovalent group C conjugate vaccines.[390] MCV4-DT, therefore, was considered insufficiently immunogenic in infants using this schedule. On the other hand, toddlers 12 to 23 months old given two doses of MCV4-DT, separated by 2 months, developed geometric mean serum bactericidal antibody responses of 1 : 300 to 1 : 400 against test strains with capsular groups C, Y, or W and about 1 : 3000 to a group A strain.[391] In other studies, a two-dose schedule of MCV4-DT given at 9 and 12 months of age was investigated in 1128 children.[392] The first dose was given alone, and the second dose was given alone; with measles, mumps, rubella, and varicella vaccine; or with pneumococcal 7-valent conjugate vaccine (PCV7). The proportions of children with SBA titers (using human complement) of 1 : 8 or more were 90.5% to 95.6% for group A, 97.8% to 100% for group C, 95.1% to 96.4% for group Y, and 81.2% to 86.4% for group W. Although there was overlap in the 95% CIs, meningococcal antibody titers were lower in children who received MCV4-DT concomitantly with PCV7 than in children who received MCV4-DT alone. In addition, in the children who received concomitant PCV7, noninferiority criteria were not met for the pneumococcal IgG GMTs for serotypes 4, 6B, and 18C. No data have been reported for concomitant use of MCV4-DT and pneumococcal conjugate vaccine (PCV13), which replaced PCV7 in the infant immunization schedule in the United States in 2010.

There is also evidence for immunologic interference between MV4-DT and Daptacel (diphtheria and tetanus toxoids and cellular pertussis vaccine, DTaP, Sanofi Pasteur). Among children 4 to 6 years old, antibody responses to each of the four meningococcal capsular groups did not meet noninferiority criteria among children to whom MCV-DT was given 30 days after DTaP. This phenomenon was not observed when MCV4-DT and DTaP were given concomitantly.[392a]

These immunogenicity and safety results formed the basis of licensure of MCV4-DT in Canada and the United States.

MCV4-CRM

For licensure of the new MCV4-CRM conjugate vaccine, its immunogenicity was compared with that of the licensed MCV4-DT conjugate vaccine. In a randomized trial of 2907 children 2 to 10 years old, the respective serum geometric mean reciprocal titers (human complement) 1 month after a dose of MCV4-DT or MCV4-CRM vaccine were similar for capsular groups A and C, and approximately twofold higher in the MCV-CRM group for capsular groups W and Y.[393] Fig. 38.8A (2- to 5-year-olds) and B (6- to 10-year-olds) show the proportions of children with protective titers 1 month after vaccination. In both age groups, the MCV4-CRM conjugate vaccine met preestablished noninferiority criteria against capsular groups C, Y, and W, whereas the responses to group A did not satisfy noninferiority criteria.[394] In a second study, the immunogenicity of MCV4-CRM and MCV4-DT was evaluated in 11- to 18-year-old children; noninferior criteria were met for all four capsular groups (Fig. 38.8C).[370] At 28 days after vaccination, clear differences in favor of MCV4-CRM were observed in the reverse cumulative distribution curves of the serum bactericidal titers against capsular groups A, W, and Y, while noninferiority criteria were met for capsular group C (Fig. 38.9).

MCV4-CRM also is highly immunogenic in infants given a variety of schedules starting at 2 months of age.[396] Fig. 38.10A summarizes the serum bactericidal antibody responses (using human complement) after immunization at 2, 4, and 6 months. Titers peaked 1 month after the third dose and declined nearly 10-fold by 6 months after vaccination (age 12 months). A booster dose at 12 months elicited high serum bactericidal titers against strains from each of the four capsular groups (Fig. 38.10B).

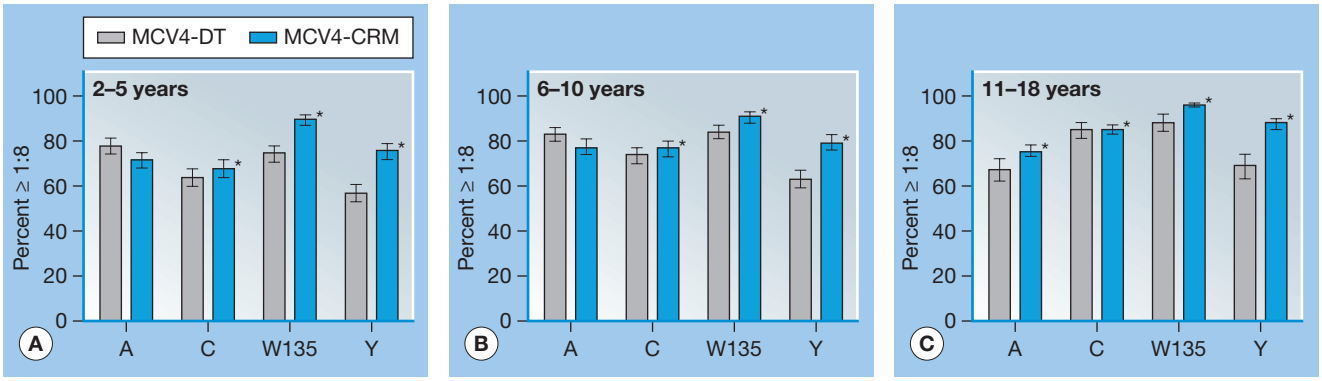


Figure 38.8. Serum bactericidal antibody responses 28 days after a dose of quadrivalent meningococcal conjugate vaccine. A and **B,** Data from a randomized study in children 2 to 10 years old.[393] **C,** Data from a randomized study in adolescents aged 11 to 18 years.[395] Bars represent the percentage of subjects with protective titers (defined as 1:8 measured with human complement). Error bars represent 95% confidence intervals. Asterisks indicate that the response to the new MCV4-CRM vaccine met preestablished noninferiority criteria compared with the licensed MCV4-DT vaccine. These criteria were not satisfied for group A in the age groups 2 to 5 and 6 to 10 years. For capsular groups W135 and Y, the new vaccine elicited superior bactericidal responses in all three age groups ($P < .05$).

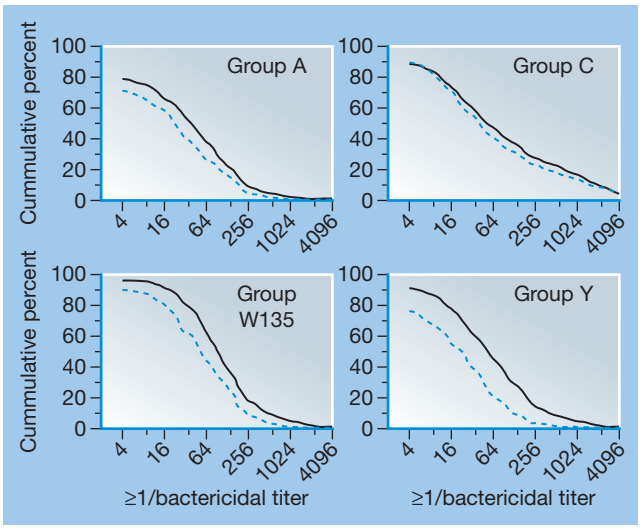


Figure 38.9. Reverse cumulative distribution of bactericidal titers (human complement) in serum samples obtained 28 days after vaccination of teenagers with MCV4-DT or MCV4-CRM. The responses were higher for the MCV4-CRM vaccine group against strains with capsular group A, W135, or Y. *(Data from Jackson LA, Baxter R, Reisinger K, et al, V59P13 Study Group. Phase III comparison of an investigational quadrivalent meningococcal conjugate vaccine with the licensed meningococcal ACWY conjugate vaccine in adolescents.* Clin Infect Dis. *2009;49:e1–e10.)*

MCV4-CRM also is highly immunogenic in toddlers.[397] After two doses given at 12 and 18 months, the percentages of subjects with protective bactericidal titers of 1:8 or more were 100% for capsular group C, W, and Y, and 84% for capsular group A.[397] The titers to group C after the second dose were more than 10-fold higher than after one dose at 12 months.

MCV4-TT

As of December 2016, this vaccine was not yet approved in the United States. A recent systematic review assessed the immunogenicity of MCV4-TT and found the vaccine to be not inferior to licensed quadrivalent meningococcal polysaccharide vaccines. When compared with MCV4-CRM, there were no differences in the proportions of individuals achieving responses over the putative protective threshold but GMTs were generally higher for MCV4-TT for A, W and Y, especially in younger children.[398]

The immunogenicity of MCV4-TT was compared with MCV4-DT in only one study, where it was found to be noninferior with 51.0% to 82.5% of adolescents and young adults mounting vaccine responses (defined as human SBA [hSBA] titer of at least 1:8 for those seronegative at baseline and a fourfold rise in titer for those seropositive at baseline) to strains with each of the four capsular polysaccharides.[399] A study in Korea also documented responses to MCV4-TT among adolescents with 79% to 99% of adolescents with hSBA responses 1:4 or greater.[400]

In a study conducted in the United Kingdom, an adolescent booster dose of MCV4-TT was compared with MCV4-CRM among teenagers who had been primed during childhood with one of the three monovalent C conjugates. Both booster vaccines induced strong MenC rabbit serum bactericidal antibody (rSBA) responses with 100% above the putative protective threshold at 1 month and 96% 9 months later. Overall the highest responses occurred after a MCV4-CRM booster dose with any MenC vaccine prime, but the strongest postbooster resposes were among those primed with MenC-TT and boosted with MCV4-TT.[401]

In younger children (9–12 months of age), 50% to 94% after one dose and 88% to 100% after two doses of MCV4-TT of infants made an hSBA response 1:8 or greater. Antibody responses waned and were substantially lower in both one- and two-dose groups for capsular group A (20.6% and 25.9%, respectively).[402]

Hib-MenC and Hib-MenCY

Hib-MenC is used as a toddler booster following infant priming with monovalent MenC in several countries, including the United Kingdom, and is highly immunogenic. Hib-MenCY is approved in the United States for primary and booster immunization beginning at 6 to 8 weeks of age, although the manufacturer recently announced that this vaccine will be discontinued. In one study of Hib-MenC, 95.6% of MenC-CRM–primed toddlers achieved postbooster Hib-MenC rSBA 1:8 or greater.[403] Interestingly, responses were greater among a group who were primed with Hib-MenCY (99% rSBA ≥1:8).

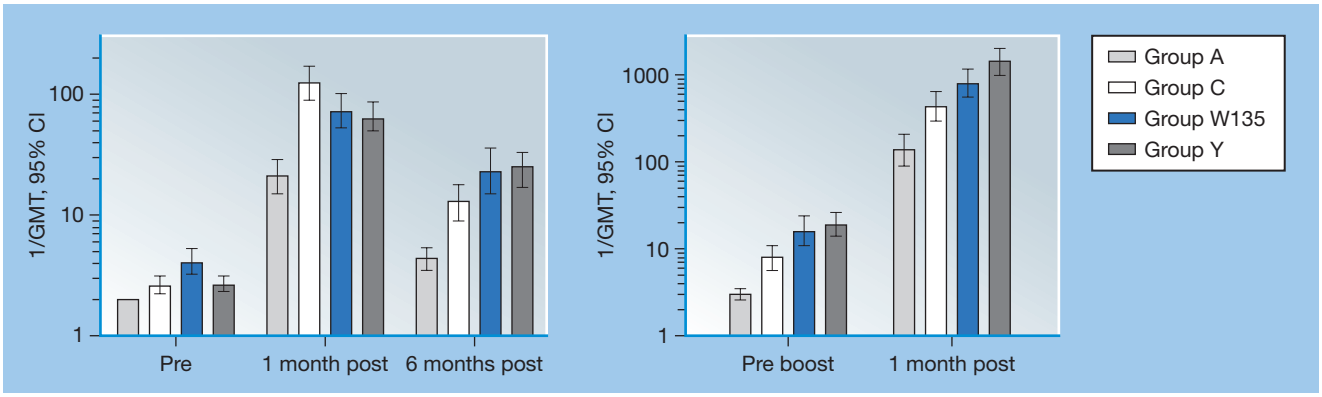


Figure 38.10. Serum bactericidal antibody responses (human complement) of infants immunized with MCV4-CRM vaccine at 2, 4, and 6 months and boosted at 12 months of age. *Left,* Reciprocal geometric mean titers (GMTs) of serum were obtained before immunization *(pre-)* and 1 and 6 months after *(post)* the third dose. *Right,* Reciprocal titers before and 1 month postbooster dose given at 12 months of age. Note that the respective preboost bars in the right panel are the same as those for the bars shown at 6 months after in the left panel, except that the *y*-axis is expanded on the right panel. CI, confidence interval. *(Data from Snape MD, Perrett KP, Ford KJ, et al. Immunogenicity of a tetravalent meningococcal glycoconjugate vaccine in infants: a randomized controlled trial.* JAMA*. 2008;299:173–184.)*

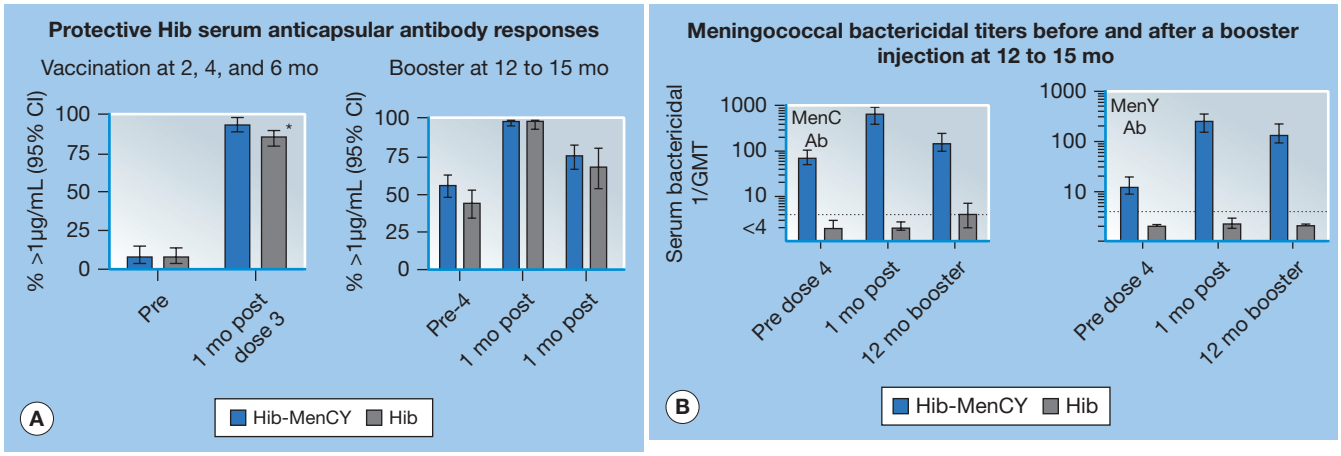


Figure 38.11. Meningococcal serum bactericidal antibody titers before (**A**) and after (**B**) a toddler booster (fourth dose) of Hib-MenCY at 12–15 months of age. Ab, antibody; GMT, geometric mean titer *(Modified from Marshall GS, Marchant CD, Blatter M, et al. Immune response and one-year antibody persistence after a fourth dose of a novel Haemophilus influenzae type b and Neisseria meningitidis serogroups C and Y-tetanus toxoid conjugate vaccine (HibMenCY) at 12 to 15 months of age.* Pediatr Infect Dis J*. 2010;29:469–471.)*

Hib-MenCY is highly immunogenic in infants and toddlers. For example, in a schedule at 2, 4, and 6 months of age with a booster between 12 and 18 months, Hib responses were higher with Hib-MenCY than with Hib-TT and more than 95% of toddlers had antibody titers above the seroprotective threshold. The vaccine was also studied in a 2-, 3-, 4-, and 12-month schedule and was found to induce strong antibody responses against the three vaccine components.[404] Antibody persistence has been studied up to 1 year after the toddler booster with anti–polyribosylribitol phosphate concentrations ≥0.15 µg/mL and MenC and Y hSBA greater than 1:8 at 100%, 96.6%, and 83.8%, respectively (Fig. 38.11).[382]

Serum Antibody Persistence After Quadrivalent Meningococcal Conjugate Vaccines

Serum samples were obtained from a group of teenagers immunized 3 years earlier with MCV4-DT.[310] Although bactericidal titers had declined compared with peak titers at 1 month, the proportions of subjects at 3 years with titers of 1:128 (rabbit complement) or greater were 95% (group A), 71% (group C), 83% (group W), and 96% (group Y). A subset of the 3-year postvaccination serum samples was reassayed with human complement using a bactericidal titer of 1:4 or greater as a measure of protection (Fig. 38.12, *right panel*).[246] As expected from previous studies, the absolute percentages of serum samples with "protective" titers with human complement were lower than with rabbit complement; with human complement, the majority of subjects lacked protective titers to capsular groups C and W and 42% lacked protective titers to group Y (titers to group A were not measured). In another study of 108 children who received MCV4-DT 5 years earlier at the age of 2 to 10 years, 55% and 94% had SBA titers (using rabbit complement) more than 1:128 for capsular groups C and Y, respectively.[405]

A third study compared serum antibody persistence 22 months after immunization of adolescents with MCV4-CRM or MCV4-DT (Fig. 38.12, *left panel*).[406] The proportions of subjects with protective serum bactericidal titers (defined as ≥1:8 with human complement) were 36% vs 25% in favor of MCV4-CRM for group A, 62% vs 58% for group C, 84% vs 74% for group W, and 67% vs 54% for group Y. These differences for capsular groups A, Y or W were statistically significant ($P < .05$ for each capsular group). Although the clinical and

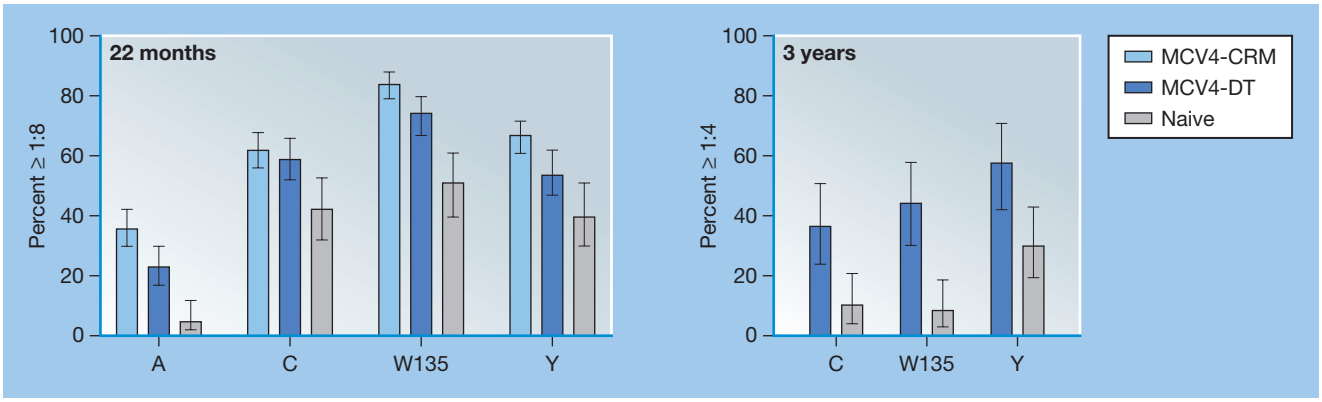


Figure 38.12. Persistence of serum bactericidal antibody (human complement) after vaccination of teenagers with quadrivalent meningococcal conjugate vaccines. Results from 22 months of follow-up of subjects immunized with MCV4-CRM or MCV4-DT *(left)*[406] or from 3 years of follow-up with MCV4-DT *(right)*.[246] At 22 months, the MCV4-CRM–vaccinated group had greater antibody persistence than the MCV4-DT group ($P < .05$ for all four capsular groups). In each study, groups of vaccine-naïve subjects were bled at the time of the respective follow-up of the vaccinated subjects.

TABLE 38.4 Recommendations for Use of Meningococcal Conjugate Vaccines Available in the United States[a]

Risk Group	Primary Series	Booster Dose
Routine immunization of healthy persons aged 11–18 y who are not at increased risk for meningococcal disease	1 dose of MCV4-CRM or MCV4-DT, preferably at age 11–12 y	At age 16 y if primary series at age 11 or 12 y At age 16–18 y if primary dose at age 13–15 y No booster needed if primary dose on or after age 16 y
Persons aged 2–23 mo with complement component deficiency,[b] human immunodeficiency virus infection, functional or anatomic asplenia, or increased risk of exposure[c]	MCV4-CRM at 2, 4, 6, 12–15 months or (only if age >9 mo) MCV4-DT at 9 and 12 mo of age (in this age group, MCV4-DT contraindicated in asplenia and human immunodeficiency virus infection[d,e])	In persons immunized at age <7 y, after 3 y In persons immunized at ≥7 y, after 5 y If continued risk, additional boosters every 5 y
Persons aged ≥24 mo with complement component deficiency,[b] human immunodeficiency virus infection, or functional or anatomic asplenia	2 doses of MCV4-CRM or MCV4-DT 8–12 weeks apart[e,f]	In persons immunized at <7 y, after 3 y In all persons immunized at ≥7 y, after 5 y If continued risk, additional boosters every 5 y
Persons aged 24 mo to 55 y with increased risk of exposure[c]	2 doses of MCV4-CRM or MCV4-DT 8–12 weeks apart 1 dose of MCV4-CRM or MCV4-DT[e,f]	In persons immunized at age 9 mo to 6 y and who remain at increased risk of exposure, after 3 y In all persons immunized at 7 y or older, after 5 y In persons immunized at age <7 y and who remain at increased risk of exposure, after 3 y In all persons immunized at ≥7 y, after 5 y If continued risk, additional boosters every 5 y

[a]See http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6202a1.htm, http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6324a2.htm, and http://www.cdc.gov/mmwr/volumes/65/wr/mm6543a3.htm for current ACIP recommendations.
[b]Such as C3, C5-C9, properdin, factor D, factor H, or eculizumab administration.
[c]For example, microbiologists routinely working with *Neisseria meningitidis* at risk because of a community outbreak caused by a vaccine capsular group, and travelers to or residents of countries where meningococcal disease is hyperendemic or epidemic.
[d]MCV4-DT contraindicated in this scenario because of interference with pneumococcal conjugate vaccine.
[e]MCV4-DT is used, it should be given either before or at the same time as DTaP. This recommendation is based on new evidence indicating that hSBA responses to all four meningococcal capsular groups did not meet noninferiority criteria when MCV4-DT was given 30 days after DTaP.
[f]If MCV4-DT is used, it should be administered at least 4 weeks after administration of all pneumococcal conjugate vaccine doses.
Modified from recommendations of the Advisory Committee on Immunization Practices.

public health importance of these differences is unknown, the greater persistence of group Y SBA in the MCV4-CRM group would be expected to translate into greater protection against capsular group Y strains, which account overall for nearly one-third of cases of meningococcal disease in the United States. Collectively, the antibody persistence data also indicate that for both vaccines, protective serum titers wane in immunized adolescents. Taken together with evidence of declining MCV4-DT vaccine efficacy over time in adolescents immunized in the United States with MCV4-DT, in 2010, the Advisory Committee on Immunization Practices (ACIP) recommended the addition of a booster dose of MCV4-DT or MCV4-CRM (Table 38.4; also see "Recommendations for the Use of Meningococcal Vaccines in the United States" below).[311] Similar findings have been reported among military personnel.[407]

Most MCV4-CRM use in the United States is currently targeted at adolescents and more recent data address persistence after a booster dose in adolescents immunized initially with either MCV4-DT or MCV-CRM. Two years after an MCV4-CRM booster, 77% to 100% of individuals had an hSBA of 1 : 8 or

greater.[408] Similarly, in a study conducted 3.5 years after a dose of MenACYW-TT (not yet FDA approved) all volunteers among a small group of adolescents maintained an rSBA of 1:8 or greater.[409]

Limited data are available on serum antibody persistence in younger children immunized with MCV4-DT. In one study, children 2 to 11 years old were given one dose of MCV4-DT or MPSV4 and were followed up for 6 months. Between 1 and 6 months, serum bactericidal titers declined in both vaccine groups, but titers waned more rapidly in the MPSV4 group.[309] When the 6-month follow-up serum samples were retested with human complement,[243] the results confirmed more rapid waning of immunity in the MPSV4 group. In a second study, serum samples were obtained at a mean interval of 2.4 years after MCV4-DT vaccination of 2-year-old children.[307] The proportions of MCV4-DT–vaccinated children with titers of 1:4 or greater (using human complement) were 15% (group A), 33% (group Y), and 45% (group W). The respective percentages for unvaccinated children were lower (2.5%, 15%, and 17.5%). In a separate analysis, serum group C antibody concentrations, which were measured by a binding assay, remained elevated for 2 to 3 years after MCV4-DT vaccination compared with unvaccinated children, but serum group C bactericidal titers of 1:4 or greater were infrequent in both groups (14.6% and 6.4%, respectively; $P = 0.3$).[259] In vaccinated children, some serum samples with bactericidal titers less than 1:4 conferred passive protective activity against group C bacteremia in the infant rat model. These results are difficult to interpret in the light of more recent studies showing that complement activation on *N. meningitidis* is not downregulated by rat fH because the molecule does not bind to meningococci.[277] Therefore, the bacteria are more easily cleared by antibodies interacting with rat complement than they would be with human complement and, therefore, the passive protection results in the rat bacteremia model in the absence of human fH are likely to overestimate protection compared with protection in humans.

At 5 years of age antibody had waned after three- or four-dose infant schedules with MCV4-CRM such that 9% to 69% had an hSBA of 1:8 or greater after three doses against the different stains, and 11% to 85% had an hSBA of 1:8 or greater after four doses, with the best responses being against W.[353] Antibody persistence was evaluated 5 years after immunization of 2- to 10-year-old children with MCV4-CRM, and about half were found to have maintained "protective" antibody levels for C, Y, and W, but persistence was poor for A.[410]

Among toddlers 12 to 23 months of age, MCV4-TT was highly immunogenic and noninferior to MenC-CRM with at least 90% of toddlers maintaining rSBA titers of 1:8 or greater 3 years after vaccination. However, in this study hSBA was also measured and shown to have waned markedly for capsular group A (only 21% maintained an hSBA of 1:4 or greater at 3 years of age).[411]

Persistence of antibody after Hib-MenC vaccine is discussed above under "Hib-MenC and Hib-MenCY."

Immunologic Memory Elicited by MCV4 Vaccines and Effect of Previous Administration of MPSV4 Vaccine

A booster dose of MCV4-DT given to adolescents 3 years after a primary MCV4-DT vaccination elicited higher groups C, Y, and W anticapsular antibody responses than in control subjects immunized with MCV4-DT for the first time (Fig. 38.13). In contrast, a dose of MCV4-DT vaccine given to adolescents who had previously been given a dose of MPSV4 vaccine elicited lower serum bactericidal antibody responses than in control adolescents immunized with MCV4-DT for the first time (Fig. 38.13). Although the impaired serum antibody response to group C was the most affected, polysaccharide-induced antibody hyporesponsiveness also was present to capsular groups A, Y, and W. Nevertheless, 100% of the subjects previously immunized with the polysaccharide vaccine who were immunized with MCV4-DT developed bactericidal titers of 1:128 or greater (using rabbit complement) to all four capsular groups, which should be sufficient for protection.

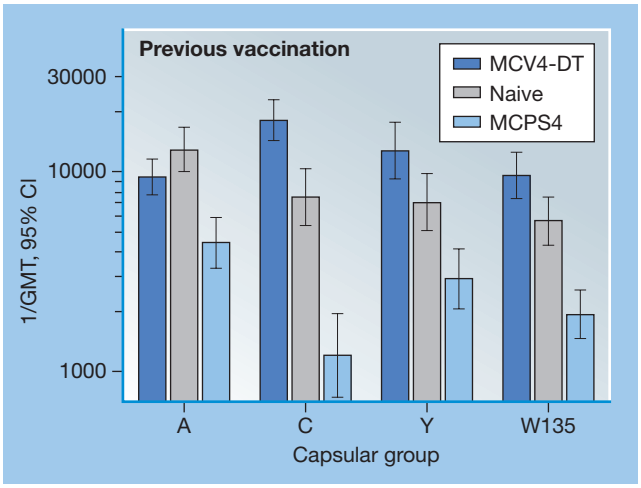


Figure 38.13. Serum bactericidal antibody titers of adolescents 8 days after a dose of quadrivalent meningococcal conjugate (MCV4-DT) vaccine in relation to previous vaccination. Three years earlier, the subjects had received a dose of MCV4-DT vaccine or quadrivalent meningococcal polysaccharide (MPSV4) vaccine as part of a randomized vaccine trial. A newly recruited control group of vaccine-naïve adolescents was given a dose of MCV4 vaccine. For capsular groups C, Y, and W135, the reciprocal serum geometric mean titers (GMTs) to the second dose of MCV4-DT were higher than those to a first dose given to the naïve subjects ($P < .05$; evidence of immunologic priming by the first injection). For all four capsular groups (A, C, Y, and W), the reciprocal serum GMTs to the dose of MCV4-DT vaccine in subjects previously vaccinated with MPSV4 were lower than those to a first dose of MCV4-DT given to the naïve subjects ($P < .05$; evidence of antibody hyporesponsiveness induced by the polysaccharide vaccine). CI, confidence interval. *(Data from Keyserling H, Papa T, Koranyi K, et al. Safety, immunogenicity, and immune memory of a novel meningococcal [groups A, C, Y, and W-135] polysaccharide diphtheria toxoid conjugate vaccine [MCV-4] in healthy adolescents.* Arch Pediatr Adolesc Med. *2005;159:907–913.)*

Adolescents previously immunized with MCV4-CRM mounted stronger responses to a booster of MCV4-CRM than was observed in both naïve individuals and those previously immunized with a quadrivalent polysaccaharide vaccine, which indicated an anamnestic response.[412]

Immunologic memory was assessed in infants immunized with MCV4-CRM vaccine by administration of a decreased dose of MPSV4 at 12 months of age. The infants had been immunized at 2 and 4 months or at 2, 4, and 6 months.[396] The percentages of the children responding with fourfold increases in human complement serum bactericidal titers to the polysaccharide probe at 12 months ranged from 65% (group C) to 95% (groups W and Y). These response rates are substantially higher than those of immunologically naïve historical control subjects who showed minimal or no detectable bactericidal responses when immunized with a dose of polysaccharide vaccine at 1 to 2 years of age.

Rationale for Two-Dose Primary Series in Certain High-Risk Groups

In the United States, a single dose of MCV4-DT or MCV4-CRM was considered sufficient for eliciting protective antibody responses in persons 2 years or older. Recently, for certain patient populations with increased risk of meningococcal disease, a two-dose primary schedule was recommended (see "Recommendations for the Use of Meningococcal Conjugate Vaccines in the United States" below). The rationale for this recommendation is that (1) persons with certain underlying medical conditions respond suboptimally to meningococcal vaccines and (2) there is evidence that a two-dose primary vaccination series given 2 months apart increases the antibody response. Also, although persons with deficiency of late complement components are reported to respond as well to MPSV4 as persons without such deficiencies, serum antibody titers wane more rapidly in complement-deficient patients.[413,414]

In one study of asplenic patients, approximately 20% did not achieve putative protective bactericidal antibody responses following a single dose of one of three group C meningococcal conjugate vaccines, and their GMTs were nearly 10-fold lower than in control subjects without asplenia (158 vs 1448).[415] Patients with a congenital or functional reason for asplenia had lower antibody responses than did patients with asplenia as a result of trauma. Among 23 persons who did not achieve a bactericidal antibody titer of 1:16 (using rabbit complement) or greater after the first dose, 61% achieved a titer of 1:16 or more after a second dose. These data suggest that a two-dose series would provide protection superior to a one-dose series.

In a study of meningococcal conjugate vaccination in adolescents with HIV infection, 86%, 55%, 73%, and 72% of patients had serum bactericidal titers of 1:128 or greater (using rabbit complement), respectively, for capsular groups A, C, Y, and W after one dose of MCV4-DT.[416] Lower $CD4^+$ lymphocyte percentages and higher HIV viral loads were independently associated with a poorer response to immunization.[417]

Postlicensure MCV4-DT Effectiveness

In 2014, MCV4-DT vaccine coverage among U.S. 13- to 17-year-olds was estimated to be 79%, although with a great deal of regional variation.[418] The small numbers of cases from the historically low rates of meningococcal disease in the United States have made it challenging to establish the effectiveness of MCV4-DT. However, several recent studies using different approaches have provided a picture of the effectiveness of this vaccine. In one study, surveillance for MCV4-DT vaccine failures was conducted and vaccine effectiveness was modeled based on the expected number of vaccine failures and estimates of MCV4-DT coverage.[419] This analysis suggested a vaccine effectiveness of 80% to 85% within 3 to 4 years after vaccination, which is similar to that expected for meningococcal polysaccharide vaccine. In a case-control vaccine effectiveness study, overall vaccine effectiveness (all capsular groups) for 0 to 8 years postvaccination was estimated to be 69% (95% CI, 51–80%). Over time, there was evidence for waning of effectiveness with estimates of 79% (49–91%) during the first year after vaccination, 69% (44–83%) at 1 to less than 3 years, and 61% (25–79%) at 3 to less than 8 years.[419a] These results are consistent with waning serum bactericidal titers and supported the addition of a booster dose (see earlier discussion and Fig. 38.12).

Based on active surveillance of meningococcal disease in regions of the United States with 13% of the overall population (ABCs[420]), the incidence of IMD among 11- to 17-year-olds caused by capsular group C, Y, or W strains fell from 0.37 per 100,000 population during 2006 to rates of less than 0.1 cases per 100,000 population in 2013. Rates have changed little in other age groups (Fig. 38.14).

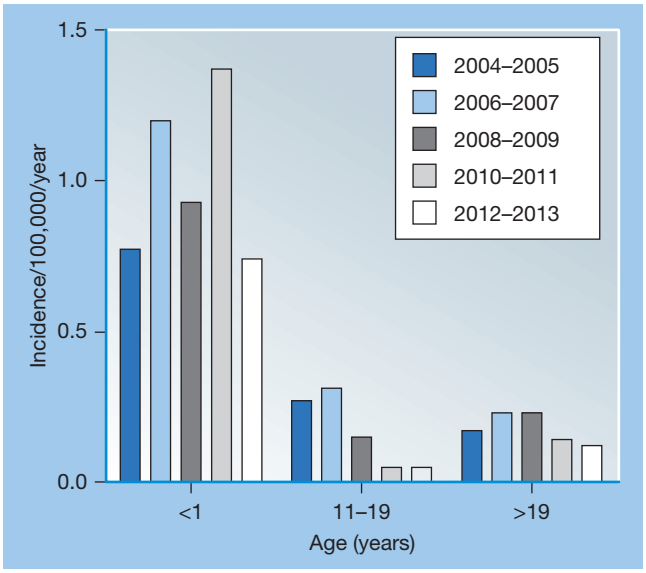


Figure 38.14. Incidence of capsular groups C, Y, and W meningococcal disease in the United States, from 2004–2013 by age group.

Although attribution of causality is not possible in this ecological analysis, these data, in combination with the vaccine effectiveness data, suggest a selective effect of MCV4-DT on the immunized population and a lack of substantial herd protection in the unvaccinated population, although this may be because the coverage was low initially. In addition, there are no data on the impact of MCV4-DT on pharyngeal carriage of vaccine capsular groups. No postlicensure effectiveness data are available for MCV4-CRM because it was licensed in the United States in 2010 and follow-up has been too brief. However, use of quadrivalent conjugate vaccines in Latin America and the United Kingdom to control recent outbreaks of ST-11 capsular group W disease will likely provide evidence in due course.

MCV4-DT and MCV4-CRM provide capsular group–specific coverage, which does not include capsular group B strains. One concern about widespread use of vaccines that do not cover all disease-causing strains is the possibility of capsular switching and subsequent clonal expansion from a virulent vaccine-capsular group strain (i.e., group C, Y, or W) to a nonvaccine capsular group strain (i.e., group B). To assess the meningococcal population structure in the United States before licensure of MCV4-DT in 2005, a study was performed on isolates from 2000 to 2005 from Active Bacterial Core surveillance (ABCs) sites throughout the United States.[51] Of 1160 isolates included in the study, 1.5% of group B, 12.9% of group C, and 0.9% of group Y isolates showed evidence of capsular switching. For example, there were one and four isolates, respectively, that expressed capsular group B polysaccharide and belonged to sequence type 11 or 103, which are both hypervirulent meningococcal lineages that typically express group C capsules.[51] An update to this study in 2015 found no new evidence of vaccine driven changes in the population.[181] It is noteworthy that although a group B vaccine was not introduced in the United States until 2015, the incidence of capsular group B disease in the United States has fallen dramatically since the 1990s.

Safety of MCV4 Vaccines

In prelicensure studies, MCV4-DT was administered to approximately 7000 persons. Vaccination was safe and well tolerated.[310,384] In randomized studies, fever (temperature, ≥38°C) was more common after MCV4-DT than MPSV4 (5.1% vs 3.0%, respectively, in immunized adolescents; and 1.5% vs 0.5% in adults). Fewer than 5% of subjects vaccinated with MCV4-DT or MPSV4 experienced serious systemic reactions (defined as high fever; headache, fatigue, malaise, chills, or arthralgia requiring bed rest; or anorexia [missing three meals]; three or more episodes of vomiting; five or more episodes of diarrhea; or the presence of rash or seizures). The respective rates of serious reactions in the MCV4-DT group met predetermined noninferiority criteria as compared with the respective rates observed in the MPSV4 vaccine group.

The prelicensure MCV4-DT studies also showed that local reactions were more common than with MPSV4, which likely reflected the presence of diphtheria toxoid as the carrier protein in the conjugate vaccine. Thus, 16.7% of adolescents given MCV4-DT and 3.9% of the MPSV4 group reported moderate pain at the injection site,[310] and 2.5% and 0.7%, respectively, reported moderate swelling. Severe pain (unable to move arm) occurred in 0.7% of the MCV4-DT group and 0% of the MPSV4 group, while severe swelling at the injection site (>2 inches) was reported by 0.7% and 0%, respectively. These local reactions generally resolved within 48 to 72 hours. The frequencies of local reactions in MCV4-DT–vaccinated adolescents were similar to those observed in this age group after Td (diphtheria and tetanus toxoids–adult) vaccination. The respective rates of local reactions in MCV4-DT–immunized adults were lower than in adolescents and similar to those observed after typhoid vaccination of adults.

In prelicensure studies, the safety and tolerability of MCV4-CRM were compared with those of MCV4-DT. In 2- to 5-year-olds, the respective rates of local reactions were similar for the two vaccines (e.g., 27% vs 25% for erythema and 33% vs 35% for pain, respectively).[393] Rates of systemic reactions were also similar (e.g., 21% vs 22%, respectively, for irritability). In the same study, there were also no substantial differences for the two vaccines in adverse reactions for 6- to 10-year-olds. In a separate study, tolerability in 11- to 18-year-olds was similar for MCV4-DT and MCV4-CRM, with, for example, 70% and 64% of subjects, respectively, reporting mild to moderate solicited reactions.[395] Tolerability of a single dose of MCV4-CRM or MPSV4 also was similar, with reported local reactions, respectively, in 63% to 71% vs 60% to 62%, and systemic in 44% to 56% vs 46% to 59%.[421]

Soon after MCV4-DT licensure in 2005, cases of GBS with onset 2 to 4 weeks after MCV4-DT vaccination were reported to the Vaccine Adverse Event Reporting System (VAERS).[422] An increased rate of GBS was not observed after vaccination with MPSV4 or diphtheria toxoids, the two principal components of MCV4-DT nor with other conjugate vaccines. Several studies conducted to clarify the association between MCV4-DT vaccination and GBS were done. A review of Vaccine Safety Datalink data revealed no GBS cases following 889,684 MCV4-DT doses. The statistical power, however, of this study alone to measure increased risk, if any, was limited. Another study was conducted to estimate the risk of GBS during the 6 weeks following MCV4-DT vaccination as compared with vaccinees at other times and in persons who had not received MCV4-DT.[423] Five health plans with a total membership of approximately 50 million persons participated. GBS cases were identified through claims data and confirmed through a review of medical records. There were 99 GBS cases confirmed and more than 1.4 million MCV4-DT immunizations observed, but no GBS cases occurred within 6 weeks following MCV4-DT immunization. The 95% upper confidence limit was 1.5 cases per million MCV4-DT doses. Taken together, these studies do not support an increased risk of GBS from MCV4-DT immunization. Based on these data, on June 23, 2010, the ACIP removed the precautionary language about the risk of GBS and this vaccine and no new safety concerns have arisen since.

Safety data have been developed during the licensure of MCV4-CRM and do not identify any unexpected adverse events signals from the trials. One study involving more than 7700 infants found that safety of MCV4-CRM is noninferior to routine vaccines after adjusting for study center.[424]

RECOMMENDATIONS FOR THE USE OF MENINGOCOCCAL CONJUGATE VACCINES IN THE UNITED STATES

The ACIP has published its recommendations on the use of meningococcal conjugate vaccines.[425–427a] Conjugate vaccines are preferred over unconjugated polysaccharide vaccines because conjugate vaccines are at least equivalent or more immunogenic than polysaccharide vaccines, can be used from early infancy, and prime for booster antibody responses. In addition, although not documented for meningococcal conjugate vaccines licensed in the United States, conjugate vaccines in general and capsular groups C and A meningococcal conjugate vaccines specifically induce herd protection through decreased pharyngeal carriage. In contrast, the meningococcal polysaccharide vaccines have minimal effect on carriage and induce antibody hyporesponsiveness to a subsequent injection. The reason conjugate vaccines, but not polysaccharide vaccines, have a substantial effect in decreasing meningococcal carriage is that the T-cell help associated with conjugate vaccines leads to higher levels of antibody that have higher avidity than those associated with polysaccharide vaccines. Therefore, except for certain circumstances, such as for those age 55 years or older for whom there are insufficient data on the use of meningococcal conjugate vaccines, a conjugate vaccine is preferred over an unconjugated polysaccharide vaccine. That said, off-label use of meningococcal conjugate vaccines could be considered for persons 55 years of age and older.

Routine Immunization

ACIP recommends routine immunization for all persons 11- to 18-years-old with MCV4-DT or MCV4-CRM, with a preference for beginning immunization at 11 to 12 years of age (see Table 38.4). For children immunized at 11 to 12 years of age, a booster dose is recommended at age 16 years (at least 3 years later). Only a single dose is recommended for those older than 16 years of age and routine vaccination is not recommended for those older than 19 years of age. This recommendation is based on an increased risk of meningococcal disease in adolescence and, as described, evidence for waning serum antibody levels and vaccine effectiveness over time.[427] Routine vaccination is not recommended for other ages in the United States.

MCV4-CRM and Hib-MenCY are approved by the FDA for use in infants as young as 6 to 8 weeks of age. However, as of September 2015, these vaccines were not recommended by the ACIP for routine immunization before age 11 years although the incidence of disease in children younger than age 1 year is more than sevenfold higher than in adolescents (Fig. 38.15).

The reasons the ACIP does not recommend routine vaccination with MCV4 beginning at 2 months of age include low

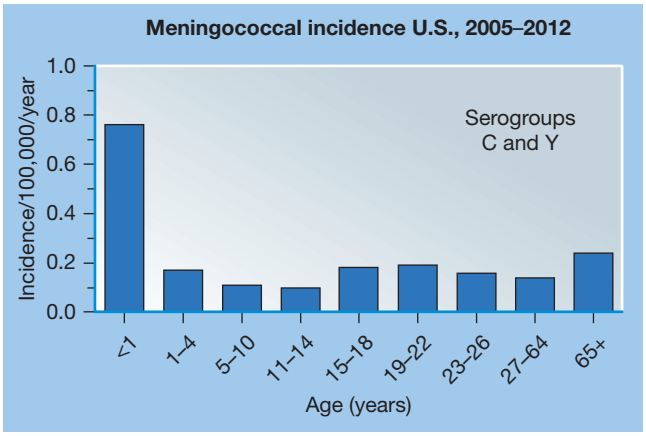


Figure 38.15. Incidence of meningococcal disease in the United States, from 2005 to 2012 by age group. *(From Centers for Disease Control and Prevention. Advisory Committee on Immunization Practices Meeting, October 2014.)*

disease burden, the majority of infant disease is caused by capsular group B strains, the high price of the vaccine, and concerns that additional injections would be unacceptable in an already overcrowded and complex infant and toddler vaccination schedule.[428]

Groups at Increased Risk of Disease, Ages 2 Months to 55 Years

Ages 2 Months to 23 Months

A four-dose course of MCV4-CRM is recommended at 2, 4, 6, and 12 months of age for infants known to be at increased risk of meningococcal disease.[426] MCV4-CRM is the only quadrivalent vaccine licensed for infants younger than 9 months of age and so infants who fall into a risk group at ages 7 to 23 months should have a two-dose course of MCV4-CRM (second dose given when infant is older than 12 months of age and at least 3 months after the first dose). MCV4-DT also can be used as an alternative for infants older than 9 months of age.

If MCV4-DT or MCV4-CRM or Hib-MenCY have been used to initiate immunization at 2 to 13 months of age, the first booster should be given 3 years later with a quadrivalent vaccine and subsequent boosters every 5 years.

Infants and toddlers with functional or anatomic asplenia should receive MCV4-CRM or wait until 2 years of age and receive MCV4-DT as MCV4-DT may interfere with PCV13-CRM responses.

Ages 24 Months to 55 Years

A two-dose primary course of MC4-DT or MCV4-CRM (8 weeks apart) is recommended for individuals with complement deficiency, or functional or anatomic asplenia, and for individuals with HIV infection who have one of the other indications for immunization (i.e., not routinely recommended in HIV infection). A single dose of MCV4 is recommended for travelers to regions with epidemic or hyperendemic meningococcal disease, college freshmen living in dormitories (younger than 22 years of age), microbiologists with routine exposure to *N. meningitidis,* and during an outbreak. For those at ongoing risk of disease, initial booster doses are recommended after 3 years of age among those younger than 7 years of age and at 5 years for children aged 7 and older and adults. Further boosters are recommended every 5 years.

Persons Age 55 Years or Older

There are no data available on the use of quadrivalent meningococcal conjugate vaccines in the 55-years-or-older age group. When meningococcal vaccination is indicated for persons 55 years of age or older, the ACIP recommends MPSV4, although the immunological advantages of MCV4 make this advice appear rather conservative. If the polysaccharide vaccine is not available or repeated vaccine doses are likely to be required as a result of ongoing risk, ACIP agrees that off-label use of one of the conjugate vaccines could be considered.

Mass Vaccination Programs to Control Outbreaks

Outbreaks of meningococcal disease can occur in institutions and communities. An outbreak has been defined by the CDC as the occurrence of three or more cases in less than 3 months resulting in a primary disease attack rate of at least 10 cases per 100,000 population in persons who have a common affiliation or who live in the same area but have no close contact.[5] Either of the quadrivalent meningococcal vaccines (MCV4-DT, from age 9 months, or MCV4-CRM, ages 2 months to 55 years) can be used for control of outbreaks caused by a strain with a vaccine-preventable capsular group.[384] For the aforementioned reasons, one of the conjugate vaccines is preferred.

Precautions and Contraindications

No clinically important adverse effects have been documented among women vaccinated with MCV4 during pregnancy or their newborns. Therefore, MCV4 can be administered during pregnancy, if indicated.[427,433–435] Meningococcal vaccines can be administered during a minor acute illness, including in the presence of low-grade fever but deferred in those with a moderate to severe illness. Meningococcal conjugate vaccines can be safely admininistered to the immunocompromised. All vaccines are contraindicated in persons with known hypersensitivity to any vaccine component.

The use of meningococcal conjugate vaccines in adolescents has occurred in the context of the addition of other vaccines in this population, including acellular pertussis, human papillomavirus, and influenza vaccines.[416,436,437] Coadministration of MCV4-DT vaccine with diphtheria combined with tetanus toxoid (dT) in adolescents and with typhoid vaccine (Vi polysaccharide) in adults is safe and immunogenic. Coadministration of MCV4-CRM with human papillomavirus vaccine and tetanus, diphtheria, and pertussis (Tdap) in adolescents also is safe and immunogenic, as is coadministration with measles-mumps-rubella-varicella (MMRV) in toddlers.[394,438,439] Studies in infants have also shown that MCV4-CRM is safe and immunogenic when given with routinely administered vaccines in Europe. In general, polysaccharide-protein vaccines can be safely and effectively coadministered with live attenuated viral vaccines or purified subcomponent vaccines. Given the risk of syncope after immunization, particularly in adolescents,[440] the ACIP recommends observation of patients for 15 minutes after vaccine administration.[441]

Hib-MenCY has been studied concomitantly with routine infant immunizations including DTaP-HepB-IPV and pneumococcal conjugate vaccine in infants and with measles, mumps, rubella (MMR), and pneumococcal conjugate vaccine among toddlers and has an acceptable profile.[442,443]

MENINGOCOCCAL VACCINES IN CANADA

There are currently two meningococcal polysaccharide vaccines (MPSV4 and MPSV2 [capsular groups A and C], both

called Menomune) licensed in Canada, and five meningococcal conjugate vaccines[444]: the conjugate products are MCV4-DT and MCV4-CRM, and three monovalent group C conjugate vaccines (P-MenC-CRM, G-MenC-CRM, and MenC-TT; see earlier section). In 2001, the Canadian National Advisory Committee on Immunization (NACI) recommended routine group C meningococcal conjugate vaccine for infants, children through 4 years of age, and adolescents and young adults.[159,445] In 2010, the NACI recommended the use of quadrivalent conjugate vaccine in place of monovalent group C conjugate vaccines in early adolescence. In addition, the NACI recommends the use of quadrivalent conjugate vaccine for persons 2 to 55 years of age who are at high risk. The NACI is an advisory organization, and immunization policy in Canada is made at the provincial level. For example, as of June 2011, Alberta recommended routine vaccination with three doses of a monovalent MenC conjugate vaccine at 2, 4, and 12 months; British Columbia recommended two doses at 2 and 12 months; and Ontario, Saskatchewan, and Quebec recommended one dose at 12 months. The diverse group of schedules used in Canada is available online.[444]

All provinces recommend a booster dose of C conjugate vaccine in adolescence but in some provinces this is administered as MCV4 and in others as a monovalent vaccine; the age of administration varies also.

MENINGOCOCCAL CONJUGATE VACCINE POLICY IN EUROPE

Between 1999 and 2006, in response to relatively high endemic rates of meningococcal C disease, multiple European countries (Belgium, Iceland, Ireland, The Netherlands, Spain, and the United Kingdom) introduced meningococcal C conjugate vaccines as part of national immunization programs. The initial regimen in the United Kingdom included immunization of infants beginning at 2, 3, and 4 months of age. Because vaccine effectiveness fell substantially after 1 year among infants immunized with this regimen, the UK vaccination schedule was changed in 2006 to include a booster dose at 12 months of age (3, 4, and 12 months of age). The 4-month dose was dropped in 2013 and replaced by a booster of MenC for adolescents to sustain herd immunity. The latter was changed to MCV4 in 2015 and the 3-month dose of MenC was dropped in 2016 so that the final schedule starts at 12 months of age (as Hib-MenC-TT) followed by MCV4 in adolescence (see below).

In several European countries where the incidence of meningococcal disease remained low and stable during the 1999 to 2006 period (≈0.2 per 100,000 population),[446] routine vaccination with meningococcal C conjugate was not initially implemented but has now become routine (Germany, Portugal, and Switzerland in 2006, and France in 2009; Table 38.5). Although waning serum bactericidal antibody titers have been observed after immunization,[349,352,373] vaccine effectiveness remains high,[188,314] although, as in the United Kingdom, an adolescent booster to maintain vaccine effectiveness is being considered or has been introduced in a number of countries either as the monovalent C conjugate or the quadrivalent product.

MCV4-CRM and MCV4-TT are both licensed in Europe but because of the low incidence of meningococcal disease in the region attributable to strains with capsular group A, W, or Y, recommendations for use of the quadrivalent conjugate vaccine in Europe are limited. In response to a marked increase in the number of cases of group W disease in the United Kingdom, a routine one-dose adolescent program of MCV4 was introduced in 2015 with catch-up for adolescents 14 to 18 years of age.

A MENINGOCOCCAL CAPSULAR GROUP A CONJUGATE VACCINE FOR AFRICA

Up to 2010, the only meningococcal vaccines available in sub-Saharan Africa were a bivalent groups A and C and a trivalent groups A, C, and W polysaccharide vaccine (MPSV2 and MPSV3). These vaccines are poorly immunogenic in infants, the age group at greatest risk of acquiring disease, and have only a transient effect, if any, on colonization and spread of

TABLE 38.5 Meningococcal Vaccine Policy in Selected European Countries[a]

Country	Current Vaccination Schedule	Initial MenC Catch-Up
Austria	MenC at 12–14 mo and MCV4 at 12 y	
Belgium	MenC at 15 mo	1–5 y/1–18 y; regional variation
Czech Republic	MenB vaccine in 3 doses at 2–5 mo with a booster at 12 mo. Two doses of MenB at 13–15 y. One dose of MCV4 at 1–2 y and at 13–15 y	
France	MenC at 12–24 mo	Catch-up to 24 y
Germany	MenC at between 12 and 24 mo	Recommended for unvaccinated through age 17 y
Greece	MenC at 2, 4, and 15–18 mo and MCV4 at 11 y	
Iceland	MenC at 6 and 8 mo	Through 20 y
Italy	MenC at 12–15 mo	
Ireland	MenC at 4 and 12 mo and 12–13 y	Through 23 y
Netherlands	MenC at 14 mo	1 through 19 y
Portugal	MenC at 12 mo	Through 18 y
Spain	MenC at 2 and 12 mo and 12 y	<6 y; regional variation
United Kingdom	MenC at 12 mo and MCV4 at 13/14 y. MenB at 2, 4, and 12 mo	In 1999 MenC <18 y; expanded to 24 y. MCV4 catch up 14–18 y initiated in 2015

[a]MenC conjugate vaccines are recommended for routine use in various countries in Europe and Hib-MenC is used at 12 months of age in the United Kingdom. MenB and MCV4 is now recommended in the United Kingdom and is under consideration elsewhere. See http://vaccine-schedule.ecdc.europa.eu/Pages/Scheduler.aspx and http://www.szu.cz/uploads/IMO/Recommendation_for_vaccination_IMD.pdf

infection. For these reasons, meningococcal vaccination in Africa was largely done in response to epidemics, which was a logistically challenging and relatively ineffective strategy. Although an emergency response plan had been developed in collaboration with the WHO and the CDC,[314] implementation was often delayed because of limitations of surveillance or logistics. One study estimated that even under optimal circumstances, fewer than 60% of outbreak-related cases were prevented by implementing vaccination after an epidemic was recognized.[314] An improved meningococcal vaccine, therefore, was needed.

In sub-Saharan Africa, most meningococcal epidemics are caused by capsular group A strains. By the late 1990s, three vaccine manufacturers had developed capsular group C meningococcal conjugate vaccines for the United Kingdom, where there had been approximately 10,000 cases of disease caused by group C organisms and 1000 deaths during the previous decade.[315] There was little commercial interest in developing a group A meningococcal conjugate vaccine for sub-Saharan Africa, where there had been more than 700,000 cases and 100,000 deaths in the same decade.[3] The countries in this region remain some of the poorest in the world and could not afford to purchase the vaccine.

In 1999, the WHO funded a feasibility study to explore options to develop and manufacture a group A conjugate vaccine that could be sold in Africa for less than US$0.50 a dose.[316,317] The results from this study formed the basis of a successful grant application to the Bill and Melinda Gates Foundation, which in 2001 awarded $70 million to the WHO and the Program for Appropriate Technology in Health to develop a meningococcal group A conjugate vaccine with the goal of eliminating group A epidemics in Africa.[316] While there were concerns at the time about the potential for epidemics caused by capsular group W strains,[225,318–320] in the previous 100 years, more than 90% of epidemic meningococcal disease in sub-Saharan Africa had been caused by capsular group A strains. The dominance of group A disease and the greater technical challenges, higher cost, and extended timelines for developing a bivalent capsular groups A and W conjugate vaccine made development of a monovalent group A vaccine more feasible as a first step.

A novel conjugation chemistry using reductive amination of group A polysaccharide aldehydes and tetanus toxoid hydrazides was developed by scientists at the FDA's Center for Biologics Evaluation and Research.[321] The process was transferred to the Serum Institute, a vaccine manufacturer in India.[322] Extensive testing of the vaccine in sub-Saharan Africa provided assurance of safety, immunogenicity, and manufacturing consistency.[323] In 2010, marketing authorization for MenAfriVac (MenA-TT conjugate vaccine) was granted by the Food and Drug Administration of India for the export of the vaccine to African countries. Mass vaccination of 1- to 29-year-olds was introduced in December 2010, as part of countrywide demonstration projects in Burkina Faso, Mali, and Niger.[324] More than 217 million people ages 1 through 29 years had received MenA-TT vaccine (containing 10 μg MenA polysaccharide) in 17 countries in the African meningitis belt by 2015.[325] It is hoped that high coverage in this age group will lead to herd protection of unvaccinated children younger than 1 year of age and elimination of epidemics of meningococcal A disease. MenA-TT led to a dramatic decline in group A carriage in African villages, suggesting that herd protection with this vaccine will be substantial.[326] Once the mass vaccination campaigns are completed, the concern will be to protect subsequent birth cohorts throughout infancy. Two strategies are recommended, one for countries where MenA coverage is high and another where MenA coverage is low (<60%).[328] In high-coverage countries, vaccination should be administered as a single dose at 9 to 18 months of age. A lower dose vaccine (5 μg of MenA polysaccharide) is available for children younger than 2 years of age. In low-coverage countries, periodic single-dose campaigns are recommended.

A major problem that has occurred since the widespread use of a capsular group A conjugate vaccine is the emergence of disease caused by strains with other capsular groups such as C, W, or X.[225,318,329–331] Currently there is no vaccine available for prevention of group X disease, which is extremely rare in North America and Europe.[332] For prevention of group C and W disease, tetravalent (A, C, Y, and W) conjugate vaccines are available in industrialized countries (see earlier discussion), but these vaccines are expensive and not available in sub-Saharan Africa. A trivalent MPSV3 vaccine is available for Africa and kept in reserve by the WHO for response to capsular groups C and W epidemics, but this vaccination strategy has many limitations, as described. Work is currently underway to produce a low-cost pentavalent MenACWXY conjugate vaccine that could (potentially) replace the monovalent vaccine.

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39 Meningococcal Capsular Group B Vaccines

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The modes of transmission, bacteriology, pathogenesis, diagnosis, and treatment of capsular group B meningococcal disease are indistinguishable from meningococcal disease caused by other capsular groups and are discussed in Chapter 38. As described below, the conjugate vaccine approach proved problematic against capsular group B strains because the capsular polysaccharide is an auto-antigen and poorly immunogenic. The protein antigens used in the new capsular group B vaccines are novel and have immunogenic properties and underlying mechanisms that are different from those associated with polysaccharide antigens. Therefore, the vaccines targeting capsular group B strains are described separately in this chapter.

The different capsular polysaccharides expressed by meningococci have traditionally been defined serologically and referred to as "serogroups." More recently, DNA sequencing of specific genes involved in capsular synthesis is replacing serologic methods to define an isolate's capsular polysaccharide.[1–3] Therefore the term "serogroup" no longer seems apt for describing an isolate's polysaccharide capsule. This chapter replaces the term "serogroup B" with "capsular group B" or, simply "group B."

CAPSULAR B DISEASE BURDEN

Capsular B disease accounts for up to 90% of sporadic meningococcal cases in certain European countries that have successful capsular group C vaccine programs in place, and approximately a third of cases in the United States in 2013.[4] Compared with group A epidemics, capsular group B epidemics generally begin more slowly, are associated with lower rates of disease, and are more prolonged, at times lasting a decade or longer. Group B epidemic disease also is associated with the emergence of hypervirulent strains.

As described in Chapter 38, meningococci are classified by multilocus sequence types (ST) and capsular group, serotype (PorB), and serosubtype (PorA). The nomenclature lists the capsular group, PorB serotype, and PorA serosubtype, each separated by a colon (e.g., B:4:P1.7,4). The VR1 (loop 2) and VR2 (loop 4) encoded epitopes of PorA are given in order after the prefix P1.x, separated from one another by a comma. For example, in the 1970s, an increase in the incidence of disease caused by ST-32 group B strains was noted in Norway (B:15:P1.7,16) and in Spain (B:4:P1.19,15).[5] In the 1980s, a severe epidemic in Cuba was also caused by ST-32 group B strains (B:4:P1.19,15), which spread to São Paulo, Brazil, in the late 1980s.[5] Group B meningococci strains from this genetic complex became established in Canada in the 1990s, and substantial increases in incidence also were noted in the United States in Oregon and Washington State (B:15:P1.7,16).[6] The incidence of disease in Oregon caused by this outbreak peaked in the 1994–96 period, but the clone of meningococcus responsible has remained an important cause of disease.[7,8] Interestingly, the Oregon clone caused disease across the United States, but only Oregon was associated with a substantial increase in disease rates.[9]

New Zealand experienced an epidemic of group B meningococcal disease that started in 1991.[10] The epidemic was caused by a strain identified as B:4:P1.7-2,4, ST-41/44 complex.[11] A strain-specific outer membrane vesicle (OMV) vaccine, described below, was developed and introduced in July 2004 for mass immunization in New Zealand,[12–15] which ultimately contributed to control of the epidemic.[16]

Over the past few years, the epidemiology of college-associated outbreaks has changed in the United States. Before the routine use of quadrivalent meningococcal vaccines in adolescents, most outbreaks in institutions of higher learning were caused by capsular group C strains, but are now predominantly caused by a diverse assortment of group B strains (Table 39.1). From 2008–16, there were at least 10 group B outbreaks on college campuses, ranging in size from two to 13 cases.[17–22] Immunization campaigns with one or both of the recently licensed group B vaccines were conducted in response to the recent outbreaks.

One of the most important epidemiologic features of group B meningococcal disease is that it is the predominant cause of disease in infants.[23] A second important feature is that for reasons that are not known, incidence rates of endemic group B disease in different countries can vary; for example, they are approximately 20-fold higher in infants in England than in the United States (Fig. 39.1). The recent licensure in Europe and elsewhere of a capsular group B vaccine for infants means that disease in infants is now potentially vaccine preventable. In the United States, however, the two licensed group B vaccines are only approved for the age group 10 to 25 years.[19] Currently, the only infant vaccine under development for prevention of infant group B disease in the United States is a pentavalent vaccine for capsular groups A, B, C, W, and Y,[24–26] which is unlikely to be available for several years.

HISTORY OF GROUP B VACCINE DEVELOPMENT

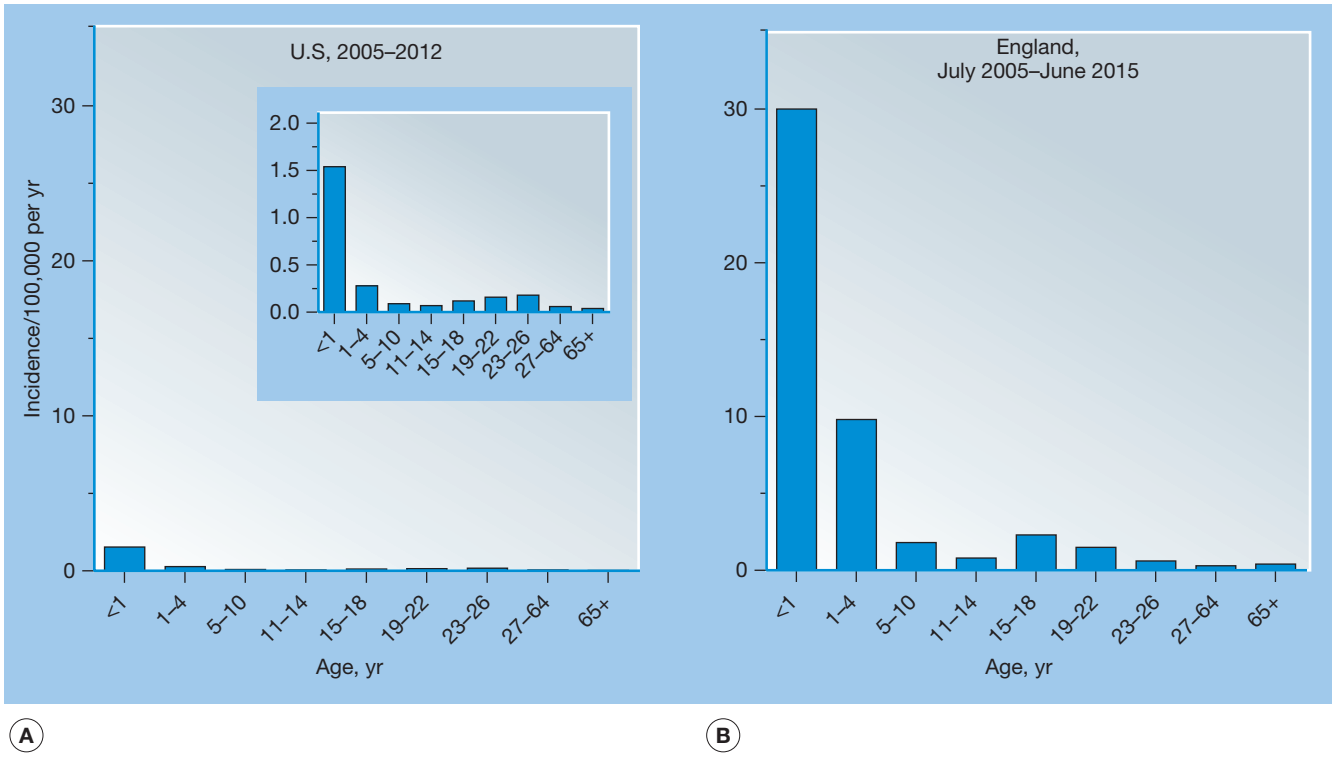
The group B polysaccharide capsule cross-reacts with sialylated proteins present on human tissues.[27] The polysaccharide also is a poor immunogen, even when conjugated to a protein carrier.[28] Also, there are potential safety concerns for a vaccine that elicits high serum titers of autoreactive antibodies to the group B polysaccharide. These concerns prompted investigators to investigate the potential of noncapsular antigens, particularly outer membrane proteins.[29,30] The idea that noncapsular antigens could be sufficiently exposed on the surface of encapsulated meningococci to be accessible to protective antibodies was at first controversial.

Compelling data showing that protein antigens could elicit protective antibodies came from pioneering studies with OMV vaccines, which were developed by several groups more than 25 years ago.[31–33] The OMVs were treated with detergents to extract lipooligosaccharide and decrease endotoxin activity. The OMV vaccines appeared to be safe and efficacious.[34] The initial OMV studies used two doses of vaccine, which were sufficient to elicit serum bactericidal antibody antibodies and protect older children and adults.[31] However, a case-control study done in Brazil in the early 1990s found no efficacy in infants younger than 24 months,[35] which for nearly a decade dampened enthusiasm for the OMV vaccine approach. A seminal finding was the publication by Tappero and colleagues in 1999 demonstrating that three doses of OMV vaccines elicited serum bactericidal antibody responses in infants

TABLE 39.1 Capsular Group B Outbreaks on U.S. College Campuses, 2008–16

Institution	Time Period	No. Cases (Fatalities)	No. Isolates Tested	Multilocus ST/CC	Vaccination Campaign?
Ohio University[218]	Jan 2008–Nov 2010	13 (1)	2	ST-269/CC269	No
University of Pennsylvania	Feb–Mar 2009	4	4	ST-283/CC269	No
Lehigh University, PA	Nov 2011	2	2	ST-1624/CC167	No
Princeton University, NJ[18,22,123]	Mar 2013–Mar 2014	9 (1)[b]	8	ST-409/CC41/44	Yes
University of California, Santa Barbara[123]	Nov 2013–May 2015	6[a]	4	ST-32/CC32	Yes
University of Oregon	Jan–May 2015	7 (1)	6	ST-32/CC32	Yes
Providence College, RI[17]	Jan–Feb 2015	2	2	ST-9069 (no assigned CC)	Yes
Santa Clara University, CA[20]	Jan–Feb 2016	3	2	New ST/CC11	Yes
Rutgers University, NJ[21]	Mar–Apr 2016	2	2	ST-11/CC32	Yes
University of Wisconsin	Oct 2016	2	2	ST-11556/CC32	Yes
Oregon State University	Nov 2016	2	2	Multilocus ST/CC, ST-32/CC32	No

[a]Includes one case in a girlfriend visiting the campus in May 2015.
[b]Fatal case occurred in a Drexel University student who developed infection with the outbreak strain after close contact with a vaccinated Princeton student (http://www.bt.cdc.gov/HAN/han00357.asp).
CC, clonal complex; ST, sequence type.



A B

Figure 39.1. Age incidence of meningococcal capsular group B disease in the (A) United States, 2005–12, and (B) England, July 2005 to June 2016. *(U.S. data are from the Centers for Disease Control and Prevention, adapted from a presentation at the U.S. Advisory Committee on Immunization Practice (J. MacNeill), October 30, 2014. Data from England are from Public Health, England.)*

younger than 1 year.[36] This finding set the stage for developing protein antigen vaccines for the age group with the highest incidence of disease.

One limitation of OMV vaccines, however, is that serum bactericidal antibody responses of infants are largely directed against the porin protein, PorA,[36] which is antigenically variable.[37] OMV vaccines, therefore, were tailor made and mainly used to control group B epidemics caused by a predominant strain. Subsequent efforts to broaden protection used OMV vaccines prepared from more than one strain[38] or from mutants expressing more than one PorA type.[39–41] While these vaccines elicited serum bactericidal antibody responses in human infants, certain PorA types were poorly immunogenic.[42] Furthermore, in the United States endemic meningococcal disease is caused by genetically diverse strains with more than 20 PorA variable region types. These factors and the discovery of more promising recombinant protein antigen approaches beginning in 2000[29] eventually led to

discontinuation of most efforts to license detergent-treated OMV vaccines as standalone meningococcal capsular group B vaccines for prevention of endemic disease. However, as described below, an OMV is one component of one of the recently licensed group B vaccines. A key lesson from the OMV vaccine trials was that a vaccine based on protein antigens could prevent disease caused by encapsulated meningococci and that a correlate of group B vaccine efficacy was serum bactericidal activity.[43,44]

The 1990s witnessed the discovery of several promising recombinant meningococcal protein vaccines capable of eliciting broad serum bactericidal antibody responses in mice.[34] These included Neisserial surface protein A (NspA)[30,45,46] and a transferrin-binding protein (TbpB).[47–49] However, in Phase I clinical trials these recombinant protein vaccines failed to elicit serum bactericidal antibody responses in humans.[50,51] NspA was subsequently shown to specifically bind human complement factor H (FH),[52] and TbpB was known to bind specifically to human transferrin.[53] The lack of serum bactericidal antibody responses to these vaccines in humans may have resulted in part from binding of the vaccine antigens to host proteins. For example, human FH transgenic mice immunized with NspA vaccines that bound human FH had lower serum bactericidal antibody responses than wild-type mice whose mouse FH did not bind to NspA.[54] Also, pigs immunized with a mutant *Haemophilus parasuis* TbpB vaccine with decreased binding to porcine transferrin elicited higher T- and B-cell responses and provided greater protection from infection than a native TbpB vaccine that bound porcine transferrin.[55] As described below, there also are data from human FH transgenic mice[56–60] and infant primate models[61–63] that show binding of human FH to Factor H binding protein (FHbp, an antigen in both licensed group B vaccines; see below) impairs protective antibody responses. Collectively the data indicate that binding of a host protein to a vaccine antigen can decrease protective antibody responses.

In 2000, the first full DNA sequence of a meningococcal genome was described[64] and was used to identify a large number of new meningococcal protein antigen vaccine candidates.[29] In the subsequent decade, Novartis Vaccines (now GSK) used three of the antigens in combination with an OMV derived from the New Zealand outbreak strain to develop a group B vaccine (Bexsero). Because the vaccine contains four components capable of eliciting serum bactericidal activity, the vaccine is referred to as MenB-4C (4C-MenB in Europe). In the United States, a second group B vaccine that contains two FHbp sequence variants was developed by Pfizer Vaccines (Trumenba; referred to herein as MenB-FHbp).

These group B vaccines provide coverage against some strains with other capsular groups such as A, C, Y, W, or X, since the protein antigens are shared by strains from all capsular serogroups.[8,65–67] However, because the capsular polysaccharide contains multiple repeating epitopes and is more abundant and accessible on the bacterial surface than most protein antigens, serum anticapsular antibodies have greater complement-mediated bactericidal activity (i.e., require lower antibody concentrations to elicit bactericidal activity than antibodies that target protein antigens). Not all meningococcal strains are susceptible to antibodies elicited by the protein vaccine antigens because of amino acid sequence diversity of the antigen, low antigen expression in some strains,[68–70] or absence or truncation of a functional gene encoding the antigens.[71–74] Also, because group B vaccines appear to have less effect on decreasing carriage of the organism than conjugate vaccines,[75] the group B vaccine probably will not provide the same degree of herd protection found with the conjugate vaccines.[76] Therefore, while the group B protein vaccines may supplement protection against some strains with other capsular groups, it is unlikely that the protein vaccines will replace polysaccharide conjugate vaccines for prevention of disease caused by non–group B strains.

VACCINE COMPOSITION

MenB-4C

The MenB-4C vaccine contains three recombinant proteins: FHbp subfamily B (as a fusion protein with GNA 2019), Neisseria Heparin binding antigen (NHba, as a fusion protein with GNA 1030), and Neisseria adhesin A (NadA), which are combined with OMV from strain NZ98/254 (Fig. 39.2). A 0.5-mL dose contains 50 µg of each the three recombinant proteins and 25 µg of OMV. The antigens are adsorbed with 0.519 mg of Al^{3+} (as aluminum hydroxide) (Table 39.2). MenB-4C was licensed in Europe in 2013 and approved for the age group 2 months to 55 years.[77,78] The vaccine was subsequently approved in Canada, Australia, and more than 34 other countries, including the United States. In the United States, MenB-4C is approved for use in the age group 10 to 25 years.

MenB-FHbp

MenB-FHbp contains two lipidated recombinant FHbp sequence variants, one from each subfamily.[79,80] The 0.5-mL dose contains 60 µg of each FHbp variant (total of 120 µg of protein) (see Table 39.2). The antigens are adsorbed with 0.25 mg of Al^{+3} (as aluminum phosphate). In the United States, the MenB-FHbp vaccine is approved for the age group 10 to 25 years.

DISCOVERY AND CHARACTERIZATION OF GROUP B VACCINE ANTIGENS

The licensed group B vaccines contain a novel antigen called FHbp, which is a lipoprotein present on the surface of nearly

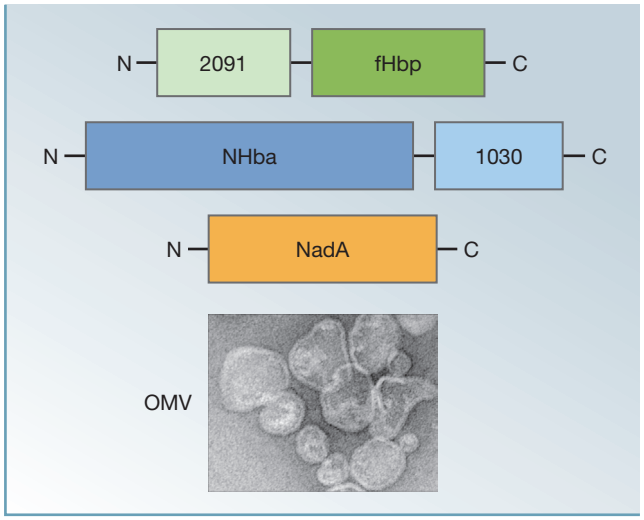


Figure 39.2. Schematic representation of the three recombinant proteins and outer membrane vesicles (OMVs) contained in the meningococcal MenB-4C vaccine. Two of the components are fusion protein (GNA2091 fused with FHbp and NHba fused with GNA 1030). The third component is recombinant NadA. N and C refer to the amino and carboxy terminal portions, respectively, of the proteins. The three recombinant proteins are combined with detergent-extracted OMV vaccine from group B strain NZ98/254 with PorA P1.4. *(Modified from Granoff DM. Review of meningococcal group B vaccines.* Clin Infect Dis. *2010;50[Suppl 2]:S54-S65.)*

TABLE 39.2 Composition of Licensed Group B Vaccines

MenB-4C Vaccine (Bexsero, GSK)	Quantity Per 0.5-mL Dose	MenB-FHbp Vaccine (Trumenba, Pfizer)	Quantity Per 0.5-mL Dose
Antigens		Antigens	
Recombinant GNA2091-FHbp (Subfamily B) fusion protein[a]	50 µg	Recombinant FHbp (Subfamily A)[b]	60 µg
Recombinant NHba-GNA130 fusion protein	50 µg	Recombinant FHbp (Subfamily B)[c]	60 µg
Recombinant NadA	50 µg		
OMV (strain NZ98/254, PorA P1.4)	25 µg		
Other		Other	
Aluminum	0.519 mg of Al^{+3} as aluminum hydroxide	Aluminum	0.25 mg of Al^{+3} as aluminum phosphate
Sucrose	10 mg	Polysorbitol 80 (PS80)	0.18 mg
Histidine	0.776 mg	Histidine buffered saline	10 mM
NaCl	3.125 mg	NaCl	Amount not reported
pH	6.4-6.7	pH	6.0

[a]FHbp peptide ID 1, as described in the public database http://pubmlst.org/neisseria/fHbp/. This sequence variant is assigned to variant group 1 in an alternative classification system.[81]
[b]FHbp ID 45 (called A05 by Pfizer using an alternative classification of amino acid sequence variants (98).
[c]FHbp ID 55 (called B01 by Pfizer; see above).

all meningococcal strains. There are, however, notable differences in the antigenic composition between the two vaccines. The Pfizer vaccine contains two recombinant FHbp lipoprotein molecules, one from each subfamily (see below), and is referred to as MenB-FHbp. In contrast, the GSK vaccine contains a subfamily B FHbp sequence variant and three additional antigens capable of eliciting serum bactericidal activity (i.e., four components: FHbp, NadA, NHba, and OMVs containing PorA serosubtype P1.4, described in detail below).

FHbp

First discovered by scientists at the former Chiron Vaccines in Siena, Italy, using genome mining,[81] the protein was designated "genome-derived Neisseria antigen 1870" or "GNA1870." Investigators at Wyeth Vaccines (now Pfizer) in New York independently discovered the same antigen using biochemical and immunologic approaches and called it "lipoprotein 2086" or "LP2086."[82] The protein was subsequently found by Sanjay Ram and colleagues to bind a human protein, called Factor H, which downregulates complement activation (particularly the alternative pathway).[83] The binding was shown to be specific for human and some nonhuman primate FH[84–86] and enabled meningococci to survive in nonimmune serum (Fig. 39.3). Rat FH does not bind to meningococci, and the organism is killed by 20% nonimmune infant rat serum after incubation of the bacteria for 60 minutes.[87] The addition, however, of as little as 3 µg/mL of human FH was sufficient to downregulate the rat alternative pathway and "rescue" the bacteria (typical concentrations of FH in human serum range from 200 to 600 µg/mL).[88] These data provide one reason why meningococci naturally infect only humans; in humans, binding of FH to the bacteria allows the organism to evade host defenses. A human genomewide association study identified variants in the FH region associated with host susceptibility to meningococcal disease.[89] Because of the importance of binding of FH to GNA1870/LP2086 in enabling the organism to evade complement, the various names of the antigen were changed to FHbp.[83]

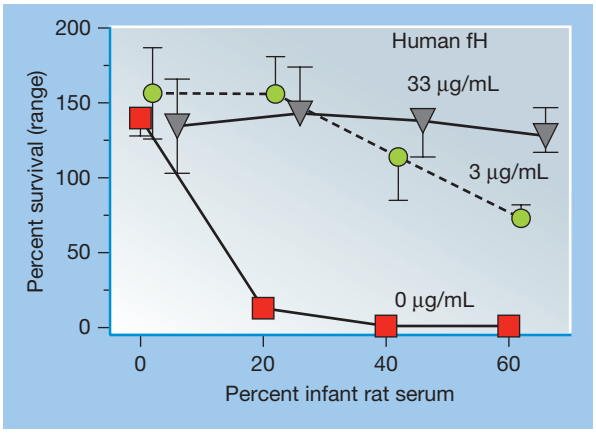


Figure 39.3. Effect of human Factor H (FH) on survival of *N. meningitidis* group B strain H44/76 in infant rat serum. *(Data from Granoff DM, Welsch JA, Ram S. Binding of complement factor H (fH) to* Neisseria meningitidis *is specific for human fH and inhibits complement activation by rat and rabbit sera.* Infect Immun. *2009;77: 764-9.)*

The FH molecule contains 20 domains.[88,90] Each domain contains approximately 60 amino acids and can be thought of as a series of individual beads on a string. Domains 1 to 4 are responsible for downregulating complement activation, and domains 6 and 7 contain the amino acid residues that interact with FHbp.[91–93] The structure of FHbp in a complex with FH domains 6 and 7 is shown in Fig. 39.4. The FHbp molecule contains two antiparallel β-barrel domains.[94] The amino acid residues in FHbp that interact with FH are located on both the N-terminal and C-terminal portions of the molecule and appear to be the most important epitopes for eliciting bactericidal anti-FHbp antibodies.[61,95] In immunized mice whose mouse FH does not bind to the vaccine antigen, serum anti-FHbp antibodies are directed at epitopes located in the FH binding site, and the antibodies block FH binding.[60] With

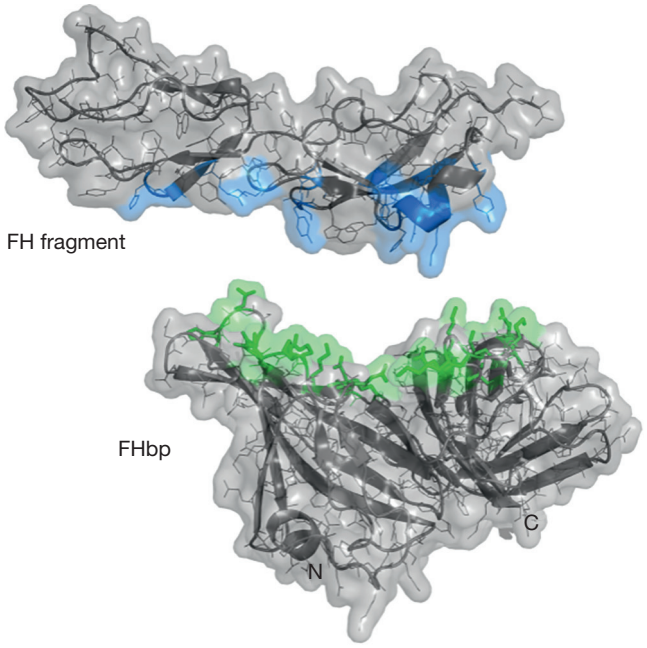
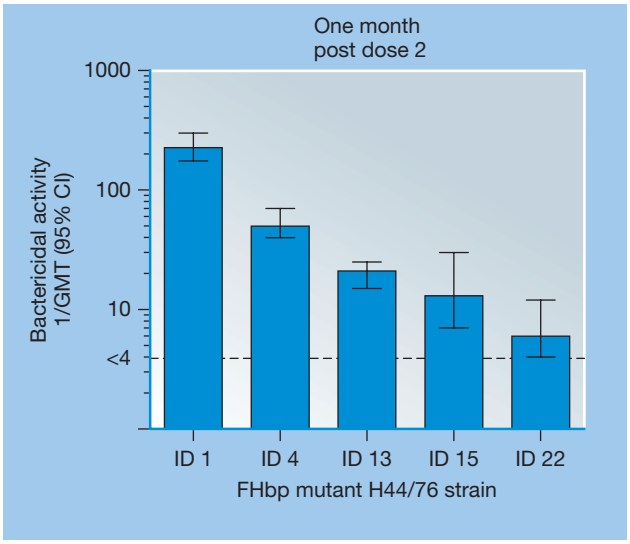


Figure 39.4. Structure of Factor H (FH) binding protein (FHbp) in a complex with a human FH fragment (domains 6 and 7). *(Data and coordinates from Schneider MC, Prosser BE, Caesar JJ, et al.* Neisseria meningitidis *recruits factor H using protein mimicry of host carbohydrates.* Nature. *2009;458:890-893.)*

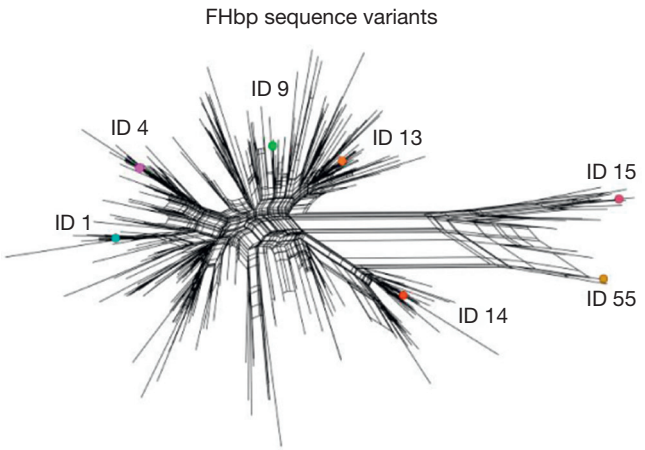
less bound FH, there is less complement downregulation and the bacteria become more susceptible to complement-mediated bactericidal activity.[96,97] In immunized humans and nonhuman primates, binding of FH to the FHbp vaccine antigen appears to mask these epitopes, and the resulting anti-FHbp antibodies are largely directed to epitopes outside of the FH binding area. As a result, the antibodies do not block FH binding.[61,62,98]

As of October 2016, more than 970 distinct FHbp amino acid sequence variants (peptide alleles) had been identified in a public database[99] and assigned individual FHbp peptide identification (ID) numbers. Based on amino acid sequence similarity, these variants can be subclassified into three variant groups (1, 2, or 3)[81] or two subfamilies (A and B).[100] Variant group 1 corresponds to subfamily B, and variant groups 2 and 3 correspond to subfamily A. Within a subfamily, FHbp amino acid sequence identity ranges from 88% to 99%. Between subfamilies, sequence identity is approximately 60%. While exceptions have been noted,[101] in general, antibodies directed at FHbp in subfamily A have greater bactericidal activity against strains expressing subfamily A FHbp, and vice versa for strains expressing FHbp in subfamily B.[81,82,102] Because of amino acid relatedness and extensive cross-reactivity between FHbp sequence variants in variant groups 2 and 3 (subfamily A),[72,102] this chapter uses the subfamily classification system.

MenB-FHbp contains two lipidated recombinant FHbp sequence variants, one from each subfamily. The lipidation is thought to act as an adjuvant and increase immunogenicity.[82,103] The subfamily A FHbp sequence variant in this vaccine is ID 45 (called A05 by Pfizer), and the subfamily B variant is ID 55 (also called B01) (Fig. 39.5). MenB-4C contains a subfamily B FHbp variant (ID 1) that is not lipidated, and three other antigens capable of eliciting serum bactericidal activity (see Fig. 39.2). The FHbp antigen in MenB-4C is a fusion



A



B

Figure 39.5. Relation between FHbp amino acid sequence variant (peptide ID) and anti-FHbp bactericidal activity in sera from infant macaques immunized with two doses of MenB-4C. A, Bactericidal activity. H44/76 is an indicator strain for MenB-4C induced anti-FHbp bactericidal activity. The mutants with different FHbp sequence variants had similar FHbp expression. FHbp ID 1, 4, 13, and 15 are assigned to subfamily B, and ID 22 is assigned to subfamily A. **B,** Split tree analysis showing relationships between different FHbp amino acid sequence variants in subfamily B. The MenB-4C FHbp vaccine antigen is subfamily B ID 1 (far left). The MenB-FHbp subfamily B antigen is ID 55 (far right). *(A, Graph prepared from published data from macaques with high binding of FH to FHbp. Data from Granoff DM, Costa I, Konar M, et al. Binding of complement Factor H (FH) decreases protective anti-FH binding protein antibody responses of infant rhesus macaques immunized with a meningococcal serogroup B vaccine.* J Infect Dis. *2015;212:784-92.)*

protein with a second antigen called GNA 2091.[104] GNA 2091 was initially thought to contribute to vaccine protection and was combined with FHbp to facilitate vaccine production. The antigen was subsequently found to be localized on the periplasmic side of the outer membrane[105] and did not elicit serum bactericidal activity. The manufacturer considers GNA 2091 an "accessory protein" in the MenB-4C vaccine, and the presence of GNA 2091 in the FHbp fusion protein may increase FHbp stability and immunogenicity.[106]

FHbp vaccines elicit broad serum bactericidal antibody responses in mouse, rabbit, and nonhuman primate models.[62,69,81,82,102,107] Both manufacturers have proposed that if there is a match between the FHbp subfamily (or variant group) of the vaccine and the strain, and if strain antigen expression is above a certain threshold, then the isolate can be considered covered by the vaccine.[69,70] However, the story actually may be more nuanced.[68,107,108] For example, within a subfamily there can be 10-fold or greater differences in susceptibility against mutants with different FHbp amino acid sequence variants (compare, for example, titers of rhesus macaques immunized with MenB-4C measured against a strain with FHbp ID 1 [an exact match with the vaccine antigen], to titers against the mutants of the strain with FHbp ID 4, 13 or 15; see Fig. 39.5).[62] There also was some unexpected cross-reactive bactericidal activity against the mutant with ID 22 in subfamily A, which is mismatched with the FHbp subfamily B antigen in MenB-4C. Fig. 39.6 shows the effect of decreasing strain antigen expression on lowering serum bactericidal activity of mice immunized with a recombinant FHbp vaccine ID 1 that matched the FHbp in the test strains.[66] Note that many wild-type meningococcal strains causing disease have similar FHbp expression to the mutant used in this study with 50% decreased FHbp expression.[66,66,69,70,98,109] Intrinsic strain resistance to anti-FHbp bactericidal activity also may be mediated by binding of FH to two other meningococcal FH ligands, NspA and a porin protein called PorB, which can downregulate complement.[110]

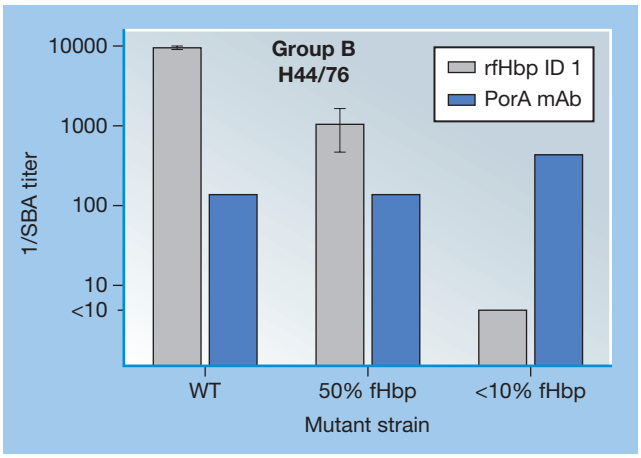


Figure 39.6. Effect of strain expression of FHbp on susceptibility of complement-mediated anti-FHbp bactericidal activity of sera from mice immunized with recombinant FHbp (ID 1). Wild-type (WT) strain H44/76 is a naturally high expresser of FHbp. Isogenic mutants of H44/76 with decreased FHbp expression exhibited increasing resistance to killing by the serum anti-FHbp antibodies (gray bars) but not by an anti-PorA monoclonal antibody (mAb) (blue bars). Titers were measured with human complement. *(From Pajon R, Fergus AM, Koeberling O, et al. Meningococcal factor H binding proteins in epidemic strains from Africa: implications for vaccine development.* PLoS Negl Trop Dis. *2011;5:e1302.)*

These experimental observations underscore the challenges for predicting strain coverage by antibodies elicited by FHbp vaccines.

Rarely (~1 : 1000), invasive meningococcal isolates with absent FHbp genes or frameshift mutations have been identified that do not express a functional FHbp.[71] These isolates express other FH ligands, such as NspA or PorB, and are completely resistant to anti-FHbp bactericidal activity.[111] Whether widespread vaccination with FHbp vaccines will select for emergence of FHbp-null strains is not known.

Effect of Factor H Binding to FHbp Vaccines on Immunogenicity

Clinical trials with meningococcal FHbp vaccines had begun before the protein was discovered to bind specifically to human and some nonhuman primate FH.[83] As noted above, FH is present in high concentrations in human serum (300–600 μg/mL). The effect of binding of FH to the vaccine antigen on immunogenicity had not been assessed by the earlier experimental animal studies because mouse or rabbit FH does not bind to FHbp. The effect of binding of FH to FHbp vaccines on immunogenicity was investigated in a human FH transgenic mouse model.[56,57,58,59,62] FHbp vaccines that bound human FH elicited 4- to 8-fold lower serum bactericidal antibody responses than in control mice whose mouse FH did not bind to the vaccines.[59,60] These findings prompted investigation of the effect of FH binding on FHbp immunogenicity in a nonhuman primate model, which may be more relevant to human immunity than mouse models. Because of a FH polymorphism (two amino acids), some rhesus macaques have FH that binds weakly with FHbp, while other animals have high avidity FH binding to FHbp.[86] The macaques with the high FH binding phenotype had lower anti-FHbp serum bactericidal titers to MenB-4C than the animals whose FH bound with low avidity to the FHbp vaccine antigen.[62] In contrast, the respective serum bactericidal antibody responses of the two groups to two other antigens tested in the MenB-4C vaccine that did not bind FH (NadA and PorA P1.4) were similar.[62] More recently, a mutant recombinant FHbp vaccine with two amino acid substitutions that decreased FH binding elicited up 15-fold higher serum bactericidal antibody responses in infant macaques than the control wild-type recombinant FHbp vaccine that bound macaque FH.[61] There also was evidence that the mutant FHbp vaccine elicited an anti-FHbp antibody repertoire directed at FHbp epitopes within the FH binding site, which resulted in greater protective activity than the antibodies elicited by the control vaccine, which targeted FHbp epitopes outside the FH combining site.

Neisseria Adhesin A

MenB-4C contains a subfamily B FHbp antigen. Vaccine coverage against meningococcal strains with subfamily A FHbp is largely dependent on protective antibodies elicited by the three other vaccine antigens. One of these is NadA, which is a surface-exposed lipoprotein discovered by genome mining and designated GNA1994.[73] Present in the outer membrane as a homotrimer, NadA has structural similarities to a family of autotransporter adhesins and may promote *Neisseria* adhesion to epithelial cells (hence the name NadA).[112] In experimental animals and humans, recombinant NadA vaccines elicited serum bactericidal antibodies.[73,113–115]

As of October 2016, there were 78 NadA amino acid sequence variants in the public database.[116] In a classification system updated in 2014, NadA was subdivided into three variant groups based on amino acid similarity (NadA-1, NadA-2/3, and NadA-4/5).[117] MenB-4C contains a variant from

group 2/3, which elicits cross-reactive antibodies to NadA-1 but not NadA-4/5.

An important limitation of NadA as a vaccine antigen is that, overall, only about 30% to 40% of serogroup B strains have genes coding for the protein (primarily strains from clonal complexes STs 8, 11, and 32).[8,118] Furthermore, the isolates for which anti-NadA antibodies might be most important for protection after MenB-4C vaccination (isolates with FHbp subfamily A, which is not present in the vaccine) infrequently have *nadA* genes (6–19%).[8,72] NadA expression levels also are largely controlled by a transcriptional regulator NadR (Neisseria adhesin A regulator).[119] Expression of NadA is repressed in many strains by NadR, which can result in resistance to anti-NadA serum bactericidal activity.[120] Experimentally, NadA expression can be induced by certain small molecules in saliva that inhibit NadR repression. In an ex vivo model of human whole blood infection, meningococci did not activate genes encoding for NadA.[121] Nevertheless, expression of NadA during human disease was sufficient to induce anti-NadA antibodies in convalescent serum.[122] In short, MenB-4C can induce anti-NadA bactericidal activity against some NadA-positive meningococcal strains (such as strain 5/99, which has naturally high NadA expression and is used by the manufacturer to assess anti-NadA serum bactericidal responses).[113–115] However, for many NadA gene-positive strains, expression of the protein is low under in vitro growth conditions used to measure bactericidal activity, and some NadA-positive strains are relatively resistant to serum bactericidal activity elicited by MenB-4C in adults (see below).[21,123] Defining the actual contribution of anti-NadA antibodies to MenB-4C vaccine coverage therefore remains challenging.

Neisseria Heparin Binding Antigen

NHba is a surface-exposed lipoprotein that also was discovered by genome mining and designated GNA 2132.[124] Subsequently the protein was found to bind heparin in vitro, which correlated with increased survival of unencapsulated meningococci in human serum.[125] While the role of heparin binding as a surrogate for interactions of *N. meningitidis* with host polyanions and pathogenesis remains uncertain, the name of the protein was changed to NHba to reflect its heparin-binding function. As of October 2016, there were 677 NHba amino acid sequence variants in the public database.[126] To date, these variants have not been classified into related families because of the lack of clear relationships between the respective amino acid sequence variants to antigenic cross-reactivity. For classification, each NHba sequence variant is designated with a specific peptide ID. MenB-4C contains NHba peptide 2, which is part of a fusion protein with a second antigen, GNA 1030 (see Fig. 39.2). GNA1030 was initially thought to contribute to vaccine-induced protection and was combined with NHba to facilitate vaccine production. However, GNA1030 was subsequently found to be a ubiquinone-8 binding protein[127] and not to elicit serum bactericidal activity. As is the case for GNA 2091 (the second antigen in the FHbp fusion protein), the manufacturer considers GNA 1030 an "accessory protein" in the NHba antigen. The NHba fusion protein expresses the expected epitopes found in the native NHba and appears to be stable.[128]

Defining the role of NHba in MenB-4C coverage has been challenging. In some strains, an endogeonous protease, NalP, cleaves NHba, which releases a C-terminal 22-kDa fragment that may contain epitopes recognized by anti-NHba antibody.[125] NHba also was found to be cleaved by protease activity of human lactoferrin. Although the investigators concluded that proteolytic cleavage of NHba did not affect anti-NHba bactericidal response, the activity was measured with rabbit complement.[125] Because rabbit FH does not bind to meningococci, the anti-NHba activation of complement was not downregulated, which would have falsely increased bactericidal activity measured with human complement.[84] Early studies of mice immunized with a recombinant NHba vaccine given with Freund's adjuvant showed that serum anti-NHba antibodies were bactericidal primarily with rabbit complement (only two selected strains were killed with human complement).[124] The vaccine manufacturer also has had difficulty finding a suitable meningococcal strain for measuring anti-NHba–specific serum bactericidal activity after MenB-4C vaccination. As a result, anti-NHba bactericidal activity was not measured in the early studies of the immunogenicity of the MenB-4C vaccine in humans.[113–115,129–132] Also, anti-NHba bactericidal activity was not included in the human immunogenicity dataset for U.S. licensure of the MenB-4C vaccine.[133]

In a recent study, Rossi et al.[123] investigated serum bactericidal activity elicited by MenB-4C against different serogroup B strains responsible for two outbreaks on U.S. college campuses. Both outbreak strains were susceptible to bactericidal activity of sera from rhesus macaques or a human immunized with MenB-4C. This result was predicted based on high expression of two of the vaccine antigens in both strains, subfamily B FHbp and NHba, and moderate expression of a third vaccine antigen, NadA, in one of the strains. Depletion of anti-FHbp antibodies from the postimmunization sera, however, resulted in loss of all of the vaccine-induced bactericidal activity. Thus, despite strain NHbp expression and high titers of anti-NHba antibody in the FHbp-adsorbed sera, there was no detectable anti-NHba bactericidal activity measured with human complement. The basis for this resistance is unknown and requires further investigation. These data, together with recent data on limited serum bactericidal responses of MenB-4C-vaccinated adults against some strains expressing NHba but mismatched for the other three MenB-4C antigens,[21] underscore the difficulty of predicting the contribution of anti-NHba antibodies to protection elicited by MenB-4C.

Outer Membrane Vesicles

OMV vaccines have been used for many years to control capsular group B outbreaks and appear to be safe[134–136] and effective.[16,31,137,138] Their major limitation is that serum bactericidal antibody responses of infants and children are largely directed at a porin protein, PorA,[36] which is antigenically variable.[139] The variability of PorA limits the utility of OMV vaccines for prevention of endemic group B meningococcal disease caused by antigenically diverse strains with multiple PorA variable region STs. The OMV in MenB-4C is prepared from strain NZ98/254, which contains a PorA serosubtype P1.4 (defined serologically) or by the variable region (VR2) ST, 1.4. This OMV component was used in New Zealand as a stand-alone vaccine (MeNZB, Chiron Vaccines) for the age group 6 weeks to 20 years to control a longstanding capsular group B epidemic.[138] Initially, three doses of the OMV vaccine were administered, with each dose separated by 2 months. A booster dose in the second year of life was added for infants vaccinated beginning at 6 to 8 weeks of age to achieve more persistent serum bactericidal responses. The vaccine was safe and immunogenic, with the strongest bactericidal activity against meningococci sharing the same P1.4 PorA antigen as in the vaccine.[140] Overall vaccine effectiveness was estimated to be between 68% and 73%.[137,138] By June 2008, routine OMV vaccination had been halted in New Zealand because of declining incidence of disease, controversies about the contribution of vaccination to decreasing disease incidence,[141,142] and the cost of the program. The OMV vaccine used in New Zealand was added to the three recombinant protein antigens

in MenB-4C to provide some anti-PorA coverage and, perhaps more importantly, as an adjuvant to enhance immunogenicity of the recombinant protein antigens.[143]

IMMUNOGENICITY

The low global incidence of meningococcal group B disease precluded performing randomized controlled efficacy trials to determine whether vaccination with the new group B vaccines decreased the incidence of meningococcal disease. Instead, efficacy was inferred from serum bactericidal antibody responses. For technical reasons (small volumes of serum, limited amounts of human complement, and strain differences in assay performance), both manufacturers tested serum bactericidal activity against a panel of three or four selected strains. Interpreting the published data also is challenging because the endpoints use in the different studies were not consistent (i.e., postimmunization reciprocal titers ≥4, ≥5, ≥8, ≥1 : 16; or 4-fold or greater increases in titer compared with the respective preimmunization titer), or a composite endpoint (i.e., how many individuals have antibodies to all strains or antigens tested).

MenB-4C

To assess antigen-specific serum bactericidal antibody responses, the manufacturer selected reference (indicator) strains that were each mismatched for three of the four antigens in MenB-4C that elicit bactericidal antibody responses. These strains also had high expression of the antigen of interest, with susceptibility to bactericidal activity by antibody to the target antigen, and resistance to antibodies directed at the other three mismatched vaccine antigens. As described below, these reference strains are more susceptible to serum bactericidal antibody elicited by MenB-4C than many wild-type, disease-causing strains.[21] Nevertheless, when the data from the reference strains are combined with data on expression and cross-reactivity of antigens expressed by isolates causing disease in the population (see below), and serum bactericidal activity measured against additional disease causing isolates, the results collectively provide important information on vaccine immunogenicity and potential strain coverage.

Immunogenicity in Infants

The immunogenicity of the three recombinant antigens in MenB-4C when administered with OMV (MenB-4C) or without OMV (MenB-3C) was investigated in two studies in infants.[114,115] In one study, two doses of the vaccines, separated by 2 months, were given beginning at age 6 to 8 months with a booster dose at 12 months.[114] In a second study, three doses were given at 2, 4, and 6 months with a booster dose at 12 months.[115] Three test strains were used to measure antigen-specific bactericidal responses to NZ98/254 (PorA), 5/99 (NadA) and H44/76 (FHbp). At the time the studies were performed, a suitable test strain for the fourth antigen, NHba, was not available (see NHba antigen section, above). Serum bactericidal responses also were measured against four disease-causing strains from the United Kingdom. Only MenB-4C, but not MenB-3C, elicited serum bactericidal antibody responses against the majority of the strain panel. Vaccine coverage therefore benefited from the addition of PorA-specific serum bactericidal antibodies elicited by the OMV component (and perhaps other antibodies elicited by minor OMV antigens) and an adjuvant effect of the OMV on enhancing antibody responses to the recombinant proteins, which was also observed in a subsequent study.[143]

Serum bactericidal antibody responses of infants immunized with three doses of MenB-4C at 2, 4 and 6 months, and boosted with a fourth dose at 12 months, are shown in Figs. 39.7 and 39.8. More than 90% of the infants developed protective titers ≥1 : 4 against the three indicator reference strains (H44/76, NZ98/254 and 5/99; see Fig. 39.7). After the 12-month booster, the majority of the infants also developed protective titers against three of the four disease-causing test isolates (MO1-1, MO1-2, and MO1-3), although the respective geometric mean titers were lower than the reference strains (see Fig. 39.8). In larger Phase IIb or III studies, the vast

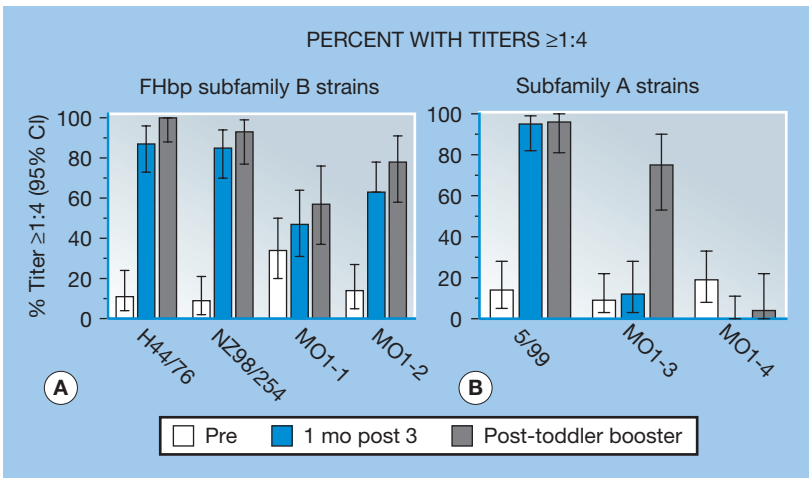


Figure 39.7. Serum bactericidal antibody responses of infants immunized with MenB-4C at 2, 4, and 6 months and boosted at age 12 months: Titers ≥1 : 4. Bactericidal antibody titers were measured with human complement in serum obtained 1 month after vaccination. **A,** Group B strains with factor H binding protein (FHbp) in subfamily B. H44/76 had FHbp ID 1, which matched the FHbp in the vaccine. The other strains had FHbp subvariants. NZ98/254 was the strain used to prepare the vaccine outer membrane vesicle (OMV) and expressed PorA 1.7-2,4. Strains designated MO1-1 and MO1-2 refer to MO1 240101 and MOO 242922, respectively (MO1-2 also had PorA 1.7-2,4, which matched the vaccine OMV). **B,** Group B strains with FHbp from subfamily A, which were not part of the vaccine. Strain 5/99 was selected to measure responses to NadA. Strains designated MO1-3 and MO1-4 refer to strains MO1 240364 and MO1 240355, respectively. CI, confidence interval. *(Data from Findlow J, Borrow R, Snape MD, et al. Multicenter, open-label, randomized phase II controlled trial of an investigational recombinant meningococcal serogroup B vaccine with and without outer membrane vesicles, administered in infancy.* Clin Infect Dis. *2010;51:1127-37.)*

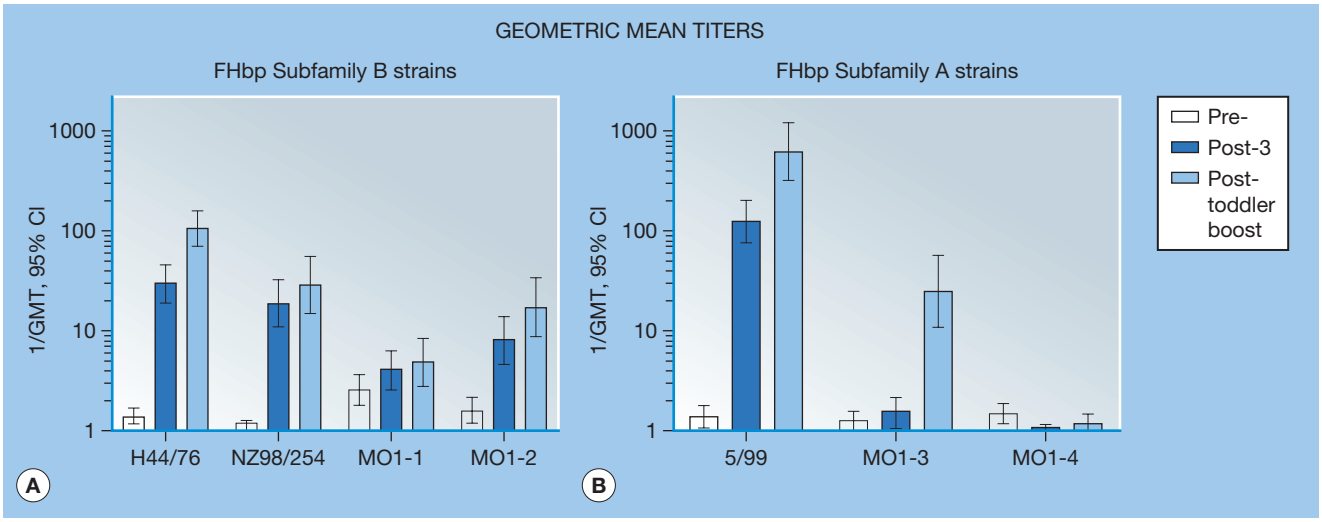


Figure 39.8. Serum bactericidal antibody responses of infants immunized with MenB-4C at 2, 4, and 6 months and boosted at age 12 months: geometric mean titers. Time points and strain designations are as described for Fig. 39.7. CI, confidence interval. *(Data from Findlow J, Borrow R, Snape MD, et al. Multicenter, open-label, randomized phase II controlled trial of an investigational recombinant meningococcal serogroup B vaccine with and without outer membrane vesicles, administered in infancy.* Clin Infect Dis. *2010;51:1127-37.)*

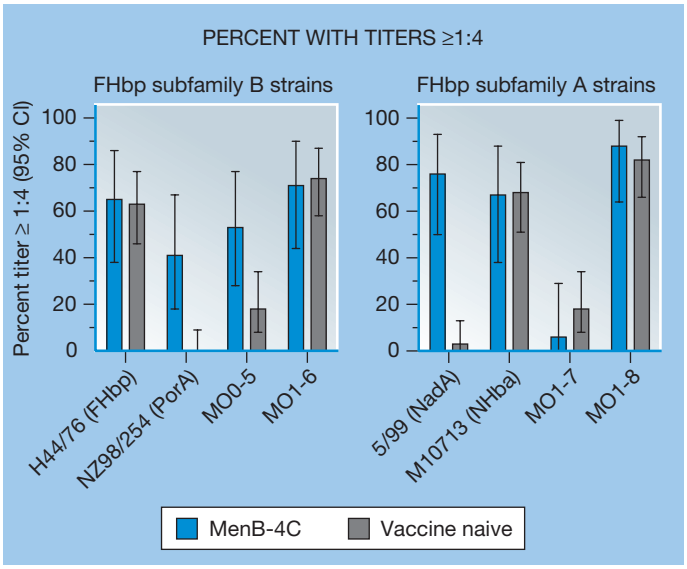


Figure 39.9. Serum bactericidal antibody persistence at age 40 months in children previously vaccinated with MenB-4C at 2, 4, 6, and 12 months. Data are shown for four indicator strains, 5/99 (NadA), M10713 (NHba), H44/76 (PorA), and NZ98/254 (PorA) and four disease-causing strains in the United Kingdom. Titers were measured with human complement. Strain MO1-7 refers to M01-240355, MO-8 refers to MO1-240364, MOO-5 refers to MOO-242922, and MO1-6 refers to MO1-24101. Strain MO1-07 is mismatched to all four antigens in the MenB-4C vaccine considered to elicit bactericidal responses. *(Data from Snape MD, Saroey P, John TM, et al. Persistence of bactericidal antibodies following early infant vaccination with a serogroup B meningococcal vaccine and immunogenicity of a preschool booster dose.* CMAJ. *2013;185:E715-24.)*

majority of infants and toddlers given two or three doses of MenB-4C also developed protective responses against the three reference antigen-specific strains.[113,144] These larger studies, however, did not include additional disease-causing strains, which are often more resistant to MenB-4C bactericidal antibody than the reference indicator strains (see Fig. 39.10).

A recent study administered a booster dose of MenB-4C in children at 12, 18, or 24 months. The children had been previously immunized with three doses at 2, 3, and 4 months, or 2, 4, and 6 months.[145] After a 12-month booster dose, 95% of the children developed titers ≥1:5 against all three indicator strains for FHbp, NadA, and PorA P1.4.

Persistence of serum bactericidal activity in infants vaccinated with MenB-4C at 2, 4, 6, and 12 months was measured at age 40 months in another study.[146] The results were compared with serum titers of control children of the same age who had not been vaccinated with MenB-4C (Fig. 39.9). For two of the indicator strains, 5/99 (NadA) and NZ98/254 (PorA P1.4), and one disease-causing strain (MOO-5), the respective proportions of children with bactericidal titers ≥1:4 at 40 months were higher in the MenB-4C-vaccinated

than in the MenB-4C-naïve children. For the remaining five test strains, the respective proportions of children with protective titers were similar in the vaccinated and unvaccinated groups.

To prolong protection, two studies administered booster MenB-4C in children at age 40 months who either had been immunized at 2, 4, 6, and 12 months ($n = 16$)[147] or at 6, 8, and 12 months ($n = 11$ to 12).[148] The percentages of subjects at 60 months with serum titers ≥1 : 4 ranged from 44% to 100% against the three group B indicator strains for FHbp, NadA, or NHba. For the PorA indicator strain, the percentages were 69% for the children vaccinated beginning at 2 months of age[147] and 17% for children vaccinated with four doses beginning at 6 months of age.[148] The reason for the discordant anti-PorA results is not known. Also, for these studies the manufacturer had recently selected strain M10713 to measure anti-NHba–specific bactericidal responses. However, interpretation of antibody persistence to NHba in the vaccinated children (45% and 88% with titers ≥1 : 4 in the two studies) was confounded by the presence of naturally acquired bactericidal activity in the majority of the 60-month-old MenB-4C-naïve control children (67% and 83%). This strain also was killed by naturally acquired serum antibactericidal antibodies in two other studies (titers ≥1 : 4 in 18% of MenB-4C-naïve infants at 12 months[144] and in 68% of children at 40 months of age[146]; see Fig. 39.9). The naturally acquired bactericidal antibodies to this strain are likely directed at antigens other than NHba, since by enzyme-linked immunosorbent assay relatively few MenB-4C-naive adults have serum immunoglobulin G antibodies to this NHba. In conclusion, additional studies are needed to determine whether infants vaccinated in the first year of life and boosted in the second year will require additional booster doses of MenB-4C to maintain protection and, if needed, the optimal age of booster dose (e.g., at adolescence).[149]

Supporting data for concomitant administration of MenB-4C with vaccines routinely administered to infants were provided for antigens in DTaP-HBV-IPV/Hib and heptavalent pneumococcal conjugate vaccine (PCV7).[113] The only immune responses that appeared to show evidence of interference by MenB-4C were the *Bordetella pertussis* protein pertactin and the pneumococcal serotype 6B antigen in PCV7, in which there were minor decreases in responses that failed to meet the prespecified 95% cutoff level. In another study,[144] supporting data for concomitant administration of MenB-4C and DTaP-HBV-IPV/Hib were provided for responses to diphtheria and tetanus toxoids, Hib polysaccharide, hepatitis B virus, pertussis toxin, filamentous hemagglutinin antigen, pertactin, and poliovirus types 1 and 3. For poliovirus type 2, there was a small decrease in the proportion of children developing neutralization titers of ≥1 : 8, which failed to meet the prespecified 95% cutoff level (MenB-4C and routine vaccines 88%, 95% confidence interval [CI] 84–92; vs routine vaccines only 94%, 95% CI 90–97). It seems unlikely that these minor decreases in immune responses are clinically important.

Immunogenicity in Adolescents and Adults

In one study conducted in Chile, 92% to 97% of 1631 adolescents immunized with one dose of MenB-4C developed serum bactericidal titers of ≥1 : 4 against the three indicator strains for NadA, PorA, and FHbp, and 99% to 100% had protective titers after two or three doses.[130] For licensure in the United States, the U.S. Food and Drug Administration (FDA) reviewed additional immunogenicity data from two studies of adolescents and young adults immunized with two doses of MenB-4C separated by 1 month. Study 1 was conducted in adolescents ages 11 to 17 years in Canada and Australia.[131] Study 2 was conducted in U.K. university students ages 18 to 24 years. The package insert summarized the results using 4-fold or greater increases in serum bactericidal titer against three indicator strains.

At 1 month after dose 2, nearly all of the adolescents in Study 1 developed responses to the indicator strains for FHbp or NadA (98% and 99%), but the response to the PorA strain was lower (39%). The respective percentages of responders in Study 2 were 78%, 94%, and 67%. Fig. 39.10A compares data from Studies 1 and 2 with a third study (Study 3) of 20 adults ages 21 to 44 years immunized with MenB-4C in the United Kingdom or Northern California.[21] Against the three indicator strains, the respective results of the three studies are similar. Study 3 also included genetically diverse clinical isolates, which were more resistant than the reference strain; only 25% to 45% of the subjects had ≥4-fold increases in serum bactericidal titers against six test strains with FHbp subfamily A sequence variants (Fig. 39.10C) or three test strains with FHbp subfamily B variants (all from U.S. college outbreaks or hyperendemic disease in Quebec[150]; Fig. 39.10D). Collectively, the data indicated that the clinical isolates with FHbp amino acid sequence variants divergent from ID 1 in the vaccine (Fig. 39.10B) were generally more resistant to MenB-4C-induced bactericidal activity than the reference strains.

A ≥4-fold bactericidal response requires a minimum postimmunization serum titer of 1 : 16. Thus, immunized persons who do not achieve ≥4-fold titer increases at 1 month can have protective serum titers of ≥1 : 4 (see Fig. 39.10E, showing post–dose 2 titers of 12 individual subjects tested against a strain from Quebec [2013]). By 4 to 6 months postimmunization, however, serum titers declined to less than 1 : 4 in the majority of subjects with available follow-up sera. A working model, therefore, is that the percentage of subjects with titers ≥1 : 4 at 1 month postimmunization may correlate with short-term protection, particularly if the titers at 1 month are relatively low, whereas the percentage with ≥4-fold titer increases represent more robust responses to vaccination, which may predict longer-term protection.

Persistence of bactericidal activity was investigated in sera obtained 18 to 24 months after MenB-4C immunization of adolescents in Chile.[129] In subjects given one dose of vaccine, the proportion with serum bactericidal titers ≥1 : 4 at 18 to 24 months against the three indicator strains ranged from 62% to 73%. For subjects immunized with two or three doses, the proportions ranged from 75% to 93%, and for the control MenB-4C-vaccine-naïve adolescents, proportions ranged from 25% to 50%. This study only evaluated indicator reference strains; if more diverse clinical isolates had been tested, the proportions of protected subjects at the different follow-up time points likely would have been lower. Also, the antibody responses of the MenB-4C vaccinated adolescents in Chile appear to be higher than those of immunized adolescents from the United States or Europe, which may reflect greater immunologic priming in Chile from natural exposure to *Neisseria meningitidis* or cross-reacting organisms.

As of October 2016, supporting data from randomized controlled studies supporting concomitant administration of MenB-4C with vaccines routinely administered to U.S. adolescents were not available.[19] In one small study, laboratory workers aged 18 to 65 years were immunized concomitantly with a quadrivalent meningococcal conjugate vaccine (MCV4-CRM) and MenB-4C at visit 1, followed by two additional doses of MenB-4C at 3 and 6 months.[151] Protective serum bactericidal antibody levels were elicited by both vaccines. The main tolerability concern was severe pain and injection site tenderness after MenB-4C (in at least 16% of subjects after each dose).

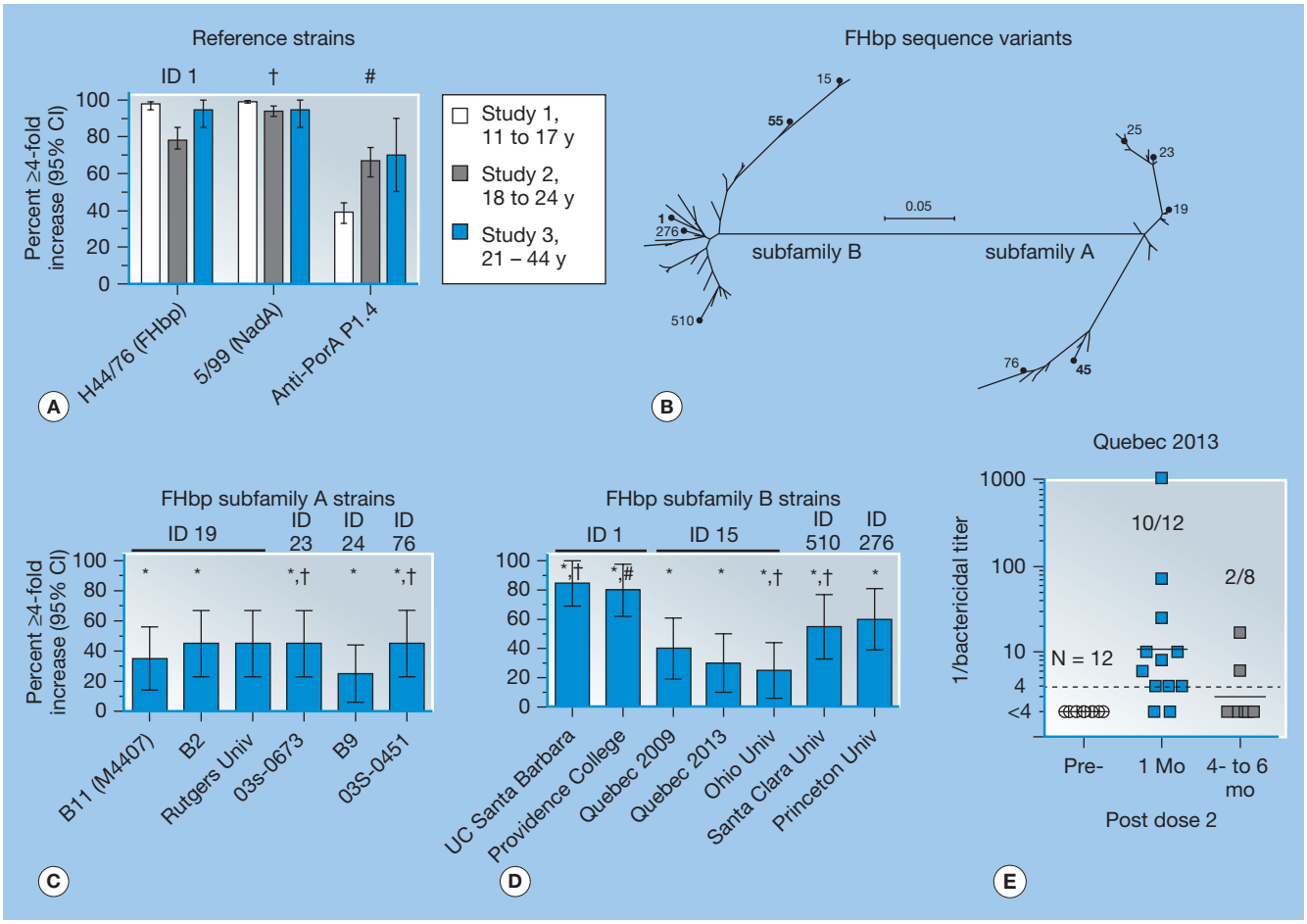


Figure 39.10. Serum bactericidal responses of adolescents and adults immunized with MenB-4C vaccine. The subjects received two doses of MenB-4C separated by 1 to 2 months. **A, C–D,** Percent of subjects with fourfold or greater increases in bactericidal titer in sera obtained 1 month after dose 2, compared with respective preimmunization titers. **A,** Reference indicator strains used to infer antigen-specific bactericidal activity (i.e., each strain is mismatched for three of four MenB-4C antigens reported to elicit bactericidal activity). Studies 1 and 2, data from U.S. Food and Drug Administration MenB-4C package insert (October 2015); Study 3 from published data.[21] Strain expression MenB-4C antigens are designated by *(NHba), †(NadA) or #(PorA P1.4). **B,** FHbp sequence variants. Unrooted maximum-likelihood phylogram of strain FHbp amino acid sequence variants.[21] ID 1 represents sequence of FHbp in MenB-4C. The scale bar indicates 5% amino acid sequence divergence. **C–D,** Serum bactericidal responses measured against six FHbp subfamily A test strains and seven FHbp subfamily B test strains.[21] **C,** Rutgers University isolate is mismatched for all four MenB-4C antigens reported to elicit serum bactericidal activity. **E,** Serum bactericidal titers of 12 subjects with pretiters <1 : 4 measured against the Quebec 2013 isolate, representative of the strain causing hyperendemic disease.[150] Although the ≥4-fold response rate was low **D,** 10 of 12 subjects had protective titer ≥1 : 4 1 month after dose 2, which were not sustained by 4 to 6 months in the majority of subjects tested. *(Data from Giuntini S, Lujan E, Gabini MM, et al. Serum bactericidal antibody responses of adults immunized with the MenB-4C vaccine against genetically diverse serogroup B meningococci.* Clin Vaccine Immunol. *2016;24:e00430-16. Reprinted with permission of the American Society of Microbiology.)*

Vaccine Effectiveness

In September 2015, the United Kingdom introduced routine MenB-4C vaccination for infants using a two-dose infant priming schedule beginning at 2 months of age. When the vaccine was introduced there was a limited catch-up for those born before May 2015. In October 2016, short-term effectiveness data became available from a national observational cohort study.[152] Vaccine uptake was high, with 95.5% of the birth cohort receiving one dose by 6 months of age and 88.6% receiving two doses. During the 10 months of follow-up, 37 cases of meningococcal serogroup B disease were identified by intense public health surveillance in the age cohort eligible for vaccination. Of these cases, 29 (78%) had been vaccinated 14 days or more before disease onset with at least one dose, with 8 (21.6%) receiving two doses. Using the screening method, which takes into consideration the proportion of the general U.K. infant cohort vaccinated, the two-dose vaccine effectiveness against all meningococcal B cases was 82.9% (95% CI, 24.1–95.2). Also, the number of cases in the vaccine-eligible cohort was 50% lower than the average number of cases in that age cohort during the 4 years before the vaccine was introduced. Collectively, these results provide clear evidence of short term-protection by two doses of vaccine.

As is the case with all observational study designs, the possibility of residual confounding cannot be excluded. Also, the small number of cases included in the U.K. study resulted in large 95% CIs about the point estimate of vaccine effectiveness. Further, the study did not address duration of protection, which is important because antibody titers can decline rapidly. The authors also did not provide strain data on the vaccine antigens (in part because most of the cases were confirmed

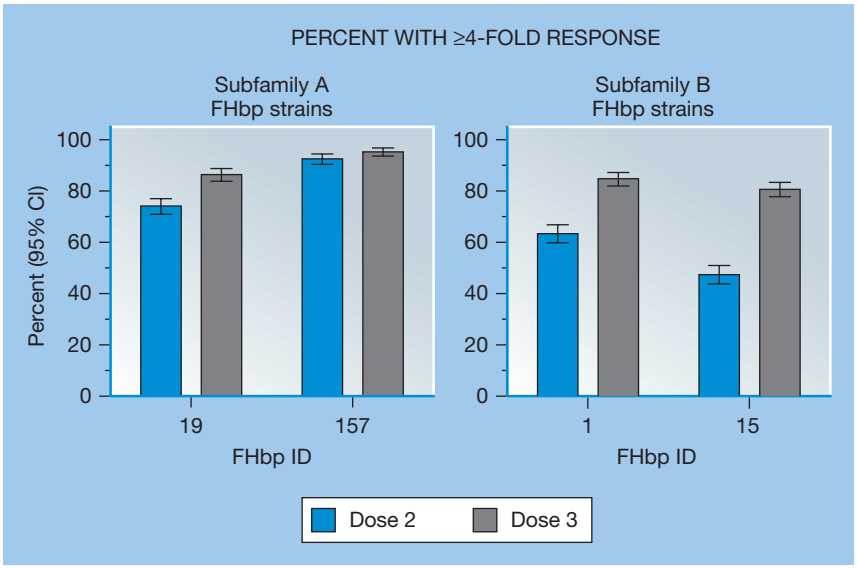


Figure 39.11. Serum bactericidal responses of adolescents immunized with MenB-FHbp vaccine. Adolescents aged 11 to 17 years received three doses of MenB-FHbp at 0, 2, and 6 months. The data shown are from the 788 subjects given MenB-FHbp in one arm and saline in the other and are expressed as percent of subjects with fourfold or greater increases in bactericidal titer in sera obtained 1 month after doses 2 or 3, compared with the respective preimmunization titers. The four serogroup B reference test strains had different FHbp amino acid sequence variants (ID). The subfamily A strains had ID 19 or 187 (referred to by Pfizer as A22 and A56, respectively) and the subfamily B strains had FHbp ID 1 (B24) or ID15 (B44; see Fig. 39.10B). *(Data from group 2 [MenB-FHbp/saline] from the Trumenba U.S package insert [October 2015].)*

only by polymerase chain reaction). Therefore, it is not possible to know whether the antigens expressed by the strains causing disease in the case patients matched those in the vaccine.

In 2013–14, two epidemiologically unrelated outbreaks of group B meningococcal disease occurred among students at Princeton University and the University of California Santa Barbara (UCSB).[18,153] At the time there was no licensed group B meningococcal vaccine in the United States, and the FDA authorized use of the European-licensed MenB-4C vaccine to control the outbreaks. Approximately 8000 students at Princeton University, and more than 10,000 students at UCSB, received at least one dose of vaccine. No further cases occurred in vaccinated persons, but two cases subsequently occurred in close contacts of students (one from each campus). In a subsequent study, serum bactericidal responses were measured in a subset of the Princeton students vaccinated with MenB-4C. Although by antigen typing the Princeton outbreak strain expressed two MenB-4C antigens and was predicted to be susceptible to serum bactericidal antibody elicited by the vaccine,[123] only 66% of the students developed protective titers ≥1 : 4.[22]

MenB-FHbp

MenB-FHbp was licensed in the United States in October 2015. For licensure the manufacturer provided immunogenicity data from a randomized trial in U.S. adolescents aged 11 to 17 years vaccinated at 0, 2, and 6 months with either MenB-FHbp + HPV4 vaccine (given as separate injections; $n = 792$), or MenB-FHbp + saline ($n = 788$).[154] Subsequently, the manufacturer provided additional data supporting a three-dose schedule at 0, 1 to 2, and 6 months, or a two-dose schedule at 0 and 6 months.[155,156] In these studies, serum bactericidal activity was measured against four capsular group B test strains with FHbp amino acid sequences representative of disease-causing isolates. Two of the strains had FHbp subfamily A sequence variants (ID 19 and ID187, referred to by Pfizer as A22 and A56, respectively), and two had subfamily B sequence variants (ID 1 [B24] and ID 15 [B44]; Fig. 39.11). After two doses of MenB-FHbp, fourfold or greater increases in bactericidal titers developed in 74% and 93% of subjects against the two subfamily A FHbp isolates and in 47% and 63% of subjects against the two FHbp subfamily B isolates (see Fig. 39.11). The corresponding percentages of responders after three doses were 86% and 95% for subfamily A and 81% and 85% for subfamily B. Similar respective response rates were observed in subjects given MenB-FHbp with a separate injection of saline or with HPV4 vaccine.

In another study, 1713 healthy adolescents aged 11 to 18 years from the Czech Republic, Denmark, Finland, Germany, Poland, Spain, and Sweden were randomized to receive one of five different MenB-FHbp vaccination schedules (0, 1, and 6 months; 0, 2, and 6 months; 0 and 2 months; 0 and 4 months; and 0 and 6 months).[157] Serum bactericidal responses were measured against four reference strains. One month after dose 3, the respective percentages of subjects with bactericidal titers ≥1 : 8 (the threshold used to define protection) immunized at the 0, 1, and 6 months or 0, 2, and 6 months schedules were not significantly different: 92% to 95% (ID 19), 99% (ID 187), 88% to 89% (ID 1), and 86% to 89% (ID 15). The respective geometric mean titers for each strain also were not significantly different. For the two-dose schedules, the longer the interval between the injections, the greater the response rate. The three-dose schedules resulted in the highest response rates, particularly against the subfamily B strains, and higher geometric mean titers. However, two doses of MenB-FHbp, separated by at least 5 months, also conferred a high level of immunity, which was similar to that after three doses at 0, 1 to 2, and 6 months. These results formed the basis of the FDA approval in October 2016 of both three-dose and two-dose schedules as long as the interval between the two-dose schedule was at least 5 months.

One recent study investigated serum bactericidal antibody responses of teenagers and young adults immunized with MenB-FHbp against a panel of 27 genetically diverse strains.[171a] One month after 2 doses of vaccine, depending on the strain,

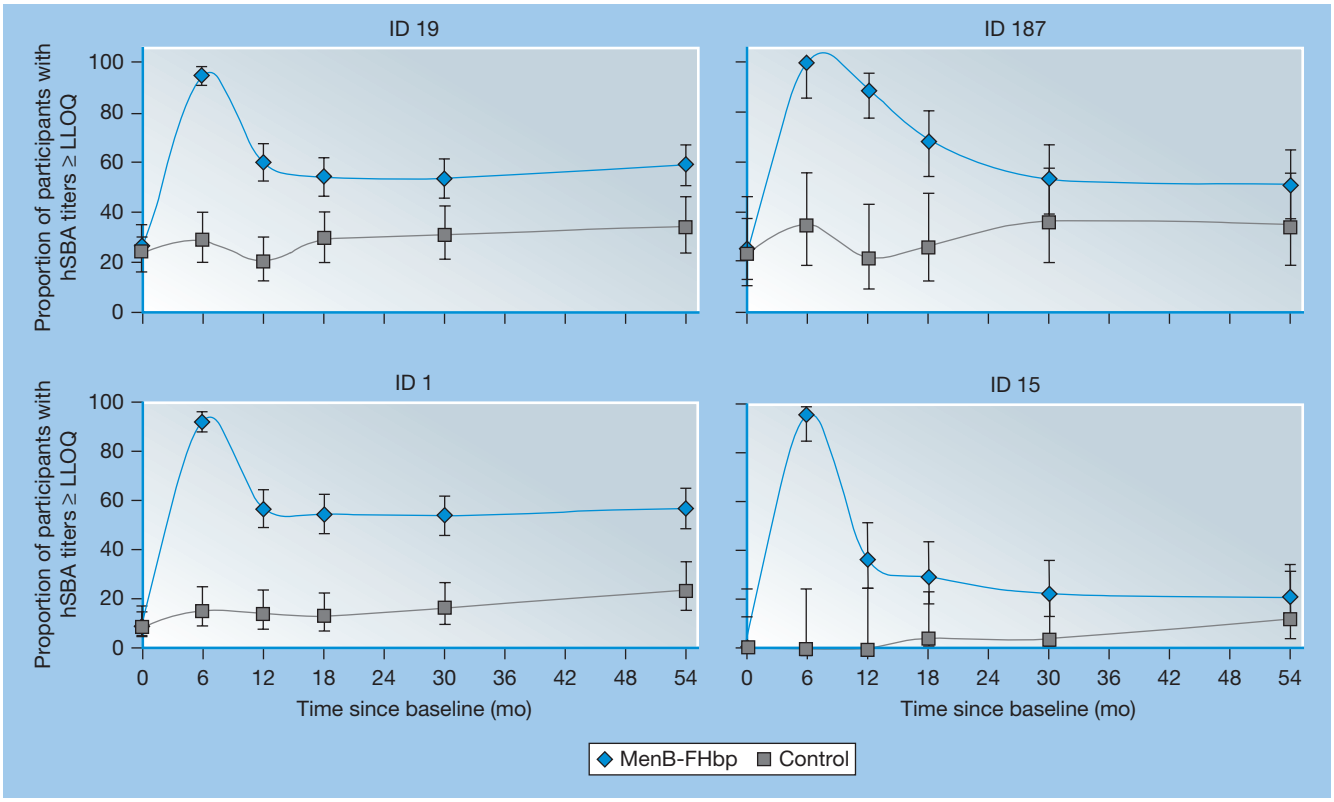


Figure 39.12. Serum bactericidal antibody persistence in teenagers immunized with MenB-FHbp. Subjects were immunized with three doses of MenB-FHbp at 0, 2 and 6 months, or a control vaccine, hepatitis A. Serum samples were obtained before and 1 month post dose 3 (7 months post baseline), and at 12, 18, 30, and 56 months post baseline. Error bars, 95% confidence interval. LLOQ, lower limit of quantification, which was 1:16 for the strain with FHbp ID 19 (A22 according to the Pfizer classification) and 1:8 for strains with ID 187 (A56), ID 1 (B24), or ID 15 (B44). hSBA, serum bactericidal activity measured with human complement. *(From Marshall HS, Richmond PC, Beeslaar J, et al. Meningococcal serogroup B-specific responses after vaccination with bivalent rLP2086: 4 year follow-up of a randomised, single-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis. 2017;17:58-67.)*

32% to 96% of the subjects had titers considered to be protective; the corresponding percentages after three doses were 56% to 100%.

Published data also became available in October 2016 on serum antibody persistence in adolescents immunized with three doses of MenB-FHbp at 0, 2 and 6 months.[158] The proportion of subjects protected by vaccination was defined by serum bactericidal titers greater than or equal to the lower limit of quantification (LLOQ), which was 1:16 for the strain with FHbp ID 19 (A22) and 1:8 for the other three test strains. One month after three doses of MenB-FHbp (7 months from baseline), 93% to 100% of the subjects had titers above the LLOQ, which by 6 months (12 months from baseline; Fig. 39.12) had decreased to 57% to 89% for three of the test strains (ID 19, ID 187, and ID 1), and to approximately 40% for the fourth test strain (ID 15). By 12 months from baseline the titers appeared to be stable through 54 months of follow-up. These data suggest that a fourth booster vaccination may be required to maintain immunity in adolescents.

Supporting data for concomitant administration of MenB-FHbp with vaccines routinely administered to U.S. adolescents are available for 4vHPV (Gardasil, Merck),[159] MCV-4-DT (Menactra, Sanofi Pasteur),[160] Tdap (Adacel, Sanofi Pasteur),[160] or dTaP/IPV (Repevax, Sanofi Pasteur)[161] vaccines.[19] With one exception, there was no interference in meningococcal antibody responses or responses to the concomitant vaccine antigens. The exception was the HPV type 18 antigen in the 4vHPV vaccine, which failed noninferiority criteria after the third dose.

Vaccine Effectiveness

There are no available data on effectiveness of MenB-FHbp for preventing disease or decreasing oropharyngeal colonization.

SURROGATES FOR PREDICTING VACCINE COVERAGE OF DISEASE-CAUSING STRAINS

The serum bactericidal data from testing three or four "indicator" strains provide useful information on functional activity of antibodies elicited by the different vaccine antigens. However, the vaccine antigens present in strains causing disease can vary in different geographic regions and over time within one region.[162] Furthermore, the isolates responsible for disease often have lower antigen expression and/or greater sequence divergence from the vaccine antigens than present in the indicator strains. Even modest FHbp amino acid sequence divergence (see Fig. 39.5)[62,68] or decreased strain FHbp expression (see Fig. 39.6)[66,67,163] can increase resistance to anti-FHbp bactericidal activity (confirmed in human adults immunized with MenB-4C; see Fig. 39.10C–D).

To estimate MenB-4C strain coverage, the manufacturer developed the meningococcal antigen typing system (MATS) that uses polymerase chain reaction to determine the strain PorA VR ST and an enzyme-linked immunosorbent assay to measure strain expression and cross-reactivity of the other three MenB-4C antigens (NHba, NadA, and FHbp).[70] Initial studies suggested that strains with the P1.4 PorA VR ST, which is present in the vaccine, or strains with a certain threshold

expression of any of the three other vaccine antigens, had 80% or greater probability of being susceptible to anti-MenB-4C bactericidal activity.[70] Since the initial study, many investigators have applied MATS to collections of disease-causing capsular group B strains from different populations.[164–170] In one study, the results of MATS predicted that 78% of 1052 group B strains collected from cases in 2007–08 from England and Wales, France, Germany, Italy, or Norway would be killed.[168] To experimentally validate this prediction, investigators compared MATS results to bactericidal activity of pooled sera from immunized infants and adolescents. The MATS results were reported to have 78% accuracy and 96% positive predictive value.[171] Other studies concluded that MATS was a "conservative" predictor of vaccine coverage.[165,171] However, these studies used pooled serum from vaccinated subjects, which may not reliably reflect activity of antibodies present in sera from individual subjects because antibodies in an individual serum that lack bactericidal activity may have cooperative bactericidal activity when combined.[95,101,163] While data supporting the use of pooled sera have been reported[172] and additional data may confirm that MATS results predict vaccine coverage, the bactericidal contribution of antibodies to individual MenB-4C antigens may be more complicated than suggested by the overall MATS analysis. This is particularly true for antibody to NHba or NadA, where strains with high antigen expression can be resistant to anti-NHba bactericidal activity[21,101,123] (see Fig. 39.10D). Thus, not all antigens in MenB-4C are necessarily equal with respect to their ability to elicit serum bactericidal antibody; additional studies are needed to confirm the hypothesis that antigen-specific thresholds measured by MATS to any one of the three antigens indicate a high probability of the strain being covered by MenB-4C.

For predicting coverage by MenB-FHbp, the manufacturer provided data that human immune sera killed 36 of 45 (80%) group B isolates tested, with the best predictor being the level of FHbp expression as measured by a flow cytometric assay that used broadly reactive anti-FHbp mAbs.[69] Unlike MATS, which uses polyclonal rabbit antisera with greater avidity for the respective vaccine antigens than for antigens with amino acid sequence divergence, the flow assay only measures strain expression (since the anti-FHbp mAbs used are reported to be broadly cross-reactive). Additional studies are therefore needed to determine whether measurement of strain FHbp expression alone without taking into account cross-reactivity with the vaccine antigen will be an accurate guide to predicting strain coverage by the MenB-FHbp vaccine.

In summary, assays such as MATS or flow cytometric detection of strain FHbp expression ultimately may prove useful for estimating vaccine coverage in different geographic areas. However, the assays remain surrogates of bactericidal activity, which is a correlate of protection. While it is true that the available volumes of serum from infants restrict the number of strains that can be tested for bactericidal activity, with adolescent and adult vaccination it should be feasible to include assays of both antigen-specific indicator strains and representative disease-causing strains to inform public health recommendations. In the United States, as part of an accelerated approval process that was used to license the vaccines, both manufacturers committed to performing studies after vaccine licensure to confirm that the group B vaccines elicit serum bactericidal antibody against diverse epidemiologically relevant meningococcal group B strains. Ultimately, however, the true impact of these two new vaccines can only be determined by carefully performed postlicensure vaccine effectiveness studies as well as other observational studies, such as was recently reported from the United Kingdom on the effectiveness of two doses of MenB-4C given to infants in preventing disease.[152]

EFFECT OF VACCINATION ON COLONIZATION

Licensed meningococcal vaccines elicit serum bactericidal activity, which protects individuals against developing invasive disease. Vaccines that also protect against acquisition of asymptomatic pharyngeal carriage confer herd protection, which decreases exposure to the organism and therefore decreases the incidence of disease in unvaccinated individuals.[76,173] Little is known about the effect of immunization with vaccines that target protein antigens on carriage. Read and colleagues investigated the effect of MenB-4C vaccination on oropharyngeal colonization in adults in the United Kingdom aged 18 to 24 years.[75] 2954 subjects were randomized to receive two doses of MenB-4C, 1 month apart, or two doses of a Japanese encephalitis vaccine (negative control), or one dose of MCV4-CRM followed by one dose of a placebo. Oropharyngeal cultures were obtained before vaccination and at five time points over 1 year. One month after dose 2 (primary endpoint), there were no significant differences in meningococcal carriage rates among the three groups. However, during the period from 3 months after dose 2 to 11 months, the authors reported a significant effect of MenB-4C vaccination on lowering carriage for any meningococcal strain (18% carriage reduction, 95% CI, 3 to 31). In general, vaccination is not expected to decrease carriage in those already colonized; the most important effect of vaccination on carriage should be on new acquisition of strains. During the period from 3 months after dose 2 to 11 months, there was a trend for lower rates of acquisition of new group B strains in the MenB-4C vaccinated group (34 of 628 [5%]), compared with the control group (50 of 674 [7%]). However, this trend was counterbalanced by a higher rate of new acquisition of group B strains in the MenB-4C vaccinated group during the 1- to 2-month period after dose 2 when vaccine-induced immunity should have been greatest (21 of 659 [3%] compared with 13 of 699 [2%] in the control group). Taking into consideration all of the data on new acquisition of group B carriage from 2 months to 11 months of follow-up after dose 2, MenB-4C vaccination did not appear to have a beneficial effect on decreasing group B colonization. However, the jury is out on this question since several factors in the study design may have limited the ability of the authors to detect an effect of vaccination on carriage, if it was present. These included high baseline meningococcal carriage rates (30%), enrollment of the students after they had arrived on campus during a period when there were relatively high rates of acquisition of meningococcal carriage (which could have resulted in acquisition of natural immunity by the control group), and progressively lower acquisition rates in both those vaccinated and controls during the 11 months of follow-up. One or more of these factors may have led to the relatively low effect of the control conjugate vaccine in this study on decreasing carriage of group C, Y, or W strains (36% reduction; 95% CI, 16–52). Whether MenB-4C provides any herd protection and the potential impact on vaccination on strain variation and carriage will need to be determined in postimplementation surveillance studies.[174]

VACCINE SAFETY AND TOLERABILITY

MenB-4C Vaccine

MenB-4C contains OMV from group B strain NZ98/254, which was used as a stand-alone vaccine in New Zealand. While more than 3 million doses of the OMV vaccine were safely administered in New Zealand to individuals younger than 20 years,[134–136] vaccination was associated with high rates of local reactions and systemic reactions such as fever, irritability, and lethargy.[140] However, not all of the reactions to

MenB-4C can be attributed to the OMV component because administration of the recombinant proteins alone without the OMV to infants also elicited high rates of local and systemic reactions (albeit less than to MenB-4c).[115]

For the European Union, the safety of MenB-4C vaccination of infants and toddlers was investigated in controlled trials involving 4843 subjects. In one study, 1885 infants were randomized to receive the MenB-4C vaccine at 2, 4, and 6 months given with routine vaccines; MenB-4C at 2, 4, and 6 months and routine vaccines at 3, 5, and 7 months; or routine vaccines alone at 2, 3, and 4 months.[113] Fever 39 °C or greater, severe irritability, and unusually severe crying occurred more frequently when MenB-4C was administered with other routine vaccines such as DTaP, Hib, hepatitis B, and pneumococcal conjugate vaccine (Fig. 39.13). The severity or frequency of reactions did not increase with subsequent doses of MenB-4C. In this study, two cases of Kawasaki disease were reported, one of which was thought by an independent panel of experts to be possibly related to MenB-4C vaccination.

Data on adverse events temporally related to MenB-4C vaccination are also available from a second large Phase III study of infants immunized at 2, 4, and 6 months with MenB-4C given concomitantly with routine vaccines (n = 2481), or routine vaccines and a control meningococcal group C conjugate vaccine (MCV-CRM; n = 659), or routine vaccines alone (n = 659).[144] For the tolerability analyses, the data for the three primary injections were pooled for each group. Relatively high rates of common local reactions at the MenB-4C injection site were observed during the 7 days of observation, such as any tenderness (87%) or erythema (93%), compared with 54% and 53%, respectively, at the MCV-CRM injection site in the control group. Severe tenderness at the MenB-4C injection site occurred in 29% compared with 5% for the MVC-CRM vaccine. The most common systemic reaction to the MenB-4C vaccine given with routine vaccines was fever, which was most prevalent on day 1 and usually had resolved by day 3 (Fig. 39.14). The higher rates of fever in the MenB-4C group resulted

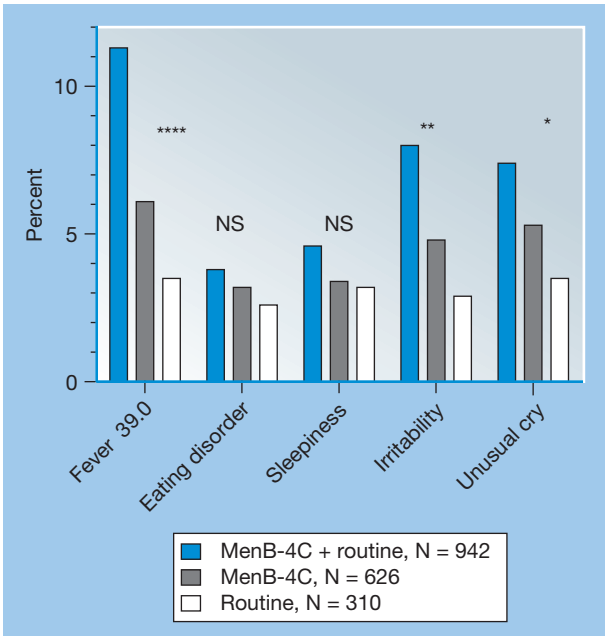


Figure 39.13. Severe systemic reactions observed in infants within 7 days of a dose of MenB-4C vaccine given at 2 months of age. "Severe" defined as follows: eating disorder (child missed more than two feedings), sleepiness (child slept most of the time and was hard to arouse), irritability (child was unable to be consoled), and unusual crying (high-pitched screaming, unlike the child's normal crying, which persisted for ≥3 hr). *P < .05; **P < .01; ***P < .001. *(Data from Gossger N, Snape MD, Yu LM, et al. Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial.* JAMA. *2012; 307:573-582.)*

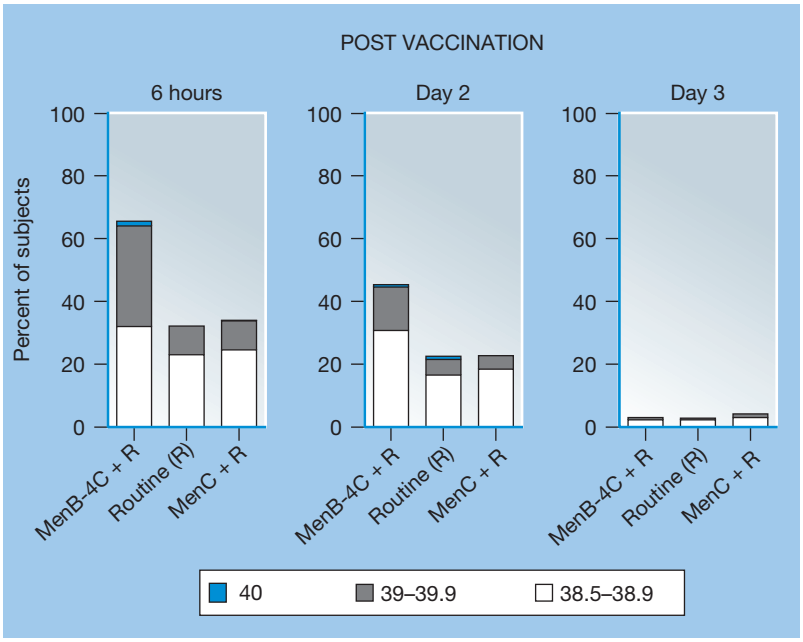


Figure 39.14. Fever observed within 3 days of MenB-4C vaccination in infants immunized at 2, 4, and 6 months of age. Pooled data for three injections. *(Data from Vesikari T, et al, and Group EUMBIVS. Immunogenicity and safety of an investigational multicomponent, recombinant, meningococcal serogroup B vaccine [4CMenB] administered concomitantly with routine infant and child vaccinations: results of two randomised trials.* Lancet. *2013;381:825-835.)*

in greater use of antipyretics (93% for the MenB-4C plus routine vaccination group, 71% for routine vaccines alone, and 66% for routine vaccines plus MVC-CRM vaccine). Other systemic reactions in the MenB-4C group such as any irritability (93%), excessive sleeping (87%), or poor appetite (72%) occurred at slightly higher frequencies than in the control groups.

In this study, two confirmed cases of Kawasaki disease and one unconfirmed case occurred 3, 7, and 14 weeks after vaccination in the 2480 infants in the MenB-4C vaccine + routine vaccines group. One confirmed case occurred at 23 weeks in 1149 control infants who received routine vaccines given with or without MCV-CRM (respective attack rates of 0.12% and 0.08%). The cases of Kawasaki disease in the MenB-4C group, and the one case in the earlier Gossger et al.[113] study considered possibly related to MenB-4C vaccination, and a subsequent case in Quebec[175] are too few to establish a relationship between MenB-4C vaccination and the risk of Kawasaki disease. However, the cases underscore the need to investigate the risk of Kawasaki disease during postlicensure surveillance.

For U.S. licensure, the safety of MenB-4C vaccination was investigated in controlled trials involving 3058 teenagers and adults aged 10 to 26 years.[133] As observed in immunized infants, there were high rates of local reactions such as injection site pain and erythema, but the rates of systemic side effects in the teenagers and young adults appeared to be less than those described for the immunized infants.

Among the 3058 teenagers and young adults in these studies, there were five serious adverse events (SAE) considered related or possibly related to MenB-4C vaccination: tremor ($n = 1$), dyspnea ($n = 1$), acute thyroiditis ($n = 1$), and juvenile arthritis ($n = 2$).[19] The overall rates of SAEs were similar in the control subjects. Information on SAEs occurring within 30 days of MenB-4C vaccination also was collected on more than 59,000 individuals who were immunized with MenB-4C as part of efforts to control group B outbreaks at two U.S. college campuses[18] and a group B outbreak in Quebec.[19] Three SAEs were considered related or possibly related to vaccination: rhabdomyolysis ($n = 1$), anaphylaxis within 30 minutes of vaccination ($n = 1$), and fever ($n = 1$), all of which resolved.

In a third study, data on adverse events were collected by a survey of 2967 students immunized with MenB-4C in response to an outbreak on a college campus in Canada.[176] About 30% of the vaccinated students responded to the survey, and the adverse events reported were consistent with those previously observed in young vaccinated adults. There were no reports of hospitalizations related to vaccination.

MenB-FHbp

A study of MenB-FHbp in infants was terminated early because of a high rate of fever.[177] The manufacturer subsequently discontinued efforts to license the vaccine for infants and toddlers. Both recombinant FHbp antigens in MenB-FHbp are lipidated, which may contribute to the vaccine's reactogenicity.

For teenagers and young adults the safety of MenB-FHbp vaccination was investigated in controlled trials involving more 4282 subjects aged 10 to 26 years.[178] The most common side effects were "any" pain at the injection site (93%), any fatigue (64%), any headache (57%), any muscle pain (41%), any chills (30%), and any joint pain (22%). Most of these reactions were mild to moderate in severity and transient, and the rates were only slightly higher than those of individuals getting similar FDA-approved vaccines such as HPV. Infrequently, the reactions to the MenB-FHbp vaccine were "severe," as defined by preventing daily activity. For adolescents given the first dose of MenB-FHbp vaccine, the rates of severe pain at the injection site (8.2%) or redness in an area greater than 10 cm (2.2%) were higher than those in controls injected with saline (0.2% and 0%, respectively). The occurrence of severe systemic reactions such as headache (1.4%), fatigue (4.3%), chills (1.3%), generalized myalgia (3.1%), or joint pain (0.9%) were uncommon but also exceeded rates of severe systemic reactions in subjects vaccinated with a control HPV vaccine (Fig. 39.15). There were trends for lower frequencies of these reactions in the MenB-FHbp group with subsequent doses.

There were no SAEs considered related or possibly related to MenB-FHbp vaccination among the 2557 subjects in the four controlled clinical trials summarized above (one published[179]). In a Phase III controlled study, 5712 healthy adolescents and young adults aged 10 to 25 years were randomized (2:1) to receive MenB-FHbp at 0, 2, and 6 months or a control vaccine (hepatitis A/saline).[180] The rate of medically attended adverse events did not differ between the two vaccines (5% to 7.0% of subjects after each dose). SAEs occurred in 1.6% of MenB-FHbp vaccinated subjects compared with 2.5% of subjects given the control vaccine. Two subjects in the MenB-FHbp group had SAEs considered to be related to the MenB-FHbp vaccine: neutropenia and an anaphylactic reaction.

In summary, vaccination with MenB-4C or MenB-FHbp is associated with higher rates of local and systemic reactions than with other commonly administered vaccines. The prelicensure and postlicensure studies did not detect an excess of SAEs related to either vaccine. One exception in infants may be Kawasaki syndrome, and one exception in adolescents and young adults may be anaphylaxis.

A theoretical safety risk of immunizing with an antigen that binds to a host protein is exposing the host to neoantigens in immune complexes that form after the antigen is injected, which can potentially elicit autoantibodies. In both human FH transgenic mice and rhesus macaques immunized with MenB-4C, a few animals developed serum autoantibodies to FH.[60,61] In the mice the antibodies were limited to immunoglobulin M, and in rhesus macaques the antibodies appeared to be transient (detected after dose 2 but not after dose 3).

Serum anti-FH antibodies are found in approximately 4% to 5% of healthy adults.[181] However, anti-FH antibodies appear to be implicated directly in the pathogenesis of atypical

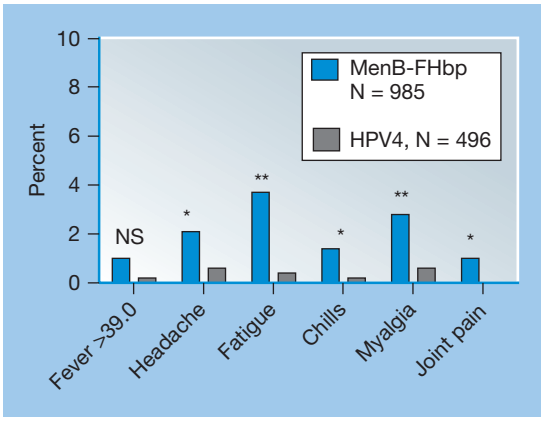


Figure 39.15. Severe systemic reactions within 7 days of dose 1 of MenB-FHbp in teenagers aged 11 to 17 years. Data shown for subjects randomized to receive MenB-FHbp or a control human papilloma vaccine (HPV4). "Severe" reaction was defined as "prevents daily activity." *$P < .05$ by Fisher exact test; **$P < .01$. *(Data from the Trumenba U.S package insert [October 2015].)*

hemolytic syndrome[182–188] and are also found in increased frequency and higher titers in patients with C3 glomerulopathies and in lupus and antiphospholipid syndromes.[182–185,188–190] The study design and sample sizes of the prelicensure and postlicensure studies of MenB-4C or MenB-FHbp and the limited postlicensure use of these vaccines are insufficient to detect rare adverse events, including autoimmune diseases. The experimental "signals" of anti-FH autoantibody in two animals models indicate the need to investigate the presence and clinical significance, if any, of these antibodies in immunized humans.

COST EFFECTIVENESS

A cost-effectiveness analysis for The Netherlands concluded that at the currently low level of disease, routine infant MenB-4C vaccination with four doses at 2, 3, 4, and 11 months was unlikely to be cost effective unless the group B disease incidence increased or the vaccine price was substantially lower than 40 euros.[191] In Germany,[192] France,[193] and Italy,[194] similar analyses concluded that with the current low incidences of serogroup B disease, universal vaccination would prevent only a small number of cases at very high overall costs. This conclusion was particularly true if vaccination did not also lower meningococcal carriage. Two cost-effectiveness analyses in the United Kingdom used modeling to investigate the potential effect of introducing MenB-4C vaccination and concluded that vaccination could decrease the disease burden and be cost effective if the vaccine were priced competitively and, in the case of adolescents, if vaccination protected against carriage.[195,196]

Given the higher disease burden in infants, the U.K. Joint Committee on Vaccination and Immunisation (JCVI) recommended introduction of routine infant vaccination if a low price could be obtained from the vaccine company, since a program for prevention of a rare disease would only be cost effective at a low vaccine cost.[197,198] To improve cost effectiveness, JCVI suggested using a three-dose schedule at 2, 4, and 12 months, which, based on some limited published post–dose 2 data and unpublished data, they believed would be sufficiently immunogenic instead of the four dose EU-approved schedule for this age group.[197] To decrease cost further the JCVI recommended eliminating one of the meningococcal C conjugate vaccine doses at age 3 months, reasoning that with the low group C disease incidence in the United Kingdom and group C herd immunity, eliminating this dose of the conjugate vaccine would have a minimal, if any, effect on the incidence of serogroup C disease (see Chapter 38).[197]

For the United States, a preliminary cost-effectiveness analysis was published as part of the Advisory Committee on Immunization Practices (ACIP) recommendations for meningococcal B vaccination for teenagers and young adults.[199] Because of the low disease incidence, even with the use of optimistic assumptions such as a decline in protective efficacy extending over 15 years, routine vaccination at ages 16 or 18 years was not cost effective (i.e., $3.7 to $4.1 million per quality-adjusted life-year).

VACCINE RECOMMENDATIONS

In the European Union, three doses of MenB-4C, each separated by 2 months of age, are approved for infants beginning immunization at ages 6 weeks to 5 months, with a fourth (booster) dose given in the second year of life. For infants aged 6 to 11 months, two doses are approved, with a third booster dose given in the second year. For adolescents and adults up to age 55 years, two doses are approved, separated by at least 1 month. As of January 1, 2017, MenB-FHbp was not licensed in the European Union. In the United Kingdom, to improve cost effectiveness, JCVI recommended using a three-dose MenB-4C infant schedule at 2, 4, and 12 months, which was implemented in October 2015. As described above, preliminary data indicate that the two-dose priming schedule provided a high level of short-term protection.[152]

As of January 1, 2017, MenB-4C vaccination was recommended in the United Kingdom and some regions of Germany, Ireland, Italy, Poland, and the Czech Republic.[200] However, with the exception of the United Kingdom, this has not been implemented in the European Union (except on the private market), partly because of recent decreases in the incidence of group B disease, lack of evidence of herd immunity from vaccination, and concerns about the high cost of a vaccination program.[191,195,196,198] In England, the annual incidence of capsular group B disease in infants in the first year of life (30 per 100,000; see Fig. 39.1) is approximately 13-fold higher than in adolescents aged 15 to 18 years (2.3 per 100,000). Because doubts remain about whether MenB-4C vaccination confers herd protection, and disease rates remain low in adolescents, MenB-4C is not recommended for routine immunization of adolescents because the economic model indicated that the vaccine could not be cost effective without the additional benefits of herd immunity.

In the United States, two group B vaccines are approved by the FDA for the age group 10 to 25 years. To date, there are no studies directly comparing the immunogenicity or tolerability of MenB-4C and MenB-FHbp. In separate studies, the reported rates of local and systemic reactions appear to be similar. For MenB-4C, two doses are recommended separated by at least 1 month. For MenB-FHbp, three doses are recommended at 0, 2, and 6 months or 2 doses at 0 and 6 months. Given the major differences in vaccine composition, the vaccines are not considered to be interchangeable; thus, the same vaccine product should be used for all the doses. The group B vaccine recommendations of ACIP are the same for both vaccines except for the different vaccine schedules.

ACIP recommends vaccination of persons who are at increased risk of acquiring group B meningococcal disease.[19] These individuals should receive two doses of MenB-4, separated by a minimum of 1 to 2 months, or three doses of MenB-FHbp at a 0, 1 to 2, and 6 months schedule. High-risk persons include those with occupational exposure (such as microbiologists exposed to *N. meningitidis*) or exposure to meningococcal group B outbreaks, or patients with functional or anatomic asplenia or complement deficiency. The latter group includes persons being treated with eculizumab (Soliris) for atypical hemolytic uremic syndrome or paroxysmal nocturnal hemoglobinuria because the drug inhibits the terminal complement pathway and increases risk of neisserial infection, including invasive meningococcal disease.[201–204] Because there are no theoretical differences in the safety of group B vaccines for persons older than 25 years, ACIP recommends off-label vaccination of persons who are at increased risk of acquiring group B meningococcal disease to include persons older than 25 years.[19] The ACIP recommendation for group B immunization of high-risk groups is Category A (i.e., intended for all persons without contraindications in these risk groups).

Certain risk groups included in the meningococcal polysaccharide-protein conjugate vaccine recommendations are not included in the group B vaccine recommendation, such as persons traveling or residing in countries where meningococcal disease is hyperendemic or epidemic (unless the strain is known to be group B), or first-year college students living in residence halls, military recruits, all adolescents, or children younger than 10 years even if they have underlying complement deficiencies or impaired splenic function (e.g., sickle cell disease). However, given the high risk of

developing invasive meningococcal disease in children younger than 10 years with complement deficiencies or impaired splenic function, physicians may consider off-label immunization with MenB-4C vaccine, which is licensed in Europe, Canada, and Australia for infants as young as 6 weeks of age. In these children the potential benefits of vaccination would appear to outweigh any theoretical risks.

The ACIP also recommends that group B vaccine be administered to adolescents and adults aged 16 to 23 years in the general population to provide short-term protection against most strains causing serogroup B disease.[199] The preferred age is 16 to 18 years because the majority of cases in adolescents and young adults occur after age 15 years (see Fig. 39.1B). For immunization of low-risk individuals in this age group, two doses of either vaccine, separated by 6 months, can be used. The interval can be as short as 1 month for MenB-4C and 5 months for MenB-FHbp. For persons immunized with two doses of MenB-FHbp at an interval of 1 to 4 months, a third dose should be given at least 4 months after the second dose. The recommendation for group B immunization of adolescents and young adults in the general population is Category B (requiring individual clinical decision by the healthcare provider).[199]

PRECAUTIONS AND CONTRAINDICATIONS

MenB-4C and MenB-FHbp are contraindicated in persons with known hypersensitivity to any of the components of the vaccine or a history of a severe reaction to a previous dose. There are no data on safety of either vaccine in pregnant women. Therefore the vaccine should be used in pregnancy with caution and only if clearly needed. There also are no data on whether the vaccines are excreted in human breast milk. While not contraindicated, the possibility that the vaccines may be present in human breast milk should be considered when immunizing nursing mothers. For MenB-FHbp, the caps of the prefilled syringes contain latex, which may cause allergic reactions in sensitive persons.

PUBLIC HEALTH PERSPECTIVE

The United Kingdom has targeted routine MenB-4C vaccination of infants younger than 1 year for group B vaccination, which is the age group at greatest risk of acquiring disease (see Fig. 39.1). Although it is not known whether a booster immunization will be required during later childhood to maintain protection in the population,[149] vaccination of infants is more cost effective than adolescents, particularly for a vaccine that is not proved to provide herd immunity.

In the United States, overall rates of group B disease are much lower than in England, and the group B vaccines in the United States are only licensed for the age group 10 to 25 years, a group with an incidence rate 10-fold lower than in the younger than 1 year age group. Thus, between 2009 and 2013, there were 36 cases and five deaths in the United States in the age group 18 to 23 years with an annual incidence is this age group of 0.14 per 100,000 population.[205] Assuming the group B vaccines have 75% or higher efficacy, about one case of disease will be prevented in the first year per 1 million persons vaccinated. The number of cases potentially prevented by vaccination will be greater if vaccination offers herd protection and/or the duration of protection is substantially longer than 1 year (e.g., declining over 15 years).[199]

The cost in the United States of immunizing a cohort of 1 million was estimated to be approximately $402 million, which included vaccine purchase, administration, and wastage.[205] To these costs must be added the cost of adverse events from vaccination. Based on observed rates of "severe" reactions reported in teenagers within 7 days of a dose of MenB-FHbp (see Fig. 39.15), in a cohort of 1 million teenagers given three doses, 31,000 would be expected to develop severe headache, 51,000 to experience severe fatigue, 38,000 to have severe myalgia, and 12,000 to have severe joint pain in excess of the number of these reactions observed in persons immunized with a HPV4 vaccine (severe defined as preventing daily activity). In short, given the currently very low group B disease burden in adolescents and young adults in the United States, the cost of prevention of one case or one death in the first year by vaccination with a group B vaccine is high and vaccine reactinogenicity is considerable. However, the major factors that appeared to influence the ACIP to decide on a category B recommendation for group B vaccination, rather than category A, were the low disease burden in the general population, unresolved scientific questions about the vaccines (including duration of protection), and the high cost of a vaccination program.[199,206] ACIP will revisit the current recommendations as additional data about these vaccines and meningococcal disease epidemiology in the United States become available.

NEXT GENERATION MENINGOCOCCAL B VACCINES

MenB-4C Combined With MCV4-CRM

MenB-4C is unlikely to become available in the United States for infants. Instead the manufacturer announced plans to combine the group B protein vaccine antigens with MCV4-CRM as a "universal" meningococcal vaccine, eventually targeting infants.[207] Results of studies defining an optimal formulation of the combination vaccine, based on immunogenicity and safety in adolescents, are promising.[24–26] Serum bactericidal antibody responses against serogroups A, C, W, and Y were higher for all of the combination MenB/MCV4-CRM formulations tested compared with MCV4-CRM alone. Two of the vaccine formulations containing OMV appeared to elicit the greatest serum bactericidal antibody responses without any identified safety concerns. Extending these studies to infants will be challenging; it will therefore be a number of years before a vaccine that prevents capsular group B disease will be available for infants in the United States.

Improving FHbp Immunogenicity

As described in the section on FHbp, after injection of FHbp, the antigen can form a complex with human FH. Data from both human FH transgenic mouse and nonhuman infant primate models indicate that binding of FH to FHbp decreases serum bactericidal antibody responses,[59,60,62] possibly by covering important epitopes. Furthermore, mutant FHbp antigens with one or two amino acid substitutions that decreased FH binding elicited higher serum bactericidal antibody responses in human FH transgenic mice,[56,58,59,208,209] or an infant macaque model[61] than control FHbp vaccines that bound human FH. In theory, the mutant FHbp antigens also may be safer by having less risk of eliciting autoantibodies to human FH.[219]

Native Outer Membrane Vesicle Vaccines

Conventional OMV vaccines such as the OMV component in the MenB-4C vaccine are treated with detergents to extract endotoxin and increase vaccine tolerability. This treatment also extracts desirable antigens such as FHbp or NHba, which are lipoproteins. To increase anti-FHbp antibody responses, native OMV (NOMV) vaccines (not treated with detergents)

have been prepared from mutant meningococcal strains engineered to overexpress FHbp.[210] A second mutation (LpxL1 knock-out) in lipopolysaccharide biosynthesis is introduced to attenuate endotoxin activity,[211,212] which avoids the need for treatment of the OMV with detergent. In mice, native (non–detergent-treated) OMV vaccines from LpxL1 KO mutants with overexpressed FHbp elicited broader serum bactericidal antibody responses than control recombinant FHbp vaccines or detergent-treated OMV vaccines prepared from wild-type strains.[212–214] By absorption studies, the principal serum bactericidal antibodies elicited by the mutant native OMV vaccines were directed against PorA and FHbp. A native OMV vaccine from an LpxL1 knock-out mutant with overexpressed FHbp also elicited broadly protective serum antibodies in a nonhuman infant primate model, which is likely to be more relevant for predicting human antibody responses than mouse models.[215] The NOMV vaccine approach can incorporate mutant FHbp antigens with low FH binding. In human FH transgenic mice, an NOMV vaccine with overexpressed mutant FHbp elicited higher serum anti-FHbp bactericidal antibody responses than a control NOMV vaccine with overexpressed FHbp that bound human FH.[58] In human FH transgenic mice, the NOMV vaccine also elicited higher and broader serum bactericidal responses than MenB-4C,[216] and in human CEACAM1 transgenic mice conferred protection against nasopharyngeal meningococcal colonization.[217]

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40 Mumps Vaccines

Steven A. Rubin

HISTORY OF THE DISEASE

The clinical picture of mumps was first described by Hippocrates in the 5th century BC in his first book of *Epidemics*, in which he described outbreaks among young males characterized by swelling about one or both ears, occasionally accompanied by painful swelling of one or both testes, referring to parotitis and orchitis, the hallmarks of this infectious disease. Recognition of neurovirulence as part of the clinical picture was made by Hamilton in his 1790 report to the Royal Society of Edinburgh[1] which included descriptions of involvement of the central nervous system (CNS). The origin of the word *mumps* is obscure, but may be related to the old English verb, which means "grimace, grin, or mumble." Mumps has since been recognized as a ubiquitous disease of childhood and continues to be so in countries that do not include mumps vaccine in their national immunization program. Where mumps vaccines are widely used, the disease has been nearly eliminated, although sporadic, and sometimes large, outbreaks still occur despite vaccination.

WHY THE DISEASE IS IMPORTANT

Mumps is generally an acute, relatively benign communicable disease. Common presentations, however, such as orchitis and parotitis, can be debilitating, and more serious complications can occur. Prior to the vaccine era, mumps was the leading cause of viral encephalitis and sudden onset deafness in the United States.[2,3] The disease is highly contagious, with nearly all persons having evidence of prior infection by age 15 years in the prevaccine era.[4–6]

Although historically a disease of childhood, mumps gained notoriety as an illness of armies during times of mobilization. During World War I, mumps was a leading cause of days lost from active duty in the U.S. army in France, with an average annual rate of hospitalization of 55.8 per 1000 for a total of 230,356 cases.[7] Gordon noted in 1949 that to the military "mumps is no passing indisposition of benign course but ranks with many of the more formidable diseases."[8] Mumps was also a major cause of morbidity among Confederate soldiers during the American Civil War[9] and during World War II,[10] and continues to occur in military settings[11–13] in addition to the general population.

Based on an analysis of data from 2009, Zhou and colleagues estimated a savings of more than $2.37 billion in societal costs in the United States through mumps vaccination.[14]

BACKGROUND

Clinical Description and Complications

Table 40.1 summarizes the clinical manifestations of mumps. Symptoms and duration vary widely among studies and are dependent on age group, sex, vaccination status, and the basis for defining a case. Complications are more frequent in adults than children; by factoring out sex-specific manifestations (e.g., orchitis, oophoritis, mastitis), rates of complications are similar in males and females, except for neurological manifestations, which have a 3:1 or greater male-to-female ratio.[15–17] The most common manifestation is salivary gland swelling, usually the parotid, which serves as the basis for the clinical case definition. Parotitis may be unilateral (Fig. 40.1) or, more commonly, bilateral. Parotitis develops an average of 16 to 18 days after exposure[18,19] and may be preceded by several days of nonspecific symptoms, including fever, headache, malaise, myalgias, and anorexia. Fever usually lasts 1 to 6 days, but enlargement of the parotid gland may persist 10 days or longer.

If viruria is used as an indication of kidney infection, then kidney involvement is common in mumps, although clinical nephritis is rarely diagnosed. Utz and colleagues found asymptomatic excretion of mumps virus in the urine of 72% of mumps cases evaluated.[20] A similar frequency of viruria was identified among military personnel with mumps admitted to a medical center.[21] Abnormal renal function can be measured in more than a third of cases; however, kidney-specific manifestations are rare.[21,22]

Orchitis, typically unilateral, occurs in up to a third of cases in postpubertal men.[5,23,24] In cases of bilateral orchitis, hypofertility and testicular atrophy have been reported; however, these are uncommon and sterility is exceedingly rare.[25] An increased risk of testicular cancer has been reported in association with mumps orchitis but remains unproved.[26–28]

In females, oophoritis and mastitis can occur, with rates ranging from 5% to 30% of cases.[5,29,30] Infertility subsequent to oophoritis has been suggested but not confirmed.[31]

Involvement of the CNS occurs in fewer than 1% to up to 62% of clinical cases, depending on the stringency of assessment. If lumbar punctures are performed routinely, greater than half of persons with mumps may show evidence of meningitis based on pleocytosis of the cerebrospinal fluid (CSF),[32,33] whereas clinical evidence of meningitis is only seen in approximately 5% of cases. Encephalitis is much more serious, occurs in approximately 0.5% of clinically apparent mumps infections, and is responsible for the majority of fatal cases.[15,16,34] Neurological manifestations are generally preceded by parotitis by 4 to 5 days but can occur before or in the absence of detectable salivary gland swelling.

Pancreatitis, usually mild, is seen in approximately 4% of cases.[29,35] Significantly higher rates have been reported in some outbreaks, although these are likely overestimates given that the diagnosis is often based on elevated serum amylase levels irrespective of clinical symptoms.[36]

Other infrequent complications include endocardial fibroelastosis[37,38] cerebellar ataxia,[39,40] transverse myelitis,[41,42] hydrocephalus,[43,44] arthropathy,[45,46] autoimmune hemolytic anemia,[47,48] thyroiditis,[49,50] thrombocytopenia,[51,52] and hearing loss. Hearing loss is typically unilateral and transient,[53–55] but sudden onset permanent deafness occurs in approximately 1 in 20,000 cases.[56,57]

Nonspecific respiratory symptoms have been ascribed to mumps, but it is not clear if such symptoms are caused by the virus or are coincidental.[58,59]

Based on assessments of seroconversion in longitudinal studies or outbreak studies, asymptomatic infections occur in approximately 20% to 40% of infected persons.[5,58,60] The high rate of subclinical mumps infections was experimentally

TABLE 40.1 Major Clinical Manifestations of Mumps[a]

Manifestation	Frequency (%)			No. of Studies
	Mean	Range	90% CI	
GLANDULAR				
Parotitis	97	83–100	96–99	40
Submaxillary and/or sublingual glands	20	1–53	8–32	8
Epididymoorchitis[b]	13	1–31	10–15	40
Oophoritis	4	<1–17	1–7	9
Mastitis[c]	10	<1–31	2–18	6
Pancreatitis	4	<1–27	2–7	18
NEUROLOGIC				
Meningitis[d]	5	<1–17	3–7	27
Encephalitis	0.5	<1–2	0.1–1	8
OTHER				
Myocarditis[e]	6	1–15	2–10	5
Nephritis	0.4	<1–1	0–1	2
Deafness (transient or permanent)	2	<1–7	<1–4	7

[a]Based on published studies of mumps outbreaks identified through a search of the PubMed database using the search term "mumps" in conjunction with terms "outbreak" or "epidemic" or "complications." Only articles on outbreak investigations involving more than 20 cases were selected for the analysis.
[b]Male patients age ≥12 years.
[c]Female patients age ≥12 years.
[d]Includes severe headache and nuchal rigidity.
[e]Based on electrocardiogram abnormalities.
CI, confidence interval.



Figure 40.1. Child with unilateral parotitis. *(Courtesy the Centers for Disease Control and Prevention.)*

demonstrated in a small study by Henle and colleagues in 1947. Of 15 initially seronegative children orally inoculated with high doses of live mumps virus, all seroconverted, yet seven (47%) showed no evidence of mumps-specific symptoms.[19]

Virology

Granata was the first to suggest a viral etiology for the disease in 1908 based on experiments in rabbits,[61] work that was replicated in monkeys by Nicolle and Conseil in 1913[62] and by Gordon in 1914.[63] A viral etiology was finally proven in 1935 by Johnson and Goodpasture in a remarkable study, although perhaps not for its time, wherein Koch's postulates were fulfilled using children residing in the neighborhood of one of the investigators.[64]

The virus is an enveloped, negative-strand RNA virus in the Paramyxoviridae family, genus *Rubulavirus*. The genome consists of 15,384 nucleotides encoding seven genes: the nucleoprotein (N), V, matrix (M), fusion (F), small hydrophobic (SH), hemagglutinin-neuraminidase (HN), and the large (L) protein genes. Each gene encodes a single protein, except the V gene, which additionally encodes the phosphoprotein (P) and I protein via cotranscriptional insertion of nontemplated guanine residues.[65,66]

The role of the N protein is to coat the viral RNA to form the ribonucleoprotein, which is the template for RNA synthesis, performed by the RNA-dependent RNA polymerase, a complex consisting of the P and L proteins.[67] The viral envelope is a host-cell–derived lipid bilayer membrane containing the M protein on its internal surface and the HN and F proteins on its outer surface. The M protein appears to be involved in the assembly of viral proteins by forming a bridge between the N proteins of the ribonucleoprotein and the cytoplasmic tails of the HN and F proteins followed by budding from the cell.[68,69] The HN protein is responsible for adsorption of the virion to its cellular receptor, sialic acid, and release of nascent virions from the cell surface.[70,71] The HN and F proteins work cooperatively to induce fusion of the virion membrane with the host cell membrane and to induce cell-to-cell fusion.[72,73] The nonstructural V protein is involved in circumventing the interferon (IFN)-mediated antiviral responses by blocking its signaling and limiting its production.[74,75] The SH protein, like the V protein, also appears to aid in evasion of the host antiviral response, but by blocking the tumor necrosis factor-α–mediated apoptotic signaling pathway.[76] Neither of these proteins are essential to virus replication, as demonstrated by the rescue of recombinant viruses engineered to not express these proteins.[77,78] The role of the putative I protein in virus replication and infection is not known.

Only antibodies directed to the F and HN proteins definitively have been shown to neutralize the infectivity of the virus in vitro and confer protection in vivo.[79–82]

Mumps virus is considered serologically monotypic; however, there is some evidence that antigenic differences between strains may allow for breakthrough infection by heterologous strains[83–89] in persons with low levels of neutralizing antibodies (see "Risk Factors for Vaccine Failure and Recent Outbreaks" below).

Pathogenesis as It Relates to Prevention

Based on knowledge of the pathogenesis of related respiratory viruses, it is presumed that mumps virus initially infects and multiplies in the upper respiratory tract before spreading to draining lymph nodes and disseminating via plasma viremia. Given the wide array of acute inflammatory reactions, there is little doubt that viremia ensues; however, primary sites of virus replication following exposure have not been demonstrated and virus has been detected in blood only rarely.[90–92]

Reported symptoms and laboratory findings indicate glandular tissues (parotid glands, testes, breasts, and pancreas) and the CNS as the most common sites of mumps virus dissemination. If viruria is used as an indication of kidney infection, then kidney involvement is also common.

Virus is shed in saliva as early as 11 days after exposure, which is about a week before symptom onset (i.e., parotitis).[19,93]

Viruria can be detected during the first 5 days of illness, and can last up to 14 days.[21,22,94] The period of virus shedding in saliva does not seem to be influenced by vaccination status[95] or symptom severity, including in those asymptomatically infected.[19] Termination of viral shedding in saliva correlates with the local appearance of virus-specific salivary immunoglobulin (Ig) A and IgM, usually appearing within a few days of symptom onset.[96,97] Fewer than 15% of patients continue to shed virus in saliva beyond day 5 of symptom onset.[95]

The pathogenesis of specific complications, such as nephritis and orchitis is uncertain. Nephritis may be the result of direct viral infection of the kidney or immune complex glomerulonephritis. Although a limited number of pathologic specimens have been examined, there is evidence to suggest that immune complex deposition may have a role in some cases.[22] In the rare cases in which renal biopsies or necropsies were performed, findings included adherent renal capsule, epithelial debris in the renal capsule lumen, interstitial mononuclear cell infiltration, edema, and focal tubular epithelial cell damage.[98,99]

Orchitis is thought to be a result of direct viral invasion, based on the recovery of virus from the semen and testis,[100,101] but some cases have been hypothesized to be caused by an immune-mediated adverse reaction.[102]

The pathogenesis of mumps infection of the CNS is almost wholly based on studies in hamsters using hamster-adapted strains of the virus.[103–105] Based on these studies, it appears that virus enters the CNS through the venous vasculature of the choroidal and ependymal epithelial cells. Once infected, these cells become a reservoir for continued production and release of virus, which can penetrate deeper into the brain parenchyma causing encephalitis and numerous neurological complications (see "Clinical Description and Complications" above).

Mumps virus–induced deafness likely involves retrograde penetration by the virus into the perilymphatic fluid following viremia via the cervical lymph nodes, leading to infection of the cochlea.[109] Although the perilymph communicates with the cerebrospinal fluid, deafness does not occur more frequently in persons with CNS complications, suggesting that mumps deafness is not a complication of CNS infection. Damage to the organ of Corti is a common finding, with degeneration of the stria vascularis and tectorial membrane being reported in some, but not other, studies.[106,107,110,113] Hearing loss caused by indirect effects of virus infection (e.g., immune-mediated damage) also has been suggested.[111]

Modes of Transmission

The demonstration by Johnson and Goodpasture[64] and by Henle and colleagues[19] that the virus can be transmitted to humans by nasal or buccal mucosal inoculation suggests that natural infection is initiated by droplet spread. Mumps virus appears to be capable of being transmitted transplacentally and infect the fetus, but this has been reported only rarely.[114,115]

The incubation period is typically 16 to 18 days after exposure, but considering that virus is shed in saliva as early as 1 week before symptom onset it is perhaps not surprising that outbreak investigations performed during the prevaccine era indicate that most transmissions occur before symptom appearance or during the first few days of symptom onset,[6] highlighting the difficulty in preventing virus transmission by case isolation.

DIAGNOSIS

A diagnosis of mumps is usually made clinically, based on the presence of parotitis; however, when disease incidence is low, laboratory testing is essential for case confirmation because other viruses, including influenza A virus, Coxsackieviruses, parainfluenza viruses 1, 2, and 3, Epstein-Barr virus, and adenovirus, can cause parotitis.[116,117,117a]

Prior to widespread vaccine use, laboratory case confirmation was relatively straightforward by demonstrating the presence of IgM or a significant rise in IgG levels between paired acute (or preexposure) and convalescent serum samples. However, in regions of high vaccine coverage, most cases may occur in vaccinees, in whom an IgM response may not be detectable as it is not a major component of the anamnestic immune response. Whereas IgM is readily detectable in unvaccinated persons, rates of IgM detection are 15% or less in two-dose vaccine recipients.[118–120] Furthermore, IgG levels in vaccinees may already be high, precluding the demonstration of a significant increase in IgG titer. In studies where a large proportion of notified cases were laboratory tested, fewer than 10% could be serologically confirmed.[116,121,122]

Considering caveats of serologic testing, reverse-transcription polymerase chain reaction (RT-PCR)–based methods are becoming more widely used for case confirmation. Viral culture is no longer commonly practiced because of its lack of specificity if not combined with laborious immunofluorescence techniques. Furthermore, a number of studies have found RT-PCR methods to be as sensitive as viral culture techniques in detecting mumps virus, obviating the need for the latter.[85,118,123,124]

Virus can be readily detected by RT-PCR and culture using oral fluid specimens (buccal swabs, throat swabs, or saliva). Virus can also be detected in the CSF, and to a lesser extent, urine. Virus rarely has been detected in blood. The relative success of virus detection by RT-PCR can depend on the timing of sample collection, sample handling, the genome target, and possibly vaccination status. Using oropharyngeal swabs obtained on the day of parotitis onset from presumably unvaccinated children, Cusi and colleagues identified mumps virus RNA in 85% of the clinical specimens.[125] Similar results were reported by others using oral specimens from one-dose vaccinees,[85,126] but success rates on samples from two-dose vaccinees were in the range of only 30%.[118,127] Rates of RT-PCR positivity were shown to be substantially greater if swabs are obtained soon after symptom onset, particularly among cases in persons with a history of vaccination. Among persons with a one- or two-dose history of mumps-containing vaccine, Rota and colleagues reported RT-PCR positivity in six of 28 (21%) samples collected 3 or more days after symptom onset compared to 103 of 152 (68%) samples obtained within 2 days of symptom onset.[128] This difference was not apparent among unvaccinated persons (50% of samples testing positive by RT-PCR when collected 3 or more days after symptom onset vs 55% of samples collected within 2 days of symptom onset), possibly suggesting that the duration of virus shedding is shorter in vaccinees, although a recent study found no difference in either the duration of virus shedding in saliva or the concentration of virus in saliva between vaccinated and unvaccinated persons.[128a]

TREATMENT

Mumps is generally a self-resolving illness. Therapy is symptom based and supportive, such as use of analgesics and the application of cold compresses to affected areas (e.g., the parotid and testes). Subcutaneous administration of IFN-α2B helps attenuate the course of mumps orchitis and preserves the organ and its function in some studies,[129,130] but other studies have questioned its safety and effectiveness.[131,132] Standard immunoglobulin or γ-globulin preparations do not appear to be effective treatment options.[60,133]

EPIDEMIOLOGY

Incidence and Prevalence

In the years before the introduction of mumps vaccine in the United States, mumps was most commonly reported among young school-age children. In a study conducted by the U.S. Public Health Service of cases reported between 1960 and 1964 in selected U.S. regions, children younger than 15 years of age accounted for 92% of cases.[134] In this and other studies conducted around this time, more than half of cases were in the age group 5 to 9 years and about a quarter in the age group 0 to 4 years.[135,136] These data are consistent with prevaccine era serosurveys from around the world. A seroepidemiologic study in St. Lucia demonstrated that 70% of children were seropositive for mumps at 4 years of age.[137] In The Netherlands, peak acquisition occurred between ages 4 and 6 years[3] and in the United Kingdom and Spain between ages 5 and 7 years.[138,139]

Table 40.2 shows annual incidences in various countries, averaging approximately 300 per 100,000 population based on passive reporting during the prevaccine era. The actual annual incidence in the general population is likely much higher, as indicated in limited prospective studies.[59,135,140] It has been estimated that less than a third of mumps cases are reported.[58]

In most countries seropositivity is high in unvaccinated populations by adolescence, indicating high infection rates in school-age children. A retrospective analysis of mumps in six European countries (Denmark, England, France, Germany, Italy, and The Netherlands) showed, in general, an inverse relationship between incidence and vaccine coverage, except in France, when at the time of analysis vaccine coverage was elevated in young children, but low in adolescents and the result was a considerable incidence of mumps.[141]

Hope-Simpson demonstrated in household contact studies that mumps is less infectious than measles or varicella.[18] Secondary attack rates among susceptible household contacts younger than 15 years were 76% for measles, 61% for varicella, and 31% for mumps. Some of the observed differences were undoubtedly a result of a higher rate of subclinical infection with mumps, but the higher average age at infection observed for mumps also supports the less-efficient transmission of mumps virus.[142]

Most epidemiologic reports suggest an interepidemic period for mumps of approximately 3 years,[138,143,144] as portrayed by Barskey and colleagues who summarized the number of reported cases of mumps in the United States in the prevaccine era (Fig. 40.2). In temperate zones, mumps exhibits seasonality, with peak incidence during the winter and spring and a nadir in the summer. Seasonality of mumps has not been noted in tropical areas.

In 1989, the Advisory Committee on Immunization Practices (ACIP) recommended all children receive two doses of measles vaccine.[145] Because measles vaccine is generally given in the United States as MMR (measles, mumps, and rubella trivalent vaccine), implementation of this recommendation has meant that most children receive two doses of mumps vaccine. Fig. 40.3 shows the effect of implementation of a two-dose vaccination schedule on disease incidence in the United States. Despite this dramatic reduction in disease incidence, large mumps outbreaks still occur even in fully vaccinated populations, indicating that complete protection against mumps using current vaccines and vaccination schedules may not be feasible (see "Effectiveness in Field Use and Duration of Immunity" below).

Surveillance

For molecular epidemiological purposes and to monitor the global distribution of mumps viruses, the genotype of an isolate is determined and is based on the viral SH gene sequence, which is the most variable in the genome. Twelve such genotypes have been assigned to date, designated A to N, excluding the erroneously designated genotypes E and M.[146] Intergenotypic nucleotide variation ranges from 3.8%

TABLE 40.2 Average Annual Reported Mumps Incidence in Several Countries in the World Health Organization European Region Before and After Introduction of Mumps Vaccine and in Two Countries With No Mumps Vaccination

Country	Years	Prevaccine Average Annual Incidence (Per 100,000 Population)	Years	Postvaccine Average Annual Incidence (Per 100,000 Population)	Reduction
TWO-DOSE SCHEDULE					
Denmark	1977–1979	726	1993–1995	1	>99
Finland		226		<1	>99
Norway		371		11	97
Slovenia		410		4	>99
Sweden		435		<1	>99
ONE-DOSE SCHEDULE					
Armenia	1983–1985	280	1993– 1995	16	94
Croatia		101		12	88
England and Wales		40		5	88
Israel		102		10	90
Latvia		141		3	98
NO MUMPS VACCINE					
Poland	1983–1985	415	1993– 1995	361	—
Romania		242		217	—

From Galazka AM, Robertson SE, Kraigher A. Mumps and mumps vaccine: a global review. Bull World Health Organ. *1999;77:5.*

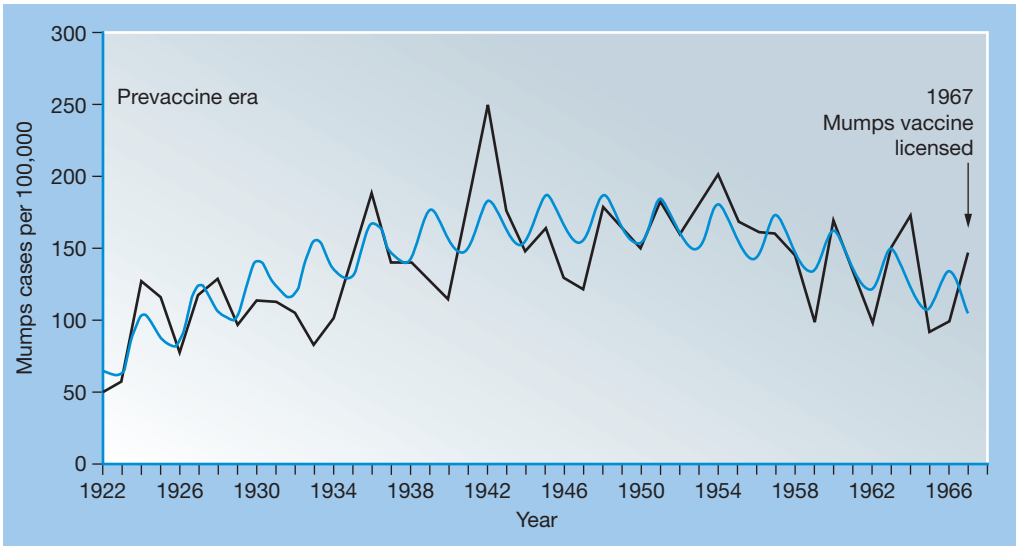


Figure 40.2. Mumps incidence in the United States in the prevaccine era, 1922–1967. Reported incidence is represented by the black line and the 3-year epidemic cycle with secular trend is suggested by the blue line. *(From Barskey AE, Glasser JW, LeBaron CW. Mumps resurgences in the United States: A historical perspective on unexpected elements.* Vaccine*. 2009;27:6186–6195.)*

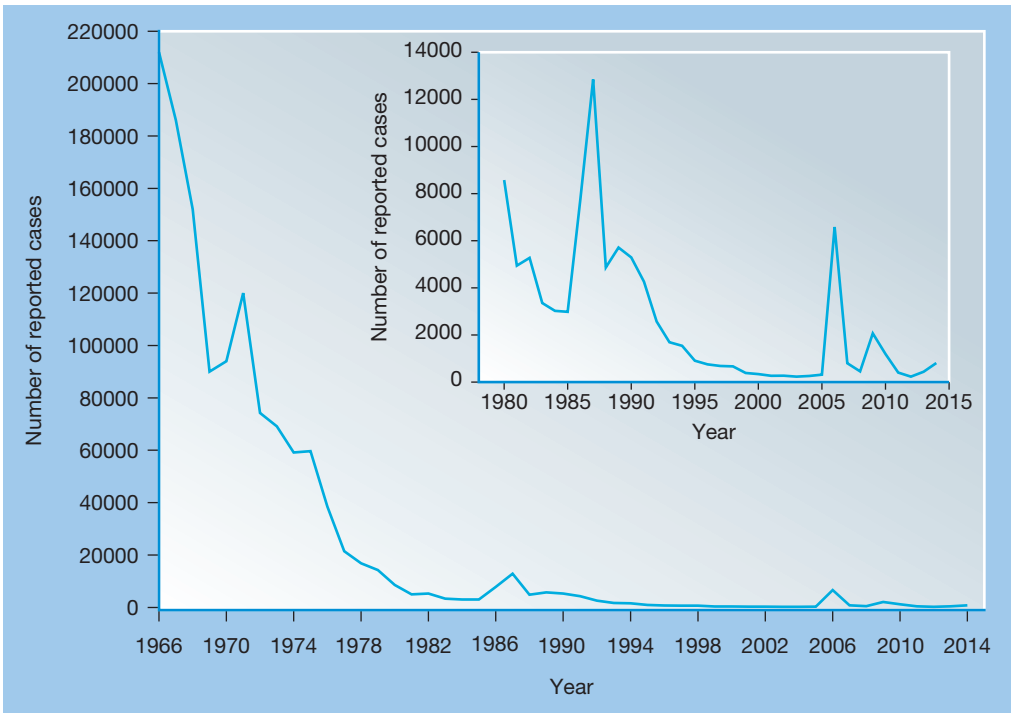


Figure 40.3. Reported cases of mumps per 100,000 population by year in the United States, 1966–2014. Data for year 2014 are provisional. *Inset*, Data for 1980–2014. *(From the National Notifiable Diseases Surveillance System, Centers for Disease Control and Prevention.)*

to 19%.[146–148] Certain genotypes predominate in certain geographic regions, but the clinical significance of such trends is unknown and cocirculation of multiple genotypes within a region is not uncommon.[149,150] As of the time of writing this chapter, members of the genotype G group of mumps viruses are responsible for the overwhelming majority of outbreaks. Historically, this genotype was mostly identified in the Western hemisphere, but its distribution is now global. Although some have suggested that certain genotypes are more virulent than others,[151–153] this remains controversial.[154–156]

High-Risk Groups

High-risk groups include international travelers, college and other post–high school students, military personnel, and healthcare personnel born during or after 1957.

Reservoirs of Infection

Although numerous mammalian species can be experimentally infected with mumps virus, humans are the only natural reservoir and only source of transmission. A virus with very

high (>90%) sequence identity to mumps virus has been identified in bats and has been determined to be conspecific to human mumps virus, but it is unclear if this virus can be transmitted to humans.[156a]

Significance as a Public Health Problem

Prior to widespread use of mump-containing vaccines, annual incidences ranged from 100 to 1000 per 100,000 population, and by adolescence, nearly everyone had serological markers of prior infection. Mumps is particularly a problem in large groups of people in close, frequent contact, such as the schools and the military. In a 1968 editorial surrounding discussion on whether a mumps vaccine is warranted, the Georgia Department of Public Health Chief Epidemiologist stated "As a cause of continuing school absenteeism and year-long disruption of teaching schedules, mumps has no equal."[157] The problem for the military was discussed earlier (see "Why the Disease Is Important"). The burden of disease in the absence of vaccination is staggering when considering direct costs for outpatient and inpatient visits, costs of outbreak control, and productivity losses incurred by days of missed work, including for provision of care for sick children and family member. Zhou and colleagues estimated that more than 2.3 million cases of mumps were prevented in the United States by vaccination in 2009, equating to a savings of greater than $1.4 billion in direct costs and more than $2.3 billion in societal costs.[14] Interestingly, whether or not to routinely vaccinate against mumps was a subject of great debate in the late 1960s, with some citing the general benignity of mumps, hazards of occult contaminating agents that might be present in vaccine, sensitization and oncogenicity that might result from use of live virus vaccine, and concerns over use in young children.[157–159] In retrospect, these concerns were for naught.

PASSIVE IMMUNIZATION

Standard immunoglobulin or γ-globulin preparations are not effective for postexposure prophylaxis against mumps or for the prevention of complications.[60,133] Mumps virus–specific immunoglobulin preparations are somewhat effective in conferring protection, but only when used early after the start of the outbreak[133] or soon after symptom development.[160] Nonetheless, the value of mumps-specific immunoglobulin to public health was considered insufficiently high to warrant routine use. The product has not been licensed for use in the United States since the 1980s.[161]

The relative success with use of specific mumps immunoglobulin is consistent with observations of the protective efficacy of maternally transferred antibody which seems to protect infants from developing mumps during infancy.[6,162] Based on the presence of antibodies, the protective effect of maternal antibody would be expected to be most effective during the first 3 months of life; beyond this time, only a small percentage of infants still possess maternal antibody.[163–165] Consistent with the measles literature, vaccine-induced maternal antibody wanes more quickly than antibody derived from maternal natural infection; however, the effect does not appear to be as significant as that for measles. The median time to loss of mumps antibodies in children of naturally immune women was 3.8 months in one study, compared to 2.4 months for children of vaccinated women ($P = .025$).[150]

ACTIVE IMMUNIZATION

History of Vaccine Development

Following his 1945 discovery that mumps virus can be cultivated in embryonated hens' eggs, (also reported by Levens and Enders the same year[166]), Karl Habel of the U.S. Public Health Service produced the first experimental vaccine (inactivated) and tested its effectiveness in 1946 among 2825 West Indian workers on a sugarcane plantation in the Everglades region of Florida.[167] Forty cases developed among 1344 vaccinees between weeks 3 and 16 postvaccination compared to 106 cases among 1481 unvaccinated controls, for a vaccine efficacy over that time period of 58%. Although inactivated vaccines were somewhat effective, the duration of immunity was short, and their use was abandoned in the United States and elsewhere in the 1950s[168,169] in favor of live attenuated vaccines, which were being developed at about that time.

The first live attenuated mumps virus vaccines for routine use were developed in the 1950s in the former Soviet Union[170,171] and in the 1960s in the United States.[172] Live vaccines have been adopted into public health practice in most developed countries, typically as part of a trivalent vaccine with measles and rubella viruses (i.e., MMR). Systematically applied, they have exerted excellent control over the disease, although not as successfully as against measles and rubella. Safety problems also have impinged on the use of some strains of mumps vaccines.

All mumps vaccines in recent use are live viruses (Table 40.3). Some strains are produced by multiple manufacturers, which may result in slight differences in their safety and effectiveness. Strains have been adapted to embryonated hens' eggs and numerous cell types as indicated in Fig. 40.4, which gives the history of passage during the attenuation of some of the more prominent strains listed in the table.

The first widely used live mumps vaccine, Leningrad-3, was produced in the former Soviet Union in 1956. The vaccine was derived from five wild-type isolates obtained from saliva and CSF specimens from children with mumps.[173] Each virus isolate was passaged in embryonated hens' eggs before being pooled for further passage in eggs to produce vaccine. In 1961, the vaccine was further passaged in guinea pig kidney cells and quail embryo fibroblast cells, with final product produced in guinea pig kidney cells.[171,174]

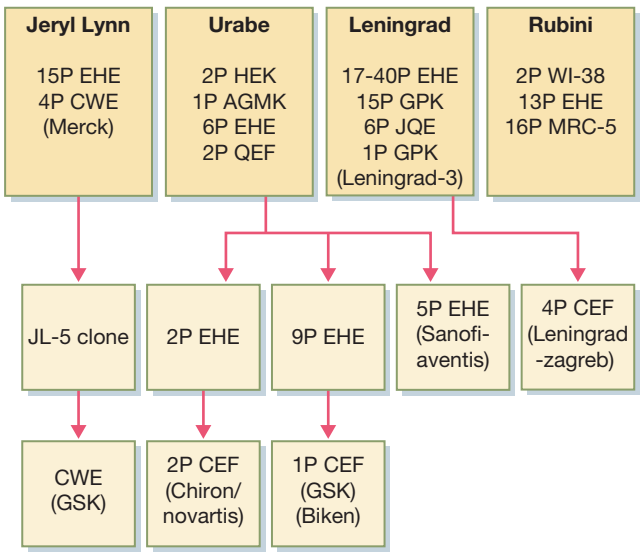


Figure 40.4. Passage history of some attenuated mumps strains. AGMK, African green monkey kidney; CEF, chick embryo fibroblast; CWE, chick whole embryo; EHE, embryonated hen's eggs; GPK, guinea-pig kidney; GSK, GlaxoSmithKline; HEK, human embryo kidney; JQE, Japanese quail embryo; MRC-5, MRC-5 human diploid fibroblast cell; QEF, quail embryo fibroblast; WI-38, WI-38 human diploid lung fibroblast cell.

TABLE 40.3 Mumps Vaccine Strains

Strain	Manufacturer (Vaccine Name)	Cell Substrate[a]	Main Area of Use
Jeryl Lynn or Jeryl Lynn-derived	Merck/Aventis Pasteur MSD (MMR-II in U.S., MMR-Vax in EU; ProQuad)	CWE	Worldwide
	GlaxoSmithKline (RIT-4385, Priorix, Priorix-Tetra)	CWE	Worldwide
	Netherlands Vaccine Institute (BMR vaccin)	CWE	Netherlands
	Sevapharma Inc. (Pavivac; Trivivac)	CK	Czech and Slovak Republics
	S79	CWE	China
Urabe Am9	Sanofi-Pasteur (Trimovax, Imovax mumps)	EHE	Worldwide
	GlaxoSmithKline (Pariorix, Pluserix, Trivirix)[b]	CEF	Europe, Canada
	Novartis Vaccines and Diagnostics (Vaxipar, Morupar)[b]	CEF	Italy, South America
	Biken[b]	CEF	Japan
Leningrad-Zagreb	Institute of Immunology of Zagreb	CEF	India, South America, Eastern Europe
	Serum Institute of India (Tresivac)	CEF	
Leningrad-3	Moscow State Facility for Bacterial Preparations	JQE	Russia, Eastern Europe
Rubini	Berna Biotech (Triviraten)[b]	HDC	Europe
Hoshino	Kitasato Institute (trivalent MPR)	CEF	Japan, Korea
Torii	Takeda Chemicals	CEF	Japan
NK-M46	Chiba Serum Institute	CEF	Japan
S-12	Razi State Serum & Vaccine Institute	HDC	Iran
Sofia-6	Center for Infectious and Parasitic Diseases[b]	GPK	Bulgaria

[a]Vaccine production level.
[b]No longer produced.
CEF, chick embryo fibroblast; CK, canine kidney cells; CWE, chick whole embryo; EHE, embryonated hen's eggs; GPK, guinea-pig kidney; HDC, human diploid cells; JQE, Japanese quail embryo.

In 1965 the Jeryl Lynn (JL) vaccine strain of mumps virus was produced, derived from virus isolated from the throat of Jeryl Lynn Hilleman, daughter of Maurice Hilleman who developed the product at Merck & Co. The isolate was attenuated by passage in embryonated hens' eggs and whole chick embryo cell culture.[175] The vaccine was licensed in 1967 and is the only mumps vaccine approved for use in the United States; it is used widely throughout the world. The vaccine has been demonstrated to contain two distinct but genetically related viruses identified as JL-major (sometimes referred to in the literature as JL-1 or JL-5) and JL-minor (sometimes referred to in the literature as JL-2), existing in an approximate 5:1 ratio, respectively.[176] These two viruses differ from each other at 414 nucleotide sites (3%), leading to 87 amino acid substitutions.[177] GlaxoSmithKline (SmithKline Beecham Biologicals, at the time) clonally isolated JL-major from a vial of Merck's JL vaccine and passaged it two times in chick embryo fibroblast cells to obtain a preparation designated RIT-4385, which was used as the virus seed for production of their mumps-containing MMR vaccine, Priorix.[178] Based on prelicensure and postlicensure experience, both vaccines have similar safety and immunogenicity profiles.[179–185] Other JL-based vaccines have also been produced and are in more limited use (see "Description of Vaccines and Producers").

The Urabe Am9, Leningrad-Zagreb, and Leningrad-3 also have been shown to contain variant virus populations, but the level of variation is not as great as with JL, and they do not represent distinct virus strains, but are rather part of the quasispecies formed by negative-strand RNA viruses.[186] The Urabe Am9 vaccine from two manufacturers has been demonstrated to contain at least two major variant virus populations existing in a 1:3 ratio.[161,187] At least two major variants were also identified in the Leningrad-Zagreb vaccine[188] and in the Leningrad-3 mumps vaccine.[189,190] The clinical significance of variants is not known.

The Urabe Am9 strain was developed by the Biken Institute in Japan from an isolate obtained from the saliva of a child named Urabe.[191] The virus was isolated in primary human embryonic kidney cells and passaged in primary green monkey kidney cells followed by passages in the amniotic cavity of developing chick embryos. Virus was then plaque purified in quail embryo fibroblast cultures and one of the purified clones, clone 9, was selected and passaged in the amniotic cavity of developing chick embryos to produce Urabe Am9 vaccine.[192]

The Leningrad-Zagreb vaccine strain was produced by limited passage of the Leningrad-3 strain in chicken embryo fibroblast culture.[174]

The Rubini mumps vaccine virus was derived from a mumps isolate obtained from the urine of Carlo Rubini in Switzerland in 1974.[193] The isolate was passaged in WI-38 human diploid cell culture, followed by passages in embryonated hens' eggs before adaptation to the human diploid cell line MRC-5, which was also the substrate for vaccine production.[193] Interestingly, sequence analysis of the SH gene demonstrated that the Rubini vaccine strain is 99% identical at the nucleotide level to the tissue culture-adapted Enders strain prepared in the United States in 1945,[88,194] and is also closely related to wild-type strains isolated in Germany from 1987 to 1992.[195] As described later, this vaccine was found to offer virtually no protective efficacy. In 2001, the World Health Organization (WHO) recommended against its use.[80] The product is no longer manufactured.

Because of the high neurotropism of wild-type mumps viruses, preclinical neurological safety testing of candidate mumps vaccines is required by national regulatory authorities. Historically, such testing has been performed in old world monkeys (Cercopithecidae) and is based on an evaluation of mumps virus–specific neuropathology (periventricular inflammation and neuronal necrosis) following intracerebral inoculation. However, two independent studies conducted in 1999 indicated monkey neurovirulence safety test results do

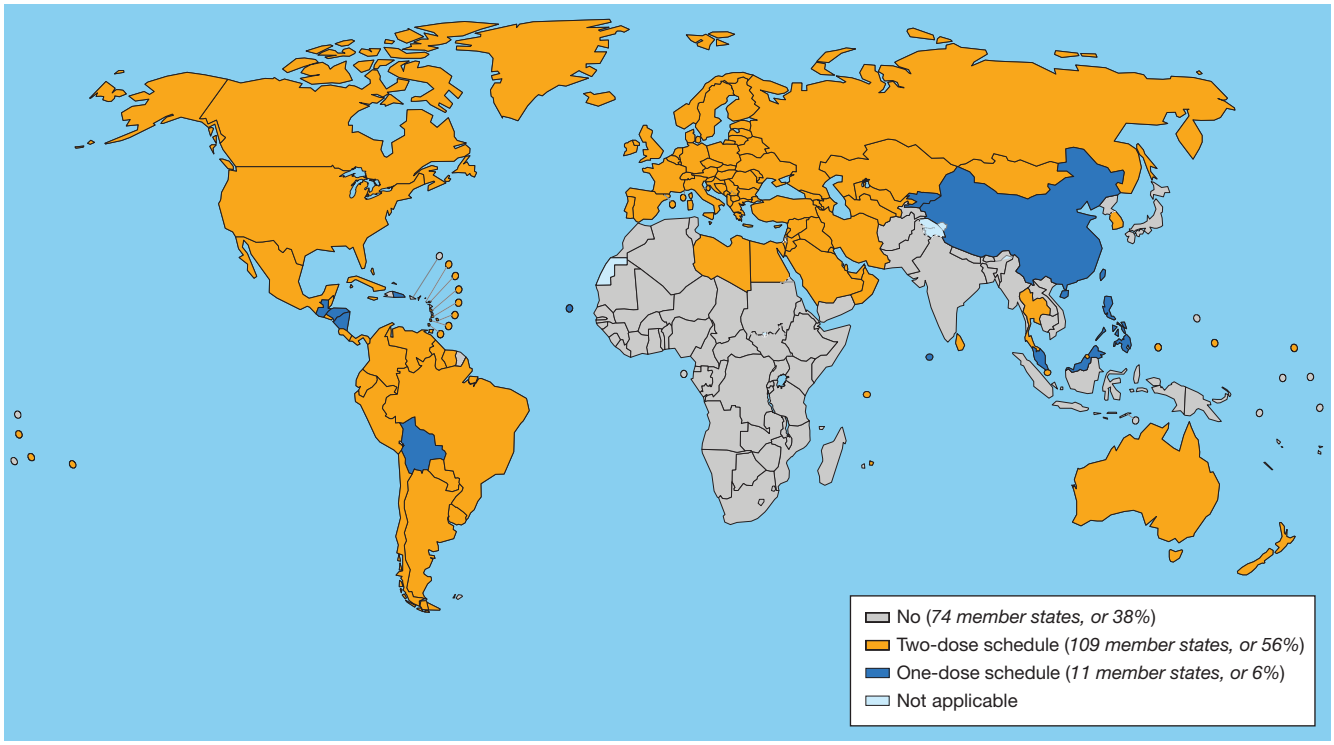


Figure 40.5. Distribution of countries using 0, 1, and 2 doses of mumps-containing vaccine. *(From data reported to World Health Organization [WHO] by 193 WHO Member States as of February 2015.)*

not correlate well with the risk of vaccine virus neurovirulence for humans.[142,196] It is therefore perhaps not entirely surprising that certain mumps vaccines presumably passing the monkey neurovirulence safety test have produced aseptic meningitis in vaccinees (see "Rare Reactions"). On the other hand, the incidence of vaccine-associated aseptic meningitis is but a small fraction of the incidence of aseptic meningitis following natural infection, and compared to natural disease with a known risk of encephalitis, postvaccine aseptic meningitis is relatively mild, suggesting that vaccine strains passing the monkey neurovirulence safety test are considerably neuroattenuated. While efforts have been made to identify specific molecular correlates of mumps virus neurovirulence, none have been identified that broadly distinguish virulent from attenuated mumps virus strains. Several groups have identified numerous genetic differences between attenuated and virulent variants of the Urabe Am9 vaccine strain, but which of these and in what combination are responsible for the observed attenuation cannot be discerned and whether any might influence attenuation of other mumps virus strains appears doubtful.[197–200] Similar conclusions have come from studies of other mumps virus strains.[154,155,201] Rather than attempting to identify point mutations that might impact virus attenuation, others have engineered mumps viruses to preclude transcription of specific genes, for example, the V gene,[78] or viruses that lack specific gene open reading frames, for example, the SH gene.[77] In both cases attenuation of these investigational recombinant viruses was achieved based on animal studies while preserving the ability of the virus to productively replicate in vitro. Whether this is a valid approach to rational vaccine design requires further study of additional wild-type clinical isolates.

Vaccine Use

In the United States, mumps vaccine was first licensed in 1967 and has been administered as MMR since 1971.[202] The ACIP first recommended inclusion of MMR in the national immunization schedule as a single dose in 1971, and then as a two-dose regimen in 1989.[145] As of February 2015, 120 of the 194 (61.9%) WHO member states were using at least one dose of mumps-containing vaccine. Of these 120 countries, 109 use a two-dose regimen. The distribution of countries is shown in Fig. 40.5 and vaccine use in select countries is given in Table 40.4.

In the United States the two-dose MMR series is administered at ages 12 through 15 months and 4 through 6 years. The second dose may be administered before age 4 years, provided at least 4 weeks have elapsed since the first dose. For international travel, the ACIP recommends vaccinating infants 6 through 11 months of age before departure from the United States, followed by doses at 12 through 15 months of age and at least 4 weeks later.[203] For older children who will travel internationally, the first dose is recommended on or after age 12 months before departure and the second dose at least 4 weeks later.

In other countries, with few exceptions, the first dose is administered at 12 to 18 months of age, but the age at which a second dose is administered varies, from as soon as 1 month after the first dose, to 10 to 12 years after the first dose. The impact of the timing of the second dose is unclear. LeBaron and colleagues found similar levels of neutralizing antibody in 17-year-old children 12 years after receiving their second dose of MMR II at 4 years of age versus antibody levels in 17-year-old children 7 years after receiving their second dose of vaccine at 10 years of age, suggesting no advantage to delaying the age at which a second dose is administered.[204]

In most countries, MMR vaccination is not compulsory; thus, mumps virus seroprevalence varies widely between countries. In the United States, where vaccine use is a requirement for school entry (see "Public Health Considerations" below), vaccine coverage is relatively high. For the 2015–16 school year (the most recent available data), median two-dose MMR vaccination coverage among children in kindergarten was

TABLE 40.4 Schedule of MMR Vaccine Use in National Immunization Plan in Select Countries

	Year of MMR Introduction		Current Recommended Schedule	
Country	**1-Dose Program**	**2-Dose Program**	**Age at First Dose**	**Age at Second Dose**
Australia	1981	1994	12 mo	18 mo
Belgium	1981	1995	12 mo	10–13 y
Brazil	1992	2003	12 mo	15 mo
Canada	1975	1996	12 mo	18 mo or 4–6 y
China	2007	–	18–24 mo	–
Croatia	1976	1994	1 y	7 y
Czech Republic	1987	1987	15 mo	2 y
Denmark	1987	1987	15 mo	4 y
Egypt	1999	1999	12 mo	18 mo
Finland	1982	1982	12–18 mo	6 y
France	1986	1996	12 mo	16–18 mo
Germany	1976	1991	11–14 mo	15–23 mo
Ireland	1988	1992	12–15 mo	4–5 y
Israel	1984	1988	12 mo	6y
Italy	1982	1999	13–15 mo	5–6 y
Macedonia, Republic of	1983	1997	13 mo	7 y
Moldova, Republic of	1983	2002	1 y	6–7 y
The Netherlands	1987	1987	14 mo	9 y
Portugal	1987	1990	12 mo	5–6 y
Russian Federation	1981	1987	12 mo	6 y
Spain	1980	1988	12 mo	3–4 y
Sweden	1982	1982	18 mo	6–8 y
Switzerland	1982	1996	12 mo	15–24 mo
United Kingdom	1988	1994	12–13 mo	3–5 y
United States	1971	1989	12–15 mo	4–6 y

From World Health Organization. WHO Vaccine-Preventable Diseases: Monitoring System. 2016 Global Summary. For a complete list of vaccination schedules by country, see http://apps.who.int/immunization_monitoring/globalsummary/schedules.

estimated at 94.6%,[205] and among adolescents 13 to 17 years of age it was estimated at 90.7%.[206] Vaccine use is also relatively high in a number of other countries, including Finland, Sweden, The Netherlands, Denmark, and Belgium.[141,207–209] In some countries with purportedly high vaccine coverage, seroprevalence is lower than expected, suggesting noncompliance with vaccine use recommendations.[141,210–212]

Description of Vaccines and Producers

Dosage, Route of Administration, and Composition

All current mumps vaccines are supplied as lyophilized powders and are administered subcutaneously following reconstitution with sterile water. The JL mumps virus vaccine produced by Merck & Co. is available in trivalent MMR formulation "MMR II" (or MMR-Vax in some countries) and in tetravalent measles-mumps-rubella-varicella (MMRV) formulation "ProQuad," both of which are preservative free. Production of monovalent Merck JL mumps vaccine (Mumpsvax) and bivalent measles-mumps vaccine (M-M-Vax) was discontinued in 2009. Each 0.5 mL dose of MMR II and ProQuad contains at least 12,500 and at least 20,000 times the median tissue culture infective dose ($TCID_{50}$) of mumps virus, respectively. Both vaccines contain sucrose phosphate, glutamate, gelatin, and albumin as stabilizers, and less than 25 μg of neomycin per dose.

Merck's JL vaccine virus has been used by other companies to produce their own vaccines. GlaxoSmithKline (then SmithKline Beecham Biologicals) clonally isolated the major component of the vaccine by limit dilution and passaged it an unstated number of times in chick embryo cultures to obtain a seed virus to produce a "new" vaccine strain in 1998 called RIT-4385,[179] which since has been incorporated with measles and rubella vaccine strains to produce the MMR vaccine Priorix or additionally with varicella to produce the tetravalent MMRV vaccine Priorix-Tetra. The Shanghai, Beijing, and Lanzhou Institutes of Biological Products in China obtained JL vaccine and passaged it three times in chicken embryo cells in 1979 to produce the S79 strain, which is used as a monovalent product, or as a bivalent vaccine with measles, or as MMR.[213] The Czech firm Sevapharma obtained the JL vaccine strain and passaged it in primary canine kidney cells to produce Pavivac. Neither S79 nor Pavivac are widely used outside the region of manufacture.

Each 0.5-mL dose of GSK's Priorix and Priorix-Tetra contains not less than 5000 $TCID_{50}$ and 25,000 $TCID_{50}$, respectively, of mumps virus. The vaccines contain sorbitol and mannitol as stabilizers and neomycin, but not gelatin. Priorix and Priorix-Tetra replace the company's discontinued Urabe

Am9 containing products Priorix (monovalent), Trivirix (MMR), and Pluserix (MMR) due to reports of Urabe Am9–associated aseptic meningitis (see "Rare Reactions"). Urabe Am9–containing vaccines produced by the Biken Institute and Novartis also have been discontinued. Vaccine containing the Urabe Am9 strain is now produced only by Sanofi Pasteur. Each 0.5-mL dose contains not less than 5000 $TCID_{50}$ of mumps virus. The trade names of these products differ by manufacturer (see Table 40.3).

The Rubini mumps vaccine strain was produced by the Swiss Serum and Vaccine Institute, Berne, Switzerland (subsequently Berna Biotech, and now Crucell) and was used to formulate the MMR vaccine Triviraten. Because of poor efficacy, this vaccine strain is no longer in use. Each 0.5-mL dose contained not less than 5000 $TCID_{50}$ of mumps virus.

The Leningrad-Zagreb strain is produced by the Institute of Immunology of Zagreb and the Serum Institute of India and is formulated with measles and rubella to produce the vaccine Tresivac. Each 0.5-mL dose contains not less than 5000 $TCID_{50}$ of the Leningrad-Zagreb mumps virus strain.

Many of the other strains listed in Table 40.3 have had limited use, often sold in only one country[214] and are not further described here.

Although the route of administration for mumps vaccine is subcutaneous, most other pediatric vaccines (which are invariably inactivated) are typically given by the less-challenging intramuscular route, making it likely that in practice, mumps-containing vaccines are occasionally given intramuscularly. However, studies found that the intramuscular and subcutaneous routes induce similar levels of antibody.[215,216]

Aerosol administration of mumps-containing vaccines has yet to be approved by regulatory bodies. Studies in mice demonstrated that this route leads to induction of neutralizing antibodies[217] and investigational studies in humans have found postaerosol vaccination enzyme-linked immunosorbent assay (ELISA) IgG titers to be comparable or superior to those measured in control groups receiving vaccine subcutaneously.[218,219] Further studies in seronegative children are awaited.

AVAILABILITY IN COMBINATIONS

Nearly all the combination MMR vaccines listed in Table 40.3 contain the RA 27/3 rubella strain, but differ in the measles and mumps strains. MMR II contains the Moraten measles strain and the Merck JL mumps strain. Trimovax, Priorix, and Trivac contain the Schwarz measles strain, but differ with respect to the mumps virus components. Trimovax contains the Urabe Am9 mumps virus vaccine strain, whereas Priorix and Trivivac contain the RIT-4385 and Sevapharma JL-based strains, respectively. Tresivac contains the Edmonston–Zagreb measles and Leningrad-Zagreb mumps virus strains. The quadrivalent vaccines ProQuad and Priorix-Tetra contain the same strains as their trivalent counterparts (MMR II and Priorix, respectively), but additionally contain the OKA varicella vaccine strain. The Shanghai, Beijing, and Lanzhou Institutes of Biological Products JL-based S79 strain is also used as a bivalent vaccine with the domestically produced Shanghai-191 (S191) or the Leningrad-4–derived Changchun-47 (C47) measles vaccine strain,[220] or as MMR additionally using either the domestically produced BRD-II rubella strain or the RA27/3 rubella vaccine strain.[221]

In general, administration of combination vaccines results in immune responses similar to those produced by the administration of individual measles, mumps, rubella, and varicella vaccines at different sites or at different times; however, there is evidence that seroconversion to mumps is somewhat higher in children receiving MMR and varicella vaccines given concomitantly at separate injection sites compared with administration of quadrivalent vaccine (ProQuad or Priorix-Tetra). In an open, randomized, controlled study among 9-month-old mumps-seronegative children, Goh and colleagues reported 92% of 118 participants seroconverted to mumps virus after concomitant receipt of Priorix and varicella vaccines, compared with 85% of 133 participants receiving Priorix-Tetra. However, seroconversion was 100% in both groups following a second dose administered 2 months later.[222] In other studies, while slightly lower responses following MMRV versus concomitant administration were apparent, differences were not statistically significant.[223–225]

The safety of combination vaccines is similar to that of individual vaccines administered at different sites or at different times, except for the occurrence of fever and febrile seizures, which have been found to occur more frequently in children 12 to 23 months old receiving ProQuad versus concomitant administration of MMR II and varicella vaccine as a first dose.[226] Consequently, administration of MMR and varicella vaccine separately is recommended for the first dose in children 12 to 47 months of age, with ProQuad given as a second dose at 4 to 6 years of age.[227,228]

SIMULTANEOUS ADMINISTRATION WITH OTHER VACCINES

MMR can be administered simultaneously with diphtheria and tetanus toxoids and acellular or whole-cell pertussis vaccine (DTP/DTaP), with oral or inactivated poliovirus vaccine, with *Haemophilus influenzae* type b conjugate vaccine, hepatitis B vaccine, varicella vaccine, or live attenuated influenza vaccine without impairing antibody responses or increasing rates of serious adverse events.[84,229–232] MMR should be given 1 month before or after administration of other live viral vaccines.

STABILITY OF VACCINE

Most lyophilized mumps virus–containing vaccines are stable for 18 to 24 months when stored and handled per the manufacturer's instructions. Exceeding the recommended storage temperature or dating period may result in accelerated potency loss to levels below those known to be efficacious. MMR II vaccine must be stored at −50°C to +8°C and be protected from light. When reconstituted to a liquid state, the vaccine should be kept at 2°C to 8°C and used within 8 hours. ProQuad is available in two formulations, one that must be stored at −50°C to −15°C, but can be kept at 2°C to 8°C for up to 72 hours prior to reconstitution and a refrigerator-stable formulation. Reconstituted vaccine may be stored at room temperature, protected from light, for up to 30 minutes, after which it should be discarded.

IMMUNOGENICITY OF VACCINES

Antibody Responses

IgM is detectable within a few days of vaccination, followed by IgG and neutralizing antibody 2 weeks later, both peaking between 1 and 3 months postvaccination.[233,234] IgM levels decline to minimal levels by 6 weeks postvaccination,[235] whereas IgG levels persist for decades, although complete loss of mumps virus-specific IgG has been reported within a few years of vaccination (see "Persistence of Antibodies" below). IgM may not be detectable in previously vaccinated or naturally infected persons who have a new infection with wild mumps virus (see "Diagnosis" above).

Virus-specific IgM and IgG antibody are typically measured by direct or indirect ELISA, rather than the more labor-intensive virus neutralization test. However, because both virus-neutralizing and nonneutralizing antibodies yield positive results in the ELISA, the ELISA can provide false-positive results in the context of assessing immunity, presuming that immunity is mediated by virus-neutralizing antibodies and not nonneutralizing antibodies.[236–238] For example, seroconversion has been demonstrated by ELISA even in the absence of demonstrable neutralizing antibody.[239] On the other hand, results of the virus neutralization assay are difficult to interpret due to the absence of an established neutralizing antibody titer that can be used as a surrogate maker of protection (see "Correlates of Protection" below). Older methods of antibody quantitation, such as complement fixation or hemagglutination inhibition, are no longer practiced.

Immunization results in lower levels of neutralizing antibody than is found following natural infection.[172,240–243] In one of the first prelicensure immunogenicity studies of the JL strain, conducted in Philadelphia in 1965, the mean neutralization antibody titers in children 6 to 8 weeks after vaccination was 1 : 9, compared with 1 : 60 after natural disease, based on hemadsorption as an end point.[172] Impressively, however, the seroconversion rate following vaccination of children who initially tested seronegative for mumps neutralizing antibody was 98.1% (355 of 362).[172] Similar results have been reported from other studies.[233,244–246] The total prelicensure experience in U.S. children showed that 97% of 6283 initially seronegative children developed neutralizing antibody after vaccination.[172] Similar seroconversion rates against mumps virus are observed following administration of trivalent formulations containing the JL vaccine strain (MMR II) or the JL-derived RIT-483 strain (Priorix),[247] as well as vaccines containing most other mumps virus strains, such as the Urabe Am9 strain,[248,249] the Leningrad-Zagreb strain,[174] the Leningrad-3 strain,[174] and the Hoshino strain.[214]

However, in contrast to the very good immunogenicity of these vaccines, significantly lower seroconversion rates have been observed with other mumps vaccines. For example, in a comparative study between MMR II (JL), Pluserix (Urabe Am9), and Triviraten (Rubini) trivalent vaccines, ELISA-based seroconversion rates were 96.5%, 100%, and 38%, respectively.[250] These data are reflected in a prospective study in Cadiz, Spain involving children within 18 months of MMR II or Triviraten vaccination. Whereas 97% of those given MMR II were mumps seropositive, only 39% of those with a history of Triviraten vaccination were seropositive.[181] Poor effectiveness of the Rubini mumps virus strain has been reported in numerous studies, ultimately leading to discontinued use of the product (see "Effectiveness in Field Use and Duration of Immunity" below). Interestingly, although the ELISA-based seroconversion rate measured for the Rubini vaccine was very low (38%), the authors stated that upon additional testing by immunofluorescence assay and plaque neutralization assay, the seroconversion rate increased to 93.4%.[250] As to how much of this increase was attributable to plaque neutralization testing was not stated, but based on data presented for a subset analysis, the geometric mean titer (GMT) of neutralizing antibody was only 2.6, which approximates the limit of assay detection. Another example is the Pavivac JL vaccine strain produced in primary canine kidney cells. In contrast to the consistently high seroconversion rates (>95%) to the JL strains produced by Merck or GSK, seroconversion after Pavivac has been found to be much lower, ranging from 75% to 82% based on limited studies.[240,250a]

Compared to measles and rubella antibody, mumps antibodies generated in response to vaccination are of relatively low avidity,[251] which might be related to the fact that of the three MMR vaccine components, mumps is the least effective. However, studies examining the relationship between IgG avidity index and protection are lacking.

Factors Influencing Seroconversion

Mild illnesses do not affect seroconversion or the magnitude of the antibody response to mumps vaccine.[252] Administration of mumps vaccine to children 6 months of age or younger is associated with vaccine failure, irrespective of the presence of maternal antibody, suggesting an intrinsic deficiency in humoral responses in this younger age group. In a study involving infants seronegative at vaccination, Buynak and colleagues reported seroconversion in 50% of infants younger than 6 months of age compared with 95% of infants 6 to 11 months old.[253] In a separate study of infants lacking maternal antibody, Gans and colleagues reported seroconversion in 91% and 93% of children vaccinated at 9 and 12 months of age, respectively, compared to 74% of children vaccinated at 6 months of age.[254] The difference in seroconversion rate at 6 versus 12 months was statistically significant, as were the GMTs of neutralizing antibody at these ages (GMT of 18 vs 51). Although this and other studies found no significant differences in rates of seroconversion between children receiving mumps-containing vaccine at 9, 12, or 15 months of age, GMTs were almost always higher in the 12- and 15-month-old age groups.[254–257] In aggregate, these studies indicate better antibody responses in children 12 months of age or older compared with responses in younger children.

Seroconversion rates among adults are similar to those observed among children, although data vary among studies. A prelicensure immunogenicity study demonstrated neutralizing antibody responses after vaccination in 95% of 20 initially seronegative adults.[258] The total prelicensure experience in adults vaccinated with the JL strain (monovalent) showed a seroconversion rate of 93% among 163 initially susceptible adults (132 men; 31 women).[246] However, Christenson and Böttiger found only 80% of seronegative 12-year-olds to seroconvert following MMR II administration compared with 92% of seronegative 18-month-olds.[259]

Primary vaccine failure occurs in approximately 5% of mumps vaccinations[260]; however, many persons not responding to the first dose of vaccine seroconvert following a second dose.[261,262] In the rare cases of inability to respond to mumps vaccination, certain human leukocyte antigen (HLA) haplotypes and cytokine/cytokine-receptor single nucleotide polymorphisms may be involved.[263–265] Genetic nonresponsiveness to measles vaccination seems to be more prevalent than genetic nonresponsiveness to mumps vaccination, as suggested by fewer HLA supertypes associated with nonresponsiveness to mumps vaccine[265] and lower heritability of these "responsiveness factors" for mumps (38.8%) than for measles (88.5%).[263]

Cellular Responses

Cell-mediated immune responses following vaccination are similar in scope and magnitude to those measured following natural infection and persist for decades. Jokinen and colleagues found mumps antigen-specific lymphoproliferative responses in 49 of 50 persons assessed 21 years after receipt of a second dose of MMR II, 14 (28%) of whom lacked detectable levels of mumps antibody.[266] The levels of IFN-γ and interleukin-10 produced in the lymphoproliferative responses were as high as those detected in a control group consisting of naturally immune persons. This was also suggested in an observational, nonrandomized cohort of 10 adults with a history of natural infection and 10 adults who received a

second dose of MMR II at least 10 years earlier. In that study, virus-specific T-cell responses were identified in 80% of naturally immune and 70% of vaccinated persons with similar amounts of IFN-γ produced by stimulated T cells in both groups.[267] Vandermeulen and colleagues reported similar results, but only for persons with relatively high antibody titers.[268] Whereas lymphoproliferation was demonstrated in 19 (79%) of 24 subjects with high antibody titer, only 13 (54%) of 24 subjects with low antibody titers demonstrated lymphoproliferative responses. The mean stimulation index for subjects with low antibody titers was approximately half that measured for subjects with high antibody titers, suggesting some correlation between antibody and cell-mediated immune responses to mumps vaccination.

In contrast with humoral immune responses to vaccination, which may be age-dependent (see "Factors Influencing Seroconversion" above) and wane with time (see "Persistence of Antibodies" below), the magnitude of cellular immune responses seems to be independent of these factors.[254,266]

As with natural infection, the role of vaccine-induced cell-mediated immune responses in protection against reinfection or attenuating the course of disease has not been established (see "Correlates of Protection"). That levels of lymphoproliferative responses induced following one and two doses of MMR II were similar to those induced following one and two doses of Triviraten,[269] a vaccine that confers virtually no protection (see "Effectiveness in Field Use and Duration of Immunity" below), suggests that the mumps virus–specific cell-mediated response following vaccination is not predictive of vaccine effectiveness, and, by extension, protection.

Studies of B-cell responses to infection are limited and deserve more attention, particularly in light of data from Latner and colleagues indicating that MMR vaccination results in substantially lower numbers of mumps virus–specific memory B cells as compared to numbers of measles- and rubella-specific memory B cells,[270] and data from Kontio and colleagues indicating that mumps antibodies (vaccine or wild-type virus–induced) are of lower avidity than measles and rubella antibodies.[251] Both of these observations might suggest some deficiency in the process of mumps virus–specific B-cell affinity maturation, which could be a factor in vaccine efficacy (see "Risk Factors for Vaccine Failure and Recent Outbreaks" below).

CORRELATES OF PROTECTION

The presence of neutralizing antibody is linked to protection; however, numerous attempts to define a level of antibody that correlates with protection have failed. For example, prevaccine era studies consistently found neutralizing antibody titers in the range of 1:4 to 1:8 to be associated with protection[237,271–273]; however, many persons with much higher titers succumbed to mumps. In a study of MMR-II vaccinees involved in an outbreak in the United States in 2006, neutralizing antibody titers of approximately 1:30 or higher were associated with protection, but titers in cases and exposed noncases overlapped and no cutoff points clearly separated these two groups.[275] In a 2014 retrospective study comparing mumps-specific IgG antibody concentrations in preoutbreak samples from cases and exposed noncases among students in two Dutch university cities, preoutbreak IgG concentrations were found to be lower among cases than noncases ($P = .005$), but no serological cutoff for protection could be established.[276]

More recently, in a retrospective study of preoutbreak sera from students in The Netherlands who were later exposed during an outbreak, mean neutralizing antibody titers against the vaccine virus strain (JL) and two wild-type strains (including an isolate from the outbreak) were lower among virus-infected individuals compared with uninfected individuals. However, there was near complete overlap in the range of antibody titers measured in the virus infected versus uninfected groups.[276a] In this and other studies, no association has been found between mumps virus–specific IgG concentrations and protection or between any of the numerous investigated cell-mediated immune responses and protection.[266,268,277–279] These data suggest that immune responses other than those measured in routinely performed in vitro assays likely play an important role in protection. Such immune mediators may include antibodies involved in antibody-dependent cellular cytotoxicity (ADCC), complement-dependent lysis, and Fc-mediated phagocytosis. Unfortunately, these antibodies are challenging to identify and quantify in vitro, and such assessments are rarely performed.[278a,278b]

IMMUNOCOMPROMISED PERSONS

The immunogenicity of mumps-containing vaccine in immunocompromised persons varies by study, but overall, there does appear to be a benefit to vaccination in this group. In studies of mumps virus seronegative pediatric patients revaccinated with MMR II 2 to 3 years after bone marrow transplantation, rates of seroconversion ranged from 37% to 80%.[280–282] In a study of children with end-stage renal disease undergoing maintenance hemodialysis, five of 10 children seroconverted after receiving MMR II.[283] On revaccination of mumps virus seronegative liver transplant recipients, all seroconverted, but titers declined to very low levels during a 7-month follow-up period.[284] No vaccine-related adverse events were reported in any of the aforementioned studies; nonetheless, MMR is contraindicated in persons with evidence of severe immunosuppression or persons actively receiving chemotherapy or long-term immunosuppressive therapy (defined as ≥2 weeks of daily receipt of ≥20 mg of prednisone or equivalent).[285]

MMR vaccination should be considered for asymptomatic HIV-infected persons and for symptomatic persons without evidence of severe immunosuppression.[285,286] The vaccination schedule in children infected with HIV is accelerated, with two doses of MMR vaccine given 1 month apart.

The majority of children receiving highly active antiretroviral therapy (HAART) seroconvert in response to mumps vaccination, although the response may be transient. Of 29 mumps virus seronegative HIV-infected children undergoing HAART, 69% seroconverted following MMR vaccination when assessed 4 weeks later, but when assessed 24 weeks postvaccination, approximately half of children who had seroconverted had either very low levels of antibody or lacked antibody. Seroconversion to measles and rubella viruses in this study were much higher, ranging from 90% to 100% when assessed at 4 weeks to approximately 80% 24 weeks postvaccination.[287] No serious adverse reactions were attributed to revaccination. These data indicate that revaccination with MMR vaccine in HIV-infected children receiving immune recovery therapy should be considered.

Safety data are not available for formulations including varicella vaccine (e.g., ProQuad, Priorix-Tetra) in HIV-infected children, and, thus, these vaccines are currently not recommended for them.[285]

EFFICACY AND EFFECTIVENESS

Protective Efficacy in Controlled Clinical Trials

Prelicensure studies of the JL vaccine strain in two clinical trials demonstrated that a single dose was 95% or more effective in preventing mumps disease. The first clinical trial was conducted in Philadelphia from 1965 to 1967 among children

and their siblings attending nursery school or kindergarten.[172,288] Of 867 children enrolled, 398 (174 vaccinees; 224 control subjects) had documented exposure to mumps during the 20-month follow-up. Five cases of mumps occurred in the vaccinated group, compared with 133 cases among the control children for an overall protective efficacy of 95.2% (95% confidence interval [CI], 88–98%). Point estimates of efficacy varied from 91.7% to 95.9% by subgroups of exposed persons (families vs classrooms) and by interval from vaccination to exposure (0–10 vs 11–20 months).

The second clinical trial was a double-blind, placebo-controlled study conducted from 1966 to 1967 of children attending 44 schools in Forsyth County, North Carolina.[245] During follow-up from 30 to 180 days after vaccination, five cases of mumps occurred among the 2965 children who received mumps vaccine, compared with 13 cases among the 316 control children, giving a vaccine efficacy of 96% (95% CI, 88–99%).

Smorodintsev and coworkers reported a small clinical trial of the Leningrad-3 strain among children 3.5 to 6 years old.[171] Of the 85 vaccinated children, two developed mumps, compared with 42 of 108 children in the control group, giving a vaccine efficacy of 94% (95% CI, 76–98%). Subsequent studies of efficacy gave protection rates from 91% to 99%, but it was not clear whether the unvaccinated control subjects were randomly chosen.[289]

Information on the protective efficacy in controlled clinical trials of other mumps vaccine strains is absent from the literature.

Effectiveness in Field Use and Duration of Immunity

The effectiveness of a single dose of the JL mumps vaccine as determined in field studies is approximately 77% and that of two doses is approximately 85%, but estimates range widely across studies (Table 40.5). These estimates are significantly lower than the efficacy determined in clinical trials, where issues such as differing case definition, incomplete surveillance with limited case ascertainment, and inaccurate determination

TABLE 40.5 Published Clinical Studies of Mumps Vaccine Efficacy and Effectiveness

Study Population	Year	Vaccine Strain[a]	Protection (%)	95% CI (%)	Reference
PROSPECTIVE CLINICAL TRIALS					
Philadelphia	1965–1967	JL	95	88–98	288
North Carolina	1966–1967	JL	96	88–99	245
Russia	1970	L-3	91–99	NA	289
OUTBREAK STUDIES					
New York	1973	JL	79	53–91	445
Yugoslavia	1974	L-Z	97–100	NA	446
Canada	1977	JL	75	49–87	447
Ohio	1981	JL	81	71–88	243
Ohio	1982	JL	85	39–94	297
New Jersey	1983	JL	91	77–93	448
Tennessee	1986	JL	78	64–87	334
Russia	1987	L-3	96	NA	449
Kansas	1988–1989	JL	83	57–94	394
Texas	1990	JL	82	77–86	335
Switzerland	1991	R U	22 93	–10 to 45 53–99	450
Switzerland	1992–1993	R	33	–77 to 75	450
Switzerland	1993–1996	JL U R	61 73 6	0–85 42–88 –45 to 40	451
Switzerland	1994	JL U R	65 76 12	11–86 36–91 –102 to 62	452
France	1995	U	76	66–83	453
United Kingdom	1996	JL	68	24–86	454
Portugal	1996–1997	U R	70 1	25–88 –108 to 53	455
Canada	1997	JL	80	29–96	456
New York	1997–1998	JL	81	46–93	457
Switzerland	1998	JL U R	78 87 –4	64–82 76–94 –218 to 15	458

Continued on following page

TABLE 40.5 Published Clinical Studies of Mumps Vaccine Efficacy and Effectiveness *(Continued)*

Study Population	Year	Vaccine Strain[a]	Protection (%)	95% CI (%)	Reference
Singapore	1998	R	0	NA	459
United Kingdom	1998–1999	JL	64 88	40–78 62–96	460
Spain	1998–1999	R	0	NA	461
Spain	1998–1999	R	40	–66 to 78	461
Singapore	1999	R JL U	–55 81 54	–122 to 9 58–91 –16 to 82	462
Switzerland	1999–2000	JL R	70 30	50–80 –30 to 60	463
Sweden	2004	JL JL (2 doses)	65 91	NA NA	464
United Kingdom	2004–2005	JL JL (2 doses)	88 95	83–91 93–96	330
China	2004–2005	JL	86	77–92	465
New York	2005	JL JL (2 doses)	80 92	42–93 83–96	298
Iowa	2006	JL JL (2 doses) JL (2 doses)	84 79 88	48–95 0–97 63–96	291
Spain	2006–2008	JL JL (2 doses)	66 83	25–85 54–94	329
The Netherlands	2008	JL JL (2 doses)	92 93	83–96 85–97	466
New York City	2009–2010	JL JL (2 doses)	83 86.3	37–95 63–95	467
France	2013	JL JL (2 doses)	49 55	NA NA	332a

[a]Given as one dose unless indicated otherwise.
CI, confidence interval; JL, Jeryl Lynn; L-3, Leningrad-3; L-Z, Leningrad-Zagreb; NA, not available; R, Rubini; U, Urabe Am9.

of vaccination status may come in to play. Furthermore, the controlled clinical trials involved mostly young children vaccinated within a few years of observation, whereas field studies often involve persons with long intervals since vaccination, setting up the possibility of waning immunity as a variable.

Although the intent of a two-dose MMR vaccination schedule was to capture the small percentage of individuals who either missed the opportunity for receipt of a first dose, or who may have failed to respond to the first dose, it is clear that two doses of vaccine are more effective than one ($P = .018$, Mann-Whitney rank sum test, Table 40.5, JL data only). Nonetheless, this level of effectiveness does not appear to be adequate to prevent outbreaks, most clearly indicated by outbreaks in the United States where, 96% to 100% of cases were found to have occurred in persons with a history of two doses of MMR II.[118,290,291,291a] Mumps outbreaks among high two-dose vaccinated populations have also been reported in other countries.[35,292–296] However, this should not be misconstrued to imply that the vaccine provided no benefit. Data from many of these outbreaks indicate that mumps vaccination was effective to a large degree and likely responsible for limiting outbreak size. In the one study in which it was possible to calculate, vaccine effectiveness was estimated at 80%,[291] demonstrating a great benefit to vaccination. Notably, attack rates in these outbreaks were low, ranging from 2% to 8%, compared with 25% to 49% calculated in unvaccinated persons before mumps vaccination became widespread.[6,18,297] One example comes from a retrospective cohort study conducted among children attending a summer camp in New York. Of the study participants, 96% had a history of at least one dose of vaccine, 91% of whom had a two-dose MMR vaccine history.[298] The mumps attack rates among two-dose, one-dose, and zero-dose vaccine recipients were 3.6%, 8.7%, and 42.9%, respectively.

Comparisons of the effectiveness of different mumps vaccines show varying results, but overall, except with the Rubini vaccine which has virtually no efficacy, the results are similar. Based on the data provided in Table 40.5, compared to a one-dose JL efficacy of 77%, that of a single dose of Urabe-containing vaccines is 76%. Demicheli and colleagues estimated the effectiveness of at least one dose of MMR to be between 69% and 81% for JL-containing vaccines and between 70% and 75% for Urabe Am9–containing vaccines.[299] The effectiveness of other mumps vaccine strains is difficult to determine owing to more limited use and fewer studies.

Influence on Symptomatology

Numerous studies indicate that mumps vaccination attenuates the course of disease, complications, and complication severity. Data from a large mumps outbreak in the New York area during 2009–2010 found that among male case patients 12 years of age or older, the frequency of orchitis was 4% in patients with a two-dose MMR history compared to 11%

TABLE 40.6 Association Between Receipt of Vaccination and Mumps Complications, Adjusted for Age and Sex, England and Wales, April 1, 2002–March 31, 2006

Complication	Vaccine Dose	% Cases (Total Cases)	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)
Hospitalization	0	3.8% (8298)	1	1
	1	1.9% (6312)	0.50 (0.40–0.61)	0.54 (0.43–0.68)
	2	1.4% (914)	0.36 (0.21–0.64)	0.45 (0.25–0.80)
Orchitis	0	7.8% (4574)	1	1
	1	3.8% (3241)	0.44 (0.36– 0.55)	0.72 (0.56–0.93)
	2	1.5% (475)	0.17 (0.08–0.37)	0.64 (0.28–1.44)
Meningitis	0	0.5% (8298)	1	1
	1	0.2% (6312)	0.31 (0.16–0.62)	0.28 (0.14–0.56)
	2	0.1% (914)	0.22 (0.03–1.57)	0.17 (0.02–1.26)
Pancreatitis	0	0.3% (8298)	1	1
	1	0.2% (6312)	0.61 (0.31–1.20)	0.95 (0.41–2.19)
	2	0% (914)	(0–1.34)	—

CI, confidence interval.
From Yung C, Andrews N, Bukasa A, et al. Mumps complications and effects of mumps vaccination, England and Wales, 2002–2006. Emerg Infect Dis. *2011;17:661–667.*

among unvaccinated case patients ($P = .04$).[300] Similar observations were made in epidemiological studies of mumps outbreaks in The Netherlands and Taiwan, which also found an association between vaccination and reduced risk for overall complications and hospitalizations.[295,301]

There is also ample evidence of fewer and less-severe complications in two- versus one-dose vaccinees. Based on a study of nearly 6000 mumps cases reported in the Czech Republic during the first 6 months of 2005, compared with two-dose vaccinees, the odds ratios for complications were 6.6 in one-dose vaccinees and 7.9 in unvaccinated persons.[35] In an analysis of mumps cases in the Czech Republic reported during 2007–12, adjusted vaccine effectiveness against specific complications was significantly higher among persons with a history of two doses of vaccine versus one dose.[301a] In an investigation of a series of outbreaks in England and Wales between 2002 and 2006, compared to unvaccinated patients, the odds of hospitalization, orchitis, and meningitis were reduced by 50%, 56%, and 69%, respectively, for one dose of vaccine, and by 64%, 83%, and 78% for two doses.[302]

Yung and colleagues analyzed data from mumps cases reported between 2002 and 2006 in England and Wales.[302] These data show significantly lower odds ratios for hospitalizations, orchitis, meningitis, and pancreatitis among one- and two-dose vaccinees than among unvaccinated persons, with a greater impact of two doses compared to one dose (Table 40.6). However, some studies have not found vaccination status to be significantly associated with complication prevalence or severity.[29,303]

HERD IMMUNITY (COMMUNITY PROTECTION)

Mathematical models of the potential impact of mass vaccination on the incidence of mumps disease predict that an 85% to 90% level of vaccination coverage by 2 years of age would be required to eliminate transmission of the virus in the Western hemisphere.[138,304] Indeed, high levels of two-dose MMR vaccine coverage in many countries resulted in dramatic reductions in disease incidence to the point of near elimination (see Table 40.2), but not elimination. In the United States, by 1996, 91% of 19- to 35-month-old children had received at least one dose of MMR II.[305] This figure has remained relatively unchanged over the past 2 decades, rising only slightly to 91.9% by 2015.[305a] Vaccine coverage among kindergardeners is higher, with a median two-dose MMR vaccine coverage of 94.6% based on data from the 2015–16 school year, a figure unchanged from the prior year.[205] Based on the 2015 National Immunization Survey–Teen (NIS-Teen), the estimated two-dose MMR vaccine coverage among adolescents aged 13 to 17 years was 90.7%, also unchanged from the prior year.[206] Despite achieving the mathematically predicted threshold for eliminating virus transmission, large outbreaks continue to occur, including in environments where vaccine coverage is at, or close to, 100%.[118,290,291a] These data suggest that herd immunity, while reducing the risk of an outbreak, can be overcome under certain conditions, such as environments where the force of infection may be high and immunity waning (see "Risk Factors for Vaccine Failure and Recent Outbreaks" below). Data from numerous studies strongly indicate that circulating wild-type mumps viruses can be spread from vaccinated individuals to close contacts who are also vaccinated.[118,290,306] Based on a serology study, Dittrich and colleagues estimated that for every case of clinical mumps in a vaccinated person, there are at least three asymptomatic cases among vaccinees.[307] Consequently, in an outbreak setting, the role of asymptomatic infections to disease spread may be substantial.

It is important to recognize that vaccination does not result in sterilizing immunity. This is evident from data on mumps reinfections[307a,307b] and the significant boosting effect revaccination has on antibody levels, indicating some ability of the vaccine virus to replicate in previously vaccinated individuals. Importantly, although viral loads in the saliva of children who contracted mumps within a few years of vaccination were found to be lower than in unvaccinated children,[306] no such difference was observed among persons with longer time intervals since vaccination.[128a] These data further raise concern as to the long-term effectiveness of the vaccine in preventing mumps virus transmission.

EXCRETION OF VACCINE VIRUS

In the Philadelphia prelicensure immunogenicity study of the JL vaccine, none of the 365 children who were classroom or household contacts of the vaccinated children seroconverted, suggesting that vaccine virus is either not shed or is not infectious under such circumstances if shed.[172] Subsequent studies failed to identify evidence of vaccine virus spread to susceptible contacts, including no evidence of virus in blood, urine, or saliva of vaccinees.[233,308] However, given that the JL vaccine

strain can cause parotitis in a small fraction of vaccinees,[248,309–311] it is reasonable to speculate that the JL strain can be excreted in saliva.

For other vaccines, evidence of vaccine virus shedding and transmission is more clear. The RNA genomes of Urabe Am9, Miyahara, Hoshino, Leningrad-3, and Leningrad-Zagreb vaccine strains have been detected by RT-PCR in throat swabs in children with parotitis 2 to 3 weeks postvaccination, and horizontal transmission of the Urabe Am9, Leningrad-3, and Leningrad-Zagreb vaccine strains has been demonstrated.[312–318] In cases of horizontal transmission of the Leningrad Zagreb vaccine, mumps symptoms appeared within 4 to 6 weeks of vaccination of close contacts, consistent with vaccine virus shedding 2 to 4 weeks postvaccination, followed by a 2- to 4-week incubation period. Nevertheless, these seem to be rare events, and compared with complications following natural infection, the course of symptoms were mild and short-lived.

DURATION OF IMMUNITY AND PROTECTION

Persistence of Antibodies

Studies of antibody persistence yield wide ranging results; however, it is clear that levels of antibody wane with time postvaccination, in some cases to undetectable levels. Whether waning of antibody levels equates to waning immunity is a subject of debate, but seems likely.

Serologic studies of children enrolled in the first clinical efficacy trials of the monovalent JL vaccine strain demonstrated that mumps virus-neutralizing antibody titers persisted for at least 12 years after vaccination, but GMTs decreased by 27% during this time, despite evidence of asymptomatic boosting of antibody levels during the follow-up period as a result of reinfection.[319–321] These longitudinal studies also demonstrated that with the passage of time antibody levels after vaccination remain consistently lower than those after natural infection (Table 40.7). In a longitudinal serologic study of 87 children who provided a serum sample 2 to 5 years after administration of the first dose of MMR II and another sample 10 years after the second dose of the vaccine, all 87 children were found to have virus-neutralizing antibody at the first time point, and all but one at the second time point, but by the latter time point, the GMT of neutralizing antibody had declined by approximately 50%.[322] Date and colleagues[323] and LeBaron and colleagues[204] also observed a 50% decline in neutralizing antibody GMTs over a similar time interval among kindergarteners, middle schoolers, and university students. In all three studies, the proportion of study subjects with low levels of neutralizing antibody progressively rose with time postvaccination.

TABLE 40.7 Persistence of Mumps-Neutralizing Antibody in Seropositive Children After Clinical Mumps or Vaccination With Jeryl Lynn Strain of Mumps Vaccine

	Mumps-Neutralizing Antibody Geometric Mean Titers	
Time	Vaccine (34 Children)	Clinical Mumps (36 Children)
1 mo	10.1	62.8
1 y	12.8	27.6
2 y	11.9	42.1
4 y	11.8	34.5
7 y	10.3	18.6
9.5 y	12.5	24.4

Waning of antibody titers to undetectable levels also has been suggested. In one study, 19% of MMR II and 13% to 16% of MMR–Urabe Am9 recipients lacked mumps virus-neutralizing antibody when assessed 4 years after receipt of a single dose of vaccine at 12 to 18 months of age.[324] Similar results were reported by Broulianne and colleagues.[325] With longer time intervals, much higher rates of mumps seronegativity were measured. Broliden and colleagues reported the lack of anti–mumps virus-neutralizing antibodies in 27% of recipients of a single dose of MMR II when assessed 10 years later.[326] Nearly the same rate of seronegativity (26% by ELISA) was reported in a different study of MMR II vaccinees tested 15 years after the second dose.[327] However, in the aforementioned studies, baseline serostatus postvaccination was not determined, and, thus, it is not known what portion of seronegative persons were indeed seropositive at an earlier time. In a study where sequential bleeds were obtained from children postvaccination, Davidkin and colleagues found the proportion of ELISA-seropositive persons to have declined from 86% at 3 months after MMR II vaccination to 73% at 1 year postvaccination.[262] Upon revaccination 4 years after dose 1, the seroconversion rate increased to 95% but then declined to 86% 4 years later. A similar pattern was observed in children receiving their first dose of vaccine at 6 years of age followed by a second dose 4 to 5 years later.[262]

RISK FACTORS FOR VACCINE FAILURE AND RECENT OUTBREAKS

Primary vaccine failure is not likely, given data from clinical trials demonstrating seroconversion in greater than 95% of vaccinees following one dose and nearly 100% after two doses.[260] Furthermore, if primary vaccine failure were responsible for outbreaks, outbreaks would likely affect younger school-aged groups, which is contrary to the currently observed epidemiology of mumps outbreaks in vaccinated populations where cases predominantly involve persons 18 to 25 years of age.[295] Failure to vaccinate or use of only one of the two recommended doses of MMR is also not a likely factor given that recent outbreaks are characterized by very high two-dose coverage.

Numerous studies identified time since last vaccination as a risk factor.[36,290,328–331] A retrospective assessment of mumps outbreaks in Belgium among recipients of a least one dose of MMR vaccine indicated that after the first year postvaccination the odds of developing mumps increased by 27% annually (Fig. 40.6).[332] A smaller odds ratio was calculated in an assessment of multiple mumps outbreaks in France in 2013. For persons with a history of at least one dose of MMR, the odds of developing mumps increased by 7% for every additional year since last vaccination. In a subset analysis of persons with a two-dose MMR history, the odds of developing mumps was found to increase by 10% per year.[332a] While factors to explain these odds ratio differences have not been explored, the preponderance of data indicate that time since vaccination is a risk factor for disease acquisition, a phenomenon also seen in vaccine effectiveness studies. An analysis of a large mumps outbreak in the United Kingdom revealed that one-dose vaccine effectiveness declined from 96% in the immediate postvaccination period to only 66% 10 years later.[330] A similar finding was reported from a matched case-control study involving 469 persons in each group wherein one-dose vaccine effectiveness steadily declined from 98.5% (95% CI, 95–100%) 1 year postvaccination to 53.0% (95% CI, 32–66%) 12 years later (Fig. 40.7). There are numerous other examples of declining vaccine effectiveness as a function of time since

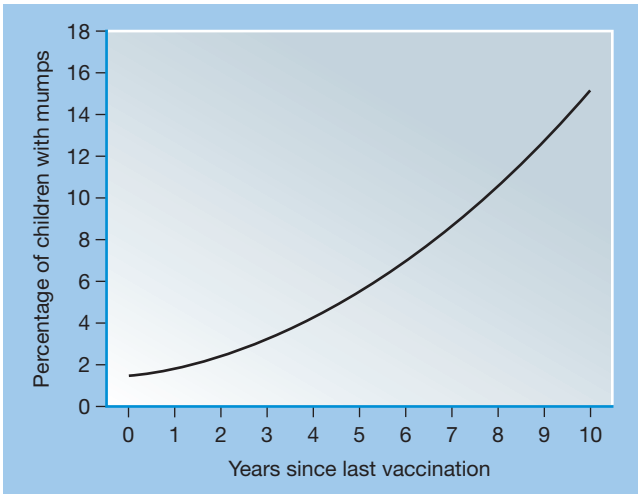


Figure 40.6. Logistic regression analysis of the percentage of children with mumps as a function of time since vaccination. *(Modified from Vandermeulen C, Roelants M, Vermoere M, et al. Outbreak of mumps in a vaccinated child population: a question of vaccine failure?* Vaccine. *2004;22:2713–2716.)*

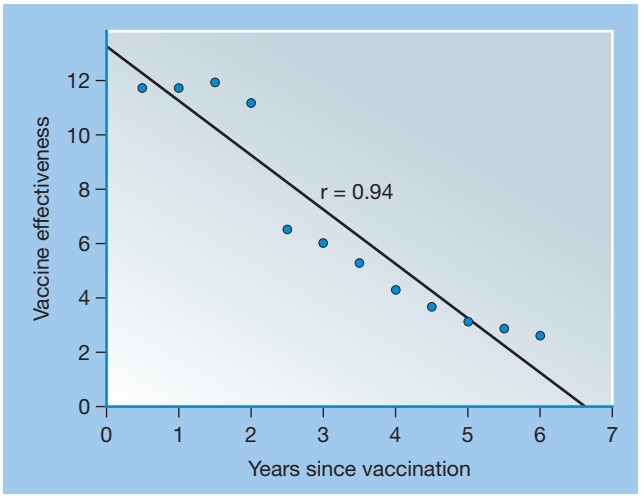


Figure 40.7. Decline in single-dose vaccine effectiveness (JL-derived S79 strain) measured during a 12-year period postvaccination. *(Modified from Fu C, Liang J, Wang M. Matched case-control study of effectiveness of live, attenuated S79 mumps virus vaccine against clinical mumps.* Clin Vaccine Immunol. *2008;15:1425–1428.)*

vaccination,[36,330,331] which dovetails well with observed declines in anti–mumps virus antibody levels as a function of time postvaccination (see "Persistence of Antibodies" above), making it tempting to link the two phenomenon. However, not all studies have found evidence of time since vaccination to be a risk factor[120,243,298,333–336] and it bears mentioning that waning immunity itself is insufficient to trigger an outbreak; otherwise mumps outbreaks would occur continually, not sporadically.

The cause of waning mumps immunity is unclear. One possibility could be, ironically, the success of the vaccine itself; that is, decreased opportunities for periodic natural boosting. However, this raises the question as to why this is not a general phenomenon across all vaccines. For example, measles outbreaks appear to almost exclusively affect isolated pockets of unvaccinated individuals within the larger vaccinated population,[337] which is quite different from the mumps experience where a high proportion of cases have occurred in vaccinees. Perhaps one clue is from a study by Latner and colleagues demonstrating very low levels of mumps virus–specific memory B cells compared to levels of measles- and rubella-specific memory B cells based on testing peripheral blood mononuclear cells isolated from persons previously vaccinated with MMR.[270] Levels of antibody-secreting cells induced following natural infection are also much greater than those induced following vaccination, suggesting that the poor B-cell responses observed in vaccinees may be an issue with the vaccine strain of the virus, not mumps virus in general; however, this difference may simply be a consequence of different routes of infection (subcutaneously in the case of vaccination and oronasally in the case of natural infection). A related finding is the in silico observation that certain amino acids at certain positions of the JL HN protein, which are unique to this virus, are predicted to impact B-cell epitopes and the ability of CD4 T-helper cells to recall antibody responses upon wild-type virus challenge.[338] Both of these observations require further study.

Another factor that is frequently cited as a possible cause of mumps in vaccinated individuals is antigenic differences between the vaccine strain and the wild-type strain, possibly permitting immune escape. This notion arose with the observation of a mismatch between the genotype of current circulating viruses (mostly genotype G of late) versus that of vaccine virus strains (genotype A: JL and JL-derived; genotype B: Urabe Am9; and vaccines of undesignated genotype, e.g., Leningrad-Zagreb)[35,36,84,292,339–343] and is supported by laboratory data demonstrating virus strain–specific differences in antigenicity.[84,85,88,89,152,344] However, these antigenic differences seem only to affect the relative potency of antibody against heterologous virus strains within a certain range as opposed to resulting in a complete inability to neutralize the virus.[322,345,345a] From a historical perspective, despite ongoing sporadic and sometime large mumps outbreaks, the disease has been nearly eliminated in countries where vaccine coverage has been consistently high.[346–349] That this could be achieved in the face of persistent circulation and importation of numerous wild-type mumps viruses of varying genotype suggests that any antigenic mismatch between circulating strains and vaccine strains is not, and has not been, a major issue.

Whatever the underlying immunologic failure, an exacerbating factor appears to be environments where the force of infection is high, such as college and university campuses where students are in prolonged, intense contact. Recent investigations of a series of mumps outbreaks in Jewish orthodox schools in New York and New Jersey revealed the number of hours spent per day in close contact with study partners and the number of study partners to be associated ($P < .05$) with classroom mumps attack rates in univariate analysis.[350] Attack rates were as high as 42% in some of the classes investigated despite two-dose MMR vaccine coverage that often exceeded 90%. Prolonged, intense contact between students was also indicated as a risk factor in a different study of the same series of outbreaks.[300] The authors of both studies postulated that in such environments inoculum exposures were sufficiently high so as to overcome vaccine-induced protection. Notably, disease transmission to non–Orthodox persons in the affected communities where contact is not as intense was rare, and when it did occur, was not sustained.

It is noteworthy that even in outbreaks in populations with high vaccine coverage, vaccine efficacy has been found to be high (see, "Effectiveness in Field Use and Duration of Immunity" above). When outbreaks occur in highly vaccinated populations, and vaccine efficacy is less the 100% (as almost

always is the case), most of the cases can be expected in vaccinees. This point is often missed by the lay media who often fail to consider the percentage of vaccinated and unvaccinated individuals within the affected population who develop disease.

POSTEXPOSURE PROPHYLAXIS

Vaccines have been used as postexposure prophylaxis (PEP) for mumps, but their effectiveness as such is unclear. During an outbreak in a Tennessee high school in 1986, MMR was given to 53 of 178 presumably unvaccinated, exposed students. Of the 53 students receiving PEP, 15 (28.3%) developed mumps compared to 51 (40.8%) of the 125 students not receiving PEP.[334] In a second study conducted during an outbreak in a New York community in 2009, MMR vaccine was given to household contacts within 5 days of the household's index case. Mumps attack rates were compared between 28 persons who received MMR as a third dose and 77 persons who had a history of two doses of MMR, but did not receive PEP. No cases occurred among the PEP recipients, compared to four cases (5.2%) among the untreated group.[351] Statistical significance could not be established in either study.

Although not specifically employed as a PEP measure, MMR was used as a control measure in school-based outbreaks in Guam,[352] New York,[353] and Illinois.[291a] In these outbreaks, MMR was used as a third dose. The mumps attack rate in students receiving the intervention in the Guam study declined by 62.5% compared to the attack rate in students not receiving the intervention, but statistical significance could not be established because of the small number of cases recorded. In the New York study, the mumps attack rate declined by as much as 97.4% after the intervention, but the intervention was initiated after the outbreak started to decline, making it difficult to evaluate the effectiveness of the intervention. Attack rates also declined precipitously following administration of a third dose of vaccine during the mumps outbreak in Illinois, but as with the prior examples, it was not clear if the reduction in cases was the result of the intervention. While there is a lack of data that clearly support use of mumps-containing vaccine to limit the scope of an outbreak, available data do not argue against such vaccine use. Based on the U.S. experience of use of a third dose of MMR vaccine as an experimental outbreak control measure, in 2013 the French health authority (Haut Conseil de la Santé Publique) recommended administration of a third dose of MMR in an outbreak setting involving schools and other semiclosed populations for individuals last vaccinated more than 10 years earlier.[345a]

SAFETY

Common Reactions

Safety data for monovalent mumps vaccines are limited. Among 402 initially seronegative subjects in the two controlled prelicensure studies of the JL vaccine strain, fever or other clinical signs or symptoms did not occur more frequently than in control groups.[354] In a different study, among 49 initially seronegative subjects, no evidence of fever or other clinical reactions referable to the JL vaccine were observed.[246] Based on diary cards completed by parents over a 3-week period postmonovalent JL vaccination, Vesikari and colleagues reported a number of mild adverse events among 58 children 14 to 20 months of age (Table 40.8), but an unvaccinated control group was not included making it difficult to determine if the reported reactions are likely attributable to the vaccine.[355]

Most other safety data for the JL vaccine strain come from studies using bivalent or trivalent formulations with measles and rubella, complicating the assessment of mumps vaccine–associated adverse reactions. However, the very low rate of vaccine-associated adverse events reported with these products attests to their excellent safety profile. A review by Lievano and colleagues of postmarketing surveillance safety data for MMR-II accumulated between 1978 and 2010, representing a time period when more than 575 million doses were distributed, identified an overall rate of 30.5 adverse events per 1,000,000 doses.[356] The most frequently reported events per million doses of vaccine distributed were pyrexia (5.49), rash (4.63), injection site reaction (2.19), febrile convulsions (1.34), syncope (0.85), and arthritis (0.67). No differences were found in comparing the safety of MMR-II

TABLE 40.8 Rates of Solicited Reactions Occurring Within 3 Weeks of Receipt of Different Vaccines in Two Different Studies Among Children Age 14–20 Months

	STUDY A: Monovalent Vaccine		STUDY B: Bivalent Vaccine With Measles	
Reported Event	Jeryl Lynn (n = 58)	Urabe Am9 (n = 58)	Jeryl Lynn- Moraten (n = 85)	Urabe Am9- Schwarz (n = 108)
Fever (>37.5°C)	13 (22.4%)	12 (20.7%)	47 (55.3%)	72 (66.7%)
Restlessness	18 (31.0%)	8 (13.8%)[a]	46 (54.1%)	74 (68.5%)[a]
Rash	4 (6.9%)	2 (3.4%)	23 (27.1%)	33 (30.6%)
Cough	8 (13.8%)	4 (6.9%)	25 (29.4%)	37 (34.3%)
Vomiting	3 (5.2%)	6 (10.3%)	4 (4.7%)	19 (17.6%)[a]
Diarrhea	5 (8.6%)	4 (6.9%)	16 (18.8%)	19 (17.6%)
Conjunctivitis	2 (3.4%)	2 (3.4%)	8 (9.4%)	19 (17.6%)
Lymphadenopathy	2 (3.4%)	0	2 (2.4%)	16 (4.8%)[a]
Local reactions	2 (3.4%)	3 (5.2%)	1 (1.2%)	9 (8.3%)[a]
No symptoms	29 (50%)	36 (62.1%)	19 (27.4%)	10 (9.3%)[a]

[a]Statistically significant difference compared to other treatment arm within the same study.

Modified from Dos Santos BA, Ranieri TS, Bercini M, et al: An evaluation of the adverse reaction potential of three measles-mumps-rubella combination vaccines. Rev Panam Salud Publica. *2002;12:240–246; and Vesikari T, Andre FE, Simoen E, et al: Comparison of the Urabe Am 9-Schwarz and Jeryl Lynn-Moraten combinations of mumps-measles vaccines in young children.* Acta Paediatr Scand. *1983;72:41–46.*

to Priorix (containing the JL-derived RIT-4385 strain), except local symptoms of injection site pain, redness, and swelling, which tended to occur less frequently in Priorix vaccinees.[179,357–359]

Data on the reactogenicity of other vaccines are more limited. In a comparative trial of monovalent JL and Urabe Am9 vaccines among 116 children 14 to 20 months of age randomized to receive either of the monovalent vaccines,[311] no significant differences among reported events were observed (see Table 40.8). Table 40.8 also shows the results of a similarly designed study of bivalent formulations with either the Moraten or Schwarz strains of measles vaccine virus.[360] In this study, statistically higher rates of restlessness, vomiting, lymphadenopathy, and local reactions were identified in recipients of the Urabe Am9–containing product. Whether these differences were caused by the mumps virus strains or the different measles virus strains used in the bivalent formulations cannot be discerned. Popow-Kraupp and colleagues conducted a prospective, randomized clinical trial wherein the safety of these same bivalent vaccines was compared in 400 children ages 9 months to 4.5 years, but found no differences in reactogenicity.[248]

Dos Santos and colleagues conducted a randomized, double-blind, placebo-controlled trial comparing MMR formulations containing the JL, Urabe Am9, and Leningrad–Zagreb mumps virus strains among 10,142 children 6 to 12 years of age.[311] These data are summarized in Table 40.9. The incidence of fever was significantly higher among Leningrad-Zagreb vaccinees than among Urabe Am9 or JL vaccinees, with no significant difference between the Urabe Am9 and JL vaccinees. Compared to JL vaccinees, the incidence of nausea, cough, lymphadenopathy, and parotitis were also statistically greater in Leningrad-Zagreb vaccinees. Local reactogenicity was similar across all three products, except for injection site redness, which was greater in Leningrad-Zagreb vaccinees (1.0%) compared to JL (0.5%) and Urabe Am9 (0.5%) vaccinees.

Comparative information on local and systemic common reactions associated with other mumps virus strains is lacking.

Rare Reactions

Less-common reactions include parotitis, aseptic meningitis, orchitis, and pancreatitis. Of these, aseptic meningitis has received the most attention and has been responsible for the discontinuation of some mumps virus vaccines. Rates of vaccine-associated aseptic meningitis vary widely, even between different studies of the same vaccine strain, likely the result of methodological differences influencing rates of detection of vaccine-associated meningitis and coincidental meningitis after vaccination due to wild-type mumps and other viruses. The most widely reported incidences of aseptic meningitis are in association with Urabe Am9–containing vaccines. Reported rates have ranged from 1 case per 336 doses to 1 case per 295,000 doses, as summarized in Table 40.10. There is some evidence that these rates may be manufacturer-related,[187] but this could be a function of differences in adverse event reporting by country, study size, method of surveillance (active vs passive), inclusion/exclusion criteria (particularly in regard to one- vs two-dose vaccine recipients), definition of vaccine-associated aseptic meningitis, and differences in background rates of aseptic meningitis. Owing to the potential of the Urabe Am9 vaccine strain to produce meningitis, MMR vaccines containing this strain were withdrawn from use in several countries in the early 1990s and mostly replaced with MMR containing the JL strain (MMR II) or the JL-derived RIT-4385 (Priorix). In Japan, mumps vaccination as part of the national immunization program was halted and has yet to resume; mumps vaccination in Japan is currently voluntary, even though the relative risk of aseptic meningitis

TABLE 40.9 Data From a Randomized, Double-Blind, Placebo-Controlled Trial Comparing Different MMR Formulations Among Children Age 6–12 Years

	MMR-II (n = 2216)	Trimovax (n = 2179)	Tresivac (n = 2226)	Controls (n = 3521)
Mumps component	Jeryl Lynn	Urabe Am9	Len.-Zagreb	Unvaccinated
Measles component	Moraten	Schwarz	Edm.-Zagreb	Unvaccinated
Rubella component	Wister RA 27/3	Wister RA 27/3	Wister RA 27/3	Unvaccinated
Headache	8.4%	8.9%	9.1%	2.2%
Fever (>37.5°C)	4.7%	4.2%	5.5%[a]	1.5%
Dizziness	1.6%	1.3%	1.3%	0.3%
Nausea	1.2%	1.7%[b]	1.7%[a]	0.3%
Vomiting	1.0%	1.1%	1.3%	0.4%
Cough	0.8%	1.0%	1.4%[a]	0.4%
Coryza	0.6%	0.8%	0.9%	0.3%
Conjunctivitis	0.1%	0.3%[b]	0.2%	0.1%
Joint manifestations	0.4%	0.3%	0.4%	0
Rash	0.4%	0.7%	0.5%	0.1%
Lymphadenopathy	0.7%	1.0%	2.2%[a]	0.3%
Parotitis	0.5%	1.3%[b]	3.1%[a]	0.2%

[a]$P < .001$ between Tresivac and MMR-II and between Tresivac and Trimovax.
[b]$P < .001$ between Trimovax and MMR-II.
Edm., Edmonston; Len., Leningrad.
Modified from Dos Santos BA, Ranieri TS, Bercini M, et al: An evaluation of the adverse reaction potential of three measles-mumps-rubella combination vaccines. Rev Panam Salud Publica. *2002;12:240–246.*

TABLE 40.10 Incidence of Postvaccine Aseptic Meningitis Following Vaccination With MMR Vaccines Containing the Urabe Am9 Mumps Virus Strain

Manufacturer	Country (Dates Covered by Study)	Rate (N)	Type of Study and Surveillance
SANOFI PASTEUR			
Rebiere et al[468]	France (1991–93)	1/28,400 (1/65,750)[a]	Retrospective: capture-recapture method
Joinville-Bera et al[469]	France (1989–92)	1/121,951	Retrospective: passive surveillance; 4 of 54 cases with laboratory confirmation of mumps virus in CSF
Dos Santos et al[311]	Brazil (1996)	0/2179	Randomized double-blind clinical trial in children 6 to 12 years old
GLAXOSMITHKLINE			
Furesz et al[470]	Canada (1986–90)	1/62,000	Retrospective: passive surveillance; 8 cases with virologic confirmation
Dourado et al[471]	Brazil (1997)	1/14,000 (452,344)	Prospective: passive surveillance of hospital admissions for meningitis; 32 cases identified
Miller et al[180]	UK (1991–92)	1/12,400 (49,585)	Retrospective: computerized hospital records for children ages 12–23 months with meningitis; 4 cases identified
SANOFI PASTEUR OR GLAXOSMITHKLINE[b]			
Miller et al[472]	UK (1990–91)	1/11,000 (78,300)	Retrospective: CSF evaluation in children discharged with diagnosis of meningitis within 15 to 35 days after MMR vaccination; 4 of 13 cases virus positive
Farrington et al[473]	UK (1988–93)	1/15,000 (77,200)	Retrospective: linkage of vaccination records and hospital discharge for meningitis; 5 cases identified
JAPAN MINISTRY OF HEALTH AND WELFARE OR BIKEN			
Fujinaga et al[474]	Japan (1989)	1/336 (11,750)	Retrospective: passive surveillance; 35 cases identified
Sugiura et al[475]	Japan (1989)	1/6,564 (630,157)	Retrospective: surveillance by physicians; 96 cases with viral confirmation
Ueda et al[367]	Japan (1990–93)	1/905 (5430)	Prospective: active surveillance by parents; 6 cases identified
Ueda et al[367]	Japan (1990–93)	0/566 (566)	Prospective: active surveillance by parents; physician confirmed
MANUFACTURER NOT SPECIFIED			
Maguire et al[476]	UK (1990–91)	1/61,000 (est. 1,650,000)	Prospective: passive surveillance by physicians with active follow up by laboratory or clinical confirmation; 7 definite cases, 17 probable cases
Colville et al[477]	UK (1988–91)	1/3,800 doses	Retrospective: 6 virologically confirmed cases in a small population
Al Mazrou et al[478]	Saudi Arabia (2000)	1/295,000 (est. 2,000,000)	Prospective: active surveillance following booster vaccination in a "catch-up" campaign; 6 cases confirmed

[a]Rate of 1/28,400 is based on an estimate of 116 cases of postvaccine aseptic meningitis by the capture-recapture method. The actual number of observed cases was 46, and no virus isolation was carried out. The rate calculation using the number of observed cases is 1:65,750.
[b]At the time of the study, Urabe Am9 vaccines manufactured by both Sanofi Pasteur (then Aventis Pasteur) and GlaxoSmithKline were licensed, and it is not possible to relate occurrence of postvaccine aseptic meningitis to a specific vaccine.
CSF, cerebrospinal fluid; MMR, measles, mumps, rubella; UK, United Kingdom.

after natural mumps is approximately 25 times greater than after vaccination with the Japanese product.[361] Based on an assessment in 2005, fewer than 25% of children 3 to 5 years old are vaccinated against mumps in Japan and it has been estimated that more than 1 million cases of mumps occur annually.[361,362]

Aseptic meningitis also has been reported in association with vaccines containing the Leningrad-Zagreb,[363,364] Hoshino,[365–367] Torrii,[365,367] Miyahara,[361] NK-M46,[368] Leningrad-3[369,370] and Sofia-6[371] strains. The rate of aseptic meningitis following MMR–Leningrad-Zagreb vaccination in Brazil and Croatia was estimated to be one case per 3390 doses and one case per 2020 doses, respectively,[363,364] but very different estimates were obtained in association with a vaccination campaign in the Bahamas where less than one case per 100,000 doses were reported.[372] In a prospective, postmarketing safety study conducted in Egypt, no cases were identified among 453,119 recipients of the Leningrad-Zagreb vaccine.[373] However, as discussed earlier (see "Excretion of Vaccine Virus"), the Leningrad-Zagreb vaccine virus RNA was isolated from Leningrad-Zagreb vaccinees presenting with meningitis, raising little doubt that this vaccine can cause meningitis.

In contrast, aseptic meningitis after vaccination with the JL strain rarely has been reported and is estimated to occur after only 1 in 1.8 million doses administered, a rate not significantly different from background.[309,374,375] In a nested case-control study, receipt of MMR II was not demonstrated to be a risk factor for hospitalization for aseptic meningitis.[376] In no case has a causal association been confirmed between the JL vaccine strain and aseptic meningitis. Similarly, aseptic meningitis has not been reported in association with the RIT-4385 strain.

In evaluating the importance of this complication, one should consider the incidence of clinical meningitis that would occur in natural mumps, conservatively estimated between 1% and 10%.[377] Assuming a conservative rate of

vaccine-associated meningitis of 1 in 10,000 and an incidence of mumps greater than 1% per year, vaccination is clearly better than not vaccinating, a conclusion also reached after evaluation of safety and effectiveness data from 64 different MMR vaccine studies involving more than 14 million children.[299]

Like aseptic meningitis, parotitis is a common complication of wild-type mumps virus infection, and therefore, it should be of little surprise that parotitis can occur after vaccination with some mumps virus strains. Parotitis following JL vaccination has been reported, although the frequency of this complication varies widely from one per 90,000 doses based on spontaneous reports from physicians[309] to 1.6% in a prospective, randomized clinical trial involving children 9 months to 4.5 years of age.[248] In this same prospective, randomized clinical trial, the incidence of Urabe Am9–associated parotitis was 2.0%, which was not statistically different from JL-associated parotitis. However, these trials lacked an unvaccinated control group; thus one cannot rule out that some of these cases of parotitis could have been caused by coincidental wild type infection in the population studied, or infection by other viruses that can cause parotitis, particularly influenza A virus (see "Diagnosis"). In the Dos Santos study (see Table 40.9), which included an unvaccinated control group, parotitis occurred in 3.1% of MMR–Leningrad-Zagreb vaccinees, 1.3% in MMR–Urabe Am9 vaccinees, and in 0.5% of MMR-JL vaccinees. The incidence in the unvaccinated control group was 0.2%.

Other infrequent mumps vaccine–associated complications include orchitis,[102,378,379] pancreatitis,[380,381] and possibly deafness,[382] although the latter is exceedingly rare. Encephalitis following vaccination does not occur more frequently than the background rate in the normal population; only 0.4 cases of encephalitis have been reported in the United States per million doses of live virus mumps vaccine distributed.

Despite the use of chicken embryo as the substrate for many mumps vaccines, few serious allergic reactions have been attributed to egg protein. Allergy to the gelatin included in some mumps virus–containing vaccines is more frequently associated with acute reactions.[383]

Immunosuppressed

Precautions regarding the safety of mumps virus–containing vaccines in immunosuppressed persons are based on very limited data or anecdotal evidence and theoretical risks based on the known biology of the live viral components. MMR and MMRV vaccines are not recommended in persons with primary or acquired immunodeficiencies (including AIDS or severe immunosuppression associated with HIV infection) or in persons with leukemia, lymphomas, or other malignant neoplasms affecting the bone marrow or lymphatic system. These vaccines should also not be used in persons receiving systemic immunosuppressive therapy, including corticosteroids equal to or greater than 2 mg/kg of body weight or equal to or greater than 20 mg/day of prednisone or equivalent for persons who weigh more than 10 kg, when administered for 2 weeks or longer.[384] Use of MMR, but not MMRV, is recommended in asymptomatic HIV-infected persons without evidence of severe immunosuppression.

In Pregnancy

Because of a theoretical risk to the fetus, MMR vaccines should not be administered to pregnant women or women attempting to become pregnant. If vaccine is administered to a woman who is attempting to become pregnant, she should be advised to avoid becoming pregnant for 28 days after vaccination. Inadvertent vaccination with MMR during pregnancy should not be considered an indication for termination of pregnancy. The vaccine can be given to eligible persons who have pregnant household contacts.

Spread to Contacts

There are no reports of transmission of mumps vaccine virus from recipients of JL-containing vaccines to susceptible contacts. Horizontal transmission of the Urabe Am9, Leningrad-3, and Leningrad-Zagreb vaccine strains, although rare, has been demonstrated.[313,315–318] In such cases, mumps symptoms appeared within 4 to 6 weeks of vaccination of close contacts, consistent with vaccine virus shedding 2 to 4 weeks postvaccination, followed by a 2- to 4-week incubation period (see "Excretion of Vaccine Virus").

INDICATIONS FOR VACCINATION

In the United States, the ACIP recommends two doses of MMR II vaccine for all children and for certain high-risk groups of adolescents and adults, including international travelers, people attending universities and other higher educational institutions, and people who work at healthcare facilities.[349] ProQuad may be given instead of MMR II, with some limitations (see "Vaccine Use" above and "Contraindications and Precautions" below).

In general, people can be considered immune to mumps who have documentation of vaccination with live mumps virus vaccine on or after their first birthday, have laboratory evidence of mumps immunity, have documentation of physician-diagnosed mumps, or were born before 1957 (although during mumps outbreaks, vaccination with mumps-containing vaccine should be considered for people born before 1957).

On June 24, 2009, the ACIP revised its recommendations for evidence of mumps immunity for healthcare personnel to include documented administration of two doses of vaccine, or laboratory evidence of immunity or laboratory confirmation of disease. For unvaccinated personnel born before 1957 who lack laboratory evidence of mumps immunity or laboratory confirmation of mumps, healthcare facilities should consider vaccinating with two doses of mumps-containing vaccine at the appropriate interval.[385]

Laboratory testing for mumps susceptibility before vaccination is unnecessary.[349] There is no increased risk of adverse reactions to vaccination of people who are already immune. In certain situations, MMR II has been given as a third dose (see, "Postexposure Prophylaxis" and "Treatment" above). Very few adverse events, all nonserious, were reported and did not differ substantially from rates seen following one or two doses.[352,386] Seronegative persons who had a history of receipt of two doses of MMR II rapidly seroconverted in response to the third dose.[323]

CONTRAINDICATIONS AND PRECAUTIONS

Most mumps vaccines are produced in chick embryo cell culture and contain small quantities of ovalbumin; however, the risk for serious allergic reactions in people allergic to eggs is extremely low and therefore egg allergy is not a contraindication for vaccination. Cutaneous testing for identifying egg-allergic children is of limited value.[387–389]

Caution should be exercised when administering MMR II or ProQuad to people who have a history of an anaphylactic reaction to gelatin. Skin testing for gelatin sensitivity could be considered, but no specific protocols for this purpose have been published.[349] Persons with a history of anaphylactic

reactions to neomycin should not receive the vaccine; a history of contact dermatitis to neomycin is not a contraindication to vaccination. Mumps-containing vaccines used in the United States and most other countries do not contain penicillin.

Mumps-containing vaccine should be given at least 2 weeks before the administration of immunoglobulin or deferred until 3 months after such administration because of the concern that passively acquired antibody may interfere with response to the vaccine, although data from coadministration studies have found no evidence of such interference.[253]

Mumps-containing vaccine should not be given to pregnant women because of the theoretical risk of fetal damage. Likewise, vaccinated women should avoid pregnancy for 1 month after vaccination.[285]

People with severe febrile illnesses should in general not be vaccinated until they have recovered. Vaccination should not be postponed because of minor illness.

The measles virus component in mumps-containing vaccines may temporarily suppress tuberculin reactivity,[285] and therefore tuberculin testing should be done before vaccination, or 4 to 6 weeks afterward.

Untreated active tuberculosis and a history of thrombocytopenia/thrombocytopenic purpura are contraindications for MMR II or ProQuad vaccination. The benefits of immunity to MMR are thought to outweigh the risk of recurrence or exacerbation of thrombocytopenia after vaccination. However, if a prior episode of thrombocytopenia occurred shortly after vaccination, avoidance of a subsequent dose of MMR II or ProQuad should be considered.[285]

MMR II or ProQuad vaccine should not be given to people with acquired immunodeficiency or suppressed immunity (e.g., leukemia, lymphoma, generalized malignancy, or therapy with corticosteroids, alkylating drugs, antimetabolites, or radiation). An exception is children infected with HIV. MMR II vaccination is recommended for all HIV-infected people who do not have evidence of severe immunosuppression and for whom measles vaccination would otherwise be indicated. Because the immunologic response to vaccines may decrease as HIV disease progresses, HIV-infected infants without severe immunosuppression should be given MMR II at age 12 months and a second dose as soon as 1 month later.[349] Data are not available on safety, immunogenicity, or efficacy of ProQuad vaccine in HIV-infected children; consequently, this vaccine should not be used in this population.[285]

Patients with leukemia in remission who have not received chemotherapy in at least 3 months may receive mumps-containing vaccine, as may people who have received short-term (i.e., <2 weeks' duration) steroid therapy.[349] There are limited data on the safety and efficacy of MMR II or ProQuad vaccine in transplant recipients receiving immunosuppressive therapy. Because the diseases prevented by MMR II or ProQuad do not result in significant morbidity (mumps, varicella) or are of a low contraction risk owing to population immunity (measles, rubella), vaccination should be avoided. However, during an outbreak, immunization may be advisable.[390] MMR II or ProQuad vaccination is recommended for persons completing immunosuppressive therapy who are determined to be immunocompetent.[285]

PUBLIC HEALTH CONSIDERATIONS

Epidemiologic Effects of Vaccination

United States

In the United States, reported cases of mumps decreased from more than 185,000 in the prevaccine era to fewer than 5000 cases within 5 years of the 1977 ACIP recommendation for inclusion of MMR in the national immunization schedule. In the years following implementation of a two-dose schedule in 1989, further declines were achieved (see Fig. 40.3). The dramatic reduction in reported cases of mumps in the United States since 1991 reflects increasing implementation of school immunization laws requiring two doses of MMR II. Between 1999 and 2005 fewer than 400 cases were being reported annually, a time period coinciding with the 1998 ACIP recommendation that all states take immediate steps to implement the two-dose MMR II schedule so that by the year 2001, all children in kindergarten through grade 12 received two doses of the vaccine.[349] Based on data from the 2015–16 school year, (the latest data available at the time of writing this chapter) median two-dose MMR vaccine coverage among kindergardeners in the United States was 94.6% and among adolescents aged 13 to 17 years was 90.7% (see "Vaccine Use").

Before widespread use of mumps vaccine, cases mostly occurred in children 5 to 9 years old. Following wider vaccine use, the average age of reported cases gradually increased, resulting in an increasing proportion of cases reported among people 15 years of age and older. From 1967 to 1971, only 8% of reported cases were among this older group, compared with more than 33% from 1987 to 1992.[392,393] This shift was an expected result of the implementation of immunization programs, which were directed at preschool-age children and led to widespread interruption of mumps virus transmission; a similar shift was previously observed for measles and rubella. National U.S. immunization policy was slow to endorse mumps vaccine as a routine immunization of childhood. Thus, a decade elapsed between licensure of the monovalent vaccine in 1967 and the recommendation at the end of 1977 that it be given routinely to all children (as part of MMR). Consequently, a cohort of children born between 1967 and 1977 (i.e., people 10 to 20 years old in 1987) grew up when the chance of exposure to wild mumps virus for a preschool-age or young school-age child was markedly declining while the opportunity to receive mumps vaccine was uncertain. The greater mobility of older children and adolescents increased the potential for contact among susceptible people in these age groups. This may explain the 1986–87 resurgence of mumps, with 7790 cases and 12,848 cases reported, respectively (see Fig. 40.3). Many of these outbreaks occurred in high schools,[334] colleges,[394,395] and the workplace.[396] Since 1989, reported cases of mumps again decreased. By 1992, the highest age-specific incidence shifted back to the 5- to 9-year-old group, but even in this group cases declined, a likely result of increasing implementation of school immunization requirements for two doses of MMR vaccine. The peak age of mumps acquisition remained in this age group in the United States until the multistate mumps outbreak in 2006 when the age-specific incidence shifted again to the older age group (Fig. 40.8).[397,398] However, unlike the 1986–1987 mumps resurgence, the mumps epidemic in 2006 was not caused by an unvaccinated cohort, as nearly all cases in many outbreaks occurred in persons with a history of two doses of vaccine and in highly vaccinated populations. In the years following the 2006 mumps outbreak, the highest age-specific incidence returned to the younger age group,[399,400] until the 2009–2010 outbreaks in New York and New Jersey, where, again, most cases occurred in young adults, many of whom had a history of receipt of two doses of vaccine.[391] The occurrence of mumps predominantly in young adults during outbreaks in highly vaccinated populations is now a common feature globally (see "Risk Factors for Vaccine Failure and Recent Outbreaks" above). Nonetheless, compared to the prevaccine era, mumps outbreaks are relatively uncommon; thus, whatever the cause of vaccine breakthroughs, the impact is small, highlighting the continued effectiveness of mumps vaccines.

From 1975 to 1984, there was approximately one death from mumps per 5000 reported cases and one case of mumps encephalitis per 500 reported cases (Fig. 40.9). Since 1984, these proportions have been more variable owing to smaller numbers of reported events, and since 1995, mumps encephalitis has not been a reportable disease in the United States.[401] Because of underreporting of mumps and mumps encephalitis, these figures probably overestimate the risk of mortality and underestimate the rate of mumps encephalitis. Indeed, before the introduction of mumps vaccination, mumps was the most commonly diagnosed cause of viral encephalitis in childhood in developed countries; introduction of routine vaccination has resulted in the virtual elimination of mumps encephalitis.[15,402] During the 2006 multistate mumps outbreak in the United States, of 1327 patients for whom clinical data were available, only four cases of encephalitis were reported, an incidence of 0.3%, which is similar to the incidence of mumps encephalitis reported before 1984.[403]

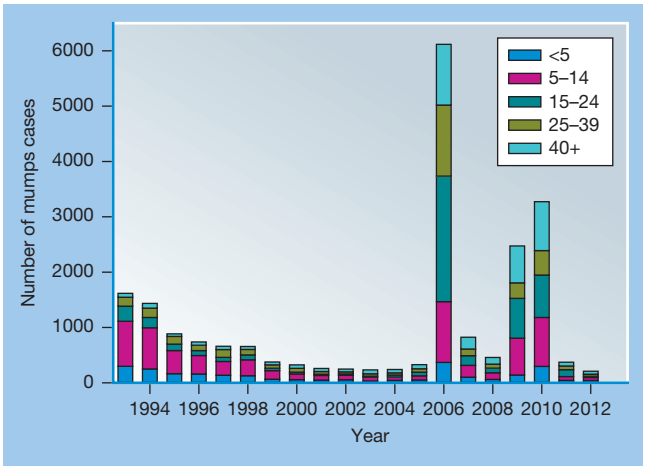


Figure 40.8. Reported cases of mumps by age group, United States, 1993–2012. In each year, the age distribution of cases of known age has been applied to the cases of unknown age. *(From the National Notifiable Diseases Surveillance System, Centers for Disease Control and Prevention.)*

Countries Other Than the United States

Although nearly all developed countries use mumps-containing vaccines, in many mumps is not a notifiable disease and vaccination is often voluntary. Nevertheless, mumps vaccination has had a tremendous impact where used, with reductions in incidence and morbidity exceeding 90%.[174,209,336,404–408] However, mumps has made a comeback in nearly all countries where the disease once had been under control. This disease, which was historically one of childhood, now predominantly occurs in young adults.[35,36,292,348,409–413] In many of these outbreaks, waning immunity has been postulated to be a major factor. However, unlike the U.S. outbreaks where it was clear that cases were occurring in fully vaccinated populations, in other countries, other factors may have had a role.

For example, at about the time of an outbreak in the Czech Republic, data reported by the public health authority indicated MMR vaccine coverage exceeded 97%; however, this did not correspond to data from a seroprevalence survey indicating that only 70% to 86% of persons 1 to 15 years old had mumps virus antibodies.[35,414] Similarly, in Austria, despite a one-dose national immunization schedule since 1974, and a two-dose schedule since 1994, nearly half of cases identified in a recent outbreak had not been previously vaccinated,[410] suggesting a lack of compliance with the recommended vaccine schedule. In Denmark, disease incidence increased 10-fold, from less than 0.1 case per 100,000 population in 2003 to nearly one case per 100,000 population in 2013, and was mostly attributed to low immunity in older age groups who grew up during a period when MMR vaccination was not routinely offered.[415] In China, mumps vaccine was introduced into the national routine immunization program in 2007, and has been used since in a one-dose regimen in infants 18 to 24 months of age; however, according to a recent publication, there is no clear evidence that disease incidence has declined.[416]

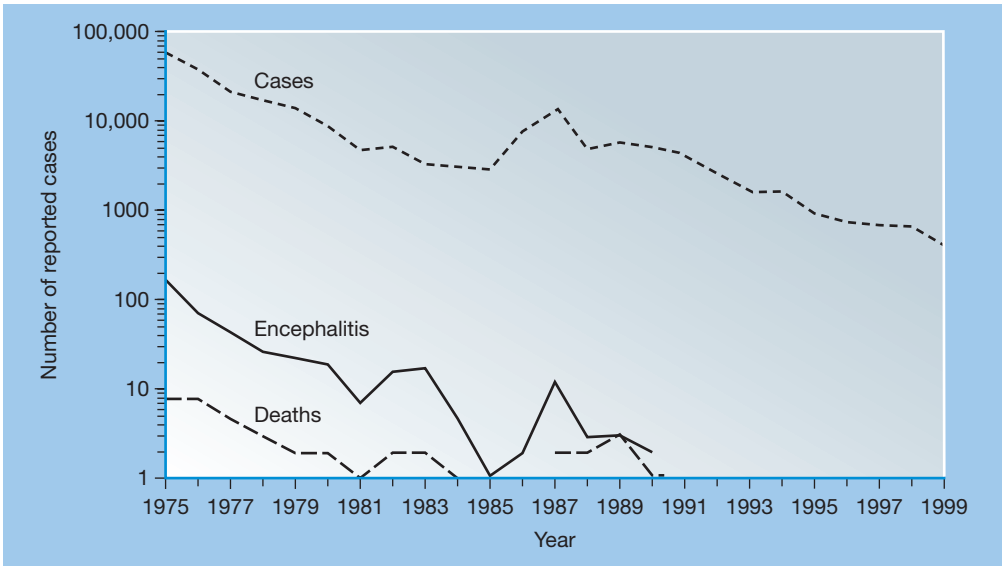


Figure 40.9. Reported cases of mumps, mumps encephalitis, and mumps deaths, United States, 1975–1999. Since 1990, two or fewer deaths reported annually, most are not likely mumps related. Data on mumps encephalitis not available after 1990. *(From the National Notifiable Diseases Surveillance System, Centers for Disease Control and Prevention.)*

In Israel and Spain, data suggest changes in population demographics may be responsible for the changing epidemiology of mumps. An assessment of mumps antibodies among Israeli military recruits conducted in 1987 revealed a seroprevalence of 94%; however, seroprevalence declined to 83% by 1999.[417] Immigration was identified as a contributing factor to the decrease in population seroprevalence over time. Similarly in the Catalonia region of Spain, a rise in the incidence of mumps in 2006–07 was thought to be partly from lower vaccine coverage in the immigrant population, which increased sixfold relative to the increase of the highly vaccinated indigenous population.[336]

In the former Yugoslav republics of Macedonia and Moldova, recent mumps outbreaks were attributed to missed opportunities for vaccination as a result of the timing of the vaccine schedule[36,412] resulting in unvaccinated or incompletely vaccinated cohorts, much as was the case in the U.S. outbreaks in 1986 and 1987. Missed opportunities for vaccination were also believed to be the primary cause of the resurgence of mumps in the United Kingdom, where routine vaccination with MMR at 12 to 15 months of age was introduced in 1988 and a second dose in 1996 at 3.5 to 5 years of age. The impact of vaccination on disease incidence was prompt, as reflected in notified cases in England and Wales, which decreased by more than 85% from 1989 to 1991, remaining at this level through 2002 (Fig. 40.10). Based on reports from sentinel surveillance, the most dramatic decrease occurred among children 0 to 14 years old. One-dose vaccine coverage reached 91% of 24-month-old children by 1996.[418] This trend of relatively low mumps incidence abruptly changed in 2004–2005, with more than 70,000 reported cases, mostly in persons 15 to 24 years old.[413,419–421] This outbreak was attributed in part to a shortage of MMR in 1994 when a measles vaccine catch-up campaign for 5- to 16-year-olds was initiated. Instead of using MMR, the bivalent measles-rubella vaccine was used.[421] Thus a large population of children born in the years 1978 to 1989 missed an opportunity for mumps vaccination. Indeed, the highest attack rate occurred in persons born between 1983 and 1986.[419] Notably, it was estimated that only 2.4% of confirmed cases in 2004 occurred in persons who would have been age-appropriate to participate in the routine two-dose MMR program.[413] This was confirmed by the data; only 3.3% of cases occurring in 2004 were reported as having received two doses of mumps-containing vaccine, and another 30% had received only one dose.[413] The number of confirmed mumps cases has declined, but still remains high with an average of 8600 cases reported annually between 2010 and 2014, a likely reflection of declining MMR coverage, which began in 1998, possibly in association with popularization of a published report linking MMR vaccination with autism.[422] The report was retracted in 2010 owing to scientific and ethical misconduct[423] and subsequent research found no evidence of such a link,[424–428] but the damage was done. By 2004, one-dose MMR coverage was down to 86% in most of England and less than 80% in London.[429] These percentages have improved since. As of the second quarter of 2016, one-dose MMR vaccine coverage among persons 2 years old in England was estimated at 91.4%, and 84.4% for London, and by 5 years of age, two-dose coverage was estimated at 87.5% and 80.2%, respectively.[430]

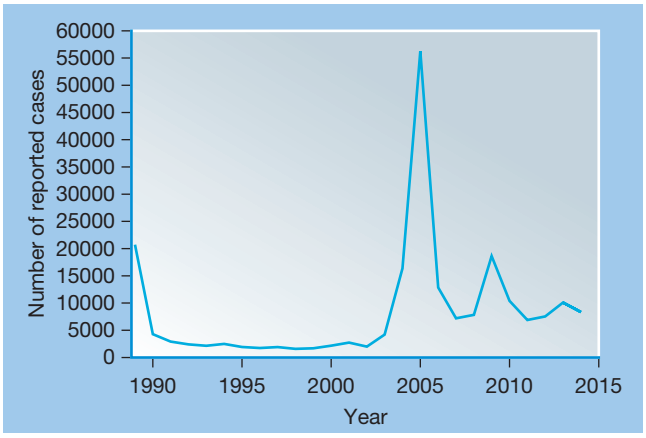


Figure 40.10. Number of mumps cases reported in England and Wales, 1989–2014. *(From Public Health England. Research and analysis: Notifiable diseases: historic annual totals. 2016. Available at: https://www.gov.uk/government/publications/notifiable-diseases-historic-annual-totals.)*

This situation contrasts sharply with that of Finland, where by 1986, more than 95% of eligible children had been vaccinated.[431] By 1994 there were fewer than 30 reported cases of laboratory-confirmed mumps and data indicate that these were probably imported.[432] By 1997, indigenous mumps disappeared from Finland, with only rare imported cases remaining.[347] Between 1997 and 2010, only 37 mumps cases had been diagnosed in Finland, despite an average of 5 million travelers to the country annually.[207] Based on data published by the WHO, no cases of mumps were reported in Finland in 2014 or 2015. In 2013, only one case was reported.[432a] The success of the Finnish program, achieved by the routine two-dose schedule of MMR vaccination, serves as a model for other countries that want to eliminate measles, rubella, and mumps.[433]

In other countries, the resurgence of mumps was found to be the cause, indirectly, of the vaccine itself. In Portugal, where routine vaccination was introduced in 1987, reports of unusually large numbers of cases began in 1994. During the first 8 months of 1996, 7620 cases were reported. Although incidence increased among all age groups, rates were highest among children 1 to 4 years old. This finding was unexpected because reported MMR vaccination coverage among children 12 to 23 months old had exceeded 90% since 1991. Three mumps vaccines—Urabe Am9, Rubini, and JL strains—were initially available in Portugal as MMR. Following recognition of aseptic meningitis after vaccination with Urabe Am9 mumps vaccine, the sale of Urabe Am9–containing vaccine was suspended in Portugal in October 1992, and subsequently only Rubini-containing MMR was used.[434] Use of the poorly efficacious Rubini strain as a substitute for the Urabe Am9 strain was followed by a mumps epidemic.[434] Similarly, despite vaccine coverage of 96% in Spain, protection against mumps was estimated to be 79% overall and was lowest in regions where the Rubini strain was used exclusively.[435]

The JL strain mumps vaccine (given as MMR II) first became available in Switzerland in 1971, and MMR vaccine including the Urabe Am9 strain was introduced in 1983. Routine vaccination of children was recommended by Swiss public health authorities in 1985. MMR containing the Rubini mumps vaccine strain (Triviraten) became available in 1986 and subsequently accounted for more than half the doses of MMR vaccine used in the country. Reports from a sentinel network of physicians demonstrated an increase in reported cases of mumps beginning in 1991. Increases in mumps cases were seen among all age groups but were most striking among children 5 to 9 years old. By 1993–94, 59% of reported cases were noted to occur in children previously vaccinated, compared with only 9% in 1986–87.[436] A 1991–92 seroprevalence survey found mumps immunity in young children to lag behind that for measles and rubella, perhaps reflecting the relatively poor immunogenicity of the Rubini strain.[437]

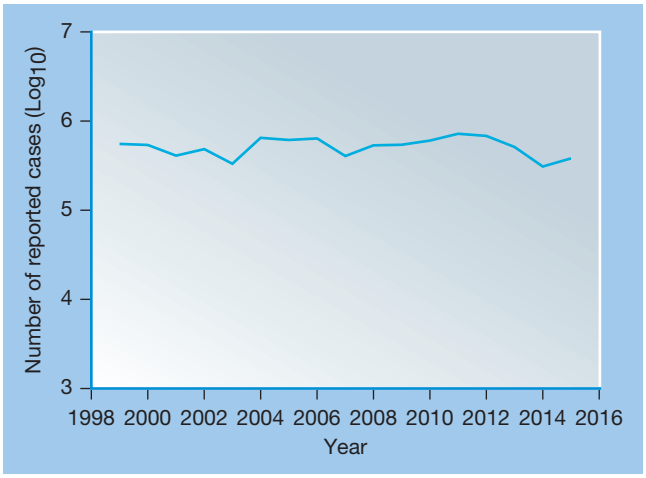


Figure 40.11. Mumps cases reported to the World Health Organization from its member states between 1999 and 2015. *(From World Health Organization. WHO vaccine-preventable diseases: monitoring system 2016 global summary. Available from: http://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencemumps.html.)*

The WHO received reports of 384,195 mumps cases during 2015 from its member states, which is a 24% decline from 2 years earlier, but not a significant deviation from case numbers on whole over the past 15 years (Fig. 40.11). The numbers are gross underestimates as many of the member states do not immunize against mumps and report no cases.[438]

Cost-to-Benefit Information

Cost-to-benefit analyses show that US$7 to US$14 are saved for every dollar spent on a program of vaccination with monovalent mumps vaccine or MMR vaccine.[439,440] Unpublished data from the Centers for Disease Control and Prevention (CDC) found similar cost-to-benefit ratios: 6.1 for direct costs and 13.0 for direct and indirect costs for use of monovalent mumps vaccine, and for MMR vaccine 16.3 for direct costs and 21.3 for direct and indirect costs. Zhou and colleagues estimated that more than US$1.5 billion in costs are averted annually by vaccinating against mumps, with a saving of US$3614 per case prevented per year.[441]

DISEASE CONTROL STRATEGIES AND POSSIBLE ERADICATION

To control the spread of disease, the CDC and the American Academy of Pediatrics recommend that persons with mumps be maintained in isolation with standard precautions and droplet precautions for 5 days after onset of parotitis.[442] In schools and colleges experiencing an outbreak, the CDC recommends that unvaccinated students be excluded and readmitted only after they have been vaccinated. Students with a history of one dose of vaccine should receive their second dose and be allowed to remain in school. Students who have been exempted from mumps vaccination for medical, religious, or other reasons should be excluded from school until the 26th day after the onset of parotitis in the last person with mumps in the affected school.[349]

A critical issue for further study concerns the extent and duration of protection conferred by two doses of mumps vaccine per the current administration schedule. Widespread use of the live virus mumps vaccine since U.S. licensure in 1967 has resulted in an exceptional decrease in the incidence of mumps, but continuing outbreaks in highly vaccinated populations worldwide illustrate the potential for continued epidemics regardless of vaccine coverage. A review of the literature shows that close to 95% of children respond to their first dose of mumps vaccine with virus-neutralizing antibodies, and nearly all those who do not will seroconvert following a second dose.[260] However, as reviewed in this chapter, a substantial proportion of vaccinees become seronegative or demonstrate significant declines in antibody titers over time (see "Persistence of Antibodies" above). In parallel, the effectiveness of mumps vaccines seems to decline with time postvaccination as well (see "Risk Factors for Vaccine Failure and Recent Outbreaks" above). These observations are suggestive of waning immunity. In the context of low levels of virus-neutralizing antibody, antigenic differences between vaccine strains and contemporary circulating viruses may be of more significance than previously appreciated. Together, these concerns indicate possible utility of an additional dose of vaccine during adolescence or later. The potential effectiveness of an additional dose later in life was suggested in studies where the MMR vaccine was used as a control measure in school-based outbreaks (see "Postexposure Prophylaxis" above). Although the mumps attack rates in students receiving the intervention declined substantially in these studies, conclusions could not be drawn because of study power as well as the caveat of the intervention being initiated after the outbreak started to decline. Nonetheless, use of an additional dose of vaccine during an outbreak would seem prudent. This notion is supported by data on the immunogenicity of a third dose of MMR among 685 young adults in a non-outbreak setting as reported by Fiebelkorn and colleagues.[443] In that study, the GMT of virus-neutralizing antibody titers was observed to significantly increase in response to the third dose when measured 1 month after vaccination. However, at 1 year postvaccination, the GMT retreated close to baseline, suggesting only a transient benefit of a third dose of vaccine, making it difficult to justify a change to the current MMR vaccine schedule for routine vaccine use. Unfortunately, other biological indicators of improved immunity, such as more robust B-cell memory or higher antibody avidity, were not examined.

FUTURE VACCINES

As a consequence of the resurgence of mumps over the past decade despite high vaccine coverage, changes to the current vaccination schedule or development of new, more effective vaccines have been suggested. As described in "Disease Control Strategies and Possible Eradication" above, changing from a two-dose to a three-dose MMR schedule was evaluated, but the results raised doubt whether this or any other change to the vaccination schedule would improve the long-term effectiveness of the vaccine. This leaves development of new vaccines as the only practical approach to improving vaccine performance; however, prospectively designing improved vaccines is predicated on knowing what is responsible for the less-than-desirable performance of existing vaccines, as well as knowledge of reliable markers of vaccine efficacy, neither of which are known. In the absence of a clear understanding of factors that influence the kinetics, robustness, and duration of meaningful immune responses to mumps vaccination, and how these parameters can be reliably measured, there is little promise of new vaccines performing any better than existing vaccines.

Some have suggested that the problem is one of a mismatch between vaccine genotype and circulating virus genotype; however, genotype-matching of the vaccine to circulating

strains is impractical, given the time required to conduct clinical trials supportive of vaccine safety and efficacy and the changing molecular epidemiology of the virus. Furthermore, as discussed in "Risk Factors for Vaccine Failure and Recent Outbreaks" above, data linking a difference between vaccine virus and circulating virus genotypes to vaccine failure are unconvincing, particularly in light of data to the contrary, including laboratory data[322,345] and empirical data, such as the fact that disease incidence in the United States and other countries has declined by well over 90% in association with the wide use of the genotype A JL vaccine, in the face of wild-type circulating mumps virus strains belonging to many different genotypes.[444]

Perhaps the best leads toward improved vaccines come from studies on B-cell responses to vaccination. Not only are levels of mumps virus–specific antibody-secreting cells following vaccination significantly lower than levels induced by natural infection, but among the viral components of the MMR vaccine, far fewer mumps virus–specific memory B cells are generated than measles- and rubella-specific memory B cells.[270] Furthermore, the avidity of MMR vaccine-induced mumps antibodies are much lower than the avidity of the measles and rubella antibodies measured in the same subjects.[251] Whether these issues are peculiar to responses to mumps viruses in general, or if they are vaccine virus strain specific, is not known. However, data from a recent study of antibody avidity among persons developing antibody in response to vaccination versus natural infection, low antibody avidity was characteristic of both groups, suggesting that mumps virus, regardless of strain (wild type vs vaccine), does not engender high avidity antibody upon initial exposure.[444a] On the other hand, an immunoinformatics study using software to predict B-cell linear epitopes and major histocompatibility complex binding affinities suggested that low antibody avidity and B-cell memory might be specific to the JL mumps virus vaccine.[338] These data come from single small studies and require further investigation, but do provide some direction for future work in this area.

Overall, however, despite less-than-ideal performance by mumps vaccines, it must be kept in mind that the preponderance of data demonstrate these products to be highly effective and solely responsible for the near elimination of this once ubiquitous disease in all countries where mumps-containing vaccine is rigorously used.

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41 Noninfectious Disease Vaccines

Aadil El-Turabi and Martin F. Bachmann

OVERVIEW

This chapter provides an overview of the challenges and latest advances in vaccine development for chronic and noncommunicable diseases. Because of space limitations it is not possible to provide an exhaustive representation of the field; consequently, we address a few major themes with examples of challenges and vaccine-based solutions. There also is a vast literature on T-cell–based cancer vaccines, which are discussed more comprehensively in Chapter 13.

The incidence of many chronic conditions increases with age and with more individuals living longer, this places operational and financial pressures on healthcare systems. More than 90% of all medications administered to patients during their lifetimes are administered in the last 10 years of life. Some estimates suggest that a staggering one-third of all healthcare costs will be expended in the final year of life.[1–3]

INTRODUCTION

Sizeable global demographic changes are leading to significant challenges for professionals charged with preserving the health and well-being of the population in the first century of the new millennium. There have been many successes in combating once prevalent global infectious diseases through vaccines and antimicrobials, leading to an estimated net increase in life expectancy of nearly 30 years in industrialized countries over the past decade.[4,5] Indeed, the "maturing" of global societies is a major achievement, with people today living longer and healthier lives than previous generations (with the World Health Organization quoting a global figure of an average life-expectancy increase of 20 years compared to 50 years ago[6]). This demographic change offers enormous social and economic benefits in opportunities to harness the experience, expertise, and creativity of a large number of older people. However, it also brings difficulties caring for an aging population, given that healthcare costs associated with treating and caring for the elderly are considerable and are likely to increase. In addition the prevalence of chronic diseases, long overlooked in the global context, has swiftly emerged as a major problem accounting for 48% of all global mortality.[7] Although much of this can be addressed by existing knowledge and cost-effective solutions, there are many as yet unmet medical challenges that require novel therapies.

One important objective must be to preserve health, prevent disease by encouraging "good" habits (e.g., exercise and nutrition), and intervene early in disease processes before they become debilitating. Of course this presumes that we have the means to detect diseases early, effective tools for medical interventions, and accessibility to the population at risk (both in terms of compliance and costs). Vaccines are highly successful in preventing infectious and communicable diseases; thus there is precedent for the goal of repeating such feats in the combating of noncommunicable diseases.

Monoclonal antibodies targeting self-proteins have provided much needed relief and life-altering therapies for several chronic diseases.[8–12] Current examples include anti–tumor necrosis factor (TNF) drugs such as adalimumab (Humira), certolizumab pegol (Cimzia), etanercept (Enbrel), golimumab (Simponi), and infliximab (Remicade) for rheumatoid arthritis, ankylosing spondylitis, and Crohn disease. These products have been extensively evaluated in preclinical and clinical efficacy trials prior to their widespread use in clinical practice.[9–12] These treatments provide rapid therapeutic effects with a high degree of specificity inherent in antibody-target interactions, but are expensive, require fairly large doses, and must be given frequently. In addition, the potential for secondary treatment failures, when recipients mount an immune response against the therapeutic agent,[8,13,14] necessitates the development of alternative therapies.

The possibility to replace the passive immunization approaches with active immunization is an attractive proposition and presents opportunities for improved compliance (fewer injections), reduced risk of secondary failure (as the immune system is encouraged to produce an antibody response), and lower cost (smaller doses and cost-effective manufacture of vaccines).[15,16] However, this entails the not insignificant task of inducing a safe and efficient immune response against self-antigens.

OVERCOMING THE IMMUNE SYSTEM'S SAFETY MECHANISMS—UNRESPONSIVENESS AND TOLERANCE

In general, humans do not generate antibody responses against self-antigens even if they are given with adjuvants, because the immune system has evolved a set of checkpoints to avoid autoreactive responses. Antiself immune responses are inhibited by several different mechanisms, including deletion of self-reactive lymphocytes (elimination of cells), anergy (functional silencing), and lack of stimulation (immune-competent lymphocytes are present in the periphery but are not stimulated, because they do not encounter the self-antigen in an immunogenic context).[17,18] This unresponsiveness toward self, also referred to as tolerance, occurs at the T- and/or B-cell level. Whereas autoreactive T cells are efficiently deleted or silenced in the thymus, B-cell tolerance is mainly induced in the bone marrow. Both B- and T-cell tolerance also occurs in the periphery. A key feature of deletion of B cells in the periphery is the crosslinking of the B-cell receptor (BCR). Hence, B cells specific for highly expressed membrane proteins are eliminated particularly efficiently, whereas B cells specific for soluble antigens typically escape negative selection and remain in circulation.[19] In contrast, because of the expression of most antigens in the thymus and the transport of soluble antigens to the thymus, T-cell tolerance, in particular T-helper (Th) cell tolerance, is a much more efficient process. Thus, while most autoreactive T cells are eliminated, a largely normal B-cell repertoire exists for secreted and soluble self-antigens. Importantly, because naïve B cells critically depend on T-cell help to proliferate, undergo isotype switching, and differentiate into long-lived plasma cells, deletion or silencing of autoreactive T cells avoids self-specific B-cell responses and maintains B-cell unresponsiveness. Nevertheless, low-affinity autoreactive T cells can escape the thymic and peripheral tolerance processes and may be activated by administration of the antigen in the presence of strong adjuvants such as complete

Freund adjuvant.[20] Bypassing or circumventing T-cell tolerance appears to be the method of choice for the induction of antibodies against self-antigens. This is best achieved by fusing a strong foreign T-cell epitope to the self-antigen or by conjugating the self-antigen to a foreign carrier protein.

INDUCTION OF B-CELL RESPONSES

For a B cell to generate a long-term response, two signals are needed: activation of the BCR and stimulation by antigen-specific Th cells. Through covalent linkage of an antigen to a carrier molecule, B cells expressing a BCR specific for the antigen will acquire carrier-specific T-help (Fig. 41.1).[21] Carrier molecules successfully used for licensed glycoconjugate vaccines are pathogen-derived proteins like diphtheria toxoid, tetanus toxoid, meningococcus B outer membrane protein C, or *Haemophilus influenzae* protein D. Other clinically tested carriers include toxin B, *Pseudomonas aeruginosa* exoprotein A, and keyhole limpet hemocyanin (KLH). Well established virus-like particle (VLP) systems (such as hepatitis B vaccine and human papillomavirus vaccine) induce excellent immune responses with increased attention to finding ways to generate responses to heterologous antigens. VLPs share many of the features of whole viruses but are not infectious. Their particulate nature, their highly ordered repetitive structure, and their Th cell epitopes are the source of their remarkable immunogenicity.[22–24]

For this reason, VLPs have gained interest as platforms for vaccines against noninfectious diseases.[25–29] Bacteriophage Qβ-derived VLPs, which are readily produced in large quantities under good manufacturing practices in *E. coli*, have been extensively tested as immunological carriers in animals and humans.[21–29] Using chemical heterobifunctional crosslinkers,[30,31] antigens can be directionally conjugated to their surface and presented in a highly ordered fashion to B cells. As a result BCRs are efficiently crosslinked, inducing potent immune responses.[32] Moreover, during self assembly in *Escherichia coli*, host RNA, a potent TLR7/8 agonist, is packaged into the VLPs acting as an adjuvant, thereby enhancing B-cell response and inducing class switching.[33] In light of these virtues, bacteriophage VLPs have been successfully used as carriers to induce potent immune responses against self-antigens and haptens in various preclinical[34–42] and clinical studies.[25–27,43] These targets include examples such as tumor necrosis factor, interleukin-1β, angiotensin II, and amyloid beta (discussed later).

LONGEVITY OF THE RESPONSE AND GENERAL SAFETY CONSIDERATIONS

High antibody titers against a given target could be maintained by yearly boosting. In contrast, the memory responses to classical vaccines are boosted with subsequent exposure to the pathogen. Patients suffering from a chronic disease or drug addiction are not naturally exposed to the antigen in an immunogenic form, because self-antigens are seen by B cells in the absence of specific Th (which had been delivered by the carrier during vaccination).[18,20] Hence, regular booster

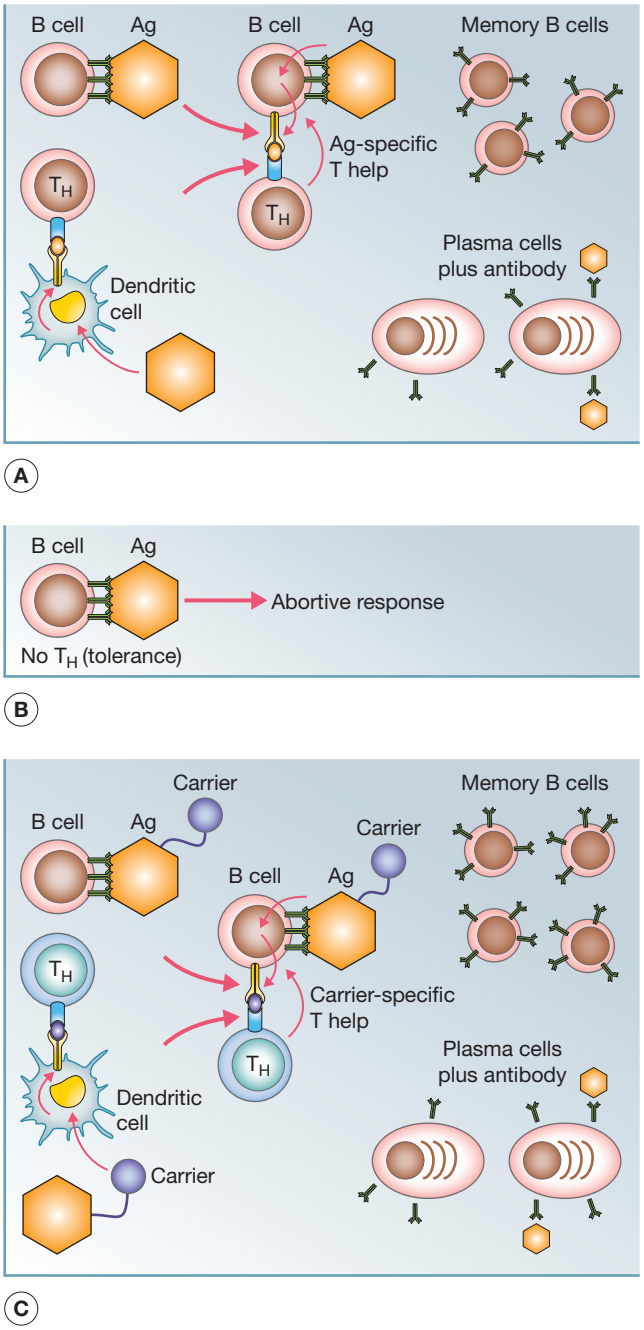


Figure 41.1. How B cells and T_H cells collaborate to circumvent T_H cell tolerance. A, After immunization with a foreign antigen, specific B cells bind the antigen (Ag), inducing an initial activation signal. In addition, B cells endocytose the antigen and display antigen-derived peptides on their major histocompatibility complex (MHC) class II molecules. B cells are not usually able to activate naïve T_H cells, and dendritic cells are an essential intermediate in the activation of T cells. Dendritic cells also take up the antigen and process it for presentation in association with MHC class II molecules. In contrast to B cells, dendritic cells are able to trigger the activation of naïve T_H cells. These activated T_H cells recognize antigen-derived peptides on B cells, leading to successful T- and B-cell collaboration: B cells proliferate, produce antibody, and undergo isotype switching. **B,** In the absence of T_H cells resulting from tolerance, antibody responses remain abortive. **C,** It is possible to circumvent T_H-cell tolerance by coupling the self-antigen to a foreign protein or peptide carrier. B cells specific for the self-antigen are able to take up the antigen plus the linked carrier and present carrier-derived peptides on their MHC class II molecules. As there is no T_H-cell tolerance to the carrier protein, a regular antibody response against the self-molecule will now be mounted. *(From Bachmann MF, Dyer MR: Therapeutic vaccination for chronic diseases: a new class of drugs in sight.* Nat Rev Drug Discov. *2004;3:81–88.)*

immunization may be required to maintain antibody titers at therapeutic levels.[44] The waning of the antibody response in the absence of continued boosting may be advantageous from a safety perspective, particularly if the vaccine targets self-antigens. In this case, lifelong immune responses may not be desired or required, and therapy could be stopped after improvement in health or if antibody-mediated adverse events occur.

For B-cell vaccines different adverse events may be seen and must be carefully assessed; toxic effects may arise and need to be addressed in detail in preclinical models and early phase clinical trials in a target-specific manner.[44] Such adverse effects include generation of immune complexes with soluble target molecules or binding to self-antigens expressed on tissue with the subsequent induction of local inflammation by activation of antibody-dependent effector cells. Consequently, not all antigens make appropriate targets for vaccine approaches. In general, rare proteins may cause fewer problems than abundant proteins, as only low amounts of immune complexes have the potential to be formed because of a decreased chance of interactions occurring. Soluble proteins are generally preferred over membrane-bound targets because of the absence of antibody-dependent cellular cytotoxicity, which may only be observed with membrane proteins.[21] Moreover, many membrane proteins are receptors, and antibodies can be either antagonistic (prevention of binding to the ligand) or agonistic (activating the receptor through crosslinking) to receptor engagement. This is problematic, as it is virtually impossible to exclude undesired agonistic antibody using a vaccination approach.[44–47] Finally, safety issues associated with the neutralization of the target molecule also have to be considered. These issues are best addressed in studies using target-specific monoclonal antibodies (mAbs), decoy receptors, or receptor antagonists.[44–46] Valuable information can also be obtained from evaluating the clinical manifestations in rare human deficiencies of the target molecule, or in animal models rendered genetically or physiologically deficient for the target molecules.[41,48–52]

TARGETS FOR THERAPEUTIC B-CELL VACCINATION

In accordance with the better safety profile of immunodrugs against secreted antigens,[29] many therapeutic vaccination approaches in humans have targeted such molecules. Meanwhile 18 self-antigens have been targeted in humans by 31 different vaccines in various indications (summarized in Table 41.1). Many of these molecules are also targets for small molecule pharmaceuticals or biopharmaceuticals and as such have been validated as appropriate therapeutic targets.

VACCINE APPROACHES FOR DEMENTIA

There is a critical need to address the rising tide of dementia in the population. The vast majority of dementia cases correlate with increasing age. This fact is of particular importance in developed countries where decreasing birth rates and increasing life expectancy are raising the average age of the population (e.g., in 2007 for the first time in the United Kingdom those older than 65 years of age outnumbered those younger than 16 years of age).[53] As a global trend, the associated healthcare costs associated with dementia represent enormous social and economic strains for both families and governments. Currently, only symptomatic treatments are available, but therapeutic strategies to prevent, mitigate, or delay the onset of dementia are being vigorously pursued.[54]

Although cognitive impairment ranges from mild to severe, neurodegenerative diseases are the most severe and are thought to arise from misfolded/aggregated proteins spreading throughout the brain.[55]

Alzheimer Disease Is a Leading Cause of Dementia

Alzheimer disease (AD) is a major contributor to dementia, with the risk of acquiring AD increasing exponentially with age. At 60 years of age approximately 5% of people are affected, while at 85 years more than 50% suffer dementias. AD and dementias currently affect 47 million people worldwide and more than 5 million Americans, numbers that are expected to increase threefold by 2050, according to the Alzheimer's Association.[56] Current estimates of the costs of therapy, particularly nursing care, are staggering, with approximately $200 billion spent in United States alone.[56,57] One of the most promising therapeutic concepts is active or passive immunization against amyloid plaques.[48]

The major pathological hallmarks of AD are plaques formed by aggregated amyloid-β ($A\beta_{1-40}$ and $A\beta_{1-42}$), cleavage products of the amyloid precursor protein (APP), and other proteins (e.g., tau) that form neurofibrillary tangles. Biochemical and genetic studies of APP and presenilin 1 mutations, all of which enhance amyloid deposition and are associated with early-onset familial AD,[49] strongly support the central role of amyloid plaque forming Aβ in the development of the disease.

In 1999, Schenk and colleagues[50] reported that active Aβ immunotherapy reduces Aβ pathology in APP transgenic mice. In these studies, immunization with aggregated Aβ peptides combined with strong adjuvants resulted in reduced plaque burden[50] and improved mental performance.[58] This finding was confirmed in several transgenic models of AD with active and passive immunization.[59] In light of these results Elan/Wyeth initiated a number of clinical studies with a vaccine consisting of $A\beta_{1-42}$ (AN1792) administered with adjuvant QS21. In Phase I trials Aβ-specific antibodies could be found in more than half of the treated AD patients without notable adverse events.[60]

Subsequently, AN1792/QS21 vaccine was tested in a Phase II trial with 372 AD patients to evaluate the efficacy of the treatment. However, this trial was discontinued prematurely[61] after 6% of the vaccine recipients developed aseptic meningoencephalitis in the clinical study.[62] Subsequent analysis revealed that the side effects did not correlate with antibody titers.[63–65] Moreover, infiltrating T cells were found in postmortem samples from the brain of one diseased patient,[66] which strongly suggested that Aβ-specific T cells were the cause for the side effects observed.[66,67] Considering that this vaccine did not contain a foreign Th cell epitope, induction of an immunoglobulin (Ig) G response had to rely on breaking tolerance of self to induce $A\beta_{1-42}$-specific $CD4^+$ Th cells (see Fig. 41.1), which presumably triggered meningoencephalitis in some of the patients. This was most likely achieved by the combination of fibrillary $A\beta_{1-42}$ aggregates with the strong adjuvant QS21. Intriguingly, individuals who had the highest antibody response in the Phase II trial showed a significantly slower decline in some tests of cognitive function when compared with controls, and despite the severe safety issues observed with AN1792, this vaccine provided some evidence that active vaccination with Aβ may provide some benefit for the treatment of AD.[61–68] Importantly, these studies highlighted the necessity to avoid antigen-specific T-cell responses, and subsequent approaches were designed to avoid inducing T-cell immunity directed against Aβ.

The second-generation vaccines are characterized by short N-terminal Aβ peptides linked to foreign carrier proteins that compensate T-help functions for the efficient induction of IgG

TABLE 41.1 Clinically Tested B-Cell Vaccines Targeting Self Antigens

Target	Indication	Vaccine	Antigen	Organization
$A\beta_{1-40/42}$	Alzheimer disease	AN1792	$A\beta_{1-42}$	Wyeth/Elan
		ACC-001	$A\beta_{1-7}$-CRM197	Wyeth/Pfizer; Elan/Janssen
		CAD106	$A\beta_{1-6}$ -Qb	Novartis/Cytos Biotechnology
		V950	$A\beta_{Nterm}$ -OMPC(?)	Merck Co
		AD01, AD02	Affitope $A\beta_{1-6}$-KLH	AFFiRis/GlaxoSmithKline
		UB311	$A\beta_{1-14}$ fused to UBITh	United Biomedical
		AC-24	$A\beta_{1-14}$/liposome	AC Immune
Tau	Alzheimer disease and MSA	ACI-35	Phospho-tau	AC Immune/Janssen
	AD	AADvac1	Tau-peptide-KLH	Axon Neuroscience
Alpha synuclein	Parkinson disease and MSA	PD01A	α-syn peptide	AFFiRiS
		PD03A	α-syn peptide	AFFiRiS
Angiotensin I/II	Hypertension	AngQb	AngII-Qb	Cytos Biotechnology
		PMD3117	AngI-KLH	Protherics
Renin			Hog renin	Academic Groups
CETP	Hyperlipidemia	CETi	$CETP^{aa461-/476}$ fused to $TT^{aa830-843}$	Avant
hCG	Contraception	HSD-hCG	bHCG-TT	Indian government
FSH		oFSH	Ovin FSH	Indian government
GnRH		LH-RH-DT	GnRH -DT	Academic group
	Prostate cancer	D17DT	GnRH -DT	Aphton
EGF	NSC lung cancer	SAI-EGF	hEGF-TT (inactive)	Micomet/Cancer Vax
HER2	Breast cancer	Her2AutoVac	Truncated HER2 fused to TT epitopes	Pharmexa
Gastrin	Pancreatic Cancer	G17DT/Insegnia	Gastrin 17^{aa1-9}-DT	Aphton
Mucin	Cancer	Theratope/STn-KLH	Sialosyl-Tn -KLH	Oncothyreon/Merck KGaD
Ghrelin	Obesity	GhrQb	$Ghrelin^{aa1-8}$-Qb	Cytos Biotechnology
IgE	Allergic asthma	RP 01	CH2-CH3-CH4 of IgE	Resistentia
IL-1β	Type II diabetes	IL-1βQb	IL-1b mutein-Qb	Cytos Biotechnology
IFN-α	HIV, AIDS	i-IFN-α	IFN-α (inactive)	University of Milan
	systemic lupus erythematosus	IFN-K	rIFN-α-KLH (inactive)	Neovacs
TNF-α	Rheumatoid arthritis	TNF-α-kinoid	rTNF-α-KLH (inactive)	Neovacs
	Crohn disease	TNF-α-kinoid	rTNF-α-KLH (inactive)	Neovacs
	Cachexia	TNFAutoVac	TNF-α with internal TT T-cell epitopes	Pharmexa
	Psoriasis	TNFQb	TNF pep.-Qb	Cytos

Aβ, amyloid beta; CETP, cholesterol ester transfer protein; CRM, cross-reactive mutant; EGF, epidermal growth factor; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; HER, human epidermal growth receptor; hCG, human chorionic gonadotropin; IFN, interferon; Ig, immunoglobulin; IL, interleukin; KLH, keyhole limpet hemocyanin; MSA, multiple system atrophy; NSC, nonsmall-cell; OMPC, outer membrane protein C; rIFN, recombinant IFN; rTNF, recombinant TNF; TNF, tumor necrosis factor; TT, tetanus toxoid.

responses without triggering cytotoxic effects categorized by antigen-specific T-cell responses. The follow-up program for AN1792 uses a vaccine consisting of the N-terminal seven amino acids of Aβ covalently linked to the diphtheria toxin cross-reactive mutant (CRM197) protein carrier, which is called ACC-001. Although this peptide is smaller than the minimum length of a T-cell epitope, strong $A\beta_{1-42}$-specific antibody responses were induced in transgenic mice that prevented plaque deposition and improved cognitive function. Several clinical trials involving ACC-001 have since concluded, albeit with limited release of results. In one study repeat administration of ACC-001 at three doses (3 μg, 10 μg, and 30 μg) with or without adjuvant (QS21) was generally safe and well tolerated.[69] In addition, it was noted that inclusion of the QS21 adjuvant was needed to generate high-titer responses. Sustained high-titer anti-Aβ IgG responses were observed, irrespective of the three ACC-001 doses tested.[69] Nevertheless, the company has discontinued the program,[70] presumably because of disappointing efficacy results.[69,71]

Novartis, using VLP technology licensed from Cytos Biotechnology, has one vaccine candidate in development for AD that also targets Aβ. This vaccine, CAD106, consists of $A\beta_{1-6}$ peptide covalently coupled to bacteriophage Qβ VLPs (Qb).[71] Several preclinical studies revealed that the vaccine induced high antibody titers and no Aβ-specific T cells when administered without adjuvant to mice, rabbits, and monkeys. Immunization with CAD106 prevented amyloid cortical plaque deposition in both APP23 and APP24 transgenic mouse models. Moreover, immunization resulted in a reduction of amyloid deposits in aged mice with advanced brain pathology.[72] Available reports from the first studies in humans with this compound suggest that the vaccine is well tolerated and induces Aβ-specific immune responses in the majority of the patients without inducing meningoencephalitis.[73–75] Novartis is planning a new study for treatment (CAD106 and a Beta site Amyloid precursor protein Cleaving Enzyme [BACE] inhibitor) on asymptomatic patients with the hope of delaying the onset of the dementia. Inhibition of BACE1 should

decrease production of $A\beta_{1-42}$, the main component of the plaques that contribute to AD pathology.[76]

Merck & Co. is also testing a vaccine consisting of an N-terminal Aβ peptide conjugated to outer membrane protein C (V950). Increasing doses of V950 formulated in aluminium adjuvant with or without ISCOMATRIX adjuvant are being tested in a multistage Phase I trial. Two other vaccines, AC-24 (AC Immune) and UB-311 (United Biomedical), have achieved preclinical proof-of-concept[77,78] and are in early clinical testing. AC-24 contains $A\beta_{1-15}$ embedded within a liposomal surface and UB-311 consists of $A\beta_{1-14}$ covalently linked to a proprietary helper T-cell technology (UBITh) and is administered together with a mineral salt adjuvant.

The AFFiRiS AD program, acquired by GlaxoSmithKline, is exploring short peptides mimicking the N-terminus of Aβ as immunogens to avoid the induction of autoreactive T cells. Two of these AFFITOPEs (AD01, AD02) demonstrated promising preclinical results[79] and subsequent Phase I trials in humans have shown the vaccine to be safe and well tolerated, but reports of antibody responses have not been published.[80,81] AD02 has proceeded to Phase II clinical testing with the goal of identifying an optimal dosage. The added value of these AFFITOPEs (they are not under patent protection by other groups so there is freedom to operate with yet another Aβ-specific vaccine) needs to be explored further for the aforementioned vaccines (AD01 and AD02) in particular, as it seems that these programs have been stopped.

Passive immunotherapies with mAbs against Aβ are in late Phase III clinical trials, and recently humanized mAb-based immunodrugs, bapineuzumab[82] and solanezumab,[83] which target the Aβ N-terminal and mid-domain, respectively, failed to meet their primary end points in enhancing cognition and improving activities of daily living.[82–84] Johnson & Johnson decided to halt their clinical development of bapineuzumab in August 2012[85] because negative trial results were likely as patients had mild-to-moderate AD and the amyloid pathology was too advanced. However, secondary analysis of solanezumab Phase III data indicated potential clinical benefits in the patient group with mild AD. An additional Phase III study is currently underway to confirm these findings (ClinicalTrials.gov NCT01900665, estimated primary completion October 2016, study completion October 2018).[86]

Using their aducanumab (BIIB037) mAb, Biogen recently observed in a Phase Ib study a statistically significant cognitive benefit in a prospectively defined (early AD) patient population. Furthermore, positron emission tomography (PET) scans indicated the highest amyloid reduction seen to date and the researchers also observed time- and dose-dependent benefits.[87,88]

In essence, what has been learned so far from a large number of clinical trials (see Table 41.2) is that (a) both the quality and quantity of antibody are critical and (b) the patients should not have advanced disease, where it may be unlikely that the damage caused by plaque formation can be reversed. It appears that plaque prevention would be easier to accomplish than to remove existing plaques and be left with the problem of "a hole in the brain," without regenerative solutions. Because suspected side effects would likely result from the interaction of antibodies with plaques, early immunization would appear to be optimal.[89]

A key advance for clinical development of AD vaccines is the ability to image the brain using PET with specific ligands such as (11)C-labeled Pittsburgh Compound-B ([11]C-PIB) to specifically visualize Alzheimer plaques.[90,91] (11)C-PIB is a radiotracer substance that recognizes Aβ plaques and therefore can be used to capture images from the living patients (as opposed to the gold standard, postmortem diagnosis). This imaging technique provides the opportunity to identify patients at early stages of disease, where pathogenesis may still be reversible. Using this technique, patients with familial AD have been demonstrated to have plaque deposition long before the onset of any symptoms.[90–92] In patients treated with mAbs, PET imaging has shown a reduction in plaque load in situ and may have a role as a surrogate marker for efficacy.[87–91] Further clinical validation of the effectiveness and safety of the imaging techniques, together with the identification of additional biomarkers or genetic markers for the disease, would help in assessing the impact of the prophylactic vaccines for the middle-aged or elderly in combating this devastating disease.

Given the mixed results of the mAb and vaccine trials, alternative targets are also being pursued. Additional targets have emerged, including the microtubule-associated protein tau. Misfolded and hyperphosphorylated tau protein aggregates extend to create the neurofibrillary tangles that are, in addition to the plaques, hallmarks of AD. Moreover, tau aggregates with Aβ and other proteins into insoluble inclusions of plaques and lesions. Small mobile forms of tau preceding aggregates are hypothesized to spread tau-related pathology among neurons. Reductions in insoluble tau and improved behavioral performance have been reported in transgenic mice[94,95] immunized with phospho tau peptides.[94–98] More recently, structural determinants of pathogenic tau–tau interactions were identified and provided data on the epitopes that would impair aggregation. A KLH-conjugated tau peptide vaccine administered in an AD experimental mouse model drastically reduced the incidence of pathological hyperphosphorylated tau.[99–101] The Axon Neuroscience SE group recently completed a clinical trial of the KLH-conjugated tau peptide vaccine (NCT1850238) with reported favorable safety results.[100,101] A Phase II study of the tau immunotherapy (AADvac1) is planned.

In addition, AC Immune and Janssen have signed an agreement to take ACI-35, designed to produce antibodies against the pathogenic, misfolded, and phosphorylated versions of tau protein, into Phase II clinical studies. In earlier preclinical testing this vaccine stimulated antibody responses that were highly specific to pathogenic tau and resulted in a reduction of both misfolded and phosphorylated tau and improvement in cognitive clinical parameters in the mouse model. ACI-35 has a T-cell–independent mechanism of action and an excellent safety profile.[102,103]

These approaches are hoped to emulate the successes and avoid the failures of current and previous anti-Aβ strategies, respectively, in addressing neurodegenerative diseases. Moreover, while active immunization with Aβ resulted in the clearance of Aβ, it did not prevent tau pathology or neurodegeneration, prompting the concern that it might be too late to employ Aβ targeting therapies once tau-mediated neurodegeneration has occurred. Tau-directed therapies might circumvent these limitations. Despite intense clinical development, key questions remain about the utility of immunotherapy in slowing or reversing disease progression with an acceptable risk-to-benefit ratio.

Parkinson Disease

Parkinson disease (PD) is a neurodegenerative condition, second in frequency only to AD, manifesting as characteristic movement disorder, associated cognitive impairment, and progressive disability. There are an estimated 7 to 10 million patients with PD worldwide, affecting approximately 1% of individuals older than 60 years.[104–106] Current treatments for PD using levodopa, a dopamine precursor, and other dopamine agonists are effective at managing the early motor symptoms of the disease. However, as the disease progresses,

TABLE 41.2 Clinical Trials With B-Cell Vaccines Against Alzheimer Disease[a]

Vaccine Name	Phase	Treatment Group	Regimen (Month/Route)	Readouts Beyond Immunogenicity, Safety, & Tolerability (Mostly Secondary End Points)	N	Trial No. (NCT)/ Completion Date
AN1792	I	QS21 (50, 100 μg) AN-1792 (50, 225 μg) + QS21 (50, 100 μg)	0, 1, 3, 6, & 16/IM		80	Not registered
	II	Placebo AN-1792 + QS21	0, 1, 3, 6, 9, & 12/IM	Pilot efficacy assessments of cognition and memory	375	00021723_Term.
ACC-001	II	Placebo (PBS & QS21) ACC-001 (3, 10, 30 μg) + QS21 (50 μg)	0, 1, 3, 6, 12/IM		120	00479557_03/2013
					240	00498602_03/2012
			0, 3, 6, 9, & 12/IM	Baseline change of: ADAS-Cog, DAD, NTB MMSE scores	40	00752232_09/2012
		QS21 ACC-001 (3,10, 30 μg) + QS21 (50 μg)	0, 1, 3, 6, & 12/IM	Baseline change of: ADAS-Cog, DAD, NTB MMSE scores	32	00959192_01/2013
		PBS ACC-001 (3, 10 μg) + QS21 (50 μg)	n.d.	Efficacy, amyloid imaging by PET, disease biomarkers, cognitive and functional difference	108	01284387_08_2014
			0, 1, 3, 6, 12, & 18/IM	Capacity to reduce brain amyloid load as determined by PET analysis of (compared to baseline): disease biomarkers, cognitive and functional difference (subjects with early AD)	108	01227564_09/2014
		ACC-001 (3,10, 30 μg) + QS21 (50 μg)	0, 6, 12, & 18/IM		120	00955409_11/2014
				Baseline change of: ADAS-Cog, DAD, NTB and MMSE scores	67	01238991_09/2014
					160	00960531_09/2014
CAD106	I	Placebo, CAD106 (50 μg) CAD106 (150 μg)	0, 1.5 & 4.5, 0.5 & 1.5	Cognitive and functional assessments	60	00411580_03/2008
	II	Placebo, CAD106	Repeated SC injections		31	00795418_11/2010 00956410[b]
					27	00733863_02/2010 01023685[b]
		Placebo (Ad1 [md] or Ad2 [md]) CAD106 150 μg + Ad1 & Ad2 (ld & md) CAD106 450 μg + Ad1 or Ad2 (ld & md)	Repeated IM injections	Biomarker changes: $A\beta_{1-40/42}$, total tau, Phospho-Tau in cerebrospinal fluid	120	I01097096_12/2012
V950	I	Placebo (IM: 0 or 16 μg) V950(0.5, 5, & 50 μg) + IM(0, 16, 47 & 94 μg) Additionally aluminum adjuvanted	0, 2, & 6/IM (n = 124)		124	00464334_02/2012
AD01	I	AD01 AD01 + Alum	0, 1, 2, & 3/SC	Exploratory clinical efficacy analysis	24	00495417_08/2009 00711139[b],01225809[b]
AD02	I	AD02 AD02 + Alum	0, 1, 2, & 3/SC Boost (n.d.)		24	00633841_09/2009 01093664[b] 0711321[b]
AD02	II	Placebo AD02 (3 different doses)	n.d.	Efficacy cognitive (ADAS-Cog) and functional (ADCS-ADL), cognitive (computerized test battery), global (CDR-sb), behavior (NPI) biomarker (volumetric MRI)	420	01117818_10/2012
UB311	I	UB311	0, 1, & 3/IM	Exploratory efficacy	18	00965588_12/2010 01189084[b]
AC-24	I/II	Placebo/AC-24, 3 different doses (n.d.)	(n.d.)	Exploratory clinical outcome	n.d	Not registered

[a]All studies are performed in mild to moderate AD patients (if not otherwise stated) and look at immunogenicity, safety, and tolerability plus different mostly exploratory readouts.
[b]Extension studies.
Aβ, amyloid-β; Ad1, adjuvant 1; Ad2, adjuvant 2; AD, Alzheimer disease; ADAS-Cog, Alzheimer Disease Assessment Scale, cognitive subscale; ADCS-ADL, Alzheimer Disease Assessment Scale–activities of daily living; DAD, diagnostic assessment of dementia; IM, intramuscular; ld, low dose; md, medium dose; MMSE, Mini Mental State Examination; MRI, magnetic resonance imaging; n.d., not disclosed; NPI, Neuropsychiatric Inventory agitation subscale; NTB, neuropsychological test battery; PET, positron emission tomography; SC, subcutaneous.

dopaminergic neurons are lost and the drugs become less effective. There are no available alternative treatments at this time. The two major neuropathologic findings in patients with PD are the loss of dopaminergic neurons of the mid brain, particularly the substantia nigra pars compacta, and the presence of Lewy bodies and Lewy neurites, consisting of aggregated alpha-synuclein (α-syn). The abnormal accumulations of fibrillar α-syn in Lewy bodies, and mutations in the gene for α-syn in familial forms of PD, have led to the hypothesis that α-syn has a central role in PD. The accumulation of α-syn correlates with the loss of dopaminergic neurons and the subsequent decline in motor performance, making it a highly relevant molecular target for diagnostic approaches.[107,108]

Similar to AD, researchers have identified mobile pathogenic clusters of protein, consisting of small oligomeric forms of native or misfolded α-syn protein.[108] In vitro studies demonstrated that these mobile pathogenic clusters of protein structures spread in an aggregated phenotype with associated neurotoxicity.[109,110] The potential for the intracerebral interception of aberrant (oligomeric) forms of α-syn might provide a treatment. Blocking the uptake of these molecules protects against α-synucleinopathies in vitro and in animal models.[111,112]

Active immunization against α-syn ameliorated the degenerative pathology of PD[112] in a murine model, paving the way for a Phase I trial of a Parkinson vaccine (PD01A). Initial reports indicate that the vaccine is safe [NCT01568099] and observational Ib and follow up (including booster) studies are underway. In addition, another Phase I study of vaccine (PD03A) plus adjuvant is ongoing [NCT02267434].

The potential also exists for synergistic involvement of tau in PD. Reports have indicated that tau can initiate α-syn aggregation and associated neurotoxicity, much as that seen with Aβ in AD. The potential for heterologous proteins to influence α-syn aggregation has been reported in vitro and cross-therapeutic benefit of drugs for AD, tauopathies, and other aggregation prone neurodegenerative conditions such as Huntington disease (which involves aggregation of the huntingtin protein) may exist for PD and other α-synucleinopathies (and vice versa).[113,114]

Vaccines Against Addiction

Expanding industrial activity in the developing world is boosting economic prosperity. In the modern era the global trend has seen populations becoming less rural and increasingly urbanized. The increasingly sedentary lifestyle, enhanced access to processed (high sugar, high fat) foods, and increased alcohol and tobacco consumption have contributed to obesity and other related health complications, including hypertension, stroke, and type 2 diabetes.

Because behavioral modifications can be very difficult to implement, vaccine approaches to smoking cessation, curbing obesity, and population control are very attractive (see Table 41.1). One example is the potential use of immunotherapy in diabetes management (discussed later in the chapter).[115,116] B-cell vaccines against noninfectious disease targets can be divided into approaches for autoimmune conditions[40–42] and allergies (see later) and for drugs of abuse.[26,43] Vaccines against drugs of abuse aim to induce antibodies that bind the drugs in the blood and prevent positive reinforcement in the brain. In particular, vaccination for smoking cessation has been extensively pursued, mainly with two vaccines: Nabi's Nicotine-exoprotein A conjugate (NicVax) partnered with GlaxoSmithKline and Cytos Biotechnology's Nicotine-Qb conjugate (NicQb or Nic002) partnered with Novartis. In a Phase II study (NCT00369616 by Cytos) with NicQb, the vaccinated subjects in the tertile with the highest antibody levels had a significant increase in continuous abstinence from smoking from months 2 to 6, when compared to the placebo recipients.[43] However, in a subsequent study (NCT00736047) performed by Novartis, the primary end point of decreased smoking, defined as a difference in continuous abstinence at weeks 8 to 12 compared to placebo, was not achieved and the program was discontinued.[117] Likewise, a second vaccine by Nabi Biopharmaceuticals, NicVax, reported a significant increase in continuous abstinence from smoking between week 19 and 26 in the highest antibody responders (top 30% individuals with the highest antibody titers) compared to placebo.[118] This vaccine was tested in two additional Phase III trials (NCT01178346 and NCT01102114) and reproduction of the reduction in smoking results were negative, and therefore Nabi stopped the further development of the vaccine, highlighting again the difficulties in tackling smoking cessation. Although the early efficacy trials appeared promising, the challenge was to induce high antibody titers of high affinity over an extended period. Despite these challenges, Pfizer has recently initiated a new program for a smoking cessation vaccine with a focus on induction of high-affinity antibodies.

Type 1 Diabetes and Tolerogenic Vaccines

Formerly known as insulin-dependent diabetes mellitus, type 1 diabetes (T1D) is a chronic autoimmune disease, caused by the loss of the insulin-producing β cells in the islets of Langerhans. Autoimmunity is the predominant mechanism of T1D, but also involves both genetic susceptibility and environmental triggers. Genetic data implicate the insulin gene and certain signaling genes (PTPN22) and several immune-related genes (e.g., human leukocyte antigen [HLA], IL-2Ra, and cytotoxic T-lymphocyte antigen [CTLA]-4) that correlate with susceptibility to T1D. Environmental triggering factors, such as microorganisms, particularly enteroviruses, have been implicated through epidemiological and other studies.[119–123] Conversely, other microorganisms such as bacille Calmette-Guérin (BCG) vaccine appear to offer protection. Timely detection and intervention are widely believed to maximize the chances for successful intervention and disease prevention. Serum autoantibodies are useful biomarkers, as well as metabolic intermediaries and regulatory T-cell populations.[124]

The following examples highlight current prophylactic trials focusing on environmental triggers. In 2015, the U.S. Food and Drug Administration approved an investigational new drug (IND) application for the conduct of a BCG vaccine trial for the treatment of T1D. The rationale for the trial was to induce elevated levels of TNF to destroy autoreactive T cells that target and destroy insulin-secreting islet cells in the pancreas. With the completion of a proof-of-concept Phase I study that confirmed vaccine safety,[125] a Phase II study is currently underway.[126]

Additional vaccine based-approaches have used synthetic controlled-release biomaterials. In a two-component system comprising of microparticles of poly(lactide-co-glycolide) (PLGA) that are used to encapsulate denatured insulin, and in the second part, peptide hydrogel (PuraMatrix) containing the inflammatory cytokine, granulocyte-macrophage colony-stimulating factor and a Toll-like receptor 9 agonist, cytosine phosphate guanine ODN1826 (CpG), acting as vaccine adjuvants.[127] In vivo tests show that this combination dual-component vaccine provides only modest protection (40% of NOD [nonobese diabetic] mice) compared to control mice that all became diabetic.[127] Based on in vitro analyses, CpG appeared to stimulate an increase in IL-10 production, suggesting the antiinflammatory action as a potential mechanism. Histological analysis of granulomas that formed in mice 1

week following the final of three injections administered subcutaneously, indicated the presence of a broad spectrum of immune cells. This localized infiltration creates opportunity for prerequisite cell–cell interactions necessary for education of immune cells.[127]

Another T1D vaccine being developed and undergoing clinical trials is the MonoPepT1De trial using a proinsulin peptide (PI C19-A3), which is being evaluated in newly diagnosed type 1 diabetic patients. Early 2015 saw completion of a Phase Ib multicenter study of this vaccine (Phase Ib Study of Proinsulin [PI] Peptide Immunotherapy in New-Onset Type 1 Diabetes—NCT01536431). The primary end point was safety of PI C19-A3 in the context of new-onset T1D, with secondary end points assessing "stimulated C-peptide production, HbA_{1c} [glycosylated hemoglobin] levels, mean glucose excursions, quality of life scores and islet cell autoantibody biomarkers of β-cell–specific immune responses."[128]

As can be seen by the above examples, the scientific rationale is rather ill-defined, so there are a number of challenges to licensure of these products.

Type 2 Diabetes and B-Cell Vaccines

Conversely in the case of type 2 diabetes mellitus (T2D) the islet cells are still functioning, but the body becomes resistant to the action of insulin, or the pancreas does not produce adequate supplies, or both. A notable characteristic of T2D is amyloid deposits of peptides derived from islet amyloid polypeptide (also known as amylin) originating from the β cells. Amylin, usually cosecreted with insulin, regulates glucagon secretion and gastric emptying (appetite), and exhibits physicochemical properties that permit it to aggregate and form amyloid fibers. Therefore, a potential strategy to eliminate the amyloid deposits (aggregated amylin)[129] through vaccination, similar to strategies being employed in tackling aggregates in AD[130] might be associated with improved production of insulin. In addition, antiinflammatory strategies have been applied in T2D.[131] Preclinical experiments using an active immunotherapy targeting IL-1β based on the full-length cytokine chemically coupled to bacteriophage Qbeta VLPs had previously demonstrated in vivo neutralization of IL-1β. Immunized animals tolerated the vaccine and exhibited attenuated disease in experimental models of rheumatoid arthritis, multiple sclerosis, and type 2 diabetes.[41,131] Cytos has since completed the Phase I/IIa trial of CYT013-IL1bQb (NCT00924105). The safety and tolerability of the vaccine was subsequently improved by introducing a single point mutation, which markedly reduces biological activity of the cytokine antigen. Cohorts of type 2 diabetes patients have been immunized in a Phase I/II study with a broad range of vaccine doses (10–900 μg) and frequencies (2–8 administrations). The vaccine was shown to be well-tolerated and IL-1β–specific antibody responses could be observed in most patients after as few as one to two injections of the vaccine. In marked contrast, IL-1β–neutralizing responses (indicating higher-affinity antibodies) could only be observed after five or six injections (MF Bachmann, unpublished). However, these neutralizing antibody responses, once induced, were long-lived and slowly declined over several months (up to 40 weeks of observation). Further studies (yet to be concluded) would be required to investigate whether it is possible to accelerate the development of neutralizing antibodies with the inclusion of adjuvants or by using higher vaccine doses.[15]

Hypertension

Persistent hypertension represents the greatest risk factor for the development of coronary heart disease and stroke with potentially lethal outcomes. As a key regulator of blood pressure angiotensin II (AngII) is a therapeutic target of many effective antihypertensive medications, including angiotensin-converting enzyme inhibitors and drugs that block the angiotensin Type I receptor. Despite the availability of these effective medicines, compliance is low with only 30% to 50% of hypertensive patients in the United States taking their medications.[132,133] The asymptomatic nature of this condition makes it difficult for patients to adhere to the therapy.[134] Active immunization represents an attractive alternative with the aim of inducing a durable antibody response to AngII. A vaccine containing the target protein (angiotensin I [AngI]) coupled to a carrier molecule, KLH was evaluated in rats and reduced blood pressure. Subsequent clinical studies (performed by Protherics), however, did not attain desired end point efficacy of reducing blood pressure,[135] likely because of suboptimal humoral responses in humans. A second vaccine study in spontaneously hypertensive rats employed the VLP Qb chemically coupled to AngII as a VLP. High levels of AngII-specific IgG antibodies and improved blood pressure were seen. A subsequent Phase I study confirmed safety[25] and demonstrated that antibody levels peaked 2 weeks after injection and declined to background levels within a few months. Efficacy of this vaccine was then tested in a multicenter, double-blind, placebo-controlled Phase II study in 72 mild to moderately hypertensive patients.[27] Two doses, low and high (100 and 300 μg), of the vaccine formulated in adjuvant (aluminium hydroxide) were used for subcutaneous injection on days 0 and 4 and at 12 weeks. Ambulatory blood pressure measurements over 24 hours were followed prevaccination and after a postvaccination treatment course consisting of the three injections. The higher dose (300 μg) significantly reduced blood pressure compared to baseline and placebo; the reduction was comparable to the response to the small molecular inhibitors of AngII. Daytime systolic and diastolic blood pressures were reduced by 9 and 4 mm Hg, respectively, when compared to placebo recipients. Early morning blood pressures were also reduced.

In an attempt to further improve the efficacy of AngQb, higher doses (up to 900 μg) at increased frequency (biweekly injection) were evaluated in additional Phase II clinical studies (NCT00701649 and NCT00710372). This new regimen induced higher antibody titers but the impact on blood pressure was less than in the previous study. The reduced efficacy correlated with reduced affinity of the antibodies,[27] demonstrating that both antibody affinity and quantity are important.

In summary, active immunization against AngII reduces blood pressure in some hypertensive patients. However, optimization is required to achieve the right balance of antibody affinity and quantity (Cytos Biotechnology Ag, unpublished data).

Anticytokine Vaccines

With increasing success of mAb therapies against different cytokines such as TNF-α, IL-12, and IL-17, several therapeutic vaccination strategies have been applied to this class of molecules. Therapies targeting TNF using mAbs have demonstrated efficacy across a wide range of inflammatory diseases, including rheumatoid arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, psoriasis, Crohn disease, and ulcerative colitis.[8–11,136,137] However, patients treated with anti-TNF mAb frequently develop treatment resistance (secondary failures) with chronic long-term use. In addition, they have cumbersome dosing regimens with frequent injections and are costly.

Tumor Necrosis Factor

Several vaccine trials targeting TNF have been performed but there are few reports in the public domain. In 2014, Neovacs reported their results of a randomized, double-blind, placebo-controlled, multicenter, 140-patient, Phase IIb clinical trial of TNF-Kinoid in rheumatoid arthritis (NCT01040715). The coprimary end points were based on rheumatoid arthritis symptoms scores (the DAS28-CRP and ACR 20 scores). The study confirmed the safety and tolerability of the product and all vaccinated subjects, except one, demonstrated significantly increased levels of anti-TNF binding antibodies. However, the patients did not produce neutralizing antibodies and had no improvement in the clinical symptoms scores. Further data analysis is ongoing.[138]

Cytos reported on the immune response and clinical benefit of a first-generation TNF peptide Qb vaccine for psoriasis.[139] The peptide-based vaccine induced strong antibodies against the peptide, but functional activity against soluble TNF was low.[140] Vaccines based on the entire protein are now being considered for further development. Finally, Pharmexa studied a TNF protein vaccine with an engineered tetanus toxoid–derived T-cell epitope in humans. Although the vaccine was immunogenic, the antibodies only recognized a partially denatured form of TNF and vaccine development was abandoned.[141]

Additional analyses of these studies and further larger placebo-controlled studies are needed to determine if vaccine-induced antibody regimens can compete with existing mAb therapies.

Interleukin-17

An additional problem with anti-TNF immunotherapy is its potential for increasing susceptibility to latent infections (such as tuberculosis and other opportunistic and neoplastic disease).

This has encouraged the search for alternative points of intervention. One attractive and very successful example has been targeting the inflammatory cytokine, IL-17A in patients with moderate to severe psoriasis. Psoriasis is a chronic inflammatory skin disease resulting in poorly differentiated, hyperproliferative keratinocytes. The scaly plaques and reddening (erythema) on the skin vary in severity from minor localized patches (lesions) to complete body coverage, and in some cases can also lead to joint inflammation (approximately 25% of psoriasis patients develop psoriatic arthritis). IL-17 is produced by Th17 cells, a subset of effector T-helper cells. Within the psoriatic lesion, activated antigen-presenting cells produce IL-23 and stimulate Th17 cells to release IL-17. Keratinocytes respond to IL-17 by upregulating proinflammatory chemokines that attract T cells, neutrophils, and mononuclear cells to the lesion. IL-17 can also induce IL-6 from fibroblasts, which stimulates differentiation of naïve Th cells to Th17 cells leading to further IL-17 production, thus creating a potential positive inflammatory feedback loop.[142]

Because it appears that IL-17 plays a key role in the inflammatory immune response in psoriasis, anti–IL-17 mAbs have been demonstrated to moderate the inflammatory response.[90] mAbs that target IL-17 are undergoing Phase III testing and are being compared with licensed anti–IL-12p40 (ustekinumab), and etanercept-targeting TNF.[136,143–145] Early in 2015, Novartis' AIN457, also known as secukinumab (Cosentyx), became the first approved anti–IL-17A monoclonal antibody on the market and is recommended as a "first-line" treatment for moderate to severe psoriasis.

The safety of neutralizing IL-17 is informed by the natural occurrence of individuals with genetic mutations in IL-17 function (lacking functional IL-17F, the IL-17 receptor antagonist IL-17RA or the downstream adaptor protein ACT1),[51] who are prone to skin-limited mild infections, but not systemic bacterial infections, tuberculosis or other mycobacterial infections, viral infections, unusual microbes, and do not have an increased risk of cancer.[51,52]

Preclinical experiments with IL-17–based vaccines in mice show strong neutralizing antibody titers and protection against the induction of rheumatoid arthritis and multiple sclerosis.[38]

These encouraging results have led to the Phase II studies currently underway. More cost-effective vaccine approaches (e.g., based on VLPs) to supplement or even replace IL-17 biologics in psoriasis and other diseases should be further pursued.

CONCLUSIONS

In the last decade a large effort was made to adapt the vaccine concept to chronic diseases that are a major health threat to modern societies. Our improved immunological understanding, together with the development of recombinant technologies, allow scientists to harness this successful therapeutic modality to target self molecules, which, if overproduced, play central roles in the etiology of disease. However, none of these approaches has become a marketed product, and further challenges remain to fully explore this therapeutic concept.

In this context it is important to remember that the quantity and the affinity of antibody needed to neutralize and inhibit self-antigens or drugs of abuse are orders of magnitude higher than those required for the protection against viral infection.[44] Hence major challenges remain to inducing antibodies in sufficient amounts and of appropriate affinity for therapeutic efficacy. Many of the specific requirements for the antibody responses will be target specific and will be influenced by the use of different adjuvants, vaccine carriers, and the vaccine regimens.

A further key consideration in pursuing active immunization strategies for neurodegenerative conditions is the natural limitation of the immune response in the elderly compared to younger adults. Finally, a general theme is bridging the relevance of preclinical data in existing animal models and expanding models to recapitulate mechanistic aspects of the human diseases being studied.

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42 Norovirus Vaccines

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Human noroviruses (NoVs) have become the leading cause of acute nonbacterial gastroenteritis in countries where rotavirus vaccination has been successfully implemented.[1–5] Overall, NoVs have been identified in 18% of reported acute gastroenteritis cases worldwide.[6] In the United States, NoVs result in approximately 21 million cases of gastroenteritis, 400,000 emergency department visits, 71,000 hospitalizations, 800 deaths, and an annual loss of an estimated 5000 quality-adjusted life years.[7–9] NoV infections are more severe in developing countries, where NoVs have been detected in approximately 5% of pediatric diarrhea episodes and result in an estimated 200,000 pediatric fatalities annually.[10,11] NoVs have also been implicated as a substantial contributor to traveler's diarrhea in tourists and in group-travel environments.[12,13] NoVs often garner attention for outbreaks on cruise ships, but these account for only about 1% of all reported norovirus outbreaks in the United States.[14] In the European Union (EU), it is estimated that NoV infection may cause up to 5.7 million illnesses, 800,000 medical visits, 53,000 hospitalizations, and 102 deaths every year in children younger than age 5 years. This burden is comparable with the burden related to rotavirus before the introduction of rotavirus vaccination and will grow in relative importance as rotavirus vaccines are rolled out progressively in the EU.[15] Simulations suggest that a NoV vaccine could reduce up to 2.2 million NoV cases annually and save up to $2.1 billion in economic burden.[16]

Financially, NoV gastroenteritis results in $284 to $355 million in healthcare costs[9,17] and an estimated $2 to $5 billion in total economic burden[8,16] in the United States and $4 billion in the EU.[16] This estimated total economic burden is only surpassed by nontyphoidal *Salmonella enterica*, *Campylobacter*, *Listeria monocytogenes*, and *Toxoplasma gondii*.[8] Even though NoV cases are believed to be underreported,[18] the known and estimated human and economic burden of NoV infection and gastroenteritis are substantial. NoVs have been referred to as the "perfect human pathogens." They are highly infectious, rapidly and prolifically shed, difficult to avoid, and without etiological treatment; taken together these considerations make NoV-related disease a good target for prevention through vaccination.[19]

NOROVIRUSES

NoVs were first identified in 1972 (Norwalk agent) from fecal isolates from a 1968 gastroenteritis outbreak in Norwalk, Ohio.[20] NoVs are nonenveloped, positive-sense single-strand RNA viruses belonging to the Norovirus genus in the *Caliciviridae* family of viruses. NoVs are divided into six genogroups (GI to GVI) and further subdivided into genotypes (Fig. 42.1A). GI, GII, and GIV NoVs infect humans; of these, GI and GII are the most important for human infection. GII and GIV viruses also infect porcines and canines, respectively, indicating hypothetical zoonotic transmission routes that have not been rigorously evaluated,[21] although antibodies against canine NoVs have been detected in humans.[22]

NoVs have a 7.5- to 8.0-kb genome surrounded by an icosahedral capsid. The genome has a 5′ VPg cap and a 3′ poly(A) tail and contains three open reading frames (ORFs). ORF1 encodes a nonstructural polyprotein. ORF2 encodes the major capsid structural protein, VP1, which self-assembles into 90 dimers to form the icosahedral capsid.[23] ORF3 encodes a minor structural protein, VP2, which increases capsid stability and VP1 expression.[24–26] VP1 is composed of two domains, shell (S) and protruding (P), separated by a short hinge sequence.[27] The P domain is further subdivided into the highly conserved P1 and hypervariable P2 subdomains.[27,28] Variation in the P2 subdomain regulates strain ligand binding and antigenicity.[29–31]

NOROVIRUS TRANSMISSION AND DISEASE

NoVs are classically transmitted by the fecal-oral route,[32] but can also be transmitted via aerosolized vomitus,[33,34] contaminated food or water,[14,35] and fomites because of the ability of NoV to remain stable for weeks on surfaces[36] and up to 2 years in water samples.[37] Chemical disinfection is the only potential intervention in the transmission cycle, but classic hygiene measures, including alcohol-based hand washing, have proved largely ineffective.[38]

Because as few as 18 viral particles are capable of causing infection and each gram of feces may contain up to 5 billion infectious doses,[39] NoVs are highly contagious and easily transmitted in semiclosed communities, such as senior care homes, cruise ships, and military barracks,[7] and are common nosocomial infections in the United States and Europe.[15,40,41] Additionally, up to 30% of NoV infections result in asymptomatic shedding; the role of asymptomatic infection in transmission and outbreaks of norovirus remains unclear.[42,43]

NoV gastroenteritis is commonly referred to as the "stomach flu" and also earned the nickname "winter vomiting disease" based on its distinct winter seasonality pattern in the Northern hemisphere.[44] NoVs have an incubation period of approximately 24 hours[45] and most people with illness are typically symptomatic for 24 to 72 hours.[46] NoV-induced gastroenteritis commonly presents as nausea, (projectile) vomiting, and diarrhea, but can also include cramps, fever, headache, and dehydration.[32,47] NoV-induced gastroenteritis induces longer duration and more frequent vomiting compared with bacterial gastroenteritis.[48,49] Approximately 10% of persons with norovirus gastroenteritis seek medical attention, which might include hospitalization and treatment for dehydration with oral or intravenous fluid therapy. Norovirus infection can also become an important factor contributing to a poor outcome in patients with underlying cardiac and renal conditions.[50]

A systematic literature review of norovirus-associated illness, hospitalization and death among individuals 65 years of age and older indicated that disease severity increased with age, presence of comorbidities, and GII.4 genotype. The burden of norovirus is anticipated to rise as an increasing proportion of society becomes elderly, with an increased need for institutionalized care. Although a self-limiting infection in an immunocompetent patient, chronic NoV infection can develop in immunocompromised hosts,[51–56] which is associated with longer durations of diarrhea and shedding.[50] In rare

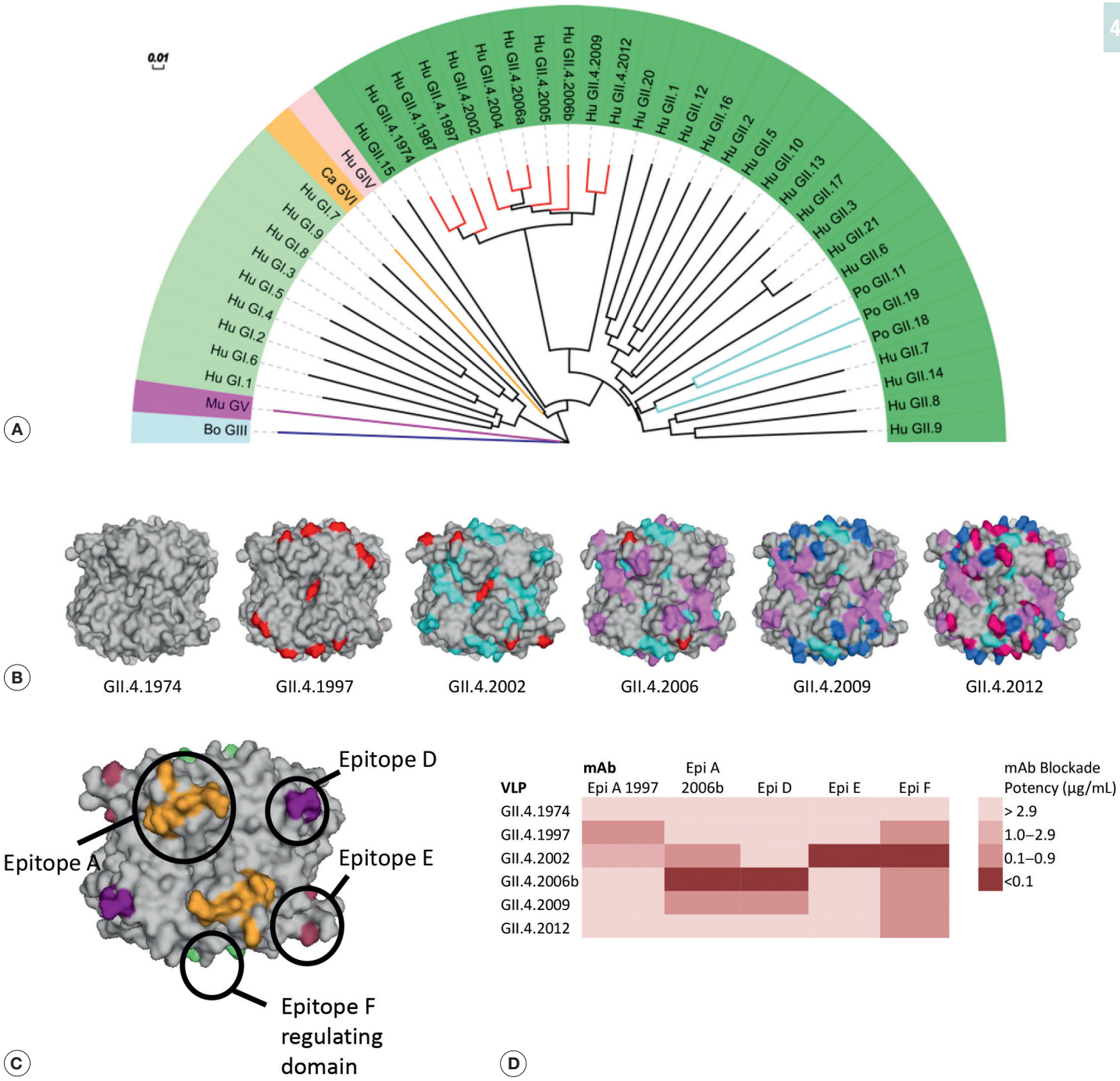


Figure 42.1. Characterization of globally significant GII.4 norovirus (NoV) strains. A, Major capsid gene phylogenetic tree of noroviruses. **B,** Structural models of the GII.4 capsid P dimer over time. Color changes represent amino acid changes compared to GII.4.1974: *red* (GII.4.1997), *teal* (GII.4.2002), *pink* (GII.4.2006), *blue* (GII.4.2009), and *dark pink* (GII.4.2012). **C,** Identified GII.4 blockade domains. Evolving GII.4 blockade epitopes (A, D, and E) and the antibody (Ab) access-regulating domain (NERK motif) of the conserved GII.4 blockade epitope (F) mapped onto the P dimer structure of GII.4.2012. **D,** Sequence changes in the blockade epitopes effect blockade Ab potency. Titers for monoclonal antibody (mAb) blockade across a panel of time-ordered GII.4 virus-like particles (VLPs) is color coded for relative potency from lowest (lightest color) to highest (darkest color) using mAbs that recognize Epitope A in GII.4.1997, Epitope A in GII.4.2006b, Epitope D, Epitope E, and Epitope F. BO, bovine; Ca, canine; Epi, Epitope; Hu, human; Mu, murine; Po, porcine.

cases, necrotizing enterocolitis and postinfectious irritable bowel syndrome also have been reported in association with norovirus infection,[57–60] although a causal link has not been established.

HISTO-BLOOD GROUP ANTIGENS

Histo-blood group antigens (HBGAs) are receptors or essential cofactors for NoV infection.[61–63] HBGAs are a large family of complex carbohydrates classified by terminal glycosylation moieties: secretor (H), Lewis (Le), and A or B antigen. In individuals who are secretor-positive, HBGAs are expressed on the surface of red blood cells and epithelial cells lining mucosal surfaces and are found free in mucosal secretions. In secretor-negative individuals, only core HBGA chains with minimal branch glycosylation are expressed and secreted.[64] Specific branch glycosylation is associated with susceptibility to NoV infection and NoVs bind HBGAs in strain-specific and genotype-specific patterns in vitro.[65–71] It is hypothesized that in individual HBGA expression levels and patterns, NoV binding patterns and virus titers may regulate infection patterns in human populations. In support, individuals expressing B antigen are more resistant to GI.1 NoV infection,[63] whereas secretor-positive individuals are more likely to be infected with certain NoV genotypes, including GII.4, the dominant genotype causing human disease worldwide. A birth cohort study in Ecuadorian children showed that secretor-negative infants experienced a similar burden of illness as secretors. However, the genotypes responsible for illness were different, indicating that even though a secretor-negative phenotype is associated with a reduced risk of infection, it is not completely protective against all NoV strains.[63,72–75]

CHALLENGES FOR NOROVIRUS VACCINE DEVELOPMENT

Several challenges remain for the development of safe, affordable, and efficacious NoV vaccines. Primarily, consistent cell culture systems and small animal models are lacking. Although advances have been made in the development of a cell culture system, viral replication in these systems remains limited. Previous studies have indicated that NoVs interact with enteric bacteria[76,77] and that murine NoVs infect immune cells.[78,79] Expanding on these observations, Jones and colleagues[80] examined B cells and enteric bacteria as a potential cell culture system. In vitro infection of human B cells with GII.4.Sydney, resulted in a 25-fold increase in genomic copies and production of viral proteins. Intestinal epithelial cells and B cells cocultured in transwell chambers were infected, while intestinal epithelial cells alone were not. Filtration decreased viral replication while culture with free synthetic H-type HBGA or bacteria expressing H-type HBGA resulted in replication similar to unfiltered NoVs.[80] These data suggest NoVs infect immune cells in vitro and commensal bacteria may act as a cofactor for viral attachment or entry. While extremely promising, these results have not yet been duplicated; other limitations to these studies are that replicating viral titers were low and the relation to in vivo infection remains unknown. Further work is necessary to develop robust cell culture systems for human NoVs.

In addition to a cell culture system, animal models that can support human NoV replication and vaccine studies have also been reported. Intraperitoneal injection of GII.4 human NoV resulted in increased viral titers and viral protein detection in splenic and hepatic macrophages of Rag-γc knockout mice.[81] These data indicate that this mouse model may serve as a starting point for production of NoV stocks and for attenuated or inactivated virus vaccine development. However, the model also has several limitations in its current state. First, mice were only susceptible to oral–intraperitoneal or intraperitoneal inoculations and developed a subclinical infection. Additionally, the immunocompromised status of these mice limits the ability to evaluate correlates to protective adaptive immunity and to test vaccine regimens. Thus, this model cannot be a suitable model for NoV pathogenesis or disease. Gnotobiotic pigs challenged with human NoV have also demonstrated utility in evaluating vaccine candidates, although viral replication in this model is limited.[82,83] Chimpanzees inoculated with GI.1 NoV did not show clinical signs of gastroenteritis, but did show similar viral shedding and immune profiles as observed in humans.[84] Both models will likely require the in vivo adaptation of virus to enhance replication and disease severity.

NOROVIRUS IMMUNITY

Understanding NoV-induced immune responses is important to vaccine development. Historically, natural NoV infection was believed to yield short-term homologous protection in humans.[85,86] Long-term memory responses were first described in 2003; bioinformatics modeling suggests memory responses may persist on average for 4 to 8 years.[63,87] These protective immune responses are genotype-specific; repeat infections with NoVs from the same genogroup or heterologous viruses are common[88–91] and aid in the emergence of novel circulating genotypes from season-to-season.[92] Importantly, previous exposure may bias vaccine immune responses toward a previous infecting strain and shape immunity to NoVs (i.e., original antigenic sin), as described for other RNA viruses.[74,75,93,94]

Several immune correlates of protection from human NoV infection and illness upon oral challenge have been identified (Table 42.1). Genetic resistance and mucosal immunoglobulin (Ig) A responses were the first identified correlates.[63,95] Additional studies have expanded these protective immune

TABLE 42.1 Correlates of Protection From Human Norovirus Infection and Disease

Species	Challenge Strain	Correlate	Infection	Symptoms	Reference
Human	GI.1	Salivary IgA	X	X	63, 96
	GI.1	Memory IgG cells		X	96
	GI.1	Blockade Ab titer	X	X	104, 105
	GI.1	HAI titer	X	X	163
	GII.4	Blockade Ab titer	X	X	97, 102
	GII.4	Serum IgA	X	X	97
Chimpanzee	GI.1	Blockade Ab titer	X		84, 103
Gn pig	GII.4.2006b	Duodenal Tregs		X	106

Ab, antibody; Gn, gnotobiotic; HAI, hemagglutination-inhibiting; Ig, immunoglobulin; Tregs, regulatory T cells.

responses against GI.1 and GII.4 NoV infection and disease. Salivary IgA has been identified as a correlate of protection against both infection and disease in unvaccinated individuals, as well as serum IgA and serum IgG memory B cells.[96,97] Both IgG and IgA antibodies that block binding of NoV capsid protein to HBGA carbohydrates (blockade Abs) were first described as potential neutralizing antibodies in 2002 after infection and vaccination[31,98–101] and are now accepted correlates of protection against infection from GII.4.2009 NoV in humans[102] and GI.1 NoVs in chimpanzees and humans.[84,97,103–105] Intestinal preinfection regulatory T cells are inversely correlated with protection from GII.4.2006b NoV in gnotobiotic pigs.[106] These data provide promise for the development of vaccine-induced immunity.

NoVs are highly diverse and capable of evading host immune responses and herd immunity,[107] complicating vaccine development. GII.4 NoVs account for approximately 60% to 90% of all NoV outbreaks worldwide,[108] with new strains emerging every 2 to 4 years.[31,109] New NoV strains emerge via several mechanisms, including quasispecies and recombination. Quasispecies are formed during viral replication with the error prone RNA-dependent RNA polymerase; antigenic variation and immune evasion primarily occur in the antibody neutralizing/blockade epitopes of the capsid P2 domain, primarily in epitope A.[110–112] NoV quasispecies may also evolve in and emerge from persistent or chronic infections in immunocompromised hosts.[113–118] There is mounting evidence that pandemic NoVs emerge from immunocompromised hosts rather than zoonotic transmission.[119] A representation of the evolution in the P domain of successive epidemic waves of GII.4 NoVs is shown in Fig. 42.1B. These sequence changes correlated to changes in blockade (surrogate neutralization) antibody epitopes (Fig. 42.1C) resulting in loss of reactivity of human monoclonal antibodies and emergence of new GII.4 strains. Fig. 42.1D shows the reactivity of virus-like particles (VLPs) against monoclonal antibodies raised against a panel of VLPs and highlights the loss of antibody reactivity associated with changes in the P2 domain of pandemic GII.4 strains over time. In comparison, monoclonal antibodies raised against a conserved GII.4-predicted neutralizing epitope (F) are highly cross-reactive between antigenically divergent GII.4 strains that have circulated since 1987, suggesting that potential targets for a broadly protective vaccine response exist.[120]

VACCINES IN DEVELOPMENT

NoV vaccine development has been an area of intense research, particularly in the development of consistent systems for viral propagation. Currently, no such cell culture system exists, hindering development of attenuated or inactivated vaccines. Because attenuated or inactivated vaccines are currently economically infeasible, NoV vaccine development has focused on recombinant capsid subunit vaccines, including VLPs and P particles. VLPs and P particles are composed of the full-length capsid and P domain, respectively. VLPs are ~38 nm in diameter, similar to the native capsid,[121] while P particles are only ~20 nm in diameter.[122] Both proteins, lacking the viral genome, are nonreplicating. Viral vectors have also been used as a vehicle to produce and deliver VLPs in vivo.[123] Several formulations of VLPs have been examined in clinical trials; as of this writing, P particles and vectored VLPs have only been studied preclinically.

VIRUS-LIKE PARTICLES

NoV VLPs were first produced for Norwalk virus in 1992[121] and are now a common virus surrogate for numerous areas of NoV research. VLPs are produced via expression of the full-length VP1 capsid protein, both the S and P domains, in an expression vector. VLPs have been produced in a variety of systems, including baculovirus,[121] alphavirus,[124] yeast,[125] tomatoes,[126,127] potatoes,[128] and tobacco.[129,130] Great strides have also been made on improving production and purification of VLPs for wide-scale vaccine production.[131–133] There are several human NoV VLP vaccines undergoing clinical trials, including different VLP formulations and routes of administration.

The most commonly studied NoV candidate vaccines have been VLPs. VLPs have been developed from a variety of strains and inoculated using a multitude of vaccine formulations and routes of administration. An intranasal GI.1 Norwalk-derived VLP vaccine regimen with monophosphoryl lipoprotein (MPL) and chitosan adjuvants developed by LigoCyte Pharmaceuticals, Inc. (now Takeda Vaccines, Inc.) reduced Norwalk-induced gastroenteritis (37% incidence compared to 69% in placebo) and infection (61% incidence compared to 82% in placebo) in adult volunteers ($n = 47$ in vaccine group and $n = 43$ in placebo).[105] The regimen also induced IgA antibody-secreting cells, serum IgG and IgA, and virus-specific memory B cells.[134,135] The only reported side effects were nasal discharge and stuffiness.[105] Although these proof-of-concept results are promising for a vaccine against Norwalk virus, monovalent vaccines do not induce broadly reactive immune responses against other NoV genogroups.[71,91,117,136] The diversity of NoVs and complex serology patterns make broadly reactive vaccine-induced immune responses of paramount importance.[75,98]

Several of the aforementioned challenges to NoV vaccine development are the unknown effect of previous exposure history, viral diversity, and viral evolution. One strategy to overcome these limitations is rational targeting of NoV strains annually, similar to the annual targeting for seasonal influenza vaccines. However, for NoVs, this approach would face significant regulatory hurdles limiting the pace of reformulated vaccine availability. An alternative approach is to broaden the vaccine-induced immune response (Fig. 42.2) to include strains not contained within the vaccine formulation, which can be achieved through several vaccine formulations, including a multivalent VLP cocktail containing VLPs from multiple distinct serotypes (Fig. 42.2A),[98,137–139] a single VLP containing epitopes from multiple strains (Fig. 42.2B),[138,140] or targeting conserved immunogenic regions within the capsid protein (Fig. 42.2C).[120,141] Thus far, multivalent and chimeric NoV vaccine regimens have only been conducted with VLPs.

Chimeric and multivalent vaccines are immunogenic and cross-reactive in rabbits[138] and mice.[140] In one study, VLPs were derived from a consensus sequence of three NoVs: GII.4.2002 Houston, GII.4.2006a Yerseke, and GII.4.2006b Den Haag. A two-dose intradermal immunization of 50-μg consensus sequence VLPs with Alhydrogel adjuvant induced strong humoral responses against GII.4 and non-GII.4 VLPs in rabbits.[138] Similarly, a two-dose regimen of epitope chimeric VLPs derived from GII.4.1987, GII.4.2002, and GII.4.2006b parental strains induced increased and similar blockade antibody responses as individual parental strains and a multivalent VLP cocktail, respectively, in mice.[140]

Chimeric and multivalent candidate vaccines also have been shown to be immunogenic in human volunteers.[93,139,142] A bivalent vaccine consisting of GI.1 and GII.4 consensus VLPs expressed in a baculovirus system and with MPL and alum adjuvants was given intramuscularly to fifty-four 18- to 49-year-old adult human volunteers in a Phase I dose escalation clinical trial. Pain and tenderness at the injection site were the most commonly reported side effects.[139] The priming dose increased mucosal homing plasmablasts and IgA antibody-secreting cells in peripheral blood mononuclear cells and serum antibodies.[139,142] A dose of 50 μg of each VLP

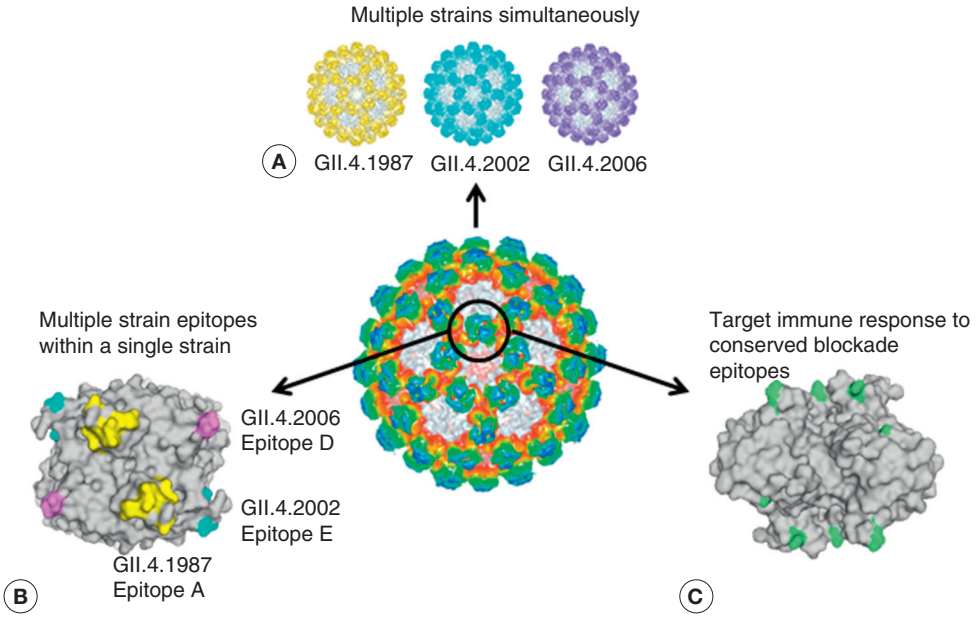


Figure 42.2. Vaccination approaches to broaden the GII.4 blockade antibody (Ab) response. Three immunization approaches lead to generation of blockade Ab responses against multiple GII.4 strains, including GII.4 strains not incorporated in the vaccine formulation: (1) Simultaneous immunization with multiple GII.4 strains **(A)**; (2) immunization with a single GII.4 engineered to contain blockade epitopes from multiple GII.4 strains (P dimer is shown from top) **(B)**; and (3) targeting of conserved blockade epitopes (P dimer is shown from an orientation that allows viewing of the epitope F regulating NERK motif of the conserved epitope in green) **(C)**.

substantially increased anti-GI.1 antibodies and anti-GII.4 antibodies by as much as 118-fold and 49-fold, respectively.[139] The 50-μg dose induced strong blockade antibody responses against NoV strains from GI and GII genotypes, including two novel GII.4 NoV strains that did not circulate in the population before sample collection was completed.[93] Importantly, the host preexisting blockade antibody titers did not appear to impair the vaccine response, and vaccination activated memory antibody responses against GII.4.1997 NoVs, the most ancestral strain VLP tested.[93] Because the boosting dose did not induce further increases in antibody titers and included responses to older NoV strains, the data suggest the first vaccine dose is restimulating the anamnestic response from previous natural exposures.

In a subsequent challenge study, the vaccine ($n = 50$) did not significantly reduce the incidence of protocol-defined illness in adult volunteers challenged with a GII.4.2003 Farmington Hills variant NoV compared to placebo ($n = 48$); however, the vaccine did significantly reduce the severity of disease when evaluated using the modified Vesikari score.[73] The vaccine was more protective against severe forms of acute gastroenteritis than against mild disease, consistent with other vaccines. Vaccination offered several potentially beneficial effects for public health, including fewer subjects with symptomatic disease, and an observed decrease in viral shedding, both in terms of duration and quantity of virus shed.[73] Interpretations of these data are complicated by the low illness rate observed in the placebo subjects, likely a result of preexisting immunity within the population that was protective upon oral challenge. Field efficacy studies are needed to confirm protection against circulating viral strains in the absence of potential protective immunity.

VECTORED VIRUS-LIKE PARTICLES

NoV VLPs can also be produced in vivo by VP1-expressing vectors. Several systems based on alphavirus,[98,123,124] adenovirus,[143,144] vesicular stomatitis virus (VSV),[145,146] and Newcastle disease virus (NDV) have been established.[147] Like VLP vaccines, vectored VLPs may require only one dose to inoculate the host and stimulate sufficient immune responses. However, preexisting host immunity to the vector backbone, such as adenoviruses or VSV, could limit induction of host immune responses.[146,147] Additionally, there are biosafety and security concerns with other vector backbones, including alphaviruses and NDV, which could limit their development and availability. Finally, these vectored NoV vaccines have not been evaluated in an animal challenge model for human NoVs or in human clinical trials.

Initial vector-based VLP delivery systems used Venezuelan equine encephalitis virus replicons (VRPs) and adenoviruses.[98,123,143,144] VRPs inoculated intradermally in mice induced stronger homotypic and heterotypic antibody responses than oral inoculation.[98,123] A three-dose intranasal adenovirus-vectored GII.4.Lordsdale regimen induced virus-specific humoral responses, balanced T-helper cell (Th)1/Th2 responses, and interferon (IFN)-γ production in BALB/c mice.[144] A combination of an adenovirus- or alphavirus-vectored VLP priming and a conventional VLP boosting regimen improved these responses.[123,143] Additionally, multivalent VRPs were the first vaccine strategy to induce blockade antibodies against NoV strains not contained in the vaccine.[98] More recently, VSV and NDV have been explored as potential vectored VLP vaccines.[145–147] Both recombinant VSV- and NDV-expressing NoV VP1 proteins are immunogenic in mice, inducing serum IgG, fecal IgA, proliferating T cells,[146,147] and splenic cytokine-expressing T cells.[147] However, the VSV backbone required coexpression of heat shock protein 70 to attenuate weight loss in mice.[145] Vectored VLPs have produced interesting preliminary immune response results, but their efficacy against NoV challenge has yet to be evaluated.

P PARTICLES

P particles were first developed at Cincinnati Children's Hospital Medical Center in 2004.[148] Expression of the P domain

in an *Escherichia coli* expression system yielded subviral particles that maintained viral binding capability, but, lacking the S domain, did not form VLPs.[149] P particles are composed of 12 P dimers and bind HBGAs.[150] Inoculation of mice with GII.4.VA387-derived P particles induced strain-specific antibodies that blocked VLP binding to HBGAs,[122] indicating their potential as vaccine candidates.

To date, P particles as vaccine candidates have been studied preclinically in murine and porcine models.[106] Studies comparing P particles and VLPs have yielded differing results in mice.[151,152] This discrepancy may be explained by the use of different preparations in the two studies and highlights the lack of understanding of how different P protein multimers may impact antigenicity.[152–154] A three-dose 100-μg intranasal GII.4.VA387 VLP or P particle vaccine with chitosan and MPL adjuvants provided similar cross-variant protection rates against diarrhea following challenge with a GII.4.2006b variant in gnotobiotic pigs.[106,155] P particles did not induce strong IFN-γ producing T cells, but did induce stronger activated $CD4^+$ T cells and intestinal IFN-γ producing $CD8^+$ T-cell responses than VLPs postchallenge.[106] P particles have provided promising immunogenic results and may be an alternative recombinant protein vaccine strategy. However, humoral immunity has not been examined outside of the murine model and P particles do not bind to several human monoclonal antibodies against the epitope A,[141] suggesting that they may present antibody epitopes somewhat differently from native virus and VLPs.

COMBINATION VACCINES

VLPs and P particles have both been examined nonclinically in vaccine regimens targeting multiple viral pathogens. Given the production cost of vaccines, simultaneous immunization against multiple pathogens is ideal and is currently used in several pediatric vaccine formulations. P particles can be manipulated to present other viral antigens in addition to the NoV P domain, while VLP-based combination vaccines have been examined in cocktails.

The first NoV combination vaccines, against influenza and rotavirus, were studied in 2011.[156–158] Parenteral VLP cocktails containing GII.4 and GI.3 NoV VLPs and recombinant rotavirus VP6 induced cross-reactive antibodies against both viruses.[156,159] The most commonly studied combination vaccines have been with the NoV GII.4.VA387-derived P-particle platform.[157,158,160–162] P-particle platforms have induced antibody and T cell immune responses against NoVs and influenza, rotavirus, and hepatitis E virus in mice.[157,158,160–162] Additionally, they have protected against influenza challenge in mice.[161,162] These combination vaccines represent promising approaches, but none have been evaluated for protection against NoV infection and all need to be evaluated in larger animal models or in clinical trials to determine their efficacy.

FUTURE VACCINES

NoVs are the leading cause of acute gastroenteritis and a growing major economic burden worldwide. Fundamental advances into NoV evolution and immunogenicity have provided new metrics and refined antibody and VLP reagents for identifying new pandemic strain emergence events, mechanism of escape from herd immunity, and the potential for refined epitope specific correlates of protective immunity. Historically, the unavailability of consistent cell culture and small animal challenge models has restricted the development of NoV vaccine candidates. Recent advances in both cell culture cultivation and animal model development may eliminate these restraints, although additional improvements are necessary to fully inform future vaccine and therapeutic interventions. The use of recombinant capsid proteins, particularly VLPs, has produced promising results. VLPs effectively mimic binding of the native virion and stimulate host immunity. Norwalk-derived VLPs and multivalent VLP preparations have shown promising results in two human challenge studies by two different routes of administration, providing a potential path forward for evaluation of protection in field efficacy trials.

Recombinant proteins are not limited in their immunogenicity against their parental strains; rather, they have been shown to be cross-reactive and induce functional antibodies against a variety of NoV strains and genotypes. The recent vaccine formulations containing consensus VP1 sequences or chimeric VLPs provide broad immune responses against numerous NoV strains. These vaccine approaches provide the most promise for protection from circulating and emerging NoV strains. Furthermore, vaccines targeting the conserved capsid regions should be investigated as an alternative to chimeric or multivalent vaccines. Although great advances have been made in NoV vaccine development, study needs to be continued. Improvements in cell culture and small-animal models for virus propagation are key to advancing our understanding of NoV disease and immunobiology. Until these systems are validated, clinical trials are the only mechanism for elucidating correlates to protective immunity and vaccine design. As the past decade and one-half was a renaissance in our understanding of norovirus evolution, epidemiology, and molecular biology, it seems likely that the field is poised to make substantial gains not only in understanding of disease processes, but more importantly, in our capacity to control the global disease burden of these important human pathogens.

Addendum

Since submission of this chapter, Ettayebi et al.[164] reported replication of several human norovirus strains in a cell culture system derived from human intestinal stem cell–derived enteroids. Human noroviruses replicated in a strain-specific, secretor-dependent manner. The addition of bile acids to the system increased replication of several NoV strains. Further, human antisera previously shown to block interaction of VLP to carbohydrate ligands neutralized virus replication. These findings provide promise for the potential development of live attenuated or inactivated NoV vaccines.

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43 Parasitic Disease Vaccines

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Parasitic diseases caused by helminths and unicellular eukaryotes (protozoa) are major causes of human disease in the resource-poor nations of the tropics. When measured in disability-adjusted life-years (DALYs, or the number of life-years lost from premature death or disability), the combined burden of disease caused by human parasites is approximately 116 million DALYs, a value that exceeds the DALYs from diarrheal diseases, lower respiratory infections, or HIV and AIDS.[1,2] Among the more critical problems in developing vaccines against these pathogens is maintaining them in the laboratory. With a few exceptions, in vitro culture methods cannot be maintained for these pathogens; frequently, laboratory animals are necessary to maintain the complex life cycles of these parasites. This problem has prevented the production of the large numbers of parasites necessary to develop live attenuated or killed vaccines, as are used for many of the viral and bacterial pathogens.

Over the last decade, investigators in academic, private, and government laboratories began applying modern biotechnology techniques to the study of human parasites. Genome projects have now been completed for most of the neglected parasitic protozoa of global public health importance,[3,4] with proteomic, transcriptomic, and metabolomic research (OMICs studies) increasingly focused on either human parasites or relevant representative species adapted to laboratory animals.[5–7] However, for several reasons, many of these OMICs studies have not yet translated into major vaccine development initiatives. First, most of the parasite genomes are not as amenable to reverse vaccinology approaches as bacterial genomes, primarily because of the absence of simplified eukaryotic expression systems that can simultaneously express hundreds of properly folded antigens.[8] In addition, there is a dearth of animal models for parasite vaccine testing.[8,9] Finally, there has been little commercial interest in the development of human vaccines for neglected tropical diseases.[9,10]

Also, over the past decade, new funds from private philanthropies such as the Bill & Melinda Gates Foundation, together with support from European governments, including the European Union, have reenergized attempts to manufacture and clinically evaluate vaccines for parasitic diseases. These funds have stimulated the formation of several product-development public-private partnerships (PD-PPPs), also known as product development partnerships (PDPs).[10] Aside from the malaria vaccines (see Chapter 35), five human vaccines are currently undergoing or are about to enter clinical development. These include four anthelmintic vaccines (one for hookworm infection and three for schistosomiasis) and several antiprotozoan vaccines for leishmaniasis (Table 43.1). There is also an extensive history of first- and second-generation leishmaniasis vaccines composed of live and killed parasites, respectively.[11,12] In addition, several veterinary vaccines are under development, which could be used to interrupt zoonotic transmission of several parasites to humans, including vaccines for bovine schistosomiasis, pig cysticercosis, canine echinococcosis, and canine leishmaniasis.

ANTHELMINTIC VACCINES

Helminth infections are among the most prevalent infections of humans.[13,14] Approximately 819 million, 465 million, and 439 million individuals worldwide harbor the soil-transmitted nematodes *Ascaris lumbricoides, Trichuris trichiura,* and hookworms (*Necator americanus* and *Ancylostoma duodenale*), respectively,[2] and an additional 252 million or more people are infected with trematodes, especially the schistosomes (primarily *Schistosoma mansoni, Schistosoma japonicum,* and *Schistosoma haematobium*), although the number of individuals infected with these trematodes is thought to be underestimated for a variety of reasons.[15] Together, the nematode and trematode helminths cause almost 10 million DALYs worldwide.[2] In much of the developing world, soil-transmitted helminth infections and schistosomiasis exhibit their highest prevalence and intensity of infection (as measured by worm burden) among children and teenagers. Children who are chronically infected with heavy worm burdens develop deficits in physical, intellectual, and cognitive growth.[14] In addition, hookworm and schistosomiasis are important health problems in pregnancy and contribute to increased maternal morbidity and mortality as well as low birth weight and prematurity.[16]

Among the global efforts to control soil-transmitted helminth (STH) infections and schistosomiasis are concerted efforts to administer either albendazole or mebendazole to target the STH infections, together with praziquantel for targeting schistosomiasis, on a yearly or twice-yearly basis. Because STH infections and schistosomiasis primarily affect school-age children, anthelmintic drugs often are administered as part of school-based health and education efforts.[14] However, although albendazole and praziquantel are often effective at removing current infections, children reacquire new helminth infections several months after treatment.[17] This potentially limits the usefulness of anthelmintic agents as a school-based public health control measure, particularly in light of new evidence for the diminished efficacy of anthelmintic drugs with frequent use or the possible emergence of anthelmintic drug resistance.[18] Moreover, single-dose mebendazole is highly ineffective as a treatment for hookworm infection and trichuriasis.[19] A recent survey of experts concluded that mass drug administration alone will not be sufficient to effect elimination either of STH infection or schistosmiasis.[20]

As an alternative approach to helminth control or elimination, efforts continue to develop anthelmintic vaccines. The goals of anthelmintic vaccination are different from those of conventional antiviral and antibacterial vaccination, as it is unlikely that immunization with defined antigens will elicit sterilizing immunity against these complex metazoan organisms, many of which have several life cycle stages in the human host. Instead, the most important goal is to reduce worm burden to below the disease-causing threshold; that is, to immunize against the disease rather than the infection. For example, the goal is to reduce hookworm burden to below the threshold that results in significant intestinal blood loss and can lead to anemia,[21] or to reduce the schistosome worm burden below the threshold that results in significant egg

TABLE 43.1 Human Antiparasitic Disease Vaccines Entering or in Clinical Development

Disease	Parasite Species Targeted	Vaccine	Major Antigens/Adjuvants	Sponsor/Lead Organization (PDP)
Hookworm infection	*Necator americanus*	Human hookworm vaccine	*Na*-GST-1 (24-kDa recombinant glutathione-*S*-transferase) *Na*-APR-1 (45-kDa recombinant inactivated aspartic protease) Alhydrogel ± GLA or other immunostimulant	Sabin Vaccine Institute PDP HOOKVAC Consortium
Schistosomiasis	*Schistosoma haematobium*	Bilhvax	*Sh28*GST (28-kDa recombinant glutathione-*S*-transferase) Alum formulation	Institut Pasteur and INSERM
	Schistosoma mansoni	*Sm*-TSP-2 schistosomiasis vaccine	*Sm*-TSP-2 (9-kDa recombinant tetraspanin) Alhydrogel ± GLA	Sabin Vaccine Institute PDP
	S. mansoni	*Sm*-14 schistosomiasis vaccine	*Sm*-14 (14-kDa recombinant fatty acid binding protein)	Oswaldo Cruz Foundation (Fiocruz)
Leishmaniasis	*Leishmania* spp	Leish-111f + MPL, or component recombinant antigens	*Leishmania* recombinant polyprotein MPL	IDRI
	Leishmania spp	LEISHDNAVAX	T-cell epitope-enriched DNA vaccine	European consortium of partners led by London School of Hygiene and Tropical Medicine
	Leishmania spp	Adenovirus-based leishmaniasis vaccine	HASPB and KMP11	European consortium of partners
Chagas disease	*Trypanosoma cruzi*	Recombinant protein-based Chagas disease vaccine	Tc24 + second antigen + stable emulsion of GLA or E6020	Sabin Vaccine Institute PDP with Mexican Consortium of partners, led by Carlos Slim Health Institute, with IDRI and Eisai Co, Ltd

APR, recombinant aspartic protease (hemoglobinase); GLA, glucopyranosyl lipid adjuvant; GST, glutathione-*S*-transferase; IDRI, Infectious Disease Research Institute (Seattle, WA); INSERM, French National Institute for Health and Medical Research (Institut National de la Santé et de la Recherche Médicale); MPL, monophosphoryl lipid; PDP, product development partnership.

deposition and subsequent granuloma formation in the liver, intestines, or bladder.[22] Still another approach to vaccination against helminth disease would be to directly block the action of parasite-induced pathogenic processes. In the case of hookworm, this would require blocking parasite-derived virulence factors that cause blood loss, and for schistosomiasis, blocking egg deposition.[22] It is likely that anthelmintic vaccination will not be used in isolation, but will probably be used in conjunction with other control efforts, including conventional chemotherapy as described above.[23]

Hookworm Infection

Human hookworm infection is a leading cause of anemia and malnutrition among children in developing countries,[24] with the greatest number of cases in sub-Saharan Africa, Asia, and tropical regions of the Americas.[14] *N. americanus* is the predominant hookworm worldwide, with *A. duodenale* responsible for most of the remaining cases. Humans become infected when third-stage larvae penetrate the skin and undergo extraintestinal migration in the vasculature and reach the heart and lungs. Migration of the hookworm larvae to the lung is associated with a mild pneumonitis, with the larvae ascending the airways and reaching the larynx before they are coughed and swallowed. The larvae molt twice in the intestine to become adult hookworms, which invade tissue and cause blood loss. Unlike other STH infections, high worm burdens with hookworms occur in both children and adults (including pregnant women),[24] so pediatric deworming has no impact on adult populations or on reducing the transmission dynamics of the infection.

Early attempts at developing a hookworm vaccine relied on the observation that numerous small doses of living third-stage infective larvae (L3) of the dog hookworm *Ancylostoma caninum* could confer resistance against challenge hookworm infections.[25] Resistance was measured by reductions in worm burden, worm size, and worm fecundity (as measured by egg counts in feces). Immunity was never sterilizing, but efficacy was between 60% and 70%. Later it was noted that larger doses of living L3 could be administered over shorter time periods if they were first damaged by ionizing radiation. This provided the basis for commercial radiation-attenuated hookworm L3 vaccines that could be administered to dogs in two doses, with an efficacy of 90%. The canine hookworm vaccine was marketed in the eastern United States during the 1970s, but ultimately it failed as a commercial veterinary product.[25]

Although it is not feasible to develop human antihookworm vaccines using living L3 (damaged or otherwise), studies have been done to identify, isolate, clone, express, and test vaccine antigens from L3 that can reproduce the reduction in worm burdens afforded by the live vaccines.[26–32] One promising class of antigens to emerge from these investigations is the *Ancylostoma*-secreted proteins (ASPs), which are released by host-stimulated L3 and contain amino acid sequences homologous to those of the major antigens from insect venoms.[27,28] In preclinical studies, it was shown that ASP-2 is an immunodominant antigen associated with the irradiated L3 vaccine,[26,29,30] and that it is protective against challenge infections with animal hookworms.[29–31] The ASP-2 from *N. americanus* (*Na*-ASP-2) was expressed in yeast and selected for subsequent process development and pilot manufacture.[30] These studies were conducted by the Sabin Vaccine

Institute PDP. *Na*-ASP-2 was formulated with Alhydrogel and underwent Phase I safety and immunogenicity testing in hookworm-naïve individuals in the United States, where it was shown to be safe and immunogenic.[32] However, a subsequent Phase I safety and immunogenicity study in a hookworm-endemic area of Brazil revealed that this vaccine could induce a generalized urticaria among chronically infected individuals with high levels of prevaccination immunoglobulin (Ig) E to the native form of ASP-2.[33] Given these safety concerns, the larval antigen program was halted in favor of the development of a different class of antigens.[21,22]

An alternative approach to hookworm vaccination currently under development by the Sabin Vaccine Institute PDP takes advantage of the fact that adult hookworms ingest blood.[21,22,24] Several proteolytic enzymes required for the parasite's ability to digest hemoglobin have been shown to line the brush-border membrane of the hookworm's gastrointestinal tract.[34] Vaccination with a recombinant aspartic protease (in this case, a hemoglobinase), aspartic protease (APR)-1 was shown to reduce worm burden, worm fecundity, and host blood loss after hookworm challenge infection in dogs.[35] By site-directed mutagenesis, an enzymatically inactivated hemoglobinase from *N. americanus* (*Na*-APR-1) was also shown to induce neutralizing antibodies against multiple hookworm species and protect dogs against heterologous hookworm infection.[36] In addition, a unique glutathione-*S*-transferase from *N. americanus* (*Na*-GST-1) was shown to exhibit a unique heme- and hematin-binding function and is hypothesized to be required for parasite heme detoxification. A recombinant *Na*-GST-1 expressed in yeast is highly protective in laboratory animals.[21,22,37,38] Phase I studies in the United States, nonhookworm endemic areas of Brazil, and hookworm (*N. americanus*) endemic areas of Brazil show that *Na*-GST-1/Alhydrogel is safe and immunogenic . The *Na*-APR/Alhydrogel has shown to be safe at an interim time point 30 days after the third immunization (unpublished observation).

These studies provide the scientific basis of an adult hookworm-vaccine antigen development program for both antigens to be coadministered. Through a new HOOKVAC Consortium, supported by the European Commission, both formulations will also undergo Phase I testing in West-Central Africa (Gabon). Ultimately, the final human hookworm vaccine will probably be composed of *Na*-GST-1 and *Na*-APR-1, combined and formulated with Alhydrogel (and possibly a second immunostimulant such as a synthetic Toll-like receptor 4 [TLR4] agonist known as GLA [glucopyranosyl lipid A]), with a vaccination strategy that may be linked either to the Expanded Programme on Immunization or to anthelmintic chemotherapy in preschool or school-age children.[21,22] A cost-effectiveness study confirms the potential economic value of a hookworm vaccine.[39]

Schistosomiasis

Schistosomes are snail-transmitted, waterborne parasitic platyhelminths (order Trematoda). High rates of infection occur near bodies of fresh water, such as tributaries of the Nile River in Egypt and the Dongting and Boyang lakes in China,[40,41] with the largest number of cases occurring in Africa. Revised estimates indicate that more than 400 million cases may occur in Africa,[15] with roughly two-thirds of the cases resulting from *S. haematobium*, the cause of urogenital schistosomiasis, and one-third from *S. mansoni*, the cause of intestinal schistosomiasis.[42] Africa's schistosomiasis accounts for more than 90% of the world's cases, with most of the remainder caused by *S. mansoni* in Brazil, and elsewhere in Latin America, or *S. haematobium* and *S. mansoni* in the Middle East, and approximately 1% caused by the *S. japonicum* complex (including *S. japonicum* and *Schistosoma mekongi*) in East Asia.[40] The human schistosomes are typically distinguished by their snail vectors, location in the host vasculature, and egg morphology. Members of the *S. japonicum* complex also have important domestic animal reservoir hosts (pigs, cattle, water buffaloes). Asexual reproduction of the parasites occurs in the freshwater snail intermediate hosts that release large numbers of free-swimming, infective larval schistosomes (cercariae) into the water (Fig. 43.1). The cercariae invade human skin, lose their tail, and then (now called schistosomula) spend the next few weeks migrating through the bloodstream and lungs until they reach the liver, where they differentiate into male and female schistosomes. Male and female worm pairs migrate through the portal vasculature until they reach their final destination in the mesenteric (*S. mansoni* and *S. japonicum* complex) or bladder venules (*S. haematobium*). The worm pairs release eggs, which exit from the body in feces or urine and then hatch in fresh water. Most of the morbidity associated with schistosomiasis occurs when the eggs fail to exit from the human host and become trapped in the intestinal or bladder wall or in the liver, where they elicit granulomas and host fibrosis. In the liver, Symmers pipestem fibrosis from chronic *S. mansoni* or *S. japonicum* infection leads to portal hypertension and hepatosplenomegaly. In the bladder, *S. haematobium* eggs stimulate formation of multiple granuloma, resulting in hematuria and an obstructive uropathy leading to chronic urinary tract infections, hydronephrosis, and kidney failure.[40] Chronic bladder fibrosis from the *S. haematobium* eggs is also associated with squamous cell carcinoma of the bladder.[43] *S. haematobium* eggs also cause granuloma formation in the uterus and cervix, leading to female genital schistosomiasis, which is an important risk factor for HIV/AIDS in Africa.[44] In addition, chronic schistosomiasis is associated with a wide variety of other sequelae, especially in children, including anemia, chronic pain, undernutrition, growth failure, and cognitive deficits. The full burden of disability-related outcomes in endemic schistosomiasis is only beginning to be fully appreciated.[45]

A number of different approaches have been taken to design antischistosomal vaccines. As in other systems, the administration of radiation-attenuated cercariae results in the best protection to date in mice. According to Wilson and Coulson,[46] the development of the Th1/Th2 paradigm for T-helper (Th) cell differentiation was extremely influential in interpreting the radiation-attenuated vaccine model. The two major patterns of cytokine synthesis correlate with the induction of cell-mediated (Th1) or humoral (Th2) immunity, thus providing a possible explanation for the separate and often reciprocal regulation of these responses in chronic schistosomiasis infection. This dichotomy in Th cell differentiation, together with subsequent identification of T-regulatory (Tr) cells and Th3/Tr1 (downregulatory interleukin [IL]-10 and transforming growth factor β production) extensions, provides the framework for the mechanisms operating in the radiation-attenuated vaccine model. It is clear that under appropriate conditions, the radiation-attenuated vaccine can elicit a partially protective Th1 or Th2 response. If such responses were directed against different parasite stages (larval and adult) and at different sites (skin, lung, mesentery), they might act additively or even synergistically to provide a very high level of protection. According to Wilson and Coulson, it is also possible that these responses to the radiation-attenuated vaccine might actually prove mutually exclusive, in which case it would be necessary to opt for the one that gave the maximal level of protection.

A second important step forward would be the identification and cloning of the antigens that mediate the respective Th1 and Th2 mechanisms induced by the radiation-attenuated vaccine.[46] The task would then be to formulate the relevant

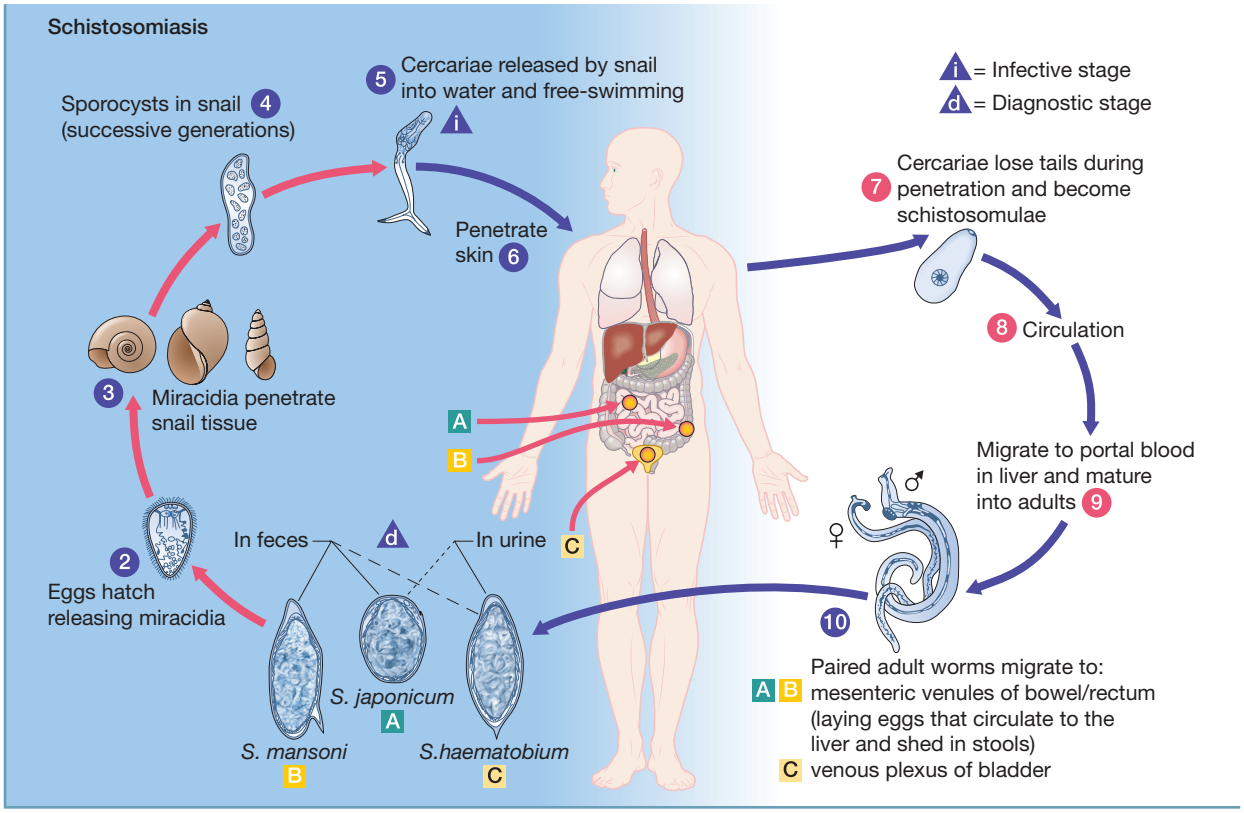


Figure 43.1. Life cycle of human schistosomes. *(From the Public Health Image Library of the Centers for Disease Control and Prevention, http://phil.cdc.gov/phil/details.asp?pid=3417.)*

antigens to invoke the desired responses, perhaps by means of two distinct and sequential vaccination procedures. In short, the radiation-attenuated vaccine model offers the possibility that, by manipulating the cytokine profile, we can bias the immune response in a protective direction. More specifically, if vaccination deflects the Th response in either a Th1 or Th2 direction, it will produce a measure of protection.[46]

Vigorous attention has been focused on potential vaccine antigens from several schistosomula stages. Vaccination with stage-specific schistosomula surface antigens has resulted in protection (reductions in worm burden or egg counts) that is usually less than 40%.[47] Somewhat better protection has been achieved using antigens shared between schistosomula and adult schistosomes, including parasite-derived myosin (63 kDa), paramyosin (97 kDa), triose phosphate isomerase (28 kDa), glutathione-*S*-transferases (GSTs; 26 and 28 kDa), a fatty acid–binding protein (14 kDa), and a 23-kDa surface protein. To date, most of these proof-of-concept vaccine studies have been conducted in mice challenged with either *S. mansoni* or *S. japonicum*. However, because Asian schistosomiasis is associated with significant animal reservoirs, including cattle, water buffaloes, and pigs, several veterinary vaccine trials have been conducted in China in these reservoir hosts, with a goal of developing an *S. japonicum* transmission-blocking vaccine.[47] To date, the recombinant antigen-based vaccine trials have been conducted with numerous adjuvants, including Freund's complete adjuvant, alum, bacille Calmette-Guérin (BCG), saponin, Quil A, and *Bordetella pertussis*. Because of the suggested role of interferon (IFN)-γ and Th1 cellular immune responses in mediating protection, there have been some efforts to bias the host cytokine profile at the time of vaccination. Some success has been reported in this regard using IL-12 as an adjuvant and with DNA immunizations.[41,47] The molecular basis of immunity with the schistosomula antigens is largely unstudied, except for new evidence that paramyosin may function as an Fc receptor for the parasite, allowing it to adsorb host immunoglobulin to shield it from the host immune response. Therefore, an antiparamyosin immune response may somehow interfere with the parasite's immune escape mechanisms.[48]

A second approach to vaccination against schistosomiasis has been to target the fecundity of female adult schistosomes so as to diminish egg excretion into target host organs. Success with this approach has been reported by immunizing mice, nonhuman primates, and large-animal reservoir hosts, including pigs and water buffaloes, with 26-kDa and 28-kDa variants of a GST.[47,49] If levels of egg excretion can be reduced by vaccination of animal reservoir hosts, this approach might be sufficient to reduce *S. japonicum* transmission.[47] Alternatively, it has been proposed that egg deposition and granuloma formation in the human host might be manipulated by immunotherapy, because granuloma formation around the schistosome egg depends heavily on host cytokines, including tumor necrosis factor and Th2-associated cytokines.

During the late 1990s, the World Health Organization's (WHO) Special Programme for Research and Training in Tropical Diseases (WHO/TDR) initiated murine trials of six vaccine candidates for *S. mansoni:*

- A 28-kDa GST, as noted earlier[49,50]
- A 97-kDa paramyosin[51]
- An irradiated larvae-associated vaccine antigen, the 62-kDa IrV-5,[52] which is a derivative of a 200-kDa molecule with extensive homology with human myosin

- A 28-kDa triose phosphate isomerase[53]
- A 23-kDa integral membrane antigen (Sm-23) that is part of a superfamily of proteins that includes CD9 and TAPA-1, first described in hematopoietic cells[54]
- A 14-kDa fatty acid–binding protein that was thought to also have protective immune cross-reactivity with the liver fluke, *Fasciola hepatica*.[55]

None of these candidate proteins reached the requirement set by WHO/TDR of generating a 40% or better reduction in challenge-derived worm burdens relative to nonimmunized controls.[56] However, it was recommended that work should continue on these antigens, including progression to clinical or veterinary trials.

In terms of vaccines now entering the clinic, a membrane-spanning *S. mansoni* surface protein (tetraspanin), known as *Sm*-TSP-2, has been identified. *Sm*-TSP-2 results in a 60% to 70% worm burden reduction in mice and is selectively recognized by IgG_1 and IgG_3 antibodies in putatively resistant individuals, but not in chronically infected individuals living in endemic areas of Brazil.[57] Furthermore, schistosomes treated with double-stranded RNA encoding *Sm*-TSP-2 display a vacuolated and disrupted tegument and are unable to fully develop in mice.[58] The extracellular domain of *Sm*-TSP-2 has been expressed in yeast, and, under the auspices of the Sabin Vaccine Institute PDP, this antigen is undergoing clinical testing as a vaccine formulated with Alhydrogel (with GLA as a possible immunostimulant) for intestinal schistosomiasis.[22] An additional membrane-spanning tegumental antigen now also in Phase I clinical trials is the *Sm*-14, a fatty acid–binding protein.[59]

For urogenital schistosomiasis, a group at the Institut Pasteur in Lille, France, has produced the 28-kDa GST from *S. haematobium* (Sh28GST) in yeast under current good manufacturing practices and has embarked on clinical testing.[49] Sh28GST was selected on the basis of protection studies in primates,[49] and studies in humans, which showed that IgG_3 to Sh28GST correlates with an age-dependent decrease in egg output in *S. haematobium* infection.[60] Among human volunteers, two subcutaneous injections of 100 μg of recombinant Sh28GST at 28-day intervals on alum did not result in any significant toxicity, and resulted in an immune response characterized by antibodies that neutralized the enzymatic activity of Sh28GST.[49,61] Studies are underway to evaluate the clinical efficacy of this vaccine, known as Bilhvax, in infected patients in sub-Saharan Africa, with an aim to determine if there is synergy with praziquantel chemotherapy. Beyond these candidate vaccines now in clinical trials, Sm-p80, the large subunit of *S. mansoni* calpain has undergone extensive preclinical testing in baboons and has been shown to cross-protect against *S. haematobium*.[62,63]

Larval Tapeworm Infections: Cysticercosis and Echinococcosis

The pork tapeworm, *Taenia solium*, is a major parasite of veterinary and medical importance. When humans serve as an intermediate host, *T. solium* can cause an important human infection known as neurocysticercosis. Because transmission of *T. solium* from pigs to humans can be interrupted, neurocysticercosis has been identified as a potentially eradicable disease, and increasing attention is being placed on efforts to control transmission of the parasite. One way to control the disease is to prevent infection in pigs by vaccinating them, thereby breaking the parasite's life cycle and removing the source of infection for humans. Several approaches to development of transmission-blocking veterinary vaccines against *T. solium* are being examined, the most promising of which is the application of recombinant oncosphere antigens.[64] Efforts are also underway to develop canine vaccines for the adult stages of *Echinococcus granulosus* as a means to interrupt transmission of the disease to humans and prevent hydatid cysts.[64]

ANTIPROTOZOAN VACCINES

Aside from malaria (see Chapter 35), the major parasitic protozoan infections include the vector-borne kinetoplastid protozoan infections, resulting in leishmaniasis, Chagas disease, and human African trypanosomiasis, which cause almost 5 million DALYs and 70,000 deaths; and the intestinal protozoan infections led by cryptosporidiosis and amebiasis, associated with approximately 10 million DALYs and 150,000 deaths.[1,2,65] Giardiasis is yet another important intestinal protozoan infections for which there are no disease burden estimates published by the Global Burden of Disease Study published in 2010. The same is true for toxoplasmosis. In terms of human vaccines, only leishmaniasis vaccines have advanced into clinical development.[66] There is also interest in a canine leishmaniasis vaccine to prevent zoonotic transmission to humans.[67]

Leishmaniasis

Leishmania species are flagellated kinetoplastid protozoan parasites transmitted by the bite of a female sandfly. There are cutaneous (CL), mucocutaneous, and visceralizing (VL) forms of human leishmaniasis. CL and mucocutaneous leishmaniasis in the Western Hemisphere (predominantly in Central and South America) are caused by members of the *Leishmania mexicana* and *Leishmania braziliensis* complex, whereas CL in the Eastern Hemisphere (predominantly in India, central Asia, and parts of Africa and the Middle East) is caused by members of the *Leishmania tropica* and *Leishmania major* complex. Recently, as a consequence of the conflict in Syria, it is estimated that more than 100,000 cases of CL caused by *L. tropica* may have occurred there. VL, also known as kala-azar, is caused by *Leishmania donovani* in India, Bangladesh, Nepal, East Africa, and China; by *Leishmania infantum* in central Asia, North Africa, and southern Europe; and by *Leishmania chagasi* in Latin America. Many, if not most, investigators now consider *L. infantum* and *L. chagasi* as identical species and sometimes refer to the organism as "*Leishmania chagasi infantum*." Members of the *L. donovani* complex are important emerging opportunistic pathogens in patients with AIDS. Recent estimates indicate that between 200,000 and 400,00 cases of VL occur annually, while 700,000 to 1.2 million cases of CL occur annually, with 10 million people living with leishmaniasis.[2,68]

Leishmanization, the ancient Middle Eastern and central Asian practice of injecting live *Leishmania* parasites as a means of actively immunizing against disease, predates vaccination and may be as old as variolation. It relies on inoculating a person with live parasites using a thorn or other sharp instrument to artificially create a cutaneous lesion. Alternatively, an arm or other body part is deliberately exposed to the bite of sandflies.[11] The procedure causes mild disease (typically 1 to 2 cm in diameter) at an unexposed site, frequently the arm or buttocks, that heals over a period of 3 to 4 months. Successful leishmanization prevents the possibility that a cosmetically disfiguring lesion ("Delhi boil" or "oriental sore") might appear on the face. Leishmanization strategies were developed in Israel by Saul Adler at Hebrew University and in the former Soviet Union.[12] One such leishmanization "vaccine" is registered in Uzbekistan.[11] In the 1980s, leishmanization was reintroduced as an emergency public health measure during the Iran–Iraq war, when approximately 2 million people were inoculated.[69] The Iranian leishmanization program was

subsequently halted when it was learned that up to 3% of the induced lesions lasted for more than a year; in some instances, the lesions never healed, even with subsequent antiparasitic antimonial chemotherapy.[69] To date, leishmanization is the only vaccine with proven efficacy in humans.[12] Several live attenuated strains of *L. major* are also under development as potential vaccines.[12] Among the hurdles that potentially block further refinement of leishmanization and related practices are the difficulties in standardizing the virulence of the vaccine and the constant risk of developing severe and persistent lesions, especially in individuals with HIV/AIDS and other immune deficiencies.[12]

In an effort to establish a safer anti-*Leishmania* immunization procedure, Iran launched a national vaccine development program during the early 1990s. A first-generation vaccine comprising killed *Leishmania* parasites was prepared under good manufacturing practices by the Razi Vaccine and Serum Research Institute (Hessarak, Iran). The cell bank for the vaccine was derived from the same strain of *L. major* used previously for the wartime leishmanization program. The *L. major* parasites were killed by autoclaving and were then formulated with BCG. In a randomized, double-blind trial, the safety and efficacy of a single injection of the vaccine was compared with a BCG control in 3637 schoolchildren.[69] Children with a prior history of CL or those with a positive skin test reaction against leishmanial antigen were excluded from the study. The Iranian trial demonstrated the vaccine's safety, although its efficacy was not apparent until 6 months after the injection. It has been suggested that there was some protective effect from BCG in the immediate postvaccination period. Also of interest was the observation that protection was better among boys who, because of Iranian dress and customs, presumably are exposed to a greater number of sandfly bites than girls.[69] Based on these results, additional trials are underway to evaluate multiple injections of the killed vaccine, as well as new formulations with alum and IL-12. A trial to evaluate the killed vaccine against *L. donovani* is also underway in Sudan. In South America, killed *Leishmania amazonensis* and *L. mexicana* vaccines were being produced by Biobras (Brazil) and Instituto de Biomedicina (Venezuela), respectively. Biobras was since purchased and the leishmaniasis vaccine program was halted.[66] In reviewing the data from several clinical trials of vaccines composed of whole-parasite antigens, Coler and Reed[70] conclude that the efficacy varies from 0% to 75% against CL, but it is only 6% against VL. A Phase III clinical trial in Ecuador and Colombia showed that the *L. amazonensis* vaccine was safe but not efficacious.[12] A metaanalysis concluded that killed whole-parasite vaccines do not confer significant protection against human leishmaniasis.[71]

Earlier studies on whole-organism leishmania vaccines have stimulated efforts by several laboratories to reproduce vaccine protection using second-generation recombinant subunit antigens. Several antigens have been identified on the basis of their surface localization, recognition by T-cell clones, and immunoscreening using patient sera.[66,70] A number of candidates have shown promise in mice, particularly when formulated with IL-12 or other costimulators of Th1 immunity. In some cases, vaccination with antigens (either alone or in combination) encoded by plasmid DNA elicits more potent and durable immunity.

Development of a human leishmaniasis vaccine has been advanced most by a group at the Infectious Disease Research Institute (IDRI), a PDP in Seattle, Washington. The IDRI group has developed several lead candidate vaccines currently undergoing human trials in the United States, Latin America (including Brazil, Colombia, and Peru), and India.[66] The most extensive testing has been done on a polyprotein composed of three priority candidate antigens, *L. major* thiol-specific antioxidant, *L. major* stress-inducible protein 1 (LmSTI1), and *Leishmania* elongation initiation factor. For VL, both Leish-111f + MPL-SE and a *Leishmania* sterol 24-C-methyltransferase formulated in adjuvant are also under development.[72,73] Some of these candidates as well as additional ones are also under development as canine vaccines to prevent zoonotic transmission to humans. In parallel, a European group based at the University of York in England, the Humboldt University, and the Robert Koch Institut in Berlin, Germany, are developing an adenovirus-derived leishmaniasis vaccine for VL composed of two antigens—HASPB and KMP11—which are in Phase I trials,[74] while through a European Union consortium of partners (LEISHDNAVAX) a multiantigen T-cell epitope-enriched DNA vaccine is being developed.[75] Finally, some additional success has been reported in the development of transmission-blocking vaccines based on immunization with sandfly salivary gland proteins.[76–78] The Sabin Vaccine Institute PDP is developing some of these candidates as potentially cost-effective CL vaccines.[79]

Chagas Disease

Chagas disease (American trypanosomiasis caused by *Trypanosoma cruzi*), like leishmaniasis, is another kinetoplastid of great public health and economic importance. An estimated 7.5 million people are infected in the Americas, where it is a leading cause of disabling cardiomyopathy,[2] including hundreds of thousands of people infected in the United States where transmission can occur.[80] Several thousand imported cases of Chagas disease are also present in Spain and elsewhere in southern Europe, Australia, and Japan. A recent cost analysis indicates that a cost-effective preventive Chagas disease vaccine would have substantial economic benefit.[81] Equally compelling are modeling studies suggesting that a therapeutic vaccine given to *T. cruzi*–infected individuals to prevent or delay chagasic cardiomyopathy would be both cost-effective and cost saving.[82] Several experimental vaccine candidates, including DNA vaccines, recombinant proteins, and some peptides and carbohydrates are under development as preventive and therapeutic vaccines,[83–86] but none have progressed to clinical trials. In mice, protective immunity is associated with strong Th1 responses to defined *T. cruzi* antigens. Because of the questionable benefit of currently available drugs for the treatment of seropositive individuals to delay or prevent chagasic cardiomyopathy, there is an urgent need for new approaches, including immunotherapy or therapeutic vaccines that stimulate *T. cruzi*–specific Th1 immunity.[87] A therapeutic vaccine is under development by the Sabin Vaccine Institute PDP, in collaboration with a consortium of Mexican partners, including the Carlos Slim Health Institute, the Southwest Electronic Energy Medical Research Institute, and Eisai Co., Ltd, together with the Japanese Government.[87]

Amebiasis

Amebiasis, secondary to intestinal and hepatic infection with *Entamoeba histolytica*, is a leading neglected tropical disease in low- and middle-income countries. Human infection occurs through the ingestion of parasite cysts and subsequent invasion by trophozoites. Amebic colitis results from ulcerating mucosal lesions that result from chemical invasion caused by the release of parasite-derived proteases and hyaluronidases. Metastatic spread to the liver occurs when the trophozoites gain access to the afferent circulation that drains the colon into the portal vein. Immunizations using both native and recombinant forms of the parasite Gal-lectin have shown great promise in protecting laboratory animals, including gerbils, against *E. histolytica* challenge infections.[88] Other candidates,

including a serine-rich protein and a 29-kDa reductase antigen, have shown promise.[88] To date, however, no human testing of a vaccine for amebiasis has been initiated.[88] In addition to vaccinating against amebiasis, there has also been some interest in developing vaccines against primary amebic meningoencephalitis caused by the free-living ameba, *Naegleria fowleri*. The nfa1 gene, which encodes a 13-kDa recombinant protein on parasite pseudopodia has emerged as a promising vaccine candidate.[89]

Toxoplasmosis

Toxoplasmosis is one of the most common infections of humans, with some estimates suggesting that 6 billion people have been infected with *Toxoplasma gondii*.[90] Currently, the highest global prevalence and incidence may occur in South America,[90] although there is a dearth of information on the true global disease burden of human *T. gondii* infection.[2] In the United States, an estimated 1 million new infections occur each year, resulting in an estimated 20,000 cases of retinal infection and 750 deaths.[90] Several research groups have developed promising approaches for a human toxoplasmosis vaccine. Because, like *Leishmania* and *T. cruzi*, *T. gondii* is an intracellular parasite, such efforts are primarily directed at stimulating *T. gondii*–specific Th1 immunity and generating long-lived IFN-γ–producing $CD8^+$ T cells.[91]

In preclinical studies, several approaches have shown promise. They include viral vectors and constructs capable of expressing *T. gondii* antigens,[92] DNA vaccinations,[93] live attenuated parasites,[94] and pairing recombinant antigens with Th1-stimulating adjuvants.[94] Transmission-blocking approaches from zoonotic reservoir hosts including cats and livestock used for food have also been proposed.[94–97] There is an urgent need to better define the target product profile for toxoplasmosis vaccines with respect to target populations—that is, preventing congenital infections versus producing therapeutic vaccines for immunocompromised individuals—and to gain consensus on lead candidate antigens, as well as to determine how to best overcome genetic restrictions to the human immune response.[97,98] No human vaccine trials are underway.

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44 Pertussis Vaccines

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HISTORY OF DISEASE

Pertussis (whooping cough) is a bacterial respiratory infection caused by *Bordetella pertussis,* a Gram-negative bacillus. Its major manifestation is a protracted cough that lasts many weeks, marked by characteristic intense paroxysms that often terminate in an inspiratory "whoop." Absent vaccination, nearly all children will contract pertussis. The first known description of an outbreak of pertussis is that of Guillaume De Baillou, who described an epidemic in Paris in the summer of 1578[1] that primarily affected infants and young children, resulting in many deaths. In Britain during the early 16th century, a disease known as chyne-cough was probably pertussis, and the terms whooping cough and chincough appeared in the London Bills of Mortality in 1701.[2] The causative organism was first grown by Jules Bordet and Octave Gengou in 1906, and the first crude vaccines appeared soon thereafter.[3]

WHY THE DISEASE IS IMPORTANT

Although pertussis affects all age groups, it is most hazardous for infants, who account for nearly all pertussis hospitalizations and deaths. Before widespread use of pertussis vaccines prepared from inactivated whole bacterial cells, there were as many as 270,000 cases of pertussis reported each year in the United States, with 10,000 deaths.[4] The occurrence of pertussis declined markedly after universal pertussis vaccination of children with whole-cell vaccine in the 1940s, to a nadir of 1010 reported cases in 1976.[5] Reported cases have since progressively increased, with more than 48,000 cases reported for 2012, the highest total since 1959.[6–10] However, because pertussis still frequently goes undiagnosed, it is likely that the actual number of cases in all age groups greatly exceeds the number reported. Globally, pertussis remains an important public health problem. The World Health Organization (WHO) estimated that despite universal vaccination programs, in 2008 some 16 million cases of pertussis occurred worldwide annually (95% of these occurring in developing countries), resulting in about 195,000 pediatric deaths.[11] In a more recent position paper, WHO estimated that pertussis was still causing around 63,000 deaths in children younger than 5 years.[11a] Both projections are based on many assumptions, but taken together they show that pertussis remains an important cause of child mortality.

BACKGROUND

Clinical Description

Infants and Young Children

The incubation period of pertussis is commonly 7 to 10 days (range: 4 to 21 days), with an insidious onset and initial symptoms indistinguishable from a minor upper respiratory infection (the catarrhal stage). Fever is usually minimal throughout the course of infection. The cough, initially intermittent, progresses within 1 or 2 weeks to become paroxysmal. The paroxysms increase in both frequency and severity, and then gradually subside over a period of 2 to 6 weeks, but sometimes longer.

During the paroxysmal stage, a series of rapid coughs without intervening inspiration will often be followed by the characteristic whoop, caused by the desperate effort to inspire through a narrowed glottis. The characteristic whoop is much less common in young infants. During a paroxysm, cyanosis may occur and vomiting may follow. After the episode, the child is often exhausted; several paroxysms may occur successively within a few minutes. Between paroxysms, the child may be playful and appear quite normal. Paroxysms can be induced by eating, laughing, crying, and a variety of other stimuli, and are usually worse at night.

For an infant with suspected pertussis, it is imperative that the clinician witnesses a paroxysm to ensure that apnea or respiratory arrest does not occur before determining that the child can be managed as an outpatient. Recovery is gradual. The paroxysms become less frequent and milder, and the whoop disappears. Nonparoxysmal cough may persist for many weeks and intercurrent viral infections can trigger a recurrence of the paroxysms.

In the absence of immunization, most children experience the full-blown disease; however, some children appear to develop either clinical immunity or serologic evidence of prior infection without a history of clinical pertussis, suggesting that mild atypical cases do occur.[12,13] Pertussis should be considered in all unvaccinated children admitted to hospital with cough and lower respiratory tract disease.[14]

One of the largest studies to characterize the symptoms associated with pertussis in infants and children was conducted from October 1990 to September 1996 in Germany, during active surveillance for a pertussis vaccine trial.[13] *B. pertussis* was isolated in 2592 patients (age range: 6 days to 41 years); 50.7% were female. In unvaccinated patients, 90.2% had paroxysmal cough, 78.9% demonstrated whooping, and 53.3% presented with posttussive vomiting. Fever was uncommon; only 5.7% had a temperature of 38°C or higher. Leukocytosis and lymphocytosis were characteristic of pertussis, and values above the age-specific mean were observed in 71.9% and 75.9% of unvaccinated children, respectively.

Winter and colleagues compared risk factors associated with fatal pertussis among 53 infant pertussis patients younger than 120 days of age in California, matched with 183 nonfatal hospitalized cases by age, county of residence, and closest symptom onset date.[15] Fatal cases had significantly lower birth weight, younger gestational age, younger age at time of cough onset, and higher peak white blood cell and lymphocyte counts. Fatal cases were less likely to have received macrolide antibiotics and more likely to have received steroids or nitric oxide and to develop pulmonary hypertension, seizures, encephalitis, and pneumonia. Additionally, exchange transfusion, extracorporeal membrane oxygenation, and intubation occurred significantly more frequently among fatal cases. In multivariate analyses, peak white blood cell count, birth weight, intubation, and receipt of nitric oxide were predictors of death. This chapter underscores that early recognition of pertussis in young infants and treatment with appropriate antibiotic therapy are important in preventing death.

Adolescents and Adults

Prior to widespread vaccination against pertussis, it was believed that only young children were susceptible to pertussis. We now understand that near-universal pertussis among children ensured that adolescents and adults were exposed to the bacterium sufficiently often to maintain their immunity through subclinical infection. Moreover, pertussis among adolescents and adults is not often associated with whoops, obscuring the correct diagnosis. The clinical characteristics of pertussis in older adolescents and adults were highlighted in a large Canadian study.[16] Females comprised 71% of the reported cases, probably reflective of their frequent contact with children. Nearly 60% of the older adolescents and adults reported prior pertussis vaccination, with fewer than 10% having had prior natural infection. Subjects with confirmed pertussis had a median of 56 days of cough and 43 days with violent cough. Vomiting was seen in 46% of the subjects, night cough in 84%, and apnea for 30 seconds after cough in 14%. Adults had higher rates of complications than adolescents, including pneumonia.

German household-contact studies conducted during active surveillance for pertussis vaccine trials also highlighted the signs and symptoms of adult pertussis.[17,18] Of 79 German adults with symptomatic pertussis, 34% (27) had been diagnosed with natural pertussis as children; 91% (72) had cough with their present illness, 80% (63) had cough lasting longer than 21 days, and 0.01% (1) had a cough for 8 months.[18] Prolonged paroxysmal cough was experienced by 63% (50), cough resulting in sleep disturbance was reported by 52% (41), cough followed by vomiting occurred in 42% (33), and whoops occurred in 8% (6). The adults often expectorated "glassy, viscous mucus." Malaise was reported in 30% (24) and arthralgia in 15% (12). Fourteen percent (11) of patients reported attacks involving flushing and sweating that lasted 1 to 2 minutes, occurring several times a day, and continuing for 2 to 8 weeks. In addition, family studies of children with culture-confirmed pertussis disease and seroprevalence studies have shown that asymptomatic infections are common in adolescents and adults.[19–21]

Complications and Sequelae

Infants and Children

In the German study cited earlier, the overall rate of major complications in infants and children was 5.8%, with 23.8% of infants younger than 6 months of age having complications.[13] The major complications of pertussis disease can be grouped into three categories: pulmonary, neurologic, and nutritional. Of these, pulmonary complications are the most frequent.[13]

Pulmonary Complications. The vast majority of full-blown pertussis patients exhibit some degree of atelectasis or bronchopneumonia. Pathologically, the pneumonia is both interstitial and alveolar, and the usual exudate is primarily mononuclear.[22] Pneumonic involvement may be sufficiently severe to compromise respiratory function and cause death. An autopsy series in 15 infants succumbing to pertussis reported necrotizing bronchiolitis, intraalveolar hemorrhage, and fibrinous edema.[23] Marked leukocytosis was noted, and most sections showed luminal aggregates of leukocytes in small pulmonary arteries, veins, and lymphatics. Immunohistochemical stains revealed abundant extracellular bordetellae in the cilia of the trachea, bronchi, and bronchioles, and intracellular bacteria in alveolar macrophages and ciliated epithelium. Although coinfections with other viral or bacterial organisms were reported in some patients in this series, the predominant histopathologic findings in the respiratory tract did not differ between those who were or were not co-infected. These observations suggest that most pneumonia associated with pertussis is caused by the organism itself. In addition, there has been an increasing appreciation for the role of pulmonary hypertension as an unexplained complication of pertussis in young infants.[24–32] Infants younger than 6 weeks of age with pulmonary hypertension have the highest mortality.

Outcomes of children infected with pertussis and admitted to intensive care units at 25 academic centers have been summarized. A total of 127 patients were identified; median age was 49 days and 105 patients (83%) were younger than 3 months old. Fifty-five patients (43%) required mechanical ventilation and 12 patients (9.4%) died during initial hospitalization. Pulmonary hypertension was found in 16 patients (12.5%) and was present in 75% of patients who died, compared with 6% of survivors ($P < .001$). Median white counts were significantly higher in those requiring mechanical ventilation ($P < .001$), those with pulmonary hypertension ($P < .001$), and nonsurvivors ($P < .001$). Age, sex, and immunization status did not differ between survivors and nonsurvivors. Fourteen patients (11%) received leukoreduction therapy (exchange transfusion [12], leukophoresis [1], or both [1]); survival benefit was not apparent.[33] Generally, children who survive pneumonia or pulmonary complications are left with no permanent lung damage.[34]

Neurologic Complications. Acute pertussis encephalopathy, generally occurring during the paroxysmal stage, is characterized by seizures and altered consciousness, and usually results from hypoxia or intracranial bleeding associated with paroxysmal cough. In a series of infants hospitalized with pertussis in Canada, seizures were seen in 21% of fatal cases but in none of the survivors.[35] A study in Denmark found that hospitalization for pertussis was associated with a 1.7-fold increase in risk of epilepsy at age 10 years.[36] Only limited data on the incidence of encephalopathy are available; estimates from population-based studies have ranged from 8 to 80 per 100,000 cases.[37,38] According to the Centers for Disease Control and Prevention (CDC), encephalopathy occurs in 0.4% of children younger than 12 months of age who are hospitalized with pertussis.[39] Approximately one-third of children with pertussis encephalopathy succumb to the acute illness, one-third survive with permanent brain damage, and one-third recover without obvious neurologic sequelae.[40]

Nutritional and Other Minor Complications. Nutritional deficiencies resulting from repeated vomiting can also be problematic. Inability to maintain adequate caloric intake in already malnourished children who develop pertussis is a particularly severe problem in developing countries.[41,42] Minor complications of pertussis include subconjunctival hemorrhages and epistaxis secondary to the paroxysms, edema of the face, and ulcers of the lingual frenulum resulting from protrusion of the tongue during paroxysms. Suppurative otitis media also occurs frequently, caused by the usual upper respiratory bacteria, not *B. pertussis*.

Adolescents and Adults

Complications of pertussis were reported in 18 (23%) of the 79 adult patients in one study.[18] These complications included otitis media (four patients), pneumonia (two patients), urinary incontinence (three patients), rib fracture (one patient), and severe weight loss (one patient). Telephone interviews of 314 adolescents and 203 adults with laboratory-confirmed pertussis in Massachusetts found that 100%

reported cough, which was paroxysmal for 74% and 84% of adolescents and adults, respectively; 77% and 84%, respectively, reported problems sleeping, 56% and 54% reported vomiting, 3% and 28% reported urinary incontinence, 33% of each group reported weight loss, 1% and 4% reported rib fracture, and 1% and 6% reported loss of consciousness.[43] Other known complications of pertussis in adults include triggering of migraines,[44] carotid artery dissection,[45] cough syncope,[46] and loss of memory.[47,48] Death from pertussis is rare in adolescents and adults, but it has been reported.[7,49,50]

Bacteriology

The causative agent of pertussis is *B. pertussis*, a small, Gram-negative, pleomorphic bacillus. Although the organism was identified before the turn of the 20th century in stained preparations of respiratory secretions and in pathology specimens from children with clinical pertussis,[51] the organism was not recovered in culture until 1906 by Bordet and Gengou.[3] The original culture medium, called Bordet-Gengou medium, is still used in some clinical laboratories, although more complex synthetic media are employed in many laboratories to grow this relatively fastidious organism. The antigens that *B. pertussis* produces are summarized below (see also Table 44.1).

Two other organisms in the genus *Bordetella* are *Bordetella parapertussis* and *Bordetella bronchiseptica*. *B. parapertussis* is responsible for a pertussis-like syndrome in humans, which usually is less severe than pertussis. *B. bronchiseptica* produces respiratory illnesses in domestic animals (e.g., kennel cough in dogs). These three species are very closely related genetically, and there is evidence that *B. pertussis* evolved from an ancestral strain of *B. bronchiseptica*.[52,53] Of all the *Bordetella* species, only *B. pertussis* synthesizes pertussis toxin (PT). Although the chromosomes of *B. parapertussis* and *B. bronchiseptica* contain the PT loci, they are transcriptionally silent because of defective promotors.[54,55] Some have suggested that the curious absence of descriptions of pertussis before the 16th century may represent the adaptation of an animal organism to humans as recently as 5 centuries ago.[52] However, the association of *B. pertussis* with humans may be more ancient than previously assumed.[56]

A *Bordetella* species first described in 1995, *Bordetella holmesii*, is associated with bacteremia, meningitis, endocarditis, pericarditis, pneumonia, arthritis, and respiratory illness with pertussis-like symptoms, mainly in asplenic and immunocompromised patients.[57–62] Infection with *B. holmesii* is frequently misidentified as being caused by *B. pertussis*, the cause of whooping cough, because routine diagnostic tests for pertussis are not species specific. Although no fatal cases of *B. holmesii* infection have been reported, secondary invasive infections caused by opportunistic organisms can cause substantial morbidities, even in previously healthy individuals. Antimicrobial treatment can be problematic because *B. holmesii* is less susceptible to macrolides (used empirically to treat pertussis) and third-generation cephalosporins (often used to treat invasive pulmonary infections). *B. holmesii*'s adaptation to human beings is continuing, and virulence might increase, creating a need for better diagnostic assays and epidemiological surveillance.[63]

Bordetella hinzii, a cause of respiratory disease in poultry, has been isolated from the blood of a patient with AIDS and from the respiratory secretions of a patient with cystic fibrosis.[64–66]

TABLE 44.1 Key Components of the *Bordetella pertussis* Organism

Component	Biological Activity
Pertussis toxin[a]	A secreted exotoxin that induces lymphocytosis, sensitivity to histamine, pancreatic islet cell activation, and immune enhancement
Filamentous hemagglutinin[b]	Involved in attachment to ciliated respiratory epithelium
Fimbriae[b]	Involved in attachment to ciliated respiratory epithelium
Pertactin[b]	An outer-membrane protein that promotes adhesion to ciliated respiratory epithelium
Adenylate cyclase	Inhibits phagocytic function
Tracheal cytotoxin	Causes ciliary stasis and cytopathic effects on tracheal mucosa
Dermonecrotic or heat-labile toxin	Causes dermal necrosis and vasoconstriction in animals
BrkA (*Bordetella* resistance to killing genetic locus, frame A)	An outer-membrane protein that mediates adherence and resists complement
Endotoxin	Contributes to fever and local reactions in animals and, probably, in humans

[a]Inactivated version is included in all current acellular pertussis vaccines.
[b]Inactivated version is included in some current acellular pertussis vaccines.

Key Components of the Organism

Pertussis Toxin. PT is a major contributor to the pathogenesis of pertussis and is generally believed to play an important role in the induction of clinical immunity. PT is an oligomeric structure composed of five different subunits, S1 through S5. Structurally, it belongs to the A-B class of bacterial toxins. The S1 component (A protomer) catalyzes the adenosine diphosphate ribosylation of guanosine triphosphate–binding regulatory proteins involved in signal transduction in the eukaryotic cell. The A protomer is largely responsible for the recognized biologic activities of PT, including promotion of lymphocytosis, stimulation of islet cells,[67] sensitization to histamines, clustering of Chinese hamster ovary cells, and heat-labile adjuvant properties. The B oligomer is a ring-shaped structure that consists of one copy each of subunits S2, S3, and S5, and two copies of S4. S5 serves to link the two dimers, S2-S4 and S3-S4.[68] The primary function of the B oligomer is to facilitate the attachment of PT to the ciliated cells of the respiratory tract.[69,70] The entire PT molecule is required for the enzymatic activities of the A protomer.[67,71] PT produced by different serotypes of *B. pertussis* appears to have a single biologic and serologic identity.[72] PT appears to play several major roles in the pathogenesis of pertussis, although the precise mechanisms are not entirely clear. First, it facilitates attachment of *B. pertussis* to ciliated respiratory cells. Second, it appears to contribute to cell toxicity. Third, it plays a role in enhancing respiratory tract colonization by inhibiting neutrophil and monocyte migration and delaying the induction of specific immune responses.[73] Thus, *B. pertussis* appears to have evolved a strategy to establish acute infection and extend the period of infectiousness, even in the face of antibody.[74–76] Interestingly, intravenous injection of substantial quantities of active PT into adult human volunteers caused no adverse effects.[77]

PT is a strong immunogen. Antibodies to it are associated with clinical immunity to pertussis, and many investigators believe these antibodies to be the most important mediators of clinical protection.[78] In the laboratory, antibodies to PT protect mice after intracerebral challenge with live *B. pertussis* (the mouse protection test) or after aerosol challenge.[67,79]

Studies in mice immunized with PT subunits or passively given monoclonal antibodies to PT and then challenged with pertussis have demonstrated that exposure to the entire molecule is required for optimal protection.[67,70,71,80] PT is chemically or genetically inactivated (toxoided) for incorporation into acellular pertussis vaccines. Chemical detoxification of PT, as opposed to genetic inactivation, alters the tertiary and quaternary structure of PT, thereby modifying the conformational epitopes. Postvaccination levels of serum antibodies recognizing clinically relevant epitopes may depend on the method of detoxification, with potential clinical implications.[81]

Many investigators have documented shifting prevalences of various PT and promoter region alleles, and in particular shifts away from the alleles contained in vaccines (both acellular and whole-cell),[82–87] but there is no convincing evidence that these changes have reduced vaccine effectiveness.

Filamentous Hemagglutinin. Filamentous hemagglutinin (FHA), a large, hairpin-shaped molecule, is synthesized as a 367-kDa precursor that is modified at both ends and cleaved to form the mature 232-kDa protein. In vitro studies suggest that FHA is an adhesin with four separate binding domains that facilitate binding to monocytes and macrophages, ciliated respiratory epithelial cells, and nonciliated epithelial cells.[88] Data suggest that FHA may also have an immunomodulatory function. The interaction of FHA with receptors on macrophages suppresses the proinflammatory cytokine interleukin (IL)-12 via an IL-10–dependent mechanism and results in persistence of the organism by curbing the protective T-helper cell type 1 (Th1) response.[89] FHA also stimulates proinflammatory and proapoptotic responses in human monocytes and respiratory epithelial cells.[90] Mutant organisms deficient in FHA adhere poorly in vitro.[88,91,92] Mice immunized with FHA are protected against lethal respiratory challenge with pertussis, but not against intracerebral challenge.[79,93] FHA is a strong immunogen, and serum antibodies to FHA are found after natural infection and after immunization. One review highlights that both FHA and fimbriae (FIM) play a critical role in adherence to respiratory epithelium and contribute to persistence in the lower respiratory tract during natural disease by modulating the host immune response.[94]

Fimbriae and Agglutinogens. *Bordetella* organisms express filamentous, polymeric protein cell-surface structures called FIM. Two serotypes of FIM, serotype 2 and serotype 3, are most commonly observed on clinical strains. Antibodies to the FIM agglutinate (clump) *B. pertussis* cells in a serotype-specific manner; for this reason, type 2 and type 3 FIM are considered important agglutinogens.[95] However, antibodies to other surface antigens, including lipopolysaccharide and pertactin, are also agglutinins. Accordingly, the terms FIM and agglutinogens should not be considered synonymous and should not be used interchangeably. More than a dozen agglutinogens are present on the cell envelope of *Bordetella*, and agglutinogen patterns differ among the three species. As many as eight are found in *B. pertussis*, six of which are unique to that species, but only agglutinogens 1, 2, and 3 are considered to be of importance in disease pathogenesis and immunity.

Serotyping *B. pertussis* on the basis of agglutination reactions has been useful in seroepidemiological studies; more recently, monoclonal antibodies to type 2 and type 3 FIM have been used for this purpose.[96] In vivo studies have shown that FIM-negative strains of *B. pertussis* are defective in their ability to multiply in the nasopharynx and trachea of mice.[97] *B. bronchiseptica* strains devoid of FIM but unaltered in expression of FHA and other putative adhesins are unable to colonize animal trachea.[98] Serum antibodies to the FIM are found almost universally after natural disease or immunization. There is accumulating evidence regarding the role of antibodies to FIM in clinical immunity. Data supporting such a role are that the efficacy of whole-cell pertussis vaccines appeared to be compromised in the absence of a match between the agglutinogens in the vaccine and those of prevalent *B. pertussis* strains.[99] There is some in vitro evidence of shifts in serotypes of *B. pertussis* on serial culture.[100]

There is also evidence that a change in serotype occurs during the course of clinical pertussis in some instances.[101] Seroepidemiologic data from the United Kingdom indicated that, between 1941 and 1953, the circulating strains of *B. pertussis* contained agglutinogens 1, 2, and 3. By 1968, however, 75% of isolated strains contained only agglutinogens 1 and 3.[102] There is suggestive evidence (but no proof) that this change resulted from the use of vaccines that contained relatively little agglutinogen 3.[101,103,104] One product that contained considerable agglutinogen 3 was far more effective than the others in preventing pertussis during this time. Subsequent manufacturing changes that incorporated more agglutinogen 3 resulted in higher clinical efficacy.[101] It is, of course, possible that serotype differences are markers for some other antigenic differences in strains of *B. pertussis;* however, the biologic activities of PT from different serotypes of *B. pertussis* did not appear to differ.[72]

Antibody to fimbrial proteins (FIM) appears to be strongly correlated with protection from clinical disease, based on the results of household contact studies among humans,[105] mouse model studies,[106] and in vitro investigations.[107] Because of the evidence that the agglutinogens play some role in the induction of clinical immunity to pertussis, the WHO has recommended that whole-cell pertussis vaccines contain FIM types 2 and 3.[108,109]

Pertactin. Pertactin (PRN), originally known as the 69-kDa protein, is a surface-associated protein that is exported to the outer membrane, where it undergoes proteolytic cleavage.[110] Similar proteins are produced by *B. parapertussis* and *B. bronchiseptica*.[111] PRN participates in attachment through its arg-gly-asp motif to facilitate eukaryotic cell binding[111–113] and invasion,[114] and is recognized as one of the nonfimbrial agglutinogens. PRN is highly immunogenic. Antibodies to it are found after natural disease and immunization with vaccines containing this protein.[115,116] Mice that have been protected passively with antibodies to PRN are highly resistant to an otherwise fatal aerosol challenge with virulent *B. pertussis*.[117] However, in the intracerebral mouse protection test, mice immunized with PRN are protected only when also immunized with FHA.[111]

Studies from The Netherlands show that genetic variations in PRN (and PT) molecules exist, with a shift over time in the circulating strains toward variants not represented in the pertussis vaccines used in the community.[118] Subsequent mouse-model studies show that the Dutch whole-cell pertussis vaccine is less effective against some PRN variants than others. In contrast, a study from France indicates that, despite lyophilization, multiple Sanofi Pasteur whole-cell vaccine lots stored since 1984 had conserved genomes and still expressed the major toxins and adhesins.[119] Studies from the same investigators confirmed that the vaccine lots were highly immunogenic in mice.[120] Additional studies from other geographic areas have been performed to determine whether selection pressure may have contributed to variation in the PRN molecule, leading to less-effective vaccines.[121,122] A study from Sweden evaluating pertussis strains from 1970 to 2003 found no evidence of a reduction in the effectiveness of the vaccination program as a result of bacterial polymorphism.[123] *B. pertussis* isolates not expressing PT or PRN were detected for the first time in 2007 in France, a country with widespread use of

acellular vaccines. Nonexpression was the result of deletion of the entire *ptx* locus, insertion of IS481 in the *prn* gene, or deletion of a part of the *prn* gene. These isolates were found to be less pathogenic in animal and in vitro models.[124]

A recent study evaluated *B. pertussis* isolates collected from four Australian states during a pertussis outbreak from 2008 through 2010. A total of 194 organisms were typed by single-nucleotide polymorphism analysis; multilocus variable number tandem repeats analysis; and fim3, prn, and ptxP sequence analyses. Results suggested increasing selective pressure favoring alleles not contained in the acellular pertussis vaccines used in Australia.[87] Another report from Australia found that 96 (30%) of 320 *B. pertussis* isolates collected from 2008 to 2012 did not express PRN. Multiple mechanisms of PRN inactivation were documented, including IS481 and IS1002 disruptions, a variation within a homopolymeric tract, and deletion of the PRN gene. These findings suggest that PRN-deficient *B. pertussis* arose independently multiple times, rather than representing expansion of a single PRN-negative clone. This pattern is consistent with continuing evolution of *B. pertussis* in response to vaccine selection pressure.[125]

Studies from other countries have also demonstrated an increasing prevalence of PRN-deficient *B. pertussis* isolates in countries using acellular pertussis vaccines targeting this virulence factor.[82,83,126–129] Laboratory studies in mice demonstrate that isolates not producing PRN are capable of sustaining longer infection as compared to PRN-producing isolates, which might thereby provide a selective advantage to PRN(−) strains.[126]

A newer study evaluated the emergence of PRN-deficient isolates in six European countries. From 2007 to 2009, very few isolates were PRN-deficient; prevalence increased in the Netherlands and Sweden from 2010 to 2012. The PRN-deficient isolates were genetically diverse and different mutations were found to inactivate the PRN gene, suggesting that PRN deficiency was subject to positive selective pressure.[127]

The prevalence of PRN-deficient *B. pertussis* has increased sharply in the United States. Screening of 1300 isolates from outbreak and surveillance studies (historical isolates collected from 1935 to 2009, isolates from the 2010 California pertussis outbreak, U.S. isolates from routine surveillance between 2010 and 2012, and isolates from the 2012 Washington pertussis outbreak) identified 306 PRN-deficient isolates. The prevalence of PRN-deficient isolates increased to more than 50% of collected isolates in 2012. Sequence analysis of PRN-deficient isolates revealed various types of mutations in the PRN gene, including two deletions, single nucleotide substitutions resulting in a stop codon, an inversion in the promoter, and a single nucleotide insertion resulting in a frame-shift mutation.[128] The prevalence of PRN-deficient *B. pertussis* has continued to increase since 2012; of 753 isolates collected from eight U.S. states between May 2011 and February 2013, 640 (85%) were PRN-negative. Patients infected with PRN-producing strains were significantly younger (more likely to be an infant or young child), more likely to report apnea, and more likely to be hospitalized (6% in the PRN-producing group compared to 3% in the PRN-deficient group). Case-patients having received at least one pertussis vaccine dose were twice as likely to have PRN-deficient *B. pertussis* compared with unvaccinated case-patients (adjusted odds ratio [OR] = 2.2; 95% confidence interval [CI], 1.3–4.0). For appropriately vaccinated case-patients at least 1 year of age, the adjusted OR increased to 2.7 (95% CI, 1.2–6.1).[129] Taken together, these data suggest that loss of PRN may confer selective advantage, while possibly decreasing clinical severity modestly.

There has been concern that the effectiveness of PRN-containing acellular pertussis vaccines might be reduced against PRN-deficient strains. However, the most recent vaccine effectiveness data from the CDC found that the effectiveness of acellular pertussis vaccine against PRN(−) and PRN(+) strains did not significantly differ.[130] It should be noted that these data were obtained in the United States, where children receive acellular pertussis vaccines containing PT, FHA, and PRN or PT, FHA, PRN, and FIM2–3. Different results might be obtained in countries using different vaccines containing fewer pertussis antigens.

All acellular pertussis vaccines developed through 2016 contain one or more of the bacterial components discussed above (PT, FHA, PRN, and FIM); none contain any of the components described below.

Adenylate Cyclase. Adenylate cyclase toxin (ACT) is a large molecule with different functional domains, including an adenylate cyclase enzyme and a hemolysin, responsible for the hemolysis observed around *B. pertussis* colonies on solid media containing blood. The toxin, present in all virulent strains of *B. pertussis,* is synthesized as a protoxin monomer that is cleaved into an active molecule, allowing it to enter a variety of eukaryotic cells. Once inside the cell, it is activated by calmodulin and catalyzes the production of large quantities of cyclic adenosine monophosphate (AMP). Purified adenylate cyclase inhibits chemotaxis, chemiluminescence, and superoxide anion generation by monocytes and neutrophils in vitro, and in vivo it augments production of cyclic AMP from adenosine triphosphate in the phagocyte, resulting in an excessive accumulation of cyclic AMP and paralysis of the various phagocytic functions.[55,131] Studies show that macrophage cytotoxicity may also result from the induction of apoptosis, and not solely from accumulation of cyclic AMP.[132] In vivo studies show that, compared with wild-type organisms, mutants in adenylate cyclase are defective in their ability to cause lethal infection. These findings suggest that adenylate cyclase serves as an antiinflammatory and antiphagocytic factor during infection. In the mouse model of aerosol infection, PT and adenylate cyclase appear to be the two most important virulence factors.[133]

Adenylate cyclase is immunogenic.[134] Mouse models of intracerebral and aerosol challenge show that prior active immunization with adenylate cyclase is similar in protective efficacy to whole-cell vaccine.[135] In addition, these models show that adenylate cyclase antibodies interfere with the multiplication of organisms.[135] Mills and colleagues also showed that adenylate cyclase promotes innate IL-1β production via activation of the NALP3 (Natch domain, leucine-rich repeat, and PYD-containing protein 3) inflammasome and, thereby, polarizes T-cell responses toward the Th17 subtype, which promotes clearance of the bacteria from the respiratory tract.[136]

ACT is not included in any current acellular pertussis vaccines because of protein stability issues and uncertainty regarding the benefit of its inclusion. Wang and colleagues immunized mice with ACT and found that the majority of resulting unique antibodies bound the C-terminal repeat-in-toxin (RTX) domain. Sera from mice immunized with the RTX domain showed similar neutralizing activity as ACT-immunized mice,[137] suggesting that RTX may present an alternative to use of the entire ACT molecule as a vaccine candidate.

Tracheal Cytotoxin. Of the virulence factors produced by *Bordetella* organisms, only tracheal cytotoxin induces paralysis and destruction of respiratory ciliated epithelium, the hallmark of pertussis disease. Tracheal cytotoxin is a fragment released from the peptidoglycan of the *B. pertussis* cell wall.[138,139] Its activities were studied in vitro in tracheal organ and cell cultures and found to induce mitochondrial bloating, disruption of the tight junctions, extrusion of the ciliated epithelial

cells, and little or no damage to the nonciliated cells.[55] There is also evidence to suggest that this cytopathology is the result of tracheal cytotoxins increasing the production of nitric oxide, which diffuses into ciliated cells, causing cell death.

Heat-Labile Toxin. Heat-labile toxin, so called because it is inactivated at 56°C, is also known as the dermonecrotic or mouse-lethal toxin because of its effects in experimental animals.[140] It is produced by all virulent *Bordetella* species. Located intracellularly, it can be recovered by disruption of *B. pertussis* cells. The mechanism of production of cutaneous lesions after injection of the toxin in animals appears to be vasoconstriction.[141] Heat-labile toxin is lethal to mice when injected intravenously. The role, if any, of heat-labile toxin in the pathogenesis of pertussis is unknown. No consistent effects on cells have been recognized in vitro. It is a weak immunogen, antibodies to it are nonprotective in animal challenge tests, and its absence does not diminish the lethality of experimental pertussis infection in mice.[133]

BrkA. BrkA (which stands for *Bordetella* resistance to killing genetic locus, frame A), another outer membrane protein of *B. pertussis* similar in structure to PRN, protects the bacterium against classical-pathway, complement-mediated killing.[142,143] It is ubiquitously expressed by highly prevalent clinical isolates of *B. pertussis*, and antibodies to BrkA augment killing of *B. pertussis*.[144] Although increased susceptibility to complement during acute bacterial growth would seem to mark the organism for elimination, antibody is needed for classical complement activation. Antibody after a primary infection takes some time to develop, and rapid multiplication might arise before killing could occur. In contrast to the primary infection, if antibody has already been stimulated by natural disease or vaccine, a secondary response can be generated rapidly and killing might occur. This may explain why pertussis tends to be a milder disease after vaccination or after earlier natural infection.[145] Vaccination of mice with BrkA alone does not protect against *B. pertussis* challenge. However, the protection provided by vaccination with a combination of PT, FHA, and BrkA is comparable to that afforded by a combination of PT, FHA, and PRN. As PRN and BrkA are similar in structure, they seem to confer comparable protection in the mouse model when combined with other pertussis antigens.[146]

Endotoxin. The endotoxin or lipopolysaccharide of *B. pertussis* exhibits many of the in vivo activities of endotoxins produced by other Gram-negative organisms, but its role in pathogenesis or in recovery is unclear.[147] Organisms with incomplete endotoxin production have shown decreased colonization of the respiratory tract in the mouse model,[148] and monoclonal antibodies to lipopolysaccharide are protective in the mouse model.[149] The endotoxin content of whole-cell vaccines may have contributed to the immediate adverse systemic and local reactions to those vaccines.[150]

Pathogenesis

Current knowledge of the components of *B. pertussis* and their actions permits construction of a reasonable hypothesis regarding the pathogenesis of whooping cough in humans.[55,151,152] *B. pertussis* overcomes the mucosal immune defenses of the upper respiratory tract and causes disease in healthy persons. The organisms have a marked tropism for, and attach strongly to, the ciliated cells through several adhesins. Although PT and FHA are important attachment proteins, fimbrial proteins, PRN, and BrkA participate in this process as well.[88,91,110,152–154] The organism can be internalized by the epithelial cells but generally does not penetrate submucosal cells or invade the bloodstream. Toxins produced by the organism enter the bloodstream and produce systemic effects.

PT, adenylate cyclase, and BrkA have marked effects on host immune function.[131,142,153,155] Adenylate cyclase induces production of high levels of cyclic AMP, disrupting the functions of several cell types of the immune system; PT inhibits chemotaxis of phagocytic cells into the site of inflammation; and BrkA protects the bacterium against classical complement attack.[142] Tracheal cytotoxin and heat-labile toxin are probably involved in the damage to the tracheobronchial epithelium that is so characteristic of the disease.[139,140] The successful persistence of pertussis appears to result from its ability to interfere with almost every aspect of the immune system, from the inhibition of complement- and phagocyte-mediated killing to the suppression of T- and B-cell responses.[156,157]

Although this pathogenic sequence may explain the respiratory manifestations of pertussis, the pathogenesis of the encephalopathy that can complicate clinical disease remains unclear.[158] Suggested pathogenic mechanisms have included anoxia secondary to severe paroxysms, metabolic disturbances, hypoglycemia, minute intracranial hemorrhages,[158] or a direct toxic effect on the brain.[159]

Animal Models

One of the greatest challenges in studying the pathogenesis of pertussis has been the development of animal models that accurately reflect human disease. Most laboratory animals do not cough and are not naturally infected with *B. pertussis*. However, the availability of many genetically distinct mouse strains and a wealth of mouse-specific reagents have made mice a commonly used model. Although mouse models do not mimic classical whooping cough disease, they have provided valuable insights into the importance of several virulence factors, the roles of various host immune factors in controlling infection, and the potential efficacy of vaccines.[160,161] Early studies in the murine respiratory infection model demonstrated that humoral immunity alone was not sufficient to confer protection against *B. pertussis* infection and that T-cell immunity was also required.[162] Using mice with targeted disruptions of the interferon gamma (IFN-γ) receptor, IL-4, or immunoglobulin heavy-chain genes, Mills and colleagues demonstrated an absolute requirement for antibody in bacterial clearance and a critical role for IFN-γ in immunity generated by previous infection or immunization with the whole-cell pertussis vaccine.[160] Passive immunization experiments suggested that protection early after immunization with acellular pertussis vaccines was mediated by antibody against multiple protective antigens. In contrast, more complete protection was conferred by previous infection or immunization with the whole-cell pertussis vaccines than with acellular vaccines and reflected the induction of Th1 cells.

Suckling pigs have also been used to model infant pertussis. The inoculation of *B. pertussis* into their lungs results in low-grade fever, mild cough, lymphocytosis, and pneumonia.[163] The administration of passive antibody to the piglets provides protection from pertussis challenge, mimicking the role of maternal antibody in disease prevention.[164,165]

Rodents, rabbits, and swine are not natural hosts for *B. pertussis* or *B. parapertussis*, but they are frequently infected with *B. bronchiseptica*. Because of the close phylogenetic relationships between all three of these subspecies and the conservation of many known virulence factors, several groups have used natural *B. bronchiseptica* infection in these animals to study features of pathogenesis that are common to all three organisms.[166,167] These models have revealed roles for several conserved virulence determinants and regulatory factors and

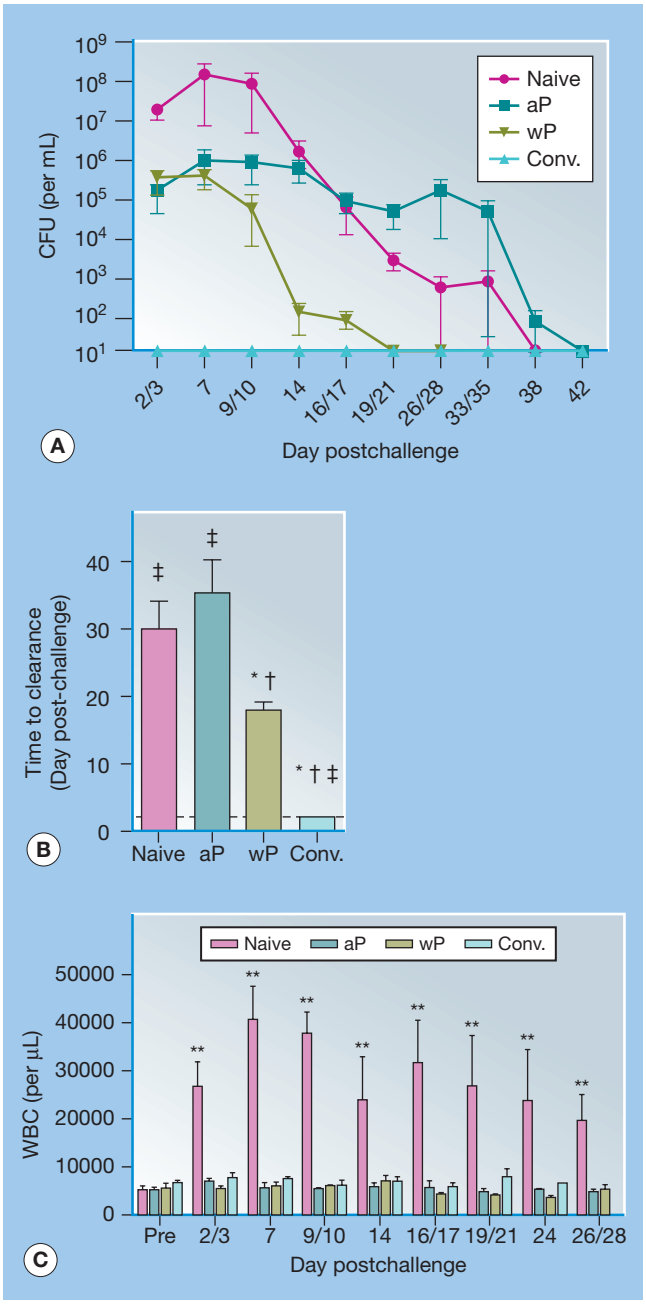


Figure 44.1. Effect of vaccination or convalescence on colonization and leukocytosis among challenged baboons. Naïve animals, acellular pertussis (aP)-vaccinated animals (half given Daptacel, half Infanrix), whole-cell pertussis (wP)-vaccinated animals, and previously infected (convalescent [conv.]) animals were directly challenged with *B. pertussis* (n = 3–4 per group). **A,** Colonization was monitored by quantifying *B. pertussis* cfu per mL in biweekly nasopharyngeal washes with a limit of detection of 10 colony-forming units (CFU) per mL. For each animal the time to clearance is defined as the first day that no *B. pertussis* CFU were recovered from nasopharyngeal washes. **B,** The mean time to clearance is shown for each group (n = 3 per group). Because no *B. pertussis* organisms were recovered from the convalescent animals, the mean time to clearance was defined as the first day of sampling (day 2, indicated by the dashed line). *P < .05 vs naïve; †P < .05 vs aP; ‡P < .05 vs wP. **C,** The mean circulating white blood cell counts before and after challenge are shown for each group of animals (n = 3–4 per group). **P < .01 vs preinfection from same group. *(From Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model.* Proc Natl Acad Sci U S A. *2014;111:787–792.)*

have also shown that several factors are functionally interchangeable between *B. pertussis* and *B. bronchiseptica*.[157,168–170]

The most exciting advance in modeling human pertussis has been the development of the baboon (*Papio anubis*) model.[171] Administration of *B. pertussis* into the nasopharynx of weanling baboons results in low-grade fever, paroxysmal cough, lymphocytosis, stimulation of PT-specific antibodies, and protection from subsequent pertussis challenge. This model has also facilitated the study of transmission of the pertussis organisms through direct contact or aerosol spread.[172,173] Baboons vaccinated with acellular pertussis vaccine (half given Daptacel, half Infanrix) and subsequently challenged with *B. pertussis* were protected from clinical illness but were still able to transmit disease to susceptible baboons caged nearby (Fig. 44.1).[173] These studies clearly show that acellular pertussis (aP) vaccination provides protection against the development of disease but not against colonization or transmission of organisms to other baboons, suggesting that asymptomatic humans immunized with aP vaccine might similarly be capable of transmitting pertussis to susceptible infants and children. This model has great appeal for the further investigations of the mechanisms of transmission and disease and for the testing of new vaccines and therapeutic drugs. In addition, carefully monitored human pertussis challenge studies also have been proposed to further the understanding of the pathogenesis of pertussis and the extent of protection afforded by aP vaccines.[165]

Modes of Transmission; Carriage

Transmission occurs when airborne bacteria reach the ciliated respiratory epithelium of a susceptible host. It was traditionally believed that transmission occurred only from symptomatic patients, and that chronic carriage did not occur.[174] However, silent transmission within families has been demonstrated[21] and the baboon model has shown that asymptomatic animals can efficiently spread infection. Further evidence supporting the applicability of this finding to humans is provided by outbreak reports within populations with prolonged close contact that found case-detection rates using sensitive polymerase chain reaction (PCR) assays to be very high, yet many "cases" identified by PCR were vaccinated, did not have classic pertussis symptoms, and experienced little morbidity. The authors reporting one such outbreak concluded that "a positive PCR result might simply have reflected transient nasopharyngeal carriage of *B. pertussis*."[175] In an outbreak among Israeli soldiers, 20% of those who were either asymptomatic or previously symptomatic but now healthy were PCR-positive for *B. pertussis*.[176]

Zhang and colleagues performed a cross-sectional study in four disparate provinces of China involving 629 schoolchildren 7 to 15 years of age. The study was conducted during the summer school break, none of the surveyed areas were experiencing known pertussis outbreaks, and all participants were asymptomatic. Of the 629 participants, two (0.3%) were culture-positive and 30 (4.8%) were PCR-positive for *B. pertussis*; for *B. parapertussis*, the corresponding results were one (0.2%) and 13 (2.1%), respectively. *B. holmesii* was not detected.[177] The extent to which these results can be generalized to other populations is unknown, absent additional

studies of the incidence, prevalence, or duration of *B. pertussis* carriage. However, these and other[178,179] studies make it clear that, contrary to traditional belief, *B. pertussis* carriage can occur and may play an important role in transmission, as suggested by the baboon model.

Diagnosis

The etiologic agent responsible for an infectious disease is generally determined by culture of the organism, detection of antigens or nucleic acids produced by the organism, or measurement of the immune response to the organism. Even when using all these criteria, the confirmation of *B. pertussis* infection is still one of the most difficult diagnostic challenges facing the clinician, particularly in previously immunized adolescents and adults. Pertussis organisms can typically be detected by culture or PCR of nasopharyngeal specimens obtained early in the illness and prior to antibiotic therapy, when the symptoms are similar to those of the common cold (and thus not likely to trigger specimen collection, except in an outbreak setting). By the time of appearance of a cough severe enough to arouse suspicion of pertussis, nasopharyngeal organisms have typically decreased in number to the point where cultures and non-PCR antigen detection methods are unlikely to be positive. The sensitivity of PCR can be sufficient to permit detection for several weeks into the illness, but detection in low copy number may represent a clinical false positive from environmental contamination from vaccine strains or other bacterial strains.[180]

Bacteriologic Diagnosis

Culture. Culture of *B. pertussis* from the nasopharynx of symptomatic patients is compelling evidence of disease and remains the gold standard for laboratory diagnosis.[181,182] The greatest likelihood of isolating the organism is achieved by immediate inoculation of a nasopharyngeal aspirate specimen obtained early in the illness onto fresh media in a laboratory experienced with handling *B. pertussis*. At best, these conditions are difficult to meet, but even under optimal circumstances, the organism is frequently not recovered because of its fastidious nature and its disappearance early in the disease process.[183–187]

Because the human nasopharynx is colonized with many other respiratory bacteria, the use of selective media containing antibiotics such as cloxacillin and cephalexin will increase the yield of positive pertussis cultures by suppressing normal flora.[185] Two media are specialized for pertussis cultures: Bordet-Gengou medium, containing defibrinated horse blood and cloxacillin; and Regan-Lowe medium, containing charcoal agar, defibrinated horse blood, and cephalexin. The latter medium may provide better yield,[188] although some have found no difference.[185] Direct plating of the specimen at the bedside or clinic has been shown to increase the yield of positive cultures, but Regan-Lowe transport medium can be used if plated by 72 hours after inoculation.[187]

It has been shown that nasopharyngeal aspirates yield better samples than nasopharyngeal swabs; Dacron swabs are better than calcium alginate because the latter inhibit PCR assays, and cotton swabs are toxic to the bacteria.[182] Incubation of the cultures for at least 7 days can increase the yield. As noted, however, "although the culture is the gold standard for the diagnosis of pertussis, its diagnostic sensitivity is insufficient even when technical conditions are optimal."[182]

Prior therapy with erythromycin or sulfamethoxazole reduces the likelihood of positive cultures. Immunized persons with pertussis have lower rates of positive cultures than unimmunized control subjects, and isolation rates are negatively correlated with increasing age,[189] further complicating the diagnosis of pertussis in partially or fully immunized adolescents or adults.

Antigen Detection: Direct Fluorescent Antibody Test and Polymerase Chain Reaction. Antigen detection tests offer the distinct advantage that organisms do not have to be viable and can be detected later in the disease and in the presence of antibiotics. The initial antigen detection test was the direct fluorescent antibody (DFA) test, which in experienced laboratories had a specificity of 99.6%, but its sensitivity was only 61% when compared with culture.[182,185,186,190–192] The development of highly sensitive and specific PCR assays have made the DFA test obsolete.

PCR assays identify unique gene sequences of various *Bordetella* species in respiratory secretions.[185,186,193–199] Although demanding of scrupulous technique to avoid cross-contamination, this rapid, highly sensitive, and specific diagnostic method has become the standard tool for laboratory identification of pertussis and permits identification of *B. pertussis* after initiation of antibiotics[200] and for several weeks later than possible by culture. However, the use of PCR must be coupled with rigorous quality-assessment programs supported both by appropriate epidemiologic investigations of suspected outbreaks and by an ongoing culture-confirmation program to which a selected subset of specimens is submitted.[201] False-positive results can arise through a variety of mechanisms, including poor technique in sampling, transport, or assay; environmental contamination with bacterial DNA (including from nearby use of vaccine); and poor choice of target.[175,180,197–199,202–206] Thus, it is important that disease categorization be based on appropriate clinical case definitions[207] and not merely on PCR positivity alone.

Numerous PCR assays have been developed, and the continued rapid evolution of this field makes a detailed review inappropriate here. However, a few principles can be delineated. Although nasopharyngeal aspirates are preferred for culture specimens of infants, Dacron or rayon nasopharyngeal swabs perform equally well for PCR for older children, adolescents, and adults and may be transported dry.[199] PCR assays typically target either specific genes or various insertion sequences; those targeting insertion sequences typically offer better sensitivity.[199] Available multiplex PCR assays that detect a variety of organisms from a single specimen often target only the IS481 sequence; the current preference for epidemiological and clinical purposes is to target two or three repeated insertion sequences (including IS481, pIS1001, hIS1001, or IS1002) to increase specificity and allow for discrimination among clinically relevant *Bordetella* species.[199,203,204]

Serologic Diagnosis

Serologic tests for antibodies to various components of the *B. pertussis* organism have been used extensively to diagnose pertussis in children, adolescents, and adults.[182,185,190,208–210] Serologic testing has improved our understanding of the clinical spectrum of pertussis, particularly by demonstrating asymptomatic, mild, or atypical infections in partially immune persons.[21,211–213] Serosurveys have been used to examine the natural history of pertussis in populations at various ages,[214–223] and they have been useful in monitoring pertussis during regional outbreaks.[224] They have also been helpful in monitoring clinical outcomes in pertussis vaccine trials, because partially immune persons may experience pertussis infection but display few or no symptoms.[189] Finally, serologic studies have enabled an understanding of the role of pertussis in the etiology of cough illness in adolescents and adults.[225,226] Serologic tests include complement

TABLE 44.2 Adolescents and Adults[a] with Prolonged Cough Illness (7–56 Days) Who Met Various Case Definitions for Laboratory Confirmation or Evidence of Pertussis

Case Definition	Criterion	No. Positive/ No. Tested	Prevalence, % (95% CI)	Mean Age, Years (Range)
1	Culture positive for *Bordetella pertussis*	2/440	0.5 (0.1–1.8)	18.4 (12.6–24.1)
2	PCR positive for *B. pertussis*	3/314	1.0 (0.2–3.0)	24.0 (12.6–37.8)
3	Fourfold increase in antibody titer	7/393	1.8 (0.8–3.8)	28.1 (12.3–70.6)
4	Antibody titer, >99.99 percentile for control values	36/440	8.3 (5.9–11.2)	36.6 (14.1–69.4)
5	Antibody titer, >3 SDs greater than GMT for control subjects	84/440	19.1 (15.6–23.0)	39.0 (12.3–87.7)

[a]Includes all subjects with at least one biological specimen.
CI, confidence interval; GMT, geometric mean titer; PCR, polymerase chain reaction; SD, standard deviation.
Modified from Senzilet LD, Halperin SA, Spika JS, et al. Pertussis is a frequent cause of prolonged cough illness in adults and adolescents. Clin Infect Dis. *2001;32:1691–1697.*

fixation, agglutination, toxin neutralization, and enzyme-linked immunosorbent assays (ELISAs),[185] but ELISA methods are used most frequently because they are the easiest to perform and standardize and they can detect specific isotype responses. Considerable effort has been expended to develop a standardized ELISA method that can be used in the evaluation of vaccine candidates and in the diagnosis of pertussis disease.[227–233]

The most conclusive serologic evidence of an infection is the demonstration of a significant rise in specific antibodies between the acute and convalescent samples. However, pertussis presents a problem because the diagnosis is often not considered early in the course of the disease; by the time it is, a substantial rise in serum antibodies has already occurred, thus compromising the likelihood of a significant increase between the acute and convalescent serum specimens. Indeed, it has been demonstrated that fourfold decreases in antibody between the "acute" and convalescent specimens can also be seen with culture-confirmed pertussis.[210] An approach that avoids this problem of specimen timing is based on comparing the result of a single serum specimen to a prespecified threshold.[211–214,234–238] Typically, the threshold is determined by assay of sera from a control population not suspected to have pertussis and is set to a value that exceeds the mean of the control population by a selected factor (typically, two or three standard deviations). This approach has been followed both in studies[211–214] and for public health purposes.[222,234,235] For many years, the Massachusetts Department of Public Health Laboratory has provided a single-sample pertussis serology assay with a specific cutoff value to diagnose pertussis in adolescents and adults[234] and CDC notes that "CDC and FDA [U.S. Food and Drug Administration] have developed a serologic assay that has been extremely useful for confirming diagnosis, especially during suspected outbreaks. Many State Public Health Labs have included this assay as part of their testing regimen for pertussis."[235]

In an attempt to standardize the serologic assays, a WHO reference preparation for human pertussis serology was made available and used by a number of pertussis laboratories throughout the world.[232] Several reports have recommended that for single-sample serology, immunoglobulin (Ig) G–anti-PT antibodies below 40 IU/mL are not indicative of recent contact, whereas levels above 100 IU/mL can be used as an indicator of recent contact with the bacteria.[239,240] IgG–anti-PT antibodies are primarily measured, with IgA–anti-PT serving as an additional test for equivocal IgG results (IgG >40 and <100 IU/mL). A 2010 study found commercial assays to be of variable quality and in need of further standardization.[241] However, in 2015 good agreement was reported between two commercial laboratories in the United States and the CDC laboratory, and between U.S. Public Health laboratories and the CDC.[242–244]

The choice of case definition, including clinical, laboratory, and epidemiological criteria, strongly influences the number of subjects diagnosed with pertussis and thus estimates of pertussis disease prevalence. The effect of laboratory criteria is illustrated by the study of Senzilet and colleagues,[16] who enrolled patients 12 years of age or older with persistent cough of 1 to 8 weeks' duration from nine health units in eight Canadian provinces. Only two cases were diagnosed by positive culture, and three additional cases were detected with PCR. When serologic measures were used, seven had a fourfold rise in titer between the acute and convalescent samples, but 36 persons had a single antibody titer that exceeded the 99.99th percentile for controls, and 84 had single antibody titers that exceeded three standard deviations from that of the control subjects. As shown in Table 44.2, the calculated prevalence of pertussis disease in this population was highly dependent on the case definition.[16] The complexities of assigning case definitions have been comprehensively reviewed.[244]

A somewhat different problem is presented by the use of serologic testing to detect pertussis in field trials of pertussis vaccine. Given the different serologic methods used among the various efficacy studies, direct comparisons of efficacy rates between such studies should be made with caution.[245] Another concern with serologic diagnosis is that, although it may be appropriate for nonimmunized control subjects, detection of antibody increases may be compromised in those who were recently immunized, because immunization itself leads to increases in antibody titers. In this situation, the evaluation of antibodies against an antigen of *B. pertussis* that was not included in the vaccine can be useful, if an appropriate antigen and assay exist. This was particularly helpful in the efficacy trials with the monovalent PT vaccine in Sweden, where antibody responses to other pertussis antigens could be used to detect pertussis infection in recipients of the monovalent vaccine.[246]

Treatment and Prevention With Antibiotics

Guidelines for the treatment and postexposure prophylaxis (PEP) of pertussis exist.[247,248] The central tenets of these guidelines are that the administration of macrolides early in the course of illness will reduce the duration and severity of symptoms, lessen the infectivity, and reduce the risk of death and that the judicious use of antimicrobial agents for PEP will eradicate *B. pertussis* from the nasopharynx of symptomatic or asymptomatic persons.[4,15,247–256] Although many persons with untreated pertussis will spontaneously clear *B. pertussis* by 3 to 4 weeks after the onset of cough, untreated and

unvaccinated infants can remain culture positive for 6 weeks or even longer.[257,258,258a] Erythromycin, a macrolide antibiotic, was originally the antimicrobial of choice for treatment or prophylaxis of pertussis, but it has unpleasant gastrointestinal side effects and has largely been supplanted by azithromycin, with reduced side effects.[259–261] For both treatment and PEP, the standard therapy is a 5-day course of azithromycin.[247,248,262] For persons intolerant of macrolides and older than 2 months, trimethoprim-sulfamethoxazole is the alternative agent.

Among young infants, erythromycin poses a risk of infantile hypertrophic pyloric stenosis (IHPS), but azithromycin has also been implicated.[263–265] In a study using the military health system database, medical records of infants born between 2001 and 2012 who were prescribed either oral erythromycin or azithromycin as outpatients in the first 90 days of life were screened for evidence of IHPS. Of 1,074,236 treated children, 2466 developed IHPS. The adjusted ORs for developing IHPS following erythromycin or azithromycin exposure in the first 14 days of life were 13.3 (95% CI, 6.80–25.9) and 8.26 (95% CI, 2.62–26.0) respectively; exposure between 15 and 42 days of life had adjusted ORs of 4.10 (95% CI, 1.69–9.91) and 2.98 (95% CI, 1.24–7.20), respectively. There was no association with IHPS for either macrolide between 43 and 90 days of life.[265] Accordingly, all infants younger than 1 month (and preterm infants until a similar postconceptional age) treated with a macrolide should be monitored for development of IHPS from initiation until 1 month after completion of therapy.[248]

Whether to administer PEP should be determined on the basis of the infectiousness of the patient, the duration and intensity of the exposure, the consequences of pertussis in the exposed person, and the possibility of contact with persons at high risk for severe pertussis, such as young infants. PEP given to asymptomatic household contacts within 21 days of onset of cough in the index patient can prevent symptomatic infection. Symptomatic contacts should be treated as if they have pertussis. PEP also should be administered in exposure settings that include infants younger than 12 months or women in the third trimester of pregnancy. A recent cost utility analysis suggested that PEP is a cost-effective strategy compared with no intervention and plays an important role in contact management.[266] From a healthcare payer perspective, azithromycin therapy had the most beneficial health outcomes among all age groups and was also the lowest-cost strategy among infants; accordingly, azithromycin therapy was recommended as the optimal therapy for all contact groups.[266]

One final concern with the use of macrolides, and particularly erythromycin, is antibiotic resistance. Erythromycin resistance was first recognized in Arizona in 1994.[267] Since then, additional erythromycin-resistant and even azithromycin-resistant isolates have been reported and new mutations described.[268–273] Resistance in some areas is high; investigators in Xi'an, China, found 14 of 16 isolates to have minimum inhibitory concentrations of greater than 256 μg/mL.[273] However, resistance appears to remain rare in Europe and North America.[274,275]

In summary, macrolides are recommended for both therapy and PEP of household and other close contacts, regardless of vaccination status, and for healthcare workers with a known or likely exposure to pertussis.[276–278] Prophylaxis reduces, but does not eliminate, the risk of pertussis.[279,280] Prophylaxis in a household is substantially more effective if given before the appearance of the first secondary case.[280] Cases and inadequately vaccinated contacts younger than 7 years should be excluded from school, daycare, and similar settings until they have received at least 5 days of prophylaxis or therapy.[276]

EPIDEMIOLOGY

Pertussis is an endemic disease with epidemic peaks occurring every 2 to 5 (typically, 3 to 4) years.[6,281–283] There is no consistent seasonal pattern.[282–288] Pertussis is acquired through transmission of large respiratory droplets generated by coughing or sneezing. Transmission by the indirect route from airborne droplet nuclei or organisms on fomites or in dust occurs extremely rarely, if ever. Persons are most infectious during the catarrhal phase and the early paroxysmal phase. Whooping cough is highly contagious: as many as 90% of susceptible household contacts acquire the disease. Rates of transmission in school settings range from 50% to 80%. Pertussis may occur at any age. Infants are susceptible to pertussis within the first few weeks or months of life, which is when mortality is highest. For many years, it was assumed that one attack of pertussis provided lifelong immunity. Before widespread vaccination, this belief was reflected by the age distribution of pertussis: approximately 20% of all whooping cough cases occurred in infants younger than 1 year, and nearly 60% occurred among children of ages 1 to 4 years.[286] However, anecdotal information indicated that second attacks of pertussis did occur in the prevaccine era, such as in older persons exposed to grandchildren with the disease, although these instances were rarely described and incompletely documented.[289] More recent data suggest that second attacks of pertussis are not uncommon.[290,291]

Widespread pertussis vaccination of children and the consequent reduction in the incidence of disease did not appear initially to have altered the periodicity of epidemic cycles,[281,284,292] suggesting that vaccination has had little impact on transmission.[285,293] However, analyses of more recent and complete data showed a lengthening of the interepidemic interval in the United Kingdom during a time of whole-cell vaccine use, consistent with a reduction of transmission.[294] It has also been argued that acellular vaccine use in Sweden reduced pertussis transmission,[295] but subsequent data show otherwise,[296] perhaps reflecting different epidemiological patterns following nationwide introduction of effective infant vaccination in a country with widespread pertussis circulation versus the steady-state situation some years later. Regardless, it is clear that endemic circulation of *B. pertussis* continues in the community despite universal childhood vaccination programs.

Incidence of Pertussis

United States and Canada

Disease caused by *B. pertussis* was once a major cause of morbidity and mortality among U.S. infants and children. From the 1920s (when pertussis was first a reportable disease) until the early 1940s, there were 115,000 to 270,000 cases of pertussis reported each year, with 5000 to 10,000 deaths (in all likelihood, pertussis infection occurred nearly universally among children, with only the more severe cases being reported). These figures represented approximately 150 cases and six deaths per 100,000 population.[4] As pertussis vaccine came to be used commonly in infants and children, the incidence of pertussis markedly decreased (Fig. 44.2).[4,282] By the 1970s, the annual incidence of reported disease had been reduced by 99%, and the lowest annual number of cases of pertussis—1010—was reported in 1976. Reported cases began to increase in the 1980s. Cyclical peaks in U.S. pertussis incidence were noted in 1983, 1986, 1990, 1993, 1996, 2002, 2004, 2010, and 2012, with each peak surpassing the last and with progressively higher inter-peak case counts.[10] For year 2012, reported cases totaled 48,277; for year 2013, reported

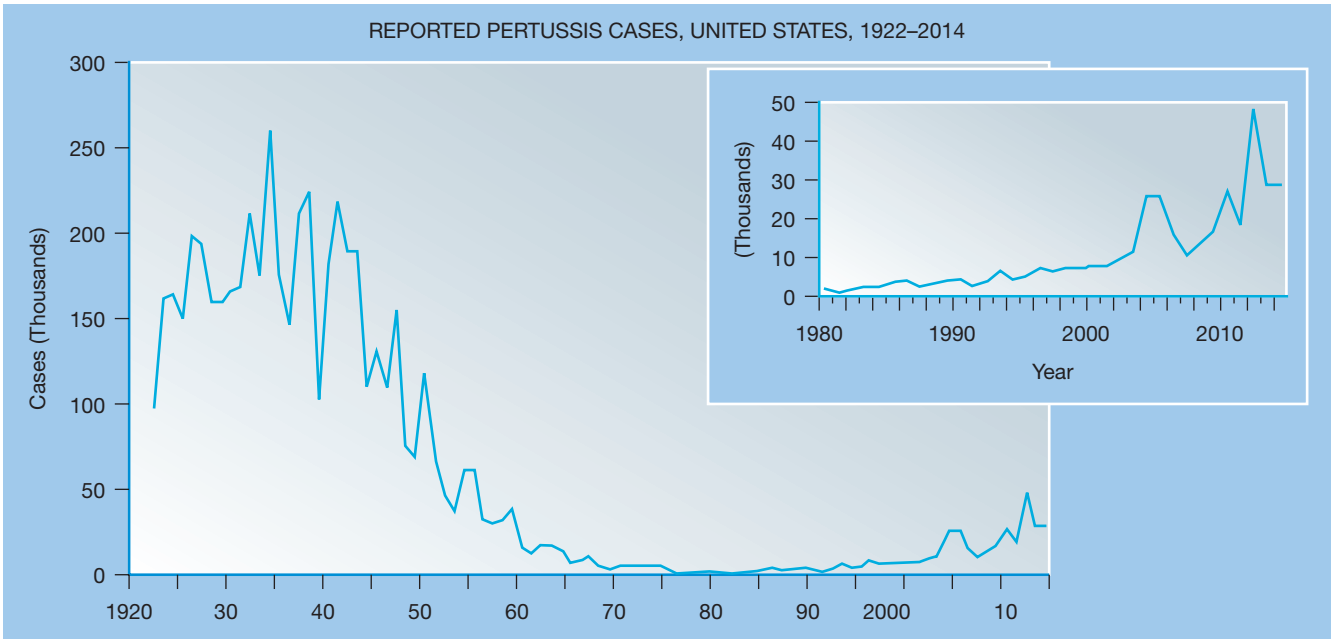


Figure 44.2. Number of reported cases of pertussis, by year, in the United States from 1922–2014. *(Data for 1922 to 1996 from W. Orenstein, personal communication, Centers for Disease Control and Prevention; data for subsequent years from the respective issues of Final Report of Notifiable Diseases, published annually in Morbidity and Mortality Weekly Report [see also www.cdc.gov].)*

cases totaled 28,639; for 2014, reported cases totaled 32,971; for 2015, reported cases totaled 18,166 (provisional).[10] The 2012 case count represented the most reported cases since 1955 and included 20 deaths, the majority of which occurred among infants younger than 3 months of age. Incidence rates were highest among infants and second highest among children 7 to 10 years of age; rates also increased among adolescents 13 and 14 years of age.

Graphs of incidence by age-group make it clear that several patterns have succeeded each other over the decades (Fig. 44.3). Prior to routine vaccination, as noted, nearly all reported cases were among infants and young children. Beginning in 2002, a disproportionate increase was seen in cases among adolescents (11 to 19 years of age), which led to institution of adolescent Tdap (tetanus, diphtheria, and aP vaccine) booster vaccination programs[7] with a subsequent decline in proportional pertussis rates among adolescents.[10] However, an increase in cases among children 7 to 10 years of age began within a few years thereafter. Fig. 44.4 shows U.S. annual childhood pertussis cases by year of age for each year from 2002 to 2009; beginning in 2005, a "marching cohort" effect can be seen in the 6- to 11-year age range, with case counts increasing annually both in each birth cohort over time and in each year of age across the years. For example, if we follow the children who were 7-year-olds in 2005 (the magenta bars in the figure), we see that they experienced increasing pertussis cases as they became 8, 9, and 10 years of age in 2006, 2007, and 2008, respectively. In 2009, many of them received their adolescent dose of Tdap vaccine as 11-year-olds, and case counts in their cohort dropped. In addition, if we look at a given age (e.g., 8-year-olds) from year to year, we see case counts are also higher in each succeeding cohort. When age-specific incidence rates are evaluated with respect to the pertussis vaccines each cohort received in infancy, a correlation can be seen between the arrival at age 7 years of the first cohort that received only aP vaccines and the progressive increase of pertussis cases in the group 7 to 11 years of age (Fig. 44.4). Numerous studies have documented this increase in pertussis

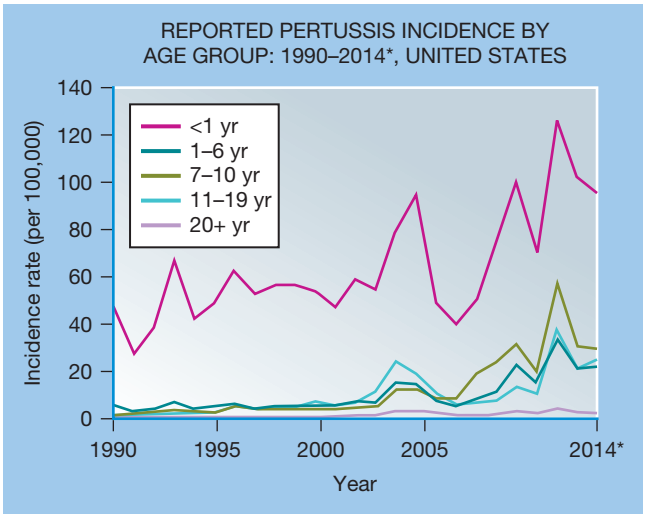


Figure 44.3. Reported pertussis incidence by age group, 1990–2014. See text for explanation. *Data for 2014 are provisional. *(From presentation by Liang JL, "Cocooning" and Tdap vaccination, Advisory Committee on Immunization Practices, Atlanta, GA, June 25, 2015. Data source: CDC, National Notifiable Diseases Surveillance System and Supplemental Pertussis Surveillance System. Available from http://www.cdc.gov/vaccines/acip/meetings/meetings-info.html.)*

activity beginning at age 7 years among the cohorts given only acellular vaccines, and also that the effectiveness of the adolescent Tdap booster has waned more quickly among this cohort than among their predecessors who were primed with one or more doses of whole-cell pertussis vaccine.[297–310]

Infants continue to experience the highest age-specific incidence of pertussis, and as noted, are at the greatest risk of morbidity or mortality. Although the Advisory Committee on Immunization Practices (ACIP) has recommended that all

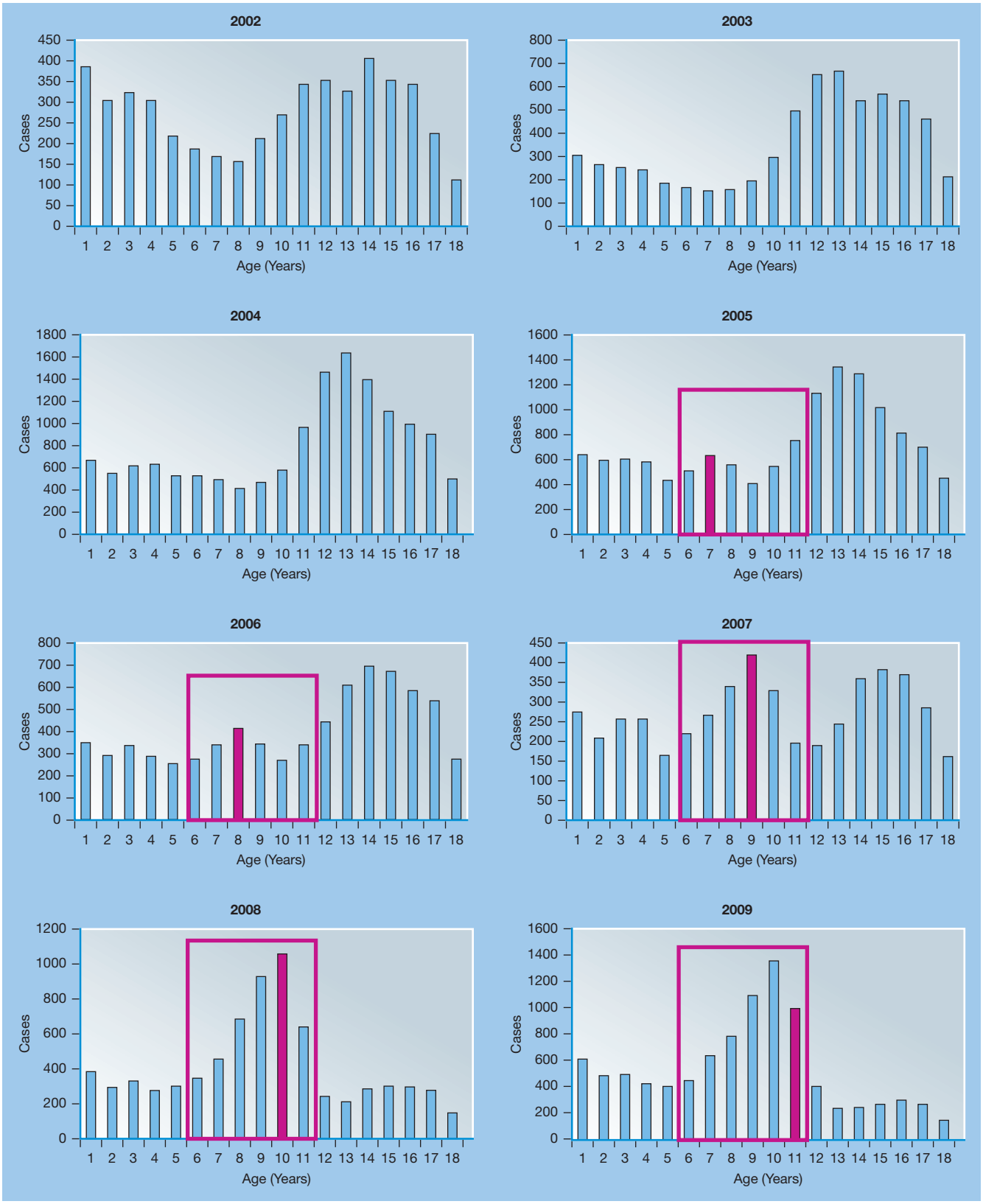


Figure 44.4. Pertussis cases by age, 2002–09. The magenta bar follows the cohort that was 7 years of age in 2005. A "marching cohort" effect can be seen beginning in 2005 in the 6- to 11-year age range, with case counts increasing annually both in each birth cohort over time and in each year of age across the years. *(From presentation by Clark T, Pertussis epidemiology and vaccination in the United States, Advisory Committee on Immunization Practices, Atlanta, GA, February 22, 2012. Available from http://www.cdc.gov/vaccines/acip/meetings/meetings-info.html.)*

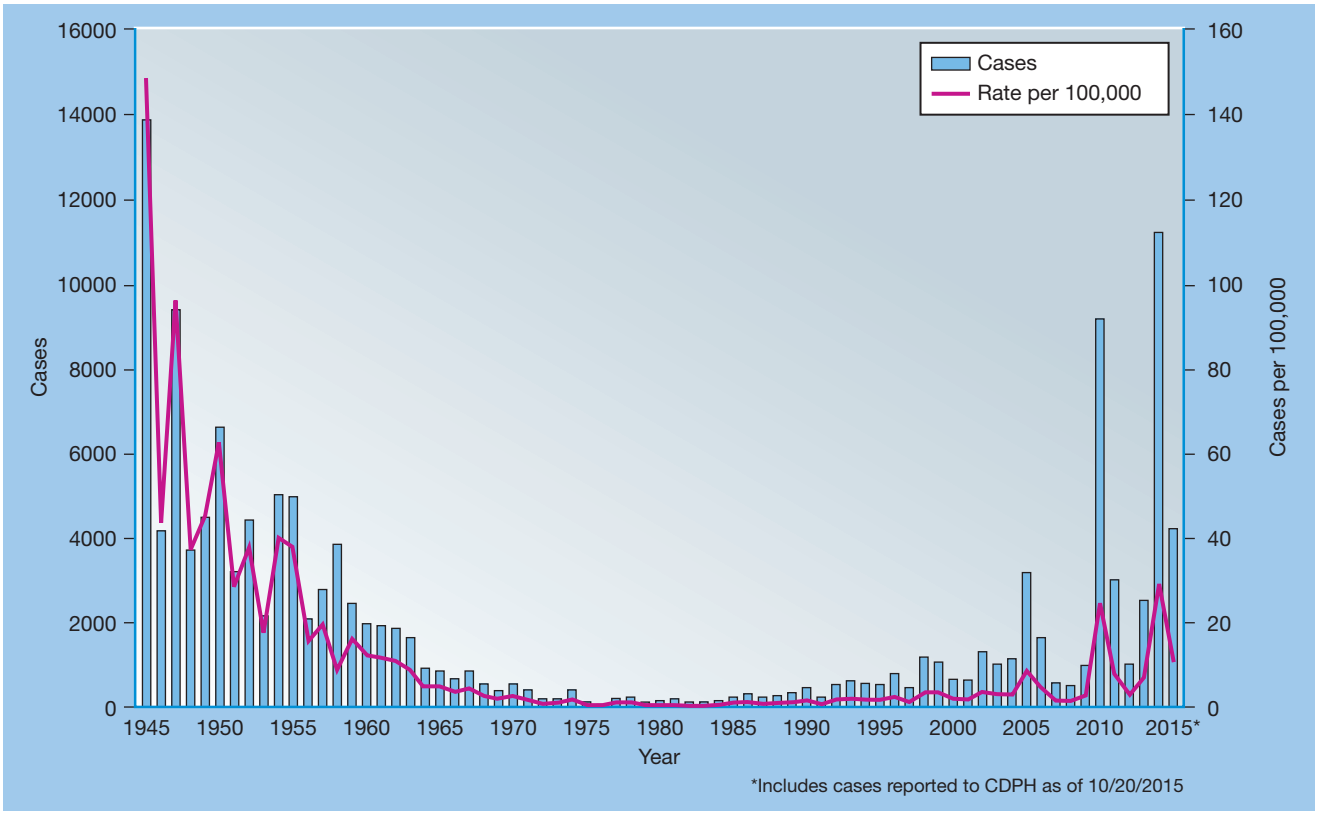


Figure 44.5. Number and incidence of reported pertussis cases by year of onset in California, 1945–2015 (through October 20, 2015). CDPH, California Department of Public Health. *(From California Department of Public Health, Pertussis Report, October 20, 2015. Available at: http://www.cdph.ca.gov/programs/immunize/Documents/Pertussis%20report%202015-10-20.pdf.)*

pregnant women receive Tdap during the third trimester of each pregnancy,[311,312] this recommendation has not yet been in place for sufficient time to show an effect on age-specific incidence rates in the youngest children (Fig. 44.3).

The increase in U.S. pertussis cases in recent years has been marked both by an increased number of endemic cases and larger, more frequent outbreaks. Since 2010, large outbreaks have been reported from multiple states, including California in 2010 and 2014 (Fig. 44.5),[313,314] Minnesota in 2012,[303] Oregon in 2012,[304] Washington in 2011,[301,315] and Wisconsin in 2012.[299] There has been considerable debate regarding the extent to which this increase in pertussis case counts reflects actual increases in disease incidence versus improved clinician awareness and recognition, more sensitive and more readily available diagnostics (e.g., PCR), and improved reporting.[283,316–318] Although it seems clear that improved awareness and diagnosis have contributed to the upsurge in reported cases, it also seems clear that these factors are insufficient to explain neither the marching cohort effect and the shifting of age-group incidences over time (see Figs. 44.3 and 44.4), nor the notable increase in large outbreaks. Concern has been raised regarding the role of vaccine refusal, which is more prevalent in certain communities and schools and may contribute to the initiation and growth of outbreaks.[319]

The epidemiology of pertussis in Canada has shown some differences from that of the United States, likely partly because of differing available vaccines and differing population characteristics. It is thought that the increase in pertussis disease in Canada in the 1980s and early 1990s was the result of whole-cell vaccines that were less protective than previously believed.[192,224,320–322] Support for this concern was provided by the pertussis vaccine efficacy trials conducted in Europe in the early 1990s, which found adequate efficacy after three doses of one U.S. whole-cell vaccine,[323] but quite low efficacy for the other whole-cell vaccine.[324,325] These results were consistent with U.S. surveillance data from 1992 to 1994, which found an overall whole-cell pertussis vaccine efficacy of 64% after three doses and of 82% after four or more doses.[326] Following introduction of aP vaccines in Canada, pertussis incidence rates dropped substantially and remained relatively low until 2012, when increased rates accompanied by regional outbreaks were observed.[327–329] Increases in incidence were noted in all age groups, with the highest rates in those younger than 1 year and those aged 10 to 14 years.[327] The lesser increase in those aged 15 to 19 years might reflect the Canadian recommendation for Tdap at 14 to 16 years of age.

Europe

Pertussis surveillance systems and procedures vary throughout Europe, although the European Centre for Disease Prevention and Control (ECDC) is seeking to standardize laboratory procedures for pertussis surveillance.[330] Because surveillance ranges from passive reporting to sentinel systems to laboratory surveillance, and case definitions range from clinical judgement without laboratory confirmation to PCR confirmation of cases meeting defined clinical criteria, neither case counts nor incidence rates can be compared from country to country and are not reported here.

Most European countries replaced whole-cell with aP vaccines during the 1990s, and many have noted increased reports of pertussis in recent years.[331–335] The ECDC noted in 2011 that pertussis reports had increased for the first time since 2008, and then 2012 saw twice as many reported pertussis cases as

in 2011.[334,336] Increases were most notable among young children and adolescents. Of note, the increase in pertussis incidence in 2012 was greater in Poland, the only included country still using whole-cell vaccine, than in the remaining countries, all of which were using acellular vaccine. England and the Netherlands experienced pertussis outbreaks in 2012. England, with a 10-fold increase in pertussis, declared a national pertussis outbreak in April 2012. The increased burden was seen particularly in adolescents and young infants, prompting the introduction in England of a national maternal vaccination program in September 2012.[332]

Africa, Asia, and Latin America

Outside North America, Europe, and Australia, national pertussis surveillance is even more variable—if it is performed at all.[337] Although it is likely that the burden of global pertussis morbidity and mortality lies most heavily on low- and middle-income countries, surveillance data are inadequate and a systematic review protocol has been proposed to evaluate the burden and trends of pertussis in those countries.[338] The WHO estimated that pertussis caused approximately 63,000 deaths in children younger than 5 years of age in 2013, but noted that "there is considerable uncertainty over these estimates in view of the paucity of reliable surveillance data, particularly from developing countries."[11a] With rare exception, no country in the African, Eastern Mediterranean, or Southeast Asian WHO regions conducts systematic pertussis surveillance; consequently, it is not possible to know whether changes in reported case counts in these countries represent fluctuations in disease recognition, types or usage of diagnostic tools, likelihood of reporting, or actual incidence. It is known that pertussis outbreaks occurred in Pakistan in 2004–06, India in 2007, and Afghanistan in 2007–08.[337]

Within the Western Pacific WHO region, pertussis is a reportable disease in countries including China, Republic of Korea, Taiwan, Japan, Australia, and New Zealand; in some of these countries, reporting is passive and largely based on clinical suspicion without laboratory confirmation. In the Republic of Korea, passive surveillance showed declining reports of pertussis from 1955 through 1999, with increases thereafter, particularly in 2009–11 (the last year available).[340] However, it has been shown that the Korean surveillance system underestimates pertussis (as is the case in most countries).[341] Outbreaks of pertussis were noted in Australia and Japan from 2008 to 2011 and New Zealand from 2011 to 2013; increased pertussis activity was also noted during this period in China and Taiwan.[337]

Australia conducts comprehensive laboratory surveillance for pertussis, and has noted large increases in pertussis rates in recent years (e.g., increasing from 47.2 to 173.3 cases per 100,000 population in 2006 and 2011, respectively).[342] The steepest increases were seen in children 2 to 4 years of age (reflecting the discontinuation in 2003 of the 18-month dose) and children 6 to 9 years of age (thought to reflect the shorter duration of protection associated with an all-acellular-vaccine schedule).[342,343] It is difficult to assess the contribution of improved laboratory methods to these increased case counts. During the same time period in which the case counts increased by some 80,000, the number of cases identified by PCR also increased by some 80,000,[318] suggesting that a positive PCR result may have led to the reporting of pertussis cases that previously would have gone uncounted.

Pertussis is a nationally notifiable disease throughout Latin America, and although surveillance is passive, laboratory support (culture and PCR) is provided in most countries. Nonetheless, surveillance system sensitivity is variable and uncertain. During the last decades of the 20th century, there were substantial improvements in vaccine coverage and corresponding declines in rates of reported pertussis.[344,345] However, in the first decade of the 21st century, pertussis case counts began rising in many countries of Latin America, including Argentina, Brazil, Chile, Colombia, Costa Rica, and Mexico.[345–349] Most countries recognize their pertussis surveillance is inadequate, and efforts to improve surveillance are ongoing.[345,349] PCR methods were introduced initially in Argentina and Costa Rica and are now used in essentially all the major countries of Latin America. As in other parts of the world where the introduction of PCR was often accompanied by a surge in reported pertussis cases, there was a surge in Latin America. However, improved detection does not entirely explain the increases in reported pertussis seen over the past decade in Latin America. For example, pertussis rates in Argentina increased from 0.7 to 5.7 per 100,000 population from 2002 to 2005; half the increase was recorded before the introduction of PCR at the end of 2004.[347] By 2011, the reported case rate was 8.3 per 100,000 population.[337] Increases were seen among adolescents and adults as well as infants. Brazil has also seen increasing pertussis cases, but the increase could be entirely related to the decline in vaccination coverage between 2006 and 2012.[345,346] Chile and Columbia also noted increases in reported pertussis during 2010–12. In contrast, no notable increases in pertussis have been observed in the Central American countries of El Salvador, Guatemala, Honduras, and Panama.[345] Of note, the public vaccination programs of all the Latin American countries mentioned thus far used whole-cell pertussis vaccines throughout this time period; only Mexico and Costa Rica were using aP vaccines (Mexico since 2007 and Costa Rica since 2010). Both countries experienced rising rates of pertussis prior to introducing aP vaccines. For the time period from introduction of aP vaccines through 2014, apart from an increase in case counts in Mexico coincident with the adoption of PCR testing in 2011,[345] neither country reported increases in pertussis activity other than the usual temporal fluctuations. However, neither country has yet used aP vaccine long enough for acellular-primed populations to reach preadolescence.

High-Risk Groups

Pertussis in Infants and Young Children

Although anyone can acquire pertussis, the highest attack rates and most hospitalizations and deaths occur among infants. More than 80% of U.S. infants with reported pertussis who are younger than 2 months of age are hospitalized.[350] From 2009 through 2014, there were 81 deaths attributed to pertussis in the United States; of these, 61 (75%) were infants younger than 3 months of age, two were infants 3 to 11 months of age, four were children 1 to 4 years of age, and six (10%) were 55 years of age or older.[351–354] Infants are also much more likely than older patients to manifest the complications described earlier. Indeed, it may be said that the primary goal of all pertussis vaccination programs is to prevent disease in infants.

Pertussis in School Children, Adolescents, and Adults

Prior to the institution of pertussis vaccination, it was believed that pertussis was solely a disease of infancy and childhood, and that the infection resulted in durable—perhaps lifelong—immunity. Those misimpressions likely arose because the circulation of *B. pertussis* in the community was so common that nearly all persons were infected as infants or children and were regularly boosted subclinically thereafter. In countries with longstanding pertussis vaccination programs, the age

distribution of pertussis has changed markedly. For example, from 1990 to 1993, 44% of U.S. cases occurred among children younger than 1 year of age, 22% occurred among children ages 1 to 4 years, 10% occurred among children ages 5 to 9 years, and 24% occurred among persons 10 years of age and older.[326] Then, during the late 1990s and early 2000s, the incidence in adolescents increased disproportionately. In 2000, the reported rate in persons 11 to 19 years of age was higher than in children 1 to 6 or 7 to 10 years of age.[350] The annual incidence of pertussis in persons aged 11 to 19 years increased from 5.5 per 100,000 in 2001, to 6.7 in 2002, and to 10.9 in 2003.[288] Some of these increases were likely the result of the growing recognition of pertussis as a cause of persistent cough in adolescents and adults and to the use of serologic methods to diagnose pertussis among adolescents and adults. Studies in the United States, Australia, Canada, and Germany in the early 1990s demonstrated that pertussis occurred commonly among adults.[16–18,212,222,237,290,355–358] Serologic evidence of recent pertussis was found in 26% of 218 Australian adults referred for investigation of chronic cough,[357] 26% of students presenting to a university health service with cough of at least 6 days' duration,[212] 26% of adults presenting to a Chicago clinic with unexplained cough,[355] and 21% of adults presenting to an emergency department in Nashville, Tennessee, with cough persisting 2 weeks or longer.[357] Of adult patients in a large California healthcare plan who were referred for chronic cough, 12.4% had serologic evidence of recent pertussis,[358] representing an incidence of 176 per 100,000 person-years—a rate greater than the overall annual incidence of reported pertussis (157 per 100,000) in the United States in the prevaccine era. Nearly 20% of 442 Canadian adolescents and adults with cough illness lasting longer than 7 days were diagnosed with laboratory-confirmed or suspected pertussis.[16] These reports highlight that chronic cough may be the sole manifestation of pertussis among adolescents and adults and that the diagnosis of pertussis in this age group often relies on serologic or PCR confirmation and rarely on culture of the organism.[357] The increase in reported pertussis among adolescents and adults was seen both in populations vaccinated with whole-cell vaccines and in populations largely unvaccinated and thus protected by natural immunity, and likely reflected several factors, including enhanced suspicion and recognition, improved reporting, and increased use of improved diagnostic tools. The most important factor, however, probably was the waning of natural or vaccine-induced immunity coupled with the reduced prevalence of pertussis in immunized childhood populations, which provided fewer opportunities for the reinforcement of immunity by casual exposure to infected children.

Further insight into the epidemiology of pertussis in a highly vaccinated population comes from a 1996 serosurvey of 600 normal healthy persons ages 1 to 65 years.[214] There was an expected peak in PT and FHA titers in the 4- to 6-year-old children, reflecting the administration of booster doses of pertussis vaccine, but also a second, larger peak in the 13- to 17-year-old group, suggesting that natural exposure to pertussis is frequent during the adolescent years. Similar findings have been obtained in a variety of countries.[215–219]

Additional data were provided by a multicenter, randomized, double-blind trial of a Tdap vaccine conducted in the late 1990s.[226] In that study, 2781 healthy subjects between the ages of 15 and 65 years received either Tdap or hepatitis A vaccine (control group) and were monitored for 2.5 years for cough illnesses. Of 1390 control subjects, 1284 (92%) experienced at least one cough illness lasting 5 days or longer. Using culture, PCR, and serologic methods, nine control subjects (0.65%) were diagnosed with *B. pertussis* infection. Based on these data, the incidence of pertussis in the control group was 368 cases per 100,000 person-years; extrapolating these rates to the total U.S. population, nearly 1 million cases of pertussis occurred in persons 15 years of age and older each year.[226]

The continued increase of pertussis in adolescents led to the institution of adolescent Tdap vaccination programs in the United States and other countries, which appeared initially to be successful.[359] However, as discussed previously, a new and unexpected epidemiological pattern emerged in the U.S. epidemics of 2010–14, which showed progressively increasing pertussis rates in schoolchildren 7 to 10 years of age, along with apparently reduced duration of protection following the adolescent Tdap dose.[350] The CDC's "2014 Provisional Pertussis Surveillance Report"[360] shows that the proportion of reported cases and the incidence per 100,000 population (in parentheses) by age were: age younger than 6 months, 10.4% of reported cases (incidence rate: 150.9/100,000); age 6 to 11 months, 2.8% (incidence rate: 40.4/100,000); age 1 to 6 years, 18.7% (incidence rate: 22.2/100,000); age 7 to 10 years, 16.9% (incidence rate: 29.5/100,000); age 11 to 19 years, 33.0% (incidence rate: 25.1/100,000); and age 20+ years, 17.8% of reported cases (incidence rate: 2.2/100,000).[354] As noted above, this pattern has also been seen in countries such as Australia, as populations primed and boosted only with aP vaccines have aged into preadolescence and adolescence.

It should not be thought, however, that a short duration of protection is seen only after acellular-vaccine usage; protection by whole-cell vaccine or even natural infection can be less durable than traditionally thought. For example, Kayina and colleagues found that pertussis prevalence rose substantially during and after the fifth year of life among Ugandan children given three infant doses of whole-cell vaccine.[361]

Reservoirs of Infection

B. pertussis is spread directly from human to human via respiratory droplets, and humans are the only natural reservoir; fomites are not known to play any meaningful role. Frequently, asymptomatic adults have been implicated in the spread of infection to susceptible children.[355,362–368] In one report, four children with confirmed pertussis infection and their 18 family members were evaluated for *B. pertussis*. The attack rate for pertussis infection in the contacts was 83%, but two-thirds of the previously immunized contacts found to be infected were asymptomatic.[21] Symptomatic infection was characterized by a higher PT antibody response, and asymptomatic infection by a higher FHA antibody response. These data suggest that, after pertussis immunization, immunity to disease is greater than protection from infection, a result consistent with the baboon data cited earlier.

An international study of laboratory-confirmed infant pertussis cases and their household and nonhousehold contacts using PCR and serology identified sources of infection for 48% of 95 infants in the primary analysis and up to 78% in sensitivity analyses. Parents accounted for 55% of source cases, followed by siblings (16%), aunts or uncles (10%), friends or cousins (10%), grandparents (6%), and part-time caretakers (2%).[366] A study in the United States conducted by the CDC during enhanced pertussis surveillance in several states determined that for the period 1999 to 2002, a source of infection was identified for 264 infant cases; the mother was the source in 32% and another family member in 43%. Of the sources with reported ages, 17% were age 0 to 4 years, 7% were age 5 to 9 years, 20% were age 10 to 19 years, 21% were age 20 to 29 years, and 35% were older than 30 years of age.[364] In 2015, the CDC updated these data with a report covering the period 2006–13.[365] Of 569 infants with an identified source of infection, the source was the mother for 21%, the father for 10%,

and a sibling for 36%. Mothers predominated as the source until 2008, after which point siblings were the predominant source. These data reflect the increasing incidence of pertussis in children 7 to 10 years old and are consistent with the hypothesis, supported by baboon data cited earlier, that children primed and boosted with acellular vaccines are protected from disease but not from colonization that can result in transmission.

Significance of Pertussis as a Global Public Health Problem

Despite global vaccination programs, pertussis remains endemic worldwide. Case counts, although far below those of the prevaccine era, are rising in many countries, adding to the already substantial human and economic burdens of pertussis. A U.S. study in 2000 evaluated 69 affected families in Monroe County, New York, and estimated the adverse financial effect of pertussis was $2115 per family.[369] Another study estimated that in the absence of a vaccination program, the medical costs of pertussis among German children 1 to 6 years of age would total nearly DM900 million (1994 prices) per year.[370] In the developing world, the greater burden is human, not economic. Crowcroft and colleagues estimated that in 2000, the disability-adjusted life-years lost globally from pertussis (12.7 million) exceeded those of other diseases such as lung cancer (11.4 million) and meningitis (5.8 million).[371] Data collected by the WHO's Expanded Program on Immunization (EPI) in 1992 indicated that 850,000 of the approximately 110 million children born annually in the developing world during the 1980s succumbed to pertussis before their fifth birthdays. In 1994, the WHO estimated that 40 million pertussis infections resulted in 5 million episodes of pneumonia, 360,000 deaths (a nearly 60% reduction from the earlier estimate), and 50,000 patients with long-term neurologic complications (including permanent brain damage).[372] In 2002, WHO estimated 45 million cases of pertussis with 301,408 childhood deaths.[371] The most recent (2008) estimates from the WHO indicated that approximately 16 million cases of pertussis occurred worldwide, 95% of which were in developing countries, and that approximately 195,000 children died from the disease, reflecting the growth in immunization programs and continued reduction in the burden of pertussis.[11]

PASSIVE IMMUNIZATION

Transplacental

Antibodies to PT and FHA readily cross the placenta and are found in infant sera in concentrations comparable to or higher than those in maternal sera.[373–375] The half-life of transplacental antibodies is approximately 6 weeks, with disappearance by 4 months of age, depending on titers.[376,377] However, despite efficient placental transfer, the low maternal pertussis antibody levels that are seen in the absence of pertussis booster vaccination soon before or during pregnancy, and the rapid decay of maternally derived antibodies in infant sera, leave infants with little humoral antibody protection against pertussis.[378] The excellent placental transfer of pertussis antibody provides the potential for neonatal and infant protection against pertussis when maternal antibody levels are high.[371]

The concept of boosting maternal antibody levels was first explored in the early 20th century, with active pertussis vaccination of pregnant women during the third trimester.[379–382] No adverse maternal or neonatal events were documented, and elevated antibody titers were seen in both mother and infant. However, high preexisting pertussis antibody levels in infants suppressed the subsequent immune responses to primary infant immunization with diphtheria, tetanus, and whole-cell pertussis (DTwP) vaccines and led to the rejection of maternal immunization at that time. In contrast, ecological studies of antibody responses to aP vaccines in infants with high versus low maternal antibody titers have not shown this suppression[376,383] (however, even the high maternal antibody levels in these studies were materially lower than those seen among women vaccinated during pregnancy).

The modern development of active immunization of the pregnant woman to protect the newborn from pertussis is discussed in detail in "Vaccination in Pregnancy" below.

Therapeutic

Before the widespread use of pertussis vaccine and the availability of antibiotics, passive immunization using whole serum was employed in an effort to prevent the spread of disease to exposed susceptible people and to modify the course of the illness in those who had already acquired whooping cough.[384] Most of the studies of preventive efficacy in exposed persons were not controlled; a few studies that did include control subjects suggested that as many as 40% of recipients were protected.[22,78,384]

The subsequent development of methods for purification of serum immunoglobulins led to the commercial production of pertussis hyperimmunoglobulin, composed largely of IgG. Although this preparation was widely used for prophylaxis and treatment, controlled studies indicated that it had no effect, and production was discontinued.[385,386] Then, Sato and Sato demonstrated that both monoclonal and polyclonal antibodies to PT improved survival of suckling mice after aerosol challenge with live pertussis organisms.[79] The ability to generate high-titer immunoglobulin by immunizing adults with less reactogenic aP vaccines led to a reassessment of the use of immunoglobulin as therapy for pertussis. In 1991, Granstrom and colleagues conducted a double-blind, randomized, placebo-controlled trial of a pertussis immunoglobulin prepared from adults immunized with an aP vaccine containing either PT or PT plus FHA.[387] A significant reduction in the duration of whoops was demonstrated in the recipients of the pertussis immunoglobulin compared with placebo recipients (8.7 vs 20.6 days; $P = .0041$). A pertussis immunoglobulin was prepared by the Massachusetts Biologic Laboratories using sera from adults immunized with monovalent pertussis toxoid vaccine. It was initially evaluated in the mouse aerosol model of pertussis and then in Phase I trials in infants. A multicenter, randomized, placebo-controlled clinical trial of the product was conducted, and 25 infants hospitalized with pertussis were enrolled in the 24 months before the study was terminated because of expiration of the product and lack of additional new product. All infants tolerated the product without safety concerns, but no differences were noted in the number or rate of improvement of symptoms between the immunoglobulin recipients and placebo controls.[388] More recently, several monoclonal antibodies that recognize protective PT epitopes were characterized and studies of these monoclonal antibodies in animal models suggest that they may confer a therapeutic benefit.[81,389]

ACTIVE IMMUNIZATION

History

The isolation and propagation of *B. pertussis* on artificial media in 1906 led to the development of a vaccine for the prevention of whooping cough. Initial steps to develop a vaccine were

empirical, but an improved understanding of the different growth phases of the organism led to refinement in the methods of vaccine production and resulted in a whole-cell vaccine prepared in a standardized and reproducible fashion. Whole-cell pertussis vaccines were first licensed in the United States in 1914 and became available combined with diphtheria and tetanus toxoids in 1948. They were recommended and widely used for routine vaccination of children. During the 1980s and 1990s, knowledge of the components of the pertussis organism and their biological roles expanded and led to the development of the aP vaccines, largely in response to concerns regarding adverse local and systemic adverse events reported after the whole-cell pertussis vaccines. In 1996, less reactogenic aP vaccines were licensed and recommended for routine use among infants and now are the only pertussis vaccines licensed in the United States.[5]

Development of Whole-Cell Vaccines

Whole-cell pertussis vaccines are suspensions of killed *B. pertussis* organisms. One of the first clinical trials of a whole-cell vaccine conducted during an epidemic of pertussis in the Faroe Islands provided evidence of efficacy.[390] In the 1930s, steps taken to improve pertussis vaccines included increasing the number of bacteria in the vaccine, using standardized culture media, inactivating the organisms by "gentler" methods, and using fresh, rapidly growing Phase I organisms as the inoculum.[51,78]

Early on, a major stumbling block in the development and assessment of pertussis vaccines was the lack of a suitable means, other than clinical trials, for assessing the potency of the vaccine. After World War II, Kendrick designed the mouse protection test, in which mice were immunized with pertussis vaccine and then challenged by intracerebral inoculation of living *B. pertussis* organisms. This test, also called the Kendrick test, reproducibly measured vaccine potency (correlated with protective efficacy in humans), and it was used for many years until the introduction of the acellular vaccines required a new standard.[391–393] Whole-cell pertussis vaccines produced in the United States varied somewhat in their methods of production and generated different levels of antibody responses.[51,321] In the United States, whole-cell pertussis vaccine was almost always administered to children in combination with diphtheria and tetanus toxoids. The vaccine was adsorbed onto an aluminum salt, which resulted in enhanced immunogenicity and reduced reactogenicity.

Development of Acellular Vaccines

Because of the common occurrence of minor but burdensome local reactions, and the less common but more severe systemic reactions, coupled with public anxiety over complications after immunization with whole-cell vaccine, new, less reactogenic pertussis vaccines were sought. An improved understanding of the biology of *B. pertussis* and the isolation of individual components of the organism important in disease pathogenesis and induction of clinical immunity led to the production of new vaccines. Sato and colleagues in Japan designed the first purified component (acellular) pertussis vaccines.[394] The initial vaccines consisted predominantly of FHA, along with smaller amounts of inactivated PT and, in some cases, fimbrial proteins and PRN, and were known as Takeda-type vaccines (named after the originating Japanese manufacturer). They were soon followed by additional acellular vaccines containing equal quantities of PT and FHA (termed Biken-type vaccines, after the originating Japanese manufacturer). Criteria for the licensure of the acellular vaccines in Japan included low toxicity in the mouse, documentation of potency in the mouse, diminished systemic and local reactivity in children, and antibody production in children similar to or exceeding that of the whole-cell vaccine. Household-contact studies and pertussis surveillance after implementation of the acellular vaccines gave clear evidence of effectiveness. Since 1981, aP vaccines have been used exclusively in Japan and have been very effective.[394–402]

The encouraging results in Japan stimulated other industrialized nations to evaluate the Japanese acellular vaccines and to develop other acellular vaccines. Nearly two dozen aP vaccines were designed, many were evaluated in immunogenicity and reactogenicity trials, and the efficacy and safety of a number were evaluated in field trials. These vaccines varied from one another with respect to their source, number of components, quantity of each component, method of purification, method of toxin inactivation, incorporated adjuvants, and excipients (Tables 44.3 and 44.4).[403] Unfortunately, identifying the optimal formulation for an acellular vaccine has proved difficult, because no simple method exists to determine the protective capability of a pertussis vaccine. The results of the mouse intracerebral protection test correlate reasonably well with the clinical protection afforded by whole-cell vaccine, but the test does not predict the efficacy of aP vaccines.[283] Guiso and colleagues found that the results of intranasal challenge in a murine model correlated with efficacy of selected aP vaccines,[404] suggesting the possible usefulness of such tests in the preclinical evaluation of candidate vaccines. Because the intracerebral mouse protection test does not provide a valid measure of the protective capability of the aP vaccines, the potency of individual lots of these vaccines produced for the U.S. market is evaluated by measurement of the antibody response to PT (plus FHA, PRN, and fimbrial proteins, as applicable) in immunized mice, using an ELISA. Although human immunologic correlates of protection have been avidly sought, these efforts have met with only partial success, as described later in this chapter. aP vaccines have entirely replaced whole-cell pertussis products in the United States, Canada, and Australia, in most of Europe, and in some Asian and Latin American markets (Table 44.5).

Vaccine Producers

The vaccine industry has undergone dramatic consolidation over the past 20 years; both long-established companies and new biotechnology startups have been acquired by, or merged into, successor companies. These changes render nomenclature problematic. For vaccines currently marketed, we use the name of the current manufacturer, even when describing studies conducted by a predecessor company. For products of historical interest, we use the name of the company that produced them, even if that company is now owned by or operates under a successor name. To further assist the reader, Table 44.6 lists the current major vaccine manufacturers along with the names of predecessor, component, or acquired companies.

Internationally Available Whole-Cell Pertussis Vaccine Preparations

Despite availability of acellular vaccines, whole-cell vaccines remain the most widely used globally for infant and childhood vaccination. Whole-cell vaccines are generally efficacious, relatively inexpensive to manufacture, and have been produced locally in many regions of the world. However, the evolving recommendations of the WHO's EPI program and the prequalification by WHO of several whole-cell based DTwP/*Haemophilus influenzae* type b (Hib)/hepatitis B (HB) vaccines have led to widespread use in EPI countries of these

TABLE 44.3 Key Characteristics of Selected Acellular Pertussis Vaccines[a]

Manufacturer or Distributor	Vaccine[b]	Evaluated in MAPT	Pertussis Antigen per Dose (μg)				Diphtheria Toxoid[c]	Tetanus Toxoid[c]
			PT	FHA	PRN	FIM		
Sanofi Pasteur (Canada)[d]	Tripacel; Daptacel	Yes	10	5	3	5	15	5
Sanofi Pasteur (Canada)[d]	HCPDT (used in Pentacel, Pediacel)	No	20	20	3	5	15	5
Sanofi Pasteur (France)	Triavax; Triaxim	Yes	25	25	—	—	15	5
Sanofi Pasteur (USA)	Tripedia	Yes	23.4	23.4	—	—	6.7	5
Baxter Laboratories	Certiva	No	40	—	—	—	15	6
Chiron Vaccines	Acelluvax	Yes	5	2.5	2.5	—	25	10
GlaxoSmithKline	Infanrix	Yes	25	25	8	—	25	10
Japan National Institutes of Health	NIH-6[e]	No	23.4	23.4	—	—	—	—
Japan National Institutes of Health	JNIH-7	No	37.7	—	—	—	—	—
SmithKline Beecham Biologicals	SKB-2	Yes	25	25	—	—	25	10
Wyeth Pharmaceuticals	Acel-Imune[f]	Yes	3.5	35	2	0.8	9	5

[a]Compositions may differ in various markets; local suppliers should be consulted as necessary. Some products are no longer available but are included because knowledge of their composition is important to understanding the results of the efficacy trials.
[b]Trade names (most common trade names, if multiple names exist) except as follows: HCPDT is the "hybrid" formulation of Tripacel, evaluated in the 1993 Stockholm trial (otherwise, used only in combinations); JNIH-6 and JNIH-7 were the acellular vaccines used in the 1986 Swedish trial; SKB-2 was an experimental two-component DTaP evaluated in the 1992 Stockholm trial.
[c]Measured in limit of flocculation units per dose.
[d]FIM component is a mixture of FIM-2 and FIM-3. In MAPT, PT was 10 μg; FHA, 5 μg.
[e]A Biken vaccine, similar to Tripedia.
[f]Contains approximately 40 μg (but not more than 60 μg) of pertussis antigen proteins, consisting of ~86% FHA, ~8% PT, ~4% PRN, and ~2% FIM-2.
DTaP, diphtheria and tetanus toxoids and acellular pertussis; FHA, filamentous hemagglutinin; FIM, fimbrial proteins; HCPDT, hybrid component pertussis-diphtheria-tetanus vaccine; MAPT, Multicenter Acellular Pertussis Trial; PRN, pertactin; PT, pertussis toxin.

TABLE 44.4 Additional Characteristics of Selected Acellular Pertussis Vaccines[a]

Manufacturer or Distributor	Vaccine[b]	How Toxoided	Aluminum (mg)	Diluent	Preservative	Trace Constituents
Sanofi Pasteur (Canada)	Tripacel; Daptacel	Glutaraldehyde	0.33[c]	PBS	Phenoxyethanol	Glutaraldehyde, PS
Sanofi Pasteur (Canada)	HCPDT	Glutaraldehyde	0.33[c]	PBS	Phenoxyethanol	Glutaraldehyde, PS
Sanofi Pasteur (France)	Triavax; Triaxim	Glutaraldehyde	0.30[d]	N/A	Thimerosal	N/A
Sanofi Pasteur (USA)	Tripedia	Formaldehyde	0.17[e]	PBS	None	Formaldehyde, gelatin, PS
Baxter Laboratories	Certiva	H_2O_2	0.50[d]	PBS	Thimerosal	None
Chiron Vaccines	Acelluvax[f]	Genetic	1.0[d]	N/A	Thimerosal	N/A
GlaxoSmithKline	Infanrix	Formaldehyde[g]	0.625[d,g]	Saline	Phenoxyethanol	Formaldehyde, PS
Japan National Institutes of Health	JNIH-6	Formaldehyde	0.08	PBS	Thimerosal	Formaldehyde
Japan National Institutes of Health	JNIH-7	Formaldehyde	0.075	PBS	Thimerosal	Formaldehyde
SmithKline Beecham Biologicals	SKB-2	Formaldehyde	0.50[d]	Saline	Phenoxyethanol	Formaldehyde, PS
Wyeth Pharmaceuticals	Acel-Imune	Formaldehyde	0.23[h]	PBS	Thimerosal	Formaldehyde, gelatin, PS

[a]Compositions may differ in various markets; local suppliers should be consulted as necessary. Aluminum content is per dose. Some products are no longer available, but are included because knowledge of their composition is important to understanding the results of the efficacy trials.
[b]Trade names (most common trade name, if multiple names exist) except as follows: HCPDT is the "hybrid" formulation of *Tripacel* evaluated in the 1993 Stockholm trial (otherwise, used only in combinations); JNIH-6 and JNIH-7 were the acellular vaccines used in the 1986 Swedish trial; SKB-2 was an experimental 2-component DTaP evaluated in the 1992 Stockholm trial.
[c]As aluminum phosphate.
[d]As aluminum hydroxide.
[e]As aluminum potassium sulfate.
[f]Pertussis components are formaldehyde-stabilized. Aluminum content was 0.35 mg in MAPT.
[g]PT component detoxified with both formaldehyde and glutaraldehyde. In MAPT, aluminum content was 0.50 mg.
[h]A mixture of aluminum hydroxide and aluminum phosphate.
DTaP, diphtheria and tetanus toxoids and acellular pertussis; HCPDT, hybrid component pertussis-diphtheria-tetanus vaccine; MAPT, Multicenter Acellular Pertussis Trial; N/A, information not available; PBS, phosphate-buffered saline; PS, polysorbate-80; PT, pertussis toxin.

TABLE 44.5 Pertussis Immunization Schedules Recommended by Selected National Authorities, as of June 2015[a]

Country	Primary Immunization Schedule	Pediatric Boosters	Adolescent-Adult Tdap[b]	Tdap in Pregnancy[c]
GLOBAL: MOST COUNTRIES IN AFRICA, THE MIDDLE EAST, AND ASIA (EXCEPT AS SHOWN OTHERWISE HEREIN)				
EPI programs	3 doses DTwP (most as DTwP-Hib-HB)	Some: DTwP at 18 mo to 4 y		
NORTH AMERICA				
Canada	2, 4, 6 mo: DTaP (in a combination)	18 mo: DTaP (in a combination) 4–6 y: TdaP-IPV or DTaP-IPV	14–16 y. If not received at that age, then 1 dose when able	Optional ≥26 wk; encouraged if outbreak or no prior Tdap
United States	2, 4, 6 mo: DTaP (alone or in combination)	15–18 mo and 4–6 y: DTaP (alone or in combination)	11–12 y. If not, then 1 dose when able	At 27–36 wk of each pregnancy
Mexico	2, 4, 6 mo: DTaP-IPV-Hib	15–18 mo: DTaP-IPV-Hib 4–6 y: DTwP		Yes
EUROPE				
Austria	3, 5, 12 mo: DTaP-IPV-Hib-HB	7–10 y: DTaP-IPV	Every 10 y, 18–60, then every 5 y	
Belgium	2, 3, 4 mo: DTaP-IPV-Hib-HB	15 mo: DTaP-IPV-Hib-HB 5–7 y: DTaP-IPV	14–16 y; 1 dose for all adults	At 24–32 wk of each pregnancy
Czech Republic	9, 13, 17 wk: DTaP-IPV-Hib-HB	18 mo: DTaP-IPV-Hib-HB 5 y: DTaP	10 y: DTaP-IPV-Hib; 1 Tdap for adults	
Denmark	3, 5, 12 mo: DTaP combination	5 y: DTaP-IPV or Tdap		
Finland	3, 5, 12 mo: DTaP-IPV-Hib	4 y: DTaP-IPV	14–15 y: TdaP	
France	2, 4, 11 mo: DTaP-IPV-Hib-HB	6 y: DTaP-IPV	11–13 and 25 y: Tdap-IPV	
Germany	2, 3, 4 mo: DTaP combination	11–14 mo: DTaP combination 5–6 y: Tdap	9–17 y: Tdap-IPV 1 Tdap as adult	
Greece	2, 4, 6 mo: DTaP combination	15–18 mo: DTaP combination 4–6 y: DTaP or Tdap	11–12 y; Tdap or Tdap-IPV once as adult	
Hungary	2, 3, 4 mo: DTaP-IPV-Hib	18 mo: DTaP-IPV-Hib 6 y: DTaP-IPV	11 y	
Iceland	3, 5, 12 mo: DTaP-IPV-Hib	4–5 y: DTaP-IPV	14 y	
Ireland	2, 4, 6 mo: DTaP-IPV-Hib-HB	4–5 y: DTaP-IPV	11–14 y	At 27–36 wk of each pregnancy
Israel	2, 4, 6 mo: DTaP-IPV-Hib	12 mo: DTaP-IPV-Hib 7 y: DTaP-IPV	14 y	At 27–36 wk of each pregnancy
Italy	3, 5–6, 11–13 mo: DTaP combination	5–6 y: DTaP-IPV	10 y after primary series	
Luxembourg	2, 3 mo: DTaP-IPV-Hib-HB 4 mo: DTaP-IPV-Hib	13 mo: DTaP-IPV-Hib-HB 5–6 y: Tdap-IPV	15–16 y and every 10 y thereafter: TdaP-IPV	
Netherlands	2, 3, 4 mo: DTaP-IPV-Hib-HB	11 mo: DTaP-IPV-Hib-HB 4 y: DTaP-IPV		
Norway	3, 5, 11–12 mo: DTaP-IPV-Hib	6–8 y: DTaP-IPV	15 y	
Poland	7–8 wk, 3–4 mo, 5–6 mo: DTwP (DTaP for risk groups)	16–18 mo: DTwP 6 y: Tdap		
Portugal	2, 4, 6 mo: DTaP-IPV-Hib	18 mo: DTaP-Hib 5–6 y: DTaP-IPV		
Russia	3, 4.5, 6 mo: DTwP	18 mo: DTwP		
Slovakia	2, 4, 10 mo: DTaP-IPV-Hib-HB	5 y: DTaP-IPV	12 y: Tdap-IPV	

Continued on following page

TABLE 44.5 Pertussis Immunization Schedules Recommended by Selected National Authorities, as of June 2015[a] *(Continued)*

Country	Primary Immunization Schedule	Pediatric Boosters	Adolescent-Adult Tdap[b]	Tdap in Pregnancy[c]
Spain	2, 6 mo: DTaP-IPV-Hib-HB 4 mo: DTaP-IPV-Hib	18 mo: DTaP-IPV-Hib 6 y: Tdap	13 y + every 10 y: Tdap	Per local epidemiology
Sweden	3, 5, 12 mo: DTaP combination	5–6 y: DTaP-IPV	14–16 y	
Switzerland	2, 4, 6 mo: DTaP-IPV-Hib	15–24 mo: DTaP-IPV-Hib 4–7 y: DTaP-IPV	11–15 y 25–29 y	
United Kingdom	2, 3, 4 mo: DTaP-IPV-Hib	3–5 y: DTaP-IPV		At 28–32 wk of each pregnancy
CENTRAL AND SOUTH AMERICA (MOST COUNTRIES NOT SHOWN FOLLOW A SCHEDULE SIMILAR TO ONE OF THE FOLLOWING)				
Argentina	2, 4, 6 mo: DTwP-Hib-HB	15–18 mo: DTwP-Hib 6 y: DTwP	11 y	At 26 wk of pregnancy
Brazil	2, 4, 6 mo: DTwP-Hib-HB	15 mo: DTwP-Hib-HB 4–6 y: DTwP		In each pregnancy
Chile	2, 4, 6 mo: DTwP-Hib-HB	18 mo: DTwP-Hib-HB 6 y: Tdap	13 y	
Columbia	2, 4, 6 mo: DTwP-Hib-HB	18 mo and 5 y: DTwP		At 21 wk of each pregnancy
El Salvador	2, 4, 6 mo: DTwP-Hib-HB	15 mo: DTwP-Hib-HB 4 y: DTwP		In each pregnancy
Nicaragua	2, 4, 6 mo: DTwP-Hib-HB	18 mo and 6 y: DTwP		
Panama	2, 4, 6 mo: DTaP-IPV-Hib-HB	15–18 mo: DTwP-Hib-HB 4 y: DTwP	10 y	In 3rd trimester of each pregnancy
Peru	2, 4, 6 mo: DTwP-Hib-HB	18 mo and 4 y: DTwP		
Uruguay	2, 4, 6 mo: DTwP-Hib-HB	15 mo: DTwP-Hib-HB 5 y: DTwP	12 y	In each pregnancy
ASIA (MOST COUNTRIES NOT SHOWN GENERALLY FOLLOW THE EPI SCHEDULE)				
Australia	2, 4, 6 mo: DTaP-IPV-Hib-HB	18 mo: DTaP 4 y: DTaP-IPV	10–15 y	In each pregnancy
China	3, 4, 5 mo: DTaP	18–24 mo: DTaP		
Indonesia	2, 3, 4 mo: DTwP or DTwP-HB			
Japan	3, 4, 5–11 mo: DTaP	18 mo: DTaP		
Korea, Republic of	2, 4, 6 mo: DTaP	15–18 mo and 4–6 y: DTaP	11–12 y	
Malaysia	2, 3, 5 mo: DTaP-IPV-Hib	18 mo: DTaP-IPV-Hib		
New Zealand	6 wk, 3 mo, 5 mo: DTaP-IPV-Hib-HB	4 y: DTaP-IPV	11 y: Tdap	
Taiwan	2, 4, 6 mo: DTaP-IPV-Hib	18 mo: DTaP-IPV-Hib 5 y: Tdap-IPV		
Thailand	2, 4, 6 mo: DTwP-HB	18–24 mo and 4–5 y: DTwP		

[a]Compiled from multiple sources. In many countries, products based on whole-cell pertussis vaccine are used in the national or public programs (shown in the above table), whereas products based on acellular vaccines often are used in private practice. In such countries, recommended schedules for private practice typically are based on U.S. or Western European schedules. As of June 2015, spreadsheets providing global immunization schedules and coverage rates were available from WHO at http://www.who.int/entity/immunization/monitoring_surveillance/data/schedule_data.xls?ua=1 and http://www.who.int/entity/immunization/monitoring_surveillance/data/coverage_estimates_series.xls, respectively. The European Centre for Disease Control offers online European vaccine schedules at http://vaccine-schedule.ecdc.europa.eu/Pages/Scheduler.aspx (click through the requests for credentials).

[b]Based on cost considerations, most jurisdictions recommend only a single adolescent-adult dose of Tdap (except for pregnant women). However, many practitioners elect to give Tdap whenever a tetanus, diphtheria, or pertussis booster is indicated.

[c]An entry is made in this column only when there is a specific recommendation for usage in pregnancy.

DTap, diphtheria and tetanus toxoids and acellular pertussis; DTwP, diphtheria and tetanus toxoids and whole-cell pertussis vaccine; EPI, Expanded Programme on Immunization; HB, hepatitis B vaccine; Hib, *Haemophilus influenzae* type b vaccine; IPV, inactivated poliovirus vaccine; Tdap, tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine.

TABLE 44.6 Current Major Vaccine Manufacturers and Their Merged, Acquired, or Component Companies

Name as of June 2015	Former, Merged, Acquired, or Component Companies or Names
AstraZeneca	MedImmune
CSL	Novartis (influenza assets only)
GlaxoSmithKline	Glaxo; Glaxo Welcome; RIT (Recherche et Industrie Thérapeutiques); SmithKline Beecham; SmithKline–RIT; Welcome. Acquired Novartis Vaccines (except influenza assets) in 2015, thus acquiring Behring; Biocine; Chiron Vaccines; Evans Vaccines; Medeva Vaccines; Powderject Vaccines; Sclavo; Zhejiang Tianyuan Bio-Pharmaceutical Co., Ltd
Johnson & Johnson	Acquired Crucell NV in 2011, thus acquiring Berna Biotech; Berna Products; Rhein Biotech; SBL Vaccin AB; Swiss Serum and Vaccine Institute (SSVI)
Merck & Co	Schering-Plough; Organon
Sanofi Pasteur	Aventis Pasteur; Connaught Laboratories; Pasteur Merieux; Pasteur Merieux Connaught; Acambis (including Berna Products; OraVax; Peptide Therapeutics); Shantha Biotechnics; VaxDesign
Pfizer	Baxter International (meningococcal serogroup C and tickborne encephalitis vaccines); Redvax GmbH; Wyeth Pharmaceuticals, including American Home Products; Lederle Laboratories; Praxis; Wyeth-Ayerst Laboratories; Wyeth Lederle; Wyeth Vaccines
Serum Institute of India	Bilthoven Biologicals (formerly, Dutch Vaccine Institute)

combination vaccines, most of which are manufactured in Asia (see Chapter 15).

In determining its pertussis immunization strategy, each country has to evaluate the costs, relative efficacy, and rate of adverse reactions of both whole-cell and acellular vaccines, in light of prevailing recommendations (see later) and its own circumstances.

Internationally Available Acellular Pertussis Vaccine Preparations

aP vaccines are available as diphtheria, tetanus, and acellular pertussis (DTaP) vaccines, Tdap vaccines, and as multicomponent combinations that add additional antigens to those backbones. The multicomponent combination vaccines are discussed in Chapter 15; in the present chapter, we provide details concerning the base DTaP and Tdap vaccines. Vaccines discussed in this section are those marketed internationally as of early 2016. In some jurisdictions, their trade names may vary from those provided here; local suppliers should be consulted as necessary.

Constituents and Stability, Storage and Handling, Dosage and Route

Details of the composition and other key characteristics of selected current or historically important DTaP vaccines are shown in Tables 44.3 and 44.4. Unless otherwise noted, all vaccines listed should be stored refrigerated at 2°C to 8°C; do not freeze. The standard dose of each vaccine is 0.5 mL given intramuscularly in the anterolateral thigh (infants and young children) or the deltoid (older recipients). It is inappropriate to give a partial dose in the hope of reducing adverse effects.

Currently Available Preparations for Pediatric Use (6 Weeks to 7 Years of Age)

Daptacel, Pentacel, and Pediacel—Sanofi Pasteur. Daptacel is the U.S. trade name for a DTaP vaccine manufactured by Sanofi Pasteur Ltd (Toronto, Canada). The aP vaccine component contains approximately 10 µg PT, 5 µg FHA, 3 µg PRN, and 5 µg of combined FIM-2 and FIM-3. Daptacel is supplied in thimerosal-free single-dose vials. Daptacel is marketed in many countries under various names (Tripacel, Triacel, or Pertacel). A version of this vaccine containing 20 µg PT, 20 µg FHA, 3 µg PRN, and 5 µg of combined FIM-2 and FIM-3 is used in combination vaccines that also incorporate inactivated polio vaccine (IPV) and HB components and marketed as Pentacel (liquid DTaP-IPV used to reconstitute lyophilized Hib) and Pediacel (a fully liquid version of the vaccine).

DTaP/IPV/Hib/HB—MCM Vaccine Co. This new fully-liquid vaccine produced by a joint venture of Merck and Sanofi Pasteur incorporates 20 µg PT, 20 µg FHA, 3 µg PRN, 5 µg of combined FIM-2 and FIM-3, and IPV from Sanofi Pasteur with Hib and HB components from Merck (see Chapter 15).

Infanrix—GlaxoSmithKline. Infanrix is a DTaP vaccine manufactured by GlaxoSmithKline Biologicals (Rixensart, Belgium). The aP vaccine component contains not less than 25 µg PT, 25 µg FHA, and 8 µg PRN. The vaccine is supplied in single-dose thimerosal-free vials or prefilled syringes. Infanrix is marketed in many countries, most often in combination with Hib, HB, or IPV (e.g., Infanrix Tetra, Infanrix Penta, Infanrix Hexa; see Chapter 15). In the United States, the combination of Infanrix, HB vaccine, and IPV is marketed as Pediarix.

Triavax—Sanofi Pasteur. Triavax is manufactured by Sanofi Pasteur SA (Lyon, France) and contains approximately 25 µg PT and 25 µg FHA. The vaccine is not available as a standalone DTaP but is marketed in many countries in combination with other components such as Hib, HB, and IPV (e.g., Tetraxim, Pentaxim, or Hexaxim; see Chapter 15).

Currently Available Preparations for Adolescent and Adult Use

The following vaccines were developed for use in adolescents and adults, but have also been found useful in some jurisdictions for preschool boosting of previously primed children (e.g., at age 4 to 6 years).

Adacel—Sanofi Pasteur. Adacel (marketed in Europe as Covaxis) is an adolescent-adult formulation of Sanofi Pasteur's five-component aP vaccine. It is identical to Daptacel (Tripacel) except that it contains reduced quantities of PT (2.5 µg) and diphtheria toxoids (2 lf [limit of flocculation]).

Adacel is licensed for use in most countries for persons 4 years of age or older (in the United States, persons 10 to 64 years of age) and is supplied in thimerosal-free, single-dose vials. Combined with IPV, the product is marketed as Repevax.

Boostrix—GlaxoSmithKline. Boostrix is an adolescent-adult formulation of GlaxoSmithKline's three-component aP vaccine. It differs from Infanrix by containing reduced quantities of PT (8 µg), FHA (8 µg), PRN (2.5 µg), diphtheria (2.5 lf), and tetanus (5 lf) toxoids, and adjuvant (<0.39 mg aluminum). Boostrix is licensed in most countries for persons 4 years of age or older (in the United States, persons 10 years of age and older). Outside the United States, it is also available combined with IPV as Boostrix-IPV.

Other Acellular Pertussis Preparations. Several additional aP vaccines were developed during the 1980s and 1990s but were not licensed, and a number of vaccines that once were licensed have been removed from the market. Interested readers are referred to the third edition of this text[405] for further details. Manufacturers in Asia have developed a variety of aP vaccines for their local markets. For example, in China, at least five locally produced aP vaccines are licensed, along with vaccines from GlaxoSmithKline and Sanofi Pasteur.[406] It is likely that some of these Asian aP vaccines will eventually be offered for export (probably as components of larger combinations; see Chapter 15).

Vaccine Schedule Deviations

Deviation from the approved vaccination schedule should be avoided for multiple reasons. The approved schedule represents the schedule best attuned to the national epidemiological situation and concerning which there is the best available information regarding safety and effectiveness. Administration earlier than recommended may result in lower effectiveness or higher risk of adverse events. Administration later than recommended increases the opportunity for intercurrent infection before vaccines can be administered. However, a delay between doses should not interfere with the final immunity achieved by any of the pertussis vaccines. There is no need to start the series over again, regardless of the time between doses. Partial doses should not be given; there is no evidence that doing so reduces the frequency of serious adverse events, and efficacy may be impaired.

Interchangeability of Vaccines

Few data are available demonstrating the effects of a change during the scheduled immunization series from one brand of DTaP to another, but there are theoretical reasons why switching formulations might reduce effectiveness. Recommendations from ACIP and the American Academy of Pediatrics Committee on Infectious Diseases (Red Book Committee) therefore have indicated a preference to use the same brand of DTaP vaccine for all doses of the vaccination series for a given child. However, the recommendations also state that, if the vaccine provider does not know, or does not have available, the brand of DTaP previously administered to a child, any of the licensed DTaP vaccines may be used to complete the vaccination series. Moreover, the one published study evaluating a change from one DTaP (Tripedia) to another (Infanrix) during the primary series found no adverse effect on safety or immunogenicity.[407] As a practical matter, interchange of the various aP vaccines has become commonplace, fueled by periodic changes in public distribution systems, the vagaries of patient migration and shifting provider purchases, and intermittent shortages of one vaccine or another.

Simultaneous Administration With Other Vaccines

DTwP and DTaP vaccines, or combination vaccines incorporating those components, are routinely administered concomitantly with other vaccines appropriate to the age and previous vaccination status of the child, with the injections given at separate sites using separate syringes. Most studies have found that the adverse reactions after multiple simultaneous vaccinations are only slightly greater than would be expected from the most reactogenic vaccine alone.[408–410] Further reductions in adverse reactions can often be obtained by using vaccines that combine multiple antigens (see Chapter 15), thereby reducing the number of injections given. Although reduced immunogenicity to the pertussis antigens has occasionally been seen when given in combination or association with one or more of the other vaccines, no data exist to suggest that concomitant administration of any of these other vaccines decreases the efficacy of pertussis vaccine. Because whole-cell pertussis vaccine has adjuvant properties, replacement of the whole-cell component with acellular vaccine has resulted in reports of diminution of the immune responses to some of the simultaneously administered vaccines (see Chapter 15), without evidence of impairment of efficacy.

RESULTS OF VACCINATION: WHOLE-CELL PERTUSSIS VACCINES

Although the results of some of the whole-cell pertussis vaccine arms in the efficacy trials in Europe demonstrated surprisingly low efficacy rates,[411,412] most authorities agree that the widespread use of the whole-cell vaccine has had enormous benefits.[252,413,414] These benefits include the rapid decline in morbidity and mortality from pertussis after implementation of whole-cell vaccine programs; the recurrence of disease in countries where whole-cell immunization was discontinued, acceptance rates declined, or vaccines became ineffective; the inverse correlation of the pertussis attack rates and the number of immunized children; and the lower attack rates in previously immunized children than in unimmunized children under both endemic and epidemic conditions.

Immune Responses to Whole-Cell Vaccines

Humoral Immunity

Data from the WHO indicate that 64% of countries use whole-cell pertussis (wP)-containing vaccines in their public programs, including 96% in the African Region and 100% in the Southeast Asia Region, with most administering a pentavalent combination DTwP-HB-Hib vaccine. Although many of the world's children are immunized with locally produced wP vaccines, few data exist to compare their safety, immunogenicity, and efficacy. Most authorities had presumed that all whole-cell vaccines were very similar and that one was as immunogenic and effective as another, given the standardization of many aspects of vaccine production and the fact that all licensed vaccines pass the intracerebral mouse challenge test (which was first introduced in 1940s). When significant differences in immune responses were noted with wP products produced by different manufacturers in the United States and Canada, it became apparent that all whole-cell vaccines were not the same.[321,415,416] The European aP vaccine efficacy trials used several different whole-cell vaccines, which differed widely in efficacy. Thus, it appears possible that some of the wP vaccines produced in various countries in the world might be less effective than others, and this could affect the global burden of pertussis disease.

Another concern with wP vaccines is the negative impact of high maternal antibody levels on infant immune responses. It has been demonstrated that the magnitude of the primary antibody response to whole-cell vaccine in infants depends on the preimmunization (transplacental) levels of antibody to PT, with higher circulating levels of maternally derived antibody being associated with significantly lower levels of postimmunization antibody.[376] In contrast, PT antibody responses to an acellular vaccine containing 12.5 µg each of PT and FHA were superior to those of the whole-cell vaccine and were not affected by prevaccination antibody levels.[376] There have been no studies in developing countries that evaluate the impact of maternal antibody on immune responses to primary immunization in young children with locally produced whole-cell vaccines. Such studies are needed if maternal Tdap is to be used in developing countries where infants will be routinely receiving wP vaccines in the primary series.

Although humoral immune responses to the pertussis antigens have been repeatedly demonstrated in recipients of the wP vaccines, as detailed below, the immune responses rapidly wane and do not provide durable protection against future reinfection. Despite considerable progress in our understanding of immune responses to *B. pertussis*, no unequivocal immunological correlates of protection against pertussis have been identified.

Controlled Clinical Trials of Whole-Cell Vaccines

It is well documented by controlled clinical trials that wP vaccine provides protection against clinical whooping cough in the majority of immunized people. The first convincing evidence was provided by studies in the Faroe Islands during two epidemics.[390] These studies showed that pertussis vaccine not only protected against disease but also ameliorated the severity of disease in immunized persons who contracted the illness. Although studies with early vaccines produced inconsistent results,[22,252,414,417] clinical trials after standardization of the vaccines by the mouse intracerebral challenge test[413] demonstrated consistent efficacy.[413,418,419]

In more recent years, a number of field trials of aP vaccines (see "Efficacy Trials, 1992 to 1997" later) have incorporated wP vaccines as controls and have provided some of the best data ever obtained about the efficacy of the conventional whole-cell vaccines. As mentioned previously, these studies showed that whole-cell vaccines vary substantially in efficacy. The following estimates reflect efficacy after three doses and, to the extent possible, consistent case definitions; however, none of these studies was fully blinded and randomized, and the estimates may be generous. The Mainz[420] and Munich[421] studies produced efficacy estimates of 98% and 96%, respectively, for the German-produced Behringwerke vaccine; the U.S.-made Wyeth-Lederle whole-cell vaccine was reported to be 83% efficacious in the Erlangen trial[323,422]; and the Senegal trial reported the French-made Sanofi Pasteur whole-cell vaccine to be 96% efficacious.[423] In each of these trials, the whole-cell vaccine was more efficacious than the acellular product.

In marked contrast, the U.S.-made Connaught whole-cell vaccine had very low rates of efficacy after three doses: 48% in Sweden and 36% in Italy.[324,325] In the Swedish study, efficacy was nearly 74% for the first 6 months after the third dose of vaccine but declined rapidly after that time.[324] A British national survey of reported whooping cough from 1989 to 1990 determined that the efficacy rates of the Wellcome whole-cell vaccine, administered at 3, 5, and 10 months of age, were 87% and 93% during epidemic and nonepidemic periods, respectively.[424] Efficacy declined with age but remained high until the age of 8 years. A repeat survey was conducted in 1994 to determine whether efficacy had been altered by the change to an accelerated schedule of immunization at 2, 3, and 4 months of age with no subsequent booster. Efficacy was not altered and was 94% overall for those subjects between 6 months and 5 years of age.[425] This accelerated schedule was also associated with a reduced rate of adverse reactions.[426]

Other Evidence of Effectiveness of Whole-Cell Vaccines

Secular Changes in Morbidity and Mortality

There is no question that the widespread use of wP vaccine in developed countries has resulted in a remarkable decline in reported pertussis.[252,284,292,427] However, it is also clear that mortality rates from pertussis were declining in some countries even before the advent of the vaccine.[252,414,428,429] The latter reductions probably reflect improved social and economic conditions, better nutrition, and declines in concomitant infections that may have enhanced pertussis mortality.

Effects of the Withdrawal of Pertussis Immunization

Strong evidence of the benefits of pertussis vaccine was provided by unintended experiments that occurred in three developed countries when vaccine use was curtailed or abandoned. Japan initiated widespread immunization against pertussis in 1950, and over the ensuing years the numbers of reported cases and the numbers of deaths declined remarkably.[284,430] However, beginning in 1975, adverse events temporally associated with administration of wP vaccine to young children led to a near-boycott of the vaccine, and epidemic pertussis recurred. Hundreds of children died of pertussis during this period.[284] A similar experience occurred in England and Wales, in the context of negative publicity surrounding adverse events associated with vaccine. Rates of vaccine acceptance fell from approximately 75% to nearly 25% during the mid-1970s, major epidemics of pertussis ensued, and numerous children died.[292,431] In Sweden, the administration of pertussis vaccine was suspended in 1979 when pertussis outbreaks occurred despite high vaccination coverage, suggesting poor efficacy of the existing vaccine. The incidence of whooping cough then increased more than fourfold from 1980 to 1985, with several major outbreaks in subsequent years.[432,433] With the advent of routine pertussis immunization using acellular vaccines after completion of the vaccine efficacy trials in Sweden, pertussis rates again declined.[434]

Pertussis Rates in Vaccinated and Unvaccinated Communities

Further evidence of the efficacy of wP vaccine is provided by the observation that the reported incidence of pertussis disease varies inversely with vaccine acceptance rates. A study in England and Wales prior to replacement of wP with aP vaccines found that communities with low pertussis vaccine acceptance rates (<30%) had a 59% higher reported incidence of pertussis among children than did areas with high (>50%) acceptance rates; areas with intermediate acceptance rates had intermediate pertussis rates.[435] These findings were not explained by differences among the communities such as crowding and social class; indeed, after adjustment for these two social indicators, the inverse correlation with immunization status and pertussis disease burden was even stronger. In the United States, where infant immunization for pertussis has been routine since the late 1940s, national surveillance demonstrated a greater than 95% reduction in pertussis; surveillance data from 1992 to 1994 found an overall wP vaccine

efficacy of 64% after three doses and of 82% after four or more doses.[326]

Pertussis Attack Rates in Immunized and Unimmunized Children During Outbreaks

Additional evidence for the efficacy of wP vaccine is provided by community outbreaks of pertussis in which the attack rates of the disease in immunized children were compared with the rates in those who were incompletely or never immunized.[292,436] Although the methods of ascertainment and analysis vary, most studies indicate that the efficacy of three or more doses of wP vaccine in protecting children against clinical disease during outbreaks is 80% to 90%, with incomplete immunization offering partial protection.[436] In children who contracted pertussis despite wP immunization, the disease was milder and complications were far less frequent, although younger infants and children are more likely to have received inadequate or no immunization.[437,438] Studies conducted in Senegal also documented that breakthrough cases of pertussis among children vaccinated with wP vaccine were of lesser severity and reduced infectivity than those seen in unimmunized children.[439,440]

Herd Immunity After Immunization

Because wP vaccine is not 100% effective, it might be considered curious that morbidity and mortality from clinical pertussis have been negligible in countries with widespread immunization programs. This is a particularly interesting finding because surveillance statistics and studies demonstrating pertussis to be a common cause of protracted cough illness in adolescents and adults suggest that *B. pertussis* remains ubiquitous in these countries.[212,214,234,356–358] Attempts to model mathematically the decline in pertussis incidence attributable to widespread immunization with vaccines of 85% efficacy have underestimated the rates of decline of the disease.[441] Although other factors, such as social and economic changes, very likely play a role, the most probable explanation is herd immunity, a complex phenomenon that varies among different infectious diseases and is difficult to measure with precision[442] (see Chapter 77). Herd immunity undoubtedly explains the cycles of outbreaks of pertussis every 3 or 4 years; after an outbreak, several years are required for the proportion of susceptible persons to increase to a level that facilitates a new wave of spread in a population. Studies describing higher rates of pertussis disease in immunized children living in communities with large numbers of unimmunized children provide additional evidence for the role of herd immunity.[443] More recently, Rohani and colleagues demonstrated an increase in the interepidemic period after the initiation of whole-cell vaccination in England and Wales, an indication of reduced transmission and strong herd immunity provided by whole-cell vaccines.[444] In addition, Broutin and colleagues, using aggregated incidence data from multiple countries, demonstrated a 1.3-year increase in the interepidemic period following the inception of wP vaccination programs.[445] These studies, and others, afford strong evidence that whole-cell vaccination reduces not only disease, but also transmission and thus induces herd immunity.[444–446]

Duration of Immunity After Whole-Cell Vaccines

A number of studies have evaluated the duration of protection after immunization with whole-cell vaccine. Those that provide the longest period of evaluation indicate that protection declines by 50% over a period of 6 to 12 years.[447–449] These data are consistent with the previously cited incidence and serosurvey data among whole-cell vaccinated subjects that suggest an increase in rates of pertussis among 13- to 17-year-olds, representing an interval of 7 to 12 years since last vaccination.[214] It is likely that the duration of protection is influenced by the specific wP vaccine used, the number of doses given, the vaccination schedule, and the level of circulating *B. pertussis* capable of stimulating an anamnestic response in previously immunized persons.

Adverse Events With Whole-Cell Vaccines

Despite their clear benefits in reducing the substantial mortality and morbidity of pertussis, the whole-cell vaccines have long been recognized as one of our most reactogenic vaccines. They commonly cause reactions that are minor but burdensome, occasionally cause reactions that are transient but frightening, and uncommonly cause more serious, generally self-limited, adverse effects. For some time, there was substantial suspicion that whole-cell vaccines might be causally related to devastating outcomes such as encephalopathy or sudden infant death syndrome (SIDS), but several careful epidemiologic studies have largely dispelled these concerns.[450–452] In addition, the identification of Dravet syndrome among some children who had the onset of seizures after wP vaccine has provided new insights into cases of encephalopathy after vaccination. Dravet syndrome is an epileptic encephalopathy linked to mutations in SCN1A (neuronal sodium channel α_1 subunit) and is characterized by the onset in infancy of seizures and developmental decline. A study in a large California healthcare system found that the causative mutation arose de novo at a rate of 1 per 20,000 live births (95% CI, 1/9600 to 1/45,700).[453] Several investigations have found SCN1A mutations among some patients previously diagnosed with alleged vaccine encephalopathy from wP vaccine.[454,455]

Untoward events after wP immunization began to be of increasing concern to the public and to physicians in the early 1970s, particularly in countries where widespread vaccination had eliminated most disease. Vaccine-associated adverse events loomed large in the eyes of young parents and physicians who had never witnessed the morbidity and mortality of whooping cough. Widespread publicity about the alleged dangers of pertussis vaccine, coupled with declining disease rates in some countries and doubts about vaccine efficacy in others, resulted in near-abandonment of wP vaccine in several countries. Consequently, pertussis disease recurred.[284,292,432] In the United States, strong school-entry immunization laws enabled vaccination rates to be maintained despite widespread publicity about these concerns. However, extensive litigation over alleged personal injuries caused by the vaccine cost millions of dollars and contributed to the cessation of pertussis vaccine production by several manufacturers.

Establishing or disproving cause and effect, particularly for events of major consequence, proved difficult. Although the original allegations of causation were largely anecdotal and based on the fallacious assumption that subsequences and consequences were synonymous, they raised great concern and stimulated the search for an improved vaccine. The relationships between wP vaccine and fatal or disabling events were difficult to evaluate because of the rarity of such events; because vaccine was administered to infants at an age when disorders such as encephalopathy, infantile spasms, neurologic conditions, and SIDS were most likely to occur; because these disorders can arise from other causes; and because an absolute negative can never be proven. Earlier estimates of the rates of adverse events were not optimal because of lack of consideration of background rates, ill-defined criteria, and uncertainty of denominators. However, in the 1980s, more rigorous epidemiologic or interventional studies greatly

improved the understanding of the incidence and spectrum of adverse events after wP vaccine. Many of these studies, particularly those related to serious untoward events, were evaluated by a special committee of the Institute of Medicine (IOM) of the U.S. National Academy of Sciences.[450–452] Although these evaluations were reassuring to healthcare providers and to parents, it was the development of less reactogenic aP vaccines and their replacement of the whole-cell products that put an end to the long debate in developed countries over adverse events associated with whole-cell vaccines. wP vaccines remain widely used in developing countries, where concerns regarding adverse events do not seem to be as common.

Because of space limitations and the replacement of whole cell vaccines by acellular ones in most developed countries, we have restricted the discussion of adverse events associated with whole-cell vaccines to a summary of the key studies and conclusions. Readers interested in a more thorough review of these data are referred to earlier editions of this text.[405,456,457]

Nonfatal, Nondisabling Reactions

Common Reactions. Minor local reactions, consisting of redness, swelling, and pain at the site of injection, occur in about half of DTwP recipients. Reactions occur five times more frequently after DTwP than after DT.[458–461] Similarly, minor systemic reactions such as fever, irritability, and drowsiness are significantly more common after DTwP than after DT (diphtheria and tetanus toxoids–pediatric).[458–461] Approximately half of the children who received wP vaccine experienced some minor fever, with less than 1% having an elevation in temperature to 40.5°C.[458–462]

Participants in the Multicenter Acellular Pertussis Trial (MAPT; see later) experienced somnolence at rates of 62% for whole-cell recipients and 43% for acellular vaccine recipients,[462] suggesting that the somnolence was, at least in part, an effect of the DTwP vaccine. Some children were reported to have an unusual, high-pitched cry. Others had a period of excessive crying, which lasted several hours or longer after an injection of whole-cell vaccine. This incessant, inconsolable crying usually begins within 12 hours. Persistent crying of 1 hour or more occurred in both the DTwP and DT groups in the study by Cody and coworkers[461] but was at least four times more common after DTwP. Among those with persistent crying, the cry was described as high-pitched or unusual in 3.5% of the children.[460–462]

These common reactions after wP vaccines varied somewhat in frequency and severity among lots[463] and manufacturers. The vaccine schedule followed may also have affected the incidence and severity of adverse reactions. In 1990, the schedule for DTwP vaccination in the United Kingdom was changed from 3, 5, and 10 months of age to 2, 3, and 4 months of age. The new schedule was associated with a substantial reduction in postvaccination fever and injection-site redness.[426] With the accelerated vaccination schedule in the United Kingdom, reaction rates for the whole-cell vaccine did not differ significantly from those of several acellular vaccines.[426]

Reaction rates also varied with the number of prior DTwP injections. In the Cody and colleagues study, local reactions increased in frequency with successive doses, including the preschool booster.[460,461,463] The incidence of fever also increased with successive doses through the 18-month booster, but was lower with the preschool booster at 4 to 6 years. On the other hand, persistent, inconsolable crying occurred most frequently with the initial dose and less often thereafter. In the MAPT, the incidence and severity of fever increased substantially with successive primary doses of the reference whole-cell vaccine.[462,464] Redness increased modestly; frequency of pain, fussiness, anorexia, vomiting, and use of antipyretics did not materially increase or decrease with successive doses; and drowsiness decreased.[462] In general, children who experienced local or systemic reactions after wP vaccine had an enhanced likelihood of experiencing the same reaction with a subsequent dose.[465]

Uncommon Reactions. DTwP was associated with febrile seizures (0.06% in the Cody and coworkers trial),[461] and seizures occurred at increased rates after DTwP in children with personal or family histories of convulsions.[461,465–472] However, simple febrile convulsions, although distressing, are considered to be benign,[473] with no evidence that seizures after DTwP induced persistent epilepsy.[474]

Another worrisome but uncommon reaction to DTwP was a strange shock-like state, termed a hypotonic-hyporesponsive episode (HHE), that usually had its onset within 12 hours of inoculation and lasted for several hours but always resolved.[475] Neither death nor adverse sequelae have been observed after HHE episodes. Cody and colleagues detected an incidence of HHE of 0.06%.[461,463] However, the mechanism of HHE is unknown, and HHE has been reported with other vaccines, including aP products, as described later.

Serious Reactions Allegedly Caused by Whole-Cell Pertussis Vaccine

Encephalopathy. The most serious reaction attributed to wP vaccine was acute encephalopathy. The National Childhood Encephalopathy Study, conducted in Great Britain from 1976 to 1979, examined whether the frequency of DTwP vaccination in children with encephalopathy was greater than expected. Based on 11 subjects who appeared to have neurologic residua 18 months after wP vaccination, it was estimated that acute encephalopathy with permanent brain damage occurred at the widely quoted rate of 1 per 310,000 doses, with a 95% CI of one in 54,000 to 5,310,000 doses. However, subsequent investigations cast doubt on most of these 11 diagnoses.[476–478] At 10-year follow-up, the rates of death or other sequelae in these subjects were similar whether or not the onset of acute neurologic illness was temporally associated with DTwP vaccination.[479]

The results of the National Childhood Encephalopathy Study have been subjected to extensive analysis, reanalysis, challenge, and debate.[478–484] None of this scrutiny has confirmed a causal relationship between pertussis vaccine and permanent neurologic damage. Several subsequent U.S. studies also failed to show a relationship between wP vaccine and acute encephalopathy leading to brain damage.[468,472] In 1994, the IOM concluded that the "balance of evidence is consistent with a causal relation between DT[w]P and chronic nervous system dysfunction in children whose serious acute neurologic illness occurred within 7 days of DT[w]P vaccination."[451] However, the IOM was not able to determine whether the wP vaccine increased the number of children with chronic neurologic illness or was simply a precipitating event in children who would have nonetheless developed chronic neurologic dysfunction as a result of underlying brain or metabolic abnormalities.[485,486] In a 2015 report from the U.S. National Vaccine Injury Compensation Program, medical records of children younger than 2 years of age with seizures or encephalopathy allegedly caused by an immunization from 1995 through 2005 were reviewed. There were 165 claims with sufficient clinical information for review. The majority of claims were associated with wP vaccines, and many of the children with alleged vaccine injury had preexisting neurologic or neurodevelopmental abnormalities, suggesting genetically determined epilepsy. The authors of the article recommended that

genotyping should be performed in all children who present with seizures and encephalopathy after vaccination.[485]

Infantile Spasms. Infantile spasms, which occur in approximately 40 per 100,000 infants,[487] have been reported in temporal association with wP vaccination.[488] Because infantile spasms typically occur between 2 and 8 months of age, an association is occasionally seen by chance alone. Four studies demonstrated no foundation for concern that DTwP causes infantile spasms.[282,477,489–491]

Sudden Infant Death Syndrome. Because SIDS occurs most often in the first 6 months of life,[492] it is to be expected by chance alone that some instances would be observed within a day or two of receipt of DTwP. Several early reports suggested clustering of SIDS cases within a few days after the administration of DTwP,[493–497] but subsequent studies found no evidence of a causal relationship between SIDS and receipt of DTwP.[22,498–505] The IOM panel, after a careful review of all studies, also concluded that no causal relationship existed.[450,506]

Other Serious Conditions. The IOM panel examined the evidence concerning an association between DTwP and a variety of syndromes. Table 44.7 summarizes their conclusions.[451,507]

TABLE 44.7 Institute of Medicine Conclusions Regarding Causation of Serious Adverse Events by Diphtheria and Tetanus Toxoids Plus Whole-Cell Pertussis Vaccines

Event	Conclusion
Evidence indicates causation	Anaphylaxis Prolonged or inconsolable crying Febrile seizures
Evidence consistent with causation	Acute encephalopathy Hypotonic-hyporesponsive episodes
Evidence does not indicate causation	Afebrile seizures Hypsarrhythmia Infantile spasms Reye syndrome Sudden infant death syndrome
Insufficient evidence to draw a conclusion	Aseptic meningitis Chronic neurologic damage Epilepsy Erythema multiforme or other rashes Guillain-Barré syndrome Hemolytic anemia Juvenile diabetes Learning or attention disorders Peripheral mononeuropathy Thrombocytopenia
No evidence available either way	Autism

Data from Howson CP, Howe CJ, Fineberg HV, eds. Adverse Effects of Pertussis and Rubella Vaccines: a Report of the Committee to Review the Adverse Consequences of Pertussis and Rubella Vaccines. *Washington, DC: National Academy Press, National Academy of Sciences; 1991; Howson CP, Fineberg HV. Adverse events following pertussis and rubella vaccines: summary of a report of the Institute of Medicine.* JAMA. *1992;267:392–396; and Howson CP, Fineberg HV. The ricochet of magic bullets: summary of the Institute of Medicine report, adverse effects of pertussis and rubella vaccines.* Pediatrics. *1992;89:318–324.*

U.S. National Childhood Vaccine Injury Act

Increasing litigation over alleged vaccine injuries and withdrawal from the marketplace of vaccines by several DTwP manufacturers prompted the U.S. Congress in 1986 to pass the National Childhood Vaccine Injury Act, which provides compensation for certain untoward events that occur within specified time periods after vaccination. In 1995, program rules were revised in light of the report of the IOM committee.[450,506] The replacement of wP with aP vaccines has markedly reduced the rates of adverse reactions temporally associated with pertussis vaccine,[507] resulting in a corresponding decline in vaccine injury claims associated with pertussis-containing vaccines.[508] Recent reports from the Vaccine Injury Compensation Program document that the claims of seizures and encephalopathy were much greater following wP than aP vaccines.[485]

RESULTS OF VACCINATION: ACELLULAR PERTUSSIS VACCINES

Immune Responses to Acellular Vaccines

Humoral Immunity

Numerous immunogenicity and reactogenicity studies have been published evaluating aP vaccines. However, making comparisons among them is difficult because of variations in study design, study populations, and serologic assays. To provide such comparisons and facilitate selection of candidate vaccines for anticipated efficacy trials, the National Institute of Allergy and Infectious Diseases (NIAID) sponsored the MAPT in six of its Vaccine and Treatment Evaluation Units from 1991 to 1992. Thirteen acellular and two whole-cell vaccines were evaluated in the MAPT, including all but one of the acellular vaccines subsequently evaluated in efficacy trials. Although that vaccine was not available for the MAPT, it was evaluated thereafter at one of the Vaccine and Treatment Evaluation Unit sites using the MAPT protocol, procedures, and data forms; sera were evaluated in one of the MAPT reference laboratories. Immunogenicity and reactogenicity results from that study are presented here, along with the MAPT results, to provide the most complete available comparison of these vaccines.

Healthy infants enrolled in the MAPT were randomized to receive one of the study vaccines (Table 44.8) at 2, 4, and 6 months of age. Two wP vaccines, one made by Lederle Laboratories (the reference or control vaccine) and the other by Massachusetts Public Health Biologic Laboratories were evaluated. Sera were obtained before the first immunization and 1 month after the third immunization. Serologic assays included ELISA antibody to PT, FHA, PRN, and fimbrial proteins; Chinese hamster ovary cell toxin neutralization and agglutination assays; and assays of diphtheria and tetanus antitoxin.[403]

Each vaccine produced significant increases in antibodies directed against its included antigens, which most often equaled or exceeded those produced by the reference whole-cell vaccine (see Table 44.8).[403] Nonetheless, postimmunization antibody levels differed substantially among the acellular vaccines. For PRN and fimbrial proteins, and to some extent for FHA, antibody levels tended to correlate with the quantity of antigen included in the vaccine. For PT, postimmunization antibody levels did not correlate well with the quantity of antigen in the vaccine, suggesting that manufacturing techniques were important in determining the immunogenicity of the particular PT component of each vaccine. As shown in Table 44.8, the two vaccines containing a recombinant genetically modified PT stimulated the highest antibody responses.[12]

TABLE 44.8 Antibody Levels 1 Month After the Third Dose of Vaccine: Results From the Multicenter Acellular Pertussis Trial and a Follow-up Trial[a]

Manufacturer or Distributor	Vaccine[b]	Geometric Mean Antibody Level (95% CI) After Immunization at 2, 4, and 6 Months			
		PT	FHA	PRN	FIM
Sanofi Pasteur (Canada)	Tripacel	36 (32–41)	37 (32–42)	114 (93–139)	240 (204–282)
Sanofi Pasteur (Canada)	CLL-3 F_2	38 (33–44)	36 (31–41)	3.4 (3.1–3.6)	230 (183–290)
Sanofi Pasteur (France)	Triavax	68 (60–76)	143 (126–161)	3.3 (3.1–3.6)	1.9 (1.6–2.1)
Sanofi Pasteur (USA)	Tripedia	127 (111–144)	84 (73–95)	3.5 (3.2–3.9)	2.0 (1.7–2.3)
Baxter Laboratories	Certiva	54 (41–71)	1.1 (1.0–1.2)	N/A	N/A
Biocine Sclavo	BSc-1[c]	180 (163–200)	1.2 (1.1–1.4)	3.4 (3.1–3.7)	1.8 (1.7–2.0)
Chiron Vaccines	Acelluvax[c]	99 (87–113)	21 (18–25)	65 (53–79)	1.9 (1.7–2.1)
GlaxoSmithKline	Infanrix	54 (46–64)	103 (88–120)	185 (148–231)	1.9 (1.7–2.2)
Massachusetts Public Health Biologic Labs	SSVI-1	99 (87–111)	1.2 (1.1–1.3)	3.4 (3.1–3.6)	2.1 (1.8–2.4)
Michigan Department of Public Health	Mich-2	66 (59–75)	237 (213–265)	3.2 (3.0–3.4)	2.0 (1.8–2.3)
SmithKline Beecham Biologicals	SKB-2	104 (94–116)	110 (99–122)	3.3 (3.1–3.5)	1.9 (1.7–2.1)
Speywood (Porton) Pharmaceuticals	Por-3 F_2	29 (25–33)	20 (17–23)	3.0 (3.0–3.1)	361 (303–430)
Wyeth Lederle Vaccines and Pediatrics	LPB-3P	39 (32–48)	144 (127–163)	128 (109–150)	19 (13–27)
Wyeth Pharmaceuticals	Acel-Imune	14 (12–17)	49 (45–54)	54 (47–62)	51 (41–63)
Wyeth Lederle Vaccines and Pediatrics	Whole-cell	67 (54–83)	3.0 (2.7–3.4)	63 (54–74)	191 (161–227)

[a]Results for Certiva are from a separate study conducted at an MAPT study center after completion of the MAPT, using the MAPT protocol, procedures, and data forms; sera were assayed at one of the MAPT reference laboratories.
[b]For those vaccines without known trade names, designation reflects source and composition of vaccine.[403] For branded products, note that the licensed vaccine's formulation may differ from that of the vaccine evaluated in MAPT.
[c]Contains genetically inactivated PT component.
CI, confidence interval; FHA, filamentous hemagglutinin; FIM, fimbrial proteins; MAPT, Multicenter Acellular Pertussis Trial; N/A, not available; PRN, pertactin; PT, pertussis toxin.
Modified from Edwards KM, Meade BD, Decker MD, et al. Comparison of 13 acellular pertussis vaccines: overview and serologic response. Pediatrics. *1995;96:548–557.*

No acellular vaccine was most or least immunogenic with respect to all included antigens.

The Connaught wP vaccine that was subsequently used in the European vaccine efficacy trials was not included in the MAPT studies, but was subsequently shown to have lower efficacy than the acellular vaccines and to be poorly immunogenic.[509]

Cell-Mediated Immunity

T-cell immune responses to aP vaccine antigens were assessed during the trials of aP vaccines conducted in Italy and Sweden in the 1990s.[324,325] These investigations documented that robust T-cell immunity was stimulated in infants after aP, that the T-cell immunity persisted longer than the serum antibody, and that T-cell responses were boosted by natural infection.[510–514] These human T-cell studies confirmed similar results obtained in mouse models.[89] Immune responses to wP vaccine more closely mirrored those seen after natural pertussis infection, with both natural infection and wP vaccination inducing Th1-polarized responses. Responses after aP vaccines were predominantly Th2.[511,513,515–518] A recent study boosted individuals immunized with either DTwP or DTaP with Tdap and demonstrated that the Th1 or Th2 preference shown after the primary series was not changed with Tdap boosting.[518a]

A small cohort of children had humoral and cell-mediated immunity (CMI) responses evaluated on multiple occasions during the Italian aP vaccine efficacy trial. A marked decline in anti-PT IgG was noted over time, whereas substantial increases were noted in the CMI responses to the same antigen more than 42 months after vaccination.[514,519] Tran Minh and Edelman in Finland evaluated pertussis-specific T-cell responses in adolescents at both 1 month and 3 years after an aP booster dose and found persistently elevated CMI responses 5 years after the boost.[520,521]

Sharma and Pichichero reported that DTaP-induced $CD4^+$ T cells in infants were functionally and phenotypically different from those of adults.[522] Smits and colleagues also demonstrated that T-cell proliferation in response to pertussis antigen stimulation was comparable for children primed with wP or aP, although the time since the last booster vaccine was significantly longer for the wP-primed children, and more children had detectable cytokine responses after wP priming than aP priming.[523] This work suggested that priming with wP induced longer-lasting immunity than priming with aP.

Recently, a more complicated picture has emerged, as summarized in an excellent review article by Fedele and colleagues, who demonstrated a robust T-cell response, with both proliferation and IFN-γ–positive $CD4^+$ T cells, in nearly one-third of vaccinees 5 years after primary vaccination with two aP vaccines.[524] Schure and colleagues showed that there is an increase in cytokine production after booster vaccination of children primed with wP vaccine but not in children primed with aP vaccine.[525] These investigators also reported that in 9-year-old children given a second aP booster 5 years after a preschool booster, T-cell responses did not increase.[526] Schure and colleagues suggested that the enhancement of T-cell immunity during the 5 years following the booster at 4 years of age was probably stimulated by natural boosting in the community by circulation of *B. pertussis*, which could explain

the persistence of protection in aP vaccine recipients despite a substantial waning of both antibody and T-cell responses induced by the primary immunization.[524] Fedele and colleagues speculated that the duration of immunity to aP vaccines introduced in Europe in the mid-1990s was overestimated initially because asymptomatic natural boosting occurred because of high rates of natural *B. pertussis* circulation. Now, with reduced circulation of *B. pertussis* in high-income countries with comprehensive vaccine coverage, T-cell responses are not boosted and pertussis has resurged.

Over the past several years, the understanding of T-cell immune responses to *B. pertussis* has expanded broadly, stimulated by observations made in animal models. In particular, murine models of *B. pertussis* infections have clearly demonstrated the cooperative role played by Th1 and Th17 T-helper cells following natural infection or wP vaccination in protection against pertussis.[161] In contrast, aP vaccines induce a mixed Th2 and Th17 response, with the Th2 component not appearing to materially improve protection.[527] Recent evidence from the baboon model of pertussis has uncovered a major deficit in protective immunity induced by aP vaccination. In this model, immune responses induced by immunization with wP vaccines prevent disease and enhance clearance after infection, although not as effectively as a previous natural pertussis infection.[173] In contrast, immune responses after aP vaccines prevent disease, but do not prevent infection or transmission of *B. pertussis* to naïve baboons. It has not yet been conclusively established in either the baboon or human that the failure of aP vaccines to prevent subsequent infection reflects a failure to induce appropriate cellular immune responses, but the mouse data suggest that this will be the case.

Because the immunological data in humans are limited, additional efforts should be made to compare the immune responses in DTwP- and DTaP-vaccinated children using current technologies, including systems biology approaches, to build models of the interactions that lead to protection, and to use next-generation RNA sequencing to highlight differences in short-term transcriptional responses to either wP or aP vaccination that could contribute to the observed differences in long-term protection.[528]

Immune Correlates of Protection

Regulatory authorities rely on end point clinical trials to establish the effectiveness of a vaccine, unless a correlate of protection (e.g., an immunological measure) has been established that reliably predicts the effectiveness of subsequently produced vaccine. The establishment of an immune correlate of protection is important for all vaccines, but it is particularly needed for aP vaccines because the old gold standard to establish the potency of vaccines, the intracerebral mouse protection test, does not appear to predict aP vaccine potency adequately. Immune correlates of protection typically are established in randomized, placebo-controlled efficacy trials in which the attainment of a particular postvaccination level of immunity is correlated with prevention of disease. Early studies from the United Kingdom had suggested that measurable agglutinin titers in serum after whole-cell vaccination correlated with protection against pertussis disease.[393,529] One of the stated goals of the 1986–87 Swedish efficacy trial evaluating the one- or two-component Japanese-made aP vaccines was to establish serologic correlates of protection. However, much to everyone's dismay, postvaccination antibody levels of PT and FHA did not correlate with clinical protection. A number of possible explanations were proposed for this disappointing result.[530] Postimmunization antibody levels may have poorly predicted antibody levels at the time of exposure, reflecting the lack of data on the kinetics of antibody decline after immunization; antibody assays may have been conducted using the wrong epitopes; or perhaps protection was conferred not by humoral antibody but by CMI or mucosal immunity.

In several of the aP vaccine efficacy trials conducted in Europe in the mid-1990s, efforts were made to determine immune correlates of protection. A nested household study was conducted as part of the 1992 Stockholm efficacy trial, which included a DT (placebo) arm, a U.S. DTwP vaccine, a two-component acellular vaccine, and a five-component acellular vaccine. Serum samples obtained at 1 and 2.5 years of age and within 4 months before exposure, or acute serum samples obtained at least 6 months after the third dose, were used as preexposure serum samples for 209 household-exposed children.[105] The results of this study indicated that vaccine efficacy against typical pertussis after household exposure to *B. pertussis* was 75% for the five-component acellular vaccine, 42% for the two-component vaccine, and 29% for the licensed U.S. whole-cell vaccine when compared with the DT placebo (it is expected that efficacy estimates from a household contact study will differ from those obtained in a prospective randomized trial, given the highly variable nature and intensity of household exposure). Logistic regression analyses of serologic data demonstrated statistically significant correlations between clinical protection and the presence in preexposure sera of IgG antibodies against PRN, FIM-2/3, and PT. Subjects with PT antibody levels exceeding the authors' analytic threshold of 5 units/mL, but with FIM and PRN antibodies below that threshold, had a computed vaccine efficacy of 46%; those with PT and FIM levels above the threshold had an efficacy of 72%; those with PT and PRN levels above the threshold, 75%; and those with all three antibody levels above the threshold, 85%. The authors concluded that multicomponent pertussis vaccines of proven high efficacy used in the Swedish efficacy trials induced higher antibody levels against PRN and FIM-2/3 than did the less efficacious vaccines evaluated in the same trials, and that anti-PRN, anti-FIM-2/3, and anti-PT may be used as surrogate markers of protection against pertussis for multicomponent acellular and whole-cell vaccines.

A different U.S.-licensed DTwP vaccine, a four-component acellular vaccine, and an open-label control group were compared in a randomized, controlled trial conducted in Erlangen, Germany.[323,531] Sera were collected from vaccinees after the third and fourth doses of vaccine and at comparable time periods in DT vaccine recipients. In addition, sera were collected from a random sample of subjects in each vaccine group at approximately 3-month intervals to construct antibody kinetics curves. This allowed estimation of the specific levels of antibody to PT, FHA, PRN, and FIM-2 at the time of exposure in the household setting. The imputed geometric mean antibody titers to PT, PRN, and FIM-2 at the time of household exposure to pertussis infection were higher ($P < .07$ or lower) in noncases compared with cases. A multivariate classification tree analysis of serologic data found that only PRN and PT were significantly associated with protection. Subjects with an imputed PRN value of 7 enzyme-linked immunosorbent assay unit (EU)/mL or less had a 67% chance of infection, regardless of the PT value. No subjects with a PRN value of 7 EU/mL or greater and a PT value of 66 EU/mL or greater were infected, but, if the PRN value was 7 EU/mL or greater and the PT value was 66 EU/mL or less, the predicted probability of being infected was 31%. Logistic regression analysis also found that high versus low PRN values were associated with prevention of illness after household exposure. In the presence of antibody to PRN, PT, and FIM-2, the additional presence of antibody to FHA did not contribute to protection.

The similarities in the findings of these two studies are noteworthy, but it must be remembered that no efficacious one- or two-component aP vaccines were evaluated in these studies; the results may inform us well as to the correlates of protection for the specific vaccines evaluated but fail to elucidate correlates that are generalizable to all aP vaccines. As Hewlett and Halperin[532] stated in their editorial accompanying the publication of these data, "Overinterpretation of these data is a significant risk and must be assiduously avoided." They cautioned that a vaccine containing PT alone was shown to be efficacious in the Göteborg trial and that the presence of PRN and fimbrial proteins in that trial were not essential to protection.[532]

Since the efficacy studies of the pertussis vaccines in Europe in the 1990s, there has been considerable progress in our understanding of the mechanisms of immune responses to *B. pertussis*, but no unequivocal immunological correlates of protection against pertussis have been identified. Serum antibody titers are important but not sufficient to represent a correlate of protection,[105,531] and the fact that maternal vaccination in pregnancy has been shown to protect the newborn is convincing evidence that IgG antibody alone can provide protection. It has also been shown that mucosal IgA antibodies can play a role in pertussis immunity.[533] Moreover, serum antibodies wane more rapidly after infection or vaccination with wP or aP vaccines than does protection,[514,517,534] and the nature of the cellular immune response appears to be important in determining protection. All these findings suggest that T-cell responses play a role in protection and should be further investigated as correlates of protection.[524]

Controlled Clinical Trials of Acellular Vaccines

Results are available from nine large efficacy trials of aP vaccines that were initiated between 1985 and 1993 in Europe or Africa. These trials differed in many ways: type of study, study population, prevalence of pertussis and other diseases in the community, number of immunizations, timing of immunizations, choice of comparison or control vaccines, methods of surveillance, case definitions, and details of the laboratory support used to evaluate fulfillment of the case definition. These differences confound interpretation of the trials and prevent a simple, direct comparison of their primary efficacy results.

Although these studies were performed several decades ago, they would be difficult or impossible to replicate today and thus continue to provide the best available information regarding the absolute and relative performance of aP and wP vaccines in the context of populations primed solely by wP or natural infection. In evaluating these efficacy trials, there are several independent goals: to position correctly the evaluated vaccines in the spectrum of available vaccines, to draw inferences concerning the influence on efficacy of various vaccine characteristics, and to draw inferences concerning the effect of various study design characteristics on vaccine evaluation. A study that poses difficulties in accomplishing one goal may nonetheless be useful for accomplishing other goals. The objectives of the pertussis vaccination program also play a role in evaluating measurements of efficacy. If the societal goal is focused on the prevention of severe disease, then a study's inability to detect mild illness (or to determine efficacy against that end point) may not be important. However, if the goal is to prevent pertussis infection, then mild illnesses must be detected, even though such illnesses may be more difficult to ascertain than classical whooping cough. To the extent that vaccines modify but do not prevent illness, the case definition chosen could have a major impact on interpretation of the efficacy, and hence the benefits, of the vaccine.

1986 Swedish Efficacy Trial

The first large-scale efficacy trial of acellular vaccine was conducted in 1986 in Stockholm, Sweden, where routine pertussis immunization had been discontinued in 1979 and pertussis had become endemic.[432] The randomized, double-blind, placebo-controlled trial (Table 44.9) evaluated a Biken vaccine containing 23.4 µg each of PT and FHA (denoted JNIH-6; see Tables 44.3 and 44.4),[433,538] a monocomponent Japanese National Institutes of Health vaccine prepared for the purposes of this study and containing 37.7 µg of PT (denoted JNIH-7),[433,538] and vaccine diluent given as a placebo.[189] There was no whole-cell control group. A two-dose schedule was used; infants were enrolled and immunized at 5 to 11 months of age, with the second dose given 8 to 12 weeks later.

Antibody responses to PT were dose dependent, being higher in the children who received the monovalent vaccine; FHA antibody rose only in the bivalent vaccine group. Efficacy for both vaccines was less than anticipated (Tables 44.10 and 44.11); for culture-confirmed cough of any duration, efficacy was 69% for the two-component vaccine and 54% for the monocomponent vaccine.[189]

During the surveillance period, four vaccinees (one JNIH-7 recipient and three JNIH-6 recipients) died of bacterial infections.[538] Review of hospitalizations for infection found no differences among the study groups. Analyses of immunoglobulins from prevaccination and postvaccination sera showed no abnormalities, nor did leukocyte counts obtained in a subsample of subjects 2 to 4 months after the second dose.

In January 1989, the Swedish National Bacteriology Laboratory withdrew the acellular vaccine licensure application, citing both the impression that efficacy was lower than that of whole-cell vaccines and the possible association with deaths resulting from serious bacterial infections. The laboratory called for studies that would directly compare acellular and whole-cell vaccines.[545]

In light of these safety concerns and of animal data suggesting that PT might enhance the susceptibility of animals to bacterial infection,[546] several studies were conducted that demonstrated, if anything, a decreased risk of severe invasive bacterial disease after pertussis vaccine[547–549] and no increased risk of minor infections.[548] Data from Japan indicated that there was no enhanced risk from the acellular vaccines.[550] Thus it appeared that the four deaths were chance events, with no causal relationship to vaccination.

Subsequent Follow-up and Analyses. Later evaluations of various case definitions and confirmatory criteria showed that the two-component and monocomponent vaccines were 81% and 75% effective, respectively, in preventing culture-confirmed disease with at least 21 days of coughing spasms.[539] Estimates of efficacy were profoundly influenced by case definition, ranging (using the monocomponent vaccine as an example) from 5% efficacy in preventing spasmodic cough lasting 1 day or longer to 100% efficacy in preventing culture-confirmed pertussis with spasmodic cough lasting 28 days or longer, with whoops on at least 1 day.[539]

It was also found that prior receipt of pertussis vaccine reduced the likelihood of positive pertussis cultures or significant antibody rises in patients with cough. Thus, pertussis infection could be confirmed more readily in placebo recipients than in vaccinees, which tended to bias efficacy estimates upward.

Although the initial results from the 1986 Swedish trial suggested that the efficacies of the monocomponent and two-component vaccines did not differ substantially, long-term follow-up showed the two-component vaccine to be

TABLE 44.9 Overview of Nine Acellular Pertussis Vaccine Efficacy Trials

Location, Year Begun, Reference	Study Groups and Vaccines Evaluated			Ages at Vaccination	Surveillance			Comments on Study Design
	Acellular[a]	Whole-Cell	Placebo		Duration[b]	Active	Passive	
RANDOMIZED, FULLY BLINDED, CONTROLLED COMPARATIVE STUDIES								
Stockholm, 1986[535]	JNIH-6, JNIH-7	None	Diluent	5–11 mo, then 8–12 wk later	15 mo[c]	Telephone every month	Parents instructed to report	Lack of whole-cell control hampered interpretation. Schedule (2 doses, relatively late in infancy) makes comparisons to other trials difficult. Later analyses revealed differential sensitivity of culture and of serology in vaccine vs placebo groups.
Stockholm, 1992[324]	SKB-2 Tripacel[e]	Connaught[d]	DT	2, 4, 6 mo	23.3 mo 23.8 mo	Telephone every 6–8 wk	Parents instructed to report	Stockholm 1992 and Italy are the benchmark studies: prospective, fully randomized and blinded, with both whole-cell and placebo (DT) control groups. Each study evaluated 2 candidate acellular vaccines head-to-head, using the same immunization schedules and nearly identical case definitions and diagnostic procedures.
Italy, 1992[325]	Infanrix, Acelluvax	Connaught[d]	DT	2, 4, 6 mo	17 mo	Telephone every month	Parents instructed to report	
Stockholm, 1993[536]	SKB-2 Acelluvax HCPDT[e]	Wellcome	None	88% at 3, 5, 12 mo; 12% at 2, 4, 6 mo	7.2 mo 21.5 mo 21.5 mo	Clinic visits at 5, 12, 18 mo	Daily check of culture reports	No placebo control group, thus no absolute efficacy estimates, hampering comparisons to other studies. Formulation of Connaught Canada 5-component vaccine changed since Stockholm 1992; however, Acelluvax arm presumably comparable to Italy.
RANDOMIZED, FULLY BLINDED, CONTROLLED STUDIES								
Göteborg (Sweden), 1991[246]	Certiva	None	DT	3, 5, 12 mo	17.5 mo	Telephone every month	Parents instructed to report	Vaccination schedule and lack of whole-cell control group hamper comparisons to other studies.
OTHER STUDIES								
Senegal, 1990[423]	Triavax	Pasteur Merieux	DT	2, 4, 6 mo	21 mo	Field worker visit every week	None	Prospective, double-blind, randomized for relative risk of DTaP vs DTP. Absolute efficacy based on case-contact study using nonrandomized DT/no vaccine group that was unblinded to parents and, probably, to field workers performing initial case detection. Investigating physicians said to be blinded.
Erlangen (Germany), 1991[323]	Acel-Imune	Lederle[d]	DT	3, 5, 7, 17 mo	25.6 mo	Telephone every 2 weeks	Parents instructed to report to PMD	Prospective, double-blind, randomized for relative risk of DTaP vs DTP. Absolute efficacy based on comparison to a nonrandomized DT group that was unblinded to parents and, occasionally, to investigators
Mainz (Germany), 1992[420]	Infanrix	Behringwerke or SmithKline Beecham	DT	3, 4, 5 mo	23 mo	None	Physician report of contact by parent	Household contact study. Vaccine assignment not randomized; parents and physicians responsible for initial case detection not blinded. Central case investigators were said to be blinded
Munich, 1993[421,537]	Tripedia	Behringwerke	DT	3, 5, 7 mo	N/A	None	Physician report of contact by parent	Nonrandomized (vaccine chosen by parents), unblinded (parents and investigators knew vaccine status) case-control study

[a]Descriptive name given for those without trade name. See Tables 44.3 and 44.4 for further details.
[b]Mean duration of surveillance. Subjects were eligible for inclusion as cases from time of last dose (Stockholm 1992, Stockholm 1993), from 28 to 30 days after last dose (Stockholm 1986, Italy, Göteborg, Senegal, Mainz), or from 14 days after last dose (Erlangen) of DTaP.
[c]Passive unblinded surveillance, augmented with inquiries mailed to parents every 6 months and follow-up of positive cultures reported to the National Bacteriology Laboratory, continued for an additional 3 years.
[d]U.S.-licensed whole-cell vaccine.
[e]Tripacel and HCPDT are similar, except that Tripacel contains 10 μg PT and 5 μg FHA, as compared to 20 μg of each in HCPDT.
DT, diphtheria and tetanus toxoids vaccine; DTaP, DT plus acellular pertussis vaccine; DTP, DT plus whole-cell pertussis vaccine; HCPDT, hybrid component pertussis-diphtheria-tetanus vaccine; N/A, not available or not applicable; PMD, private physician.

TABLE 44.10 Results of Nine Acellular Pertussis Vaccine Efficacy Trials, Based on Case Definition Most Similar to That of the WHO[a]

Study	Case Definition[b]	Vaccine[c] (No. of Components)	Efficacy (95% CI)			Comment
			Cases	Absolute %	Relative Risk	
RANDOMIZED, FULLY BLINDED, CONTROLLED, COMPARATIVE STUDIES						
Stockholm 1986[535]	≥21 days cough + ≥9 coughing spasms on at least 1 day + positive culture	JNIH-6 (2) JNIH-7 (1)	10 12	81 (61–90) 75 (53–87)	— —	Differential sensitivity of culture, lack of serologic or epidemiologic link criteria may bias estimates upward
Stockholm 1992[324]	≥21 days paroxysmal cough, plus either: positive culture, confirmed by SA or PCR; 2-fold PT or FHA IgG rise; or epidemiologic link to culture-positive case	SKB-2 (2) Tripacel[d] (4) Connaught DTP	159 59 148	59 (51–66) 85 (81–89) 48 (37–58)	0.83 (0.66–1.1) vs DTP 0.29 (0.21–0.40) vs DTP —	Because of unusually low DTP efficacy, relative risks difficult to compare to other studies
Italy 1992[325]	≥21 days paroxysmal cough, plus either: positive culture, confirmed by SA or PCR; 4-fold CHO or 2-fold PT or FHA IgG or IgA rise; no epidemiologic link criterion	Infanrix (3) Acelluvax (3) Connaught DTP	37 36 141	84 (76–89) 84 (76–90) 36 (14–52)	0.25 (0.17–0.36) vs DTP 0.25 (0.17–0.36) vs DTP —	Because of unusually low DTP efficacy, relative risks difficult to compare to other studies
Stockholm 1993[536]	≥21 days paroxysmal cough plus positive culture (no information on confirmation); no serologic or epidemiologic link criteria	HCPDT[d] (4) Acelluvax (3) SKB-2 (2) HCPDT[d] (4) SKB-2 (2)	13 21 99 38 99	— — — — —	0.85 (0.41–1.79) vs DTP 1.38 (0.71–2.69) vs DTP[e] 2.3 (1.5–3.5) vs DTP 0.62 (0.31–1.2) vs Acelluvax 2.0 (1.4–2.8) vs Acelluvax[e]	Comparison is to the Wellcome DTP used in this study (see Table 44.10). Acelluvax, unchanged from Italy, provides a link to the Italy study (and thus to Stockholm 1992)
RANDOMIZED, FULLY BLINDED, CONTROLLED STUDIES						
Göteborg 1991[246]	≥21 days paroxysmal cough plus either: positive culture confirmed by SA, PCR; 3-fold PT or FHA IgG rise; epidemiologic link	Certiva (1)	72	71 (63–78)	—	Lack of FHA in vaccine enhanced sensitivity of serologic criteria, improving accuracy of estimate
OTHER STUDIES						
Senegal 1990[423]	≥21 days paroxysmal cough plus either: positive culture confirmed by DIF; 2-fold PT or FHA IgG rise; epidemiologic link	Triavax (2) PMC-Fr DTP	24 7	74 (51–86) 92 (81–97)	2.42 (1.4–4.3) vs DTP —	Few cases, thus broad CIs. Open DT group may bias absolute efficacy estimates upward
Erlangen 1991[323]	≥21 days cough with paroxysms, whoop, or vomiting, plus confirmation[f]	Acel-Imune (4) Lederle DTP	≤45 ≤18	78 (60–88) 93 (83–97)	1.5 (0.7–3.4) vs DTP[258] —	Open DT group, incomplete case ascertainment may bias absolute efficacy estimates upward
Mainz 1992[420]	21 days paroxysmal cough plus either: positive culture confirmed by DIF or SA; 2-fold PT or FHA IgG or IgA rise	Infanrix (3)	7	89 (77–95)	4.7 (0.6–37.3) vs DTP	Few cases, thus broad CIs. Note how misleading relative risk is if DTP result is close to 100%
	Behring, SKB DTP	1	98 (83–100)	—	—	

Continued on following page

TABLE 44.10 Results of Nine Acellular Pertussis Vaccine Efficacy Trials, Based on Case Definition Most Similar to That of the WHO[a] *(Continued)*

Study	Case Definition[b]	Vaccine[c] (No. of Components)	Efficacy (95% CI)			Comment
			Cases	Absolute %	Relative Risk	
Munich 1993[421,537]	21 days paroxysmal cough plus either: positive culture confirmed by SA; epidemiologic link; no serologic criteria	Tripedia (2)	4	93 (63–99)[g]	2.0 (N/A) vs DTP	Few cases, thus broad CIs. Lack of serologic criteria, blinding, or randomization may bias efficacy estimates upward
	Behring DTP	1	96 (71–100)	—	—	—

[a]Results shown are for complete primary infant immunization series (3 doses, except Stockholm 1986, 2 doses); effects of any booster dose are not included. Some results obtained by recalculation from data provided in the referenced source; such results may repr esent crude, rather than adjusted, efficacies. Blank cells: not applicable or no data. WHO case definition: ≥21 days paroxysmal cough, plus bacteriologic, serologic, or epidemiologic confirmation that cough is caused by *Bordetella pertussis*.

[b]Unless otherwise noted: All cultures were nasopharyngeal; FHA rises were considered diagnostic only if culture (and PCR, if done) were negative for *Bordetella parapertussis*; EPI link means documented contact with a culture-confirmed (PCR-confirmed, for Senegal) case within 28 days before or after illness onset in the subject (for Erlangen or Munich, no time limit was specified). Criteria shown are for the case definition most similar to that of the WHO; different criteria may have been used for alternative case definitions.

[c]Descriptive name given for those without trade name. Letters before hyphen indicate source; characters after hyphen indicate number and type of components. All one-component vaccines contain PT; all two-component vaccines contain PT and FHA; three-component vaccines contain PT, FHA, plus either PRN or FIM (as indicated by the letter P or F, respectively); four-component vaccines contain PT, FHA, PRN, and FIM. "F2" signifies that two fimbrial proteins are included. See Tables 44.3 and 44.4 for further details.

[d]*Tripacel* and HCPDT are similar, except that *Tripacel* contains 10 μg PT and 5 μg FHA, as compared to 20 μg of each in HCPDT.

[e]For time from first dose to soon after the third dose, when SKB-2 recipients were unblinded and boosted with another vaccine.

[f]Confirmation criteria: Positive culture (confirmed by PCR in last year of study); "significant" PT IgG or IgA result; or household contact to a culture-confirmed case. "Significant" serologic result defined as a ratio of convalescent to acute antibody level that exceeded the 95th, 99th, or 99.9th percentile of a distribution of similar ratios determined among randomly selected subjects at roughly the same time postimmunization. Selection of the percentile limit was determined by how many, and which, antibody ratios were elevated. Efficacy rates reflect adjustment for single adult households and households in which all siblings were unimmunized. Other published efficacy estimates[323] may have used FHA, PRN, or FIM IgG or IgA values or fourfold agglutinin rises as well, and may not have reflected such adjustment.

[g]Differs from efficacy reported in FDA-approved patient package insert, which is based on the primary case definition (≥21 days of any cough) rather than the WHO definition.

CHO, Chinese hamster ovary cell assay; CI, confidence interval; DIF, direct immunofluorescence; DTP, diphtheria and tetanus toxoids and whole-cell pertussis vaccine; epi link, epidemiological link; FHA, filamentous hemagglutinin; FIM, fimbrial proteins; HCPDT, hybrid component pertussis-diphtheria-tetanus vaccine; Ig, immunoglobulin; N/A, not available or not applicable; PCR, polymerase chain reaction; PMC-Fr, Pasteur Merieux Connaught-France; PRN, pertactin; PT, pertussis toxin; SA, slide agglutination; SKB, SmithKline Beecham; WHO, World Health Organization.

significantly more efficacious (Fig. 44.6).[433] The study was unblinded after 15 months of active observation, and then passive surveillance continued for an additional 3 years, during which time vaccine efficacy ranged from 77% to 92% for the bivalent vaccine and from 65% to 82% for the monovalent vaccine, depending on the case definition (see Table 44.11).[433] Clinical protection after aP vaccine was maintained for at least 4 years.[433]

The monocomponent vaccine, with 50% more PT, appeared to be more effective than the two-component vaccine in preventing the most severe manifestations of disease. Its efficacy exceeded that of the two-component vaccine for every case definition involving whoops (see Table 44.11), a difference that was statistically significant for 28 or more days of cough with whoops.[539] On the other hand, the two-component vaccine appeared to be more effective in preventing mild or moderate disease (e.g., shorter durations of cough or cough without whoops).[433,521,535,551] Thus, efficacy was influenced by both the choice of antigens and the quantity of antigen included.

The initial efficacy results[189] gave the impression that the acellular vaccines were substantially less efficacious than whole-cell vaccine.[189,433,535] As a result, Japan remained the only country in which they were licensed. However, results of later studies suggested that efficacy would have been higher had a standard three-dose schedule been used. Importantly, inclusion of a whole-cell control group might have led to

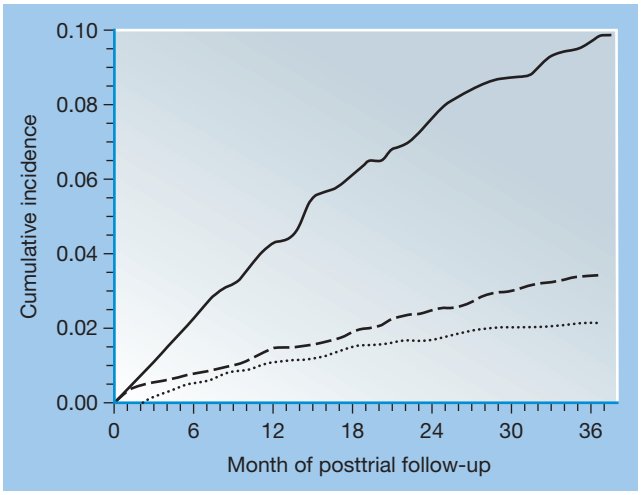


Figure 44.6. Cumulative incidence curves for culture-confirmed pertussis during unblinded posttrial follow-up, in the placebo group *(solid line)*, in the monocomponent (JNIH-7) vaccine group *(dashed line)*, and in the two-component (JNIH-6) vaccine group *(dotted line)*. Unblinded follow-up started August 27, 1987, and ended September 9, 1990. *(From Storsaeter J, Olin P. Relative efficacy of two acellular pertussis vaccines during three years of passive surveillance. Vaccine. 1992; 10:142–144.)*

TABLE 44.11 Results of Nine Acellular Pertussis Vaccine Efficacy Trials for Various Case Definitions or Surveillance Periods[a]

Study	Vaccine(s)[b]	Case Definition[c]	Efficacies (95% CI)	
			Absolute (%)	Relative Risk
RANDOMIZED, FULLY BLINDED, CONTROLLED, COMPARATIVE STUDIES				
Stockholm (1986)	JNIH-6, JNIH-7	≥1 day of any cough + positive culture[189]	69 (47–82), 54 (26–72)	—
		Same; but including cases from date of first dose[189]	65 (44–78), 53 (28–69)	—
		Same; but for first 60 days after first dose only[535]	41 (0–79), 42 (0–79)	—
		≥1 day cough with spasms (defined in Table 44.10)[539]	16 (3–27), 5 (–10, 17)	—
		≥1 day cough with spasms + ≥1 day whoops[539]	39 (16–56), 51 (30–65)	—
		≥1 day cough with spasms + culture[539]	75 (54–86), 60 (33–76)	—
		≥1 day cough with spasms + whoops + culture[539]	85 (67–94), 89 (72–96)	—
		≥21 days cough with spasms[539]	41 (23–55), 27 (6–43)	—
		≥21 days cough with spasms + whoops[539]	60 (37–75), 62 (39–76)	—
		≥21 days cough with spasms + culture[539]	81 (61–90), 75 (53–87)	—
		≥21 days cough with spasms + whoops + culture[539]	84 (63–93), 90 (73–97)	—
		≥28 days cough with spasms + whoops + culture[539]	82 (57–92), 100 ("…")	—
		3 additional years of passive surveillance: positive culture[433]	77 (65–85), 65 (50–75)	—
		3 additional years: ≥30 days cough + positive culture[433]	92 (84–96), 79 (67–87)	—
		3 additional years: ≥9 cough spasms/day + positive culture[433]	89 (76–97), 82 (67–90)	—
Stockholm (1992)	SKB-2, Tripacel	**≥21 days paroxysmal cough, confirmed as in Table 44.10**[324]	59 (51–66), 85 (81–89)	0.83 (0.66–1.1), 0.29 (0.21–0.40) vs DTP
		Same; but including cases from date of first dose[324]	59 (51–66), 84 (80–88)	0.83 (0.66–1.1), 0.30 (0.22–0.42) vs DTP
		≥1 day any cough, confirmed as in Table 44.10[324]	42 (33–51), 78 (73–82)	—
		≥21 days any cough, confirmed as in Table 44.10[540]	54 (46–62), 81 (76–85)	—
Italy	Infanrix, Acelluvax	≥21 days paroxysmal cough + positive culture[325]	85 (N/A), 87 (N/A)	—
		≥21 days paroxysmal cough, confirmed as in Table 44.10[325]	84 (76–89), 84 (76–90)	0.25 (0.17–0.36), 0.25 (0.17–0.36) vs DTP
		Same; but including cases from date of first dose[325]	82 (73–87), 84 (76–89)	0.28 (0.20–0.39), 0.25 (0.17–0.36) vs DTP
		Same; but from 30 days after first dose to 29 days after third dose[325]	19 (0–84), 83 (0–98)	0.49 (0.17–1.44), 0.10 (0.01–0.79) vs DTP
		≥7 days any cough, confirmation as above[325]	71 (60–78), 71 (61–79)	0.38 (0.30–0.49), 0.38 (0.29–0.48) vs DTP
		≥21 days any cough, confirmation as above[325]	79 (70–85), 77 (68–84)	0.29 (0.21–0.39), 0.31 (0.24–0.42) vs DTP
Stockholm (1993)	HCPTD, Acelluvax	Positive culture, with or without (±) cough[536]	—	1.40 (0.78–2.52), 2.55 (1.50–4.33) vs DTP
		≥21 days paroxysmal cough + positive culture[536]	—	0.85 (0.41–1.79), 1.38 (0.71–2.69) vs DTP
		Positive culture, ± cough; from date of first dose[536]	—	1.25 (0.90–1.75), 1.84 (1.36–2.51) vs DTP
		≥21 days paroxysmal cough + positive culture; from first dose[536]	—	1.25 (0.82–1.89), 1.65 (1.12–2.45) vs DTP
		Same; but from date of first dose to date of second dose[541]	—	1.49 (0.80–2.77), 1.42 (0.76–2.65) vs DTP
		Same; but from date of second dose to date of third dose[536]	—	1.42 (0.54–3.74), 3.14 (1.34–7.34) vs DTP
RANDOMIZED, FULLY BLINDED, CONTROLLED STUDIES				
Göteborg	Certiva	≥7 days any cough, confirmed as in Table 44.10[246]	54 (43–63)	—
		≥7 days any cough, confirmed by Göteborg criteria[246]	62 (51–70)	—
		≥21 days any cough, confirmed as in Table 44.10[246]	63 (52–71)	—
		≥21 days any cough, confirmed by Göteborg criteria[246]	69 (60–77)	—
		≥21 days paroxysmal cough, confirmed by Göteborg criteria[246]	77 (69–83)	—
		Same; from 30 days after second dose to 29 days after third dose[542]	39 (0–66)	—
		≥21 days paroxysmal cough, confirmed as in Table 44.10[246]	71 (63–78)	—
		Same; from first dose to 29 days after second dose[246]	≤16 (0-≤64)	—
		Same; from 30 days after second dose to 29 days after third dose[246]	55 (12–78)	—
		Same; from 18.5 to 24.5 months after third dose[543]	77 (65–85)	—

Continued on following page

TABLE 44.11 Results of Nine Acellular Pertussis Vaccine Efficacy Trials for Various Case Definitions or Surveillance Periods[a] *(Continued)*

Study	Vaccine(s)[b]	Case Definition[c]	Efficacies (95% CI)	
			Absolute (%)	Relative Risk
OTHER STUDIES				
Senegal	Triavax	≥21 days any cough, confirmed as in Table 44.10[423]	31 (7–49)	1.54 (1.23–1.94) vs DTP
		As above, plus epi link cases confirmed by PCR[423]	53 (23–71)	1.87 (1.38–2.52) vs DTP
		≥8 days paroxysmal cough, confirmed as in Table 44.10[544]	—	3.26 (2.08–5.10) vs DTP
		≥21 days paroxysmal cough, confirmed as in Table 44.10[423]	74 (51–86)	2.42 (1.35–4.34) vs DTP
		As above, plus epi link cases confirmed by PCR[423]	85 (66–93)	2.80 (1.36–5.74) vs DTP
Erlangen	Acel-Imune	≥7 days any cough, confirmed as in Table 44.10[323]	62 (38–77)[d]	3.1 (N/A) vs DTP
		≥21 days paroxysmal cough, confirmed as in Table 44.10[323]	78 (60–88)	1.5 (0.7–3.4) vs DTP
Mainz	Infanrix	≥1 day paroxysmal cough[420]	64 (51–73)	—
		≥7 days paroxysmal cough, confirmed as in Table 44.10[420]	81 (68–89)	—
		≥21 days paroxysmal cough, with or without confirmation[420]	83 (71–90)	—
		≥21 days paroxysmal cough, confirmed as in Table 44.10[420]	89 (77–95)	4.7 (0.6–37.3) vs DTP
Munich	Tripedia	≥21 days any cough, confirmed as in Table 44.10[421]	80 (63–89)[e]	4.0 (N/A) vs DTP
		≥21 days paroxysmal cough, confirmed as in Table 44.10[421]	93 (63–99)	2.0 (N/A) vs DTP

[a]Results shown are for complete primary infant immunization series (3 doses, except Stockholm 1986, 2 doses); effects of any booster dose are not included. Some results obtained by recalculation from data provided in the referenced source; such results may represent crude, rather than adjusted, efficacies. Lower confidence limits less than zero are shown as zero. Blank cells: not applicable or no data. WHO case definition: ≥21 days paroxysmal cough, plus bacteriologic, serologic, or epidemiologic confirmation that cough is due to *Bordetella pertussis*. See Tables 44.9 and 44.10 for additional details, including confirmation methods used by each study.

[b]Descriptive name given for those without trade name. Letters before hyphen indicate source; characters after hyphen indicate number and type of components. All one-component vaccines contain PT; all two-component vaccines contain PT and FHA; three-component vaccines contain PT, FHA, plus either PRN or FIM (as indicated by the letter P or F respectively); four-component vaccines contain PT, FHA, PRN, and FIM. "F2" signifies that two fimbrial proteins are included. See Tables 44.3 and 44.4 for further details.

[c]Definition most similar to that of the WHO (≥21 days paroxysmal cough, confirmed as pertussis by culture, serology, or epidemiologic link to a culture-confirmed case) is shown in boldface; see Table 44.10 for details. Göteborg criteria: Positive culture; or IgG against both PT and FHA ≥6000 in a single convalescent specimen; or two major criteria; or one major and one minor criterion. Major criteria: 3-fold rise in PT or FHA IgG; household contact with confirmed pertussis. Minor criteria: 3-fold change in PT or FHA IgA or IgM; positive PCR. Unless otherwise specified, subjects were eligible for inclusion as cases from time of last dose (Stockholm 1992, Stockholm 1993), from 28 to 30 days after last dose (Stockholm 1986, Italy, Göteborg, Senegal, Mainz), or from 14 days after last dose (Erlangen) of DTaP (see Table 44.9 for vaccination schedules).

[d]Not adjusted for single-adult households and households in which all siblings were unimmunized (see Table 44.10).

[e]This result, which is based on the primary case definition rather than the WHO definition, is the one reported in the FDA-approved patient package insert.

CI, confidence interval; DTP, diphtheria and tetanus toxoids and whole-cell pertussis vaccine; epi link, epidemiological link; FDA, U.S. Food and Drug Administration; FHA, filamentous hemagglutinin; FIM, fimbrial proteins; HCPDT, hybrid component pertussis-diphtheria-tetanus vaccine; Ig, immunoglobulin; N/A, data not available; PCR, polymerase chain reaction; PRN, pertactin; PT, pertussis toxin; WHO, World Health Organization.

markedly different conclusions regarding the relative efficacies of these vaccines.

World Health Organization Case Definition of Pertussis. In response to the demonstration in the 1986 Swedish trial of the strong influence of pertussis case definitions on estimates of vaccine efficacy, and in expectation of the efficacy trials that would be conducted to evaluate the new acellular vaccines being developed, the WHO convened a group of pertussis experts in January 1991 to develop a consensus case definition of pertussis for use in clinical trials. The resulting WHO case definition required the presence of paroxysmal cough for at least 21 days plus confirmation that the cough illness actually was caused by pertussis. This confirmation could be based on laboratory results (e.g., a positive culture for *B. pertussis* or a significant rise in a specific antibody, such as IgG or IgA, against PT, FHA, or FIM-2 or -3) or on an epidemiological link (e.g., a household contact with a laboratory-confirmed case within 28 days before or after the onset of illness in the subject).[552] The consensus statement recognized that future laboratory developments might add to the tools available for bacteriologic or serologic confirmation (e.g., PCR, assay of antibody to PRN).

Although the WHO case definition played an essential role in improving comparability of subsequent field trials, its limitations must be recognized. First, it improved but did not eliminate the problem of differential sensitivity of the case definition with respect to detecting pertussis in the various arms of a comparative trial. The yield of positive pertussis cultures was lower in immunized than in unimmunized pertussis patients and varied inversely with the efficacy of the vaccine.[324,540,543,551,553] Thus, a cough illness that was really pertussis was more likely to be confirmed by culture in the unvaccinated group than in the vaccinated group. This disparity led to a disproportionate discarding of true pertussis cases in the vaccinated group, which resulted in falsely elevated estimates of vaccine efficacy. Similarly, the differences between a less-effective and a more-effective vaccine were likely to be exaggerated because more cases would have been confirmed by culture in the group that received the less-effective vaccine. Those subjects who already had a substantial increase in the level of a pertussis antibody, as a result of the receipt of a highly

immunogenic vaccine, were less likely to show a fourfold (or other diagnostic) rise in antibody titers after infection than those subjects who did not receive a vaccine containing that antigen (or a vaccine substantially less immunogenic with respect to that antigen).[543,551] When comparing two vaccines—one that stimulates the antibody being tested and one that does not—there will be a diagnostic bias favoring the vaccine that stimulates the antibody. If both vaccines contain the antigen in question, the bias will favor the more immunogenic vaccine.

Second, setting the clinical cutoff at "paroxysmal cough of 21 days or more" has been shown to result in the removal of a substantial number of laboratory-confirmed pertussis cases from efficacy calculations.[433,539,554] Because mild cases are more common in vaccinees than in control subjects, it might be argued that the effect is to inflate efficacy estimates, reducing the ability to discriminate among vaccines. However, if the intent is to measure the vaccine's efficacy in preventing classical whooping cough, and mild disease is of lesser interest, then this quality is not a defect.

Third, the case definition permits the use of a variety of serologic assays for confirmation as well as the future use of additional serologic assays or other bacteriologic techniques to detect *B. pertussis* organisms. Although commendable, this flexibility means that studies that differ in the laboratory tools employed for case confirmation, and thus in the sensitivity and specificity of case confirmation, nonetheless may characterize themselves as using the WHO case definition. Thus, as always, the burden is on the reader to consider carefully the methods used in each trial.

Efficacy Trials, 1992 to 1997

After the 1986 Swedish clinical trial, worldwide efforts to develop additional aP vaccines continued, culminating in eight additional efficacy trials initiated between 1990 and 1993 (see Table 44.9). Four of the trials were randomized, prospective, and fully blinded; of these, three incorporated a whole-cell control arm and evaluated several candidate acellular vaccines head to head. The four remaining trials each evaluated only a single vaccine, using a variety of study designs; all made use of placebo (DT or no vaccine) groups that were neither randomized nor fully blinded.

Key characteristics of all nine efficacy studies are summarized in Tables 44.10 and 44.11. For each trial, Table 44.10 presents results for the case definition that we consider most similar to the WHO standard case definition, and Table 44.11 presents selected results reflecting a variety of alternative case definitions or surveillance intervals. For studies not incorporating a placebo control group (e.g., DT), only relative risks (usually calculated relative to a DTwP vaccine used in the same trial) are available. However, relative risks must be interpreted with great caution and can be directly compared across trials only if the reference vaccines (e.g., DTwP) are identical. Unfortunately, no two studies conducted without placebo control groups incorporated a common DTwP vaccine, and thus relative risks can be compared meaningfully only within the studies that evaluated multiple acellular vaccines.

Sweden and Italy, 1992 to 1993. Of the 13 acellular vaccines evaluated in the MAPT, four were selected, on the basis of evaluations of their safety, immunogenicity, and purity, for evaluation in two NIAID-funded efficacy trials conducted in Sweden and Italy.[324,325] These two NIAID-sponsored studies were of rigorous design and served as the benchmarks against which others were compared. The studies were prospective, double-blinded, and randomized, and they incorporated both placebo (DT) and whole-cell control arms. The two studies used closely coordinated protocols, serologic assays, and case definitions (although the Italian case definition did not include epidemiologically linked cases, and preexposure sera were not collected routinely in Italy as they were in Sweden). Infants were immunized at 2, 4, and 6 months of age, and no booster was given. Each study compared two candidate acellular vaccines head to head. The control whole-cell vaccine used in both was produced by Connaught Laboratories and was licensed for use in the United States. Unfortunately, this was not one of the wP vaccines studied in the MAPT trial.

During the main follow-up period of the Swedish study (hereafter referred to as Stockholm 1992), 737 cases of pertussis were diagnosed that met the primary case definition. Efficacy was 59% for the SmithKline Beecham bivalent vaccine (SKB-2), 85% for the Aventis Pasteur five-component vaccine (Tripacel), and 48% for the Connaught whole-cell vaccine (see Table 44.10).[324] The efficacy of the five-component vaccine was sustained during the 2 years of follow-up, whereas the efficacy of the whole-cell vaccine declined substantially.

The performance of the two-component vaccine was much lower than had been anticipated. (JNIH-6, a similar vaccine, was 81% effective in the 1986 trial, using the most similar case definition.) It was noted that the SmithKline Beecham two-component vaccine produced significantly less antibody to PT in Sweden than it had in the MAPT,[555] prompting the investigators[324] and other reviewers[555] to speculate that the vaccine's performance in Stockholm 1992 may, in part, have reflected characteristics unique to the batch of vaccine used in that trial.

The five-component vaccine was 78% effective against laboratory-confirmed pertussis with at least 1 day of cough, suggesting substantial protection against mild or atypical pertussis. In contrast, for this case definition the two-component and Connaught whole-cell vaccines were 42% and 41% effective, respectively.[324]

During the main follow-up period of the Italian trial, 288 cases of pertussis met the primary case definition. The GlaxoSmithKline (Infanrix) and Chiron Vaccines (Tri Acelluvax) three-component acellular vaccines were both 84% effective; efficacy for the Connaught whole-cell vaccine was 36%.[325] After completion of the main follow-up period, unblinded surveillance of the study cohort continued. During the first 9 months of extended follow-up, efficacy of the two vaccines differed significantly: 36 cases were detected among recipients of the GlaxoSmithKline vaccine, as compared with 18 among recipients of the Chiron vaccine (efficacies of 78% and 89%, respectively).[495] However, a more complete analysis that incorporated results through April 1997 found no material differences in long-term efficacy. For the GlaxoSmithKline and Chiron vaccines, respectively, efficacies by year of observation were as follows: for 1994, 82.7% and 82.1%; for 1995, 81.5% and 85.9%; and for 1996, 87.6% and 87.7%.[556] Additional follow-up through 6 years of age demonstrated continued high efficacy of these vaccines.[557] (In retrospect, one wonders about the impact of circulation of wild-type pertussis organisms on persistence of immunity in these vaccinated subjects.)

The similarity in efficacy of the three multicomponent vaccines is noteworthy, particularly given their substantially different compositions (see Tables 44.3 and 44.4). Tri Acelluvax had only one-fifth the PT, one-tenth the FHA, and one-third the PRN content of Infanrix (but the PT was genetically inactivated rather than being a toxoided natural protein and immune responses to this recombinant PT were robust in the MAPT trial). The Canadian five-component vaccine, Tripacel, had far less than half the PT, FHA, and PRN of Infanrix but contained FIM-2 and FIM-3. These facts strongly suggest that the efficacy of an aP vaccine is influenced by both the choice of components and the particular characteristics of those components.

Sweden, 1993 to 1996. After completion of the immunization phase of Stockholm 1992 (but before completion of the surveillance phase), the Swedish investigators launched another NIAID-supported, prospective, randomized, double-blind trial (referred to as Stockholm 1993) that compared four vaccines: (a) the two-component SmithKline Beecham vaccine evaluated in Stockholm 1992; (b) a five-component Sanofi Pasteur Canada vaccine (hybrid component pertussis-diphtheria-tetanus vaccine [HCPDT]) similar to the one evaluated in Stockholm 1992 (Tripacel) but reformulated to contain more PT and FHA; (c) the three-component Chiron acellular vaccine (Tri Acelluvax) evaluated in Italy; and (d) a different whole-cell vaccine, the Medeva Wellcome whole-cell vaccine (now produced by Evans Medical) used in the United Kingdom.[536] Because of the safety and efficacy of aP vaccine demonstrated in Stockholm 1992, Stockholm 1993 did not incorporate a placebo group. Conducted in 22 of 25 Swedish counties, the trial randomized 82,892 children to be immunized at 3, 5, and 12 months of age (the Swedish schedule for DT). Surveillance was predominantly passive; detected rates of pertussis were markedly lower than in Stockholm 1992. Surveillance was terminated early for the group receiving the two-component SmithKline Beecham vaccine, and the subjects were immunized with the Canadian five-component acellular vaccine when results from the Stockholm 1992 study showed the two-component vaccine to be of substantially lower efficacy than the others.

The relative performances of the evaluated vaccines are shown in Tables 44.10 and 44.11 (the absence of a placebo control group precludes calculation of absolute vaccine efficacies). Relative risks of culture-confirmed *B. pertussis* infection with at least 21 days of paroxysmal cough were 0.85 and 1.38 for the five-component and three-component vaccines, respectively, as compared with whole-cell vaccine. The relative risk of pertussis occurring between administration of the second dose (at 5 months of age) and third dose (at 12 months of age) of vaccine was 1.42 for the five-component vaccine, 3.13 for the three-component vaccine, and 7.81 for the two-component vaccine (as compared with whole-cell vaccine). Efficacy of the two-component vaccine differed significantly from that of the other three.

The Stockholm 1993 study provides important new data, but several factors complicate the direct comparison of its results to those of other trials. As noted, there was no placebo control group, precluding calculations of absolute efficacy. The Wellcome whole-cell vaccine was not used in any other efficacy trial. The five-component Aventis Pasteur Canada vaccine evaluated was the "hybrid" formulation developed for use in combination vaccines, containing twice the PT and four times the FHA as in the "classical" formulation. The SmithKline Beecham two-component vaccine was used in Stockholm 1992, but surveillance of this group was terminated early in the second Stockholm study. Thus the Chiron three-component vaccine represents the only link to the prior trials.

Based on the Stockholm 1992 and Italy 1992 trials (see Table 44.10), Infanrix, Tri Acelluvax, and the classical (CLL-4 F_2) formulation of Tripacel appear to be of equal efficacy. In Stockholm 1993, however, the relative risk of typical pertussis with the reformulated (hybrid or HCPDT) version of Tripacel was 0.62 (95% CI, 0.28–1.29) compared with Tri Acelluvax. For combined mild and severe pertussis, the hybrid five-component vaccine was equal to the whole-cell vaccine but significantly better than Tri Acelluvax.[558] Taken together, these observations suggest that the hybrid formulation of Tripacel is more efficacious than the classical formulation, and accordingly, that increasing the amount of PT and FHA in a vaccine can increase its efficacy. Of note, serologic studies performed on small subsets of subjects in the 1992[540] and 1993[543] Stockholm trials found significantly higher FHA responses with the hybrid formulation used in 1993 compared with the classical formulation used in 1992. There were no differences in antibody responses to the SmithKline Beecham bivalent vaccine lots used in the 1992 and 1993 trials.

Göteborg, Sweden. A randomized, double-blind, placebo-controlled trial sponsored by the National Institute of Child Health and Human Development (NICHD) was conducted in Göteborg, Sweden, from September 1991 to July 1994. This trial evaluated the monocomponent (PT toxoid only) acellular vaccine (Certiva) developed by the NICHD and manufactured at that time by North American Vaccine.[246] Children were immunized with DTaP or DT at 3, 5, and 12 months of age; there was no whole-cell control group.

During the period from 30 days after the third (12-month) vaccination to the end of the study, 72 DTaP and 240 DT recipients met the WHO case definition, for an efficacy of 71%. Other case definitions produced efficacy estimates ranging from 54% to 77%, depending on the stringency of the definition. A nested analysis of subjects with household exposure to pertussis found 66% efficacy after two doses and 75% efficacy after three doses.[559] During an additional 6 months of surveillance after unblinding of the study (representing a period averaging 18.5 to 24.5 months after the third injection), efficacy was 77% for the full cohort and 76% in a nested household-contact study.[543]

Because this vaccine included only PT toxoid, serologic rises of antibodies to FHA were an unbiased indicator of possible pertussis. Thus, pertussis could be identified more readily among vaccine recipients than if the vaccine had contained FHA. Other vaccines to which this vaccine is compared may have benefited from a lower case-ascertainment rate in the vaccine groups, biasing their efficacy estimates upward.

The only other efficacy trial that immunized subjects at 3, 5, and 12 months of age was Stockholm 1993. However, comparing results with that study cannot readily be done; the two studies evaluated no acellular vaccines in common, and there was no DT arm in Stockholm 1993 or whole-cell arm in Göteborg.

Follow-up for 2 years after immunization showed no diminution in protection among the DTaP group, as compared with the DT group.[543] Subsequent mass immunization in the Göteborg area using the PT toxoid vaccine resulted in significant decreases in *B. pertussis* isolates and hospitalizations in all age groups.[295] However, pertussis returned to Greater Göteborg during the second half of 1999, with a peak in 2004 (see "Duration of Immunity After Acellular Vaccines," later).[560,561] A recent surveillance report from Sweden (largely reflecting use of multicomponent aP vaccines) indicates that pertussis has decreased in nonvaccinated infants during the period from 2003 to 2012 ($P < .001$), indicating either reduced circulation of *B. pertussis* or herd immunity both in those too young to be vaccinated and those older than three months. Hospitalization rates have also decreased in this age group ($P < .001$). In the period 1998 to 2012, 70% of all cases in infants younger than age 3 months and 99% of cases with apnea caused by pertussis were admitted to hospital. Median duration of hospitalization was 7 days for unvaccinated versus 4 days for vaccinated infants 3 to 5 months of age.[562]

Munich, Germany. An unblinded, nonrandomized, case-control study sponsored by Sanofi Pasteur was conducted in 63 German pediatric practices from February 1993 to May 1995.[421] A cohort of children was enrolled prospectively to receive, according to parental choice, either the Aventis Pasteur-Biken two-component DTaP (Tripedia), the Behringwerke

DTwP, a DT vaccine, or no vaccine at approximately 3, 5, and 7 months of age.

All infants 2 to 24 months of age who came to a participating pediatrician with cough for 7 days or more, or with suspected exposure to pertussis, had nasopharyngeal specimens obtained for culture (whether the children were part of this cohort or not). There were 11,237 such children, including 3245 who were part of the prospective vaccine efficacy cohort. Children whose cultures were positive and whose cough persisted for 21 days or more were each matched with four control patients from the same practice who were born within 30 days of the subject. Pertinent clinical, demographic, and immunization data were entered into a conditional logistic regression analysis to calculate the relative odds of pertussis while controlling for confounding factors.

Eighty-seven subjects met the case definition of 21 or more days of paroxysmal cough plus either positive culture or household contact with a laboratory-confirmed case. These persons were matched to 344 control subjects. Eighty-one of the case subjects and 186 of the control subjects had received no pertussis vaccine; 4 and 55, respectively, had received three doses of DTaP; and 1 and 61, respectively, had received three doses of DTwP. Adjusted estimates of efficacy were 96% for the DTwP and 93% for the DTaP.[421]

That this study was not randomized or completely blinded raises certain issues. Although its efficacy estimates reflect adjustment for recognized risk factors that differed between cases and controls, it is impossible to identify or adjust for unrecognized bias or confounding. The analysis rested on comparing the proportions of vaccinated patients in two groups: those patients who were brought in because of possible pertussis, and control subjects who were selected in a systematic manner. If unvaccinated children were more likely than vaccinated children to be brought to the pediatrician with a suspect cough, they would be overrepresented in the case (but not control) groups, biasing upward the estimates of efficacy for DTaP and DTwP. It is reassuring that 76% of cultures in the prospective cohort were from infants receiving DTaP, and infants receiving DTaP comprised 75% of the cohort, suggesting that any bias was negligible. We know that vaccinated children who develop pertussis have a milder illness, so it is less likely that these children would have been brought in for culture. Because case confirmation depended on obtaining a positive culture, this may have also increased the risk of diagnostic bias (as described after the 1986 Swedish trial).

These factors, coupled with the small number of cases detected, suggest that the point estimates of efficacy from the Munich trial should be interpreted with caution. Some reassurance, however, is derived from the fact that the Munich and Mainz studies generated similar estimates of efficacy for the control whole-cell vaccine that was common to both.

The Munich investigators continued to follow the study cohort through the end of 2001. During the period from 2 years through 6 years (1997–2001) after the fourth dose of pertussis vaccine (given at 15 to 24 months of age), the crude overall efficacy against typical pertussis (defined as 21 or more days of paroxysmal cough) was 93% (95% CI, 79–98%) for those receiving Tripedia, and 96% (95% CI, 76–99%) for those receiving the Behring whole-cell vaccine; adjusted for various confounders, the respective efficacies were 96% (95% CI, 85–99%) and 97% (95% CI, 81–99.6%). Protection was materially lower among those not receiving four doses: crude and adjusted efficacies for those receiving three or fewer doses of Tripedia were 82% (95% CI, 7–96%) and 83% (95% CI, −5% to 97%), respectively. Adjusted efficacy against milder pertussis (defined as ≥7 days of any cough) was 71% (95% CI, 45–84%) for those receiving four doses of Tripedia and 80% (95% CI, 40–93%) for those receiving four doses of Behring whole-cell vaccine. (Note that long-term follow-up results from the various efficacy trials should not be compared directly to the results obtained in the original trials, given the substantial differences in surveillance methods and sensitivities.)

Erlangen, Germany. A prospective, randomized, double-blind clinical trial conducted from May 1991 to December 1994 in Erlangen, Germany, compared two vaccines produced by study sponsor Wyeth-Lederle: the Lederle-Takeda four-component DTaP (Acel-Imune) and the U.S. DTwP that served as the control whole cell vaccine in the MAPT.[323,422] Participants were immunized at approximately 3, 5, 7, and 17 months of age. Infants whose parents declined pertussis immunization received a German DT vaccine at approximately 3, 5, and 17 months of age and served as the control group for estimates of absolute efficacy.

Based on a case definition of 21 or more days of cough with paroxysms, whoop, or posttussive vomiting plus either positive culture, positive serology, or household contact with a culture-proven case, efficacies after three doses of vaccine were 78% for DTaP and 93% for DTwP.[323] (The abstract of this trial's published report cites an efficacy of 83% for the DTaP, but that figure reflects the benefit of a fourth booster dose of vaccine and thus is not comparable to the data presented for the other trials.) As reported in the FDA-approved labeling for Acel-Imune, the relative risk of pertussis was 1.5 for DTaP compared with DTwP.[422] A household-contact study nested in the overall trial found efficacy rates similar to those reported from the cohort study.[563]

Although this study also used an unblinded, nonrandomized placebo control group for estimates of absolute efficacy, the potential for ascertainment bias may have been reduced by the use of active surveillance (telephone calls every 2 weeks) to detect possible cases. In a follow-up analysis that has implications for all the efficacy studies, the local physicians who served as study investigators were stratified into three groups on the basis of the proportion of their patients who underwent investigation for pertussis.[564] For subjects attended by physicians who sought pertussis diligently, efficacy of the DTaP in preventing laboratory-confirmed pertussis with 7 or more days of cough was only 40%, whereas efficacies were 78% and 75% in the physician groups of moderate and low diligence, respectively. These results are not surprising, in that the less-diligent physicians detected only the more obvious (i.e., more severe) cases, and it has long been known that the efficacy of pertussis vaccine appears higher when one uses case definitions that focus on more severe disease. Nonetheless, this analysis has illuminated the extent to which fairly subtle differences in diagnostic diligence can markedly alter estimates of efficacy, confounding efforts to compare the results of different studies.

The Lederle-Takeda acellular vaccine was a four-component vaccine, but it predominantly consisted of FHA (86%; approximately 35 μg), with very little PT, fimbrial proteins, or PRN, as reflected by both its formulation (see Table 44.3) and its immunogenicity (see Table 44.8). It appeared to be of somewhat lower efficacy than the three-component vaccines evaluated in Italy (although the 95% CIs do overlap). If so, this represents further evidence that the quantity of the individual components can be as important as the number of components in determining the efficacy of the vaccine.

After unblinding of the study, physicians and parents of approximately one-third of study participants agreed to respond to semiannual questionnaires regarding cough illness and pertussis among study participants. Surveillance from 1995 through 2000 showed no diminution in pertussis protection in either the DTaP or the DTwP group, as compared with the DT group.[565]

Niakhar, Senegal. A prospective, randomized, double-blind study conducted in Senegal from 1990 to 1994 compared a two-component DTaP produced by study sponsor Aventis Pasteur (Triavax) with that company's European DTwP in children immunized at 2, 4, and 6 months of age.[423] Estimates of absolute efficacy were derived from a nested case-contact study that compared rates of pertussis (after exposure to an index case) among study subjects and nonstudy children (who thus had received either DT or no vaccine) living in the same villages and housing compounds. Surveillance detected 197 DTaP and 123 DTwP recipients who met the primary case definition of confirmed pertussis (see Table 44.10) with cough for 21 or more days, yielding a relative risk of pertussis 1.54 times higher in the DTaP than in the DTwP group. Requiring the cough to be paroxysmal (the WHO definition) reduced the case counts to 41 and 16, respectively, for a relative risk with DTaP of 2.42 compared with DTwP. When cases were stratified by age, the relative risk of meeting the primary case definition was 1.16 for children younger than 18 months versus 1.76 for older children, suggesting that protection waned more quickly among DTaP than DTwP recipients.

The case-contact study included 197 DTaP recipients, 190 DTwP recipients, and 17 unvaccinated children exposed to pertussis, of whom 24, 7, and 8, respectively, met the WHO case definition; the absolute efficacy estimates were 74% for DTaP and 92% for DTwP. Because of the small number of cases, confidence limits for these estimates are wide (95% CIs, 51–86% and 81–97% for DTaP and DTwP, respectively). In addition, although field surveillance workers and physician evaluators were blinded to the vaccination status of the randomized children, it is clear that parents knew whether their children had been vaccinated, a situation that may have increased case detection among unimmunized children. For both reasons, the relative rates are likely to be more reliable than the absolute efficacy estimates.

Initial reports from the investigators cited efficacies of 85% to 86% for DTaP and 95% to 96% for DTwP,[566] and some early reviews echo those figures.[567,568] However, these higher efficacies are based on a requirement that epidemiologically linked cases be confirmed by PCR, a case definition more strict than that of the WHO.

Mainz, Germany. A prospective household-contact study was conducted from October 1992 to September 1994 in six areas of Germany, in which 22,505 children had been immunized with study sponsor GlaxoSmithKline's three-component aP vaccine (Infanrix) in a prior safety and immunogenicity trial. Other children in these regions were unimmunized against pertussis or had been immunized with the Behringwerke whole-cell vaccine at 3, 4, and 5 months of age, in accord with the standard German schedule. Passive surveillance for pertussis identified households that contained both an index case of pertussis and at least one contact 6 to 47 months old who could be evaluated. Prospective surveillance of the 360 eligible contacts identified 104 secondary cases of pertussis (defined as 21 or more days of spasmodic cough plus either culture or serologic confirmation of *B. pertussis* infection): 96 of the 173 unvaccinated children, 7 of the 112 DTaP recipients, and 1 of the 75 DTwP recipients. The corresponding estimates of vaccine efficacy were 89% for the DTaP and 98% for the Behringwerke DTwP.[421]

The use of a household-contact study design largely eliminated the potential for ascertainment bias that would otherwise arise from the fact that study groups were not blinded or randomized, because family members were intensely surveyed by blinded field supervisors for pertussis in households with an index case. The simplest explanation for the higher efficacy estimate for Infanrix in this study (89%) than in the more rigorous, prospective Italian trial (84%) is that the small number of cases in this study reduced the precision of the estimate, as reflected by the wide confidence limits (95% CI, 77–95%).

Conclusions From the Efficacy Trials

Although the efficacy trials and other studies greatly enriched our understanding of pertussis vaccines, they left many important questions unresolved. Despite attempts to standardize key variables, no two studies are perfectly comparable. For example, as shown in Fig. 44.7, not only is the estimate of efficacy affected by case definition (being higher with stricter case definitions), but the various vaccines differ profoundly in the degree to which their efficacies vary with changes in case definition. Moreover, no study has evaluated a multicomponent vaccine directly against versions of itself that contain alternative components or different quantities of each component. Because of the trend toward combination vaccines, the drive to eliminate thimerosal use, and continued consolidation in the vaccine industry, there are only three broadly distributed aP vaccines: the French two-component, the Belgian three-component, and the Canadian five-component vaccines. National surveillance data have clearly demonstrated the ability of each vaccine to accomplish excellent control of pertussis during the ages covered by the childhood vaccination series.[123,569]

Onset of Protection and Effect of Vaccine Schedule. It appears that some protection (perhaps 15% to 20%) accrues with the first dose, and substantially more with the second dose (Table 44.12). A reanalysis of data in the Technical Report of the Stockholm 1993 trial indicates that the incidence of pertussis was markedly lower after the second dose than after the first dose, and it was reduced somewhat further by the third dose (Table 44.12).[541]

In general, three doses appear to be necessary for good protection and, for most of the vaccines and schedules, a booster dose at around 15 months of age provides substantial additional benefit. There is probably little difference between schedules that immunize at ages 2, 4, and 6 months; 3, 5, and 7 months; or even 2, 3, and 4 months (unless maternal Tdap vaccination is practiced). Immunizing at 3, 5, and 12 months of age gives a little less protection in the second half-year of life and better protection thereafter, as one would intuitively expect.

After conclusion of the efficacy trials, Sweden implemented nationwide aP vaccination, using a variety of vaccines in various counties over time. Gustafsson and colleagues reported the results of 7 years of follow-up of national pertussis surveillance.[570] From 2001 to 2004, pertussis rates per 100,000 person-years were 225 in the unvaccinated, 212 after one dose (3 months), 31 after two doses (5 months), and 19 after three doses (12 months). These studies suggested that the incidence of confirmed pertussis was increasing in children older than 6 years and suggested that a booster dose (now recommended) was warranted between 5 and 7 years of age.[570] Reports from both Göteborg and Stockholm, Sweden, supported these conclusions.[560,561,570]

Choice of Vaccine. For those providers free to choose among vaccines, we recommend consideration of five factors (some may not apply in every situation): efficacy, rate of adverse effects, cost, convenience (e.g., how the vaccine is supplied), and service (e.g., reliability of supply, provision of educational materials). Choices can then be made on the basis of the relative weights assigned to each of those factors.

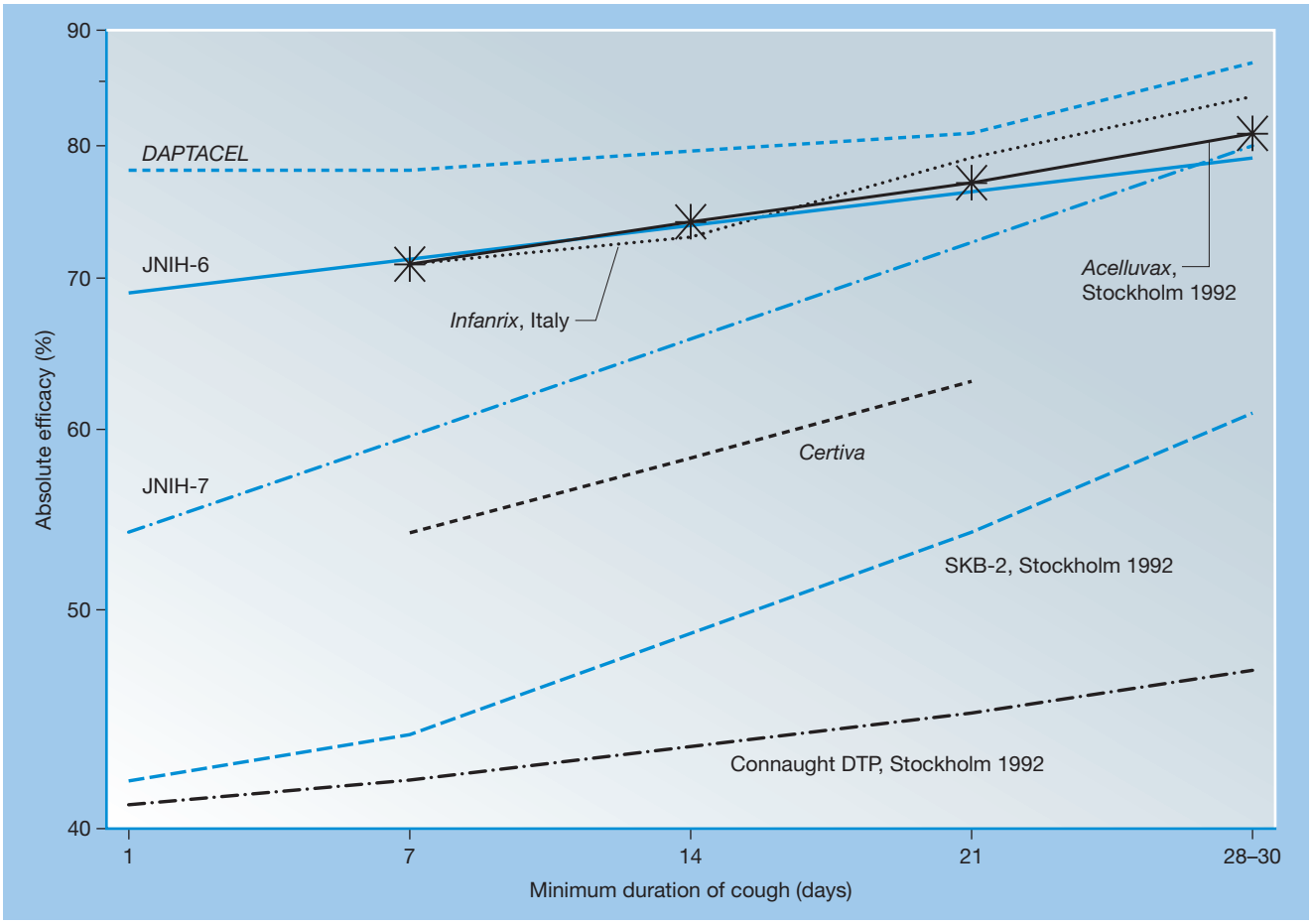


Figure 44.7. Efficacy vs minimum duration of cough (as case definition). Efficacy of pertussis vaccines evaluated in prospective, randomized trials (names defined in Table 44.3) for preventing various durations of any cough associated with culture-confirmed pertussis. A slope of zero (horizontal line) would indicate equal protection for mild and severe disease; steeper slopes reflect less protection for mild than for severe disease. Because estimates were obtained in separate studies, only the slopes, and not the actual values, should be compared. *(Courtesy Michael D. Decker.)*

TABLE 44.12 Incidence of Pertussis (Per Million Days at Risk) by Dose, Stockholm 1992 Efficacy Trial

Interval	SKB-2	Acelluvax	HCPDT	Evans-Medeva WCV
PRIMARY ANALYSES (FOLLOW-UP FROM STUDY START UNTIL OCTOBER 1996)				
Dose 1 to dose 3	N/A	8.1	6.7	4.4
Dose 3 to study end (~21 mo)	N/A	1.8	1.1	1.3
SECONDARY ANALYSES (FOLLOW-UP FROM STUDY START UNTIL JULY 28, 1995, WHEN SKB-2 GROUP WAS REIMMUNIZED WITH HCPDT)				
Dose 1 to dose 2	19.6	16.0	16.9	11.3
Dose 2 to dose 3	14.3	5.8	2.6	1.8
Dose 3 to early termination (~7 mo)	4.2	1.3	1.0	0.5

HCPDT, hybrid component pertussis-diphtheria-tetanus vaccine; SKB, SmithKline Beecham; WCV, whole-cell vaccine.
Data from Olin P, Gustafsson L, Rasmussen F, et al. Efficacy Trial of Acellular Pertussis Vaccines: Technical Report Trial II, with Preplanned Analysis of Efficacy, Immunogenicity and Safety. *Stockholm, Sweden: Swedish Institute for Infectious Disease Control; 1997.*

Duration of Immunity After Acellular Vaccines. Long-term follow-up of subjects in the 1986 Swedish efficacy trial did not demonstrate any decline in efficacy of the JNIH-6 or JNIH-7 vaccines through the end of the fourth year after immunization (although it did make clear the higher efficacy of the JNIH-6 vaccine).[433] In the Stockholm 1992 trial, efficacy of the Aventis Pasteur five-component vaccine was sustained above 80% during 2 years of follow-up, whereas that of the control whole-cell vaccine declined sharply.[324] Gustafsson and colleagues, also reported that pertussis incidence remained low for 6 years after aP vaccination in Stockholm.[570] During 6 years of extended follow-up in the Italian trial, efficacies of both the Chiron and the GlaxoSmithKline three-component vaccines were fully maintained.[325,556] Follow-up of subjects given Certiva in the Göteborg trial showed that protection remained unchanged for at least 2 years after the third dose,

but an increase in disease was noted in late 1999, with a peak in 2004.[560,561] Follow-up of the Erlangen cohort for 6 years showed no diminution in protection for either the Lederle-Takeda DTaP or the Lederle DTwP. Five-year follow-up results for the Aventis Pasteur–Biken vaccine used in the Munich cohort also demonstrated persistent protection. As these studies were all conducted in areas with high rates of circulating natural pertussis, one cannot rule out the impact of potential boosting by asymptomatic infection.

When pertussis incidence rose in Sweden for those who were 7 and 8 years old,[570] a booster dose was recommended at 5 to 6 years of age. In addition, the long-term effects of aP vaccines, monitored in an enhanced surveillance project, were discussed at an international workshop in Stockholm in November 2008.[560,561] The Swedish experience clearly demonstrated that aP vaccines were effective but immunity waned and booster doses were needed.

Follow-up studies in the Göteborg area, where the monovalent PT vaccine was used, demonstrated a reemergence of pertussis from the end of 1999, with a peak in 2004, followed by a decrease when booster doses were introduced to both 6- and 10-year-old children from 2005 to 2006. Pertussis was prevalent in all age groups, with the highest documented incidence in infants younger than 12 months. Many of the pertussis-infected subjects had received three doses of the pertussis toxoid vaccine in the mass vaccination project, with a mean time from the last dose of monovalent PT vaccine of 5 years. In total, there were 128 hospitalizations, 106 of which were of infants. The authors concluded that pertussis had not been eliminated from the Göteborg area, and that booster doses were needed to circumvent waning immunity after vaccination.[560,561] Currently, booster doses of pertussis vaccine are routinely recommended in Sweden at 5 to 6 years and again at 14 to 16 years of age.

Recent reports from Japan, where only aP vaccine is used, suggest that the incidence of pertussis in adults has gradually increased since 2002, with outbreaks reported in college campuses, high schools, and offices.[571–573] In 2009, the incidence in infants younger than 1 year and in adults both increased.[574] The current immunization schedule in Japan incorporates a three-dose primary schedule with a booster 6 months after the primary series. No pertussis booster is given in adolescence or adulthood.

Since 2010, a number of pertussis outbreaks have been noted in Australia and the United States. These outbreaks resulted in the largest numbers of pertussis cases in over half a century and were remarkable for the large number of cases among older children and young adolescents, the age groups representing the first cohorts vaccinated solely with aP vaccine. Although many have pointed out the potential roles in this increased reporting of greater awareness of pertussis and widespread availability of more sensitive pertussis diagnostic methods, epidemiological features such as the "marching cohort" effect argue that the switch from DTwP to DTaP has played a major role.

During Australian pertussis outbreaks from 2009–11, high rates of pertussis were seen in 5- to 14-year-old children.[575] In Queensland, the highest age-specific notification rates of pertussis were noted among children 6 to 11 years old, higher than rates noted in infants.[576] To assess the reasons for this change, Sheridan and colleagues compared pertussis reporting rates to the pertussis vaccines used in the primary immunization series in infants in the 1998 birth cohort in Queensland, a group that might have been primed either with DTwP or DTaP.[577] A total of 58,233 children were born in 1998 and identified in the regional vaccine registry. Of these, 40,694 (69.9%) received at least three doses of any pertussis-containing vaccine during their first year of life. There were a total of 267 pertussis cases reported from this cohort between 1999 and 2011. Children who received a three-dose primary series with DTaP had higher rates of pertussis than those who received a three-dose primary series with DTwP (Fig. 44.8). In those children who received mixed courses that included both DTwP and DTaP, rates of pertussis were highest for children receiving DTaP as their first dose. Children who received a mixed course with DTwP as the initial dose had incidence rates that were between rates for the pure DTwP and DTaP cohorts. The impact of this difference in priming persisted for more than a decade.[577]

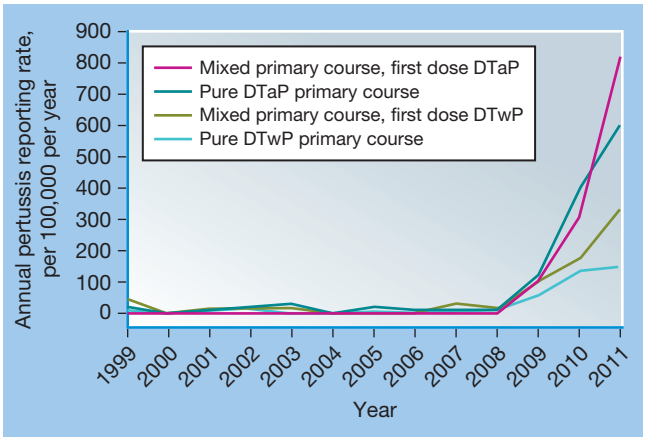


Figure 44.8. Queensland, Australia pertussis reporting rates from 1999–2011 by primary course of pertussis vaccination for children born in 1998. DTaP indicates diphtheria-tetanus-acellular pertussis; DTwP, diphtheria-tetanus-whole-cell pertussis. Children who received a three-dose primary series with DTaP had higher rates of pertussis than those who received a three-dose primary series with DTwP. In those children who received mixed courses that included both DTwP and DTaP, rates of pertussis were highest for children receiving DTaP as their first dose. Children who received a mixed course with DTwP as the initial dose had incidence rates that were between rates for the pure DTwP and DTaP cohorts. *(From Sheridan SL, Ware RS, Grimwood K, et al. Number and order of whole cell pertussis vaccines in infancy and disease protection. JAMA. 2012;308:454–456. Copyright 2012, American Medical Association.)*

In the United States, outbreaks have also been reported from California, Wisconsin, Oregon, and Washington, with the emergence of increasing numbers of cases in children between 7 and 10 years of age, many fully vaccinated with aP-containing vaccines.[313,316,578,579] During 2012, nearly 50,000 cases of pertussis were reported in the United States, the highest number in 57 years.[10] Infants had the highest rate of reported disease, followed by children 7 to 10 years old, and then adolescents.

A case-control study from Kaiser Permanente Northern California was conducted among children who were vaccinated with DTaP, comparing children with PCR-confirmed pertussis with two sets of controls, those who were PCR-negative and closely matched controls from the general population of health-plan members.[580] A total of 277 PCR-positive children, 4 to 12 years of age, were compared with 3318 PCR-negative controls and 6086 matched controls. PCR-positive children were more likely to have received the fifth DTaP dose earlier than PCR-negative controls ($P < .001$) or matched controls ($P = .005$). These data indicated that after the fifth dose of DTaP, the risk of acquiring pertussis increased by an average of 42% each year.

In the context of the California 2010 outbreak, Misegades and colleagues conducted a case-control study of 682

suspected, probable, or confirmed pertussis cases in children 4 to 10 years of age versus 2016 controls and found that receipt of all five recommended doses of aP vaccine was 88.7% (95% CI, 79.4–93.8%) effective. Efficacy declined with time since receipt of the fifth dose, from 98.1% within 1 year to 71.2% 5 or more years later.[581]

Witt and colleagues reviewed data from Kaiser Permanente Northern California to determine whether prior vaccination with wP-containing vaccines provided better protection compared to receipt of aP-containing vaccines.[305] Their data showed a much higher risk of pertussis associated with an aP-only series, which was somewhat lessened by the administration of a sixth dose of aP vaccine. In addition, receipt of one or more wP doses markedly augmented the durability of immunity from subsequent aP doses. They concluded that "a wholly acellular pertussis vaccine series was significantly less effective and durable than one that contains at least one dose of the traditional whole cell vaccine." These findings are strikingly similar to those obtained from Australia, which found high pertussis notification rates corresponding to the first birth cohorts that received aP vaccines for both their primary and booster vaccinations. It is also important to note that these outbreaks have occurred within the context of high vaccine coverage. In Australia, 92% of children 5 years of age in 2013 had received four doses of pertussis-containing vaccine,[582] and in the United States, 83% of children 19 to 35 months of age in 2012 had received four doses of pertussis-containing vaccine.[583] More recently, Klein et al.[583a] analyzed data from their large integrated healthcare system for the period 2010–15, during which two large pertussis outbreaks occurred. By comparing pertussis rates among adolescents who did or did not receive Tdap, they found that Tdap effectiveness declined from 69% during the first year after vaccination to 9% by 4 or more years after vaccination.

Adverse Events With Acellular Vaccines

Numerous safety and immunogenicity studies of the acellular vaccines have been conducted in infants and children[584–590] and have invariably found the acellular vaccines to be associated with lower rates of adverse reactions than whole-cell vaccine. Although most trials have not compared one acellular vaccine with another to detect differences in the rates of adverse reactions, the MAPT evaluated 13 acellular and two whole-cell vaccines and thus offers the best comparison of common reactions with these vaccines.[462] The various efficacy trials maintained surveillance for severe adverse events among much larger numbers of infants and thus supplement the MAPT data by providing more precise estimates of rates for these less common events.

Common Adverse Events: Comparative Rates. The MAPT included all acellular vaccines studied in the efficacy trials except one, a monovalent PT vaccine similar to Certiva, produced by North American Vaccine. Fortunately, this vaccine was evaluated using the MAPT protocol in a subsequent trial at one of the MAPT study sites, which allows it to be directly compared with the other vaccines (Table 44.13). Among the acellular vaccines, there were significant differences with respect to redness, swelling, pain, and vomiting but not with respect to fussiness, drowsiness, anorexia, or antipyretic use.[462] No acellular vaccine was consistently the most or least reactogenic. Compared with the reference whole-cell vaccine, all the acellular vaccines were associated with significantly lower rates and severity of every reaction except vomiting.

Common and Severe Adverse Events: Data From the Efficacy Trials. Table 44.14 summarizes the adverse reaction data from the efficacy trials, organized by vaccine. Definitions and methods may have differed from study to study, and thus caution should be used in comparing rates between trials. Note also that Table 44.14 presents reaction rates per dose of vaccine, whereas the rates presented in the following paragraphs are per subject (these two approaches will produce different rates unless every child with a reaction has the same reaction with every dose). Detailed information is provided only for currently available products; readers desiring information regarding other products are referred to the fourth edition of this text.[457]

Infanrix—GlaxoSmithKline. In the Italian trial, adverse events were significantly more common among recipients of DTwP than DTaP or DT. Temperature of 40.5°C or greater was seen in 6.8% of recipients of DTwP, 0.8% of recipients of Infanrix, 1.1% of recipients of Tri Acelluvax, and 1.3% of recipients of DT; crying for 3 or more hours in 11.5%, 1.9%, 1.3%, and 0%, respectively; and HHE in 1.7%, 0.2%, 0%, and 1.3%, respectively.[591] Seizures within 48 hours of vaccination occurred in one Infanrix recipient and in three DTwP recipients.[325]

Triavax—Sanofi Pasteur, France. In the Senegal efficacy study, Triavax and Sanofi Pasteur's French whole-cell vaccine were each given to approximately 2200 children. Persistent crying was significantly more common among whole-cell recipients (eight vs zero episodes). Two subjects in each group experienced febrile seizures within 48 hours of vaccine administration. No episodes of HHE or anaphylactic reactions were seen. In the pilot study preceding this trial, fever, crying, and local reactions were significantly more common with whole-cell than acellular vaccine.[423]

Daptacel (Tripacel)—Sanofi Pasteur Ltd, Canada. Of the 2552 children given the classical (CLL-4 F_2) formulation of Tripacel in Stockholm 1992, one (0.04%) had HHE and another was withdrawn as a result of pronounced local reactions.[324] There were seven episodes of convulsions, but none occurred within 48 hours of vaccination, making a causal association unlikely. Of 17,686 children given the hybrid (HCPDT) formulation of Tripacel in Stockholm 1993, 0.04% had fever of 40°C or greater, and 0.02% had convulsions within 48 hours of vaccine administration.[536] Minor local and systemic reactions were significantly less common with HCPDT than with whole-cell vaccine. The investigators made a special effort to detect HHE in Stockholm 1993, and thus higher HHE rates were recorded for vaccines evaluated in that study than for the same, or similar, vaccines evaluated in other studies. HHE rates were, for Tripacel, 0.47 per 1000 in Stockholm 1993 (hybrid formulation) versus 0.13 per 1000 in Stockholm 1992 (classical formulation); and for Tri Acelluvax, 0.26 per 1000 in Stockholm 1993 versus 0.07 per 1000 in Italy 1992.

Tripedia—Sanofi Pasteur, USA. Of 12,514 children given Tripedia in the Munich trial, 2.2% experienced the following adverse events related to vaccination: fever, 0.9%; local reactions, 0.4%; unusual crying, 0.3%; irritability, 0.3%; somnolence, 0.2%; crying for more than 3 hours, 0.04%; HHE, 0.02%; and febrile seizure, 0.01%.[289,421] Ten children given Tripedia had culture-confirmed invasive bacterial disease, but the rate of disease was not significantly higher than that seen in the control group.

Severe Adverse Events: National Surveillance Data. The claims paid by the Japanese Vaccine Compensation System provide a comparison of severe neurologic reactions and deaths with whole-cell versus acellular vaccine and with immunization beginning at 3 months versus 2 years.[397,398,592] Changing the age of administration of whole-cell vaccine from 3 months to 2 years was associated with a dramatic reduction

TABLE 44.13 Adverse Reaction Results from the Multicenter Acellular Pertussis Trial and a Follow-up Trial[a]

		Reported Incidence of Reaction by the Third Evening After Vaccination at 2, 4, or 6 Months of Age												
		Temperature (°C)		Redness (mm)		Swelling (mm)								
Manufacturer or Distributor	**Vaccine[b]**	**37.8–38.3**	**>38.4**	**1–20**	**>20**	**1–20**	**>20**	**Pain[c]**	**Fussiness[d]**	**Drowsiness**	**Anorexia**	**Vomiting**	**Crude Overall Average[e]**	**Values Exceeding MAPT DTaP Average[f] (N)**
Sanofi Pasteur (Canada)	Tripacel	29.2%	3.6%	32.8%	3.6%	21.9%	4.4%	5.1%	18.2%	42.3%	19.0%	12.4%	17.5%	6
Sanofi Pasteur (Canada)	3-component	22.4%	3.2%	44.0%	2.4%	22.4%	8.0%	12.0%	21.6%	45.6%	27.2%	21.6%	20.9%	8
Sanofi Pasteur (France)	Triavax	24.1%	4.6%	42.9%	4.5%	28.6%	5.3%	8.3%	12.0%	42.1%	20.3%	7.5%	18.2%	7
Sanofi Pasteur (USA)	Tripedia	19.3%	5.2%	27.4%	5.2%	16.3%	3.7%	9.6%	19.3%	41.5%	22.2%	7.4%	16.1%	5
Baxter Laboratories	Certiva	20.0%	2.5%	20.0%	2.5%	7.5%	2.5%	7.5%	22.5%	30.0%	20.0%	2.5%	12.5%	2
Biocine Sclavo	1-Component	18.6%	3.6%	28.3%	5.3%	24.8%	3.5%	3.6%	20.4%	52.2%	25.7%	17.7%	18.5%	6
Chiron Vaccines	Acelluvax	19.0%	1.6%	29.4%	1.6%	17.5%	2.4%	1.6%	16.7%	41.3%	19.0%	9.5%	14.5%	0
GlaxoSmithKline	Infanrix	28.3%	3.3%	35.0%	4.2%	24.2%	5.8%	10.8%	15.0%	46.7%	19.2%	12.5%	18.6	7
Massachusetts Public Health Biologic Labs	1-Component	21.2%	4.1%	36.3%	1.4%	21.9%	6.2%	8.2%	16.4%	48.6%	22.6%	14.4%	18.3%	8
Michigan Department of Public Health	2-Component	22.1%	2.2%	30.1%	5.9%	19.1%	3.7%	13.2%	16.2%	46.3%	23.5%	14.0%	17.8%	5
SmithKline Beecham Biologicals	2-Component	18.8%	3.1%	31.3%	2.1%	23.4%	4.2%	6.2%	17.2%	37.0%	17.7%	10.9%	15.6%	2
Speywood (Porton) Pharmaceuticals	3-Component	17.6%	5.0%	36.1%	2.5%	21.0%	4.2%	4.2%	24.4%	45.4%	18.5%	10.9%	17.3%	5
Wyeth Lederle Vaccines and Pediatrics	3-Component	16.0%	5.9%	15.1%	2.5%	10.9%	0.8%	5.9%	12.6%	29.4%	22.7%	12.6%	12.2%	2
Wyeth Pharmaceuticals	Acel-Imune	16.6%	3.2%	23.5%	2.8%	12.4%	3.2%	3.7%	14.3%	40.6%	24.9%	13.4%	14.4%	2
Average for all DTaPs in MAPT	—	**20.8%**	**3.7%**	**31.4%**	**3.3%**	**20.1%**	**4.2%**	**6.9%**	**17.1%**	**42.7%**	**21.7%**	**12.6%**	**16.8%**	**5**
Wyeth Lederle Vaccines and Pediatrics	Whole-cell	44.5%	15.9%	56.3%	16.4%	38.5%	22.4%	40.2%	41.5%	62.0%	35.0%	13.7%	—	—

[a]Results for Certiva are from a separate study conducted at an MAPT study center after completion of the MAPT, using the MAPT protocol, procedures, and data forms.
[b]Number of pertussis components, for those vaccines without known trade names. For branded products, note that the licensed vaccine's formulation may differ from that of the vaccine evaluated in MAPT. See Table 44.3 and its footnotes for details.
[c]Moderate (cried or protested to touch) or severe (cried when leg moved).
[d]Moderate (prolonged crying and refused to play) or severe (persistent crying and could not be comforted).
[e]An unweighted average of the 11 specific rates shown to the left of this value. Has the effect of giving more weight to less serious reactions but provides a crude overall comparison that may be useful.
[f]Of the 11 specific rates, the number for which this vaccine's reaction rate exceeded the average for all DTaPs evaluated in the MAPT (see the next-to-last row of the table). Treats small differences and large differences as though they were the same, but provides a crude overall comparison that may be useful.
DTaP, diphtheria, tetanus, and acellular pertussis; MAPT, Multicenter Acellular Pertussis Trial.
Modified from Decker MD, Edwards KM, Steinhoff MC, et al. Comparison of 13 acellular pertussis vaccines: adverse reactions. Pediatrics. *1995;96:557–566.*

TABLE 44.14 Incidence (Per 1000 Doses) of Major Adverse Reactions After Primary Immunization: Data From Efficacy Trials, 1992–1997[a]

Product	Trial	Vaccine	Doses	High Fever[b]	HHE	Persistent Crying[c]	Seizures[d]
Acel-Imune	Erlangen[323]	DTaP	16,644	0.06	0	2.0[c]	0.06
		DTP	16,424	0.19	0.06	8.8[c]	0.18
Tripedia	Munich[421]	DTaP	41,615	N/A[b]	0.05	0.12	0.02
Infanrix	Italy[325]	DTaP	13,761	0.36	0	0.44	0.07
		DTP	13,520	2.4	0.67	4.0	0.22
		DT	4,540	0.44	0.44	0	0
Acelluvax	Italy[325]	DTaP	13,713	0.29	0.07	0.66	0
		DTP	13,520	2.4	0.67	4.0	0.22
		DT	4,540	0.44	0.44	0	0
	Stockholm 1993[536]	DTaP	61,219	0.24	0.26	N/A	0.03
		DTP	60,792	0.61	0.56	N/A	0.21
Certiva	Göteborg[246]	DTaP	5,124	2.6	0	0[c]	0.4
		DT	5,130	1.9	0	0[c]	0
Tripacel	Stockholm 1992[324,540]	DTaP	7,699	0.26	0.13	0.9	0
		DTP	6,143	4.4	0.81	4.8	0.16
		DT	7,667	0.39	0	0.52	0.26
HCPDT	Stockholm 1993[536]	DTaP	61,220	0.11	0.47	N/A	0.06
		DTP	60,792	0.61	0.55	N/A	0.21
Triavax	Senegal[423]	DTaP	6,881	N/A	0	0	0.29
		DTP	6,595	N/A	0	1.2	0.39

[a]Note that duration of surveillance or definitions of adverse reactions may vary from trial to trial. Thus, comparisons within trials are more valid than comparisons across trials.
[b]Temperature ≥40°C, except: ≥40.5°C for Erlangen; for Munich, rate of "fever" was 2.8/1000 but "fever" was not defined.
[c]Crying persisting ≥3 hours, but duration not specified for Erlangen and Göteborg.
[d]Within 48 hours of vaccination.
DT, diphtheria and tetanus toxoids vaccine; DTaP, DT plus acellular pertussis vaccine; DTP, DT plus whole-cell pertussis vaccine; HHE, hypotonic-hyporesponsive episode; N/A, data not available.

in compensable adverse neurologic events and deaths; a further reduction occurred with the change from whole-cell to acellular vaccine. The data strongly suggest that this low rate of serious adverse events was maintained after the initiation of immunization with acellular vaccine at the age of 3 months in 1989. Severe neurologic events and deaths were both reduced more than eightfold during 13 years of acellular vaccine use, as compared with the preceding 11 years of whole-cell vaccine use.

The public health system provided all immunizations and conducted uniform surveillance for postimmunization adverse events in Alberta, Canada during the years preceding and following the transition from whole-cell to aP vaccines. Before July 1, 1997, a pentavalent DTwP-IPV-Hib combination vaccine was used in the province; beginning July 1, a DTaP-IPV-Hib combination was used (this combination, Pentacel, is based on the HCPDT version of Tripacel that was studied in Stockholm 1993). The switch to the acellular combination was associated with an 89% reduction in fever greater than 40°C, an 82% reduction in unusual cry, and a 74% reduction in HHE.[593] These results were confirmed by Scheifele and Halperin, who evaluated emergency department visits and hospitalizations at tertiary-care pediatric centers across Canada and found an 86% decline in febrile seizures and a 75% decline in HHE coincident with the transition from whole-cell-based to acellular-based combination vaccines in Canada.[594]

Similar data, albeit less dramatic, are available from the U.S. Vaccine Adverse Event Reporting System (VAERS), maintained by the CDC and the FDA.[595] From 1991 to 1993, rates of reported adverse events after the fourth and fifth doses in the pertussis immunization series were significantly lower for DTaP than for DTwP. These events included fever (1.9 vs 7.5 events per 100,000 vaccinations), seizures (0.5 vs 1.7), and hospitalization (0.2 vs 0.9), for a total of 2.9 versus 9.8 cases per 100,000 vaccinations for DTaP and DTwP, respectively.[596] There were three reports of encephalopathy after DTwP, but none after DTaP. A follow-up report, covering the period between January 1, 1995 (when whole-cell vaccine was in exclusive use), and June 30, 1998 (when acellular vaccine was in predominant use), revealed steadily declining annual numbers of VAERS reports involving immunization against pertussis, from 2071 in 1995 to 491 in the first half of 1998.[597] Another subsequent report demonstrated the lack of association of DTaP vaccines with seizures in early childhood using data compiled from 1997 to 2006 in the seven managed-care organizations that participate in the Vaccine Safety Datalink.[598]

Finally, the U.S. National Vaccine Injury Compensation Program reported in 2015 that most claims between 1995 and 2005 of seizure disorders or encephalopathy following vaccination were associated with DTwP receipt. In addition, many of the children with alleged vaccine injury had preexisting neurologic or neurodevelopmental abnormalities.[485,486,599] These data offer additional evidence of an improved safety profile for acellular vaccines.

Safety of Booster Doses Given to Children Primed With Acellular Vaccine. A continuation study of the MAPT evaluated the safety and immunogenicity of a fourth dose of acellular vaccine given at 15 to 20 months to children who had been primed with either aP or wP vaccine at 2, 4, and 6 months of age.[600] For children who received four consecutive doses of an acellular vaccine, fever and injection-site redness, swelling, and pain were seen more frequently with the fourth dose than with the primary series. Of children given a booster with acellular vaccine, those who had been primed with

acellular vaccine had local redness and swelling significantly more frequently than did those who had been primed with whole-cell vaccine. However, children who received four consecutive doses of whole-cell vaccine had significantly higher rates of irritability, redness, swelling, and pain after the booster than did either of the groups that were given a fourth dose of acellular vaccine. None of 1293 evaluated children experienced seizures, HHEs, or fever greater than 40.6°C. Also of note, several cases of swelling of the entire limb were observed after administration of the fourth dose of acellular vaccine to children previously primed with DTaP.

A report from Germany of entire-limb swelling occurring after the fourth dose of one three-component DTaP appeared in 1997.[601] Among children from whom adverse reactions were specifically solicited, the rate of entire-thigh swelling was 2.4%, whereas it was reported to occur in only 0.5% of those not specifically followed for reactions. With this report in mind, Rennels and colleagues evaluated the cases of entire-limb swelling after the fourth dose of DTaP vaccine in MAPT.[602] Although extensive swelling reactions had not been anticipated in MAPT and had not been prospectively studied, parents in MAPT had been provided with diary cards that contained a section for them to report other reactions of concern. Parents of 20 (2%) of the 1015 children who had received the same DTaP vaccine throughout the primary and booster series described swelling of the entire thigh after the fourth dose. In contrast, none of the 246 children primed with DTwP and boosted with DTaP were reported to have entire-thigh swelling. Differences in rates between the whole-cell– and acellular-primed groups were significant (P = .02). An interesting observation was that 40% of these children with whole-limb swelling were judged by their parents to have experienced no pain, and 40% were judged not to have erythema, despite the extensive swelling reactions. The only significant association between rates of entire-thigh swelling after dose 4 and the composition of the associated vaccine was with the quantity of diphtheria toxoid (P = .02). Antibody levels to pertussis toxoid, diphtheria toxoid, and tetanus toxoid were evaluated for subjects with extensive swelling and three controls per case in an attempt to determine whether the swelling might be the result of an Arthus reaction. No difference was noted in the comparative distribution of prevaccination and postvaccination antibody titers between cases and controls, but sample sizes were small. In contrast, a large study of children given a fifth dose of Tripedia found that higher prevaccination antibody titers were associated with a higher frequency of large local reactions.[603] Thus, there is some evidence that high IgG antibody levels before booster vaccination might play a role in the large limb swelling and may be related to excessive antigen content.

Children enrolled in MAPT were also studied after the fifth dose of DTaP.[604] Given the 5-year interval between initial enrollment and the fifth dose, many children were not located for enrollment in the study. None of 121 children given five doses of the same DTaP, versus four (2.7%) of 146 children receiving a mixed DTaP series, were reported to have experienced swelling of the whole upper arm after the fifth dose (P = .13). For children with swelling after the fifth dose, the onset of swelling was noted by parents on days 1 and 2 after vaccination and generally resolved by day 4, with no sequelae.

Rennels and colleagues reanalyzed data from the fourth-dose and fifth-dose MAPT studies and found no consistent relationship between the quantity of aluminum in various DTaP vaccines and their rates of extensive swelling.[605] Further review of the literature regarding extensive swelling reactions revealed other sporadic reports in early studies of DTaP vaccines in Japan[606] and Sweden.[607] The proposed explanation at that time was that the immunizations had been given by the deep subcutaneous and not the intramuscular route. This observation was supported by the report that shorter needle length was associated with increased local reactions.[608] However, in the MAPT studies, extensive swelling was seen with nine of the 12 DTaP vaccines (containing from one to five pertussis antigens), which were given intramuscularly by experienced vaccine-study nurses.[602]

In a randomized, blinded study of DTaP-IPV and Tdap vaccines (similarly formulated except for the concentrations of PT, diphtheria toxoid, and IPV) among children 4 to 6 years of age, Scheifele and colleagues found that rates of large injection-site reactions and of pain were lower by half to two-thirds in the group receiving the Tdap vaccine.[609] Children with large reactions were more likely to have relatively elevated prevaccination antibody levels to PT, PRN, or diphtheria toxin. Similar findings were reported by Knuf and coworkers in children receiving booster doses of DTaP during the second year of life: local reactions were reduced after vaccines containing smaller quantities of antigens.[610]

Rennels and colleagues enrolled children who had suffered large swelling reactions after the fourth dose of DTaP and assessed whether large local reactions occurred after the fifth dose of DTaP.[611] Of 20 children with extensive local reactions after dose 4, only four experienced entire upper arm swelling and seven had swelling greater than 5 cm after dose 5. These reactions were well tolerated and supported revaccination.

Summary of Adverse Events. Almost every study has found minor local and systemic adverse reactions to be less common with acellular than with whole-cell vaccine. Although HHEs and seizures are seen after acellular vaccine, they occur less frequently than after whole-cell vaccine.

Rates of rare adverse effects cannot be determined reliably even with large efficacy trials, and they require postmarketing surveillance for their determination. Of course, adverse events temporally but not causally associated with vaccination will continue to occur at their background rates regardless of the vaccine used. Among children primed and boosted with acellular vaccine, reaction rates increase successively with each booster dose but remain lower than those seen among children primed and boosted with whole-cell vaccine.

Vaccination of Adolescents and Adults

Numerous aP vaccines have been evaluated in adolescents and adults and shown to be safe and immunogenic.[460,612–616] As noted previously, Tdap vaccines have been licensed in various jurisdictions for immunization of adolescents and adults under the brand names Adacel, Boostrix, Covaxis, and (combined with IPV) Repevax. Many national advisory bodies have recommended universal adolescent Tdap booster vaccination and substitution of Tdap for tetanus-diphtheria (Td) for some or all adult booster immunizations. Adolescents and adults in close contact with babies, including parents and other close family members, healthcare workers, and daycare workers have also been recommended to receive Tdap.

In some jurisdictions, Tdap is licensed or recommended for use instead of DTaP at the 4- to 6-year booster, to reduce the adverse reactions.

Immunogenicity in Adolescents and Adults

A multicenter trial sponsored by the U.S. National Institutes of Health (NIH) evaluated the immunogenicity among adults of varying strengths of five aP vaccines (variants of Certiva, Tri Acelluvax, Infanrix, Acel-Imune, and a PT-FHA vaccine supplied by the Massachusetts Public Health Biologic Laboratories).[616,617] These studies and others demonstrated excellent

immunogenicity of the acellular vaccines in adults, even with dosages substantially lower than the standard pediatric dose, and no interference was noted with diphtheria or tetanus antibody responses in any of the groups. In the NIH dosage-ranging study, dosage-related increases in serum antibody levels against known pertussis vaccine antigens were seen in all vaccine groups.[616,617] For several vaccines, significant antibody responses were seen against antigens not known to be present in the vaccines, suggesting that the vaccines contained trace quantities of these antigens and were stimulating an anamnestic response in these adults, who had been immunized in childhood with whole-cell vaccine. An immunogenicity study in adults older than 65 years of age found Tdap to be immunogenic when administered alone or coadministered with influenza vaccine or conjugate meningococcal vaccine.[618,619]

Efficacy in Adolescents and Adults

An NIH-sponsored study, the APERT trial, evaluated the efficacy of an aP vaccine in adolescents and adults.[226] This prospective trial, conducted at eight centers across the United States, randomized 2781 healthy subjects ages 15 to 65 years to receive either an aP vaccine (Boostrix minus the Td components) or hepatitis A vaccine. For 2 years, subjects were actively followed for pertussis illness with phone calls every 2 weeks (a total of more than 58,000 person-months of surveillance). Serum specimens were obtained at routine intervals; for any cough illness lasting 5 days or longer, nasopharyngeal aspirates were obtained for culture and PCR.

The aP vaccine was well tolerated, with fewer than 5% of subjects reporting minor local or systemic adverse reactions. The study and control groups did not differ in the rate of severe adverse events, none of which were considered vaccine related. Local reactions were detected more commonly in women than in men.

Cough illnesses lasting 5 or more days were reasonably common (more than one per every 2 person-years) and were seen equally in both study groups. There were four well-documented pertussis infections per 1000 patient-years of follow-up; the incidence was highest in subjects 15 to 30 years of age and in those with cough illnesses of longest duration. A variety of case definitions were evaluated based on the strength of the laboratory evidence (positive culture, PCR, or rise in pertussis-specific antibodies in paired acute-convalescent specimens). There was a significantly lower incidence of pertussis in aP vaccinees than controls; the point estimate of vaccine efficacy was 92% (95% CI, 32–99%). The rate of pertussis seen in the APERT trial is quite consistent with rates identified in other studies of adult pertussis (see "Epidemiology," earlier) and suggests that between 1 and 1.5 million pertussis illnesses occur per year in the United States among adolescents and adults.[226]

The effectiveness of Tdap was also evaluated in an outbreak of pertussis in a school in the U.S. Virgin Islands. There were 51 confirmed or probable cases of pertussis among 499 students, for an attack rate of 10%. Disease clustered in grades 6 to 12, with a peak attack rate of 38% among 10th graders. Of 266 students 11 years of age with complete immunization data, 31 (12%) had received Tdap. Forty-one unvaccinated students (18%) had confirmed or probable pertussis, compared with 2 (6%) of the vaccinated students (relative risk, 2.9). Vaccine effectiveness was determined to be 65.6% (95% CI, 35.8–91.3%). This study suggested that Tdap provided protection against pertussis, but that increased coverage would be needed to realize the full benefit of the vaccine program.[620]

The effectiveness of a three-component Tdap was also evaluated among high-school students 12 to 19 years of age in Australia in 2005.[297] Vaccine effectiveness, measured 2 years after Tdap receipt and adjusted for potential confounding factors, was 85.4% (95% CI, 83.0–87.5) for laboratory-confirmed notified cases. Nearly all these students would have received DTwP for some or all of their prior childhood vaccinations.

A case-control study was also conducted from January 2006 to December 2011 at Kaiser Permanente among PCR-confirmed cases of pertussis in members 11 years of age and older.[298] Tdap vaccination rates were 24.0% in PCR-positive cases and 31.9% in PCR-negative controls, with an adjusted estimate of effectiveness of Tdap vaccination against pertussis of 53.0% (95% CI, 41.9–62.0%) when compared with PCR-negative controls and 64.0% (95% CI, 55.5–70.9%) when compared with Kaiser Permanente Northern California controls.

Thus, the efficacy of Tdap vaccines has been demonstrated, and their routine use to boost immunity in adolescents and adults has been recommended (see below; see also discussion below regarding waning protection). Healthcare workers, child-care workers, adolescents and adults in households containing children (particularly newborns), and pregnant women are among the high-priority target groups for immunization.[621,622]

Cocooning

"Cocooning," the vaccination of postpartum mothers and other close infant contacts with Tdap booster vaccine, has been proposed as a method to prevent infant disease and has been recommended by many national advisory groups.[623–626] However, implementation of cocooning is challenging.[626a] Those responsible for medical care of a newborn may be unable or unwilling to provide vaccinations to persons not otherwise their patients. Even when efforts are made to overcome structural barriers, outcomes can be disappointing, as noted below. In addition, the data showing that aP-vaccinated baboons colonized with *B. pertussis* were protected from pertussis disease but able to transmit pertussis to susceptible baboons may mean that protected family members nonetheless can provide a pathway for infant infection. Even if this is true, however, cocooning still would benefit the infant by reducing the risk of exposure to actively infected, coughing family members.

A number of published studies have assessed its impact. An evaluation of the impact of a cocoon program during a pertussis epidemic in New South Wales, Australia found that cocooning reduced the risk of pertussis in infants younger than 4 months of age by 51% (95% CI, 10–73%).[627] A study conducted during an Australian pertussis outbreak from 2011–12 was unable to demonstrate a benefit for the infant of cocoon vaccination.[628] A study in Basel, Switzerland found that despite vigorous educational efforts, cocooning remained incomplete in 93% of families and in most families less than 50% of the infant's close contacts received pertussis vaccination.[629] Healy and colleagues assessed the impact of a cocooning program at a metropolitan hospital in Houston, Texas and found postpartum immunization and cocooning did not reduce pertussis illness in infants 6 months of age and younger.[630]

Vaccination in Pregnancy

The difficulties of cocooning further stimulated interest in pertussis vaccination during pregnancy, which has never been a contraindication to Tdap or Td vaccination. Studies of Tdap vaccination during pregnancy show that the infant benefits from high levels of transplacentally derived maternal antibody, which provides protection during the period of greatest risk, before the infant vaccination series begins. Maternal Tdap vaccination during pregnancy results in some modest

reduction in pertussis immune responses to the first and second infant DTaP doses, but shrinking—and probably immaterial—differences in infant pertussis antibody levels after the third and fourth (toddler) doses, as detailed below.

One study of maternal Tdap vaccination during pregnancy collected maternal and cord sera at birth. Newborns of mothers who received Tdap during pregnancy had significantly higher concentrations of diphtheria antitoxin ($P < .001$), tetanus antitoxin ($P = .004$), and antibodies to PT ($P < .001$), FHA ($P = .002$), PRN ($P < .001$), and FIM-2/3 ($P < .001$) than newborns from mothers who did not receive Tdap.[631]

A double-blind, randomized, active-controlled maternal pertussis vaccination trial conducted by the NIH documented the safety of vaccine administration to both mother and infant, the antibody titers achieved in the mother and the infant, and the relatively modest impact of maternal vaccination on the infant responses to primary DTaP vaccination.[632] Preliminary data from a similar maternal pertussis vaccination trial in Canada also were encouraging.[633] In light of these data and the ongoing problem of infant pertussis, in 2011 ACIP recommended Tdap vaccination of all pregnant women.

To evaluate vaccination of pregnant women in Wisconsin following the recommendation that all pregnant women receive Tdap during each pregnancy, health insurance claims data for approximately 49% of Wisconsin births were analyzed.[634] The percentage of women who received Tdap during pregnancy increased from 13.8% of women delivering during January 2013 (63.1% of whom received Tdap 2 to 13 weeks before delivery) to 51.0% of women delivering during March 2014 (90.9% of whom received Tdap 2 to 13 weeks before delivery).

In response to a large pertussis outbreak in England with several infant deaths, a national vaccination program for Tdap vaccination of pregnant women was implemented in October 2012. To evaluate effectiveness, laboratory-confirmed cases and hospital admissions for pertussis in infants between January 1, 2008, and September 30, 2013 were collected.[635] Vaccine effectiveness was 90% (95% CI, 82–95%) when the analysis was restricted to cases in children younger than 2 months of age. The authors concluded that this effectiveness probably resulted from protection of infants by both passive antibodies and a reduction in maternal pertussis exposure. In addition, a separate case-control study determined that maternal pertussis vaccination was 93% effective (95% CI, 81–97%) in preventing pertussis infection in infants younger than age 8 weeks.[636] Vaccination during the third trimester was found by Winter et al.[637a] to be 85% more effective than postpartum vaccination of the mother at preventing pertussis during the first 8 weeks of life. Moreover, among pertussis-infected infants, hospitalization was significantly less likely and hospital stays were significantly shorter if the mother had been vaccinated during pregnancy.[637b]

Adverse Reactions in Adolescents and Adults

A NIH-sponsored multicenter trial evaluated the immunogenicity and reactogenicity in adults of varying strengths of five aP vaccines (variants of Certiva, Tri Acelluvax, Infanrix, Acel-Imune, and a PT-FHA vaccine supplied by the Massachusetts Public Health Biologic Laboratories).[617] All vaccine dosage strengths were well tolerated, although dosage-related increases in the rates of injection-site symptoms and the duration of injection-site discomfort were seen in some subjects. Late-onset or biphasic reactions, generally minor, were noted in this study, as they had been in some prior studies. The frequency of these late reactions seemed to be greater for the higher-strength dosages and for vaccines with more antigens.

Large-scale clinical trials of the Tdap vaccines demonstrated generally similar reaction profiles for Tdap as for Td vaccines; in other words, the addition of purified aP components to Td added essentially nothing to the adverse reaction profile. Biphasic or late reactions were generally not seen, and large local reactions were rare or not seen.[637–639] However, it should be noted that these studies were conducted in adolescents and adults who had been primed with whole-cell vaccine and who had received zero, one, or at most two prior injections of acellular vaccine. The safety of a Tdap vaccine was evaluated in adolescents previously vaccinated with five doses of aP-containing vaccine. Pain (63.6%), redness (51.7%), and swelling (41.4%) were more frequently reported after the sixth dose than after earlier doses. In contrast, large injection-site swelling (swelling of >100 mm, arm circumference increase of >50 mm, or diffuse swelling interfering with daily activities) occurred in only three adolescents and resolved without sequelae.[640]

The question of the minimal safe interval between prior diphtheria, tetanus, or pertussis vaccination and Tdap was addressed in a large-scale study conducted in Prince Edward Island, Canada.[641] All consenting provincial schoolchildren ($N = 7156$) in grades 3 to 12 (except 10th graders, who received the vaccine the prior year) were given Tdap, and reactions were compared according to time since prior vaccine (range, 18 months to >10 years, the latter serving as the comparison group). No variation in adverse reactions was found based on time since last Td; a slight increase in redness and swelling was found for those who had previously received DTaP, compared with those who had previously received only DTwP.

These findings were confirmed in another study reported from an outbreak in a hospital where more than 4000 healthcare workers were immunized with Tdap, half of whom completed a safety survey.[642] Adverse events after Tdap were not significantly greater in those vaccinated with other tetanus-containing vaccines less than 2 years versus 2 or more years previously. The authors concluded that a short interval between Td/tetanus toxin and a single dose of Tdap was safe.

A very large safety study was conducted among 13,427 10- to 18-year-old adolescents enrolled in the Northern California Kaiser Permanente Health Care Plan who received Tdap vaccination as part of their normal healthcare. No increased risk for medically attended neurologic reactions (OR, 0.962; 95% CI, 0.533–1.733) or allergic reactions (OR, 1.091; 95% CI, 0.441–2.729) was observed after Tdap vaccination. When compared with matched historical Td recipients, no increase in new-onset chronic illnesses (OR, 0.634; 95% CI, 0.475–0.840) was seen after Tdap, and no deaths were reported.[643]

A large study was reported from the CDC-funded Vaccine Safety Datalink in which the safety of Tdap vaccine was monitored weekly among subjects ages 10 to 64 years from 2005 to 2008. Encephalopathy-encephalitis-meningitis, paralytic syndromes, seizures, cranial nerve disorders, and Guillain-Barré syndrome were selected as outcomes based on previous reports and biologic plausibility. The risk after Tdap was not significantly higher than the risk after Td, providing reassurance that Tdap was similar in safety to Td for the outcomes studied.[644]

A Vaccine Safety Datalink study conducted in seven large U.S. health maintenance organizations evaluated the safety of Tdap in persons 65 years of age and older. Although there was a small increased risk of medically attended inflammatory or allergic events in the first 6 days following Tdap compared to other time periods, it was not more common than following Td.[645]

SAFETY OF TDAP DURING PREGNANCY

After Tdap vaccines were recommended for routine administration to pregnant women, the CDC implemented ongoing

collaborative studies to evaluate whether vaccination with Tdap during pregnancy adversely affected the health of the mothers or their offspring. In mid-2015, the CDC reported that "Data from the various monitoring activities support the safety of Tdap during pregnancy."[646] The Vaccine Safety Datalink site in California evaluated whether maternal Tdap vaccination during pregnancy was associated with increased risks of adverse obstetric events or adverse birth outcomes.[647] Of 123,494 women with singleton pregnancies ending in a live birth between January 1, 2010, and November 15, 2012, a total of 26,229 (21%) received Tdap during pregnancy and 97,265 did not. Receipt of Tdap during pregnancy was not associated with increased risk of hypertensive disorders of pregnancy, preterm birth, or being small for gestational age, but a slightly increased incidence of chorioamnionitis was observed. This finding has not yet been replicated in another study.

The most comprehensive data on the safety of maternal Tdap administration come from the United Kingdom following implementation of routine maternal vaccination.[648] In an observational study, 20,074 pregnant women (median age, 30 years) received the pertussis vaccine and were matched with a historical unvaccinated control group. There was no evidence of an increased risk of any of an extensive predefined list of adverse events related to pregnancy. In particular, there was no evidence of an increased risk of stillbirth. These safety data are reassuring but ongoing safety assessment will continue.

INDICATIONS, RECOMMENDATIONS, AND COVERAGE RATES

Immunization of Infants and Children

DTaP vaccines, given alone or as part of larger combinations, have entirely replaced DTwP vaccines in North America, Europe (except for Poland), and some Asian and Latin American countries, and are commonly used in the private sector worldwide. DTwP combination vaccines are the standard of care in Africa, much of Asia, and many Latin American countries. Table 44.5 provides details regarding pertussis vaccination schedules for many countries around the world.

The September 2015 pertussis guidance document from the WHO recommended that national programs currently administering wP vaccination should continue to use wP vaccines for the primary series, because surveillance and modeling data suggest that the use of aP vaccines may result in a resurgence of pertussis after a number of years and such resurgence may also lead to an increased risk of death in those too young to be vaccinated. WHO further recommended that a switch from wP to aP for the primary infant immunization should only be considered if the inclusion in the national immunization schedules of additional periodic booster or maternal immunization can be assured and sustained.[11a]

United States and Canada

In the United States and Canada, unless specifically contraindicated (see "Contraindications and Precautions" later), every infant should receive pertussis vaccine at ages 2, 4, and 6 months and at 15 to 18 months (in Canada, 18 months is preferred). A booster (based on DTaP in the United States and either DTaP or Tdap in Canada) is indicated at 4 to 6 years of age.[5,649] Dose 4 can be administered as early as 12 months of age, provided at least 6 months have elapsed since dose 3. The timing of infant vaccination is determined by birth age, without regard to prematurity.[408,650] Children whose vaccination series has been interrupted need not have prior doses repeated but should have the series resumed at the earliest opportunity. Combination vaccines incorporating DTaP and other antigens are detailed in Chapter 15.

Europe

There are nearly as many vaccination schedules used in Europe as there are countries. Most countries recommend three doses of a DTaP combination vaccine in the first 6 months of life, followed by a booster in the second year of life. Those following the "Scandinavian schedule" recommend doses at 3, 5, and 12 months of life, with no booster in the second year. Most countries in Western Europe recommend a booster at 4 to 7 years of age, whereas most in Eastern Europe do not.[651]

Asia, Africa, and Latin America

Among the countries in the WHO's Western Pacific and Southeast Asian regions, DTaP vaccines, alone or in combination, were used as of 2016 in Australia, Brunei, China, Japan, Malaysia, the Marshall Islands, Micronesia, New Zealand, Niue, Palau, Republic of Korea, and Singapore; all other countries in those regions were using whole-cell vaccines, mostly DTwP-Hib-HB combinations. All countries give three doses in the first 6 months of life, but only a few give a dose of pertussis-containing vaccine in the second year of life. Only Australia, India, the Republic of Korea, and some of the Oceanic republics offer a booster at 4 to 6 years of age.

In the WHO Africa Region, only Mauritius and South Africa use aP vaccines. Every country gives three doses of vaccine in the first 6 months of life (most following the EPI 6-, 10-, 14-week schedule). Only Algeria, Cape Verde, Equatorial Guinea, Gambia, Mauritius, South Africa, and Zimbabwe give a dose in the second year of life, and none give a dose at age 4 to 6 years.

In Latin America, DTaP combination vaccines are used in the public programs of Argentina (but only for premature babies), the Bahamas, Costa Rica, Mexico, and Panama. Every country gives three doses of vaccine in the first 6 months of life (most at 2, 4, and 6 months). All countries except Belize and Haiti give a dose in the second year of life; all except Antigua and Barbuda, Chile, Cuba, Ecuador, Grenada, Saint Lucia, and Saint Vincent and the Grenadines give a dose at 4 to 6 years of age (at 3 years of age for Dominica).

Percent of Children Receiving Three Doses of DTwP or DTaP

The WHO provides estimated vaccine coverage rates at http://www.who.int/immunization/monitoring_surveillance/data/en/. These estimates are derived from reports submitted by countries, estimates of WHO country managers, and the literature. For countries with well-established surveillance systems, the WHO data track closely with other available estimates. However, WHO estimates may reflect unduly optimistic reports from countries with less robust vaccination and surveillance programs.

The WHO reports that 2014 DTP3 coverage rates were 90% or greater for most countries in the Americas, but below 80% for Guatemala, Haiti, and Venezuela. In Europe, all but five countries had coverage rates of 90% or greater; only Ukraine (78%) was below 80%. In the Western Pacific region, about half of countries have rates of 90%; rates in the Philippines and four small island nations fall below 80%. In the eastern Mediterranean and Southeast Asian regions, rates in about half of countries are 85% or greater, but Iraq, Pakistan, Somalia, and Syria fall below 75%. Finally, rates in most countries in Africa fall between 65% and 85%, but rates in the

Central African Republic, Chad, Equatorial Guinea and South Sudan fall below 50%.

Immunization of Adolescents and Adults

Tdap vaccines, developed expressly to allow pertussis immunization of adolescents and adults, are now widely used for a variety of indications: among adolescents, to address the increased risk of pertussis seen in many countries in that age group; for the preschool booster dose at ages 4 to 6 years, instead of DTaP vaccine, to reduce adverse events; during the third trimester of pregnancy, to augment transplacental antibodies and reduce the risk of pertussis among neonates; among those age 7 years or older who are pertussis vaccine-naïve or are at high risk of pertussis because of medical, occupational, or other characteristics; for close contacts of high-risk persons, including healthcare workers and those with anticipated contact with infants; and in lieu of Td vaccine, for any persons desirous of augmenting their pertussis protection.

United States and Canada

In 1999, Newfoundland and Labrador implemented a school-based program to provide Tdap vaccine in Grade 9.[652] In 2003, the Canadian National Advisory Committee on Immunization (NACI) recommended that Tdap replace Td in the adolescent booster programs and that all adolescents and adults should receive a dose of Tdap in lieu of Td.[653] ACIP made similar recommendations beginning in 2005[7] and progressively extended their recommendations to cover groups such as healthcare workers, persons in close contact with infants, postpartum women, the elderly, and so on.[9,311,623,654] In 2011, ACIP recommended Tdap during pregnancy for women who had not previously received Tdap,[655] and in 2012 recommended a dose of Tdap in the third trimester of every pregnancy.[656] NACI does not recommend universal Tdap during pregnancy, but does recommend Tdap for all women beyond 26 weeks of pregnancy who have never received Tdap or who are at risk from a pertussis outbreak.[649]

Presently, ACIP recommends a single dose of Tdap for all persons age 11 years or older; all women in every pregnancy, as early in the third trimester as possible; and for initiation or completion of a primary pertussis vaccination series in persons age 7 years or older with an unknown or incomplete tetanus and diphtheria vaccination series.[657,657a] In 2014, 88% of adolescents 13 to 17 years of age had received a Tdap.[658] NACI recommendations are the same except that the adolescent dose is recommended at 14 to 16 years of age, Tdap is an optional alternative to DTaP at age 4 to 6 years, and the pregnancy recommendations differ as detailed above.[659] Apart from doses recommended during pregnancy, neither ACIP nor NACI recommend more than a single life-time dose of Tdap. However, because those recommendations rest on economic considerations rather than any actual contraindication, many practitioners elect to use Tdap whenever Td might otherwise be indicated.[660]

Europe

In 2002, the Austrian Standing Commission on Immunization noted the increasing incidence of pertussis in adolescents and adults and called for routine use of Tdap at 7 years, 14 to 15 years, and every 10 years thereafter. As of 2015, the recommendation has changed to call for a Tdap at 7 to 9 years, 13 years, then every 10 years between 18 and 60 years, and every 5 years from age 65 years on. This represents the most extensive currently recommended Tdap schedule; some Western European countries recommend one dose in adolescence and another in adulthood, most recommend only one or the other, and most Eastern European countries have no adolescent or adult Tdap recommendation.[651] As of September 2015, vaccination during pregnancy was recommended by Belgium, Ireland, Israel, and the United Kingdom, and under consideration by several other countries.

Asia, Africa, and Latin America

As of September 2015, Tdap is not recommended by any country in the Southeast Asian or African WHO regions for uses other than maternal vaccination. In light of the September 2015 WHO recommendation that "Vaccination of pregnant women is likely to be the most cost-effective additional strategy for preventing disease in infants too young to be vaccinated and appears to be more effective and favourable than cocooning,"[11a] Tdap is recommended during pregnancy in India and Thailand and under consideration elsewhere.

In the Western Pacific region, Tdap is recommended during early adolescence in Australia, Hong Kong, the Marshall Islands, Micronesia, New Zealand, Palau, Republic of Korea, and Singapore, and is recommended during pregnancy in Australia, the Philippines, Republic of Korea, Taiwan, and New Zealand.

In Latin America, adolescent Tdap is recommended in Argentina, the Bahamas (also every 10 years), Chile, Panama, and Uruguay. Tdap during pregnancy is recommended in Argentina, Brazil, Colombia, Costa Rica, El Salvador, Mexico, Panama, Paraguay, Suriname, and Uruguay. Additional Tdap recommendations include: Argentina, healthcare workers; Costa Rica, women of childbearing age; Panama, high-risk groups and healthcare workers; and Paraguay, high-risk groups.

Use of Vaccine in Outbreaks: Children

Immunization initiated after a pertussis exposure does not protect from that exposure; in an outbreak, however, exposure opportunities are ongoing and the existence of an outbreak should reinvigorate efforts to ensure that potentially exposed persons are fully vaccinated for age. An accelerated infant vaccination schedule (e.g., at 4, 8, and 12 weeks of age) may be indicated.[276] There is no evidence that supplemental doses of pertussis vaccine are required during an outbreak for the protection of most children who have received pertussis immunization in accord with the recommended schedule, and ACIP does not recommend supplemental pertussis vaccination during an outbreak for persons already fully immunized for age. However, this approach implicitly assumes that DTaP or Tdap has been given within the preceding 6 to 10 years, a condition that will become progressively less true over time. Accordingly, local public health authorities will need to consider their specific circumstances during an outbreak and may wish to recommend a repeat Tdap for those whose last prior Tdap is considered too remote. In addition, supplemental immunization for children who have immunologic or cardiopulmonary compromise, however, may be considered if the benefits of vaccination are believed to outweigh the risks of adverse reactions.

CONTRAINDICATIONS AND PRECAUTIONS

Most Infants and Children

Older recommendations regarding contraindications and precautions for pertussis vaccination reflected concerns stemming from the wP vaccine and are not supported by recent data with acellular vaccines. At present, there are but two true

contraindications to the use of pertussis vaccine: anaphylaxis or encephalopathy after administration of a vaccine containing a pertussis component.[248,458]

An anaphylactic reaction occurring immediately after administration of DTaP or DTwP is a contraindication to further vaccination with separate diphtheria, tetanus, or pertussis (acellular or whole-cell) components, absent proof as to which of these three components was responsible. Given the importance of tetanus vaccination, referral to an allergist for tetanus toxoid desensitization may be indicated. Acute encephalopathy occurring within 7 days after administration of DTwP or DTaP, not attributable to another identifiable cause, is a contraindication to further pertussis immunization. DT vaccine should be administered for the remaining doses in the vaccination schedule, although it may be appropriate to delay vaccination until the patient's neurologic status clears.[248,458]

The following are now considered precautions, not contraindications, to pertussis vaccine: temperature 40.5°C or greater within 48 hours of pertussis vaccination and not attributable to another identified cause; collapse or shock-like state (HHE) within 48 hours; persistent, inconsolable crying lasting 3 or more hours within 48 hours after vaccination; and convulsions occurring within 3 days, with or without fever. Decisions regarding the further administration of pertussis vaccine should be guided by an individualized evaluation of benefits and risks.[248,458] The risks are likely to be substantially lower if acellular, rather than whole-cell, pertussis vaccine is used.

A family history of seizures, of adverse reactions to vaccine, or of allergy to vaccine is not a contraindication to the receipt of DTwP or DTaP. Administration of pertussis vaccine should be deferred briefly for children with moderate or severe acute illnesses, with or without fever; they may be vaccinated as soon as they recover. Children who are immunocompromised or are receiving immunosuppressive therapy may be vaccinated with DTwP or DTaP. If immunosuppressive therapy will be discontinued soon, deferral of vaccination until 1 month after therapy may permit better immune responses.

Children with Neurologic Disorders

As discussed (see "Adverse Events with Whole-Cell Vaccines" earlier), pertussis vaccine may precipitate febrile convulsions and may unmask neurologic disorders that would soon have become evident anyway, but it does not appear to cause or worsen chronic neurologic disorders. Because children with neurologic impairments are as needful—indeed, perhaps more needful—of protection from pertussis disease, any decision to decline pertussis vaccination should reflect a careful consideration of risks and benefits, particularly in light of the increasing incidence of pertussis.[248,458] It may be appropriate to delay pertussis vaccination of infants with neurologic disorders until their status is clarified, but again careful consideration is required. The highest risk of pertussis is in the first 6 months of life, whereas the risk of febrile convulsions is higher thereafter; both factors therefore argue for adherence to the standard primary immunization schedule.

Children with prior convulsions should probably have pertussis vaccine deferred until the cause of the convulsions is assessed and any necessary treatment is established. Febrile seizures unrelated to pertussis vaccine are not a contraindication to further vaccination, nor is a family history of seizures.[458] Administration of acetaminophen at a dose of 15 mg/kg should also be considered at the time of vaccination and every 4 hours for the ensuing 24 hours to reduce the risk of fever associated with vaccination.[458]

The prophylactic use of antipyretic drugs was evaluated to determine whether they decreased fever in children after vaccination and whether they impacted the immune response to the vaccines. The percentage of children with temperature 38°C or higher after at least one dose was significantly lower in the prophylactic paracetamol (acetaminophen) group than in the no-treatment group. Antibody geometric mean concentrations were significantly lower in the prophylactic paracetamol group than in the no-treatment group after primary vaccination for all 10 pneumococcal vaccine serotypes, protein D, antipolyribosyl-ribitol phosphate, antidiphtheria, antitetanus, and anti-PRN. After boosting, lower antibody geometric mean concentrations persisted in the prophylactic paracetamol group for antitetanus, protein D, and all pneumococcal serotypes apart from 19F. The authors recommended that "although febrile reactions significantly decreased with prophylactic antipyretic drugs at the time of vaccination, they should not be routinely recommended since antibody responses to several vaccine antigens were reduced."[459]

False Contraindications to DTaP

A number of situations have been considered incorrectly to be contraindications to DTaP, and deferral of pertussis vaccination on these bases is inappropriate: redness, swelling, or pain at the injection site; temperature less than 40.5°C; mild acute illness, even involving diarrhea or low-grade fever; current antibiotic therapy; recent exposure to an infectious disease; prematurity; personal or family history of allergies; and family history of SIDS, convulsions, or adverse event after pertussis vaccination.[458] Prior pertussis infection is not a contraindication to vaccination. Going ahead with vaccination would provide protection if the prior illness was not in fact pertussis.

Adolescents and Adults

For adolescents and adults, the only contraindications to Tdap vaccine are a history of serious allergic reaction to a component of the vaccine and a history of encephalopathy not attributable to another identifiable cause within 7 days after pertussis vaccination. Precautions include a history of a vaccine-related Arthus reaction, Guillain-Barré syndrome, or progressive neurologic reaction; in such cases, individualized consideration should be given to risks and benefits.

Other reactions after pediatric vaccination, including high fever, syncope, seizure, HHE, and large local reaction, are not contraindications or precautions, nor is a prior diagnosis of pertussis.

PUBLIC HEALTH CONSIDERATIONS

Disease-Control Strategies

There is but one disease-control strategy for pertussis: vaccination. But with which vaccine? Numerous wP and aP vaccines have been evaluated in efficacy trials. Which ones should be used?

In North America and Europe, acellular vaccines are widely used and have largely or entirely replaced the whole-cell vaccines. Despite a high rate of vaccine coverage, pertussis is the only vaccine-preventable disease currently increasing in the United States and in other developed countries. Expanded Tdap booster vaccinations in adolescents and adults are recommended, but it is not clear that this will control pertussis disease. In most countries, multivalent combination vaccines are used (see Chapter 15).

Outside North America and Europe, many countries use acellular vaccines (usually as combinations) in the private market, but few countries have implemented them in their

national vaccination programs. Such countries will have to apply their own relative weights when evaluating the comparative costs, efficacies, and adverse effects of the acellular and whole-cell vaccines. Many countries may decide that their present whole-cell vaccine offers unsurpassed efficacy at low cost; if the vaccine is well accepted by the public, no change is indicated. However, with the increasing availability of combination vaccines, these decisions are likely to become even more complex (see Chapter 15).

Eradication or Elimination

Humans are the only reservoir for pertussis, and chronic carriage is not known to occur. In principle, then, pertussis can be eradicated. Acellular vaccines are suitable for use among all age groups, but it remains to be seen whether their widespread use can interrupt transmission of pertussis in a region. Global eradication, although perhaps possible, is clearly many years away.

FUTURE VACCINES

Different approaches have been proposed during the last decade to achieve better pertussis control. These approaches include returning to the whole cell vaccine, the addition of new pertussis antigens to the current acellular vaccines, the addition of adjuvants to the current vaccines, and the development of live-attenuated pertussis vaccines. None of these new options have yet led to a new licensed pertussis vaccine, but the various efforts will be briefly reviewed.

Adjuvanted Vaccines

Currently licensed DTaP vaccines contain alum as an adjuvant that elicits a strong Th2-type immune response. However, the observation that a strong Th1 response appears to play a crucial role in the clearance of *B. pertussis* has led to the proposal of combining new novel adjuvants with DTaP to elicit a more Th1-skewed response.[661,662] A Canadian study tested the impact of various adjuvants (polyphosphazene microparticles containing pertussis toxoid, cytosine phosphate guanine [CpG] oligodeoxynucleotides, and a synthetic innate defense regulator peptide) on pertussis immune responses in mouse models and found significantly higher levels of proinflammatory cytokines in macrophages.[663] Mouse-challenge studies demonstrate significantly lower bacterial loads with adjuvanted vaccines compared to unadjuvanted vaccine formulations (additional adjuvants evaluated include MF59, monophosphoryl lipid A, and other Toll-like receptors, and immunostimulatory molecules).[663–665]

Gracia and colleagues evaluated, in mice, CpG oligodeoxynucleotides, polyphosphazenes, and cationic innate defense regulator peptides as adjuvants when combined with genetically detoxified PT. The adjuvants induced increased serum antibody titers that persisted for longer than 22 months.[662] UK investigators have also demonstrated that CpG oligodeoxynucleotides, alone or combined with aluminum hydroxide, enhanced humoral and T-cell responses.[666]

Another report demonstrated that the addition of CpG oligonucleotides could overcome suppression of infant immune responses to PT by maternally derived antibodies, supporting the potential for novel vaccine adjuvants to improve protection.[667]

Finally, an Australian group assessed the immune enhancement provided by a nanoparticle formulation of PT and IgA, which induced significantly higher serum antibody titers when compared with the conventional alum-adjuvanted PT formulation. The authors concluded that this formulation had potential application as a nasal vaccine delivery system.[668,669]

The effect of adding novel adjuvants to the current DTaP vaccines has not been studied in humans to date, but offers the potential to improve immune responses.

New Antigens

Argentine investigators have reported the evaluation of two unique pertussis proteins as vaccine candidates, IRP1–3 and AfuA. These surface-exposed, highly immunogenic pertussis proteins are involved in iron uptake and are expressed under physiological conditions. Inclusion of IRP1–3 and recombinant AfuA in an experimental vaccine enhanced greater protection against pertussis challenge in a murine model, compared to vaccine without these proteins, suggesting that they might be good candidates for inclusion in a vaccine.[670,671]

Adenylate cyclase has been proposed as another antigen that might be added to an aP vaccine. Studies in a mouse model of this antigen showed that a recombinant form of adenylate cyclase lacking enzymatic activity enhanced protection against intranasal administration of *B. pertussis* and that both Th1 and Th2 immune responses to *B. pertussis* antigens were induced.[672]

Another intriguing approach is the use of outer membrane vesicles containing naturally incorporated bacterial surface antigens such as PRN and PT.[673] Roberts and colleagues showed that the administration of intranasal or intraperitoneal outer membrane vesicles derived from *B. pertussis* induced protection against pertussis challenge in a mouse model.[674]

De Gouw and colleagues have evaluated *B. pertussis* biofilm-derived membrane proteins.[675] It was found that a protein termed *Bordetella* intermediate protein A (BipA) was the most abundant surface-exposed such protein and that antibodies to BipA were present in convalescent serum of whooping cough patients. Vaccination of mice with recombinant BipA generated opsonizing antibodies and significantly reduced colonization of the mouse lung.

Many experts have noted the attractive qualities of the genetically inactivated PT, such as used in the three-component vaccine *Acelluvax* evaluated in the Italian efficacy trial, and it is likely to be incorporated into newly developed aP vaccines. Robbins and colleagues have proposed a new vaccine that would conjugate genetically inactivated PT to a *Bordetella* oligosaccharide, to provide more durable protection.[676]

Detoxified or Live-Attenuated Pertussis Vaccines

B. pertussis genetically modified to inactivate or eliminate certain toxic moieties might offer a vaccine with immunologic benefits approaching those of wP vaccine while resembling aP vaccines in safety.[677,678] The most advanced candidate at present is a live-attenuated pertussis vaccine termed BPZE1, which is an approach that may have a number of advantages over the current parenteral inactivated vaccines. First, nasal administration to young children is appealing with the avoidance of injections, although tetanus and diphtheria toxoids would still need to be administered by injection. Second, mucosal administration stimulates local immunity that could counteract pertussis organisms in the nasopharynx at the site of infection.[667] In a mouse model, BPZE1 stimulated comparable antibody responses to those seen after two parenteral administrations of aP.[679] Protection mediated in this model was found to be associated with both antibody and Th1 and Th17 $CD4^+$ cells, but not $CD8^+$ T cells.[680] Protection of mice against *B. bronchiseptica* has also been shown with this vaccine, invoking similar mechanisms as seen with pertussis.[681] Mice immunized with the live-attenuated strain were protected for

1 year after immunization, suggesting that the live-attenuated vaccine induced long-lasting antibody responses.[682] Finally, a prime-boost regimen in mice of intranasal BPZE1 followed by aP injection resulted in enhanced immune responses with a strong Th1–Th17 response that was maintained after repeated aP administrations.[683] Human studies with live-attenuated strains have begun.[684,685] A Phase I study found successful colonization of 7 of 36 adults given BMZE1 intranasally, with good cellular immune responses. A second clinical trial is underway.[685]

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45 Plague Vaccines

E. Diane Williamson and Petra C.F. Oyston

During the last two millennia, the bacterium *Yersinia pestis* has been responsible for social and economic devastation on a scale unmatched by other infectious diseases or armed conflicts. The first reliable reference is to the Justinian plague (542–750 AD), which originated in central Africa and spread throughout the Mediterranean Basin. The second pandemic, the Black Death, which started on the Eurasian border in the mid-14th century, may have caused 25 million deaths in Europe (25–30% of the population), persisted on the continental land mass for several centuries, and culminated in the Great Plague of London in 1665. The third pandemic started in China in the mid-19th century, spread east and west, and caused 10 million deaths in India alone. Credible estimates indicate that overall almost 200 million deaths could be attributed to plague,[1] which swept across Europe in these three major epidemics.[2] The disease occurred in both bubonic and pneumonic ("Black Death") forms. The bubonic form spreads as a result of transmission of the bacterium from rodents to humans via the bites of infected fleas (usually the rat flea, *Xenopsylla cheopsis*[2]). The close contact of humans with infected rats undoubtedly contributed to the spread of the disease by this route. In some instances, bacteremic spread of plague bacilli to the lungs leads to the development of the secondary pneumonic form of the disease; subsequent person-to-person transmission by respiratory droplets can result in rapid epidemic spread of primary pneumonic plague.[3] It is the pneumonic form of the disease that is most feared and that is associated with a mortality rate approaching 100% when untreated.[4,5] A recent report has suggested that the plague-causing bacterium existed long before previous estimates.[6] Bacteria morphologically similar to *Y. pestis* have been identified on the proboscis and compacted in the rectum of a flea that had been preserved in amber, and estimated to date back 20 million years. If these bacteria are indeed *Y. pestis,* they may be ancient strains that evolved as rodent parasites that are now extinct, as genomic data indicate that the flea–animal–human transmission cycle for *Y. pestis* evolved some 20,000 years ago. Wagner and colleagues retrieved teeth from two victims of the Justinian plague and compared the DNA sequences obtained against a database of more than 130 *Y. pestis* strains from the second and third pandemics.[7] This study identified differences in genome sequences, indicating that the transmission of *Y. pestis* from rodents to human beings was a lineage of *Y. pestis* independent of the lineage that caused the Black Death some 800 years later.

For reasons that are not fully understood, epidemics of urban plague have dramatically waned, but data from the 1990s reported by the World Health Organization (WHO) indicate that plague was still a significant public health problem, especially in Africa, Asia, and South America (Fig. 45.1).[8,9] During 2005, an outbreak of pneumonic plague at a diamond mine in Northern Congo caused 114 confirmed infections among the miners, 54 of whom died.[10] The outbreak was limited only by the dispersal of miners fleeing in panic from the area.[10] Between 2005 and 2009, more than 12,500 cases of plague were reported to the WHO, with a fatality rate of 6.7%, from 16 countries.[11] The majority of these cases (>97%) were reported from eight African countries, with fewer than 150 cases each from Asia and the Americas, resulting in 23 and six deaths on these continents, respectively. During 2009, the United States reported 27 plague cases resulting in five deaths, while an outbreak in the Qinghai Province of China, associated with the marmot hunting season, resulted in 12 cases of pneumonic plague with three deaths.[11] During 2010, more than 30 cases of plague, predominantly bubonic but with some pneumonic and septicemic cases also, were reported in Peru.[12] Between January 1, 2010, and December 21, 2015, 3,248 cases of plague in humans were reported, resulting in 584 deaths.[12a] As of February 2016, the three most endemic countries are reported to be Madagascar, Democratic Republic of Congo, and Peru.[12a] During 2014, 119 cases of plague were reported in Madagascar with at least 40 deaths.[13]

During 2015, there was a greater-than-usual incidence of plague reported from the United States, with a total of 11 cases reported from Arizona, California, Colorado. Georgia, New Mexico, and Oregon, and three deaths reported.[14] These cases were predominantly bubonic and were attributed to exposure to fleas that had bitten infected wild animals.

Thus, even in the 21st century, plague still occurs episodically in parts of the world where environmental conditions permit. In plague-endemic areas of the world, vaccines may be useful in preventing plague in people at high risk, as well as in those who are involved in research and diagnosis of the disease.

BACKGROUND

Clinical Description

Bubonic Plague

Bubonic plague is the form of disease typically transmitted to humans by an infected flea, that flea having previously fed on an infected rodent. In some circumstances, infection can occur via open wounds that are exposed to infected material through handling and other direct contact.[4] Within 2 to 6 days of infection, the patient develops a fever, headache, and chills.[15] Occasionally, lesions develop at the site of inoculation. The classic feature of bubonic plague is the development of swollen and tender lymph nodes called *buboes,* from the Greek word *bubon,* meaning groin. The buboes are often located within the inguinal and femoral lymph nodes, which drain the original site of infection on the lower extremities.[4] Bacteremia is common in patients with bubonic plague, typically resulting in blood culture counts ranging from fewer than 10 and up to 4×10^7 colony-forming units (CFU)/mL.[4] High levels of bacteremia are often associated with gastrointestinal symptoms such as vomiting, nausea, abdominal pain, and diarrhea.[4] Early intervention during the course of disease with antibiotics such as streptomycin, gentamicin, tetracycline, chloramphenicol, or the fluoroquinolones, usually leads to rapid recovery.[16]

Septicemic Plague

Primary septicemic plague is a clinical diagnosis in a patient who has acute toxic illness, large numbers of *Y. pestis* in the bloodstream, and no identifiable anatomical site of infection, such as a peripheral bubo.[15] Clinically, the disease appears

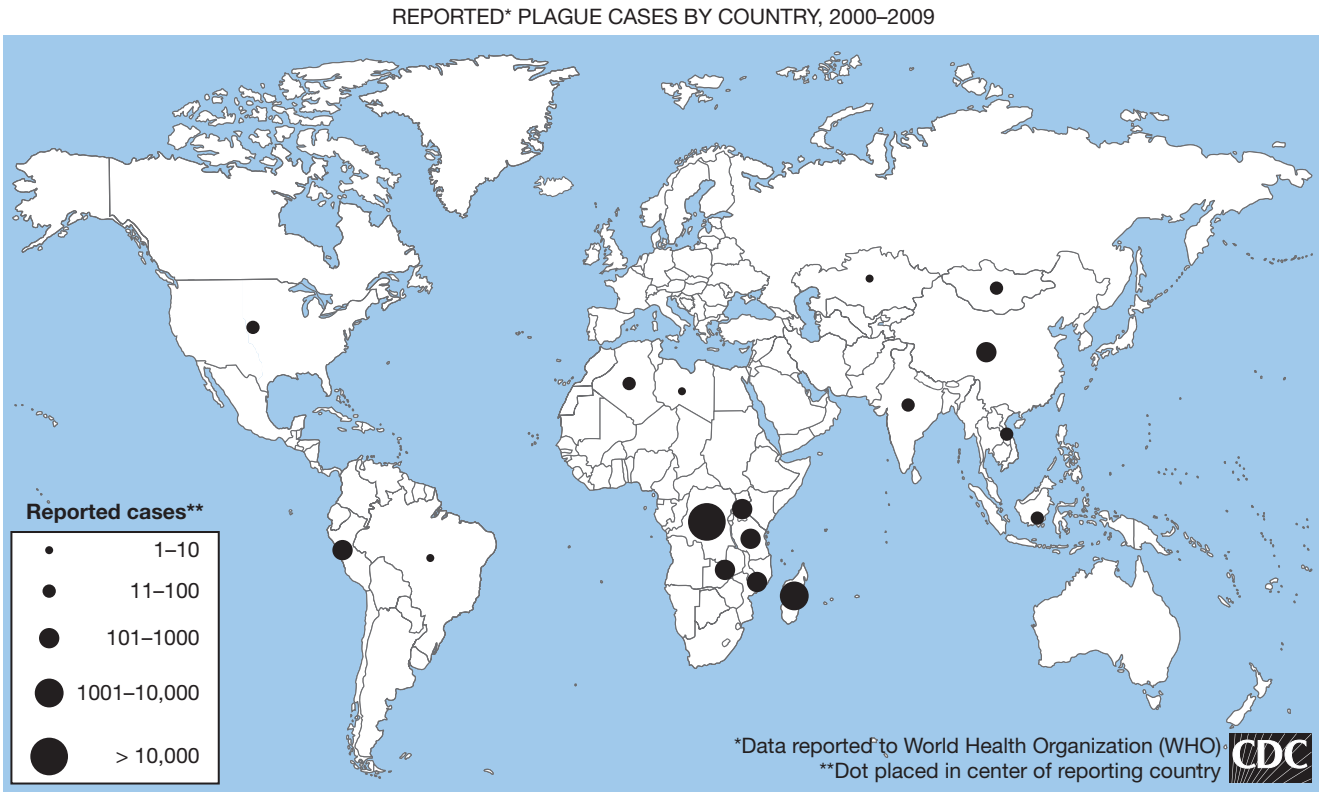


Figure 45.1. Occurrence of plague in the modern world. *(Modified from Division of Vector-borne Diseases, Centers for Disease Control and Prevention [CDC], Atlanta, GA, 1998.)*

similar to other gram-negative septicemias, with elevated temperature, chills, headache, malaise, and gastrointestinal disturbances. In the absence of aggressive treatment, life-threatening complications of the systemic inflammatory response syndrome occur, such as disseminated intravascular coagulation with bleeding, adult respiratory distress syndrome, shock, and organ failure. Owing to the absence of localizing signs, the diagnosis of primary plague sepsis may be delayed and result in a high case fatality rate. The overall case fatality rate for persons with plague sepsis approaches 20% to 40%.

Pneumonic Plague

Some colonization of pulmonary tissues occurs in most untreated fatal cases of plague, with the potential to develop into a secondary pneumonic plague. However, most of these patients do not develop a transmissible plague pneumonia.[15] However, when there is colonization of the alveolar spaces after a respiratory exposure, a suppurative pneumonia develops, and during the terminal stages of disease there is coughing with the production of a highly infectious, watery, and bloody sputum. Pneumonic plague is the most widely feared form of the disease because it is suggested that *Y. pestis* can be spread from person to person as respiratory droplets formed during coughing.[17] A number of experiments with animals (guinea pigs, lemurs, and nonhuman primates) have demonstrated the potential for the cross-infection of control animals from infected animals showing the symptoms of pneumonic disease.[18–22] More significantly, good evidence supporting the potential for the airborne spread of infection in humans has been derived from an analysis of an outbreak of pneumonic plague in Madagascar.[23] In this outbreak, four cases of pneumonic plague were attributed to contact with one patient who had developed secondary pneumonic plague. These four infected individuals apparently then transmitted the disease to 11 others, one of whom transmitted the disease to two further individuals.[23] In total, 18 individuals contracted pneumonic plague resulting from this single individual and eight died.

The available evidence indicates that the inhalation of airborne droplets containing *Y. pestis* by susceptible individuals leads to the development of pneumonic plague within 1 to 3 days.[15] The rapidity with which the infection spreads between individuals, along with the relatively short incubation period, makes control of the disease difficult and antibiotic therapy may be ineffective once the pulmonary symptoms have developed.[4,15,16] Primary pneumonic plague is now rare; in the United States, the few cases that have been reported in recent years have been in veterinarians and owners of domestic cats who have contracted pneumonic plague from their pets[4,5] or in individuals who have been exposed to infected wild animals. Nevertheless, the potential for pneumonic plague to spread quickly in human populations is evident (e.g., from the 2005 outbreak in the Northern Congo diamond mine[9] and in the later outbreak in Peru in 2010).[12]

Bacteriology

The etiological agent of plague is *Y. pestis*, a gram-negative bacterium that is a member of the family Enterobacteriaceae. The bacterium is able to grow at temperatures between 4°C and 40°C and has nutritional requirements for L-isoleucine, L-valine, L-methionine, L-phenylalanine, and glycine. The species has been subdivided into three biovars (*Orientalis, Mediaevalis,* and *Antigua*) on the basis of the ability to convert nitrate to nitrite and to ferment glycerol; however, all three biovars show similar virulence in animal models.[4] The genus *Yersinia* also includes *Yersinia enterocolitica* and

Yersinia pseudotuberculosis species, both of which are pathogens of humans but rarely cause disease with a fatal outcome.[1,16] Newer studies show that *Y. pestis* evolved from *Y. pseudotuberculosis* (most probably serotype 1b) between 1500 and 20,000 years ago.[24] Recent attempts to reconstruct the genome sequence of the medieval *Y. pestis* from DNA isolated from the teeth of victims of the Black Death, using the contemporary CO92 strain as a reference, have not indicated major genetic differences between medieval and the CO92 strain of *Y. pestis*, although this approach could not rule out the loss of genomic regions that may have existed in the ancient strain.[25] The results from independent studies reporting the analysis of DNA from the eras of the Justinian and Black Death plagues classify these samples as most similar to the Orientalis biovar.[26–28] An intermediate form, termed *Pestoides*, has been identified. Genome sequence analysis reveals that Pestoides strains do not contain the full complement of plasmids associated with *Y. pestis* and retain chromosomal loci found in *Y. pseudotuberculosis* but lost in *Y. pestis*.[29,30] Pestoides isolates are virulent, particularly by aerosol routes,[29,30] but can show attenuation by systemic routes[30] and are enzootic strains maintained in rodents of the superfamily *Muroidea* comprising rats, mice, hamsters and gerbils.[31]

Evolution of *Y. pestis* has been by the inactivation of a range of genes that are thought to be required for an enteric lifestyle and acquisition of new virulence factors.[32] The acquired virulence genes appear to be located primarily on two plasmids. Both *Y. pestis* and *Y. pseudotuberculosis* possess a 70-kilobase (kb) virulence plasmid called pYV that carries a type III secretion system operon.[33,34] *Y. pestis* possesses two further unique plasmids, a 9.5-kb plasmid, pPCP1, and the 100- to 110-kb pFra plasmid.[1] The *pla* gene on pPCP1 encodes a surface-bound protease (plasminogen activator), which has potent fibrinolytic activity (Fig. 45.2).[1] The possession of the pPCP1 plasmid has recently been used to date the evolution of *Y. pestis* from *Y. pseudotuberculosis*.[35] Although the oldest Pestoides strains (E and F) do not possess the pPCP1 plasmid, Angola and Pestoides A strains, which arose later, do contain the pPCP1 plasmid. Furthermore, Angola and Pestoides A strains do not possess a later sequence change in the Pla protein which optimizes its function in disseminating bacteria in vivo; this indicates that the pPCP1 gene was acquired by *Y. pestis* relatively early in its evolution from *Y. pseudotuberculosis*, and before it was fully adapted to its flea-vectored lifestyle. The pFra plasmid shows extensive sequence homology with plasmid pHCM2, possessed by some strains of *Salmonella enterica* serovar Typhi.[36] However, some regions of pFra appear to be unique to *Y. pestis*, and one of these regions includes the *caf* operon responsible for synthesis of the protein capsule expressed by *Y. pestis* at 37°C.[37]

Pathogenesis

Plague has a complex lifestyle, cycling between arthropod and mammalian hosts, with the primary mammalian hosts being rodents: humans are accidental hosts. As a result of this lifestyle, the organism has had to develop sophisticated strategies to ensure transmission from infected rat to the flea, then from the flea to a new host. The rat flea (*Xenopsylla cheopis*) ingests bloodborne bacteria from the infected rodent, and growth of the bacteria leads to blockage of the flea's foregut. The hemin storage system is thought to play an important role in the formation of this blockage,[38] with the result that mutants of *Y. pestis,* lacking the hemin storage locus, established long-term infection of the flea's midgut but failed to colonize the proventriculus, preventing the efficient transmission of bacteria.[38] The blockage prevents digestion of the blood meal, and further ingestion of blood leads to regurgitation of bacteria-contaminated material from the flea into a mammalian host.[39] In achieving its transmission, the pathogen results in the death of both mammalian host and flea vector. High levels of septicemia in the infected mammal are required to allow the infection of a subsequent flea vector, and efficient transmission from the flea is required to infect the mammal.[40] However, in situations in which humans and rodents are in close proximity or when the rodent population is reduced, either as a result of the disease or rodent control measures, humans and other warm-blooded mammals serve as alternative hosts. In this respect, *Y. pestis* differs from the other human pathogenic *Yersinia* species in that it is an obligate parasite. The organism circulates in a "sylvatic" form in wild rodent populations, typically causing a fatal disease in mice and squirrels and a milder, subclinical infection in gerbilline and dipodids[41] such as the Jerboas. Other reservoirs of infection include prairie dogs, rabbits, and members of the cat family, including the domestic cat.[4]

Thus the infection of humans usually occurs as the result of a bite from an infected flea and it has been suggested that as many as 24,000 bacteria are delivered into the host with a single bite.[4] However the "blocked" flea model alone cannot account for the rates of spread of plague during plague endemics and it is thought that unblocked fleas are also able to transmit infection, by a so-called early phase transmission process.[42] This would allow the flea to transmit bacteria to its

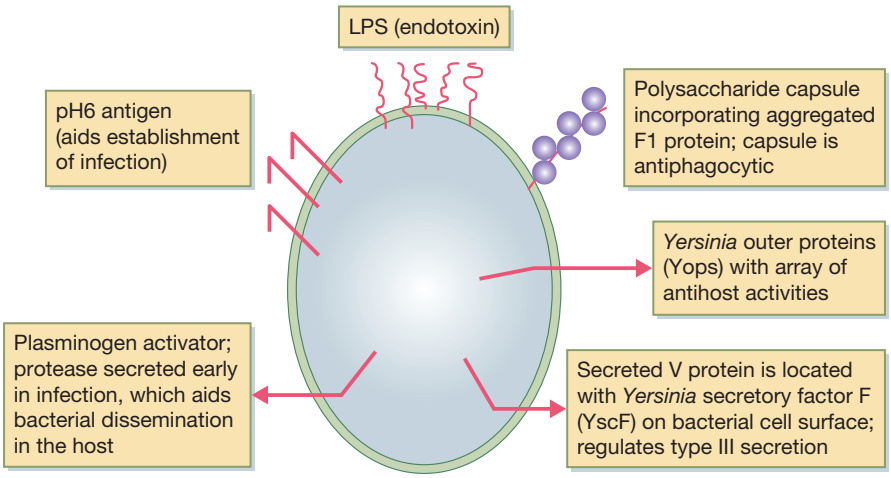


Figure 45.2. Virulence factors expressed by *Yersinia pestis*. LPS, lipopolysaccharide.

host in a much shorter time interval after its latest blood meal and would promote sylvatic epizootics and possibly also maintain endemic disease in man at times of low flea burdens or where human flea populations *(Pulex irritans)* predominate. To date, only three factors have been positively confirmed as essential for transmission from flea to mammal: a murine toxin, an extracellular polysaccharide, and a lipopolysaccharide core modification locus. The murine toxin has phospholipase D activity that is essential for survival in the flea midgut,[43] whereas the extracellular polysaccharide and lipopolysaccharide (LPS) core modifications are required for biofilm formation and blockage of the flea.[38,44] However transcriptional analysis of *Y. pestis* in the flea gut identified a wide range of genes, such as the insecticidal-like toxin genes, that were differentially regulated, expression of which meant that the bacteria regurgitated into a new host had increased resistance to innate immune effectors.[45]

Upon infection of a new host, the plague bacilli are vulnerable to phagocytosis by polymorphonuclear leukocytes or monocytes. However, bacteria from fleas showed significantly lower levels of phagocytosis than bacteria grown in vitro, indicating the importance of the complex influence of growth in the flea on pathogenesis.[45] The insecticidal-like toxins were key components in resisting phagocytosis in the early stages of infection,[45] before the antiphagocytic type III secretion system could be induced.

There is controversial evidence that preferential phagocytosis in monocytes/macrophages may be facilitated by the chemokine receptor CCR5,[46–48] prevalent on monocytes/macrophages and dendritic cells, but not on polymorphonuclear leukocytes. A specific role for Ccr5 in plague pathogenesis remains uncertain, appearing possible from in vitro bacterial uptake studies, but less likely from in vivo susceptibility studies in Ccr5 knockout mice. Ccr5 is a coreceptor with CD4 facilitating entry of another infection, HIV, into host cells.[46–48]

The plague bacteria are killed within polymorphonuclear leukocytes, but persist within monocytes and express various virulence determinants, thereby allowing growth and eventual release from the monocytes.[49] One such virulence determinant is the pH6 antigen, a fibrillar adhesin induced by the low phagosomal pH (pH of 4.5),[50] which enhances resistance to phagocytosis.[51] The F1 capsule likely also plays a key role in avoiding phagocytosis.[52] However, mutants of *Y. pestis* that are unable to produce F1 antigen are still able to cause disease in the mouse, although they have reduced virulence.[53] It appears that possession of the locus encoding the F1 antigen increases transmissibility from the flea,[54] although this is not observed following needle challenge in a laboratory setting.

The dominant antiphagocytic effect is a result of induction of the type III secretion system carried on the large virulence plasmid. Type III system effectors, historically called *Yersinia* outer membrane proteins (Yops), are secreted into host target cells and result in phagocyte killing. The function of many of the Yops has been determined in this well-characterized secretion system, which serves as a paradigm for all type III secretion systems.[33] For example, the YopE protein is a cytotoxin that is transferred into host cells after bacterial contact[55]; the YopH protein is a tyrosine phosphatase that has antiphagocytic cell activity.[55] The V antigen component of the type III system appears to play several roles in the pathogenesis of disease. It is present at the tip of the injectisome that delivers Yops into the host cell.[56] Additionally, V antigen secreted from *Y. pestis* exerts a local immunomodulatory effect in the host, by downregulating the production of interferon-γ and tumor necrosis factor-α.[57,58]

Another major virulence factor required for dissemination from the site of infection is the plasminogen activator (Pla). Pla is an outer membrane located protease, required to break down the physical barriers of connective tissue in the host and thus Pla is essential in the dissemination away from the injection site of the bolus of *Y. pestis* injected by the flea; this has driven the selection of a "rough" phenotype of LPS which lacks an O antigen.[59,60] While rough LPS is rare among gram-negative bacteria, it allows for the functionality of Pla. On the other hand, when O antigen expression is forced, it blocks the activity of Pla, and is thus attenuating by both dermal and respiratory routes of infection,[61,62] but not following intravenous inoculation.[63] Pla interacts with the DEC-205 receptor on antigen-presenting cells to promote uptake, and it appears that dissemination only occurs after phagocytosis.[62]

The bacteria disseminate from the site of primary infection into regional lymph nodes that drain these tissues. Within the lymph node, further growth of the bacteria, accompanied by a massive inflammatory reaction, leads to lymphadenopathy and the formation of buboes. In the bubo, bacteria are predominantly extracellular, mainly because of the type III secretion system, which is highly expressed in the lymph node.[54] An ability to proliferate in the bubo requires efficient iron-acquisition systems. *Y. pestis* possesses 10 iron-acquisition systems, which are actively used in the bubo.[54]

Eventually, the bacteria are disseminated by the lymphatic system, gain access to the bloodstream, and colonize pulmonary tissues, which may lead to development of the pneumonic form of the disease. Untreated, pneumonic plague is almost invariably fatal, inducing an overwhelming septicemia that triggers septic shock in the host. However, the precise mechanisms that lead to death of the host have not been identified. Systemic induction of nitric oxide synthase may be the terminal event, as seen with other gram-negative septicemias.[64]

Although pigmentation-negative strains of *Y. pestis* are usually avirulent and attenuated, the risk of reversion to virulences was recently highlighted by the fatal case of a laboratory worker who was unknowingly suffering from hemochromatosis and who was exposed to the attenuated pgm^- (pigmentation) *Y. pestis* laboratory strain KIM. This individual developed and died from plague, presumably from the complementation of the haem deficiency by the individual's medical condition.[65]

Models of Disease and Protection

Y. pestis causes disease in a wide variety of laboratory animals,[4] including mice, Brown Norway rats,[66] guinea pigs, and nonhuman primates.[67] Additionally, several species, such as the black-footed ferret[68] and the prairie dog,[69] have been actively immunized against plague. Most experimental work has been carried out in the mouse model of disease, however the model may not faithfully mimic human disease because of the unique susceptibility of mice and rats to the murine exotoxin.[70] Although this limitation might be resolved by using the guinea pig model of disease, the protracted nature of the disease in the guinea pig[71] suggests that the mouse model is a better indicator of the infection that occurs in animal reservoirs. In a comparative study with mice and guinea pigs, it was concluded that mice were more suitable for the evaluation of plague vaccines[72] and this species was approved by the U.S. Public Health Service for the testing of plague vaccines. Disease arising from the delivery of *Y. pestis* by the subcutaneous route (median lethal dose [LD_{50}]: 1–2 CFU[73]) is considered to mimic bubonic plague, whereas the exposure of mice to the bacteria via inhaled aerosols results in the pneumonic form of the disease (LD_{50}: 2×10^4 CFU[74]). The efficacy of the original Cutter Killed Whole Cell (KWC) vaccine was determined by measuring the ability of sera passively transferred from immunized mice, guinea pigs, monkeys, or humans to protect naïve

mice against *Y. pestis*, with the derivation of a mouse protection index (MPI).[75] A potency test based on active immunization and organism challenge in the mouse model is being developed to test the efficacy of a next generation subunit plague vaccine.

Diagnosis of Plague

The clinical diagnosis of plague is supported by laboratory tests. Bacteriological diagnosis of plague is usually made on the basis of the analysis of aspirates taken from a bubo, or from blood or sputum samples. The most straightforward analysis involves Gram or Wayson staining of air-dried smears on microscope slides.[76,77] After staining, *Y. pestis* cells appear as small gram-negative rods with a characteristic bipolar staining (safety-pin appearance). Culture on Congo Red agar, ideally at 26°C to 28°C, results in so-called bull's-eye colonies, which have a red pigmented central region and paler margins.[77] However, culture methods are generally considered to be too slow (typically taking 48 hours) in the context of the rapid progression of the disease (especially pneumonic plague) and a presumptive diagnosis should be made before such tests are completed.

A variety of confirmatory tests for *Y. pestis* have been proposed, including the use of fluorescent antibody to the capsular F1 antigen, passive hemagglutination tests, the use of the polymerase chain reaction, and enzyme-linked immunosorbent assay (ELISA).[73,78–80] These tests can be used with samples such as bubo aspirates for the rapid diagnosis of disease.[78] An exciting development is the demonstration that an immunogold-chromatography dipstick can be used for the direct analysis of bubo aspirates, serum, or urine samples.[81] Such tests have subsequently been sent to endemic countries to evaluate their use for the rapid detection of plague.[82] Conceivably, such tests might be used outside the laboratory and at the patient's bedside for the rapid diagnosis of plague.

Treatment and Prevention With Antimicrobials

The successful treatment of plague is dependent on the prompt commencement of therapy during the early stages of the disease. In cases of bubonic plague treated with antibiotics, the fatality rate is generally less than 5%. In contrast, the successful treatment of septicemic or pneumonic plague is less certain because of the rapidity with which the disease develops and the difficulties of making an early diagnosis.

A number of antibiotics, including streptomycin, tetracycline, and ciprofloxacin, are approved by the U.S. Food and Drug Administration (FDA) for the treatment of plague.[76,83] For the treatment of plague meningitis, intravenously administered chloramphenicol is the antibiotic of choice because of its ability to cross the blood-brain barrier.[76,83] However, alternatives include streptomycin (or gentamicin) with or without doxycycline or a fluoroquinolone such as ciprofloxacin or levofloxacin.[84–87] The fluoroquinolones ciprofloxacin and levofloxacin have been licensed by the FDA for the indication of inhalational plague.[88]

For all antibiotics, a 10-day course is recommended. In mice infected with *Y. pestis* by the airborne route, successful treatment of disease with ciprofloxacin was dependent on the commencement of antibiotic dosing within 24 hours of exposure to the pathogen.[83]

Improvements in the condition of patients suffering from bubonic plague are seen within 2 to 3 days, but buboes may remain for several weeks after successful treatment. A strain of *Y. pestis* showing plasmid-mediated resistance to tetracycline, streptomycin, chloramphenicol, and sulfonamides has been isolated from a case of bubonic plague in Madagascar.[89] The failure of a patient to respond to treatment with one or more of the antibiotics named above, should alert the clinician to the possibility of an antibiotic-resistant strain of *Y. pestis*.

Family members and other close contacts with individuals suffering from plague should be maintained under surveillance for at least 7 days after their last possible exposure to *Y. pestis*. It may be appropriate to use tetracycline or doxycycline prophylactically for 7 days in these individuals, especially if the primary case is pneumonic plague. The prophylactic use of antibiotics is recommended even in individuals who have been previously vaccinated with the KWC vaccine and who might have been exposed to airborne *Y. pestis*[5] because of the limited ability of this vaccine to provide protection against pneumonic disease.[90]

EPIDEMIOLOGY

Endemic foci of animal plague occur mainly in semi-arid regions of the world (see Fig. 45.1). The main foci have been identified in the southwestern United States, the former Soviet Union, South America, South Africa, and Asia. Not surprisingly, these are the regions of the world that also report the highest incidence of human plague. Each year there are several thousand reported cases of the disease worldwide,[91] with a fatality rate between 5% and 15%,[8,9] and plague has been classified as a reemerging disease by the WHO with endemic regions as described above. There was an apparent increase in the incidence of disease during the 1990s, although it is possible that this increase was the result of more efficient diagnosis and reporting of cases. However, recent statistics on the global incidence of plague serve as a reminder that the disease can suddenly reemerge in endemic regions.[11] A significant outbreak of plague occurred at Surat in India in 1994. Serological testing of individuals during this outbreak showed that there were 876 presumptive cases of plague and 54 fatalities.[4,92] In the last few decades, however, the major incidence of plague activity in the world has shifted from Asia to Africa[10] where plague is now endemic in many countries.[93] As of February 2016, the three most endemic countries were reported to be Madagascar, Democratic Republic of Congo, and Peru.[12a] An outbreak of plague cases in northeast Tanzania was reported in 1980[94] and by 2004 more than 7000 cases, predominantly of bubonic plague, had occurred, illustrating a prolonged incidence of disease in this country. Data from this outbreak indicated that the risk of infection for children 10 to 14 years of age was twofold higher than in adults and women were more often infected than men.[93] This female-to-male susceptibility was also reported for outbreaks in Kenya and Mozambique,[95] although in Madagascar the opposite was true.[96] Whether there are true gender effects on susceptibility or result from social, occupational, or environmental risk factors is unclear. In Tanzania, plague outbreaks appear to be associated with the wet season, whereas in Madagascar the occurrence of plague cases correlates with the cool dry season.[93] In the United States, a new trend in plague epidemiology appears to be related to the residential encroachment on former rural areas that contain enzootic foci,[4] putting humans at risk of exposure to flea bites or in close contact with infected wild rodents or other animals.

Potential Use as an Agent of Bioterrorism

Plague vaccines have also attracted considerable attention because of the potential for *Y. pestis* to be used as an agent of biological warfare or bioterrorism.[76] It is likely that *Y. pestis* used in this way would be disseminated by the airborne route, and would cause pneumonic plague. A 1970 report by the WHO[97] indicated that, in a worst-case scenario, the

airborne release of 50 kg of *Y. pestis* over a city of 5 million would result in 150,000 cases of plague. Of these, 36,000 would be expected to die. The released bacteria would remain viable for 1 hour. Disease that resulted from the deliberate release of *Y. pestis* in this way would appear within 1 to 6 days of exposure and most probably within 2 to 4 days.[76] The initial symptoms would resemble other severe respiratory illnesses,[76] which might make early diagnosis and appropriate treatment difficult. The indicators of an attack with *Y. pestis* would include the clustering of cases, the death of rodents in the area, the incidence of disease in individuals without known risk factors, and disease in areas where plague is not known to be endemic.[76] The unpredictable nature of such attacks and the potential for epidemic spread of disease from the index case would make the availability of vaccines and immunoprophylactics important and is discussed later in this chapter.

PASSIVE IMMUNIZATION

Early studies showed that serum from human volunteers immunized with purified F1 antigen or with a KWC vaccine would provide passive protection of mice against a parenteral challenge with 100 LD_{50} of *Y. pestis*.[98,99] Newer studies have reported that the passive administration of antiserum or monoclonal antibodies against the F1 and V antigens protects mice previously challenged parenterally with *Y. pestis*,[100–102] demonstrating the potential of postexposure immunoprophylaxis. However, there is currently no licensed immunoprophylaxis or immunotherapies for plague.

ACTIVE IMMUNIZATION

Killed Whole-Cell Vaccines

Killed *Y. pestis* organisms have been used as a vaccine since 1897, when Waldemar Haffkine inoculated himself with an experimental vaccine. A KWC vaccine for human use was first produced in the United States in 1946 (Army Vaccine). Improvements to this vaccine led to the plague vaccine United States Pharmacopoeia (USP), which was produced from the virulent 195/P strain of *Y. pestis*.[103] During the 1990s, there were several commercial suppliers of KWC vaccines against plague. Plague vaccine USP, which contains formaldehyde-killed bacteria, was initially manufactured by Cutter Laboratories, then in 1994 was manufactured by Greer Laboratories, and in 1999 production of this vaccine was discontinued. An alternative KWC vaccine was manufactured by the Commonwealth Serum Laboratories (CSL Australia)[104] and until November 2005 was licensed for clinical use in Australia. This vaccine contained heat-killed bacteria (*Y. pestis* strain 195/P) resuspended in saline containing 0.5% weight per volume of phenol to a concentration of 3×10^9 organisms/mL. The vaccine was given subcutaneously as two 0.5-mL doses separated by an interval of 1 to 4 weeks, with some modifications for children (Table 45.1). Booster doses at 6-month intervals of this vaccine were also recommended. However, currently there is no licensed plague vaccine for general use, although the recombinant subunit vaccine candidates discussed below, have investigational new drug status.

TABLE 45.1 Schedules and Dosages of the Commonwealth Serum Laboratories Killed Whole-Cell Vaccine

Age of Recipient	Volume Administered			
	First Dose	Second Dose	Third Dose	Booster Dose
6 mo–2 y	0.1 mL	0.1 mL[a]	0.1 mL[a]	0.1 mL[b]
3–6 y	0.2 mL	0.2 mL[a]	0.2 mL[a]	0.2 mL[b]
7–11 y	0.3 mL	0.3 mL[a]	0.3 mL[a]	0.3 mL[b]
>12 y	0.5 mL	0.5 mL[a]	—	0.3 mL[c]

[a]One to 4 weeks after previous dose.
[b]At 6-month intervals after dose 3.
[c]At-6 month intervals after dose 2. Dose can be reduced to 0.1 mL intradermally for individuals who have reactions to previous doses of the vaccine.

Live Attenuated Vaccines

The first live attenuated *Y. pestis* strain used as an experimental vaccine was produced in 1895 by Yersin, but was never tested in humans for fear of reversion to virulence. Subsequently, the EV76 strain was developed from the EV strain which had been isolated in Madagascar from a human bubonic plague victim in 1926. Attenuation was achieved following 6 years of in vitro passage.[105] The EV76 strain and its derivatives have been used to vaccinate humans since 1908, especially in the former Soviet Union and the French colonies. However, the genetic lesion that results in attenuation of EV76 has not been defined. The strain is known to be a pigmentation *(pgm)* mutant that prevents the bacterium from assimilating hemin[4] and also results in changes in the surface properties of the bacterium.[38] Other live vaccine strains include the Tjiwidej smooth strain developed in the 1930s,[106] where attenuation resulted from the loss of V antigen expression.[107] This strain was used in limited field trials in Madagascar and Java.[105] At present, however, no live vaccine is commercially available or licensed for use in humans because of the risks of reversion and severity of disease caused by these strains.[105]

The safety of these vaccines was also assessed. Inoculation with the EV strain resulted in reactions ranging from local swelling and erythema of up to 15 cm diameter, with a rise in temperature accompanied by headache, weakness, and malaise, severe enough to require hospitalization in some cases. Although immunization with these strains protected against both bubonic and pneumonic infections, the risks associated with the vaccines appeared to outweigh the benefits.

Subunit Vaccines

Vaccines consisting of purified recombinant proteins are now in advanced development and represent the next generation of plague vaccines. One such vaccine, including recombinant F1 and V proteins administered in an alhydrogel-adjuvanted liquid formulation, has successfully undergone Phase II clinical trials. The F1 and V proteins, naturally produced by the parent organism as virulence factors, have been cloned and expressed under good manufacturing practices conditions in avirulent *Escherichia coli*[108–111] and purified prior to coadsorption to alhydrogel. The resulting vaccine has been demonstrated safe and immunogenic in a total of 145 clinical trial volunteers to date. A variation of this vaccine, where the C terminus of F1 is genetically fused to the N-terminus of V to produce a recombinant fusion protein (rF1-V), is currently in advanced development.[112] A major advantage of such subunit formulations enriched for protective antigens, compared with KWC vaccine formulations, is the reduced immunization regimen required with the rapid induction of protective immunity and reduced reactogenicity. Such subunit vaccines are also versatile and could be formulated for administration by noninvasive routes. To date, formulation of the rF1 and rV subunits for oral delivery has achieved approximately 60% protection against challenge in the mouse model.[113]

RESULTS OF VACCINATION

Immune Responses

The immune responses to KWC vaccine formulations were mainly directed against the F1 antigen, as the content of V antigen in these vaccines was low or undetectable.[114] The titer of the F1 antibody, determined by passive hemagglutination, correlated with protection against plague challenge in animal models.[115] Ninety percent of animals with anti-F1 passive hemagglutination titers of 128 survived challenge with 1×10^3 to 5×10^5 CFU of *Y. pestis*, whereas the proportions of animals with titers of 32 to 64 or 16 that survived were 46% and 6%, respectively. Passive immunization studies with sera from immunized animals[75] or humans[116] also suggest that anti-F1 passive hemagglutination titers of 1 : 128 or greater were protective. However, the accepted standard involves passive immunization of mice with antisera and the demonstration of an acceptable MPI. Studies of the Greer Laboratories' vaccine suggest that 55% to 58% of individuals develop an antibody response with an acceptable MPI after two vaccinations[5]; however, even after multiple vaccinations, averaging five per subject, 8% of individuals failed to develop any antibody response.

The rationale for combining the F1 and V antigens in the next-generation vaccine is to induce an immune response to counteract the key virulence mechanisms demonstrated during natural infection. Evidence from animal models[114] and from clinical trials[117] indicates that both antigens are immunogenic and that they are additive in their effect. The current lead vaccine candidate is a recombinant genetic fusion of the F1 and V antigens (rF1/V) adsorbed to alhydrogel.[112] Earlier published work outlined the combination of recombinant F1 and V antigens, adsorbed to alhydrogel (rF1+rV),[114] and is presented below.

Clinical Immunogenicity

The human immune response to the rF1-V vaccine has been assessed in more than 400 healthy volunteers; the vaccine has been shown to be safe and immunogenic.[118] Immunogenicity of the rF1-V vaccine has been assessed by titration of antibody titer and assay of antibody functionality, including the ability to protect naive animals by passive transfer of antiserum before plague challenge.[119] Similarly, the rF1 + rV vaccine has been assessed during Phase I safety and immunogenicity trials in 32 healthy volunteers progressing to Phase II trials.[117] The data from the first trial have been published and show that all the subjects produced F1 and V-specific immunoglobulin (Ig) G after the initial dose, which peaked after the booster dose (day 21), with the exception of one individual in the lowest dose-level group, who responded to rF1 only.[117] The assessment of functional antibody has been aided by the identification of a mouse monoclonal antibody (mAb) directed against a protective B-cell epitope in V antigen, which provides solid protection against live organism challenge in the mouse.[117] Human antibody as well as a reference macaque antibody, was found to compete with this mAb for binding to rV in vitro and also to protect naïve mice by passive transfer.[117] This suggests that recognition of this protective B-cell epitope in the V antigen is conserved between mouse, macaque, and humans. Total IgG to rV in human volunteers and the titer of IgG competing for binding to rV, correlated significantly at days 21 and 28 ($P < .001$).[117] Protection afforded by passive transfer of immune human sera into mice also correlated significantly with the IgG titers to both rF1 and rV at days 21 ($P < .001$) and 28 ($P < .03$), as determined by regression analysis (Fig. 45.3).[117] Thus potential serological immune correlates of protection have been identified and

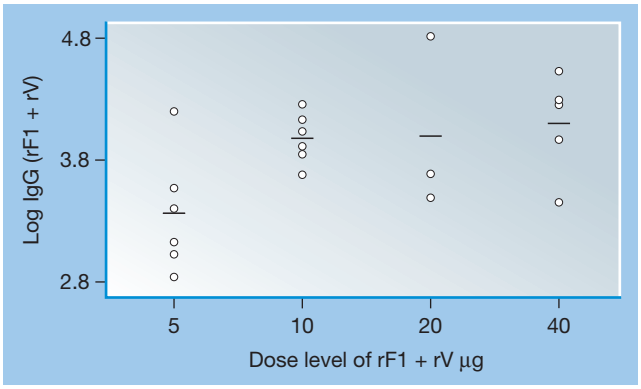


Figure 45.3. Correlation between vaccine dose level and response in Phase I clinical trial of rF1+rV vaccine. Volunteers were administered a priming immunization and a booster dose on day 21. The IgG response was measured by combining areas under the curve for Log_{10} IgG responses to rF1 and to rV, measured in the period between day 21 (prior to the booster dose) through day 70 of the immunization schedule. *(From Williamson ED, Flick-Smith HC, LeButt CS, et al. Human immune response to a plague vaccine comprising recombinant F1 and rV antigens.* Infect Immun. *2005;73:3598–3608.)*

can be used as surrogate markers of protection in future clinical trials of the rF1/V vaccine, for which conventional Phase III efficacy trials will not be possible.[119] Surrogate markers of protection, which rely on the induction of cell-mediated immunity (CMI) mechanisms, may also be required, based on the indications from animal models.[67,120] However, assays of CMI during large trials rely on fresh whole-blood samples, which can be difficult to obtain. Flow cytometric analysis of such samples in subjects responding to vaccination showed no significant changes in markers of cellular activation, but advances in flow cytometric technology may well improve sensitivity of detection, making it a viable approach to monitor CMI development.[117]

INTEGRATION OF CLINICAL AND NONCLINICAL DATA

None of the plague vaccines that have been developed have been subjected to a randomized, clinically controlled field study in humans.[121] Although controlled clinical studies are desirable, the sporadic and relatively low incidence of plague means that such studies would be difficult to conduct. Thus, evidence for the efficacy of vaccines must be based on comprehensive studies in several animal models and on the evidence that the vaccines can induce antibody in humans that can passively protect challenged animals. Additionally, anecdotal data from Vietnam have proven informational. From 1961 to 1971, many thousands of Vietnamese civilians developed plague (333 cases/10^6 person-years of exposure), but the incidence of disease in KWC-vaccinated U.S. troops based in Vietnam during this period was low.[122] (The total of eight cases represents one case per 10^6 person-years of exposure.[5,122]) Although this difference in the incidence of disease might be attributed to different exposures of these populations to *Y. pestis*, it is worth noting that many military personnel developed murine typhus, which is also transmitted by *X. cheopis*.[5] Furthermore, serological studies indicated that some immunized individuals were exposed to *Y. pestis* and developed subclinical infections.[122] The effectiveness of KWC vaccines against the pneumonic form of the disease is more questionable, as pneumonic plague has been reported in vaccinated individuals.[97,123]

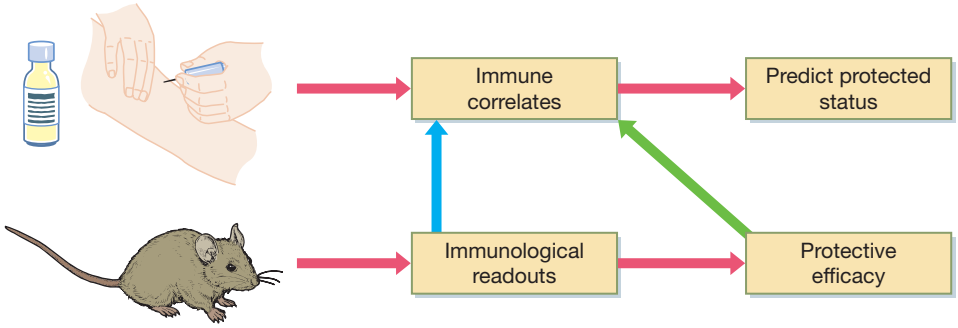


Figure 45.4. Integration of nonclinical and clinical studies with the identification of immune correlates of protection.

Thus for diseases such as plague, where the logistics of a clinical field trial are difficult and efficacy testing is neither practical nor feasible, the only recourse to predict efficacy in humans is to compare the immunological readouts from the clinic with those gained from nonclinical studies, particularly where the latter can be related to a measurement of protective efficacy, to derive immune correlates of protection (Fig. 45.4). Based on these data, surrogate markers, which would correlate with protective immunity, can be sought from clinical studies.[124] The nonclinical evidence that indicates the efficacy of the subunit vaccines in relevant animal models is reviewed in the next section.

Protective Efficacy in Nonclinical Studies

In the murine model of disease, immunization with two doses of a KWC vaccine provided solid protection against a subcutaneous challenge with 5000 LD_{50} of *Y. pestis* and partial protection (60% survival) against a subcutaneous challenge with 50,000 LD_{50} of *Y. pestis*.[73] In contrast, when mice were challenged with approximately 18 LD_{50} of *Y. pestis* by the airborne route, none of the mice that had been immunized with two doses of the KWC vaccine survived.[90] Although these data alone do not necessarily indicate a deficiency of the vaccine to protect against pneumonic plague in humans, when viewed alongside the reports previously cited, the overall evidence is that a KWC vaccine is more effective against bubonic plague than pneumonic plague. All these studies, however, have been carried out using $F1^+$ strains of *Y. pestis*. The efficacy of this vaccine to protect against $F1^-$ strains of *Y. pestis*, which are rarely encountered in natural infections, has not been fully tested.

Immunization of mice with the EV76 live attenuated vaccine strain induced protection against both subcutaneous and inhalation challenges with virulent strains of *Y. pestis*,[73] and appeared superior to the performance of the KWC vaccine (Table 45.2). The enhanced protection offered by the live vaccine over a KWC vaccine, might be the result of three factors. First, the KWC vaccine does not contain detectable levels of V antigen[114] and no ex vivo lymphoproliferative memory response or serum IgG to the V antigen could be detected in KWC vaccinees.[125] Second, the chemical or heat inactivation of the bacterium required in KWC vaccines might have caused structural changes in other surface components such as the F1 antigen.[74] Third, local responses to vaccination with EV76 in mice suggest that a limited local infection is initiated and that the duration of exposure to the antigenic complement is therefore prolonged. The immune response induced by the live attenuated vaccine may be more similar to a natural infection than that induced by KWC preparations.

A significant amount of work has now been carried out in the mouse,[126] guinea pig,[71] and macaque[127–132] models from which immune correlates have been proposed. Although a titer of neutralizing antibody provides protection against exposure, it is clear that the development of CMI is also critical to full protection and eventual clearance of the bacterial challenge from the host.

TABLE 45.2 Protection Afforded by Killed Whole Cell Vaccine, EV76 Vaccine, or F1- and V Antigen Subunit Experimental Vaccine Against Parenteral or Inhalation Challenge With *Yersinia pestis* Strain GB

Vaccine	Protection (% Survival) Against Challenge (LD_{50})	
	Subcutaneous Route	Inhalational Route
Control	0 (10^1)[a]	0 (10^2–10^3)[b]
Killed whole cell vaccine	60 (2×10^6)[a]	80 (19)[d]
EV76	100 (2×10^9)[a]	100 (150)[c]
F1 + V	100 (2×10^9)[a]	100 (10^2–10^4)[b,d]

[a]Tested in Balb/c strain mice.[104]
[b]Tested in Balb/c, CBA, C57Bl6, and CB6F1 strain mice.[121]
[c]Tested in Porton strain outbred mice by intranasal route of challenge.[69]
[d]Tested in Porton strain outbred mice.[115]
EV76, attenuated live vaccine strain of *Yersinia pestis*; F1 + V, subunit vaccine containing the F1 and V antigens of *Y. pestis*.

Intraperitoneal or intramuscular immunization with native F1 or rF1 antigens adjuvanted with alum induced an immune response that protected mice against a subcutaneous challenge with as many as 10^5 CFU of virulent *Y. pestis*.[74,114] Although the F1 antigen also induced protection against inhalation challenge with 100 LD_{50} of an $F1^+$ strain of *Y. pestis*,[74] there was concern that a vaccine based solely on the F1 antigen would not provide protection against naturally occurring but virulent $F1^-$ strains of *Y. pestis* (although only one such naturally occurring strain has been reported). Immunization of mice intraperitoneally with the V antigen adjuvanted with alum induced protection against a subcutaneous challenge with 4×10^6 CFU of *Y. pestis*.[108] A significant advantage over the F1 antigen is the ability of the V antigen to induce protection against virulent $F1^-$ strains of *Y. pestis*; protection provided by the V antigen vaccine against 1000 or more LD_{50} of either virulent $F1^+$ or $F1^-$ strains of *Y. pestis* given by the inhalation route has been reported.[133]

To protect against $F1^-$ virulent strains of plague, recombinant truncated V protein (rV10, lacking residues 271 to 300), has been tested and demonstrated to provide protection against pneumonic plague in mice, rats, guinea pigs and nonhuman primates.[134] In the mouse model, immunization with a combination of 80 μg F1+ 80 μg V subunits rapidly induced

protective immunity so that protection against 100 LD_{50} of *Y. pestis* KIM could be achieved up to 1 day after immunization, while postexposure immunization gave partial protection.[135]

The protection afforded individually by the F1 or the V antigens was overcome by very high subcutaneous challenges (10^9 CFU of *Y. pestis*) in the immunized mouse.[114] However, protection against this challenge was achieved after intraperitoneal immunization in multiple doses with a mixture of both the F1 and V antigens.[114] The combined vaccine also protected mice against 100 LD_{50} of *Y. pestis* given by the inhalation route (see Table 45.2), suggesting that the vaccine might provide protection against pneumonic plague in humans.[90] The advantages of such a combined subunit vaccine lie not only with the enhanced level of protection afforded against disease but also with the ability of the vaccine to confer protection against both $F1^+$ and $F1^-$ strains of *Y. pestis*.

A systematic study using mice of four different haplotypes and both genders showed that all animals responded to immunization to the rF1 and rV antigens with high antibody titers.[136] The response to either antigen was affected by the conformation of the protein[110,137] and the ratio of rF1 to rV, with an optimal response when rF1 was in a twofold molar excess to rV.[138] Mice could also mount an antibody response to both of the antigens when the C-terminal of the F1 antigen was genetically fused to the N-terminus of the V antigen.[112]

The rF1+rV vaccine has also been used successfully to immunize guinea pigs and nonhuman primates. Antibody titer to rF1+rV in guinea pigs also correlated with protection and passive transfer of specific antibody (IgG) from the guinea pig into naïve mice, protected the latter against challenge.[71] Cynomolgus macaques immunized with the combination of rF1 and rV antigens produced high titers of specific antibody which competed with the V-specific and protective mAb7.3 for binding to V antigen and also passively protected naïve mice against challenge[127] and was used as a positive control in assays assessing the impact of passive transfer of human serum.[117]A newer study shows that the immunization of cynomolgus macaques with rF1+rV or rF1–V in alhydrogel fully protected them against pneumonic plague following inhalation exposure to *Yersinia pestis*.[128,130,131]

MECHANISM OF PROTECTIVE IMMUNITY

The mechanism by which the rF1+rV vaccine induces protective immunity has been the subject of considerable investigation during the past few years. The F1 capsule is thought to inhibit phagocytosis of the bacterium by preventing complement-mediated opsonization.[52,139] In addition, it is also possible that antibody induced to the F1 antigen promotes antibody-dependent cellular cytotoxicity. Some vaccinated animals do appear to harbor the pathogen even in a mutated $F1^-$ form.[74] The KWC vaccines were less effective than purified F1 antigen in inducing high titers of antibody to F1.[74,114] Antibody against the V antigen may also be of overriding importance in protection against plague, as immunization with V antigen restores the ability of the host to produce tumor necrosis factor-α and γ-interferon in response to infection.[58] Therefore, immunization with the V antigen would enable the host to mount a vigorous inflammatory response and enable host phagocytes to clear bacteria opsonized by antibody to the F1 antigen. Additionally because V antigen forms part of the injectisome of the type III system[56] and is exposed on the bacterial surface,[140] it remains extracellular for sufficient time to be targeted by antibody induced by vaccination or passively transferred.[100–102] Antibody to V antigen can inhibit the translocation of Yops by *Y. pestis* into the host cell,[141] and this inhibitory effect is dependent on the binding of antibody to the bacterial cell surface and attributed to the promotion of phagocytosis of *Y. pestis* by polymorphonuclear leukocytes.[141] Although protection against plague in the mouse model correlates with the specific IgG titer to the subunits (specifically of the IgG_1 subclass in the mouse, in response to this alhydrogel-adjuvanted vaccine),[138] there is no doubt that the mechanism of protection following immunization with the rF1+rV proteins also involves T-cell memory, which has been demonstrated in the mouse model.[125,138,142] In the mouse, the T-cell response to alhydrogel-adsorbed F1+V is biased toward T-helper type 2 (Th2) cells and is highly protective. In contrast, delivery of the F1+V proteins formulated in the Ribi adjuvant system to IL4T mice (genetic knockouts for the interleukin [IL]-4 receptor) induced predominantly a Th1 response. The passive transfer of this antibody into B-cell–deficient knockout mice, with no intrinsic ability to produce antibody, protected the mice fully against live organism challenge.[143] Furthermore, a targeted gene deletion in the Stat4 signaling pathway (preventing the ligation of the Stat4 receptor with IL12 signaling) and abrogating a Th1 response, resulted in reduced protection, whereas mice with a targeted gene deletion in the Stat6 pathway and no Th2 activity, were fully protected against challenge.[144] Thus, it appears that antibody is highly protective against this predominantly extracellular pathogen, but CMI,[145] and particularly a balanced Th1/Th2 response,[144] provides an optimal strategy for protection. Recent data supports this assumption as mice immunized with the rF1-V vaccine and depleted of tumor necrosis factor-α and γ-interferon just prior to challenge had very poor survival compared with immunized-only controls,[120] indicating key roles for these Th1 cytokines in the development of protective immunity.[146,147]

Correlates of Protection and Surrogate Markers of Efficacy

In summary, correlates of protection to the rF1/V vaccine reside in both antibody-mediated and cellular mechanisms, which do not act independently. Knowledge of mechanisms derived from nonclinical models of vaccination and infection can lead to the identification of potential surrogate markers of efficacy.[148] Assays of surrogate markers of efficacy might include the in vitro inhibition of the cytotoxicity of a *Y. pseudotuberculosis* construct expressing *Y. pestis* V antigen,[149] the passive transfer of human immune serum into a naïve animal model,[117] and an assessment of CMI using ex vivo peripheral blood mononuclear cells and a secondary recall response in an enzyme-linked immunosorbent spot or similar format. Much work is ongoing to identify statistically valid immune correlates of protection for plague and to use these to establish surrogate markers of efficacy. With an increasing understanding of the molecular basis of pathogenicity and of the innate and adaptive immune response mechanisms required to counter *Y. pestis*, progress will continue to be made in this area and expedite the development of next generation vaccines.

Indications for Vaccine Use

Vaccination is recommended for use in individuals who are working with fully virulent strains of *Y. pestis* (e.g., researchers and laboratory workers) and for persons in regular contact with the wild animal hosts of plague or their fleas in endemic areas. It may also be considered for use in personnel who are deployed to work in endemic areas (e.g., emergency workers, military personnel, field workers, and agricultural consultants), especially if they might be exposed to epizootic plague arising from some acute geological disturbance.[5] However, vaccination against plague is not a statutory requirement for travel to any country. The vaccination of populations in

plague-endemic areas of the world is not routinely indicated, because the incidence of disease is relatively low and most cases of disease are of the bubonic form, which, once diagnosed, can be treated with antibiotics. The previously available KWC vaccines had limited utility in controlling sporadic plague epidemics, because they required several months to induce protective immunity. However, the response to the new rF1/V vaccine is more rapid, so that once licensed, it would be feasible to use it in outbreaks or in the indigenous populations in areas with intense plague activity that cannot be controlled by other measures. Additionally, a licensed subunit vaccine for plague would be of benefit as a deterrent to the use of *Y. pestis* as a potential bioweapon. Although antibiotics can provide protection against disease, it is not clear if sufficient quantities could be stockpiled to allow the treatment of large populations for long periods of time. In addition, there is concern over the appropriateness of long courses of broad-spectrum antibiotics being used in large numbers of individuals. It seems likely that antibiotics will have a clear utility for the protection of relatively small numbers of individuals while vaccination would be more appropriate for entire populations. At the present time it seems unlikely that routine mass vaccination of entire populations, in the absence of any clear indication of an impending attack, would be undertaken. However, if vaccines were to provide protection of the civilian population, they would need to be available on short notice and to induce a rapid protective response.

Postexposure Prophylaxis

The previously available KWC vaccines were not suitable for postexposure use because several months were required to complete the primary vaccination schedule and induce optimal immunity. Even rF1/V vaccine, which induces relatively rapid immunity, is unlikely to provide postexposure protection in an infected and previously unvaccinated individual. The use of antibiotics should be considered in the case of possible exposure to *Y. pestis,* even in vaccinees who had previously completed the full KWC vaccination schedule, as this vaccine type offered poor protection against pneumonic plague. However, real prospects exist for the future development of passive immunotherapy for postexposure prophylaxis.

Precautions and Contraindications

The KWC vaccine formulations were contraindicated in individuals who had a history of hypersensitivity to any of the vaccine components (e.g., beef protein, soya, casein, sulfite, phenol, or formaldehyde). The safety and efficacy of the KWC vaccine formulations in people younger than 18 years or in pregnant women was not known, as is the case for the rF1/V vaccine.

Persistence of Immunity

Few studies have examined the duration of immunity to *Y. pestis* after immunization with KWC vaccines. However, it is generally considered that immunity is short lived and that booster immunizations were needed at 6-month intervals to ensure that a protective response was maintained. A single dose of the live attenuated vaccine derivatives of strain EV76 could protect against bubonic and pneumonic plague for up to a year.[150] Nonclinical data indicate that immunization with the combined rF1+rV vaccine induces durable immunity up to 9 months after a single immunization in the mouse model.[125] Cynomolgus macaques immunized with rF1+rV were observed to have functional IgG titers able to compete with mAb7.3 for binding to the V antigen more than 1 year after immunization.[127] The establishment of an immunization regimen and the need for a booster immunization requires similar monitoring of volunteers during clinical trials of the rF1-V vaccine for the persistence of immunity.

Safety

The KWC vaccines were known to be reactogenic in humans.[151,152] The manufacturer of the Cutter vaccine reported that reactions such as malaise, headaches, local erythema, and induration, or mild lymphadenopathy occurred in approximately 10% of vaccinees; this frequency of side effects was also reported by other workers.[105] Data provided by the manufacturer of the Greer vaccine suggested that more than 10% of vaccinees suffered side effects. Allergic reactions induced by immunization with KWC vaccines, evidenced mainly as urticaria, occurred infrequently.[151] One study found that the frequency of side effects was much greater in individuals who had previously been immunized with the live EV vaccine.[105]

In a review of the use of live, attenuated vaccines such as EV76, Meyer and associates,[153] reported that in one study in the former Soviet Union, a febrile response was reported in 20% of human vaccinees, accompanied by headache, weakness, and malaise. Erythema surrounding the site of vaccination was frequently reported, reaching dimensions of 15 × 15 cm. Some severe systemic reactions required hospitalization. Numerous unsuccessful attempts were made to reduce the incidence of side effects in clinical studies, by administering the vaccine by different routes, including scarification, inhalation, and intraocularly.[153] Of equal concern is the finding that the EV76 strain is capable of causing disease in some animal species. Russell and colleagues reported that immunization of mice with *Y. pestis* EV76 induced severe side effects and occasional death (approximately 1%).[73] The vaccine was reported to also cause fatal infections in vervets.[153]

By comparison, the rF1+rV vaccine was shown to be safe and well-tolerated in the first reported clinical Phase I trial in 32 healthy volunteers.[117] Four dose levels were assessed, and no serious or severe adverse events attributable to the vaccine were reported. Further clinical trials of the rF1/V formulation vaccine are ongoing in larger numbers of subjects to investigate the safety and immunogenicity of the formulation, as well as to predict its efficacy through surrogate markers of protection.[119]

FUTURE DEVELOPMENTS

With the discontinuation of production of a KWC vaccine formulation, the most likely vaccine replacement in the medium term is a formulation of a combination of the recombinant F1 and V antigens, which is expected to induce more rapid and comprehensive immunity. Indeed, this subunit vaccine may be the first nonlive vaccine to protect against the most dangerous form of the disease, pneumonic plague. Currently being developed as an injectable alhydrogel-adsorbed formulation, there is potential for alternative formulation of the recombinant fusion protein for self-administration and/or for mucosal delivery. A considerable body of work has now been conducted on the polymeric microencapsulation of the F1 and V antigens and the mucosal delivery of these formulations to achieve protective immunity.[142,154]

As well as permitting more flexibility in route of administration, polymeric encapsulation is advantageous in that it enhances vaccine stability and removes the need for cold chain storage. Many refinements have been made to optimize these formulations; the antigens have been either mixed for

coencapsulation or they have been separately encapsulated and mixed after encapsulation. In each case, both subunits have retained immunogenicity[155] and protective efficacy.[137,156] Further refinements have included the substitution of polymers conferring particular properties of stability and hydrophobicity and controlling particle size and loading.[126] A microsphere formulation suitable for nasal or parenteral administration has been derived that is fully protective against an inhalational challenge with *Y. pestis* after two separate[157] or even a single immunizing dose[158] in the mouse model. Liposomal encapsulation of the plague subunit vaccine offers an alternative approach to mucosal delivery.[154] Alternatively, a spray-dried powder formulation of rF1/V has been shown highly immunogenic in mice by intradermal or intramuscular delivery.[159] The delivery of vaccines by advanced transdermal patch is a possibility[160] and would provide an alternative noninvasive approach to vaccination with the rF1 and rV subunits.[161]

The most promising approach to an oral vaccine for plague is the use of *S. enterica* serovar *Typhimurium* expressing the *caf* operon, which induced a high level of protection against subcutaneous challenge with *Y. pestis*.[162,163]

Additional Subunits

There have been many unsuccessful attempts to identify additional protective subunit proteins. *Yersinia* secretory protein F (YscF) along with V antigen form the injectisome of the type III system and immunization with YscF provides some protection against plague in the mouse.[164] Additionally, the vaccination of mice with a Yop translocon complex comprising Yops B, D, and E protects against infection with F1$^-$ *Yersinia pestis*.[165] Thus it may be beneficial to add YscF to a future subunit vaccine formulation to maximize the protective efficacy achievable.

Additionally, a newer study shows that immunization with *Y. pseudotuberculosis,* which lacks the Lcr plasmid, can provide protection against plague, suggesting that protective antigens other than F1 antigen and components of the type III system exist.[166]

Live Attenuated Vaccines

The finding that the live EV76 vaccine induces protection against plague, although its use is accompanied by unacceptable local and systemic side effects,[153] has prompted additional work to develop stably attenuated strains of *Y. pestis* for vaccines. Work toward this goal has been based on the finding that other gram-negative pathogens can be attenuated by the introduction of mutations into genes essential for bacterial growth, virulence, or survival in the host, and that the nature of the attenuating mutation can influence the immunogenicity and reactogenicity of these vaccines. Strains of *Y. pestis* with mutations in the *aroA*,[167] *phoP*,[168,169] or *htrA*[170] genes have been constructed and tested but show only a modest reduction in virulence in mice and would not be suitable for development of a live attenuated vaccine in humans. However mutation of the *dam* gene resulted in a 2000-fold attenuation of *Y. pestis* and mice exposed to sublethal doses of the mutant were protected against a subsequent challenge with fully virulent *Y. pestis*.[171] Even more impressively, a *pcm* mutant of *Y. pestis*, which is believed to be defective in its response to environmental stresses, was more than 10^7-fold attenuated in mice.[172] The immune response induced after immunization with the *pcm* mutant was superior to that induced by the EV76 strain, suggesting that this might be an efficacious and safe alternative to the EV76 vaccine. The elucidation of the *Y. pestis* genome sequence has identified a wide range of other genes that might be inactivated to yield a live attenuated vaccine.[32] For example, organisms with genes encoding mutations in lipoprotein *(lpp)* and an acyltransferase involved in modification of LPS *(lpxM)* are attenuated and might be useful vaccine candidates.[150,173] For a live attenuated vaccine to be safe, multiple mutations in independent pathways are required to reduce any risk of reversion to virulence. The effect of combining attenuating mutations is hard to predict, as the multiple mutations may overly attenuate and induce poor immune responses.

An alternative strategy would be to use a closely related species with lower virulence and exploit cross-immunity. For example, an attenuated *dam* mutant of *Y. pseudotuberculosis* induced a robust protective immune response against challenge with *Y. pestis*.[174] A naturally arising attenuated strain lacking the high pathogenicity island, the *Y. pseudotuberculosis* superantigens, and type IV pili was similarly able to induce a protective response against plague.[175] In addition to reduced risk as a result of using a less-virulent strain, another advantage of this approach is that the immunity was induced following oral dosing, indicating that a needle-free vaccine is achievable.

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46 Pneumococcal Conjugate Vaccine and Pneumococcal Common Protein Vaccines

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The original demonstration by Avery and Goebel, in 1929, that capsular polysaccharides were immunogenic when linked to a protein[1] and, 60 years later, the demonstrated efficacy of *Haemophilus influenzae* type b (Hib) conjugate vaccines in the 1990s (see Chapter 23) led to an extraordinary public health intervention in the year 2000 when conjugated pneumococcal polysaccharide vaccines were introduced for the prevention of pneumococcal disease in infants. Acute respiratory infections are the major global cause of death in young children and led, in 2013, to an estimate of 935,000 (with a range of uncertainty of 817,000 to 1,057,000) deaths annually, in children younger than 5 years of age.[2] A second study of lower respiratory tract mortality in children younger than 5 years of age projected a similar mortality estimate in 2013 (905,100 deaths, with a 95% uncertainty interval of 797,900 to 1,015,900 deaths).[3] Although pneumonia remains the leading infectious cause of death in infants, pneumonia deaths are declining with nearly half of the reduction in child mortality from 2000 to 2013 attributable to reductions in pneumonia, diarrhea, and measles.[2] An updated estimate of lower respiratory tract mortality in children younger than 5 years in 2015 suggests that deaths are now 703,900 (95% uncertaintly range, 651,000–763,000).[3a] While increased access to antibiotics likely played a major role, the introduction of both Hib and pneumococcal conjugate vaccines (PCVs) has also had a substantial impact. Advances in methodology, using efficacy data from the trials of pneumococcal conjugate vaccination conducted in both developed and developing countries, allow an estimate of residual mortality attributable to the pneumococcus in children younger than 5 years of age in 2013 of 264,000 (uncertainty range, 155,700–365,800). The attribution of pneumococcal deaths, however, was likely underestimated as the effectiveness of PCV against pneumococcal pneumonia may be less than its effectiveness against invasive disease, which was used in the calculation.[3] A revised 2015 estimate of the pneumococcal pneumonia mortality in children younger than 5 years taking this observation into account provides an estimate of 393,000 pneumococcal deaths, with an uncertainty range of 228,400 to 532,300 deaths.[3a]

This chapter reviews pneumococcal polysaccharide-protein conjugates, isolated pneumococcal proteins, and whole-cell pneumococcal vaccines as avenues for wider coverage and increased efficacy compared with pneumococcal polysaccharide vaccine (PPV). Conjugate vaccines are the most advanced of these approaches with large-scale, randomized clinical trials in multiple countries confirming their efficacy against invasive disease[4–11] and otitis media.[11–13] Conjugate vaccines are also widely available in most developed countries, with a rollout to more than 50 of the 73 Global Alliance for Vaccines and Immunization (GAVI)–eligible developing countries by the end of 2015. The overall effectiveness of conjugate pneumococcal vaccines has resulted in large part from the impact on vaccine serotype-specific carriage and thus reduced transmission of vaccine types in the community. In the United States, immunization of young children has led to the virtual elimination of vaccine-type disease in both children and adults,[14,15] and more than a 90% reduction in hospitalization for nonbacteremic pneumonia is attributable to herd protection immunity of adults resulting from the vaccination of children.[16] At the same time as protection is largely limited to the specific vaccine serotypes, vaccine effectiveness has been reduced by replacement carriage resulting from nonvaccine serotypes and replacement disease resulting from the disease potential of those serotypes. The impact of PCVs on herd protection in developing countries is also being seen.[17] The first randomized trial of PCV in the elderly has demonstrated efficacy against both bacteremic and nonbacteremic pneumonia caused by vaccine types.[18] The development of pneumococcal proteins as vaccines is still at an early stage, and other approaches, such as vaccines targeting the development of T-cell immunity, including a killed whole-cell vaccine, are either in preclinical or early clinical development. A vaccine that combines conjugated pneumococcal polysaccharides with additional nonconjugated pneumococcal proteins finished a Phase II trial in 2015. The vaccine was safe and immunogenic, but there was no impact of the proteins on pneumococcal carriage.[18a]

PATHOGENESIS

With the possible exception of the great apes,[19] humans are the only natural host of the pneumococcus. However, other mammals, such as pets (e.g., horses, dogs, cats, and guinea pigs), zoo animals (e.g., dolphins), and laboratory animals (e.g., mice and rats)[20] can acquire the organism from their handlers. The organism lives in the nasopharynx of humans, from whence it can be transmitted by respiratory droplets to other individuals. In most cases, pneumococci are carried asymptomatically before being cleared from the nasopharynx. The duration of carriage is age- and serotype-dependent.[21–23] In some cases, usually shortly after the acquisition of carriage, pneumococci can cause mucosal disease by local spread to the middle ear (causing otitis media), the sinuses (causing sinusitis), or the lungs (causing pneumonia). In addition, pneumococci may cause systemic infections with considerable risk of mortality, including bacteremia and meningitis and less commonly infections in joints, bones, and soft tissues.

The pneumococcal capsular polysaccharide is the most important virulence factor for *Streptococcus pneumoniae*,[24,25] although not the only one.[26] The polysaccharide capsule significantly increases the resistance of pneumococci to phagocytosis by several mechanisms, including shielding from antibodies and complement. The capsule promotes adherence to epithelial cells in animal models. Strains of pneumococci that lack a detectable capsule can cause conjunctivitis,[27] but are infrequently isolated in cases of invasive disease. Pneumococcal virulence depends in part on several serotype-specific characteristics, such as the chemical composition and molecular size of the capsular polysaccharide.[23,28] The different pneumococcal serotypes vary in virulence, depending on their ability to activate the alternative and classical complement pathway, to deposit and degrade the complement components on the capsule, and to resist phagocytosis[29,30]; they also differ

in their ability to induce antibodies. Antibodies to the capsular polysaccharides are protective, as has been amply demonstrated in animal models and in clinical trials of conjugate vaccines.[31,32]

BACKGROUND

The reader is referred to Chapter 47 for a discussion of the clinical and bacteriologic aspects of pneumococcal disease.

EPIDEMIOLOGY

Pneumococcal Infections in Children Prior to Conjugate Vaccines

Before the introduction of seven-valent PCV7 in the United States, *S. pneumoniae* caused approximately 17,000 cases of invasive disease per year in children younger than 5 years of age, resulting in 700 cases of meningitis and 200 deaths.[33] The annual incidence of invasive pneumococcal diseases was highest in children 6 to 11 months of age, at 235 per 100,000,[33] declined to 35.2 per 100,000 in children between 2 and 4 years of age, and reached its lowest levels in children 5 to 17 years of age (3.9 per 100,000).[34] A similar distribution of incidence by age was seen in Canada, although the rates for the youngest children were somewhat lower, peaking at 161.2 per 100,000 for children 6 to 17 months of age.[35]

In Europe, the highest levels of infection were in children in the second 6 months of life, then in those 2 years of age or younger, and declining steadily thereafter into the teenage years.[36,37] However, the measured incidence of pneumococcal infections has generally been much lower in Europe than in the United States.[38] For example, in England and Wales, the annual incidence of invasive pneumococcal diseases in infants between 6 and 11 months of age was 35.8 per 100,000 in the late 1990s,[39] compared with 235 per 100,000 in the United States. In Finland, the annual incidence of invasive pneumococcal diseases was 45.3 per 100,000 among those younger than 2 years of age.[37] This difference in incidence was thought to be largely a consequence of the common practice of obtaining blood cultures in outpatients in the United States, whereas in Europe they generally only get blood cultures during hospitalization.[40]

Bacteremia without focus is more common in young children than in older persons,[33,36,37,41] and this diagnosis comprises 60% to 70% of invasive pneumococcal disease diagnosed in young children in the United States.[33,42,43] Pneumonia and meningitis are the next most common, but meningitis remains the diagnosis with the highest case fatality rate.[36,41,44] After the introduction of the Hib vaccine causing a marked decline in Hib disease, *S. pneumoniae* became the leading cause of bacterial meningitis and occult bacteremia in the United States.[45,46] As children grow older, the proportions of pneumococcal infections classified as meningitis and bacteremia without focus decline, and the proportion of pneumonia increases.[42,44,47,48]

In the United States before the introduction of PCV, there were approximately 15 million office visits annually for the diagnosis of acute otitis media (AOM),[49] with costs estimated at US$5 billion per year.[50] *S. pneumoniae* was the most commonly reported bacterial cause of AOM, with the proportion of the pneumococcus as an etiologic agent recovered from middle ear fluid by tympanocentesis varying from 18% to 55% in published studies.[51–54]

The burden of pneumococcal disease has been greatest for those populations in which the plain PPV has been the least immunogenic: children younger than 2 years of age; the elderly (≥65 years); immunocompromised individuals, including those infected with HIV, and those with malignancies; there is also a significant burden in individuals with functional or anatomic asplenia and those with chronic cardiovascular, pulmonary, or liver diseases.[33,55–57] Among individuals without underlying illness, children younger than age 2 years are most vulnerable to pneumococcal infections. There is evidence that this level of risk is increased further by spending time in crowded or communal settings, such as daycare centers.[43,58]

Children from specific minority groups suffer disproportionately. Although the overall incidence of invasive pneumococcal disease in children younger than 2 years of age in the United States before licensure of PCV7 was 167 cases per 100,000 person-years, black children of the same age had an incidence of 400 per 100,000 person-years, Alaskan Native children had an incidence of 624 per 100,000 person-years, and Native American children had an overwhelming incidence of 2396 cases per 100,000 person-years (Table 46.1),[33] although rates in Navajo children have declined.[47] Asians, blacks, and Hispanics also had a greater risk of pneumococcal pneumonia than whites.[59] Similar to the United States, indigenous people in Australia had much higher rates of pneumococcal disease with indigenous children younger than 2 years of age having an incidence of invasive pneumococcal diseases of 2053 cases per 100,000 person-years.[60] Nonindigenous children in Australia had rates intermediate between those of Europe and the United States.[61] Minority populations in Israel, including the Bedouins, also had a higher incidence of pneumococcal infections, although the difference was not as pronounced as in Australia and the United States.[62] This difference may be socioeconomically based because minority groups in Israel are not a single ethnic population but, as a group, have higher birthrates, more crowded living conditions, less education, and lower socioeconomic status than the majority Jewish population.[62,63]

Boys are more likely than girls to contract pneumococcal infections,[47,63–66] and there are often seasonal peaks in incidence with infection more likely in the winter.[48,62] Recently documented influenza or another respiratory viral infection has been reported to be a risk factor for severe pneumococcal pneumonia[67] and invasive disease.[68] Recent ear infections, indicating the middle ear is an important route of *S. pneumoniae* invasion, and a lack of breastfeeding are also associated with increased incidence of invasive pneumococcal diseases.[43]

TABLE 46.1 Incidence of Invasive Pneumococcal Disease in Children in the United States Before the Introduction of Pneumococcal Conjugate Vaccine by Age and Ethnic Group

	Incidence per 100,000 Person-Years			
Age (yr)	All Races	Blacks	Alaskan Natives	Native American (Navajo or Apache)
<2	167	400	624	2396
2–4	36	116[a]	98[b]	227[b]
5–9	6	9	23	54
10–19	3	5	5	35

[a]Age 2–3 years.
[b]Age 2–5 years.
Modified from Advisory Committee on Immunization Practices. Preventing pneumococcal disease among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Recomm Rep. *2000;49(RR-9):1–35.*

Children with cochlear implants have a risk of pneumococcal meningitis approximately 40 times higher than the rate in the general population of children.[69] The risk was highest among children using a specific spacer, which was subsequently discontinued, and in those with middle ear malformations associated with cerebrospinal fluid leaks. A study in Denmark suggested that deaf children without cochlear implants have a fivefold increased risk of meningitis relative to hearing children.[70]

Most deaths among children from pneumococcal disease occur in developing countries.[71] In developing countries, carriage of pneumococci frequently occurs before 2 months of age[72] (in contrast, children in the United States acquire their first strain on average at 6 months of age), a larger proportion of invasive diseases occur by 6 months of age than in industrialized countries,[73–75] and neonatal infections are not uncommon.[74]

A compromised immune system, whether caused by HIV infection, malignancy, asplenia, or primary immunodeficiencies, is a major risk factor for pneumococcal disease. Infection with *S. pneumoniae* occurs more than 100 times more frequently in HIV-infected children than among the general population.[76–78] Meningitis or recurrent bacterial sepsis can be the first indicator of AIDS in pediatric patients,[79,80] and otitis media is reported to be three times more frequent in HIV-infected children than in uninfected children.[81] In the United States, rates of invasive pneumococcal diseases for HIV-infected children younger than 3 years of age are 12.6 times higher than in HIV-negative children,[71] and in children younger than 5 years of age are 2.8 times higher than in HIV-negative children. In both children and adults, the introduction of highly active antiretroviral therapy (HAART) reduced the incidence of pneumococcal infections in HIV-infected patients, but rates remain elevated compared with HIV-uninfected persons.[57,82–86]

In South Africa, the incidence of pneumococcal bacteremia was 36.9-fold higher in HIV-infected children than in HIV-negative children before introduction of antiretrovirals and PCV.[85] The rates of pneumococcal bacteremia doubled between 1987 and 1997 as the rates of HIV infection rose.[86] Overall, the disease spectrum is similar in HIV-positive and HIV-negative populations, except that pneumonia is more common in HIV-infected children.

Individuals with sickle cell disease and other hemoglobinopathies resulting in functional asplenia are also at increased risk for serious infections with *S. pneumoniae*.[87–89] Without immunization, the rate of invasive pneumococcal disease in children with sickle cell disease is 30 to 100 times greater than that of healthy children of similar age. Even with the use of PCV and PPV, invasive pneumococcal disease among children with sickle cell disease who are younger than 5 years of age is 10-fold more frequent than in children without sickle cell disease,[90,91] and the case-fatality rate is much higher in children with sickle cell disease[92] or other forms[92] of anatomic or functional asplenia than in children with normal splenic function.

Children with nephrotic syndrome also are more susceptible to pneumococcal disease, with the majority of infections occurring within the first 2 years after presentation. *S. pneumoniae* is responsible for 50% to 60% of sepsis and peritonitis in children with nephrotic syndrome.[93] Patients who have received a bone marrow transplant are also more vulnerable to pneumococcal disease than the general public,[94] in part because they often have impaired opsonization function and lose protection from previous immunizations because of T-cell and B-cell depletion[95]; in addition, solid-organ transplant also confers a higher risk of invasive pneumococcal disease.[96] Of interest, the increased risk of pneumococcal infection in patients with Hodgkin disease can persist long after the underlying condition has been successfully treated.[97]

Serotype Distribution

Forty serogroups comprising 97 serotypes[98] have been described for *S. pneumoniae*. Among this large number of serotypes, a relatively small number account for most of the invasive disease in young children around the globe (Fig. 46.1).[99] Candidates for conjugate vaccines have been designed based on the relative percentages of disease-causing serotypes. To date, conjugate vaccines containing the saccharides of 7, 10, and 13 serotypes have been licensed; the first to be licensed in 2000 was a seven-valent vaccine (PCV7), sold under the trade name Prev(e)nar by Pfizer. The 10-valent vaccine (PCV10, Synflorix, sold by GlaxoSmithKline) is licensed in many other countries, but not in the United States. The 13-valent (PCV13, Prevenar 13, Pfizer) was licensed in the United States in 2010 and replaced PCV7. Table 46.5 (see later) lists the serotypes included in each of these vaccines.

Although comprehensive information about serotypes is not available for some regions, a recent systematic review summarized the information available from children younger than age 5 years before introduction of pneumococcal conjugate vaccines.[99] The review included 169 studies in the final analysis, representing 70 countries and 60,090 serotyped isolates. According to this analysis, serotypes 14, 6B, 1, 23F, 5, and 19F, in that order, were the most common serotypes found among sterile-site isolates from around the world. However, the distribution of disease-causing serotypes varied somewhat with location, age, and clinical presentation. Before the introduction of PCV7, serogroups in the seven-valent vaccine were responsible for more than 80% of invasive pneumococcal diseases in young children in the United States and Canada, and for 50% or more in all other regions; 10-valent and 13-valent vaccine formulations covered more than 70% of the invasive strains in all regions. Serotype 6A accounts for a substantial portion of disease, but within-serogroup cross-reactivity with serotype 6B in PCV7 and PCV10 provides protection.[100–103]

Serotypes 1 and 5 are encountered much more frequently in the developing world than in the developed world, although in the last decade serotype 1 disease has increased in Western Europe and has also been found among children living in

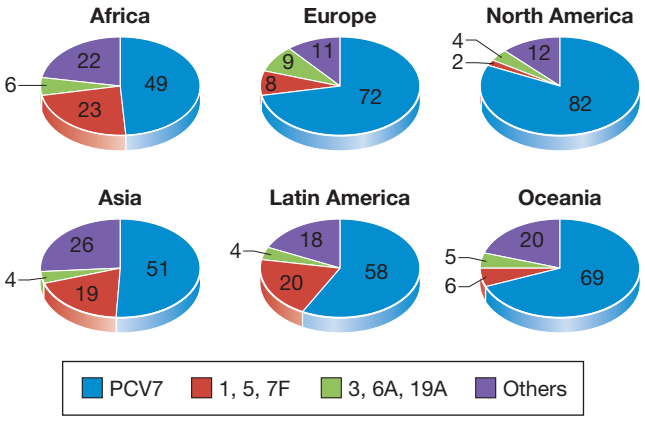


Figure 46.1. Systematic evaluation of serotypes causing invasive pneumococcal disease among children <5 years: the pneumococcal global serotype project. *(From Johnson HL, Deloria-Knoll M, Levine OS, et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project.* PLoS Med. *2010;7:e1000348.)*

Utah.[104,105] Differences in blood culture practice may also affect the observed distribution of serotypes: virulent serotypes that are more likely to cause severe disease requiring hospitalization would have a higher apparent prevalence in regions that perform blood cultures only on hospitalized patients. This may be the case for serogroups 1, 5, and 7, which were isolated at similar (low) rates in the United States and Europe, although the most common serotypes/groups (14, 6, 19, 18, 23, 9, and 4) had a much higher incidence in the United States before PCV use.[40] For example, serotype 1 caused 5% of reported invasive pneumococcal diseases in Western Europe and only 0.5% of invasive pneumococcal diseases in the United States before conjugate vaccine introduction, yet the rate of serotype 1 disease was approximately 0.9 per 100,000 person-years in each region.[40] This pattern indicates that serogroups 1, 5, and 7 cause severe rather than mild disease in both regions and supports the idea that certain serogroups are intrinsically highly virulent.[40] This could also account, in part, for the high prevalence of serotypes 1 and 5 in the developing world, where, as in Europe, blood cultures are rarely performed on an outpatient basis.

Serotype distributions change with age. A narrower range of serotypes are responsible for disease in young children[106,107] than in older children and adults, and in most regions, 50% of invasive pneumococcal disease is caused by three serotypes in young children and by four or five serotypes in older children and adults.[106] Serotypes 1, 5, and 18C are responsible for a larger portion of invasive pneumococcal disease in children.[47,63,108–110]

In addition to varying with age, certain serotypes are more likely to be associated with particular clinical presentations, although none of the most prominent serotypes is found exclusively in one clinical site, and all have the potential to invade multiple sites.[106] Note that age and site of isolation may not be independent because, for example, young children are much more likely to present with meningitis than older children, and serotypes isolated from young children are therefore more likely to be found in the cerebrospinal fluid (CSF) than serotypes isolated from older children. Generally speaking, serogroups 6, 10, and 23 are more likely to be isolated from CSF, whereas serogroups 1, 4, and 14 are more often isolated from blood than from CSF.[106,111] Severe pneumonia is more likely to be associated with serotypes 1 and 3,[112] although both pneumonia and these serotypes are more common in older children than the very young. Serotypes 19F, 23F, 14, and 6B are consistently among the six serotypes most frequently isolated from children with AOM, although their relative frequency varies by study. Serotype 3 is also a frequent AOM isolate in some areas, but not others.[48,66,113–116]

Antibiotic Resistance

Antibiotic resistance also varies with age, race, and geographic location. Globally, the northern European countries of Norway, Sweden, Denmark, The Netherlands, and Switzerland have consistently low rates of resistance; in contrast, France, Spain, Hong Kong, Singapore, and South Africa have high rates of resistance.[117] In the United States, resistant strains are found more frequently in the southern states of Tennessee and Georgia than elsewhere.[118,119] The highest percentage of resistant strains occurs in children younger than 5 years of age with high rates of antibiotic use since the 1990s.[118,119] After 2000, increases in nonvaccine serotype 19A strains resistant to multiple antibiotics were seen in countries using seven-valent PCV7, such as the United States,[119,120] but also in countries that had not yet introduced PCV7.[121] The amount and types of antibiotics used have been correlated with increased resistance.[119,122,123] The serotypes most likely to become resistant (6A, 6B, 9V, 14, 19A, 19F, and 23F)[119,121,124] were those that were frequently carried and most likely to cause AOM, and resistance rates are higher in isolates from middle ear fluid and sinuses than from normally sterile sites.[125] This may be because pneumococci in these regions are exposed to antibiotics more extensively than serotypes that cause invasive disease, both because they are carried for longer periods and because AOM is frequently treated with antibiotics. The serotypes with the highest rates of resistance were included in the PCV7 vaccine or were within the same serogroups, and introduction of PCV7 reduced disease caused by resistant infections in the United States and in developing countries, both among children and among older persons through reduced transmission.[17,126]

HISTORY OF VACCINE DEVELOPMENT AND VACCINE FORMULATIONS

Over the past 25 years, several PCV formulations have been tested in clinical trials; these vaccines differ in their included serotypes and carrier proteins (see Table 46.5).

The number of pneumococcal serotypes included in current vaccines and vaccine candidates in clinical development ranges from seven to 15, fewer than the 23 serotypes included in PPV. Although it would be preferable to include a larger number of different polysaccharides in a conjugate vaccine, doing so is technically challenging. Moreover, the total amount of carrier protein in the final vaccine may need to be limited because too much of some carrier proteins can impair the antibody response to the polysaccharide antigens.[127–129] In many countries, the incremental benefits in coverage for young children gained by increasing the number of serotypes remains low after 10 serotypes have been included; however, the 10 serotypes selected for inclusion may vary geographically and over time.

Prevnar 13 (Pfizer), a pneumococcal 13-valent vaccine, is a sterile suspension of saccharides of the capsular antigens of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, individually linked to nontoxic diphtheria CRM_{197} protein. Each serotype is grown in soy peptone broth. The individual polysaccharides are purified through centrifugation, precipitation, ultrafiltration, and column chromatography. The polysaccharides are chemically activated to make saccharides, which are directly conjugated by reductive amination to the protein carrier CRM_{197}, to form the glycoconjugate. CRM_{197} is a nontoxic variant of diphtheria toxin isolated from cultures of *Corynebacterium diphtheriae* strain C7 (β197) grown in a casamino acids and yeast extract-based medium. CRM_{197} is purified through ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography. The individual glycoconjugates are purified by ultrafiltration and column chromatography and analyzed for saccharide to protein ratios, molecular size, free saccharide, and free protein. The individual glycoconjugates are compounded to formulate Prevnar 13. Potency of the formulated vaccine is determined by quantification of each of the saccharide antigens and by the saccharide to protein ratios in the individual glycoconjugates. Each 0.5 mL dose of the vaccine is formulated to contain approximately 2.2 μg of each of *S. pneumoniae* serotypes except 4.4 μg of 6B saccharides, 34 μg CRM_{197} carrier protein, 100 μg polysorbate 80, 295 μg succinate buffer, and 125 μg aluminum as aluminum phosphate adjuvant.[130]

The first licensed PCV7 from Wyeth, also conjugated to CRM_{197}, included 2 μg each of the polysaccharide serotypes. Several conjugate vaccines that were tested in clinical trials are no longer in development. Merck developed a seven-valent vaccine containing polysaccharides of the same serotypes as the Wyeth product, conjugated to a meningococcal outer membrane protein (OMP) modified from *Neisseria meningitidis,*

and Wyeth produced a CRM_{197}-conjugated nine-valent vaccine containing 2 μg each of polysaccharide from serotypes 1 and 5 in addition to the serotypes in the seven-valent vaccine. Sanofi Pasteur developed an 11-valent vaccine that added serotypes 3 and 7F; in this vaccine (PncD/T), some of the saccharides (for serotypes 3, 6B, 14, and 18C) were conjugated to diphtheria toxoid and the others (for serotypes 1, 4, 5, 7F, 9V, 19F, and 23F) were conjugated to tetanus protein. Other potential carrier proteins that have been tested in animal studies include bovine serum albumin, human immunoglobulin (Ig) G, complement C3d, keyhole limpet hemocyanin, flagellar protein of *Salmonella*, pertussis toxoid, and pneumolysin toxoid.[131–134] The conjugation chemistry and manufacturing protocols vary for each preparation.

GlaxoSmithKline designed an 11-valent vaccine in which 1 μg of each saccharide was conjugated to protein D (9–16 μg per dose of vaccine) derived from nontypeable *H. influenzae* as a carrier protein for eight of the 10 serotypes (1, 4, 5, 6B, 7F, 9V, 14, and 23F), while serotypes 18C and 19F are conjugated to tetanus toxoid (5–10 μg) and to diphtheria toxoid (3–6 μg), respectively. All conjugates are adsorbed onto 0.5 mg aluminum phosphate. This vaccine protected against both pneumococcal and *H. influenzae* otitis media. However, serotype 3 proved not to be protective against AOM in the clinical trial[12]; consequently, GlaxoSmithKline proceeded with the clinical development of a 10-valent version, containing serotypes 1, 5, and 7F in addition to those in the seven-valent vaccine. This 10-valent version (PCV10, sold under the name Synflorix) is currently licensed in many countries globally and is prequalified by the World Health Organization (WHO). This vaccine is not licensed in the United States, but was used for a time in Quebec. Merck has an investigational 15-valent vaccine that has completed Phase I trials in adults and toddlers. A Phase II trial in infants comparing immunogenicity to PCV13 was completed and the immunogenicity was reported in infant macaques.[135] This vaccine is also conjugated to CRM_{197}, and in addition to the serotypes in the 13-valent vaccine, includes serotypes 22F and 33F. The vaccine contains 2 μg of each serotype, except for 4 μg of serotype 6B, and had aluminium phosphate as an adjuvant. It is currently undergoing reformulation.

The Serum Institute of India has a 10-valent CRM_{197} conjugate PCV in human trials in India and The Gambia. The vaccine has been formulated to include important serotypes in developing countries and includes serotypes 1, 3, 5, 6A, 6B, 7F, 14, 19A, 19F, and 23F. Panacea in India has a vaccine in human clinical trials which is conjugated to CRM_{197} and comprises the same serotypes as the GlaxoSmithKline PCV10, while SK Chemicals in South Korea is also testing a number of CRM_{197}-based conjugates in human trials. A seven-valent PCV that includes serotypes 1 and 5 has been tested in humans in Cuba.[136]

IMMUNOGENICITY

Measurement of Immunogenicity

For many encapsulated bacterial pathogens, antibodies against capsular polysaccharides are protective against disease. In pneumococcal infections, this has been demonstrated indirectly by the increased incidence of serious pneumococcal infections in groups with lower antibody concentrations (e.g., infants and the elderly, patients with hypogammaglobulinemia or agammaglobulinemia, and patients recovering from bone marrow transplantation). Direct evidence of the protective effect of anticapsular antibodies comes from studies where passively administered antibodies have provided protection from otitis media or invasive pneumococcal disease.[137,138] Furthermore, vaccines that induce antibodies to pneumococcal capsular polysaccharides are protective against invasive pneumococcal infections (see Chapter 47).

The two most important serologic methods used to quantify and evaluate the function of antibodies induced by vaccination are IgG quantification by enzyme immunoassay (EIA) and measurement of opsonophagocytic activity (OPA) of antibodies from the sera of vaccinated individuals. The EIA method has been validated extensively,[140] a link between antibody concentration and efficacy against invasive pneumococcal disease has been established,[141,142] and a process for cross-laboratory standardization of the assay has been completed.[143] The OPA assay provides important information about the functional activity of the antibodies and has also been the focus of intensive standardization efforts.[144]

Additional tests, such as isotype and subclass analysis, determination of the avidity of antibodies and serotype cross-reactivity, provide qualitative information about the immune mechanisms evoked by the vaccines, but they have not yet been standardized, and their value in predicting protective efficacy still needs to be evaluated and/or validated. Further information about the characteristics of the immune response to the vaccines can be obtained through demonstration of the induction of immunologic memory as well as by analyzing cellular and mucosal immune responses to the vaccination. Important information about immunity can also be obtained through challenge studies in animal models.

Enzyme Immunoassay

Immune responses to pneumococcal vaccines have been evaluated by radioimmunoassay and EIA, both of which measure the binding of antibodies to pneumococcal polysaccharide antigens. The radioimmunoassay technique is no longer in use. EIA is a sensitive, convenient, and rapid method even for a large number of samples. Concentrations of IgA, IgM, and IgG isotypes,[145] as well as of IgG subclasses,[146] can be determined using a reference serum. The United States Food and Drug Administration (FDA) previously made available a reference serum from pooled human sera (89SF). More recently, a new serum pool was formulated by the FDA (007Sp) and validated by the WHO; it replaced 89SF as the pneumococcal reference standard.[143]

All current EIA protocols have an absorption step to remove anti–C-polysaccharide antibodies,[140,147–149] the presence of which was the main limitation of radioimmunoassays. However, it has recently been recognized that, to some extent, similar problems exist with EIA, in that some sera contain polyreactive antibodies that recognize pneumococcal polysaccharides of different serotypes.[147,150,151] These antibodies are especially prevalent in the sera of individuals who have naturally acquired antibodies and are less prevalent in the sera of those immunized with pneumococcal vaccines. They are most probably directed to contaminants in the pneumococcal polysaccharide preparations that are used as antigens in the assays or directed to polyreactive epitopes present in the polysaccharide antigens. Inhibiting antibody binding using soluble polysaccharides of another serotype (e.g., of serotype 22F) removes these polyreactive antibodies, improves the specificity,[147] and increases the linear correlation between anticapsular polysaccharide antibody concentration and OPA.[147,152] Consequently, neutralization with 22F is now included in the EIA protocol. This change has caused some confusion regarding the need to change threshold values for protection as discussed below. Further complicating the picture for the Merck 15-valent vaccine in development,

which contains serotype 22F, is an electrochemiluminescence assay that was developed using serotypes 25 and 72 for preadsorption.[153]

The IgG antibody response in early infancy is mainly of the IgG_1 subclass. After the PCV booster dose in the second year of life, IgG_2 antibodies also start to appear, and the response in adults to both pneumococcal polysaccharide and conjugate vaccines is mainly of the IgG_2 subclass.[146,154–156] In adults, some differences have been found in the IgG_1:IgG_2 ratio elicited after vaccination with pneumococcal polysaccharide and various conjugate vaccines.[155] However, the relevance of these findings for estimating the T-cell dependence of the response in infants and the functional activity of antibodies remains to be elucidated.

Avidity

Antibody avidity describes the strength with which an antibody binds to a complex antigen. Several methods have been described for determining relative antibody avidity to different types of antigens.[157–162] In EIA techniques, the binding of antibody to the coated antigen may be prevented by competitive inhibition using decreasing concentrations of free antigen or, more usually, by eluting the antibody from the antigen by a dissociating agent, such as thiocyanate, urea, or diethylamine. Thiocyanate anion and urea interfere with the antibody-antigen binding primarily through disrupting the hydrophobic bonds; diethylamine is a protein-denaturing agent. Two avidity assays based on pneumococcal EIA and using sodium thiocyanate elution have been developed. Because the elution assays are based on dissociation of antibody-antigen complexes of low avidity, they allow the ranking of the antibodies by their avidity.

The avidity of antibodies induced in infants after pneumococcal conjugate vaccination increases from postprimary samples to prebooster samples and increases further over time.[157,163,164] Experience with Hib conjugate vaccines suggest that measurement of antibody avidity is also useful in assessing the induction of memory.[160]

Opsonophagocytic Activity

Because opsonin-dependent phagocytosis is the primary host defense mechanism against *S. pneumoniae*, a variety of techniques to measure the OPA of antibodies to pneumococci have been developed.[152,165–167] The intent of measuring OPA is to assess the function of antibodies rather than just their quantity because a proportion of antibodies measured in EIA have no functional activity. In support of the likely improved utility of OPA over EIA is the observation in animal models that OPA provides better correlation with protection than the concentration of IgG antibodies as measured by EIA.[168,169] In humans, sera from the Finnish otitis media trial, reanalyzed using an OPA assay, revealed that the OPA titers predicted the observed low efficacy for serotype 19A, whereas the enzyme-linked immunosorbent assay (ELISA) titers did not.[170] Furthermore, the cross-reactive IgG response to serotype 19A in the seven-valent vaccine, which was not associated with protection against invasive pneumococcal disease, lacked significant OPA activity.[171] Of importance, EIA results may have limited relevance in older adults, who often have more nonfunctional anticapsular antibodies than infants and children. Interest in OPA has increased in recent years. It is the primary assay to evaluate the functional activity of the immune response to PCV, and OPA responses are an essential part of the registration of new conjugate vaccines.

The classic OPA is an assay to determine the titers of sera that reduce the number of live bacteria by more than half through opsonophagocytosis. Early studies were performed using human polymorphonuclear leukocytes as the effector cells, but recent assays have also been adapted to use cultivable phagocytes (e.g., HL-60 or NB-4 cells). Romero-Steiner and associates[165] developed an OPA assay for pneumococcal antibodies, in which they used rabbit complement and HL-60 cells as phagocytes. This method has become a reference against which other assays are compared. Additional OPA techniques, including radioisotopic, flow cytometric, microscopic, and viability (or killing) assays, have been tested. The killing OPA method, which measures the killing of live bacteria by effector cells, is regarded to be the most biologically relevant.[152,172] There is also more information about the validation and performance of the killing-type OPA than about the flow cytometric OPA. In general, the results obtained by the various techniques correlate well, and an assessment of assays from six laboratories showed acceptable agreement between their assays.[144]

With increasing need for high-throughput and automated functional assays, multiplexed methods for OPA have been developed. The Romero-Steiner assay has been validated to measure seven serotypes simultaneously, using fluorescent dye to quantify the bacterial killing.[173] Another approach is to measure phagocytosis by flow cytometry, using fluorescently labeled bacteria or latex particles,[166] and a multiplexed phagocytosis assay for three to four different serotypes has been developed using bacteria or latex beads coated with different polysaccharides.[174] Still another type of multiplexed OPA is based on a killing-type OPA, using pneumococcal strains resistant to clinically irrelevant antibiotics.[173,175–177]

The correlation between the IgG EIA antibody concentration and OPA results is relatively strong in immune sera[156,165,178,179] but is weaker at low antibody concentrations.[155,168,178,180] In general, the correlation between antibody concentration and OPA seems to be good in infants who have been immunized with conjugate vaccines,[31,154] but is weaker in unimmunized children and in adults.[155,181,182] Correlations between EIA and OPA results are high for vaccine serotypes but lower for cross-reacting serotypes such as 6A and 19A.[183] One reason for the poor correlation is that antibodies detected by standard EIA are not functionally active.[150,181,184]

As discussed below, a recent indirect cohort method was used to attempt to identify an aggregate correlate of protection using OPA.[142] The variation of serotype-specific OPA values that correlated with protection was too great, however, and thus no such value could be defined.

Immunologic Memory

Memory is traditionally characterized by the presence of IgG antibody elicited in a previously naïve individual (such as an infant), by evidence of priming after immunization, or by affinity maturation. For the purposes of vaccine evaluation, the simplest method of demonstrating memory is often the increased concentration and IgG dominance of an antibody response after a booster dose.[185] The increase of geometric mean concentration (GMC) in a primed individual with immunologic memory is much stronger than that seen if the same vaccines were given at the same age without precedent priming.[184,186–191]

The maturation of antibody avidity and increased OPA have also been evaluated in studies of pneumococcal vaccines.[160,179] The avidity of antibodies is increased in children who receive a pneumococcal conjugate booster but not a polysaccharide vaccine booster.[157,192] This suggests that the conjugate triggers a T-cell response, but the T-cell–independent PPV only triggers the existing memory B cells.[155,189,192]

Antibody response may be higher after the polysaccharide booster than after the conjugate booster.[193,194] Whether this has any clinical advantage is not clear. Theoretically, a conjugate booster may stimulate the generation and expansion of high-affinity clones of B memory cells, whereas use of a polysaccharide booster could result in depletion of the memory pool.[195] Long-term (5-year) follow-up of children receiving polysaccharide vaccine did not, however, show reduction in PCV responses or the pool of polysaccharide-specific memory B cells.[195a] Evidence in support of this idea was found in a comparison of memory B cells following polysaccharide versus conjugate pneumococcal vaccination of adults.[196] Therefore, some have argued that boosting with a conjugate may prove important for the persistence of immunity, although this has not been conclusively shown. It is also important to note that the use of the polysaccharide booster may be associated with more pronounced reactogenicity.[193]

The response to the booster dose to either PCV or PPV is rather vigorous at 24 months of age, as seen in the increase in GMCs of anticapsular antibodies in samples taken 7 to 10 days (but not yet at 4 days) after immunization, provided the children had received primary doses with the conjugate.[187,188,197] A comparison of a booster dose at 12 months of age between PCV13 and PCV10, given to those previously primed with the same vaccine in infancy, suggests that the memory B cell response to PCV13 appeared superior to PCV10 for most serotypes common between the vaccines.[198] The clinical relevance of this observation, if any, related to impact on carriage or duration of protection has not been established. The priming effect is less impressive in healthy adults,[134,199] maybe because adults often have already encountered the antigen and become primed, either through carriage of the bacterium or other, cross-reacting antigens. Some indications of priming can be seen also in patients with HIV,[200,201] after bone marrow transplant,[97,202] and sickle cell anemia.[87]

Whether mucosal memory is induced by PCVs is still under examination. In one United Kingdom study, the salivary IgA concentrations against pneumococcal serotypes were only slightly higher in the vaccinated group than in the control group that did not receive PCV7, but there was a significant increase in mucosal antibodies after a booster dose of polysaccharide.[203] Mean-fold rises after the polysaccharide vaccine were lower for serotypes not included in the conjugate, suggesting that the conjugate vaccine had primed children for memory responses.[203] However, two other studies found no clear differences in salivary antibody responses between children primed with conjugates and those who received their first pneumococcal vaccine during the second year of life.[184,204] Follow-up data from the Finnish Otitis Media Vaccine Trial (FinOM), an AOM efficacy trial, showed that PCV7 induced both salivary IgG and IgA responses to the vaccine serotypes. By 4 to 5 years of age, IgA concentrations were similar in both the vaccinated group and the nonvaccinated control group,[205] most probably reflecting natural stimulation through repeated episodes of colonization.

Whether immunologic memory plays an important role in vaccine-induced protection against pneumococcal disease remains controversial. The experience with Hib immunization in infants supported the view that, in addition to the potent herd immune effect, priming may have provided some protection against disease because very young children were protected despite low antibody concentrations.[206] However, priming and memory to the Hib polysaccharide induced by natural exposure in the prevaccine era[207] or Hib conjugate vaccine[208,209] may not be sufficient for protection against disease, as the United Kingdom's experience with Hib strongly suggests. To explain the observation of a rise in failures of the Hib vaccine between 1999 and 2003, the introduction of an acellular pertussis–containing Hib conjugate vaccine without a booster suggested that this vaccine primed well but was associated with lower immunogenicity.[210,211] In contrast, no such increase in failures was noted in countries that adopted this new vaccine but did not eliminate the booster dose. Similarly, studies of the serogroup C *N. meningitidis* conjugate vaccines in the United Kingdom demonstrate lower effectiveness 1 year after vaccination, suggesting that priming of the immune response and memory responses may not be sufficient to prevent invasive disease.[212] It is possible that the lack of efficacy against serotype 1 pneumococcal disease in the PCV9 trials in Africa may also reflect failures in the second year of life after a primary vaccination schedule without a booster.[213] Because of the observed delay in antibody response in primed subjects after exposure, a memory response may not be rapid enough to protect against an isolate that has the capacity to rapidly invade the bloodstream soon after establishing nasopharyngeal colonization. Thus, the role of immunologic memory in conferring long-term protection against invasive bacterial disease remains controversial.[209]

Animal Models

In addition to in vitro assays, many animal models have been developed to study the immunogenicity and efficacy of pneumococcal conjugate and novel protein vaccines, including rodent (mice and rats), rabbit, chinchilla, or infant monkey models.[214–223] In the immunogenicity studies, animals are bled before and after active immunization with an experimental vaccine to obtain sera or cells for various analyses.[217–219,221,224] The functionality of antibodies can also be evaluated in animal models.[168,214,220,225–227] Animals may be passively or actively immunized and subsequently challenged with bacteria to determine protective efficacy.[214–216,224]

Many different strains of pneumococci can be used for colonization and challenge studies, although there are marked serotype-dependent differences with respect to their capacity to colonize the nasopharynx or cause invasive disease in animal models. Various routes of challenge have been employed, in attempts to mimic the human situation of nasopharyngeal colonization, otitis media, bacteremia, pneumonia, and meningitis. For nasopharyngeal colonization, inocula using clinical isolates can result in sustained colonization for several days to weeks. Using the same challenge protocol but under anesthesia and with a larger inoculum (50 µL or more), mice aspirate the inoculum, which subsequently leads to lung infection and, depending on the strain used, possibly also sepsis (particularly if lower numbered serotypes are used). Adult and infant mice and rats have been used in passive-protection models to determine the protective capacity of human antipneumococcal antibodies against bacteremia, meningitis, or death.[168,169,224,228–230]

In addition, special mention should be made of a human model of intentional pneumococcal exposure that permits an evaluation of the immune responses that are either associated with resistance to colonization or response to exposure.[231,232] Use of this model demonstrated the nasal inoculum required to establish colonization in healthy adults (50% colonizing dose estimated at 10^3–10^4 colony-forming units [CFU]), defined the duration of colonization (range: 0–122 days) and analyzed the mucosal and systemic humoral response to a single carriage event. Such studies could be particularly useful in the future for testing of vaccines that target carriage. Indeed, this model has been used for evaluation of the protective effect of PCV13 against carriage[233] and is, at the time of this writing, being used for the evaluation of a protein-based vaccine candidate being developed by Genocea Biosciences.

Infants and Young Children

Infants are the main target group for PCVs; consequently, most clinical trials have focused on the safety and immunogenicity of candidate vaccines in infants. Various dosing schedules have been examined and the available literature on immunogenicity, efficacy, and effectiveness of different schedules has been systematically reviewed.[234] Although aggressive schedules starting with vaccination at birth were studied,[235–237] they have not been approved. In early infancy, passively acquired maternal antibodies seem to interfere with the response to the first doses of conjugates, although there is no evidence of interference for any serotype after completion of the entire immunization series.[238] In the Expanded Programme on Immunization (EPI) schedule, the conjugate is given at the ages of 6, 10, and 14 weeks. The schedule that has been studied most extensively is a three-dose primary immunization series either at 2, 3, and 4 months or at 2, 4, and 6 months of age, followed by a booster dose during the second year of life. Reduced vaccination schedules at 2, 4, and 12 months or at 3, 5, and 11 to 12 months of age have also been studied. Although three doses were originally considered necessary for an optimal immune response to conjugate vaccines,[235,239] studies indicate that even one dose of conjugate vaccine may be sufficient, at least in circumstances where carrier priming and early stimulation with high carriage of pneumococci is common.[240–242] Direct studies comparing different schedules are few, but in general, some conclusions can be drawn: (a) a short interval between doses (i.e., 1 month) often results in lower antibody response than a longer one (i.e., 2 months)[243]; (b) for some serotypes (in particular serotypes 6B and 23F), reduced schedules may show significantly lower immunogenicity, when immunogenicity prior to the booster dose in a two-dose regimen is compared with a three-dose regimen[234,242–249]; and (c) although a single dose in infancy is immunogenic, it is inferior to a two- or three-dose regimen for all serotypes.[242] In this regard, further evaluation of the quality of antibodies and induction of memory, as well as of the determination of the optimal age of vaccination, would be of interest.[250,251] Although most of the early data on immunogenicity were derived from the seven-valent and nine-valent CRM_{197}-conjugated vaccines (PCV7 and PCV9), data on other early PCVs (conjugated to diphtheria toxoid, tetanus toxoid, and OMPC) are available. Recently, studies conducted for the licensure of *H. influenzae* protein D (PHiD-CV, PCV10), and the 13-valent CRM_{197} conjugate vaccine) suggest that they elicit antibody responses somewhat lower than PCV7 in infants and toddlers (Table 46.2).[4,12,13,171,194,252–260] Thus, the addition of more serotypes contained in PCV10 or PCV13 results in a wider spectrum of immune response to the additional serotypes with generally minor compromise to the response of the seven serotypes in PCV7.

In general, anticapsular antibody GMCs increase fivefold to 10-fold after the initial series, relative to the preimmunization concentrations. The antibody concentrations achieved are usually only sustained for a few months and decline during the second year of life to about the preimmunization levels. However, a dose of the pneumococcal vaccine, either polysaccharide or conjugate, administered during the second year of life to children primed with any of the conjugates, generally induces an approximately 10-fold increase in antibody concentrations. Typical ways to describe the immunogenicity results are reverse cumulative distribution curves (Fig. 46.2) or radial diagrams ("logograms") (Fig. 46.3). Table 46.2 compiles representative immunogenicity data after the primary immunizations from selected pivotal trials (either efficacy trials or trials used for licensure of new vaccines after PCV7 introduction [relevant to PCV10 and PCV13]).

Two doses of PCV7 given at either 2 and 4, 3 and 5, or 4 and 6 months of age as a primary series, together with a booster at 11 to 12 months, seem to induce an immune response and induction of immunologic memory comparable to those noted with the standard four-dose schedule in both normal and preterm infants.[242,244–250,261–263] Thus, the marked booster response, after two doses in infancy, even for the least immunogenic serotypes, has led many countries to adopt a two-dose infant series and a booster rather than the initially licensed schedule of three infant doses and a booster. The WHO has now recommended the two primary doses plus a booster series as an alternative to the three-dose primary series typically used in EPI schedules.[264]

In addition to the vaccination schedule and number of doses, the characteristics of the target population also affect the immune response. Direct comparisons are difficult, however, because both the vaccination schedules and the laboratories performing the serologic assays are usually not consistent in all the studies. Similar comparisons were conducted with PCV7 and PCV9 vaccines containing either seven or nine serotypes, which were evaluated in studies conducted in the United States, Finland, and South Africa and showed greater immunogenicity in South Africa than in Finland and the United States.[4,265–267]

PCV immunogenicity is clearly associated with geographic region and vaccine protection, but the magnitude varies with serotype.[268,269] Children in studies from Asia, Africa, and Latin America had significantly higher antibody responses compared to those from Europe and North America.[269] The reasons for these differences could derive from many factors, such as the differences in the nasopharyngeal microbiome of the children, priming effect of tetanus toxoid given to pregnant women, earlier pneumococcal acquisition, differences in concomitant vaccine administration, and genetic differences among populations. Similar differences were noted in immune responses to Hib conjugate vaccines in earlier studies.

The IgG responses of children with invasive pneumococcal disease (IPD) have been measured and values compared between those previously immunized with PCV versus unimmunized children.[270] Vaccinated children with IPD are less likely to have greater than 0.35 μg/mL of antibody to the infecting serotype compared with other serotypes. These children with IPD proved hyporesponsive to their infecting serotype in subsequent exposure to PCV, although recurrent disease was rare (0.2%).[270]

Preterm Infants

Preterm infants seem to respond to the PCV satisfactorily, although the immunogenicity of the vaccine in preterm infants is usually lower than in term infants.[252,261,271–273] The Advisory Committee on Immunization Practices (ACIP) in the United States recommends in its guidance document that PCVs be given to preterm infants at their chronologic age, consistent with the schedule recommended for full-term infants.[274] It may be particularly important to offer a timely booster dose of PCV13 to such infants.[268,273–276] The response of preterm infants after booster depends on the number of doses in the primary series; infants who received only two doses in the primary series had lower rates of seroprotection after the primary series than children who received a three-dose primary series but had higher levels of seroprotection after boost.[276a]

Neonatal Immunization

Neonatal administration of PCV does not lead to tolerance of subsequent doses[277] and postprimary responses are similar[277] or slightly lower[238] than PCV administered to older infants.

TABLE 46.2 Antibody Concentrations (GMCs, μg/mL) 1 Month After Last Dose of the Primary Series of Pneumococcal Conjugate Vaccines

Company	Vaccine	Site/ Population	N	Schedule	1	3	4	5	6B	7F	9V	14	18C	19F	23F	6A	19A	Reference
Wyeth/Pfizer	PCV7	U.S.	88	2, 4, 6 mo	–	–	1.46	–	4.70	–	1.99	4.60	2.16	1.39	1.88	–	–	247
Wyeth/Pfizer	PCV7	U.S.	75	2, 4, 6 mo	–	–	1.34	–	2.14	–	1.23	5.04	1.88	1.52	1.20	–	–	4
Wyeth/Pfizer	PCV7	Finland	57	2, 4, 6 mo	–	–	1.70	–	2.00	–	2.50	6.30	3.60	3.30	2.50	–	–	13
Wyeth/Pfizer	PCV7	Navajo and Apache, U.S.	223	3 doses at age 6 wk–7 mo	–	–	3.21	–	8.25	–	2.47	6.81	2.60	2.74	2.59	–	–	–[a]
Wyeth/Pfizer	PCV7	Israel	715–782	2, 4, 6 mo	0.02	0.04	2.02	0.23	2.30	0.05	1.44	6.46	1.47	2.23	1.57	0.36	0.70	255
Wyeth/Pfizer	PCV9	South Africa[b]	63	6, 10, 14 wk	7.55	–	4.09	5.79	1.76	–	3.35	3.62	4.55	6.02	3.15	–	–	234
Wyeth/Pfizer	PCV9	South Africa[c]	30	6, 10, 14 wk	3.45	–	2.77	3.32	1.21	–	2.36	2.23	1.87	3.59	1.78	–	–	234
Wyeth/Pfizer	PCV9	The Gambia	217	2, 3, 4 mo	6.94	–	4.90	5.84	4.93	–	4.07	4.45	4.89	2.91	2.85	–	–	248
Merck	PncOMPC	Finland	376	2, 4, 6 mo	–	–	3.45		0.35	–	1.79	3.23	1.02	3.19	0.67	–	–	190
GSK	PncPD	Czech Republic	140	3, 4, 5 mo	1.58	3.78	2.16	1.92	0.62	2.34	1.60	3.00	1.49	2.60	0.90	–	–	12
GSK	PCV10	Finland, France, Poland	1107	2, 3, 4 mo	1.05	–	1.45	1.70	0.33	1.72	1.32	2.90	1.66	1.84	0.53	–	–	249
GSK	PCV10	Germany, Poland, Spain	173	2, 4, 6 mo	1.00	–	1.70	1.69	0.71	2.25	1.58	3.76	2.34	3.81	0.96	–	–	250
GSK	PCV10	Philippines	285	6, 10, 14 wk	3.23	–	4.96	4.87	1.19	4.84	4.04	6.45	11.56	10.46	2.23	–	–	251
Wyeth/Pfizer	PCV13	U.S.	252	2, 4, 6 mo	2.03	0.49	1.31	1.73	2.10	2.57	0.98	4.74	1.37	1.85	1.33	2.19	2.07	166
Wyeth/Pfizer	PCV13	U.S.	258–264	3, 5 mo	2.30	1.15	2.38	1.27	0.41	2.06	1.68	2.84	1.72	3.42	0.61	1.17	2.87	252
Wyeth/Pfizer	PCV13	Germany	285	2, 3, 4 mo	1.83	1.55	2.18	1.31	0.98	2.59	1.65	4.14	1.94	1.73	1.26	1.33	3.26	253
Wyeth/Pfizer	PCV13	U.K.	107	2, 4 mo	1.69	0.63	1.37	0.95	0.26	2.14	0.87	1.83	1.37	2.38	0.53	0.86	1.90	254
Wyeth/Pfizer	PCV13	Israel	741–765	2, 4, 6 mo	2.08	0.97	2.16	1.38	2.26	3.34	1.40	5.72	1.49	2.90	1.13	2.53	1.81	255

[a]K.L. O'Brien, personal communication.
[b]HIV-negative children only.
[c]HIV-infected children only.
–, Indicates that the serotype was not present in the vaccine; GMCs, geometric mean concentrations; GSK, GlaxoSmithKline; OMPC, outer membrane protein conjugate; PncPD, pneumococcal protein D (conjugate); Ref, reference.
Data derived from pivotal trial (either efficacy trials or trials used for licensure of new vaccines after PCV7 introduction). Note that the studies were conducted in different populations, during different time periods, and the antibody assays were performed in different laboratories; consequently, results from separate studies are not directly comparable.

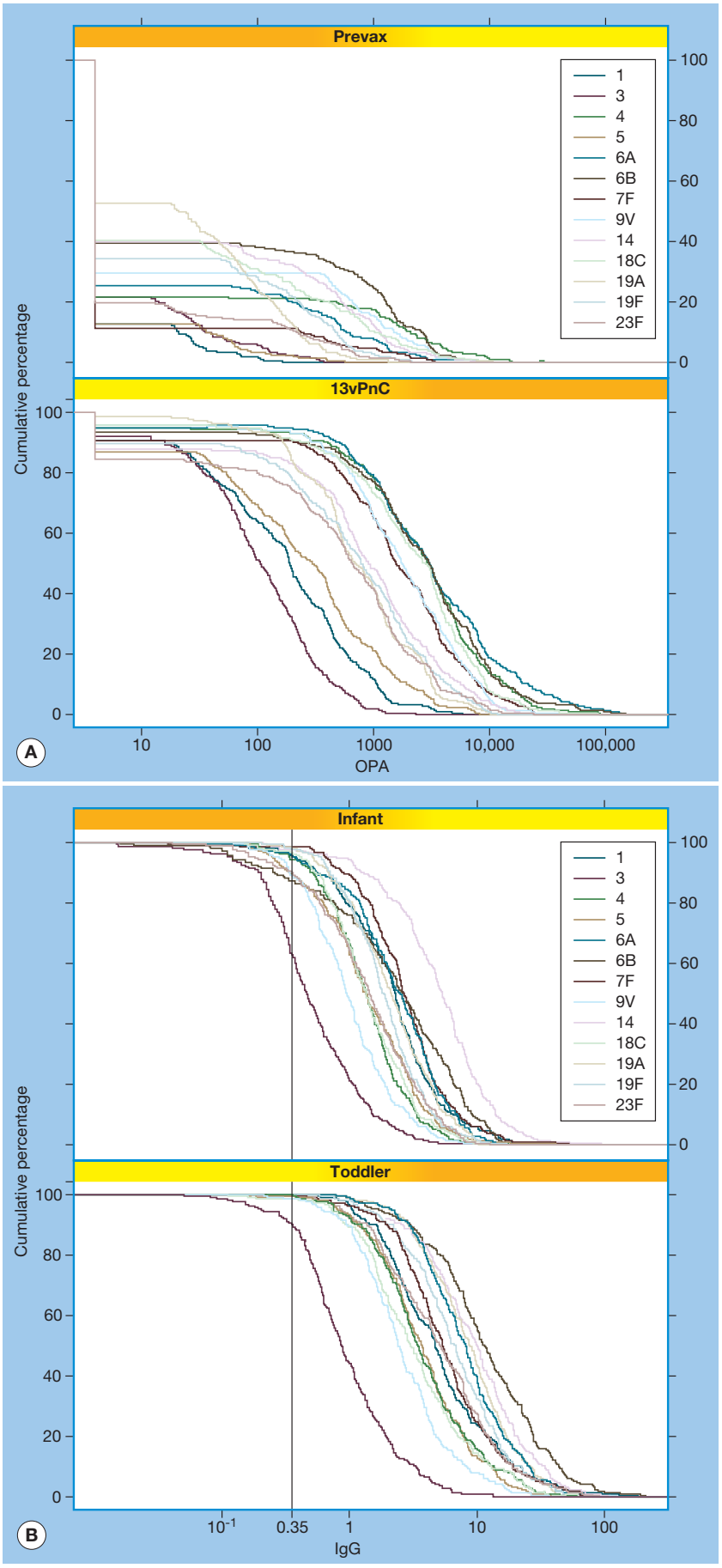


Figure 46.2. **A** and **B,** Reverse cumulative distribution curves of 13-valent vaccine. **A,** OPA values pre– and post–single-dose PCV13 in adults. **B,** IgG post–primary series in infants and toddlers. IgG, immunoglobulin G; OPA, opsonophagocytic activity. *(Data courtesy Pfizer.)*

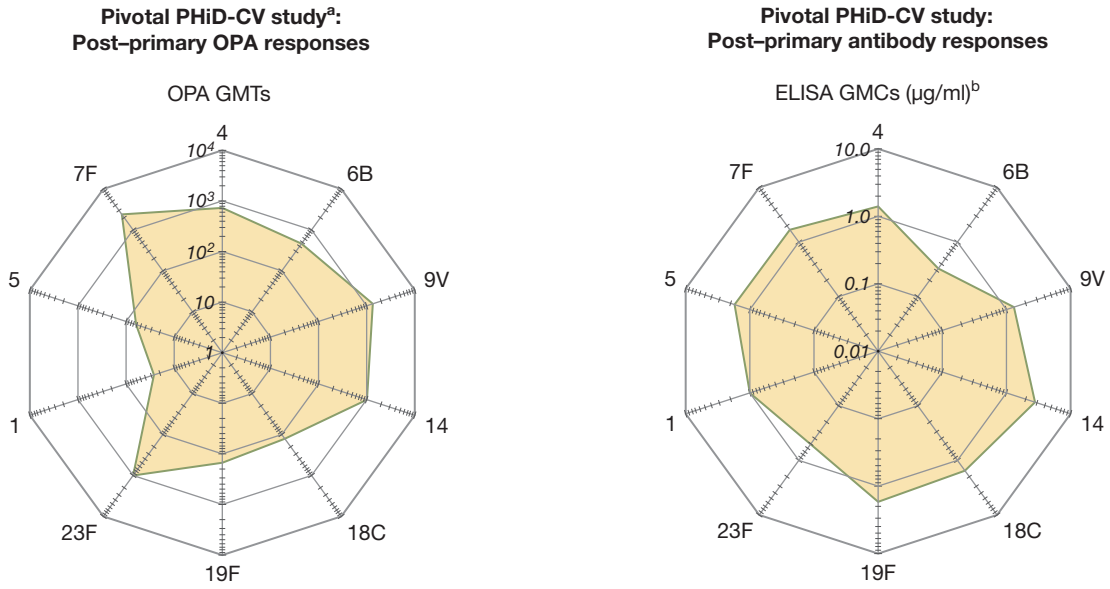


Figure 46.3. Spider web plots for licensed 10-valent study conducted in Finland, France, and Germany according to a 2-, 3-, 4-months-of-age schedule with Infanrix hexa coadministered. ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean concentration; GMT, geometric mean titer; OPA, opsonophagocytic activity; PHiD-CV, pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine. *(Modified from Vesikari T, Wysocki J, Chevallier B, et al. Immunogenicity of the 10-valent pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine [PHiD-CV] compared to the licensed 7vCRM vaccine.* Pediatr Infect Dis J. *2009;28[4 Suppl]:S66–S76.)*

Impact of Maternal Immunization on Immunogenicity in Infants

In a randomized trial of PCV9 in pregnant women, administered at 30 to 35 weeks of pregnancy, serotype-specific IgG was significantly raised in infant cord blood compared to infants whose mothers received placebo.[278] Infants then received PCV7 at 2, 4, 6, and 12 months. At 7 months of age, the IgG responses to the initial three-dose series of PCV7, were significantly reduced for all serotypes among infants of immunized mothers, compared to infants of mothers who received placebo. Robust booster responses to PCV7 were measured 1 month after the 12-month dose. The mean levels of IgG remained lower in infants of vaccinated mothers compared to those born to control mothers for serotypes 6B, 18C, 19F, and 23F. These data suggest that high levels of passively acquired maternal antibody suppress the primary responses to those serotypes in their infants. Responses to serotypes 1 and 5, not given to the infants, remained elevated compared to infants of controls through 6 and 13 months of age. Data from infants born to mothers receiving tetanus, diphtheria, and pertussis (Tdap) during pregnancy had reduced responses to CRM based PCV conjugate compared to historical controls whose mothers did not receive Tdap.[279]

Older Children, Adults, and the Elderly

GMCs for most serotypes in infants 1 year of age receiving two doses of PCV and older children receiving a single dose of PCV7 were comparable to the immune responses of infants who received three doses of conjugate in the Northern California Kaiser Permanente (NCKP) efficacy study.[4] Based on these data, the official recommendation in the United States is to use PCV7 at 2, 4, 6, and 12 to 15 months of age. For children 12 to 23 months of age, two doses were recommended at least 2 months apart, and for those 24 months through 9 years of age, one dose was considered sufficient. Similar recommendations have been made for PCV13 for the primary schedule.[274] Vaccination is recommended for all children 2 to 59 months of age and those 60 to 71 months of age with underlying medical conditions (Table 46.3). Children who received one or more doses of PCV7 were recommended to complete the schedule with PCV13. A supplemental dose of PCV13 was recommended for those children 14 to 59 months of age who had completed their four-dose PCV7 schedule. For children with underlying medical conditions, a supplemental dose of PCV13 was recommended up to age 71 months (the recommendation for PPV23 remained for children 2 to 18 years of age who have received their full schedule of PCV13).[274] For older children not previously given PCV7, those 7 to 11 months of age were recommended to get three PCV13 doses, the first two at least 4 weeks apart and the second two at least 8 weeks apart, with the third dose at 12 to 15 months. Children 12 to 23 months of age should receive two doses at least 8 weeks apart, and those 24 months of age or older should receive a single dose, or two doses at least 8 weeks apart if they have underlying medical conditions (Tables 46.3 and 46.4).[274] In February 2013, the ACIP recommended routine use of PCV13 for children 6 to 18 years of age with immune-compromising conditions, functional or anatomic asplenia, CSF leaks, or cochlear implants who have not recently received PCV13, regardless of whether they previously received PCV7 or PPV23.[280] The recommendation was for a single PCV13 dose first, followed 8 or more weeks later by a PPV23 dose. A second PPV23 dose is recommended 5 years after the first PPV23 for children with anatomic and functional asplenia,

TABLE 46.3 Medical Conditions or Other Indications for Administration of PCV13[a] and Indications for PPSV23[b] Administration and Revaccination for Children Aged 2–18 Years[c]

		PCV13	PPSV23	
Risk Group	**Underlying Medical Condition**	**Recommended**	**Recommended**	**Revaccination 5 y After First Dose**
Immunocompetent persons	Chronic heart disease[d]	X	X	
	Chronic lung disease[e]	X	X	
	Diabetes mellitus	X	X	
	Cerebrospinal fluid leaks	X	X	
	Cochlear implants	X	X	
	Alcoholism	X	X	
	Chronic liver disease	X	X	
	Cigarette smoking	X	X	
Persons with functional or anatomic asplenia	Sickle cell disease/other hemoglobinopathies	X	X	X
	Congenital or acquired asplenia	X	X	X
Immunocompromised persons	Congenital or acquired immunodeficiencies[f]	X	X	X
	Human immunodeficiency virus infection	X	X	X
	Chronic renal failure	X	X	X
	Nephrotic syndrome	X	X	X
	Leukemia	X	X	X
	Lymphoma	X	X	X
	Hodgkin disease	X	X	X
	Generalized malignancy	X	X	X
	Iatrogenic immunosuppression[g]	X	X	X
	Solid-organ transplantation	X	X	X
	Multiple myeloma	X	X	X

[a]13-Valent pneumococcal conjugate vaccine.
[b]23-Valent pneumococcal polysaccharide vaccine.
[c]Since 2010 the recommendation is that all healthy children younger than 5 years of age receive PCV13; PPV23 is not recommended for healthy children younger than 2 years of age.
[d]Including congestive heart failure and cardiomyopathies.
[e]Including chronic obstructive pulmonary disease, emphysema, and asthma.
[f]Including B-(humoral) or T-lymphocyte deficiency, complement deficiencies (particularly C1, C2, C3, and C4 deficiencies), and phagocytic disorders (excluding chronic granulomatous disease).
[g]Diseases requiring treatment with immunosuppressive drugs, including long-term systemic corticosteroids and radiation therapy.
From Advisory Committee on Immunization Practices, 2010 and 2013. Frenck R, Thompson A, Senders S, et al. 13-Valent pneumococcal conjugate vaccine in older children and adolescents either previously immunized with or naïve to 7-valent pneumococcal conjugate vaccine. Pediatr Infect Dis J. *2014;33(2):183–189.*

TABLE 46.4 Recommended Schedule for Administering Doses of 13-Valent Pneumococcal Conjugate Vaccine (PCV13) to Children <24 Months by PCV Vaccination History and Age

Age at This Visit (mo)	**Vaccination History: Total Number of PCV7 and/or PCV13 Doses Received Previously**	**Recommended PCV13 Regimen[a]**
2–6	0	3 Doses, 8 wk apart; fourth dose at age 12–15 mo
	1	2 Doses, 8 wk apart; fourth dose at age 12–15 mo
	2	1 Dose, 8 wk after the most recent dose; fourth dose at age 12–15 mo
7–11	0	2 Doses, 8 wk apart; third dose at 12–15 mo
	1 or 2 before age 7 mo	1 Dose at age 7–11 mo, with a second dose at 12–15 mo, ≥8 wk later
12–23	0	2 doses, ≥8 wk apart
	1 before age 12 mo	2 doses, ≥8 wk apart
	1 at ≥12 mo of age	1 dose, ≥8 wk after the most recent dose[b]
	2 or 3 doses before age 12 mo	1 dose, ≥8 wk after the most recent dose[b]
	4 doses of PCV7 or other age-appropriate vaccine, complete PCV7 schedule	1 supplemental dose ≥8 wk after the most recent dose

[a]Minimum interval between doses is 8 wk except for children vaccinated at <1 y of age, for whom minimum interval between doses is 4 wk.
[b]No additional PCV13 doses are indicated for children aged 12 to 23 mo who have received two or three doses of PCV7 before age 12 mo and at least one dose of PCV13 at age ≥12 mo.
PCV7, 7-valent pneumococcal polysaccharide-protein conjugate vaccine.
From Advisory Committee on Immunization Practices, United States, 2010. Frenck R, Thompson A, Senders S, et al. 13-Valent pneumococcal conjugate vaccine in older children and adolescents either previously immunized with or naïve to 7-valent pneumococcal conjugate vaccine. Pediatr Infect Dis J. *2014;33(2):183–189.*

HIV infection, or other immunocompromising condition. If these children previously received one or more doses of PPV23, they should be given a single PCV13 dose 8 or more weeks after last PPV23 dose. These recommendations are based on immunogenicity studies in both immunocompetent and immunocompromised children 6 to 18 years of age.[281–285]

To date, there is some evidence that conjugate vaccines are more immunogenic than the PPV vaccine in adults, although this has not been consistently shown.[286] In early studies, a five-valent PCV vaccine elicited a greater response than PPV to some serotypes (6B, 18C, and 23F) but not others (14 and 19F).[133,198] In more recent studies, the IgG and opsonophagocytic responses to PCV7 and PCV13 were either similar or higher than those to PPV.[287–291] In a dose ranging study by Jackson and colleagues,[287] administration of a 1-mL dose (double the infant dose, so 4 μg of all serotypes except 8 μg of serotype 6B) demonstrated an enhanced immune response, but no further immunologic advantage was seen with a 2-mL dose (fourfold greater than the infant dose). In adult Alaskan natives, PCV did not induce higher IgG and opsonophagocytic responses when compared with PPV and provided no benefit when given before PPV.[292] There is also some evidence that the baseline level of antidiphtheria antibodies is associated with the response to the PCV7 vaccine: adults with higher diphtheria antibody levels have higher responses to the conjugate.[293] Repeated PCV doses in adults seem to induce comparable immunogenicity to the first dose.[288,290] Receipt of PCV 6 months[294] or 1 year after PPV led to a diminished response to PCV compared with PPV-naïve adults.[288,294] The order of immunization of adults with the conjugate versus polysaccharide vaccine appears to matter, with conjugate vaccination first conferring a booster response to a later dose of polysaccharide, while an initial dose of polysaccharide vaccine leads to a reduced response to PCV.[288,294–296]

Because most adults have preexisting antibodies to pneumococcal polysaccharides and to the carrier protein, the initial vaccination with the conjugate acts as a booster.

Maturation of pneumococcal antibody avidity is prominent after the vaccination of infants, but the presence of mature antibodies before vaccination of adults results in only modest increases in avidity. In a study of different pneumococcal conjugates, the avidity index was stable in adults, whereas clear increases occurred in children after both primary and booster immunizations with PCV.[155] Other reports have noted no avidity maturation in elderly subjects vaccinated with PPV,[297] or young adults vaccinated with either PPV or PncD/T conjugate.[298]

Although both IgG and OPA responses to the PncT conjugate and to the polysaccharide vaccine appeared to be similar in a group of patients with chronic obstructive lung disease and in healthy adults[299]; in patients recovering from pneumonia, there was better persistence of antibody after PCV7 than after PPV23 when assessed 6 months after immunization. This study also found little utility in combining PCV and PPV regimens.[300] In patients with chronic obstructive pulmonary disease there was an improved persistence at 1 to 2 years postimmunization of functional activity for the common types except 19F in individuals given PCV7 compared to those given PPV23.[301] Improved OPA persistence was also seen in hospitalized frail elderly a year post PCV followed by PPV23 given 6 months later, compared to 1 year post-PPV23 alone, but these data are confounded by the shorter time post-PPV23 in the combined immunization group.[302] These data do suggest that in nonimmunocompromised adults, there may be an advantage of PCV over PPV, particularly in the duration of antibody response and the response to revaccination. A conjugate vaccine of comparable valency to PPV23 would thus likely be a significant advance, but for the reasons outlined earlier would pose technical and financial challenges.

Immunocompromised Individuals

In the absence of immunogenicity data in immunocompromised individuals, the immunogenicity cutoffs for immunocompetent individuals have been applied to the immunocompromised population.

HIV. PCVs have been tested in HIV-positive children in different populations. It appears that the immune status of the child (measured by the CD4 count) and the existing viral load impact the response to the immunization.[303] The responses after the first two PCV vaccinations are rather modest in children with advanced HIV, but are higher than those seen in a similar population after the polysaccharide vaccine.[304,305] Three doses of PCV7 are immunogenic in young HIV-infected children; children with milder HIV disease tend to have better responses.[253,304] Among HIV-infected infants with a CD4 percentage greater than 25, there was no impact of antiretroviral therapy on IgG responses, although functional responses were better in those receiving antiretroviral therapy.[306] Yet, the functional OPA responses remain less than those seen after PCV in HIV-unexposed, uninfected infants. There also may be some functional deficits in the quality of antibodies made by HIV-exposed but uninfected children.[306] One study suggested that more than one dose of conjugate vaccine was needed to elicit immunologic memory in children with HIV infection (see Table 46.3).[304] Memory is retained in most HIV infected children on HAART 4 to 5 years after sequential exposure to two doses of PCV followed by a single PPV dose.[307]

In a study from Uganda,[308] HIV-infected adults received two doses of the PCV7 vaccine and postvaccination antibody concentrations were directly correlated with the CD4 cell count, particularly after a second dose. Past receipt of PPV did not impact the response to the conjugate. Similarly, PCV13 given to HIV infected adults previously exposed to PPV23 had significant immune responses regardless to the number of their previous PPV exposures.[309] In a trial among HIV-infected Malawian adults, PCV7 vaccine induced both systemic and mucosal responses, similar to the responses seen in HIV-uninfected subjects. A second dose of conjugate did not provide any additional benefit.[310] The antibodies were functionally active, as measured by OPA.[311] Supplementation with zinc or vitamin A before immunization did not change the response in HIV-positive adults.[312] Among HIV-infected adults with CD4 counts between 200 and 500/μL and a viral load of less than 10,000 virus/mL, priming with PCV increased the titer and duration of response to PPV.[313] Furthermore, this enhanced response was correlated with Th1 lymphoproliferative responses to the CRM carrier.[314] Conversely, immunization with PCV after previous PPV among HIV-infected adults resulted in higher IgG responses than those randomized to receive a second dose of PPV, but the duration of difference in antibody response was limited, with no differences seen between the two groups when titers were measured 6 months postvaccination.[315] There was no variance in vaccine responses by black or white ethnicity among trial participants.[316] PCV appears to induce higher levels of antibody with longer persistence in HIV-infected adults on antiretrovirals than in those receiving PPV.[317]

Other Immunocompromising Conditions. In infants with sickle cell disease, PCV7 vaccine induced significant and comparable increases in serum antibody concentration compared to control infants without sickle cell disease.[90] In older children with sickle cell disease, two doses of the conjugate vaccine

followed by a PPV booster resulted in higher GMCs and OPA titers than one dose of PPV alone.[87] Patients with ataxia-telangiectasia, who usually have low T-cell and B-cell counts, as well as low levels of pneumococcal antibodies, responded to PPV vaccination with a booster-type response if polysaccharide was given after a dose of the conjugate. However, the antibody concentrations were lower than those observed in control subjects.[318,319]

Asplenic patients with β-thalassemia who have previously received PPV23 have significant B memory cell responses producing IgG, but not IgM. Of particular concern, though, was the observation that B-cell responses post-PCV13 in these patients were inversely related to the number of previous PPV23 doses and to the time since the last PPV23 dose, consistent with the idea that PPV23 commits existing B cells to antibody production but depletes the B-cell memory pool.[320] Two studies examined the immune response to PncOMPC in adults previously treated for Hodgkin disease. Although the response to a single dose of seven-valent PncOMPC vaccine was lower than to a polysaccharide vaccine,[97] the conjugate appeared to induce immunologic priming in this patient group.[201] In adults with chronic lymphocytic leukemia, responses to PCV were detected in 40% of cases before the onset of hypogammaglobulinemia associated with chemotherapy.[321]

Adults with allogeneic or autologous stem cell transplantation have an antibody response to a three-dose schedule of PCV7.[322] Immunization of the donor before transplantation (or the patient before stem cell collection) enhances antibody responses pretransplantation,[323,324] and revaccination posttransplantation induces a greater than 80% immune response to PCV7.[325] Among adult allogeneic bone marrow transplant recipients, responses to PCV and PPV are generally poor, but PCV resulted in longer periods with detectable antibody when compared with responses to PPV.[326] Although early immunization with PCV at 3 months posttransplantation was as immunogenic as waiting for 9 months, antibodies at 24 months posttransplantation were lower for the early immunization group, supporting waiting 6 to 9 months after transplantation to vaccinate.[327] Current guidelines suggest three to four doses of PCV a month apart starting at 6 to 9 months posttransplantation followed by one dose of PPV.[328]

In pediatric solid-organ transplant recipients, two doses of PCV7, followed by one dose of PPV, increased pneumococcal antibody concentrations. The first conjugate dose seemed to do the job; neither the second dose of conjugate nor the dose of polysaccharide increased the levels further, suggesting that additional closely spaced PCV or PPV does not add significant benefit. Final responses were, however, significantly lower in solid-organ transplant recipients than in the healthy control group.[329] In renal transplant recipients, PCV7 was marginally more immunogenic than PPV; OPA responses were not different,[330] antibodies waned significantly over 3 years, and PCV did not increase the durability of the immune response when compared with PPV receipt.[331] The prime boost strategy was no better than PPV alone in terms of immunogenicity among liver transplant recipients.[332] There may be some heterogeneity in responses with the different types of solid-organ transplants since multiple doses of PCV and a prime boost strategy improved the immunogenicity after cardiac transplantation.[333]

Duration of Immunity

Over a 5.3-year follow-up after primary immunization with PCV9 of South African infants at 2, 4, and 6 months of age with no booster in the second year of life, antibodies to the seven-valent vaccine serotypes remained significantly elevated compared with placebo-control subjects.[334] Titers greater than 0.35 μg/mL (see "Correlates of Protection" later) were detected among 44% to 95% of children who received placebo in infancy, depending on serotype. The lowest titers were noted for serotype 4, suggesting that long-term immunogenicity for types rarely carried, such as types 1, 4, and 5, could be problematic, particularly in the absence of a booster dose in the second year of life. Even among HIV-infected children, higher antibody titers were detected 5.3 years postimmunization than in HIV-infected unimmunized control subjects, but the percentage of children with titers greater than 0.35 μg/mL was lower in HIV-infected than in HIV-uninfected children and ranged from 19% to 81%.[334]

Variables Influencing the Immunogenicity of Conjugate Vaccines

Composition of the Vaccine

The immunogenicity of a conjugate vaccine is dependent on its chemical characteristics.[336–338] In pneumococcal conjugates, polysaccharides seem to be more immunogenic than oligosaccharides.[189,339,340] Other variables influencing immunogenicity include the inherent properties of carrier proteins, the nature of the chemical linkage of the carbohydrate to the protein carrier,[341,342] conformational changes in epitopes after conjugation,[327,335] the availability of antigenic structural components,[337,339] the presence of free polysaccharides or carrier proteins in the vaccine,[343,344] and the carbohydrate-to-protein ratio.[345]

Clear-cut differences exist in the antibody responses to different capsular-type polysaccharides in the conjugate vaccines.[13,197,252,267,345,346] In studies with Finnish children, the magnitude of the IgG response to serotype 6B appears to be rather consistent between the different vaccines, but large differences can be found for other serotypes, particularly 14 and 19F.[347] In general, in infants, serotypes 6B and 23F appear to be poorly immunogenic, whereas serotype 19F induces a relatively high antibody concentration, although higher OPA titers are not seen. Serotypes 3 and 18C are usually satisfactory immunogens even after the first dose, although a poor booster response is seen for serotype 3 antibodies. No marked increase in anti-6B IgG can be seen in infants after a single dose, and even two doses are markedly inferior to three doses.[242–244] For serotype 4, there is a marked response after the first dose and a further increase after the second dose, but not after the third dose. Serotype 19F induces an antibody response after the second dose, and the third dose does not increase the mean antibody concentrations.

Dose-responses have been published for four-valent PncT and PncD vaccines (containing 1, 3, or 10 μg of each polysaccharide). For PncT vaccine, no dose dependency was seen after the primary immunization, whereas the booster response was highest in the group primed with the lowest dose of the conjugate vaccine.[191] In contrast, the highest dose of PncD induced the strongest response after primary immunization, but, as with the PncT vaccine, the booster response was greatest in the group primed with the lowest dose.[190] When a tetravalent *H. influenzae* protein D PCV was tested in three doses (0.1, 1.0, and 10.0 μg of each polysaccharide), the 0.1 μg dose was superior to the 10 μg dose; for serotype 14, the response increased with increased polysaccharide content, and for serotype 19F the 1.0 and 10.0 μg were equal and superior to that of the 0.1 μg.[348] Thus, the dose response in the various PCVs is not always predictable and may well depend on the carrier and the serotypes.

The optimal carrier may be different for each serotype. Thus far, direct comparisons have been made only between diphtheria and tetanus carriers. Polysaccharides from serotypes 3, 9V, and 14 evoke a better response in infants when conjugated to

diphtheria toxoid, and polysaccharides from serotype 4 induce a stronger response when conjugated to tetanus protein.[266,349] No carrier-specific differences in immunogenicity have been shown for serotypes 6B, 18C, 19F, and 23F. Furthermore, the conjugates seem to have different capacities to induce a mucosal immune response: whereas diphtheria toxoid conjugates induce a better mucosal response, tetanus conjugates seem more efficient in inducing systemic responses.[184]

Immunity to carrier proteins is beneficial for the improved immunogenicity of conjugate vaccines. Early priming with carrier proteins enhances the immune response to polysaccharides in subsequent immunizations with conjugates.[344,350–352] In a study in the Philippines,[240] antibody concentrations at 9 months of age were similar after one dose or three doses of an 11-valent tetanus/diphtheria conjugate vaccine.[240] The most plausible explanation for the good immunogenicity and persistence of antibodies, despite the lower number of doses, is carrier-priming with previous diphtheria-tetanus-pertussis vaccine. Although the T-cell immunity against carrier proteins is essential for responses to polysaccharides, B-cell immunity to carrier protein may become detrimental upon induction of anticarrier antibodies.[353,354] In a situation where polysaccharide- and protein-specific B cells compete to capture conjugates, high amounts of antibodies to carrier proteins may result in a suppressed immune response to polysaccharides.[343,344,352,354–356] This is a reasonable argument for minimizing the amount of antigen and for developing mixed-carrier vaccines.

Aluminum adjuvants, used in all marketed conjugate products, except in two standalone Hib vaccines, bias the immune system to a T-helper cell type 2 (Th2) response and enhance the immune response. The addition of an aluminum hydroxide adjuvant to the PncT/PncD vaccine increased the IgG response and functional activity of antibodies in studies in Finland and Israel.[357]

Table 46.5 describes the composition of the three PCVs licensed to date.

Concurrent Administration With Other Vaccines

High coverage of infant and toddler populations with PCVs and Hib-conjugated vaccines has partly been fostered by their ability to be coadministered or mixed together with other routinely administered vaccines. However, conjugate vaccines can induce immune interference (enhancement or impairment) of humoral immune responses to coadministered antigens.[355,356] Two common interference mechanisms are (a) carrier-induced epitopic suppression, whereby preexisting immunity to a carrier (conjugate protein) suppresses subsequent responses to the hapten/saccharide linked to the same carrier and (b) bystander interference, whereby coadministration of vaccine combinations containing a given conjugate protein induce interference that extends to unrelated antigens that are part of the combination vaccine.[355]

The best recognized form of immune enhancement occurs when vaccines using tetanus toxoid (TT) as the conjugate protein are coadministered with Hib-TT. Anti-PRP (polyribosylribitol-phosphate) antibody concentrations increase when Hib-TT is coadministered with meningococcal serotype C TT conjugate (MenC-TT), or with protein D PCV.[358–363] Conversely, immune responses to TT-conjugated pneumococcal polysaccharides and MenC-TT decrease in the presence of excessive amounts of coadministered TT through carrier-induced epitopic suppression.[364,365] Immune enhancement mechanisms also contribute to increased responses to vaccines using diphtheria toxoid variant (CRM_{197}) as conjugate proteins. In general, CRM_{197} and TT, when engaged as carrier, lead to higher antipolysaccharide responses than does DT, as exemplified by Hib-TT/CRM versus Hib-DT,[366] and meningococcal ACWY-TT/CRM versus ACWY-DT.[366–368] However, unlike TT, DT/CRM_{197} conjugates need T-cell enhancement via DT/CRM_{197} priming or coadministration to maximize immune response.[241]

Conjugate vaccines using CRM_{197}, although less likely than those using TT or DT to have reduced responses to polysaccharides through carrier-induced epitopic suppression, are more likely to induce interference through another yet unclear mechanism, termed *bystander interference*.[355,356] This phenomenon affects vaccines coadministered with the CRM_{197} conjugate vaccines, with Hib-TT and hepatitis B vaccines being particularly vulnerable to this interference. Bystander effects are probably mediated by T-cell mechanisms shared between DT and CRM_{197} and are spreading to other coadministered antigens.

TABLE 46.5 Composition of the Three Currently Licensed Pneumococcal Conjugated Vaccines[a]

Vaccine	Commercial Name	Polysaccharide Content (μg) and Protein Carrier													Adjuvant
		1	3	4	5	6A	6B	7F	9V	14	18C	19A	19F	23F	
PCV7 (CRM_{197} conjugate)	Prevnar/ Prevenar	(–)	(–)	2.0	(–)	(–)	4.0	(–)	2.0	2.0	2.0	(–)	2.0	2.0	Aluminum phosphate
PCV13 (CRM_{197} conjugate)	Prevnar13/ Prevenar13	2.2	2.2	2.2	2.2	2.2	4.4	2.2	2.2	2.2	2.2	2.2	2.2	2.2	Aluminum phosphate
PCV10 (serotypes 1, 4, 5, 6B, 9V, 14, 23F conjugated to nontypeable *Haemophilus influenzae*–derived protein D; serotype 18C conjugated to tetanus toxoid; serotype 19F conjugated to diphtheria toxoid)	Synflorix	1.0	(–)	3.0	1.0	(–)	1.0	1.0	1.0	1.0	3.0	(–)	3.0	1.0	Aluminum phosphate

[a]Information as described in product monographs presented for licensure.

Immune interference in general, and that observed with conjugate vaccines in particular, are most marked when vaccines are coadministered with combination vaccines containing acellular pertussis antigens (i.e., diphtheria, tetanus, and acellular pertussis [DTaP]), as compared with whole-cell pertussis combinations (i.e., diphtheria and tetanus toxoids combined with whole-cell pertussis [DTwP]).[355,356,364] PncD/T was immunogenic during infancy when given in the presence of DTwP.[269,369] However, when DTaP was present in the concomitantly administered vaccine, a reduction in the immunogenicity of the primary response to pneumococcal polysaccharides conjugated to TT was observed.[364] No similar reduction was seen in relation to the polysaccharides conjugated to DT in the same vaccine. The phenomenon was attributed to a disturbance in the rather complicated balance between the immunogenic properties and amounts of carrier protein and the adjuvant effect of whole pertussis vaccine, which was removed when switching to acellular pertussis vaccines.[364] Because of this finding, Sanofi Pasteur decided to abandon the development of the PncD/T vaccine.

When studies are compared, CRM_{197}-conjugated vaccines coadministered with DTaP vaccines and Hib-TT consistently demonstrate reduced anti-PRP responses compared with schedules where CRM_{197} is not coadministered, as is exemplified in Table 46.6. In contrast, coadministration of Hib-TT with conjugate vaccines that do not contain CRM_{197} demonstrate anti-PRP responses that remain unaffected or are enhanced.[358–362]

In addition to the potential interference induced by the introduction of PCV into the infant immunization program, the effect of increasing the DT or TT component of the pneumococcal vaccine on immunogenicity needs to be considered, especially when introducing extended-spectrum PCVs with additional conjugated polysaccharides. Head-to-head studies of PCV13-CRM (34 μg CRM_{197}) versus PCV7-CRM (20 μg CRM_{197}) show a modest reduction in post–dose-three anti-pneumococcal GMCs in PCV13-CRM for at least four of the seven serotypes in PCV7, in particular serotype 6B.[258,259,370] When PCV7-CRM was administered concomitantly with a tetravalent conjugated meningococcal-CRM vaccine (Menactra, meningococcus A, C, Y, W_{135} conjugated to DT), a marked reduction in the immune response to all pneumococcal seven polysaccharide antigens was observed when compared to the arm receiving PCV7-CRM alone (FDA statistical review evaluation BLA 125084/395). These observations suggest dose-dependent carrier-induced epitopic suppression in the pneumococcal response by CRM_{197}. The mechanisms involved in suppression of immune responses are not explained. Table 46.7 depicts some of the observed interference phenomenon related to conjugate vaccines.

In addition to the complexity of multiple vaccines coadministered in infants, it has been observed that some coadministered drugs, such as acetaminophen, can also markedly interfere with the immune response to PCV, Hib, and diphtheria, tetanus, and pertussis vaccines.[371]

With the introduction of new conjugated vaccines using CRM_{197}, there is a need to further evaluate potential interference either by carrier-induced epitope suppression or by the bystander interference mechanism. However, although interference may often be seen when vaccines are coadministered in infancy, it is rarely of clinical importance. In the setting of less immunogenic vaccine combinations and their coadministration with other vaccines, the booster dose assumes greater importance. The role of the booster dose of Hib in the second year of life has been highlighted in postmarketing surveillance studies[372] and by public health experience in the United Kingdom. However, the extent to which carrier-induced epitopic suppression or bystander effects are negated or persist upon boosting is not well studied.

Correlates of Protection

Challenges in the Search for Correlates

Determination of serologic correlates or surrogates of protection against pneumococcal diseases has proven complicated.

TABLE 46.6 Effect of CRM_{197} Bystander Interference on the Immune Response to Coadministered Hib-TT

Vaccines Administered	Schedule (mo)	No. of Participants	Anti-PRP[a] Response (μg/mL)		Reference
			% ≥0.15	GMC (95% CI)	
DTaP5-IPV-Hib-TT	2, 4, 6	324	97.9	4.86 (4.21–5.62)	362
DTaP5-IPV-Hib-TT+MenC-TT	2, 3, 4	53	94.2	5.17 (3.53–7.58)	350
DTaP5-IPV-Hib-TT+MenC-CRM	2, 3, 4	50	87.6	2.17 (1.34–3.53)	350
DTaP5-IPV-Hib-TT+MenC-CRM+PCV7[b]	2, 3 or 2, 4	126	89	1.75 (1.29–2.38)	351
DTaP5-IPV-Hib-TT+MenC-CRM+PCV77[c]	2, 3 or 2, 4	126	85	1.76 (1.29–2.39)	351
DTaP5-IPV-Hib-TT+MenC-CRM+PCV7	2, 3, 4	53	85	0.77 (0.51–1.16)	363
DTaP5-IPV/Hib-TT+PCV7	2, 3, 4	248	91	1.38 (1.14–1.66)	364
DTaP3-HBV-IPV/Hib-TT	2, 3, 4	138	95.7	2.27 (1.78–2.90)	365
DTaP3-HBV-IPV/Hib-TT+PCV10	2, 3, 4	542	97.4	2.13 (1.93–2.36)	354
DTaP3-HBV-IPV/Hib-TT+PCV	2, 3, 4	141	93.6	1.59 (1.23–2.05)	365
DTaP3-HBV-IPV/Hib-TT+PCV7	2, 3, 4	178	97.2	1.18 (0.97–1.42)	354
DTaP3-IPV/Hib-TT+PCV7	2, 3, 4	242	80.8	0.59 (0.49–0.71)	364
DTaP3-IPV-HBV/Hib-TT+PCV13	2, 3, 4	285	89.5	1.23 (1.03–1.46)	253

[a]Anti-PRP measured by enzyme-linked immunosorbent assay.
[b]Manufactured by Wyeth.
[c]Manufactured by Novartis.
CRM, Carrier protein in conjugate vaccine; DTaP, diphtheria-tetanus–acellular pertussis vaccine; DTaP3, 3-component DTaP vaccine; DTaP5, 5-component DTaP vaccine; GMC, geometric mean concentration; HBV, hepatitis B virus; Hib, *Haemophilus influenzae* type b; IPV, inactivated polio virus; PCV, pneumococcal conjugate vaccine; PRP, polyribosyl-ribitol-phosphate; Ref, reference; TT, tetanus toxoid.

TABLE 46.7 Observed Immune Interference Phenomena After Conjugate Vaccination in Children and Suggested Mechanisms

DTP Vaccine	Coadministered Conjugates	Direction of Effect	Mechanism	References
DTaP3, DTaP5	Hib-TT + MenC-TT	↑ PRP	Enhancement via TT	350
DTaP3	Hib-TT + PCV10	↑ PRP	Enhancement via TT or neutral	354
DTaP3	Hib-TT + PCV7	↓ PRP	Bystander interference	227, 354, 365
DTaP3	PCV7	↑ Pn	Enhancement via DT	258, 354
DTaP3	PCV13	↓ Pn	Carrier-induced epitopic suppression	252, 253, 366
DTaP5	PCV-TT + MenC-TT	↓ Pn ↓ MenC	Carrier-induced epitopic suppression	128, 344, 356
DTaP5	Hib-TT + MenC-CRM	↓ PRP	Bystander interference	348, 350
DTaP5	Hib-TT + PCV7 + MenC-CRM	↓↓ PRP ↓ Pn ↓ MenC	Bystander interference	237, 362
DTaP2	PCV7	↓ HBs (unconfirmed)	Bystander interference	347
DTwP	PCV9-MenC-CRM	↓ MenC	Carrier-induced epitopic suppression	367
(–)	MenA, C, Y, W-135-DT + PCV7	↓ MenA ↓ MenB ↓ MenY ↓ MenW-135 ↓Pnc	Carrier-induced epitopic suppression	FDA statistical review and evaluation BLA125089/395

CRM, carrier protein in conjugate vaccine; DTaP2, DTaP3, or DTaP5 refers to 2-component, 3-component or 5-component diphtheria-tetanus–acellular pertussis vaccines, respectively; DTwP, diphtheria-tetanus–whole-cell pertussis vaccine; HBs, anti–hepatitis B surface antibody; Hib, *Haemophilus influenzae* type b; MenA, meningococcal A; MenC, meningococcal C; MenY, meningococcal Y; MenW-135, meningococcal W-135; PCV, pneumococcal conjugate vaccine; Pn, pneumococcal polysaccharide; PRP, polyribosyl-ribitol-phosphate; Ref, reference; TT, tetanus toxoid.

First, even if the level of a specific antibody is considered to be an adequate surrogate in a particular setting,[185,373] the level may vary between serotypes and the disease in question. Second, the quantitative assessment of the antibody titer alone may be insufficient, and measurement of the qualitative characteristics of the antibodies should also be included in the analyses.[347,374,375] Finally, induction of immunologic memory must be considered as immunologic memory can be induced even with a weak antibody response.[160,209,376,377]

Traditionally, methods for assessing the association between immunogenicity and the prevention of disease have relied on comparisons of aggregate measures, such as comparison of vaccine efficacy and GMCs of antibodies. An alternative possibility for the definition of a protective level is to combine information about the incidence of disease and antibody concentrations in different subgroups of the study population. Correlates of protection should ideally be sought on the individual level by collecting longitudinal follow-up data on children who contract pneumococcal infection despite vaccination. They should also be sought in populations by comparing cross-sectional information on antibody concentrations and estimates of protection in the same group of subjects.

Two comprehensive reviews have been published on the principles and methods of correlates of protection and their use in the licensure of PCVs. One was written by WHO experts and academic researchers,[185] the other by scientists from the FDA and National Institutes of Health.[378] A universally stable and accepted method of measuring OPA antibody titers is practically impossible to achieve, as this is a bioassay with many potential variables. Furthermore, OPA does not measure IgG only and can, for example, be influenced by high IgM levels. On the other hand, when measured in infants and young children post–PCV administration, serotype-specific anti-IgG concentrations measured by ELISA correlate well with OPA titers in many studies with a few exceptions (e.g., anti-19A ELISA antibodies obtained post–serotype 19F administration may increase considerably, but the respective OPA titers usually will not).

Serologic Correlates

Once an effective vaccine has been licensed and routinely recommended, it is difficult to conduct additional controlled studies to demonstrate the efficacy of new vaccines directed against *S. pneumoniae*. Then, immunologic correlates of protection become critical for predicting the efficacy of new vaccines.[141]

For this purpose, noninferiority of immunologic correlates based on efficacy against IPD was chosen. Establishing noninferiority based on serologic criteria becomes difficult, however, because there is no clear agreement on the concentration of antibody or titer in vaccines that may be used in a noninferiority model to predict whether vaccine is effective.

Pneumococcal disease is multifaceted. IPD is the first outcome against which the PCVs were aimed, and efficacy against IPD is considered the gold standard when calculating or assuming serologic correlates. However, the most common pneumococcal disease outcome is not IPD, but mucosal infections such as pneumonia or otitis media. Furthermore, nasopharyngeal carriage is the main player in pneumococcal spread and development of antibiotic resistance. Even if serologic correlates of efficacy against IPD were established, they would not accurately predict efficacy against mucosal infections. Effectiveness of PCVs is greatly dependent on reduction of carriage and spread; consequently, correlates that predict protection against IPD may not necessarily accurately predict effectiveness.

The first assumption needed to establish a threshold concentration of antibody that predicts protection is that antibody concentration after the primary series will be the reference used to predict protection. The second assumption is that the relationship between risk of disease and antibody concentration is expressed in a stepwise manner rather than as a continuous function. Neither of these assumptions actually holds completely true. The multiplicity of antigens in the current vaccines and the fact that newer formulations contain additional antigens for which no licensed product is available for comparison adds complexity to the decision process.[185]

One proposed possibility for addressing the multiple immunologic end-point comparisons is to assign a weighted average based on the ranking of serotypes by their epidemiologic and clinical significance.[379] This has resulted in a pooled model based on three efficacy studies, the NCKP PCV7 study in the United States,[4] the American Indian PCV7 study,[5] and the PCV9 South Africa study.[7] These three studies showed efficacy of 97.3%, 78.8%, and 90.0%, respectively, and the lowest serologic estimate for PCV7 serotype protection was 0.2, 1.0, and 0.68 μg/mL, respectively. The pooled antibody concentration from these three studies was 0.35 μg/mL.[141,379] A subsequent controlled PCV9 study from The Gambia showed vaccine efficacy of 86% for IPD, with protective antibody estimate of 1.20 μg/mL. The three studies in high-risk populations (American Indians, South Africa, and The Gambia) suggest that such populations may require higher antibody levels to achieve an equivalent protective efficacy against IPD than do Californian infants, or that more antibody is needed to protect against meningitis and pneumonia than is needed to protect from bacteremia without a focus.[141] Variations between laboratories and different analysis caused some disagreement with regard to the pooled antibody concentration cutoff for the protective antibody levels. Thus, some use the value of 0.35 μg/mL, while others use the cutoff value of 0.20 μg/mL.[380] More recently, the indirect cohort method has been used to develop serotype-specific correlates for protection from IPD in England and Wales.[142] This work suggested a correlate of 0.6 μg/mL as an aggregate for the PCV7 types and 0.8 μg/mL as an aggregate for all PCV13 types excluding serotype 3 for which the correlate was 2.8 μg/mL and where the protective effectiveness for serotype 3 was not significant. These data also suggest that correlates of immunity for serotypes 6B and 23F are lower than 0.35 μg/mL, at 0.16 and 0.2 μg/mL respectively. Finally, OPA values varied so much between serotypes that no aggregate correlate of protection using OPA was attempted.

The practical approach for licensure of new PCVs containing additional serotypes beyond the PCV7 is as follows: For the common 7 serotypes, noninferiority is established for each serotype by direct comparison of the response in the new PCV compared with the licensed vaccine. For each of the six additional serotypes in PCV13, the difference in proportions above the cutoff level between the new vaccine and the established vaccine is calculated using the serotypes with the lowest proportion above the cutoff level (usually for serotype 6B) among the established vaccine as the reference group. For each serotype, noninferiority would be described if the lowest boundary of the 95% confidence interval for the difference is greater than −0.10%.[379]

The protective level is best applied to the population rather than individuals; thus, if a high proportion of individuals in a population achieves anticapsular antibody concentrations above the protective threshold, one can predict a high level of protection for IPD in that population. In a particular individual, the outcome of an encounter with the pneumococcus may vary depending on multiple host and pathogen factors.[141]

As stated above, the situation with mucosal infections and carriage is not identical to that of IPD. Several studies suggest that there is a need to achieve higher concentration after primary immunization series to protect against AOM and carriage than against IPD. In AOM, generalized linear modeling has been used to study the association between the antibody concentrations to serotypes 6B, 19F, and 23F and the risk of AOM caused by the homologous serotypes in the 5 months after immunization.[31] The association was most clear-cut for serotype 23F and poorest for serotype 19F. Even low antibody concentrations (GMC 0.5 μg/mL) predicted vaccine efficacy of 50% for serotype 6B, whereas a GMC of 2 μg/mL was required for the same efficacy for serotype 23F. For serotype 19F, the results suggested that much larger concentrations (GMC >10 μg/mL) might be required to achieve protective efficacy. Thus, these results indicate different behavior for different serotypes, discouraging the setting of a unique serologic correlate of protection for all serotypes. In addition, the concentration needed to protect against AOM seems to be higher than the concentration that is protective against IPD.

For protection against carriage, it has been clearly demonstrated, like the case of AOM, that the concentrations needed for protection are higher than those needed for IPD. Furthermore, complete protection may be associated with concentrations higher than those attained after PCV administration with the current vaccines.[32,381] In addition, serotype-to-serotype variation in protection, in relation to achieved antibody concentrations, has been observed.

To date, no studies have been able to demonstrate any correlation of antibody concentrations with protection against pneumonia or nonpneumonia lower respiratory infections. The lower efficacy of PCV13 in adults against vaccine-type nonbacteremic pneumococcal pneumonia than against vaccine-type IPD[18] also suggests that the cutoff of 0.35 μg/mL may be too low to predict efficacy against nonbacteremic pneumococcal pneumonia.

In summary, although correlates of protection were drawn in IPD, and have been used for licensure, many questions still remain. As of this writing, no correlations have been determined for mucosal disease and carriage responsible for most of the *S. pneumoniae* pneumococcal disease burden. Furthermore, post-PCV introduction effectiveness is greatly dependent on indirect protection conferred by reduced carriage and spread of vaccine serotype *S. pneumoniae*, but no correlates predicting such an effect are known. However, it is suggested that if higher functional antibody concentrations are achieved after the primary immunization series, a higher reduction in mucosal disease carriage will take place, resulting in higher and faster population protection through herd immunity.

Mucosal Antibody Response

Although it is generally assumed that host defense at a mucosal site contributes to protection, it has been difficult to determine the relative importance of mucosal antibodies in preventing pneumococcal invasive disease and/or colonization. Nonetheless, analysis of mucosal immunity has been an important focus for the understanding of the protection provided by the conjugate vaccine,[382] and also is being evaluated for protein-based or whole-cell vaccines (see "Human Experiences with Natural or Vaccine-Derived Pneumococcal Protein Antigens" later). These antibody responses can be analyzed either by measuring anticapsular or noncapsular antibodies from mucosal membranes and secretions, or through demonstration of specific antibody-secreting cells (ASCs) in lymphatic tissues or in the peripheral blood.

ELISAs can be applied to mucosal secretions and saliva, but there are two specific challenges. One is the instability of the secretions as there are many degradative enzymes present, especially in saliva. The other challenge is the wide individual variation in the concentration of the samples because of unknown dilutions of the samples. The first challenge can be dealt with through the use of enzyme inhibitors and by storing the samples at −70°C, and the second by relating the concentration of specific antibodies to the total antibody concentration in the sample.

Parenterally administered pneumococcal capsular vaccines, both polysaccharide and conjugate, induce mucosal antibody responses. Serotype-specific salivary antibodies

are found rarely after primary immunization but more frequently after a booster either with the conjugate or with polysaccharide vaccine.[184,204,383–385] The IgG detected in saliva is thought to be derived from the serum, with high serum IgG concentrations transudating into the mucosal secretions and is supported by comparable serum and salivary IgG concentrations.

As B cells are continuously circulating through the lymphatics and blood and back to the peripheral tissues, cells committed to mucosal sites are also present in the peripheral blood for a limited period before homing in to different sites. The appearance of these ASCs can be measured with an enzyme-linked immunospot assay (ELISPOT) in peripheral blood after an antigen challenge, with the magnitude of this response thought to reflect the immune response at mucosal sites.[386–391]

A high number of IgA ASCs detected by ELISPOT is considered to be an indicator of the mucosal IgA response after parenteral pneumococcal vaccination. The response to parenteral protein vaccines is generally dominated by IgG-producing cells,[390] whereas the response to polysaccharide antigens is dominated by IgA-producing cells.[390–392] The IgA/IgG ratio of the ASC response to a polysaccharide that is conjugated to a protein carrier is altered toward a pattern typical of T-cell–dependent antigens.[386,392] For the PPV vaccine, the response is dominated by the IgA ASCs,[388] but after the conjugate vaccines, the IgG ASC responses are higher.[216,389] The ASC responses to the PncT and PncD conjugate vaccines in toddlers are lower than those seen in adults after conjugate vaccine but comparable to those in adults after PPV vaccine. In toddlers, the ASC response is clearly dominated by IgA-secreting cells.[388]

Cellular Responses

The improved immunogenicity of the conjugates compared with the pure polysaccharide vaccines is thought to be caused by the engagement of $CD4^+$ lymphocytes because of the presence of the carrier protein. This cellular response has been evaluated by measuring lymphocyte proliferation and the production of different cytokines (e.g., interferon [IFN]-γ and various interleukins [ILs]) after stimulation with the polysaccharide and protein components of the conjugated vaccines.[346,393] These studies demonstrate, as expected, that conjugate vaccines can induce proliferative and cytokine responses to the carrier proteins but not to the polysaccharide components.

The importance of the cellular component of immunity is well illustrated by animal experiments and more recent human studies. Age-dependent Th2-cytokine responses can be observed in mice immunized with the conjugate; the lack of response to the polysaccharide alone in neonatal mice seems to be associated with absence of carrier-specific IL-5 production. These results suggest that immunization with pneumococcal conjugate induces carrier-specific T-cell responses that increase with age and determine the levels of the antibody response to the polysaccharide component.[394] A randomized controlled trial in Papua New Guinea demonstrated that neonatal immunization with the seven-valent conjugate primes T-cell responses to the protein carrier and polarizes T-cell responses toward Th2.[236]

At the same time, data from humans and animal models have emerged to provide support for the existence of an antibody-independent T-cell response that confers resistance to pneumococcal colonization. In adult patients with chronic obstructive pulmonary disease, naturally acquired and vaccine-induced antibodies to pneumococcal antigens (capsular and noncapsular) do not predict resistance to pneumococcal colonization, suggesting the existence of other mechanisms.[395] In mice, acquired immunity to colonization after mucosal exposure to either live or killed unencapsulated whole-cell pneumococcal vaccine[396] was antibody independent and $CD4^+$ T-cell dependent. Mice that genetically lack antibodies, IFN-γ, or IL-4 maintained whole-cell vaccine-induced immunity to colonization, but mice treated with neutralizing anti-CD4 or genetically lacking the IL-17A receptor were not protected by these exposures. A likely effector cell is the IL-17A–producing $CD4^+$ Th17 cell,[397,398] which was previously implicated in resistance to pathogens such as *Candida albicans*,[399] *Bordetella pertussis*,[400] *Mycobacterium tuberculosis*,[401] and *Staphylococcus aureus*.[402,403] Memory $CD4^+$ Th17 cells are absent in individuals with autosomal dominant hyperimmunoglobulin E (Job) syndrome because of a mutation in the *STAT3* gene.[404] Consistent with murine data, Job syndrome patients are at high risk of candidal, staphylococcal, and pneumococcal infections.[405,406] A proposed mechanism of protection by Th17 cells involves the release of IL-17A, IL-22, and other related cytokines, which help recruit and activate neutrophils to the site of infection, enhancing clearance of the organism.[407] Whether such cellular responses demonstrated in mice also mediate protection to pneumococcal colonization in humans remains to be proven, but it is noteworthy that systemic and mucosal IL-17A responses to pneumococcal antigens have been demonstrated in humans.[397,408]

Schedules

Schedules of PCV are an area of intense debate, and the arguments around selecting them—in terms of immunogenicity; impact on carriage; and effectiveness in prevention of the of IPD, pneumonia, and otitis media—are made under separate headings. The gold standard vaccination schedule of 3+1 (three doses in infancy, generally 2 months apart with a booster in the second year of life) was used in the licensure trials for PCV in the United States.[4,5] A dose-sparing schedule of 2+1 (two infant doses, generally 2 or more months apart with a booster in the second year of life) has been extensively studied for effectiveness. It appears effective in conferring protection, with the caveat that before herd protection is established, there may be a period of increased susceptibility to the less immunogenic serotypes, such as serotype 6B, between 6 and 12 months of age. A variant of 2+1 (two infant doses with a booster at 9 months) has also been proposed to bring it in line with the WHO infant EPI schedule. This could also be considered a variant of the 3+0 schedule that was tested in two large efficacy studies in Africa. In the South African study, three doses were given at 6, 10, and 14 weeks of age, and in The Gambia study, at 2, 4, and 6 months of age because of delays in presentation for vaccination.[6,7] A limitation of the shortened 3+0 schedule for countries with a significant burden of disease caused by serotype 1 is that no evidence of protection was found in the African randomized trials for serotype 1 using this schedule for serotype 1 episodes occurring after 18 months of age.[213] This observation led to the introduction of a 6-week, 14-week, plus 9-month (modified 2+1) schedule in South Africa. Serotype 1 has declined in South Africa (by 57% in infants younger than 2 years of age and by 33% in adults 25–44 years of age) since this schedule was introduced,[17] but given the epidemic nature of serotype 1, confirmatory data of long-term serotype 1 effectiveness using a booster at or after 9 months of age remains under investigation. A systematic review of the data compiled through 2012 on dosing schedules found that, in general, three- and four-dose schedules were comparably effective against carriage, invasive disease, and pneumonia in the setting of an established vaccination program with high coverage rates.[409–411]

EFFICACY AND EFFECTIVENESS

Invasive Disease

Several studies have now examined the efficacy of PCVs in preventing invasive disease. In a double-blind pivotal licensure study conducted in northern California, 37,868 infants were randomized to receive either the PCV or a meningococcus group C conjugate vaccine the control.[4] Study vaccine was given at 2, 4, 6, and 12 to 15 months of age, along with other routine childhood vaccines. The primary outcome measure was the incidence of IPD defined by culture from a sterile site caused by vaccine serotypes. To be included in the main efficacy analysis, invasive disease must have been caused by a vaccine serotype, occurred more than 14 days after the third dose of the vaccine, and occurred in a subject vaccinated according to protocol. Children younger than 16 months were considered to be fully vaccinated if they had received three or more doses of vaccine; children 16 months old or older were considered to be fully vaccinated after receipt of a fourth dose. In addition to the per-protocol analysis, an intention-to-treat (ITT) analysis was performed that included all invasive disease caused by any pneumococcal serotype occurring after randomization, regardless of vaccination status.

In the per-protocol analysis, 40 cases of IPDs were seen in the study population, with 39 in the control group. This represents a vaccine efficacy of 97% (95% confidence interval [CI], 83% to 100%; $P < .0001$; Table 46.8). The one vaccine failure was a child who had received four doses of vaccine, yet developed bacteremic pneumonia caused by serotype 19F. In the ITT analysis, there were 52 cases of vaccine-serotype IPDs, three of which were in vaccinated children, representing a vaccine efficacy of 94%. The three failures included the one described above, a case of serotype 19F disease in a child who developed leukemia after vaccination and was receiving immunosuppressive chemotherapy, and a partially vaccinated child who developed a serotype 6B infection 317 days after a single dose of vaccine. Point estimates of serotype-specific efficacy were obtained for four vaccine serotypes and ranged from 85% for serotype 19F to 100% for serotypes 14, 18C, and 23F.

In this prelicensure study, there was no evidence of an increased risk of disease caused by nonvaccine serotypes. Nine cases of IPDs resulting from nonvaccine serotype were detected, six in the control group and three in vaccinated children. Only one case was caused by a potentially cross-reactive serotype in a vaccinated child. Overall, in an ITT analysis, including disease caused by both vaccine and nonvaccine serotypes, an 89.1% reduction was seen in the burden of IPDs in children who had received at least one dose of conjugate vaccine.

Efficacy of PCV7 also was analyzed separately for the low-birth-weight and preterm infants who had been included in the NCKP study.[271] No difference in mean age of administration of vaccine was seen in the low-birth-weight and preterm infants compared with normal-birth-weight and full-term infants. For low-birth-weight infants who received the control vaccine, the relative risk of IPDs was 2.6 compared with normal-birth-weight infants, and for preterm infants, the relative risk was 1.6 compared with full-term infants. However, no low-birth-weight or preterm recipient of pneumococcal vaccine contracted an IPD, as compared with six low-birth-weight infants and nine preterm infants in the control group.

A community-randomized, controlled efficacy trial of PCV7 was conducted between April 1997 and October 2000 among Navajo and White Mountain Apache children,[55] who had at that time, some of the highest rates of IPD in the world. As in the NCKP study, meningococcal conjugate vaccine was used as a control. A per-protocol analysis was performed of the 5792 children younger than 2 years who were enrolled in the primary efficacy study. Eight cases of vaccine-serotype invasive disease occurred in the control group, compared with two in the PCV7 group, yielding an efficacy of 76.8% (95% CI, –9.4% to 95.1%). In an ITT analysis, 11 cases of vaccine-serotype disease occurred in the control group, compared with two cases in the PCV7 group, for an efficacy of 82.6% (95% CI, 21.4% to 96.1%). This study was stopped early when PCV7 was approved for licensing in the United States, resulting in lower statistical power than planned; however, these efficacy estimates demonstrate that the vaccine provided significant benefit, even in this high-risk population.

The efficacy of PCV9, containing serotypes 1 and 5 in addition to the PCV7 serotypes, was tested in a large randomized, double-blind study in South Africa in children with and without HIV infection.[77] A total of 39,836 children received vaccine or placebo at approximately 6, 10, and 14 weeks of age, and follow-up was continued until 15 cases of vaccine-serotype IPD occurred in fully vaccinated HIV-negative children. The vaccine was highly efficacious in this per-protocol analysis: 13 of the cases were in children who received placebo, and two were in children who received PCV7, for an efficacy of 85% (95% CI, 32% to 98%). The efficacy was similar in an ITT analysis (83%; 95% CI, 39% to 97%; $P = .003$). Efficacy was lower, but still highly significant, against vaccine-serotype IPD in HIV-infected children (65%; 95% CI, 24% to 86%; $P = .006$) and against any IPD in all children (50%; 95% CI,

TABLE 46.8 Efficacy of PCV Against Invasive Pneumococcal Disease and Pneumonia

Study	Efficacy Estimate (%)	95% CI	Notes
INVASIVE DISEASE CAUSED BY 7-VALENT VACCINE SEROTYPES			
NCKP 3+1	94	80 to 98	PCV7
Intent to treat			
The Gambia 3+0	77	49 to 89	PCV9
Intent to treat			
Navajo and Apache 3+1	83	22 to 96	PCV7
Intent to treat			
South Africa 3+0	77	19 to 93	PCV9
HIV-negative children			
Intent to treat, prolonged follow-up			

TABLE 46.8 Efficacy of PCV Against Invasive Pneumococcal Disease and Pneumonia *(Continued)*

Study	Efficacy Estimate (%)	95% CI	Notes
Intent to treat			
South Africa, 3+0, HIV-infected children, intent to treat, prolonged follow-up	28	–22 to 58	PCV9
Invasive disease caused by 10-valent vaccine serotypes, Finland 2+1, modified intent to treat	92	58 to 100	PCV10
Finland 3+1, modified intent to treat	100	83 to 100	PCV10
Latin America, per protocol	100	74 to 100	PCV10
INVASIVE DISEASE CAUSED BY ANY SEROTYPE			
NCKP 3+1	89	75 to 95	PCV7
Intent to treat			
The Gambia 3+0	45	19 to 62	PCV9
Intent to treat			
Navajo and Apache 3+1	46	–6 to 73	PCV7
Intent to treat			
South Africa 3+0	35	–31 to 68	PCV9
HIV-negative children			
Intent to treat, prolonged follow-up			
Intent to treat			
South Africa 3+0	46	19 to 64	PCV9
HIV-infected children			
Finland 2+1, and 3+1 combined, modified intent to treat	93	75 to 99	PCV10
Latin America, 3+1 per protocol	65	11 to 86	PCV10
RADIOGRAPHICALLY DEFINED PNEUMONIA			
NCKP 3+1	26	7 to 40	PCV7
Intent to treat			
The Gambia 3+0	37	27 to 45	PCV9
Per protocol			
South Africa 3+0	20	3 to 35	PCV9
HIV-negative children intent to treat			
Navajo and Apache 3+1	-21	–62 to 9	PCV7
Intent to treat			
Philippines 3+0	23	–1 to 41	11-Valent PncD/T
Per protocol			
Latin America 3+1	23	9 to 36	PCV10

CI, confidence interval; NCKP, Northern California Kaiser Permanente; PCV, pneumonococcal conjugate vaccine; PncD/T, pneumococcal diphtheria-tetanus vaccine.

Invasive pneumococcal disease data from Klugman KP, Cutts F, Adegbola RA, et al. Meta-analysis of the efficacy of conjugate vaccines against invasive pneumococcal disease. In: Siber GR, Klugman KP, Makela PH, eds. *Pneumococcal Vaccines*. Washington, DC: American Society for Microbiology Press; 2008:317–326.

Pneumonia data from Madhi SA, Klugman KP. Efficacy and safety of conjugate pneumococcal vaccine in the prevention of pneumonia. In: Siber GR, Klugman KP, Makela PH, eds. *Pneumococcal Vaccines*. Washington, DC: American Society for Microbiology Press; 2008:327–337.

Data for Philippines from Lucero MG, Nohynek H, Williams G, et al. Efficacy of an 11-valent pneumococcal conjugate vaccine against radiologically confirmed pneumonia among children less than 2 years of age in the Philippines: a randomized, double-blind, placebo-controlled trial. *Pediatr Infect Dis J*. 2009;28:455–462.

Data for Finland from Palmu AA, Jokinen J, Borys D, et al. Effectiveness of the ten-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV10) against invasive pneumococcal disease: a cluster randomised trial. *Lancet*. 2013;381:214–222.

Data for Latin America from Tregnaghi MW, Sáez-Llorens X, López P, et al. Efficacy of pneumococcal nontypable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) in young Latin American children: A double-blind randomized controlled trial. *PLoS Med*. 2014;11:e1001657.

23% to 68%) and HIV-positive children (53%, 95% CI, 21% to 73%). The incidence of IPD caused by antibiotic-resistant pneumococci also decreased by 56% (95% CI, 21% to 77%). The decrease was most prominent in HIV-infected children, who nonetheless continued to have a higher rate of infection with resistant strains than HIV-negative children. In a further analysis of long-term efficacy after 6.16 years of follow-up, efficacy remained high at 77.8% (95% CI, 34% to 93%) but declined from 65% to 39% among HIV-infected children (95% CI, −8% to 65%). Vaccine efficacy was, however, retained overall against serotype 6A, and this protection was clear even in the HIV-infected children (vaccine efficacy: 79%; 95% CI, 39% to 93%).[334]

The same nine-valent vaccine was evaluated for efficacy against IPD in a large, randomized, double-blind study in The Gambia.[6] Infants between 6 weeks and 1 year of age were given three doses of vaccine or placebo mixed with diphtheria, pertussis, and tetanus DPTHib vaccine; hepatitis B and oral polio vaccines were given concurrently. Vaccine efficacy against any vaccine-serotype IPD was 77% (95% CI, 51% to 90%) and against vaccine-serotype meningitis or sepsis was 92% (95% CI, 44% to 100%). Efficacy against IPD of all serotypes was 50% (95% CI, 21% to 69%), reflecting an absolute reduction of approximately two episodes per 1000 child-years. Although there was a relative reduction in IPD caused by vaccine-related serotypes and a relative increase caused by nonvaccine serotypes, neither difference was significant. In the placebo group, 65% of IPD was caused by the nine vaccine serotypes and 48% by serotypes contained in PCV7.

Efficacy of the PCV10 conjugate vaccine against IPD has been demonstrated in a large, double-blinded, cluster randomized trial in Finland. There were 78 clusters within which 47,369 children younger than 19 months of age were enrolled. Among children younger than 7 months of age at enrollment, the children in 26 control clusters received hepatitis B vaccine as a control; among these children there were 12 cases of IPD caused by vaccine types for an incidence of 56.4 cases of vaccine type IPD per 100,000 children. Among 30,528 vaccinated children in the 52 clusters where PCV10 was given, there were no cases among vaccinees in 26 clusters receiving 3+1 doses and only one case of serotype 7F disease among the 26 clusters receiving a 2+1 schedule at 3, 5, and 12 months. The single failure was just 12 days after the first dose of vaccine. These data translate to a vaccine efficacy of the 3+1 schedule of 100% (95% CI, 83% to 100%). The efficacy of the 2+1 schedule was 92% in the ITT analysis (95% CI, 58% to 100%).[412]

Pneumonia and Other Respiratory Infections

Several of the IPD studies also examined vaccine efficacy against pneumonia (see Table 46.8). In the NCKP trial, pneumonia was a secondary outcome measure. Cases of pneumonia were identified by the presence in the NCKP database of a diagnosis of pneumonia, "bronchopneumonia," or "viral pneumonia" by the treating physician in the outpatient, emergency room, or hospital where the patient was seen. Based on the interpretations of radiologists at the point of care, the conjugate vaccine was found to significantly reduce the number of episodes of pneumonia in which there was a positive chest film to 8.3 per 1000 person-years, for an estimated efficacy of 17.7% (95% CI, 4.8 to 28.9; $P = .01$).[59] Radiographs obtained for this study were then reevaluated using the criteria established by the WHO in 2001 for interpreting chest radiographs, which are intended to standardize radiologic diagnoses for epidemiologic purposes.[413] Of the 2841 original sets of radiographs, 2446 were digitized and analyzed according to the WHO criteria. The standardization increased the vaccine efficacy estimate to 25.5% (95% CI, 6.5% to 40.7%; $P = .011$).[8]

In the South African trial of PCV9,[77] the efficacy of the vaccine in reducing radiographically defined pneumonia was a primary end point and was 20% (95% CI, 2% to 25%; $P = .03$) in the ITT analysis and 25% in fully immunized children without HIV infection (95% CI, 4% to 41%). This reduction was in addition to any protection afforded by the Hib vaccine, which was provided to all subjects. The vaccine efficacy did not reach statistical significance in children infected with HIV (13%; 95% CI, −7% to 29%; $P = .19$), but subsequent secondary analyses showed that only 39% of the pneumonia prevented by the vaccine among HIV infected children was included in the radiographically defined end point. In an ITT analysis of clinical pneumonia, excluding bronchiolitis, vaccine efficacy among HIV-infected children remained at 15%, but because of the larger number of children meeting this definition (379 vaccinees vs 446 control subjects), the difference was significant (95% CI, 5% to 24%; $P = .004$).[414]

Pneumonia was also a primary outcome measure of the Gambian PCV9 trial.[6] It was defined radiographically as the presence of a dense or fluffy opacity that occupied some or all the lung, presence of fluid in the lateral pleural space, or both and defined clinically as a history of cough or breathing difficulty of less than 14 days' duration, with a raised respiratory rate or chest-wall in-drawing. Overall, the vaccine reduced first episodes of radiologic pneumonia by 37% (95% CI, 27% to 45%) and reduced first episodes of clinical pneumonia by 7% (95% CI, 1% to 12%). The absolute reduction in radiographic pneumonia was 15 cases (95% CI, 10% to 19%) per 1000 child-years in the per-protocol analysis, which was more than seven times higher than the absolute reduction in IPD (≈2 per 1000 child-years), indicating a high burden of preventable pneumonia. In addition, the vaccine resulted in a 16% reduction in mortality in young children, suggesting that pneumococcal pneumonia and bacteremia contribute to a substantial number of deaths.

PCV9 has also been shown to reduce respiratory infections other than pneumonia. In a study of children 12 to 35 months old attending daycare in Israel,[415] vaccinated children had a relative risk of upper respiratory infection, including rhinorrhea, pharyngitis, and sinusitis, of 0.85 (95% CI, 0.76–0.96; $P = .009$) and of lower respiratory infection, including bronchitis, cough, and asthma as well as pneumonia, of 0.84 (95% CI, 0.72–0.98; $P = .024$). The effectiveness of the vaccine in preventing these infections indicates that *S. pneumoniae* plays a role in diseases more commonly associated with viral infection. This role has been supported by evidence from the large South African efficacy trial[416,417] in which vaccination with PCV9 significantly lowered the risk of confirmed viral pneumonia by 22% to 58%, depending on the causative virus, presumably because pneumococcal coinfection contributes to the pathogenesis of a large proportion of pneumonias thought to be viral. The vaccine prevented 45% of confirmed hospitalizations caused by influenza,[416] which suggests a potential role for PCV in protection from pandemic influenza, in which postinfluenza pneumococcal mortality may be significant.[418] A similar reduction (48%; 95% CI 1% to 76%) in hospitalization associated with the 2009 pandemic influenza was found in PCV7 recipients in a case-control study of PCV7 effectiveness from Spain. In the following season during which extensive herd protection would be expected, there was no further evidence of PCV7 effectiveness against influenza hospitalization.[419] Another secondary analysis of the South African trial data suggested that children with culture-confirmed tuberculosis may also be at increased risk of pneumococcal pneumonia, which was also prevented by the PCV9 vaccine.[420]

In the only randomized trial to date of pneumococcal conjugate vaccination in Asia, an 11-valent Sanofi Pasteur investigational vaccine conjugated to tetanus and DT, which has not been licensed, was compared with placebo in 12,191 children in Bohol, Philippines. The vaccine efficacy against radiologically confirmed pneumonia in fully immunized children (three doses at least 1 month apart between 6 weeks and 6 months of age) was 23% (95% CI, −1% to 41%; $P = .06$). Significant protection was seen in the first year of life (34%; 95% CI, 5% to 54%; $P = .02$) but not the second year (3%; 95% CI, −44% to 34%; $P = .88$).[9] A secondary spatial analysis of that study showed increasing vaccine efficacy from −14% (−13% to 38%) to 55% (11% to 79%) as the distance of the child from the primary hospital increased from less than 1.5 km to greater than 8.5 km, suggesting that less access to healthcare increases the fraction of X-ray–confirmed pneumonia attributable to the pneumococcus, and that ensuring good PCV coverage in areas with poor access to care may maximize program benefits.[421]

The PCV10 vaccine conjugated to protein D was evaluated in a clinical trial in Argentina, Colombia, and Panama, and demonstrated vaccine efficacy against the WHO-confirmed definition of pneumonia[413] in the ITT analysis of 23.4% (95% CI, 9% to 36%) and 26% (95% CI, 8% to 40%) in the fully immunized cohort.[11]

Acute Otitis Media

S. pneumoniae, together with nontypeable *H. influenzae* (NTHi), is the most commonly reported bacterial pathogens in AOM. *S. pneumoniae* is isolated from the middle ear fluid in 20% to 60% of patients.[51,52,422] By choosing seven to 13 common serotypes, approximately 50% to greater than 90% of the AOM pneumococcal serotype isolates could be covered.[422] Thus in theory, pneumococcal vaccines could cover between 10% and greater than 50% of all cases of AOM. However, several sometimes contradictory factors play a role in the overall effectiveness of PCV against AOM.

Large-scale efficacy trials of PCV were conducted in three sites, each with different designs, and thus not comparable for many outcomes. One trial using PCV7-CRM conjugate was conducted in the NCKP Health Services.[4,423] The second was conducted in Finland (the FinOM study) with two vaccines: PCV7-CRM and PCV7-OMPC.[13,194] The third was conducted in the Czech Republic, using PCV11–protein D vaccine (PCV11; PHiD-CV) (the Pneumococcal Otitis Efficacy Trial [POET] study).[12] All three studies were double-blinded, randomized, controlled studies, with an arm that did not receive any PCV. In all three studies, healthy infants in the PCV arms received three PCV doses during the first 6 months of life and a booster early in the second year of life (2, 4, 6, and 12 months or 2, 4, 5, and 12 to 15 months of age).

In the NCKP trial, infants were followed between 8 months and 3.5 years of age. Although the trial was not primarily intended to look at AOM, computerized data on AOM visits could be retrieved to assess the effect of PCV7-CRM on AOM visits. In contrast, the FinOM and POET studies were designed primarily to determine the effect of PCV on AOM. In the FinOM study, the children were followed up to age 24 months, and diagnosis of AOM was standardized as follows: AOM was defined by the presence of a tympanic membrane that was visibly abnormal in terms of color, position, or mobility, suggesting middle-ear effusion; plus at least one of the following symptoms or signs of acute infection: fever, earache, irritability, diarrhea, vomiting, acute otorrhea not caused by otitis externa, and other symptoms of respiratory infection. The FinOM study was somewhat complex because one arm received PCV7-CRM, another arm received PCV7-OMPC, and a third arm received no PCV (the control arm). Furthermore, the PCV7-OMPC arm was further randomized after three PCV7-OMPC doses to receive either a fourth PCV7-OMPC or a 23-valent polysaccharide vaccine (PS-23) dose as a booster.[13,194] In the POET study, follow-up continued until age 24 to 27 months. In this study, the diagnosis of AOM was standardized, but the follow-up in the POET study was passive because parents were advised to consult their pediatrician if the child was sick, had ear pain, or had spontaneous ear discharge. Furthermore, the trigger to be defined as a case was performance of a tympanocentesis after the child was referred to the otolaryngologist. Thus, the study population in the POET study was enriched with complex, severe, recurrent, or nonresponsive otitis media (OM). This point is important, as complex OM is a more advanced and a late-stage form of OM that cannot be compared to AOM. In contrast, the studies in California and Finland included all OM cases and most were uncomplicated. Therefore, the POET study cannot be directly compared with the two others.[424]

The NCKP and FinOM studies suggested that PCV7-CRM could reduce overall AOM by 6% (95% CI, −4% to 16%) to 7% (95% CI, 4% to 9%) (Table 46.9). In contrast, the PCV7-OMPC did not show any reduction in overall AOM.

The POET study, which was enriched with complex OM cases, showed an overall reduction of 34% in AOM episodes. The reduction in AOM caused by vaccine serotypes, determined by tympanocentesis or culture of spontaneous ear drainage, was quite similar among all three studies, ranging between 56% and 67%. PCV7-CRM vaccine also reduced the cross-reactive serotype 6A, whereas the PCV7-OMPC did not show any cross-protection, and PCV11 was not conclusive. In the FinOM study, the administration of both PCV7-CRM and PCV7-OMPC was associated with a higher occurrence of AOM caused by nonvaccine pneumococcal serotypes, *Moraxella catarrhalis* and *H. influenzae*. This was not the case in the POET study, where AOM caused by NTHi decreased by 35% and nonvaccine pneumococcal type did not increase.[12] In recurrent OM, PCV7-CRM had an efficacy of ≈9%,[4,13] while in the NCKP study there was a more marked reduction of 20.1% (95% CI, 1.5–34.2)[4] and in the FinOM study there was a 39% reduction[425] in frequent recurrences. The greatest impact of PCV7-CRM was seen on chronic middle ear effusion, a form of chronic OM, necessitating insertion of ventilation tubes.[379] Furthermore in a follow-up study,[426] the effectiveness of PCV7-CRM and PCV7-OMPC in reducing tympanostomy tube placement in patients 2 to 13 years of age was assessed. PCV-CRM showed a 34% reduction in tube placement (95% CI, 1% to 52%) versus only a 6% reduction (95% CI, −28% to 31%) for PCV7-OMPC. After age 5 years, none of the vaccines showed any more impact but the benefits achieved by the PCV7-CRM were sustained up to age 13 years. The PCV11–protein D showed an efficacy of 56% against all-case recurrent AOM, but this finding was not statistically significant because of insufficient sample size. Whether the results of the POET study that included reduction of *H. influenzae* cases of OM, culture-negative cases, and recurrent cases were different than those of other studies because of the use of a PCV conjugated to *H. influenzae* protein D or because of a different study design, potentially capturing more chronic cases, was not clear. The PCV11–protein D vaccine did not protect against OM caused by serotype 3, and this was the reason behind changing the formulation of the protein D conjugate vaccine to contain only 10 instead of 11 serotypes. More recently, the efficacy of the PCV10 vaccine (similar to PCV11, but with two serotypes conjugated to CRM_{197} and absence of serotype 3 antigen) was studied in Panama.[11] This double-blind, placebo-controlled study suffered from several methodological problems, such as the very small outcome events available for analysis, the

TABLE 46.9 Double-Blinded Efficacy Studies Against Acute Otitis Media Before the Introduction of Vaccine to Widespread Use

Reference	Location	Vaccine	Subjects	Reduction in Vaccine-Type Serotypes (95% CI)	Reduction in Pneumococcal AOM (95% CI)	Reduction in Overall AOM (95% CI)	Cross-Reacting (95% CI)	Nonvaccine Serotype (95% CI)	Recurrent AOM (95% CI)	Remarks
4	California	PCV7	Healthy infants	67% (*P* = .077): PP 65% (*P* = .035): ITT	ND	ND	ND	ND	9% (3–15): PP; 9% (4–14): ITT	
13	Finland	PCV7	Healthy infants	57% (44, 67): PP 54% (41, 64): ITT	34% (21–45): PP	6% (–4, 16): PP	51% (27–67): PP	–33% (–80 to 1): PP	16% (–6 to 35): PP; 9% (–12 to 27): ITT	
420	California	PCV7	Healthy infants	ND	ND	7% (4–9): PP; 6% (4–8): ITT	ND	ND	ND	
190	Finland	PCV7-OMP	Healthy infants	56% (44, 66): PP	25% (11–37): PP	–1% (–12, 10): PP	5% (–47 to 25): PP	27% (–70 to 6)	ND	
12	Czech Republic	PCV11-protein D	Healthy infants	58% (41, 69): PP	52% (37–63): PP	34% (21–44): PP	66% (22–85): PP	9% (–64 to 49): PP	56% (–2 to 80): PP	Outcome measured in children attending an otorhinolaryngologist for tympanocentesis. Probably enriched with capsular OM cases.
11	Panama	PCV10	Infants	70 (30, 87) ITT	56% (22–75) ITT	19 (4–31) ITT	29 (–124 to 76)	15 (–154 to 71)	ND	Small number of events analyzed. Probably enriched with capsular OM cases.
422	Israel	PCV9	Toddlers attending daycare centers	ND	ND	17% (–2, 33): ITT	ND	ND	ND	
423	Netherlands	PCV7	Toddlers and young children with recurrent AOM	52% (*P* = –.21)	34% (*P* = .02): PP	–29% (–62 to –2): PP; –25% (–57 to 1): ITT	ND	21% (*P* = .44): PP	ND	
424	Belgium	PCV7	Toddlers and young children with recurrent AOM	ND	ND	–16% (–96 to 3): PP	ND	ND	ND	

AOM, acute otitis media; CI, confidence interval; CRM, protein carrier in conjugate vaccine; ITT, intention to treat; ND, not determined; PCV, pneumococcal conjugate vaccine; PP, per protocol; Ref, reference; OMP, outer membrane protein.

relatively late age of onset of the OM cases, and the relatively higher proportion of culture-negative events, which suggest, like the POET study, a higher proportion of complex OM cases. In the context of these limitations, the study demonstrated that PCV10 was efficacious against pneumococcal (vaccine efficacy 56% [95% CI, 22% to 75%]) and overall AOM (vaccine efficacy 19% [95% CI, 4% to 31%]), probably mainly complex cases, on the same order of magnitude as PCV11.

An additional trial was conducted in an underprivileged and somewhat more compromised population in the United States, the Navajo and White Mountain Apache population.[427] In this double-blind study, infants were randomized to receive either PCV7-CRM or meningococcus C-CRM vaccine. The methodology of the study did not allow it to reach statistically significant conclusions, but its findings support those of the three larger-scale studies conducted with PCV7-CRM described above. The ITT efficacy findings were: overall OM, −3.3% (95% CI, −21.4 to 12.0); number of medical visits resulting from severe episodes, 13.7% (95% CI, −3.4 to 49.5); and ventilation tube insertion, 22.2% (95% CI, −215.7 to 83.0). Thus, although not statistically significant, the trend toward higher efficacy with more complex outcomes was seen in this study, as in the previously described studies. Furthermore, when cultures were taken from spontaneously draining ears, 11 isolates were of vaccine-type (VT) serotypes, eight of which were in the control group (64% efficacy, 95% CI, −34% to 90%), similar to previously observed findings. Thus, when combined these five studies suggest only moderate efficacy against all-cause OM cases, and higher efficacy against more-complex OM.

Another relatively small study in Sweden lends support to this notion. Ninety-six children with AOM occurring before 6 months of age (implying high risk of recurrent OM and complex OM) were assessed.[428] The subjects were randomized in a nonblinded manner to receive either PCV7 ($n = 46$) or no vaccine ($n = 50$). The children were followed up closely. A total of 363 OM episodes were recorded. In PCV7 vaccinated subjects, the incidence of OM was reduced by 26% ($P = .03$), emergency visits resulting from suspected OM were reduced by 36% ($P = .01$), and the proportion of children needing ventilation tube placement dropped by 50% ($P = .02$). These findings support the above described greater efficacy of PCV7 against complex and chronic OM versus simple AOM.

Three prelicensure efficacy trials were conducted in toddlers. The first was a double-blind, randomized study in healthy Israeli daycare center toddler attendees who received PCV9 or meningococcus C–CRM vaccine (two doses for toddlers 12–17 months old and one dose for toddlers 18–35 months old). The children had approximately 18 encounters per child during the successive 2 years (monthly visits in first year and bimonthly visits the year after).[14] In addition, the parents responded to a questionnaire that included data on respiratory infections, as well as AOM episodes. Although the AOM diagnosis was not confirmed by study physicians and not standardized, the double-blind approach permitted a comparison between the two arms. There was a 17% reduction (95% CI, −2% to 33%; $P = .078$) in the number of episodes in the PCV9 recipients and a reduction of 20% (95% CI, 14% to 26%; $P < .001$) in the length of time the children were on antibiotic treatment for AOM.[415] Two additional studies were conducted in The Netherlands and in Belgium in toddlers and young children (1–7 years of age) with a history of two or more AOM episodes in the year before enrollment (i.e., children with established complex OM before enrollment).[429,430] The children were randomized in a double-blind manner to receive either PCV7-CRM (two doses in children 1–2 years old and one dose in children 3–7 years old, followed by PPV23 6–7 months later) or hepatitis A and B vaccines. The children were followed for 24 weeks by the family physicians or specialists, when symptomatic. In both studies, PCV7-CRM did not reduce overall AOM cases, despite the reduction seen in pneumococcal AOM.[429] In the Dutch study, there was a reduction of 33%, with a 56% reduction in vaccine serotypes and a 17% reduction in nonvaccine serotypes. However, none of these values were statistically significant, probably because of the small numbers. The findings from the three studies on toddlers suggested that PCVs are effective in toddlers, especially in those with higher risk conditions such as daycare attendance, but when a recurrent AOM is already established, PCVs may not be effective against disease, or even against development of effusion.[431]

A single trial of PCV9 administration to pregnant women at 30 to 35 weeks gestation was designed to prevent clinical visits for OM in their infants during the first 6 months of life. Infants also received PCV7 at 2, 4, and 6 months. As mentioned above, the immunogenicity of PCV7 was reduced in these infants, and while 89% of infants in the placebo group were free of clinically diagnosed AOM by 6 months of life, only 74% of the infants of vaccinees remained free of the diagnosis by 6 months of age ($P = .03$). There was thus an excess of OM associated with maternal PCV which was ascribed by the authors to the lower immunogenicity of PCV given to the infants of mothers who received maternal PCV compared to the controls, who also got PCV7 but whose mothers did not get PCV9.[278]

It is not easy to assess the reduction of AOM burden once PCVs are implemented, for several reasons. First, pneumococci are not the only pathogens in OM and, in most cases, tympanocentesis is not performed, and thus the contribution of the pneumococcal component to the overall OM reduction cannot be assessed. Second, AOM is difficult to diagnose with the current skills of many practicing physicians, and overdiagnosis, as well as underdiagnosis, are common. Third, because tympanocentesis is not accepted as a routine procedure in most practices, it is often performed in selected cases (i.e., nonresponsive OM or complex OM). On the other hand, the indirect protection through reduction in vaccine serotype carriage (herd protection) may positively impact disease reduction because reduction of circulation of VT serotypes in the community means reduced early exposure of VT serotypes, resulting in protection against diseases even in the nonvaccinated infants who have not yet reached the age of PCV vaccination.[432,433] Preventing early otitis potentially reduces the resulting recurrent and complex OM.[434]

Because AOM may be mild or even asymptomatic and its diagnosis is difficult, assessing true OM incidence after vaccine implementation is impractical. Therefore, studies on post-PCV implementation impact of OM almost exclusively examined complex cases of OM (recurrent, nonresponsive, spontaneously draining or chronic representing mostly sequelae of AOM), or simply measured visits resulting from OM or OM-related morbidity (e.g., otolaryngologist visits, ventilation tube insertion, hearing tests) that are easier to detect. As described above, in this context, it is important to remember that in the prelicensure studies, the efficacy/effectiveness of PCV against complex and chronic OM (more likely to be captured by post-PCV surveillance than all-cause AOM), ranged from approximately 10% to 39% of all-case OM, representing a significantly higher efficacy than that against overall OM incidence (ranging from −1% to 7%).

Several studies looking at OM after PCV7 implementation reported reduced proportions of VT serotypes, increased proportions of non-VT serotypes, increased nonpneumococci organisms (i.e., *H. influenzae* and *M. catarrhalis*), and reduced antibiotic-resistant pneumococci.[51,435–439] However, because

these increased proportions could also be observed because of reduced proportions of VT pneumococci, the studies could not appropriately document the true vaccine effect on OM incidence.[438,439] On the other hand, a nationwide study in the United States showed that by 2002–03 (2–3 years post–PCV7-CRM introduction), rates of OM-related outpatient visits of children younger than age 2 years had decreased by 20%.[440,441] For some high-risk populations, such as the American Indians and Alaska natives, the figures were a bit more confusing.[331] When complex otitis was measured in the United States, most studies showed a significant reduction compared to the pre-vaccination era, although with wide variability. One study found a 17% and 28% decline in frequent OM in two birth cohorts from Tennessee and upstate New York shortly after PCV7-CRM introduction.[442] A study made up of more than 500,000 person-years of children younger than 2 years of age found reductions of 42.7% and 41.9% in ambulatory visits related to OM and antibiotic-prescribing related to this entity, respectively.[443]

PCV7 was introduced in other countries a few years later than in the United States, but results from the other countries support the post-PCV7 implementation findings from the United States. In Canada, a 13.2% reduction in claims for OM attributable to PCV7 was reported 3 years after implementation of PCV7 in children younger than age 5 years.[444] In Italy, PCV7 was implemented in the Liguria administrative region in 2003, with a claimed 38% reduction in hospitalizations associated with AOM in children younger than 2 years.[445] In Australia, in the 2.5 years after routine PCV7 introduction, a significant adjusted reduction in myringotomy with ventilation tubes insertion was seen in 23%, 16%, and 6% of children (the CIs were not reported) younger than 1, 1, and 2 years of age, respectively.[446] In France, carriage of PCV7 serotypes during OM (an indirect measure of PCV7 impact on OM) was reduced by 16% and 35% in year 1 and year 3, respectively, after PCV7 introduction.[439] In Greece, in years 3 to 5 after PCV7 introduction, overall rates of otorrhea visits per 10,000 pediatric emergency room visits decreased by 38%, mainly as a result of the decline in pneumococcal cases (48%), all results being significant at less than 0.001.[447] A study including children participating in the Norwegian mother and child cohort study, recruiting pregnant women between 1999 and 2008 and using maternal reports of AOM among others, showed an adjusted relative risk for OM of 0.86 (95% CI, 0.81 to 0.91) for children who received three or more PCV7 doses by 12 months of age.[448] Between 12 and 18 months of age the relative risk for AOM was 0.86 (95% CI, 0.81 to 0.91), and for ages 18 to 36 months, it was 0.92 (95% CI, 0.90 to 0.94). Thus, there is growing evidence that widespread implementation of PCV7 reduced AOM disease burden, at least in developed country populations.

Despite the apparent success of PCV7, there were several outstanding problems and uncertainty with this vaccine. First, the extent of replacement disease was not determined in OM. Numerous reports pointed to at least two serotypes that have been important in OM, even before PCV7 implementation and which became dominant postimplementation: serotypes 19A and 3.[422,432,433,435,438,449–451] Furthermore, serotype 19A became more multidrug resistant during the decade of PCV7 use. It seems plausible that serotype 19A increased substantially because of dual pressure. The first was "vaccine pressure" by PCV7, by reducing competition of 19A with PCV7 serotypes, and the second was "antibiotic pressure" promoting the spread of resistant and multiresistant serotype 19A. These conditions, coupled with the relative high virulence of serotype 19A, made it one of the most prominent causes of OM after PCV7 introduction (and especially complex OM and its complications, in particular mastoiditis).[432,433,438,449,451–457]

PCV7 was replaced by the more extended-spectrum PCV13. The importance of the latter to OM was that it contained three additional serotypes that are common in OM (serotypes 3, 6A and 19A). Serotypes 6A and 19A were the most common in OM after the PCV7 effect, although serotype 6A was reduced but not eliminated by PCV7. In addition, these serotypes are commonly antibiotic multiresistant. The following studies from the United States, France, and Israel demonstrated the additional effect of PCV13 beyond that of PCV7.

A study assessed trends in OM-related healthcare use in children younger than 6 years old in the United States during the PCV era (2001–2011) by analyzing claims in a large managed healthcare plan.[458] An overall downward trend in OM-related cases from 2001 to 2011 was observed. The significant reduction in visit rates in 2010–2011 in children younger than 2 years old coincided with the uptake of PCV13. These early post-PCV13 promising results were supported by findings from France,[459] where pneumococcal carriage during OM episodes (as an indirect measure of pneumococcal OM effect) was determined. The study was conducted during the transition between PCV7 and PCV13 (October 2010 through March 2011) by 58 pediatricians who obtained 943 nasopharyngeal cultures in children 2 to 24 months old. Of these children, 69% received one or more PCV13 doses. Among PCV13 recipients, overall pneumococcal carriage and carriage of the additional PCV13 serotypes was lower than that in PCV7 recipients (54% vs 65%, respectively, $P = .002$; and 10% vs 21.1%, respectively, $P < .001$). Significantly lower carriage was found for single serotypes 19A, 7F, and 6C (cross-reacting with 6A in PCV13). A third population-based prospective study in Israel[460] assessed children, mainly with complex OM, using middle ear fluid cultures. The unique study design permitted longitudinal assessment of the population-based incidence of the various conditions studied. The study, initiated 5 years before PCV7 (2004–08) introduction, described the initial impact of PCV7 (2010–11) and then the PCV13 impact (2012–13) on overall OM, pneumococcal OM, and serotype-specific pneumococcal OM. Compared to the pre-PCV period, OM caused by PCV7 serotypes +6A decreased by 73%, but this was associated with an increase in the additional PCV13 serotypes (1, 3, 5, 7F, and 19A) by 25% ($P < .001$). However, after introduction of PCV13, compared to pre-PCV period, rates of PCV7+6A and additional PCV13 diseases dropped by 96% and 85%, respectively ($P < .001$). The overall pneumococcal OM rates decreased by 31% during the PCV7 period, and then during the PCV13 period there was a 77% decrease ($P < .001$). The respective figures for the reduction in all-cause OM with middle ear fluid culture was 60% ($P < .001$).[460,461] As early post–PCV13 introduction data accumulated, it became clear that PCV13 added significantly to PCV7 in the impact on OM, especially when complex OM was measured.

As mentioned previously in the chapter, the efficacy of PCVs against simple, AOM is significantly lower than that against complex OM, expressed as recurrent/nonresponsive, spontaneously draining, and chronic OM (measured by the impact on ventilation tube insertion). The current understanding it that early occurring OM is most often caused by *S. pneumoniae*, whereas complex OM, especially in its more chronic manifestation, is not simply pneumococcal, but rather a mixed infection, expressed as a biofilm, with NTHi as the main pathogen.[462,463] This might explain why early vaccination with PCVs successfully reduces occurrence of complex OM, including chronic manifestations, which are only rarely caused by VT serotype,[462] through prevention of early *S. pneumoniae* disease leading to secondary infections, where NTHi and potentially other bacteria outnumber *S. pneumoniae*. The studies from The Netherlands and Belgium[429,431] showing that after recurrent OM is already established PCV7 could not

reduce further diseases, lend support to this notion. Also, the success of the protein-D conjugated PCV11 (POET study)[12] against *H. influenzae* otitis might be explained by the high likelihood that most captured children probably had complex OM that might have been prevented by early vaccination and prevention of the primary pneumococcal infections. Recent post PCV7 introduction observations also support this notion. In Greece, visits to clinics because of spontaneously draining ears were measured (rates per 10,000 all-culture visits).[447] The pre-PCV7 (2000–03) and post-PCV7 (2005–08) years were compared. The reduction in all-cause OM was 38% and 48% in *S. pneumoniae* OM, with *H. influenzae* cases also reduced by 20% (all $P < .001$).

In southern Israel, a population-based, prospective, observational study compared rates of OM episodes for which middle ear fluid was obtained in the pre-PCV period (2004–08) to that after PCV7 and PCV13 introduction.[460,461] In the fourth year after PCV7 introduction (and 2.5 years after it was replaced by PCV13), the incidence reductions were: overall OM, 60% (95% CI, 55% to 64%); PCV13 serotype OM, 93% (95% CI, 55% to 64%); non-PCV7 vaccine serotype, 14% (95% CI, –41% to 11%); all-pneumococcal OM, 77% (95% CI, 70% to 82%); NTHi OM, 63% (95% CI, 55% to 64%); and culture-negative OM 48% (95% CI, 36% to 57%). Although these are observational studies comparing pre- and postvaccine introduction, they strongly support the notion that early pneumococcal vaccination, by reducing early pneumococcal OM, prevents all-cause complex OM, including those caused by organisms beyond VT serotype pneumococci. Longer follow-up and more observations are needed to confirm these important early findings.

Effect on Carriage

Direct Effect of PCVs on Colonization

The first study demonstrating that PCV could reduce colonization of the serotypes included in the vaccine was conducted in Israel and published in 1996.[464] In this study, one and two doses of PCV-OMPC7 were compared with a single PPV23 dose in toddlers 12, 15, or 18 months of age. The PPV23 group did not have any effect on carriage; in contrast there was a marked reduction in carriage in the group receiving PCV-OMPC7, which persisted for 1 year. However, at the same time an increase in carriage of serotypes not included in the vaccine (non-VT serotypes) was observed. This study and similar observations from The Gambia[465] led to the assumption that PCVs can reduce carriage of VT serotypes and may increase the carriage of non-VT serotype (replacement phenomenon). The study also supported previous observations that nonconjugate polysaccharide vaccines are incapable of reducing carriage.[466] Further clinical trials confirmed that observation, using a variety of conjugates, age groups, locations, and schedules. Table 46.10 summarizes some representative early studies.[254,265,465,467,468] The overall pattern to be observed in these studies was a reduction in VT carriage, sometimes seen only after the booster dose, and an increase in the carriage of non-VT, so that the overall carriage rate did not change significantly or was slightly reduced.

Several trials were conducted in toddlers in the 1990s using either PCV7 or PCV9. In a series of studies in daycare center attendees in Israel,[32,469,470] a reduction in both acquisition and prevalence of VT (and serotype 6A) serotypes were seen in PCV9 recipients, compared with control subjects (receiving meningococcus C–CRM vaccines). At the same time, a marked increase of prevalence and acquisition of non-VT serotypes was also observed. No effect on duration of carriage was seen. The effect of the vaccine on carriage persisted for the duration of the follow-up (2 years). Serotype-specific protection against increased carriage of serotypes 6A, 6B, 9V, 14, and 23F was demonstrated.[32] In The Netherlands and Belgium,[429,430] PCV7, followed by a PPV23 booster, administered to children 1 to 7 years old with recurrent AOM, resulted in a marked reduction of VT carriage with an increase in non-VT carriage.

One study from Israel assessed the potential additional reduction of carriage by PCV13 as compared with PCV7.[471] In this randomized, double-blinded study, 881 children received PCV13 and 873 received PCV7 (per-protocol analysis) at ages 2, 4, 6, and 12 months, and nasopharyngeal cultures were obtained at ages 2, 4, 6, 7 12, 13, 18, and 24 months. Blood was drawn at ages 7 and 12 months. There was a 43% (95% CI, 44% to 51%) reduction in the cumulative acquisition of the additional PCV13 serotypes, combined (1, 3, 5, 6A/6C, 7F, or 19A): 21.8% versus 38.4% in the PCV7 versus PCV13 group, respectively. Serotype 5 was not prevalent enough for evaluation, and for serotype 3 no reduction was observed. Similar findings were seen when prevalence of carriage was assessed. For the common PCV7/PCV13 serotypes excluding 19F, no significant difference in carriage between the two groups was found. Thus, PCV13 showed an added benefit in reducing carriage of VT pneumococci in vaccine recipients.

An important point is to determine whether postvaccination antibody concentrations (serotype-specific) correlate with acquisition of VT nasopharyngeal carriage. Two studies, conducted in Israeli toddlers with PCV9[27] and in American Indian infants and toddlers with PCV7[343] showed an inverse correlation between serotype-specific anticapsular IgG and the risk for nasopharyngeal acquisition of the corresponding pneumococcal serotypes. As part of the analysis of a study comparing PCV13 with PCV7 on the effect on pneumococcal carriage,[462] an additional analysis was conducted on 1410 to 1530 serum samples to investigate the potential correlation between nasopharyngeal acquisition and serum IgG concentrations.[461] PCV7/PCV13 was given at ages 2, 4, 6, and 12 months. IgG concentrations were measured after dose 3 and nasopharyngeal cultures were obtained at ages 7, 12, 13, 18, and 24 months. New acquisition rates decreased as IgG concentrations increased for all studied serotypes ($P < .001$ for 6A, 14, 23 F; $P = .010$ for 6B; $P = .089$ for 19F). For example, for serotype 6B, the calculated proportion of children who will acquire this serotype before age 2 years, if the antibody concentration was 0.35 μg/mL was 9.1% (95% CI, 6.4% to 12.8%); the respective figures for 2.54 μg/mL (50th antibody percentile) and 10.15 μg/mL (90th antibody percentile), were 5.8% (95% CI, 4.7% to 7.2%) and 4.2% (95% CI, 2.9% to 10.2%). The respective figures for serotype 19F for 0.35 μg/mL 50th antibody percentile (2.74 μg/mL) and 90th antibody percentile (7.37 μg/mL) were carriage in 14.0% (95% CI, 9.4% to 20.3%), 10.0% (95% CI, 8.6% to 11.7%), and 8.5% (95% CI, 6.5% to 11.0%). In this study, two populations were enrolled—Bedouin and Jewish infants. The Bedouin infants had significantly higher carriage rates than the Jewish infants, but the immune responses were similar across ethnic groups and the decreased acquisition rates of the studied serotypes by increasing IgG concentrations with each group were similar across the ethnic groups for most serotypes. Thus, the efficacy against acquisition and carriage of VT-serotype pneumococci inversely correlated with the serotype-specific antipolysaccharide antibody concentrations achieved by PCV administration.

Recently, because of the desire to reduce the number of PCV doses in various universal programs, the potential effect of a lower number of doses (compared with the gold standard of three doses in infancy and one booster dose in the second

TABLE 46.10 Selected Early Clinical Trials Testing PCV Effect on Colonization When Administered in Infancy

Location	Reference	Vaccine	Schedule	Assessed (mo postprimary)	No. of Children		Colonization VT (%)			Colonization Non-VT (%)			Comments
					PCV	Control	Vaccine	Controls	*P* Value	Vaccine	Controls	*P* Value	
Israel	464	PnTT4	2, 4, 6, 12 mo[a]	1, 6, 7 combined	25	25	10	27	.014	No clear pattern		(–)	VT including 6A
Israel	464	PnDT4	2, 4, 6, 12 mo[a]	1, 6, 7 combined	25	25	5	27	.001	No clear pattern		(–)	VT including 6A
United States	468	PCV7	2, 4, 6, 12 mo	1	54	31	11	7	NS	17	11	NS	
				6	54	31	17	18	NS	17	11	NS	
				7	54	31	10	21	NS	10	7	NS	
The Gambia	254	PCV9	2, 3 mo	1	102	100	54	64	NS	45	33		
			4 mo	5	102	100	61	74	NS	28	16		
South Africa	265	PCV9	6, 10, 14 wk	1	242	239	No difference			No difference			
				6	242	239	18	36	.001	36	25		
The Gambia	465	PCVCRM5	2, 3, 4, 18 mo[a]	20	26	160	50	90	< .001	77	43		
			2, 3, 18 mo[a]	20	30	160	67	90	.002	43	43		
Czech and Slovakian Republics	12	PCV protein D-11	3, 4, 5, 12 mo	10–13	177	175	6	11	NS	7	8		

[a]Booster with a PPV23.

NS, not significant; PnTT4, tetravalent PCV (6B, 14, 19F, 23F) conjugated to tetanus toxoid; PnDT4, tetravalent PCV (6B, 14, 19F, 23F) conjugated to diphtheria toxoid; PCV-CRM5, pentavalent PCV (6B, 14, 18C, 19F, 23F) conjugated to CRM_{197}; VT, vaccine type.

year) raised some concerns because of the correlation between postvaccination antibody concentrations and acquisition and carriage of VT serotypes. Several studies were conducted to evaluate this question.[246,409] Four studies with head-to-head comparisons of reduced versus standard dosing regimens were conducted. A study, conducted in Fiji, compared zero doses to one, two, and three PCV7 doses (given at 6 weeks, 6 and 14 or 6, 10, and 14, respectively), with and without a PPV23 booster at 12 months.[472] A significant difference in VT-carriage prevalence between the two- and three-dose groups at 9 months was found: the three-dose group had significantly lower VT carriage (odds ratio [OR] 0.30; 95% CI, 0.09 to 0.9). At 6, 12, and 17 months of age, VT carriage among the two- and three-dose groups was not significantly different. The PPV23 booster did not have any impact on VT carriage measured at age 17 months. Another study, conducted in The Gambia, compared one, two, and three PCV7 doses (given at 2 months; at 2 and 3 months; and at 2, 3, and 4 months); all children received a PPV23 booster at 10 months.[473] At 11 months, the group receiving three PCV7 doses showed a borderline significant reduction in VT carriage compared with the group receiving two PCV7 doses (10.0% vs 16.7%, $P = .056$). However, at 5 and 15 months, the VT-carriage prevalence was not significantly different among children who received two or three doses. The third study, conducted in Israel with PCV7, compared a 3+1 schedule (2, 4, 6, and 12 months) with 2+1 schedule (4, 6, and 12 months), a 0+2 schedule (12 and 18 months), and a 3+0 schedule (2, 4, and 6 months).[474,475] Between 7 and 12 months of age (prebooster), the mean VT prevalence was nonsignificantly lower in the 3+1 group when compared with the 2+1 group (22.6% vs 28.4%, $P = .089$). The 0+2 group between 7 and 12 months, before children were vaccinated, had a mean VT prevalence of 35.2%, which was significantly higher than prevalence in the 3+1 group ($P < .001$). Between 13 and 18 months (i.e., after the booster dose) and between 19 and 30 months, the 3+1 and 2+1 groups had equivalent VT prevalence, but both groups had significantly lower VT prevalence than the 0+2 group. When the 3+1 and the 3+0 schedules were compared, at 13 months, all GMCs were significantly lower in the 3+0 compared to 3+1 ($P < .001$). During the first year of life, carriage of VT serotypes was similar in the two groups, but lower than in unvaccinated controls ($P < .001$) (0+2 who received the final dose at 12 months of age). From age 12 months to 30 months, despite the absence of booster in the 3+0 group and the significantly higher GMCs in the 3+1 group, no differences in carriage rates were seen between the groups. A fourth study, conducted in The Netherlands, compared children who received PCV7 at 2 and 4 months (2+0), at 2, 4, and 11 months (2+1), and unvaccinated controls.[475,476] At 12, 18, and 24 months of age, children in the 2+0 and 2+1 groups had significantly less VT carriage than unvaccinated controls. Although children in the 2+1 group had a significantly lower prevalence of VT carriage at 18 months (16%) than the 2+0 group (24%, $P = .01$), no difference was found at 24 months.

The question with regard to the duration of protection from carriage acquisition is still not fully answered. A study was conducted in the United Kingdom to assess whether the reduction in nasopharyngeal carriage of VT serotypes induced by PCV7 given to infants persisted beyond 2 years of age. The study was performed as an unblinded controlled study in children 2 to 5 years old who had received three PCV7 doses in infancy and a PPV23 booster at 13 months of age; they were compared with unimmunized control children. Nasopharyngeal cultures were taken in the summer and the winter. In the summer, the rates of VT-serotype carriage in vaccinated and control subjects were 10.0% and 13.5%, respectively, and in the winter, the rates were 30.0% and 31.5%, respectively. Thus, no real difference could be found between vaccinated and control subjects in VT serotypes (or overall pneumococcal) carriage at this age. However, the role of PPV23 versus a PCV as booster was not assessed. In South Africa, long-term carriage after a primary series of three PCV9 doses (6, 10, and 14 weeks of age) or placebo was assessed. A random subset of children, who had received three doses of study vaccine per protocol during the trial, was enrolled in the long-term carriage study in a fashion blinded to the study personnel. It was found that by age 5.3 years, the PCV9 no longer significantly reduced nasopharyngeal colonization by VT serotypes. In addition, HIV-infected children had a higher prevalence of colonization by *S. pneumoniae* (71.6%) than did HIV-negative children (50.9%). A nested study in American Indian children 1 to 7 years of age who had participated in a group-randomized efficacy trial reported that 27 months postvaccination children from communities randomized to receive PCV7 had lower carriage prevalence than the unvaccinated control subjects: 10.3% versus 17.1% ($P = .01$).[477] Thus, it seems that protection from acquisition of carriage can be achieved for some limited time (probably 2–3 years) after a full PCV schedule, but additional studies are needed.

PCV impact on carriage likely is mediated by protection against acquisition, reduction in the density of carriage by antibody, and induction of mucosal memory B cells.[478] In the mouse, the duration of carriage appears to be antibody-independent, Th17 cell-mediated immunity.[408]

Thus, the direct effect of PCV7 is an overall reduction of carriage of VT serotypes, with a parallel increase in non-VT serotypes (termed *replacement*). This phenomenon is expected to be of great magnitude, resulting in herd immunity against vaccine serotype disease on the one hand, but a potential for replacement disease on the other hand, and the magnitude of the effect is dependent on vaccination coverage. Future studies of PCV impact on carriage will include a broader analysis of PCV impact on the nasopharyngeal microbiome. Initial data of PCV impact show a short-term increase in bacterial diversity in the nasopharyngeal microbiome with an impact on a number of genera including other streptococci, *Haemophilus* species, *Staphylococcus* species, and anaerobes.[479]

Indirect Effect of Pneumococcal Conjugate Vaccine on Pneumococcal Colonization

The reduction of VT serotypes and the replacement by non-VT serotypes, instigated in the nasopharyngeal flora by vaccination, does not exist in isolation.[466] Pneumococci are constantly on the move in the population, being transmitted and acquired at high rates, especially in young children. The impact of vaccination on the transmission of pneumococci were first demonstrated in Israel in a series of studies where vaccination of daycare center attendees reduced carriage of VT pneumococcus in their younger siblings.[470,480] In a group-randomized efficacy trial of PCV7 conducted in the Navajo and Apache reservations,[481] there was a significantly lower carriage rate of VT serotypes among the vaccinated children compared with the placebo recipients in the communities allocated to no vaccination.[481] Furthermore, even when the vaccinated children were carriers of VT serotypes, they were less densely colonized with VT serotypes than unvaccinated control children (OR 0.61 [95% CI, 0.38 to 0.99]).[482] Adults and unvaccinated children younger than 5 years of age living in households with a PCV7 vaccinee were less likely to be colonized with VT serotypes (OR for adults 0.57 [95% CI, 0.33 to 0.99]; OR for children 0.57 [95% CI, 0.26 to 0.98]) than were those living in a household with unvaccinated control children. No such effect was seen in children 5 to 17 years of age. Decreases in VT

serotypes were offset by increases in carriage of non-VT. Among adults, those living in a household with a trial participant who was colonized with VT pneumococcus and randomized to receive PCV7 were less likely to be colonized with the same serotype than those in the households randomized to the unvaccinated control group (OR 0.34 [95% CI, 0.11 to 0.99]), suggesting that even with carriage of VT, children receiving PCV7 were less infectious than unvaccinated children.

An initial study in The Gambia[483] found no reduction of carriage of VT-related serotypes and no increase in the carriage of non-VT serotypes in the younger siblings of PCV9 recipients compared with siblings of placebo recipients (relative risk 0.95 [95% CI, 0.78 to 1.16], 0.97 [95% CI, 0.73 to 1.29], and 1.03 [95% CI, 0.87 to 1.22] for VT serotypes, VT-reduced serotypes, and non-VT serotypes, respectively). However, a follow-up study in The Gambia provided evidence of herd immunity in adults when the children received PCV7.[484] There was a significant, but only limited, additional benefit on VT carriage when vaccinating adults with PCV, and there were no data presented on the extent, if any, to which vaccination of adults contributed to herd protection. As time elapsed after introduction of PCV vaccination, a new postvaccination type of flora becomes prevalent in the population and spread beyond the vaccinated age group to younger infants and adults. After the introduction of PCV7 in the United States, the first demonstrations of indirect effect on a population level were published from Massachusetts and Alaska.[485–487] Additional studies from other developed countries showed a decline in carriage of VT serotype and an increase in non-VT serotypes in children younger than 5 years of age, after introduction of PCV7.[488–493] In addition, herd protection against carriage following PCV7 introduction in infants was observed among both HIV-infected and HIV-uninfected adults in South Africa.[494] Similarly, reduction in carriage of VT in neonates and infants too young to be vaccinated was also reported.[495] In addition, unvaccinated children in the age targeted for vaccination also showed similar trends.[492] Following PCV13 introduction in the United States, significant herd protection against the additional six types was demonstrated at 65% to 75% coverage with PCV13.[496] Herd protection against carriage in unvaccinated individuals has now been documented as a result of the use of PCV10 in both developed and developing countries.[497–499] However, in all reports of indirect effects of PCV7 on reduction of VT serotypes, an increase in carriage of non-VT was seen at the same time. The coverage of PCV in infants required to induce herd protection is likely to vary by the vaccine used, number of doses given, and the pattern of transmission in the community. For PCV13, using a 3 + 1 schedule, data from Massachusetts showed herd immunity (defined as significant a drop in PCV13 type carriage in underimmunized children) when full vaccination reached 75% of children, while similar drops in carriage among the Navajo were identified at 58% full immunization coverage.[499a,499b]

The replacement serotypes were the non-VTs seen in the area before vaccination and included serotypes/groups 3, 11A, 15 (A, B, and C), 35B, and the vaccine-related serotype 19A.[120,488,489,491] Now that both PCV13 and PCV10 have rolled out globally, it remains to be seen which additional non-VT serotypes or clones will evolve and spread globally.

Effect of Dosing Schedule on Pneumococcal Vaccine Effectiveness

The two commercially available conjugate vaccines, PCV10 and PCV13, are licensed in the United States, Europe, and elsewhere on four-dose schedules, including three primary doses plus a booster at approximately 1 year of age.[130,500] In Europe, and increasingly elsewhere, PCV13 and PCV10 are approved for use on a schedule of two primary doses plus a booster at 1 year of age when part of a "routine immunization program." Both conjugate vaccines are prequalified by the WHO for use in developing countries. The WHO initially recommended that conjugate vaccines be used on a schedule of three doses without a booster, the standard EPI schedule[264]; more recently WHO added a recommendation for a schedule of two primary doses plus a booster as an alternative.[501]

In practice, schedules used in routine immunization programs differ. In the United States and most Canadian provinces, the vaccine is given to infants on a four-dose schedule, with doses at 2, 4, 6, and 12 to 15 months, identical to the schedule used in the United States vaccine trials.[4,5] In other settings, three doses are used, either given during the first 6 months of life without a booster (e.g., Australia) or as two doses during that period, followed by a booster after 1 year of age (e.g., the Canadian Province of Quebec and many Latin American and European countries). In Africa, schedules of three doses given at 6, 10, and 14 weeks (e.g., Rwanda) or at 6 and 14 weeks and 9 months (South Africa) are used.

The number and timing of doses that will provide optimal prevention of disease is unclear, and may be different for different settings.[502] Data from early adopting countries all show substantial disease reduction using several different schedules. Three doses given at 6, 10, and 14 weeks were efficacious, except for serotype 1, in clinical trials in Africa.[6,7] The Australian schedule of three doses at 2, 4, and 6 months without a booster has been shown to quickly reduce invasive disease both in young children and in adults through herd effects.[503] Likewise, the four-dose schedule led to rapid and sustained reductions in the United States and Canada,[14,504] and marked reductions in pneumonia hospitalizations in children occurred quickly after introducing a "2+1" (two primary doses plus a booster) schedule in Quebec Province in Canada[505] and in the United Kingdom.[506] In these settings, the true impact of the recommended vaccine schedule alone is difficult to discern because the initial national programs included catch-up campaigns that offered vaccine to all children younger than age 2 years, which likely helped to quickly reduce disease burden. Data are becoming available on the effectiveness of different schedules in developing country settings, including a rapid reduction in invasive disease among HIV-infected and -uninfected children in South Africa on a schedule of doses at 6 and 10 weeks with a booster at 9 months,[17] and a rapid reduction in pneumonia hospitalizations in Brazil on a schedule of three primary doses with a booster.[507]

Comparable to immunogenicity studies, several from the United States shortly after seven-valent conjugate vaccine introduction found small but statistically significant differences between the effectiveness of a two- or three-dose primary series. One study of invasive disease occurring in vaccinated children showed a disproportionate number of serotype 6B cases among children who had only received one or two primary doses compared with children receiving three primary doses or a full four-dose series.[508] A study of lower respiratory tract infections found that compared with children who had received two primary doses, children who had received three doses had 7.8 fewer hospitalizations and 57 fewer outpatient visits (per 1000 children) among those born in 2002; no difference in hospitalization rate was seen after the booster dose for the 2002 birth cohort or at any time for the 2003 birth cohort, a finding the authors attributed to herd effects that were more impactful later as more children became vaccinated.[509] Other studies also found a booster dose to be beneficial, including a large case-control study from the United States. In this study, a variety of schedules were found to be

protective against invasive disease compared with no vaccine, but a schedule of three doses plus a booster dose after 12 months was significantly more protective than three doses without a booster.[103] A systematic review of the literature found some differences in immunogenicity between three- and four-dose schedules, but few differences in effectiveness in routine program settings, although few data are available from head-to-head studies of vaccine effects on clinical outcomes.[234]

SAFETY

Children Younger Than 2 Years of Age

The safety of PCVs has been most extensively examined in children younger than 2 years of age because most of the pivotal licensure trials were performed in this age group. For seven-valent conjugate vaccine, local reactions (including swelling, redness, and induration) were observed but were generally mild and self-limiting. The rates of local reactions after receipt of PCV13 were similar to the rates observed in recipients of PCV7,[370] whereas the rates of local reactions after PCV10 vaccine were somewhat higher than seen for PCV7.[510]

Fever is generally more frequent in infants receiving PCV than in control subjects.[4,271,339,370,511] The incidence of fever (>38°C) after receipt of PCVs varies widely but appears to be somewhat higher with both PCV10 and PCV13 than PCV7.[378,511] Generalized systemic symptoms, such as irritability, drowsiness, and crying more than usual, have been seen in recipients of all three licensed conjugate vaccines,[378,510,512] but these symptoms have usually been mild. Other events that could possibly be attributed to conjugate vaccine include urticaria, rash, excessive crying, hypotonic-hyporesponsive episodes, cyanosis, and transient granulocytopenia.[13,236,267,513]

In the NCKP efficacy trial of PCV7,[4] the frequency of uncommon events requiring medical attention after vaccination was evaluated through the use of comprehensive hospitalization and emergency room utilization databases. The events in 92 diagnostic categories were followed for 30 days after vaccination in hospitalized children and for 60 days after in children were seen in the emergency department. Adverse events that were severe, unexpected, or possibly had been caused by the study vaccine were followed up through chart review, parent contact, or both. Significant differences were seen only for febrile seizures and elective admissions. Febrile seizures requiring hospitalization were more common in recipients of PCV7 than control subjects, but only in those patients who had received DTwP at the same time; there was no difference in rate of seizure between recipients of PCV7 and infants in the control group, who had received a concomitant DTaP vaccine. There was no clustering of febrile seizures within the 3-day period after vaccine administration, and the rates of seizure in this study were below the historical rates of seizure seen after DTwP vaccination.[33] Rates of sudden infant death syndrome (SIDS) also were similar to or lower than those expected from historical data. Elective admissions, including ventilatory ear tube placement, occurred more frequently in the control group.[4] In an analysis of selected categories of outpatient clinic visits, control subjects had significantly more seizures overall than PCV7 recipients, but no specific seizure subcategory was different between the two groups, and there was no temporal clustering of the seizures relative to vaccination. Similarly, although there was an excess of generalized seizures among vaccinees in the South African trial, there were less seizures in the "unspecified" seizure category and no excess of seizures overall.[7] In the trial in South Africa, hyperactive airway disease and asthma were noted 79% more frequently in recipients of PCV9 (nominal P = .009), but no temporal association to receipt of vaccine was apparent.[77] This was not observed in trial of PCV9 in The Gambia nor in the NCKP or Navajo trials of PCV7.[514]

There was an excess of visits to the clinic or hospital associated with respiratory infections in the first week after the first PCV compared to controls vaccination in the South African, The Gambian, and Philippine trials,[6,7,9] but this excess was not noted in the Navajo[5] or in the NCKP trial.[4]

Seizures were also specifically examined among cases reported to the Vaccine Adverse Event Reporting System (VAERS), a repository for reports of adverse events seen after vaccinations given in the United States. The reports are voluntarily submitted by manufacturers, healthcare workers, and patients; consequently, the reports must be interpreted with caution. Wise and colleagues[515] reported on data submitted to VAERS in the first 2 years after licensure of PCV7, during which time an estimated 31.5 million doses of the vaccine were administered in the United States. Of 4154 reports made within 3 months of PCV7 vaccination of children 17 years of age and younger, there were 393 reports of seizures; 159 of these were considered serious (including three deaths). Fifty-seven (14.5%) of the total seizures and 15 of the serious seizures (9.4%) occurred in children who had received PCV7 alone, without concurrent vaccinations. Additional details of case history and follow-up were obtained for the first 98 reports of seizure. Seventy-nine of these cases (80.6%) either had fever at the time of the seizure or had a history of seizures. The three children who died had seizures without known cause, although one had a previous diagnosis of "gross motor delay"; the other two were fraternal twins.

The 4154 reports in the VAERS database represent a rate of 13.2 adverse events per 100,000 vaccinations temporally, although not necessarily causally, related to receipt of the vaccine. Serious adverse events comprised 14.6% of these reports (n = 608, including 117 deaths). This is consistent with the rate of serious adverse events (14.2%) reported for other vaccines.[516] The majority of reports involved minor adverse events that had been observed in clinical trials. In 23 cases, rechallenge with a subsequent dose of vaccine produced the same event encountered after a previous dose, suggesting a causal relationship. These 23 cases included seven cases of fever, irritability, or other nonspecific symptoms; four cases of respiratory symptoms; four cases of prolonged or abnormal crying; three cases of gastrointestinal disturbance; two possible allergic reactions; two seizures; and one case of alopecia. In addition, there were 14 reports of anaphylactic reactions, 78 cases with possible thrombocytopenias (12 with platelet counts less than 20,000/mm^3), and six cases of serum sickness. For each of these categories, alternative explanations were present in some cases, but not in all cases. Of the 117 deaths, 73 (62.4%) did not have an identified cause, although 59 of these were classified as SIDS or possible SIDS. Of the 44 deaths in which the cause was identified, seven were caused by pneumococcal infections (six in partially vaccinated children).

More recently, an excess of febrile seizures was reported to the Vaccine Safety Datalink in the United States, when the 2010–11 trivalent influenza vaccine was administered with PCV13 to children 6 months to 2 years of age. The Centers for Disease Control and Prevention (CDC) estimated an excess of 60 such seizures per 100,000 children receiving both vaccines simultaneously, with the seizures typically occurring the day after immunization.[517]

Older Children and Adults

In the United States and some other countries, catch-up vaccination is recommended for healthy children up to 23 months of age who did not receive pneumococcal vaccine as

infants,[33] but vaccination of older children without previous vaccination or who did not respond to vaccination with PPV could prevent considerable additional disease. In 115 Spanish children who received a single dose of vaccine between 24 and 36 months of age,[518] local reactions of erythema, induration, and tenderness occurred in 32% to 40% of the vaccinees. Approximately one-third of these reactions were severe, but most resolved within 3 days of vaccination. Eight children (7%) had fever of 38°C or higher within 72 hours of vaccination, a lower rate than seen in younger children.[4,236,513]

Pain at the injection site was the most common adverse reaction in children 2 to 18 years of age, occurring in 20% to 56% of patients,[293,519] but there were no significant differences in those receiving the conjugate vaccines and control vaccines. The incidence and severity of local reactions may be reduced by using a deep intramuscular route to inject the vaccine,[275] and a study eliminating alum adjuvant from the vaccine appeared to decrease the incidence of local reactions in toddlers,[275] although it increased the incidence in adults.[520] Reactogenicity does not increase with repeated doses.[519,521] Fever is seen more frequently in children receiving PPV vaccine than conjugate vaccines, but temperature higher than 38°C is infrequent in any group.[64,324,519]

The safety of various doses of PCV7 was studied in older adults who had previously received PPV.[288,522] Increases in PCV7 dose were associated with increases in both local and systemic adverse events, but among systemic symptoms, only general muscle aches and pains were significantly different between the lower and higher PCV7 doses. Swelling, pain, and limited movement all increased significantly with dose of PCV7, but tended to be encountered less frequently after PCV7 than after PPV vaccination. Earlier studies[133,199] found no differences in symptoms produced by PCV7 and PPV in older adults, although younger adults experienced more pain and headache after PCV7 than after PPV.[199] Thirteen-valent conjugate has been evaluated in adults. In this population, fever was infrequent, and the most common reaction observed was mild local tenderness.[289] The Phase III trial bore out these earlier data with no excess of serious adverse events in the PCV recipients and no serious adverse events associated with the vaccine administration, although minor systemic and local adverse events were increased.[18] Any local redness occurred in 4.9% of vaccinees, an excess of 3.7% over controls ($P < .001$) with moderate redness (5–10 cm) occurring in 1.7%, a 1.4% excess over the controls ($P = .007$). Any local swelling occurred in 6.8% of vaccinees, an excess of 5.6% over controls ($P < .001$), with moderate swelling (5–10 cm) occurring in 2.6%, an excess of 2% over controls ($P < .001$). Local pain was common at 36.1%, an excess of 30% over controls ($P < .001$) with moderate pain (interfering with activity) occurring in 7.7%, an excess of 7.1% over controls ($P < .001$). Some limitation of arm movement was found in 14.1% of vaccinees, an excess of 10.9% over controls ($P < .001$), but moderate limitation, defined as inability to raise the arm above the head but able to raise it above the shoulder, was less common at 1.7% in vaccinees, an excess of 1.2 % over controls ($P < .02$). In terms of systemic adverse events that were mild or moderate, there was a small excess of any fever at 2.9%, an excess of 1.7% over controls ($P = .02$). There was a small excess of fatigue reported (3.9%, $P = .03$) and a small excess of rash (2.5%, $P < .001$). New generalized muscle pain that was sufficient to interfere with activity occurred in 5.9% of vaccinees, an excess of 3.1% over controls ($P < .001$).[18]

Special Groups

Several studies examined reactogenicity of PCVs in children with immunodeficiencies. In a study of children infected with HIV, those receiving PCV7 did not have significantly more reactions than those receiving control vaccine,[511] but swelling, redness, pain, fever, and irritability of at least moderate severity were significantly more frequent after the first and second doses of PCV7 than after the corresponding doses of placebo (56.7% vs 20.0% [$P = .02$] and 53.3% vs 13.3% [$P = .01$], respectively). Among the 30 HIV-positive children randomized to receive PCV7, three had severe reactions to vaccination (severe induration, erythema, and limited leg movement; limited leg movement and high-pitched crying; and temperature of 39.9°C). Among 15 HIV-positive children randomized to placebo, one had a fever of 39.5°C. All four children with severe symptoms had symptomatic HIV disease at baseline. In an earlier study of five-valent PCV vaccine in HIV-positive and HIV-negative children older than 2 years of age,[305] only minor adverse reactions were observed.

The reactogenicity of PCV7 is not different between children with and without sickle cell disease[87,90]; however, children with sickle cell disease who had a three-dose primary series of PCV7 had slightly stronger reactions to a PPV booster than those who had a single dose at 12 months.[110] Children with solid-organ transplants experienced less pain at the injection site than healthy control children, but no serious adverse events were seen in either group.[313]

Preterm infants receiving PCV7 had fever higher than 38°C, swelling, tenderness at injection site, irritability, loss of appetite, vomiting, diarrhea, and hives more frequently than preterm infants receiving control meningococcal vaccine.[276] However, these reactions were not severe enough to preclude the use of PCV7 in preterm infants. A smaller study[261] found no significant differences between preterm and full-term infants in local or systemic reactions when vaccine was given at 3, 5, and 11 months after birth, although both groups had fewer adverse events after the third (booster) dose than after either of the two primary doses. A recent study on safety and immunogenicity of PCV13 conducted among 100 preterm and 100 full-term neonates found similar safety profiles in both groups.[273]

Few data are available on the safety of PCV administration during pregnancy. However, PCV9 was well tolerated in 74 pregnant women in a single trial.[278]

PUBLIC HEALTH CONSIDERATIONS

Epidemiologic Effects of Vaccination

Effect of Vaccination in Children Younger Than 5 Years of Age

Use of PCV has had a profound effect on pneumococcal disease in the United States, the first country to include PCV as part of the routine childhood immunization schedule. The effects were seen very quickly after vaccine introduction in 2000. In a multicenter study conducted in 2002 of children requiring hospitalization for IPD, 77% fewer cases in children younger than 2 years of age were caused by vaccine serotypes than seen during 1994 to 2000.[523] Surveillance in the NCKP population identified no cases of invasive disease caused by vaccine serotypes among children younger than 1 year of age between April 2002 and March 2003, compared with rates between 51 and 98 cases per 100,000 person-years before vaccine licensure.[524] According to the CDC's Active Bacterial Core Surveillance (ABCs), a population-based surveillance system measuring IPD in approximately 20 million people in eight states, overall rates of IPD (both VT and non-VT) among children younger than 5 years of age had dropped 75% from prevaccine levels by 2003. Rates of disease caused by all serotypes in this age group fell from 96.7 cases per 100,000

population during 1998 and 1999 to 23.9 cases per 100,000 population in 2003 and after introduction of PCV13 in 2010, all serotype disease reduced below 20 per 100,000 (Fig. 46.4).[525] Between 2004 and 2007, overall disease rates among children younger than 5 years remained relatively stable; invasive disease caused by PCV7 serotypes became very unusual (0.4 cases per 100,000 population in 2007), and an increase in disease caused by nonvaccine serotype 19A occurred, which accounted for the lack of change in overall disease rates between 2004 and 2007.[14] In 2010, the U.S. immunization program changed from PCV7 to PCV13, and for the period July 2012 to June 2013, the incidence of total invasive disease and invasive disease caused by serotypes in PCV13 but not in PCV7, declined by 64% (95% interval estimate 59% to 68%) and 93% (95% interval estimate 91% to 94%), respectively, compared with incidence rates expected among children younger than 5 years old if PCV7 had been continued.[487]

Use of conjugate vaccine in children has also reduced the differences in risk of IPD between certain racial and ethnic groups. According to ABCs data, rates of invasive disease fell 77% overall in white children younger than 2 years of age between 1998 and 2002, and fell 89% in black children in the same interval; incidence among black children younger than 2 years of age went from 3.3 times the rate among white children in the prevaccine period to 1.6 times higher in 2002.[526] Among Navajo children living in the American Southwest, rates of vaccine serotype disease between 1995 and 1997 were 210 and 263 cases per 100,000 children younger than 1 year of age and 1 to 2 years of age, respectively. Between 2004 and 2006, no vaccine serotype cases occurred in this age group.[527] In addition, rates of disease caused by nonvaccine serotypes did not change significantly after vaccine introduction. In Alaska, surveillance by CDC's Arctic Investigations Program identified a large drop in disease caused by vaccine serotypes among the Alaska Native population.[486] After vaccine introduction, VT invasive disease fell 91% among Alaska Native children younger than 2 years of age (from 275 cases per 100,000 children to 25 cases per 100,000 children) and by 80% among non-Natives the same age (from 101 cases per 100,000 children to 20 cases per 100,000 children), eliminating the disparity in disease caused by vaccine serotypes. However, after the initial report, an increase in non–vaccine-serotype disease was seen among Alaska Natives, primarily among those living in the rural Yukon–Kuskokwim region and reduced the overall benefits of pneumococcal vaccination in this population.[528]

A large amount of data on vaccine impact are now available from outside the United States. Surveillance from 12 centers across Canada (the IMPACT [Incident Management of Patients, Actions Centered on Treatment] Program) found a 56% decrease in the number of cases among children younger than 5 years of age, from 269 in 2000 to 119 in 2007, including a 92% decrease in PCV7 cases in children younger than age 5 years.[503] In Australia, the incidence of invasive disease decreased 74% between 2002 and 2007 (from 98.1 to 25.1 per 100 000 children); the reduction was more pronounced in nonindigenous children (84.7 to 19.5 per 100 000 children) than indigenous children (129.1 to 82.3 per 100 000 children).[529] In Europe, results of studies from the first few years after vaccine introduction are demonstrating findings similar to those seen in other regions,[530] although in some settings, the emergence of serotype 1 disease has partly offset reductions seen by PCV7 implementation.[531] United Kingdom researchers have shown effectiveness against invasive disease for PCV13 overall and serotype-specific effectiveness for four of the vaccine antigens, including 84% effectiveness of the first two primary doses or the booster dose against serotype 1.[142] Longitudinal data on PCV13 impact in England and Wales show significant reductions in all IPD across all ages, including an impact on serotype 3 despite the lack of impact on serotype 3 found in the indirect cohort study.[142,532] Data are now becoming available showing benefit of PCVs for reducing invasive disease in developing countries, including effect of PCV7 and PCV13 on invasive disease in South Africa.[17] In Israel, the IPD impact of PCV7 was more pronounced in children with sepsis and meningitis than in those with pneumonia, with IPD among pneumonia

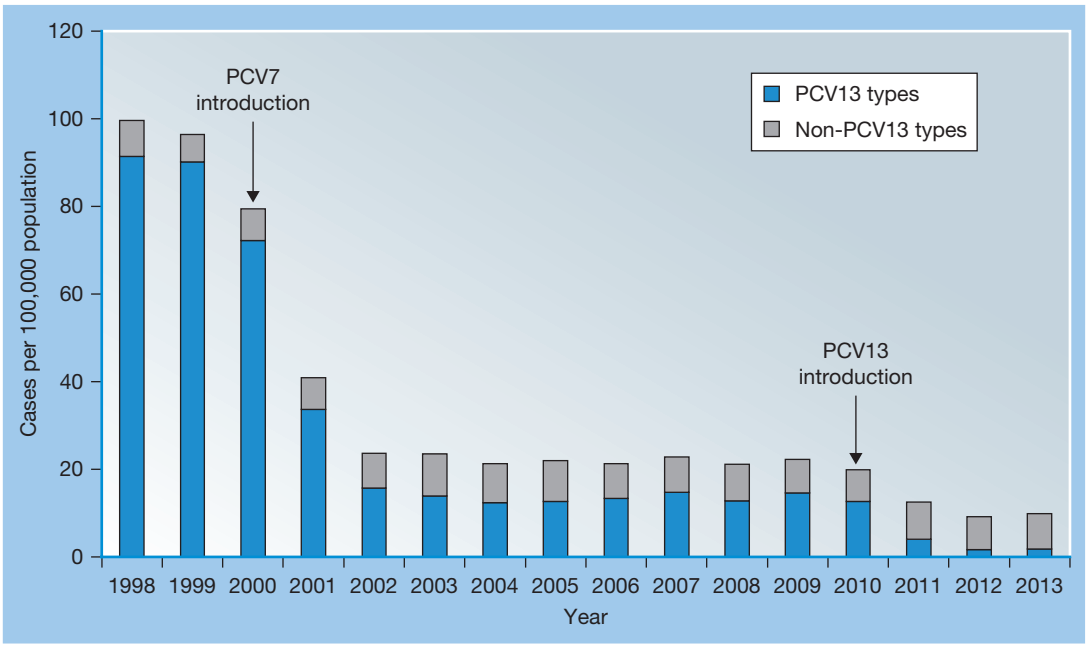


Figure 46.4. Rates of invasive pneumococcal disease among U.S. children <5 years, 1998–2013, showing PCV13 and non-PCV13 serotypes. *(From Moore MR, Whitney CG. Use of pneumococcal disease epidemiology to set policy and prevent disease during 20 years of the Emerging Infections Program.* Emerg Infect Dis. *2015;21[9]:1551–1556.)*

cases reducing only after protection was provided for the additional serotypes in PCV13.[534]

While there are increasing reports on PCV impact on IPD from many countries, the specific impact of PCV on pneumococcal meningitis has been less clearly described. The reduction of pneumococcal meningitis resulting from PCV7 led to group B streptococcus emerging as the major numerical cause of bacterial meningitis in children younger than 5 years of age in the United States.[535] In contrast in France, PCV7 had impact on meningitis in children younger than 2 years of age, but there was an increase in pneumococcal meningitis during the PCV7 period in children 2 to 5 years of age because of the additional PCV13 types 7F and 19A.[536] Early data on PCV13 impact on pneumococcal meningitis in the United States suggests that vaccine types and ceftriaxone resistance are decreasing after PCV13, but that nonvaccine types are increasing. These data suggest that nonvaccine types remain a risk for pneumococcal meningitis in susceptible patients.[536a]

Studies from multiple countries now also show that changes have occurred in the epidemiology of noninvasive pneumococcal syndromes, in particular pneumonia, after vaccine introduction. A study using the Nationwide Inpatient Sample, the largest available inpatient database in the United States, and an interrupted time-series analysis found large reductions in hospitalizations coded as all-cause and pneumococcal pneumonia among children when comparing 1997–1999 (pre-PCV7 years) to 2001–2004 (post-PCV7 years).[537] By the end of 2004, all-cause pneumonia hospitalization rates had declined 39% in children younger than 2 years of age, a drop of 506 hospitalizations per 100,000 children younger than 2 years of age, or approximately 41,000 pneumonia hospitalizations prevented per year. Pneumococcal pneumonia rates (2% of episodes) declined by 65% in children younger than 2 years of age, or approximately 17 fewer hospitalizations per 100,000 children per year. In Australia, hospital discharges with the primary diagnosis of pneumonia fell 38% (95% CI, 36% to 40%) and 29% (95% CI, 26% to 31%), respectively, among children younger than 2 years of age and 2 to 4 years of age during the 30 months after universal funding for seven-valent conjugate vaccine, representing approximately 4700 fewer hospitalizations for pneumonia over the 30-month period.[538] Likewise, hospitalizations fell 69.4% for lobar pneumonia and 8.2% for all-cause pneumonia in children younger than 2 years of age in the 15-month period after provision of seven-valent vaccine for all children younger than 5 years in Quebec Province in Canada.[505] Early data since the introduction of pneumococcal conjugates are showing benefits in pneumonia, including reports of reductions in pneumonia hospitalizations in Brazil following PCV10 introduction,[507] and in the United States following PCV13 introduction.[539]

Indirect Effects

There is increasing global evidence that herd effects are an important part of the public health benefit of PCV use. The first report demonstrating herd effects was from the NCKP population early after vaccine licensure in the United States. Data from this population indicated that the reduction in disease was greater than the percentage of children who had been vaccinated.[540] By early 2001, only 34% of children younger than age 5 years had received one or more doses of conjugate vaccine, and only 14% were fully vaccinated. Despite this low coverage, disease caused by vaccine serotypes had dropped to 18 cases per 100,000 children from a baseline incidence of 42 to 60 cases per 100,000 children in this age group. The indirect effect of infant vaccination on IPD among persons 5 years of age and older has been clearly demonstrated in the United States during the period 2000–13 during which the IPD burden was halved (Fig. 46.5). Analyses of CDC data through 2007 showed that invasive disease caused by vaccine

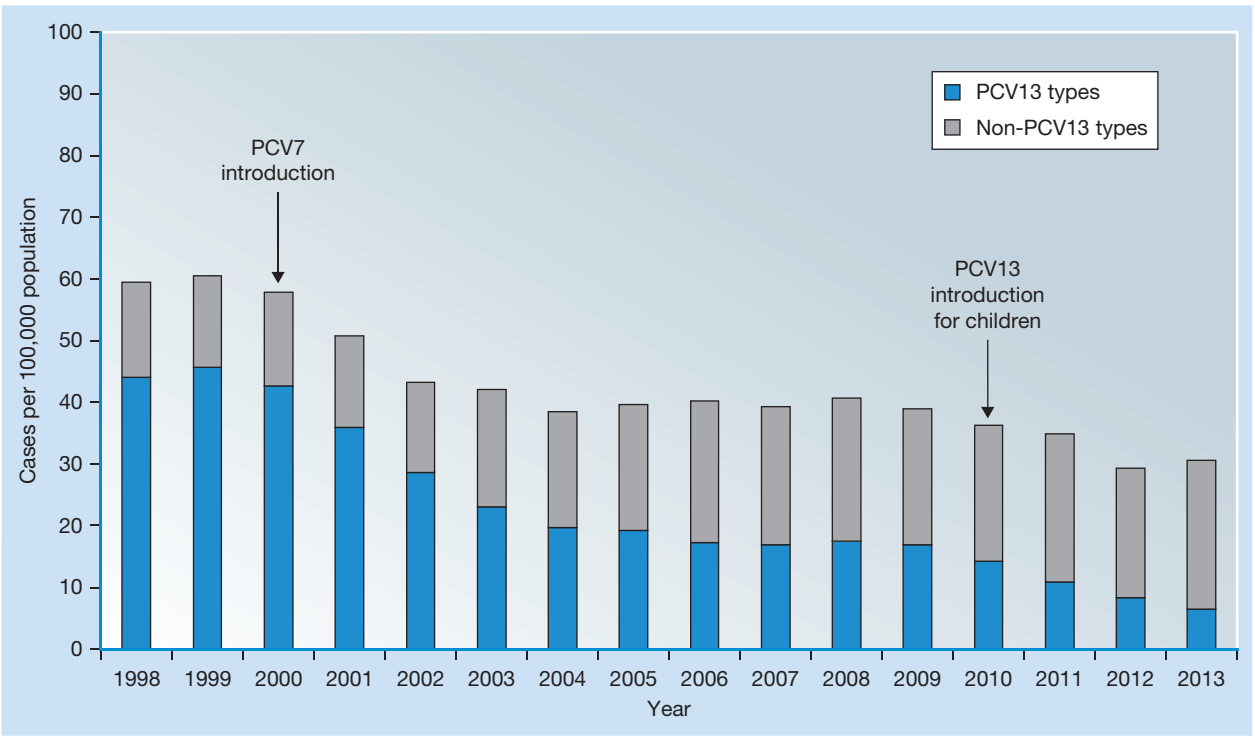


Figure 46.5. Rates of invasive pneumococcal disease among adults >18 years, Active Bacterial Core surveillance 1998–2013 showing PCV13 and non-PCV13 serotypes. *(From Moore MR, Whitney CG. Use of pneumococcal disease epidemiology to set policy and prevent disease during 20 years of the Emerging Infections Program.* Emerg Infect Dis. *2015;21[9]:1551–1556.)*

serotypes had dropped by 87% to 94% among age groups 5 years of age and older.[14] Between the 1998/1999 baseline years and 2007, the overall disease rates fell 43%, 40%, 18%, and 37% among persons ages 5 to 17 years, 18 to 49 years, 50 to 64 years, and 65 years and older, respectively.[14] An examination of adults in the conjugate vaccine era indicated that disease was seen more commonly in persons with comorbid conditions, indicating that the population of adults benefiting from herd effects were somewhat healthier than those who continued to get invasive disease.[541] Infants too young to be vaccinated also are protected through herd effects.[542]

A random effects metaanalysis revealed significant reductions in invasive disease caused by vaccine serotypes among adults 2 years after PCV7 vaccine introduction in children, but data have been mixed on whether there is an overall benefit for disease rates when disease caused by both VT and non-VT serotypes is included. Point estimates suggest reductions in overall IPD among adults from 2 years post PCV introduction in infants, but the CIs have included 1 to 6 years postintroduction.[543] While herd impact on PCV7 types has been thus reliably demonstrated, the extent of replacement disease in adults has varied geographically presumably reflecting disease among adults with comorbidities who remain at risk for disease with non-VTs. A lack of overall disease reduction in adults after conjugate vaccine introduction for children in some studies was the result of a low baseline percentage of invasive disease caused by conjugate vaccine serotypes, such as was seen among Alaska Native or Navajo adults,[486,527] and a large disease outbreak caused by a non-VT serotype in Canada.[544] In England and Wales where there was initial skepticism about an overall impact on IPD in all ages, as the baseline measure of IPD was increasing pre-PCV, modeling to adjust for case ascertainment revealed a 56% overall reduction in IPD post-PCV13 compared to the pre-PCV7 baseline (15.63 vs 6.85 per 100,000; incidence rate ratio [IRR] 0.44, 95% CI, 0.43–0.47).[532]

A study from the United States was the first to document significant herd effects for prevention of nonbacteremic pneumonia in older adults.[16] In this study, which used a very large administrative database, investigators found a reduction of 54% (95% CI, 53% to 56%) for nonbacteremic pneumonia hospitalizations among adults 65 years of age and older. A model included in this report estimated a total reduction of 788,838 (95% CI, 695,406 to 875,476) hospitalizations for pneumococcal pneumonia between 2000 and 2006, with 90% of the reduction in hospitalizations and mortality attributed to herd effects among adults 18 years of age and older. Reductions in all-cause pneumonia hospitalization among adults have been documented in Australia without a booster given to infants in the second year of life.[538] In the United States with a 3+1 immunization schedule, significant pneumonia hospitalization reductions have been confirmed a decade after PCV7 introduction, with an estimate of 168,000 fewer hospitalizations annually.[545] Further significant reductions in pneumonia hospitalizations encoded as pneumococcal have been demonstrated post-PCV13 in the United States.[546]

Effect on Resistance

There are several mechanisms by which widespread use of the new conjugate vaccine might affect antibiotic-resistant pneumococci. Serotypes included in the vaccine cause the majority of disease caused by resistant strains,[547] so reducing infections caused by these serotypes reduces resistant infections.[7] In addition, the vaccine reduces acquisition of vaccine serotypes in the nasopharynx,[465,470] reducing the opportunity for vaccine-serotype strains to be exposed to the selective pressures of antibiotics. As the vaccine reduces transmission of vaccine-serotype strains from vaccinated children to others, vaccinating a subset of the population reduces the overall number of infections caused by resistant vaccine-serotype strains.[548] Finally, by preventing infections, vaccination reduces the need for antibiotics mainly for respiratory tract infections,[412,415,423,549,550,550a] thereby reducing the selective pressure that antibiotic use provides. On the other hand, the acquisition of non-VTs in the nasopharynx exposes them to the selective pressure of antibiotics, and resistance emerges in the residual non-VTs. Overall, the burden of resistance is reduced, but may evolve with time to the same proportion of resistant strains or higher than previously encountered.[547]

Initial reports after PCV introduction in the United States demonstrated a reduction in the proportion of resistant strains in both children and adults.[126] In northern California, penicillin nonsusceptible strains with minimum inhibitory concentrations greater than or equal to 2 μg/mL were seen in 15% of pneumococcal isolates causing disease in all age groups in 2000, compared with 5% of strains in 2003; erythromycin resistance was prevalent in 28.9% of strains at baseline, compared with 15% after vaccine introduction.[4] Stephens and associates[548] evaluated trends in macrolide-resistant invasive disease in an ABC site in Atlanta, Georgia. Large decreases in the incidence of macrolide-resistant disease were seen in children younger than age 2 years (82%) and in children ages 2 to 4 years (71%) after vaccine introduction. The predominant clone causing macrolide-resistant disease in Atlanta had a *mefE* (macrolide efflux gene)-mediated mechanism for resistance and was serotype 14, a seven-valent PCV7 serotype. The decrease in macrolide-resistant pneumococcal disease was largely due to a reduction in disease caused by this clone.

Data from the ABCs Emerging Infections Program Network found that rates of IPD caused by penicillin-nonsusceptible strains dropped 78% in children younger than 5 years of age from a peak of 33.8 cases per 100,000 in 1999, before conjugate vaccine introduction, to 7.5 cases per 100,000 in 2004.[126] Rates of invasive disease caused by penicillin-nonsusceptible strains and strains not susceptible to multiple antibiotics among persons older than 65 years peaked in 1999 at 16.4 per 100,000 and then decreased by 49% to 8.4 cases per 100,000 in 2004; effects on resistance were most pronounced among children younger than 2 years of age (see Fig. 46.4). Although the incidence of nonsusceptible strains fell as VT pneumococci began to disappear from the community, the proportion of resistance among residual strains causing disease in children began to increase.[126]

In Alaska, rates of disease caused by penicillin-resistant, cotrimoxazole-resistant, erythromycin-resistant, and multidrug-resistant strains dropped in children younger than 5 years of age, consistent with the reduction in disease caused by vaccine-serotype strains.[486] Studies of carriage in young children in Alaska however, showed that although carriage of cotrimoxazole-resistant strains in children fell after vaccine introduction, carriage of penicillin-nonsusceptible strains was not significantly changed.[486,487]

In a multisite study, the proportion of pneumococcal strains from children with OM caused by vaccine serotypes dropped from 76% in 1999 to 52% in 2002, but no change was seen in the proportion of isolates that were resistant to penicillin.[435]

The reversal in the trend to a reduction in the proportion of resistant strains in the United States and elsewhere after PCV7 introduction was related to the emergence of a small number of antibiotic-resistant nonvaccine serotypes. In the United States the most important was serotype 19A, the burden of which increased from 0.8 cases per 100,000 per year from 1998 to 2.5 cases per 100,000 per year in 2003.[120]

Serotype 19A seen in the United States was part of a global clone that had undergone a capsular switch from 19F to 19A, acquired the *ermB* gene in addition to *mefA*, conferring high-level macrolide resistance, and high-level resistance to β-lactams, including injectable third-generation cephalosporins such as ceftriaxone.[120] The only oral agents that could be used to treat OM caused by these resistant strains were fluoroquinolones.[433] Multiresistant serotype 19A post-PCV7 introduction was also a problem in other parts of the world, such as France and Spain.[552–554] Other serotypes not included in the PCV7 vaccine also emerged as antibiotic-resistant in the United States, including serotype 6C[555] as well as serotypes 15A, 23A, and 35B.[556] Although the emergence of antibiotic-resistant pneumococci has occurred over the past half century independent of PCV selection, and will continue to do so, the changes in serotypes carried in the nasopharynx of PCV-immunized children contributed to the selection of resistance in these serotypes. The introduction of PCV13 in 2010 had a major impact in reducing this trend. In a double-blinded, randomized study comparing efficacy of PCV13 and PCV7 on carriage of antibiotic-nonsusceptible *S. pneumoniae*, Dagan and colleagues described the superiority of PCV13 over PCV7 against acquisition and carriage of serotypes 6A, 19F, and 19A, all three with high rates of antibiotic resistance. PCV13 administration resulted in significantly lower carriage than PCV7 during infancy and toddlerhood of penicillin, macrolide, clindamycin, tetracycline, and multidrug nonsusceptible *S. pneumoniae*.[557] This predicted reduction in diseases caused by antibiotic-nonsusceptible *S. pneumoniae*. Furthermore, indirect protection in all ages regardless of vaccination status was also predicted. Indeed, reduction of disease caused by antibiotic-resistant *S. pneumoniae* of the additional PCV13 serotypes was observed as early as within 3 years postintroduction of PCV13, in the United States, France, South Africa, and Israel.[15,17,439,558,559] A trend of increasing antibiotic-susceptibility with each serotype, including both PCV13 and non-PCV13 serotypes, was observed.[559,560]

Serotype Replacement

At best, conjugate vaccines as currently designed can only protect against a limited number of the 97 known pneumococcal serotypes. Carriage studies have shown that PCV vaccination has reduced carriage of VT pneumococci among children in the community.[485–487,561] The concomitant increase in carriage among children of pneumococcal serotypes not covered by the vaccine means that nonvaccine serotypes may be transmitted and potentially cause disease more often than they did before routine use of conjugate vaccine. An increase in disease caused by nonvaccine serotypes in the wake of a reduction in VT disease is known as "replacement disease." To what extent replacement disease occurs depends in part on the ability of the nonvaccine serotypes to cause disease; studies of invasiveness that have analyzed distributions of serotypes associated with carriage and with invasive disease have shown that certain serotypes are much more likely to cause invasive disease than others.[562]

The first evidence for replacement disease came from a clinical trial in Finland evaluating two conjugate vaccines for prevention of AOM among children younger than 2 years. The first analysis evaluated the seven-valent PCV7; compared with a control group receiving hepatitis B vaccine, children receiving PCV had 57% fewer OM episodes caused by serotypes in the vaccine and 51% fewer episodes caused by cross-reactive serotypes, but these changes were accompanied by a 33% increase in episodes caused by other pneumococcal serotypes and an 11% increase in those caused by *H. influenzae*.[13] In the analysis of seven-valent PncOMPC, the efficacy against OM caused by vaccine serotypes was similar (56%) to that of PCV7 (57%).[195] More episodes of OM caused by non–vaccine-serotype pneumococci and *H. influenzae* occurred among children receiving pneumococcal vaccine (121 and 315 episodes, respectively) than receiving hepatitis B vaccine (95 and 287 episodes, respectively), but the differences were not statistically significant between the two groups. A shift in pneumococci causing OM was also seen in Pennsylvania, where in 2001 children receiving at least two doses of PCV were noted to be more likely to have AOM caused by non-VTs (47%) than children who had not received conjugate vaccine (21%).[537]

The first report of significant replacement invasive disease among children came from an eight-center study monitoring hospitalizations for invasive disease among children younger than 2 years in the United States.[523] While noting a 77% decrease in VT disease between baseline (1994–2000) and 2002, they also found a 66% increase in non–vaccine-serotype disease. Isolates of nonvaccine serogroups 15 and 33 had the largest increases. In Utah, an increase in cases caused by serogroup 3 was noted among children enrolled in a large healthcare system, along with an increase in the incidence of empyema and severe invasive disease from 2001 to 2003 compared with prevaccine years.[105]

Data from the CDC's ABCs program have shown that rates of invasive disease caused by non-VTs in children younger than 5 years increased 128% between the 1998–99 average and 2007.[14] In this large sample, the main serotype causing replacement disease in children was serotype 19A, a serotype structurally similar to vaccine serotype 19F but for which the vaccine provides no cross-protection.[563] An analysis comparing pneumococcal genotypes before and after conjugate vaccine introduction noted that the increase in serotype 19A was a result of the serotype 19A clone most prevalent in the United States becoming more common as a cause of disease and also to the emergence of new clones with capsular serotype 19A that were previously identified as having only vaccine serotypes.[563] This analysis suggests that pneumococci may have adapted to vaccine use by acquiring genetic material that allowed a switch in expressed capsular type.

Surveillance data from Alaska Native children indicate that this group is particularly prone to replacement disease. An increase in non-VT disease among Alaska Native children younger than 2 years of age began as early as 2003, 2 years after universal introduction of PCV7. Between 1995 and 2000 and 2004 to 2006, among children younger than 2 years of age, rates of invasive disease caused by nonvaccine serotypes increased from 95 to 229 cases per 100,000, substantially reducing the overall benefit of PCV7.[564] In contrast, replacement disease has not been as evident among non–Native Alaskans, suggesting that environmental, socioeconomic, or genetic factors may explain some of the differences seen in the two groups. Replacement disease has not been problematic among other indigenous populations, such as Australian Aboriginals[565] or Navajo residing in the American Southwest.[527] Although disease rates among Alaska Native children are among the highest reported, the overall size of the population is small, such that rate calculations are based on relatively few cases; therefore, more data are needed to better understand the trends in this population.

Evidence suggests that nonvaccine serotypes are more likely to cause disease in persons with comorbidities including immune system disorders. Children with comorbidities were more likely to be infected with non-VTs post-PCV13 in Massachusetts and these children with comorbidities were more likely to be hospitalized.[566] Some of the most dramatic increases in nonvaccine serotypes documented after conjugate vaccine introduction have been among adults 18 to 64 years

of age with HIV or AIDS.[567] In this very-high-risk population, overall rates of IPD (all serotypes) declined 25% between 1998–99 and 2007. The overall decline was the result of an 88% reduction in disease caused by the seven serotypes in the vaccine and a 60% increase in disease caused by nonvaccine serotypes. A comparison group of adults 18 to 64 years of age without HIV or AIDS noted a very similar percent reduction in vaccine-serotype disease (91%), but this group had a smaller (28%) increase in non-VT disease.

In July 2010, the WHO convened a meeting of experts to discuss the issue of replacement disease and to review data on invasive disease trends from several countries that had introduced conjugate vaccine.[568] The latest data were reviewed from Australia, Canada, England and Wales, South Africa, and the United States and included information from both indigenous and nonindigenous populations. The data were collected through systems that varied in size, methods, background incidence rates, the prevaccination proportion of invasive disease caused by vaccine serotypes, vaccination schedules, and the use of catch-up programs for older children. In each setting, children benefited overall from vaccine introduction; the overall incidence of IPD among children targeted for vaccination declined after vaccine introduction. The overall reduction in each case was the result of large declines in vaccine-serotype disease and, in some but not all datasets, smaller increases in disease caused by serotypes not covered by conjugate vaccine. The increases in non–vaccine-serotype disease were seen mostly in hospitalized patients. Among older children and adults, an overall indirect benefit was not seen in all settings. In some sites, overall rates were lower after conjugate vaccine introduction; all sites saw reductions in vaccine-serotype invasive disease, but increases of variable size in non–vaccine-serotype disease resulted in either no change or an increase (one site) in the overall rate of IPD in others. After reviewing the data, participants concluded that vaccine-induced serotype replacement likely explains some of the observed increases in non–PCV-serotype disease but that other factors, such as population immunity, secular trends in disease, and changes in surveillance system sensitivity, also contributed to the pattern observed. The group concluded that they could not discern the relative contribution of vaccine-induced replacement disease versus observed trends in non–vaccine-serotype disease related to these other factors and that more data are needed as the newer conjugate vaccines were implemented. A follow-up analysis using 21 datasets, largely from high-income settings, found that replacement disease occurred but that overall benefits in young children were seen quickly after PCV7 introduction and remained up to 7 years after vaccine introduction; benefits were also seen among adults but were less consistent and delayed relative to benefits among children.[543] The first long-term observation post-PCV13 from England and Wales, which has documented an overall reduction in IPD in all ages, has identified replacement of non-PCV13 serotypes across many age groups, but the early impact is in children younger than 5 years of age as reductions in PCV13 serotypes were largely complete by 2015, thus emphasizing continuing replacement in that age group. The significant increases in any age group were serotypes 8, 10A, 12F, 15A, and 24F.[532]

Disease Control Strategies

Pneumococcal disease is endemic globally. Nearly all young children will have one or more mild infections caused by *S. pneumoniae.* Severe infections most often occur sporadically rather than in outbreaks clustered together by place and time. Therefore, including PCV in the routine childhood vaccination schedule is likely the optimal strategy for controlling pneumococcal disease.

In 2000, the first year seven-valent PCV7 was licensed, the United States introduced the vaccine for all children younger than 2 years.[569] Beginning in 2006, with expansion of supply, a large number of countries began introducing seven-valent PCV7. With the licensure of PCV10 and PCV13, beginning in late 2009, national programs using seven-valent PCV7 began switching to PCV10 or PCV13 as they became available, and additional national programs began adopting the newer vaccines. As of September 2014, 110 countries have included PCV in their routine infant immunization programs (Fig. 46.6).[570] As of December 2014, 44 low-income countries had introduced PCV10 or PCV13, using funding from a unique donor program called the Advance Market Commitment.[571]

Outbreaks of severe pneumococcal disease, including bacteremia, sepsis, and pneumonia, can occur. Most outbreaks occur in closed settings where people are in prolonged close contact, such as jails, long-term care facilities, childcare centers, or homeless shelters.[572] In these settings, control measures include improved infection control coupled with vaccination; conjugate vaccine or 23-valent polysaccharide vaccine might be appropriate, depending on the serotype and ages of persons involved. Use of antibiotic prophylaxis might be helpful, depending on the susceptibility profile of the strain causing the outbreak and whether the nature and timing of cases suggests that the at-risk population requires protection while vaccines are taking effect. Care must be taken with use of antibiotic prophylaxis, however, because widespread antibiotic use can lead to circulation of resistant strains.[551] In some outbreaks, the presence of a viral cofactor may suggest that other prevention measures might be helpful, such as use of influenza vaccine.

Pneumococcal Conjugate Vaccine Immunization of Adults

Adult PCV13 vaccination has been recommended for adults with underlying immunodeficiency and most recently for all adults 65 years of age or older by the ACIP in the United States.[573] There has been significant debate about the advantages of conjugate vaccine use in adults in terms of immunogenicity and potential effectiveness compared with PPV vaccination.[286] A large-scale, randomized, double-blind trial, CAPITA (Community-acquired Pneumonia Immunization Trial in Adults), has provided data on the efficacy of PCV in adults.[18] This trial was performed in The Netherlands and 84,496 adults, 65 years of age or older were randomized to receive a single intramuscular dose of the same PCV13 vaccine formulation given to infants or placebo. Patients with evidence of being immunocompromised were excluded, and the per-protocol analysis excluded patients who developed an immunocompromising illness during the trial. VT disease was assessed by the culture of strains from sterile sites, or the detection of serotype-specific antigen in urine.[574] X-rays confirmed that community-acquired pneumonia caused by VTs occurred among 49 vaccinees and 90 placebo recipients for a vaccine efficacy of 45.6% (95% CI, 22% to 63%). Efficacy against IPD was 75% (7 vs 28 cases; 95% CI, 41% to 91%). Cumulative efficacy against VT community-acquired pneumonia, nonbacteremic and noninvasive community-acquired pneumonia, as well as VT IPD are shown in Fig. 46.7. Overall impact on x-ray confirmed pneumonia in a modified ITT analysis was modest and not significantly different in PCV13 vaccine recipients and controls (5.1%; 95% CI, −5% to 14%). The immunization of infants with PCV7 followed by PCV10 in The Netherlands may have reduced the contribution of VTs to x-ray–confirmed pneumonia in the adults, which is consistent with the low overall impact on pneumonia. There was no impact on mortality. In supplementary analyses, there was no impact on the

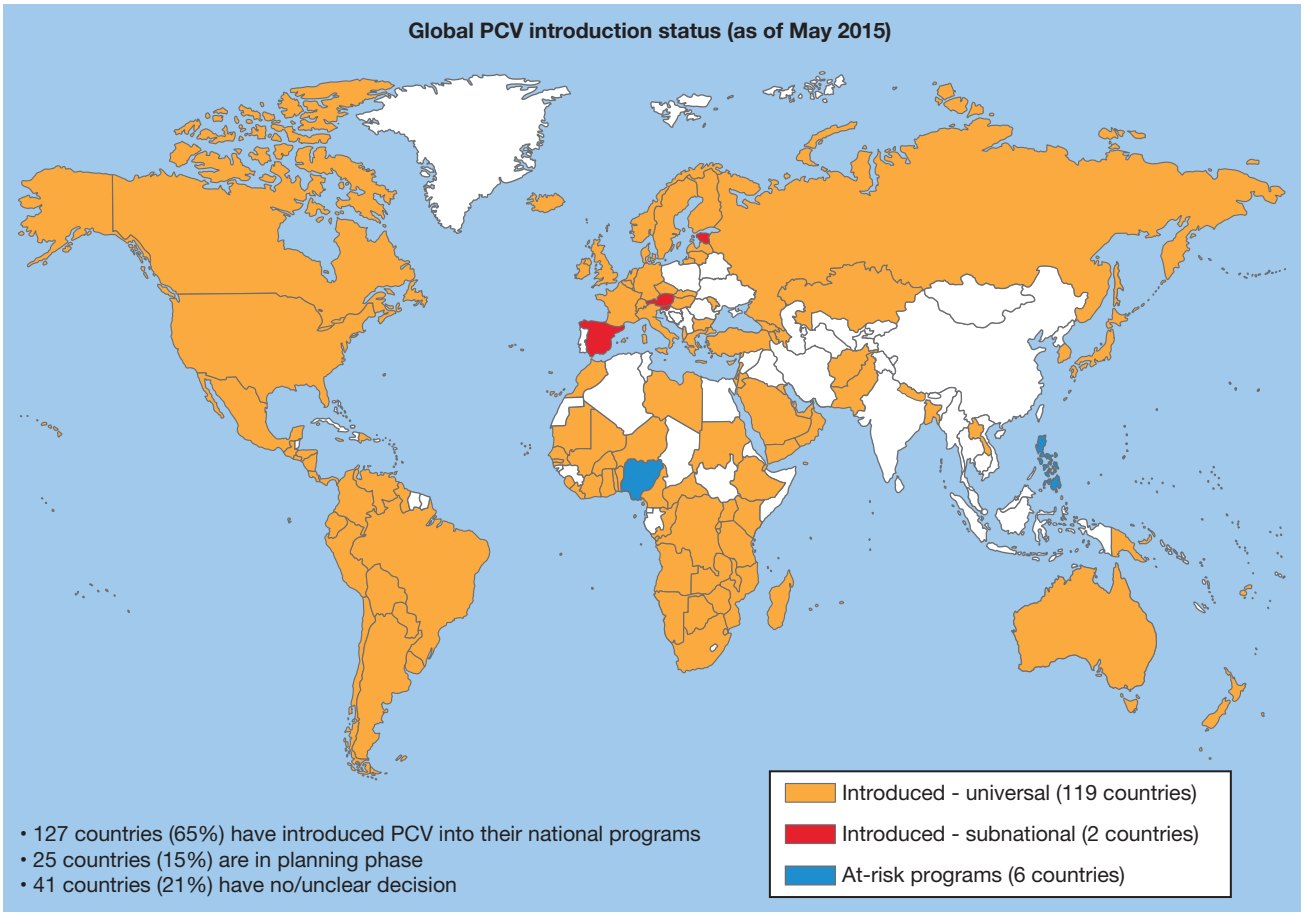


Figure 46.6. Countries that have introduced pneumococcal conjugate vaccines (PCVs) in their national immunization programs—worldwide, 2015. As of May 2015, 127 countries (65%) have introduced PCV into their national programs, 25 countries (15%) are in planning phase, and 41 countries (21%) have no/unclear decision. *(From International Vaccine Access Center [IVAC, Johns Hopkins Bloomberg School of Public Health. Vaccine Information Management System [VIMS] Global Vaccine Introduction Report, May 2015.)*

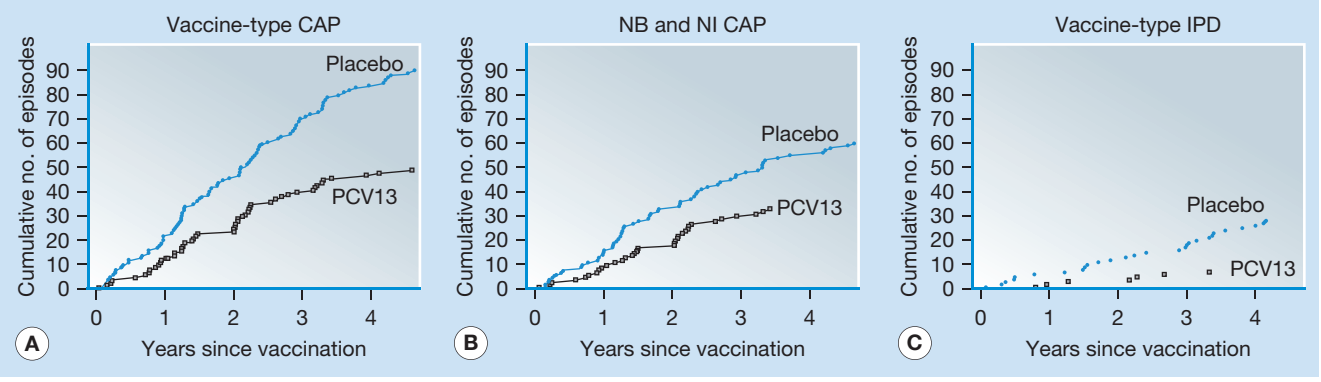


Figure 46.7. Post hoc analysis of the cumulative episodes of the primary and secondary efficacy end points in the per-protocol population. CAP, community-acquired pneumonia; IPD, invasive pneumococcal disease; NB, nonbacteremic; NI, noninvasive. *(Modified from Bonten MJ, Huijts SM, Bolkenbaas M, et al. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. N Engl J Med. 2015;372[12]:1114–1125.)*

primary end point of VT community-acquired pneumonia in those older than age 85 years (six vaccinees vs three controls); nor in those who developed immunodeficiency during the trial (14 vaccinees vs 11 controls). In terms of serotype-specific protection, most efficacy was seen against serotypes 3, 7F, and 19A (of the overall difference of 51 cases between vaccinees and controls, 36 were to these three serotypes). As mentioned in the "Safety" section above, there were more local reactions, but no excess of severe adverse events in the vaccine group. The data suggest that PCV prevents nonbacteremic pneumonia in otherwise healthy 65- to 85-year-olds, but that the efficacy against all-cause community-acquired pneumonia is limited

when children are receiving PCV in the same community. The magnitude of the impact on VT IPD was similar (75%) to that seen in infants given PCV, but the data suggest that PCV impact on VT nonbacteremic pneumonia is less than that against IPD (45% vs 75% in this study).

There are data supporting the efficacy of PCV in the prevention of IPD in HIV-infected adults who have already experienced a previous episode of IPD.[575] Because the PCV7 serotypes have almost disappeared as a cause of IPD in adults in the United States after PCV immunization of adults, the impact of PCV13 immunization in infants on the preventable disease burden of PCV13 immunization in adults in the United States may be time limited. The recommendation for use in adults was therefore time-limited with a review expected in 2018 once the residual burden of vaccine-preventable disease is clarified. The impact of PCV13 on herd protection of the additional six serotypes requires further study, although an early report showed decreasing rates among adults of serotypes 19A and 7F, the two most common serotypes, after PCV7 introduction in the United States.[15] Little is currently known about the risks of imported carriage of PCV13 serotypes on disease among susceptible adults in the United States or other countries where these types are close to elimination. The risks of travel by susceptible adults to countries where these serotypes remain common also have not been evaluated. These factors need to be considered both in the licensure decisions around PCV13 vaccines for adults and in their potential indications for use.

Cost-to-Benefit Information

The cost of PCV was considered quite high when it was initially introduced, although since then other vaccines of higher cost, such as human papillomavirus vaccine, have been introduced. In the developing world, the unit cost remains high compared with EPI vaccines, and only subsidies through the GAVI and other programs have allowed its use in this setting.

Vaccine cost-effectiveness is dependent on many factors, including disease incidence, vaccine efficacy, herd effects, disease sequelae, number of doses, and cost per dose of the vaccine. Any of these factors may change over time as prices change, as more is learned about vaccine efficacy or herd effects, or when standards of medical care improve. Consequently, any determination of cost-effectiveness is limited by the time-specific and place-specific variables that were used for the analysis. In addition, there are significant nonmedical costs associated with pneumococcal disease, most notably the cost of work-time lost by parents caring for ill children.[576]

In the year 2000, Lieu and associates[576] were the first to analyze the potential cost-effectiveness of the seven-valent PCV using data from the NCKP randomized trial as well as the opinions of a panel of experts.[576] The analysis was based on a comparison of "vaccination" with "no vaccination," assuming the use of four doses for routine vaccination of healthy infants and one dose for catch-up vaccination of children older than age 2 years. In addition, the conservative assumption was made that a vaccinated infant would experience reductions in pneumococcal diseases only until the child's fifth birthday and that vaccination of children would not protect unvaccinated persons (no herd effects). From the societal perspective, vaccination of healthy infants would result in savings if the vaccine cost $46 or less per dose. From the healthcare payer perspective, net savings would occur if the vaccine cost $18 or less per dose.

An updated analysis looked at cost-effectiveness in the United States for the first 5 years of routine use and showed that the actual cost-effectiveness has been better than predicted by this earlier estimate because of the "herd effect" and consequent reduction in disease among unvaccinated children and adults.[577] Without incorporating herd effects in this more recent model, conjugate vaccine was estimated to have averted 38,000 cases of invasive disease during its first 5 years of use, at a cost of US$112,000 per life-year saved. After incorporating the reductions in invasive disease for unvaccinated persons, the vaccine prevented 107,000 cases of invasive disease at a cost of US$7800 per life-year saved. Similar analyses have been performed for other countries, which have demonstrated similar results.[578,579] An analysis of PCV cost-effectiveness in developing countries suggests the vaccine would greatly reduce mortality and be highly cost-effective at between US$1 and US$5 dollars per dose.[580]

A recent analysis was conducted of the impact of the PCV10 manufactured by GlaxoSmithKline as compared with PCV7.[581,582] This simulation model predicted, as expected for a higher-valency vaccine, that there would be a higher impact of PCV10 on invasive disease than has been observed for PCV7. For OM, the contribution of protection against nontypeable *H. influenzae* increased the impact of this vaccine against OM significantly. Since nontypeable *H. influenzae* is a major cause of OM in the developed world, this has been the most important cost-saving determinant of the PCV10 vaccine in this setting. In another study comparing the impact of PCV13 against both PCV10 and PCV7, PCV13 was predicted to be most cost-effective.[583] This study did not take into account any impact of the 10-valent PHiD-CV vaccine against OM caused by nontypeable *H. influenzae*. In reality, whether the 13-valent or 10-valent vaccine is more cost-effective will depend on the relative importance of the additional serotype coverage provided by the 13-valent vaccine versus the impact of PHiD-CV against nontypeable *H. influenzae*. At this point, it is too soon after the introduction of these two vaccines to reliably make this assessment.

Efforts are underway to find financing to cover costs of introducing new vaccines, including PCV, in developing countries. In an analysis that estimated the funding needs for childhood immunization in developing countries during the 2004 to 2014 period, the total costs of providing immunization for the poorest countries would be approximately US$14 to US$17 billion, accounting for phasing in of new vaccines. Vaccine-related costs represented the largest share of this amount (US$7.1 to US$9.3 billion), with US$4.3 to US$6.5 billion for a set of new vaccines, including PCV. These estimates were for all 75 countries eligible for support from GAVI and covered existing vaccines that were considered priorities (diphtheria/tetanus/pertussis, hepatitis B, Hib, and yellow fever) as well as future vaccines likely to be available during 2004 to 2014 (meningococcal A and C, rotavirus, human papillomavirus, malaria, *S. pneumoniae*, and tuberculosis).[584] The sustainability of these programs will depend both upon the ability of developing countries to assume responsibility for paying for PCV and the ability of manufacturers to supply vaccine to these countries at an affordable price.

FUTURE VACCINES

Problems With Conjugates

Despite the good safety record of PCVs and their demonstrated high efficacy against invasive infections, several limitations remain. One of the issues is that conjugate vaccines are only capable of protecting against infection with bacteria that express polysaccharide capsule types that are included in the vaccine. Current formulations in use or in clinical development cover up to 75% to 90% of strains prevalent

in children.[38,40,99,106,112] However, this figure is lower in certain geographic areas and in older age groups. Another issue is the potential for replacement disease with nonvaccine serotypes to reduce the overall benefit seen from reduction in disease caused by vaccine serotypes.[585] The third major issue is the complexity of conjugate vaccines production, the major reason only a limited number of companies are manufacturing conjugate vaccines and the higher price than for other vaccines.

To improve and expand the immunogenicity of the conjugate vaccines, new components in the conjugate and novel adjuvants have been tested in animal studies.[32,569,586,587] Innovative administration routes are being evaluated. Intranasal immunization with various adjuvants induces strong antibody responses both in serum and on mucosal surfaces in mice, protecting them against lethal infections.[214,586,587] Even though these strategies may circumvent some of the problems of conjugates, they do not address the issues of coverage, replacement, or complexity of vaccine production. Therefore, other types of immunogens, including whole-cell pneumococcal bacteria, DNA vaccines, and protein antigens, are being evaluated as novel pneumococcal vaccine candidates, both in animal models and in humans. One killed whole-cell–based vaccine has proven effective in a mouse model,[569] successfully completed a Phase I clinical trial in healthy U.S. adult volunteers, and is undergoing a Phase II trial in Kenyan adults and toddlers. Attempts to develop pneumococcal DNA vaccines are still limited by the generic problems of immunogenicity associated with naked DNA in humans. The extensive work performed on protein vaccine candidates will be discussed in more detail below.

Protein Antigens

Protein-based vaccines are attractive for several reasons. They are expected to be immunogenic in early infancy and in the elderly because of their T-cell–dependent nature and, if the proteins are well conserved across all pneumococcal strains, the afforded coverage theoretically could be broader than that of conjugate vaccines. Enhanced protection against colonization and avoidance of the replacement phenomenon are also potential advantages. In addition, the cost of goods would likely be lower than that of conjugates because they would have fewer components than multivalent conjugate vaccines, allowing wider global use.

The list of pneumococcal proteins that have been evaluated for their potential as vaccine candidates is extensive. Although exceptions can be found, these candidates tend to have several characteristics in common: significant contributions to bacterial virulence, conservation across all or most sequenced pneumococcal strains, and little homology to human proteins. In general, the candidate antigens have been promising when used as immunogens in a rodent models of invasive disease, colonization, or both. These proteins have been studied alone, in combination with other candidates, such as carrier proteins for pneumococcal conjugates. Some examples are provided below and the field has been reviewed.[588]

Pneumococcal surface protein A (PspA) is a surface protein with variable molecular size, ranging from 67 to 99 kDa in different pneumococcal strains. It is present on the surface of pneumococci and is required for full virulence in animal models.[589,590] It is serologically highly variable, and the structure of the *pspA* gene is mosaic.[591] The high degree of variability exhibited by PspA may indicate the importance of this surface protein as a natural target for host defense. Based on the relatedness of the nucleotide and amino acid sequences, the different PspA molecules are grouped into three families, which are further subdivided into clades.[591] More than 98% of the PspA molecules typed to date are members of families 1 and 2.

Because of the variability of the PspA protein, a potential PspA-based vaccine would likely need to contain PspAs of more than one pneumococcal strain or be based on conserved regions of the molecule to be able to protect against all pneumococci.[592]

The mechanism of action of PspA has been carefully investigated. The aminoterminal region of PspA functions as a specific receptor for lactoferrin. It has been suggested that by binding lactoferrin, pneumococci are able to interfere with the host's immune functions.[593] PspA inhibits the activation of complement by *S. pneumoniae* facilitating pneumococcal survival and host invasion.

PspA is immunogenic and has been shown to elicit protective immunity in mice[594–598]; after intranasal immunization mice are protected against pneumococcal carriage and subsequent invasive disease.[599] Animal models show that PspA has the ability to elicit cross-protection against heterologous strains.[590,596,598] In addition, human antisera from a Phase I vaccine trial protected mice against challenge with pneumococcal strains of various PspA types.[600] However, further development of a PspA vaccine was halted because of concerns about areas of low-sequence homology with human cardiac myosin with the potential to generate cardiac disease or autoimmunity.[601] *Pneumococcal surface protein C (PspC)* plays an important role in pneumococcal pathogenesis by functioning as an adhesin.[602] It also binds soluble host factors, such as the secretory component, the third component of complement, and complement factor H.[603–606] The gene for this protein is present in approximately 75% of *S. pneumoniae* strains.

PspC is one of a dozen pneumococcal choline-binding proteins (CBPs) that are attached to the bacterial surface by docking noncovalently with the phosphorylcholine of the cell wall. These CBPs also include PspA, CbpA (*C*3-*b*inding *p*rotein *A*), SpsA (*S. pneumoniae s*ecretory Ig*A* binding *p*rotein), Hic (factor *H*-binding *i*nhibitor of *c*omplement), and LytA (major pneumococcal autolysin).[602,603,607,608] Sequence analysis has shown that PspC, CbpA, Hic, PbcA, and SpsA are variants of the same CBP.[609]

When used as an immunogen in a mouse model,[610] PspC, protects against challenge with pneumococcal strains expressing both PspA and PspC, as well as those expressing only PspA, suggesting cross-protection.[609]

Pneumococcal choline-binding protein A (PcpA) is a bacterial surface antigen thought to play a role in pneumococcal adherence to pulmonary epithelial cells.[611] Immunization with PcpA is protective in murine models of sepsis and pneumonia. and it is being considered as a component of a three-valent pneumococcal protein-based vaccine, as discussed below.

Pneumococcal surface adhesin A (PsaA) is a 37-kDa surface lipoprotein that contributes to pneumococcal virulence. Initially, PsaA was thought to be a pneumococcal adhesin, but genomic sequence comparison[612] and studies on the crystal structure of PsaA[613] revealed that it is a component of an adenosine triphosphate–binding cassette-type (ABC-type) permease membrane transport system, and responsible for the uptake of Mn^{2+} (and possibly Zn^{2+}) into the bacterium.[614] Many genes for such metal transporters exist in the pneumococcal genome. Some of these proteins, such as Adc and PsaA, are particularly important for pneumococcal virulence and may be attractive candidates for further evaluation.[614]

The loss of host adhesion observed in $PsaA^-$ mutants is thus probably indirect evidence that PsaA appears to have a regulatory role in adhesion by affecting the expression of CBPs on the surface of pneumococci; mutants lacking the *psa* operon

demonstrate a complete absence of PspC/CbpA.[615] It has been suggested that PsaA might play an important role in regulating the expression of oxidative stress response enzymes and intracellular redox homeostasis.[616]

In animal models, PsaA is immunogenic and protective against IPD.[617–619] It is highly effective against nasopharyngeal carriage in mice when administered intranasally; however, as with other proteins, combinations of antigens containing PsaA are far more effective than when PsaA is used alone.[620]

Pneumolysin (Ply) is a 53-kDa intracellular toxin produced by all clinical pneumococcal isolates. This toxin has several properties that may contribute to virulence: Ply inhibits neutrophil bactericidal activity by reducing chemotaxis, phagocytosis, and the respiratory burst.[621] It also inhibits the lymphocyte proliferation response to mitogens, the synthesis of all Ig classes,[622] and ciliary function in human respiratory tract.[623,624] Ply induces separation of the tight junctions of alveolar epithelial cells.[625] Ply also activates the classic complement pathway, which leads to complement consumption, allowing pneumococci to evade opsonophagocytosis, the essential mechanism for clearance of pneumococci from the lung.[626] Ply also induces Toll-like receptor 4–dependent inflammatory responses and apoptosis in eukaryotic cells.[627] What role each of these properties plays in pathogenesis is unclear; a serotype 1 strain causing invasive disease in Scotland was found to express a nonhemolytic Ply.[628]

Mutants deficient in Ply are less virulent than parental wild-type strains in some animal models.[629] Immunization with Ply prolongs the survival of mice after challenge with pneumococci of different serotypes.[630–632] Mice immunized with combinations of pneumococcal virulence proteins including Ply have significantly longer survival times than mice immunized with any of the antigens alone.[619] Conjugation of pneumolysoid toxoids or recombinant Ply with pneumococcal capsular polysaccharides may confer broader protective immunity than conjugates using nonpneumococcal proteins.[131,211,212,633,634] Constructs of pneumolysoid–pneumococcal protein fusions conjugated to polysaccharides confer enhanced protection against pneumococcal disease and colonization.[635] As discussed below, vaccines containing detoxified Ply are currently undergoing clinical trials.[636–638]

Neuraminidase enzymes (NanA and *NanB)* cleave sialic acid residues from host glycolipids and gangliosides, causing damage to host tissues. This action may reveal new receptors for pneumococcal adhesins, facilitating both adhesion and invasion.[639] NanA is a cell-surface–associated enzyme with sialidase activity produced by all clinical isolates of pneumococcus.[640,641] NanA has the potential to cause great damage to the host by cleaving the terminal sialic acid residues from a wide variety of glycoproteins, glycolipids, and oligosaccharides present on host cell surfaces.[642] It has been suggested that NanA plays a role in pneumococcal carriage and AOM. NanA may enhance pneumococcal colonization by decreasing the viscosity of mucus.[642] or by exposing new host cell receptors for pneumococcal adherence.[643–645] NanA does promote brain endothelial cell invasion.[646,647] Immunization of mice with purified NanA confers some protection against challenge with virulent pneumococci.[648] In a chinchilla model, immunization with NanA resulted in significant reduction in nasopharyngeal colonization and in OM with effusion.[649]

A more recently described group of pneumococcal vaccine candidates, the *pneumococcal histidine-triad proteins,* contain similar surface-exposed proteins capable of protecting mice against fatal pneumococcal infection. This protein family has various names based on the conserved histidine triad motif (HxxHxH) repeated several times in the proteins. The proteins have been named the Php family (for pneumococcal histidine protein; PhpA, PhpB, and PhpC),[650] the Pht family (for pneumococcal histidine triad; PhtA, PhtB, PhtD, and PhtE),[651] and BVH3 and BVH11.[652]

These proteins are immunogenic and protective against pneumococcal challenge in animal models. The immunization of mice with PhtA (BVH11–3), PhtB/PhpA (BVH11), or PhtD (BVH11–2) induced protection against IPD.[651] Immunization of mice with recombinant PhpA protein significantly reduced nasopharyngeal colonization and protected mice against bacteremia and death after intranasal challenge with pneumococci.[650] Another study reported that immunization of mice with the recombinant proteins BVH3 and BVH11 elicited protection against both experimental pneumococcal sepsis and pneumococcal pneumonia.[653] More recently it was shown that natural anti-PhtD human antibodies transferred to mice protected against lethal intranasal challenge.[654] As discussed below, vaccines containing PhtD have been studied in humans, with favorable safety profiles in adults and toddlers.[636–638]

The discovery of a mobile genetic element encoding a *pilus-like* structure in pneumococcus and the demonstration of a role for the pilus in virulence in mouse models have led to the consideration of pilus antigens as vaccine candidates.[655,656] In *S. pneumoniae*, pili are encoded by two genomic islets (pilus islet 1 and pilus islet 2), but these are present in only a subgroup of pneumococcal isolates. Mutants of pilus islet 1 demonstrate reduced virulence in different models. The pilus appears to be an inflammatory structure, but it remains to be determined whether the resulting inflammation promotes or reduces the ability of the bacterium to colonize or invade.[656]

Pilus islet 1 was found to be associated with certain serotypes and correlates very tightly with genotype.[657] After the introduction of the conjugate vaccine in the United States, the prevalence of pilus islet 1 in clinical isolates dropped dramatically,[658] but rebounded to pre-PCV7 prevalence levels in Massachusetts within 7 years.[659]

Mice immunized with the three structural proteins that constitute pilus islet 1 (encoded by *rrgA, rrgB,* and *rrgC*) were protected against invasive disease resulting from piliated strains.[660] The low prevalence of piliated pneumococci may limit the usefulness of these antigens, but it has been proposed that they may be used in combination with more conserved pneumococcal proteins in a multicomponent vaccine.[661]

Two newer antigenic discovery approaches have been described. In one, investigators screened pneumococcal protein libraries for immunogenic targets by identifying antigens recognized by antibody from convalescent sera of patients recovering from IPD.[662] Two antigens were identified, "protein required for cell wall separation of group B streptococcus" (PcsB) and serine/threonine protein kinase (StkP). Another strategy used a proteomic approach to identify antigens recognized by $CD4^+$ Th17 cells from murine or human hosts exposed to pneumococcus. This approach identified different targets than those selected by antibody-mediated approaches and demonstrated that animals could be protected from colonization with a $CD4^+$ T-cell and IL-17A–dependent approach.[408]

Human Experiences With Natural or Vaccine-Derived Pneumococcal Protein Antigens

Over the past few years, there have been numerous reports on the natural development of antibodies to many of these pneumococcal proteins in humans, including children.[663–672] Concentrations of antibodies to PsaA are lower in children with

pneumococcal AOM than in those with nonpneumococcal AOM [664] and in children with pneumococcal carriage than in noncarriers.[663] Similarly, a low concentration of PspA antibodies was detected in children with invasive pneumococcal infection as compared with children with nonpneumococcal invasive infection.[668] The presence of salivary IgA antibodies (but not serum IgG antibodies) to PspA was associated with a decreased risk of pneumococcal OM during the following 6 months.[673] Increases in anti-PsaA and anti-Ply concentrations are seen in adults with pneumococcal pneumonia, the increase being more evident in those with bacteremic pneumonia. On the other hand, anti-PspA levels showed a twofold increase in only 15% of Finnish children with IPDs. Studies in Filipino children did not support the notion that natural immunity protects against OM, although there were some hints that antibodies to Ply were associated with a reduction of pneumococcal carriage.[674]

Human adults have been shown to naturally produce antibodies to PhtA and PhtB.[653] Likewise, pneumococcal colonization has been shown to induce the development of natural antibodies in infants.[675] Antibodies to PhtA and PhtD have been detected in convalescent-phase sera from patients with pneumococcal bacteremia.[651]

McCool and associates[231] provided evidence that serum IgG and/or secretory IgA targeting the variable terminal region of PspA were associated with reduced likelihood of carriage and that experimentally induced nasal colonization in human adults induced serum IgG antibodies to both PspA and CbpA.[232] Finnish researchers showed that high serum anti-PsaA levels predicted low risk of progression to OM in colonized children older than 9 months of age, but predicted a greater likelihood of subsequent OM in younger infants.[664] In a British study, anti-CbpA and anti-Ply serum levels and salivary IgG antibody levels were higher in children undergoing adenoidectomy who were culture negative for pneumococci than in those obtained from children who were colonized with pneumococcal organisms.[676]

Whether these antibodies provide protection to children against pneumococcal colonization or are just associated with resistance to colonization remains uncertain. As described above, data in mice and humans suggest that, independent of antibodies, $CD4^+$ T cells directed against pneumococcal antigens may provide resistance to colonization.[397,398] Adenoidal cells obtained from children at the time of surgery demonstrated that children colonized with pneumococci had lower $CD4^+$ T-cell proliferative responses to Ply than those who were not colonized.[677] It has also been shown that children in Sweden and Bangladesh readily develop Th17 responses to a pneumococcal proteins.[678]

Despite decades of research in this area, there have been only a few trials of pneumococcal proteins in humans, with even fewer published results. A Phase I clinical trial demonstrated that a recombinant family 1 PspA protein was safe and elicited persistent antibody to the PspA protein in vaccinated volunteers.[679,680] These antibodies, when transferred to immunocompromised mice, protected against challenge with pneumococci expressing either family 1 or family 2 PspAs. As noted above, clinical trials of combination vaccines containing PcpA, detoxified Ply, and PhtD have been performed in adults and toddlers[636–638] with favorable safety profiles to date. PhtD and Ply are being studied in combination with PCVs in collaboration among GlaxoSmithKline, Programs for Appropriate Technology in Health (PATH), the Gambian Medical Research Council, and the London School of Hygiene and Tropical Medicine. A Phase II clinical study in The Gambia of this combination PCV+protein vaccine candidate to be safe and immunogenic but without impact of the proteins on pneumococcal carriage.[18a]

A protein-based candidate pneumococcal vaccine consisting of two lipoproteins (SP_2108 and SP_0148) and a protein of unknown function (SP_1912) was evaluated in a Phase I clinical trial. These proteins were identified by Genocea Biosciences in collaboration with PATH and Boston Children's Hospital, based on a proteomic analysis of the Th17 responses from immune mice or human adult volunteers.[408,681] The combination of these three proteins elicited robust Th17 responses and TH17-dependent protection against colonization in mice.[408] The lipid motif on the two lipoproteins, which activates Toll-like receptor 2, appears to be critical for optimal immunogenicity and protection in the murine model.[682] Following these studies, lipidated versions of the three proteins were formulated on aluminum hydroxide and successfully evaluated in a double-blind, dose-escalating Phase I clinical trial. This vaccine was studied in a Phase IIa clinical trial of intentional human challenge in healthy adults to evaluate the effect of the vaccine on the frequency, magnitude and duration of pneumococcal colonization, with nonsignificant results presented, but not published to date.

Killed Whole-Cell Vaccine

In general, because different virulence factors of S. *pneumoniae* may act at different stages of pneumococcal colonization and/or infection, it is likely that immunization with a combination of proteins may be the best approach. An extension of this idea involves the possibility of using a killed whole-cell pneumococcal vaccine, where a large number of pneumococcal antigens would be presented at once. Published reports of numerous trials using this type strategy date back to 1911[683] and a metaanalysis of these studies suggests efficacy of this approach.[683] Since then, work in mice has demonstrated that immunization with killed whole cells, using a variety of routes (intranasal, oral, sublingual, transcutaneous, subcutaneous, and intramuscular) and with adjuvants (enterotoxins, enterotoxin mutants, or nontoxic subunits or Toll-like receptor agonists by the mucosal route, and aluminum hydroxide by the parenteral route), provides robust and reproducible protection against nasopharyngeal colonization (via $CD4^+$ T cells) and invasive disease (via antibodies).[684] A good manufacturing practices–grade killed whole-cell vaccine administered parenterally with aluminum hydroxide conferred protection in mouse models of carriage or aspiration pneumonia/sepsis.[224] This vaccine was studied in a Phase I safety and immunogenicity trial in healthy adult volunteers in the United States at three different concentrations. The vaccine was well tolerated and induced both antibody and Th17 responses. Passive transfer experiments in mice, using sera pre- and postimmunization, showed vaccine-induced protection against type 3 invasive disease and sepsis.[685] Following these promising results, a Phase I/II age deescalation clinical study was begun in Kenya, to evaluate, in addition to safety and immunogenicity outcomes, the potential impact of the whole-cell vaccine on pneumococcal carriage in toddlers. Although the ultimate target of the vaccine is infants, an early sign in toddlers of a possible impact on reduction of carriage, or duration of carriage, would be very promising. At the same time, it is important to recognize that further development of this vaccine will require more direct evaluation of efficacy against pneumococcal disease. Several issues will need to be considered, such as a careful definition of the outcome of interest (pneumonia, sepsis, meningitis), the ethical considerations surrounding the evaluation of the whole-cell vaccine versus PCV, as well as the setting of the trial (either in a PCV-experienced or -naïve pediatric population).

Combination Strategies

These above considerations will determine the feasibility and size of any pivotal trial of both the whole-cell and the protein-based pneumococcal vaccines as correlates of protection for whole-cell vaccine and protein-based vaccines are not established. The development of these vaccine candidates will likely require large double-blind, randomized trials with a precisely defined disease outcome in infants and children. Estimates of the size of the trial vary according to whether the population is PCV-experienced or -naïve, and whether both arms of the study would also be receiving PCV.

As an alternative approach, the inclusion of conserved pneumococcal proteins as components of polysaccharide-protein complexes has been considered. In particular, Plys and PspA have been evaluated as potential carrier proteins in various conjugates in mice,[216,634,686] with promising results. These strategies, which may offer broader coverage against the pneumococcus than traditional conjugates wherein the protein carrier is an irrelevant protein (such as CRM_{197}, diphtheria or TT), still represent very complex and expensive manufacturing processes, potentially limiting their applicability in the developing world setting. The possibility of protein-epitope damage by chemical conjugation to diverse polysaccharides has also been raised.

To address these issues, alternative strategies for associating polysaccharides to proteins in a more efficient and less complex fashion have been studied. Liquidia Technologies described its Particle Replication In Non-wetting Templates (PRINT) technology as a tool to form nanoparticle-based virtual conjugates[687] and initiated preclinical evaluations of this vaccine in rodent and rabbit models. Another approach in which affinity interactions between biotinylated polysaccharides and rhizavidin-fused pneumococcal proteins are used to form polysaccharide-protein macromolecular complexes has been presented.[688] The manufacture of these constructs is highly efficient and significantly less complicated than chemically coupled polysaccharide–protein vaccines, which may offer important advantages for the developing world. Immunological responses to the polysaccharides in these affinity complexes are notable for $CD4^+$ T-cell dependence, as well as for the generation of boostable antibody responses and affinity maturation. In addition, both antibody and $CD4^+$ Th17 responses to the proteins are generated, thus raising the possibility of broad, serotype-independent antibody- and Th17-mediated protection against invasive disease and colonization, respectively. The MAPS (Multiple Antigen Presentation System) technology was licensed by Boston Children's Hospital to a biotechnology company (Affinivax, Cambridge, MA) for the development of novel vaccines for both the developing and developed world. In particular, a program to design, manufacture, and test a low-cost, multivalent pneumococcal vaccine received support from the Bill and Melinda Gates Foundation and PATH. Clinical trials of a MAPS-based multivalent pneumococcal vaccine are planned to start in 2017.

CONCLUSIONS

Since its rollout in children, pneumococcal conjugate vaccine has provided significant protection against invasive disease and pneumonia due to the vaccine serotypes. These benefits have extended to all age groups through the induction of herd protection by immunization of infants and toddlers. The reduction in disease has provided significant public health benefits to the populations where these vaccines have been used and there is a priority for additional populations, especially in Asia, to receive these vaccines. The benefits appear to extend beyond the previously known burden of pneumococcal disease to prevention of previously suspected, but unmeasured secondary pneumococcal infections that complicate disease due to influenza, respiratory syncytial virus, and other copathogens. At the same time, the limited serotype coverage of the vaccines have allowed replacement disease with non-VTs. The extent of the replacement has been limited in most settings, but continued surveillance is needed. Further expansion of the serotypes in these vaccines and consideration of protein based vaccines on their own, or as adjuncts to pneumococcal conjugates, have moved beyond preclinical studies in animals and are now in human clinical trials.

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47 Pneumococcal Polysaccharide Vaccines

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Since the late 19th century, the study of *Streptococcus pneumoniae* and pneumococcal infections has occupied a central position in developing a scientific basis for controlling infectious diseases.[1–7] The organism was first isolated and grown in the laboratory almost simultaneously by Sternberg and Pasteur in 1881. During the next decade, the pneumococcus was shown to be the chief cause of lobar pneumonia. In the early 20th century, the importance of humoral immunity in prevention of infectious diseases was recognized, in large measure as a result of studies of *S. pneumoniae*, and there was the beginning of an understanding of the potential protective effects of antisera and vaccines.

The first large-scale clinical trial of a pneumococcal vaccine, which evaluated a crude whole-cell formulation, was conducted in 1911, at a time when the importance of serotype-specific immunity was not understood.[7] During the following decades, scientists discovered the pivotal role of serotype-specific antibody to pneumococcal capsular polysaccharides and began developing effective polyvalent pneumococcal polysaccharide vaccines (PPSVs).

PPSVs are considered poorly immunogenic in children younger than 2 years of age, the age group traditionally with highest incidence of invasive pneumococcal disease (IPD). To improve the immune response to the capsular polysaccharide in young children, following the model established for a vaccine to prevent infection caused by *Haemophilus influenzae* type b, vaccines in which capsular polysaccharides are conjugated to one of several proteins were developed. A protein-conjugate pneumococcal vaccine (PCV) that contained conjugated capsular polysaccharides from seven common pneumococcal serotypes (PCV7) was administered to children in the first year of life and shown to be highly effective in preventing IPD caused by corresponding serotypes.[8,9] Because PCV also stimulates mucosal immunity and prevents nasopharyngeal colonization, widespread use of the PCV7 has also provided substantial protection for children who were not vaccinated, as well as for unvaccinated adults, a phenomenon that is called an indirect (also called herd or community) effect.[10–13] After evaluating six additional conjugated polysaccharides, PCV13 was licensed for children in the United States in 2010, replacing PCV7. In 2011 and 2012, PCV13 gained an indication for use in adults in various countries.[14]

As in prior editions of this book, PCVs are discussed in Chapter 46. PCVs are routinely recommended for children throughout the world. Consequently, that chapter includes a discussion of pneumococcal infections in children. This chapter focuses on the use of unconjugated PPSVs and their effect on pneumococcal infections in adults, but the epidemiologic impact of PCV in children informs the use of PPSV23 and is reviewed here briefly.

BACKGROUND

Clinical Pneumococcal Infections

S. pneumoniae is a commensal that asymptomatically colonizes the nasopharynx, and is also a major cause of a diverse array of infections. The organism causes infection either by direct extension from the nasopharynx (e.g., pneumonia, otitis media, sinusitis) or by invasion with bacteremic spread into normally sterile fluids (e.g., bacteremia, septic arthritis). Meningitis or spontaneous bacterial peritonitis may occur by direct spread or from hematogenous spread. IPD is defined by isolation of *S. pneumoniae* from a normally sterile site, such as blood, cerebrospinal fluid (CSF), pleural fluid, or synovial fluid.

Bacteriology and Pathogenesis

The microorganism initially observed by Sternberg and Pasteur was called *Pneumococcus* by Fränkel in 1886.[1] It was renamed *Diplococcus pneumoniae* in 1920 because of its distinctive morphology. Its name was changed again to *Streptococcus pneumoniae* in 1974 because of its many similarities to other streptococci.

Avery, MacLeod, and McCarty used the pneumococcus in 1944 to demonstrate that DNA is the active substance responsible for the genetic transformation of pneumococci from rough (nonencapsulated) to smooth (encapsulated) strains. This is one of the cornerstones of modern molecular biology.[15–17] Other biologic markers, such as type specificity and antibiotic resistance, are genetically transferable.

Pneumococci are relatively fastidious, facultative anaerobic organisms that grow in short chains in broth culture. Microscopically, they appear as lancet-shaped gram-positive diplococci or chains of cocci (Fig. 47.1). Growth requires a source of catalase, obtained by adding red blood cells to the growth medium, to neutralize the hydrogen peroxide produced by the bacteria. On blood agar, colonies characteristically produce a zone of α (green) hemolysis, which differentiates *S. pneumoniae* from group A (β-hemolytic) streptococci, but not from α-hemolytic (viridans) streptococci. Pneumococci are distinguished from other α-hemolytic streptococci by two key reactions: optochin susceptibility and solubility in deoxycholate (bile). Pneumococci, but not most other streptococci, are inhibited by optochin and undergo lysis by bile salts. Some pneumococcal isolates, however, are resistant to optochin.[18] Therefore, solubility of the colony in bile is usually regarded as definitive for routine diagnostic purposes.

Capsular Polysaccharides

The capsular polysaccharide on the cell surface (Fig. 47.2) is the major factor responsible for the virulence of *S. pneumoniae*.[19–22] The capsule interferes with phagocytosis by preventing complement C3b–mediated opsonization of bacterial cells. Antibodies to the capsular polysaccharides are protective. More than 90 pneumococcal serotypes have been identified on the basis of antigenic differences in the capsular polysaccharide. Current antipneumococcal vaccines are based on formulations of various capsular polysaccharide antigens derived from selected serotypes; selection is based on the frequency with which specific capsular polysaccharides cause disease in the target population.

Traditionally, capsular serotypes were defined by the Quellung reaction, using polyclonal rabbit sera. The Quellung reaction is, however, not sufficiently sensitive to distinguish

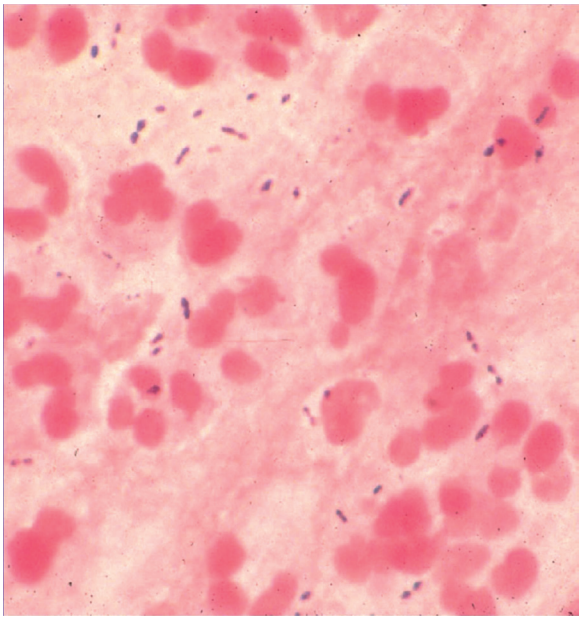


Figure 47.1. Microscope image (×440) of a Gram-stained specimen of sputum showing proteinaceous background, polymorphonuclear cells, and gram-positive, slightly elongated cocci singly and in pairs. Culture yielded *Streptococcus pneumoniae* as the predominant organism. *(Courtesy Daniel M. Musher.)*

serotypes with small structural differences, and more recently, molecular techniques have been used to identify new pneumococcal serotypes, such as 6C, which was indistinguishable from serotype 6A using the Quellung reaction.[22a]

The cell wall of *S. pneumoniae* consists primarily of a peptidoglycan backbone that is covalently linked to teichoic acid on the outer surface of the cell wall and is present in all isolates.[19,20,23] C-polysaccharide plays a major role in stimulating the inflammatory response associated with pneumococcal infections.[24,25] It reacts with a specific β-globulin in human serum (C-reactive protein) in the early stages of the inflammatory response and activates the alternative complement pathway. Antibody to C-polysaccharide appears early in life and can be found in most children and adults, but antibodies directed against C-polysaccharide do not protect against pneumococcal infection.[26]

On the basis of functional genomic analysis, it is estimated that the pneumococcus contains more than 500 surface proteins. The role of several pneumococcal proteins in the pathogenesis of *S. pneumoniae* infections has been extensively studied. These proteins, such as pneumolysin (not a surface protein, but a well-recognized virulence factor),[19,21] pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), pneumococcal surface antigen A (PsaA), neuraminidase enzymes, and histidine-triad proteins, are the targets for a new generation of protein-based vaccines. The potential advantages of such vaccines include greater cross-protection among different capsular serotypes. More information on these virulence proteins and the current state of pneumococcal protein vaccine development may be found in Chapter 46.

Diagnosis

The diagnosis of IPD is established by isolation of the organism from blood or another normally sterile site (e.g., cerebrospinal, pleural, or synovial fluid). The diagnosis of noninvasive pneumococcal infections, such as nonbacteremic pneumococcal pneumonia, is more complicated. Approximately 75% of cases of pneumococcal pneumonia are not associated with detectable bacteremia. The finding of *S. pneumoniae* in sputum by Gram stain and culture in a patient who presents with a pneumonia syndrome enables the diagnosis of presumptive pneumococcal pneumonia. However, there are significant limitations to diagnostic tests of sputum. At least 40% of patients are unable to produce an adequate expectorated sputum specimen at time of hospitalization for pneumonia, and, once antibiotics have been administered for longer than 18 hours, the diagnostic yield from sputum Gram stain and culture is less than 25%.[27,28]

A rapid immunochromatographic assay reliably detects cell-wall polysaccharide from *S. pneumoniae* in urine from 60% to 80% of patients who have bacteremic pneumococcal pneumonia and 50% to 60% of those with pneumococcal pneumonia but with negative blood cultures.[29–31] In adults, there are few false-positive results. The assay is less reliable in children who have a heavier colonization burden and who, therefore, have many false-positive results.[32–35] This test is also approved for the rapid diagnosis of pneumococcal meningitis through testing of CSF samples, with a sensitivity of at least 95% and a specificity of 100%.[36–38] The test has also been used to detect *S. pneumoniae* antigen in pleural fluid specimens,[39–41] bronchoalveolar lavage fluid samples (with utility limited by contamination via nasal colonization),[42] and blood cultures.[43]

The newest approach to diagnosing pneumococcal infections has been to use nucleic acid–amplification techniques.[44] Although polymerase chain reaction (PCR)-based assays are highly sensitive, their use to detect *S. pneumoniae* in respiratory samples can be limited by nonspecificity of the gene targets, some of which may also be present in other organisms, such as *Streptococcus pseudopneumoniae*[45] and nonpneumococcal viridans group streptococci that are oropharyngeal colonizers, leading to false-positive results. More importantly, the test may also detect asymptomatic pneumococcal colonization; consequently, a positive test may not indicate pneumococcal infection.[46–54] The first concern may be addressed by the use of gene targets found only in *S. pneumoniae*.[55] The second concern may potentially be addressed by the use of quantitative real-time PCR methods to prevent false-positive results resulting from colonization with small numbers of organisms.[56,57] In patients with meningitis, PCR is highly sensitive and specific for detection of *S. pneumoniae* in CSF samples.[58,59]

Treaent and Resistance

The definition of penicillin susceptibility of pneumococci depends on the site of infection, specifically whether central nervous system infection is thought to be present.[60] In the absence of meningitis, susceptible isolates are inhibited by less than 2 μg/mL penicillin (minimum inhibitory concentration [MIC]). Isolates with a MIC of 2 μg/mL are called *intermediate* and those with a MIC greater than 4 μg/mL are called *resistant*. In cases of central nervous system (CNS) infection, organisms with a MIC of 0.06 μg/mL or less are called *susceptible* and those with a MIC of 0.12 μg/mL or greater are regarded as *resistant*, even though that may be considered an overly strict definition. At present, in cases of non-CNS infection, greater than 95% of pneumococcal isolates are susceptible.[61] In cases of meningitis, 10% to 15% of pneumococcal isolates are penicillin-resistant. Consequently, infections outside the CNS that are known to be caused by the pneumococci can be treated with parenteral penicillin, if appropriate, or other recommended antibiotics.

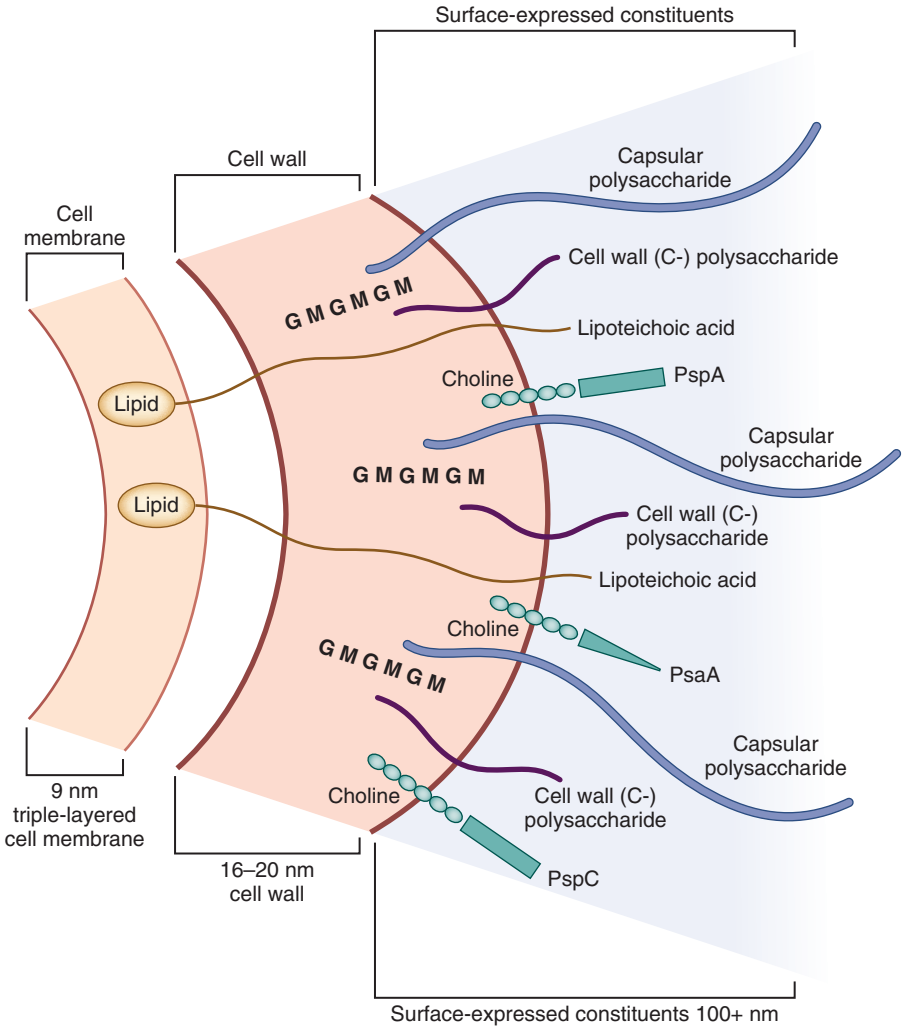


Figure 47.2. Representation of the cell membrane, cell wall, and capsule of *Streptococcus pneumoniae*, emphasizing constituents that play, or that may play, a role in virulence. PsaA, pneumococcal surface adhesin A; PspA, pneumococcal surface protein A; PspC, pneumococcal surface protein C. *(Modified from Musher DM. Streptococcus pneumoniae. In: Mandell GL, Bennett JE, Dolin R, eds.* Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, *6th ed. Philadelphia: Elsevier; 2004:2392–2411.)*

EPIDEMIOLOGY OF PNEUMOCOCCAL INFECTION

Invasive Pneumococcal Disease

As with many other infectious diseases, rates of IPD are highest at the extremes of age. In 2013, the Centers for Disease Control and Prevention (CDC) Active Bacterial Core surveillance (ABCs) program estimated the following rates (per 100,000 population) by age in the United States: 15.2 for children younger than 1 year; 15.1 for 1-year-olds; 6.9 for 2- to 4-year-olds; 1.6 for 5- to 17-year olds; 2.8 for 18- to 34-year-olds; 8.0 for 35 to 49-year-olds; 17.2 for 50- to 64-year-olds; and 30.2 for persons 65 years of age and older (Fig. 47.3).[13,62] In 2013, the case-fatality ratio of IPD in persons 65 years of age and older was 14%, compared with 1.6% in children younger than 5 years. A total of 33,500 cases and 3500 deaths resulting from IPD are estimated to occur in the United States annually. Pneumonia with bacteremia accounts for the majority (67%) of cases of IPD. Bacteremia without a focus and meningitis account for 19% and 6% of cases, respectively.

Although IPD occurs all 12 months of the year, the incidence in adults increases in the winter. This seasonal variation is closely associated with viral respiratory infections that are also more common in winter. Exposure of children to respiratory viruses and bacteria during the school year is probably a major factor. This increase is not clearly attributable to lower ambient temperature or air pollution. Factors such as photoperiod-dependent variation in host susceptibility may also be involved.[63,64] Although most IPD is community acquired, IPD can be nosocomially acquired. A population-based study in Finland reported that nosocomial pneumococcal bacteremia accounted for 10% of hospitalized pneumococcal bacteremia cases.[65] Numerous pneumococcal outbreaks have been reported, most often from settings of clustered living or working conditions.[66–69]

Pneumococcal Pneumonia

In the preantibiotic era, *S. pneumoniae* caused 95% of cases of pneumonia. This number has steadily declined, such that recent studies document pneumococcus as a cause in approximately 8% to 10% of patients who are hospitalized for pneumonia.[70,71] In most studies of the etiology of hospitalized community-acquired pneumonia (CAP) in adults, *S. pneumoniae* ranks first among bacterial causes.[72–105] However, an etiologic agent is not identified in 50% or more of patients with CAP.

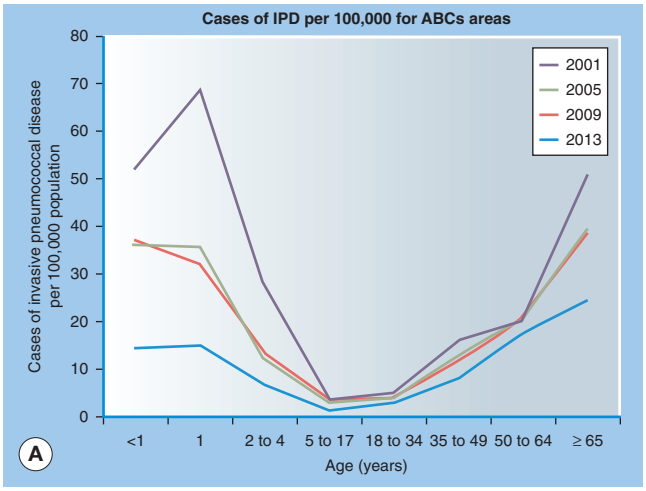


Figure 47.3. A and **B,** Incidence of invasive pneumococcal disease (IPD) caused by any serotype by age cohort for selected calendar years in Active Bacterial Core surveillance (ABCs) areas, cases, and deaths. Data for 1997 not shown in this graphic because extreme values would make interpretation of the other annual reductions difficult to discern. For 1997, in the pre-PCV7 era, the incidence rate of IPD case per 100,000 population were 143 for <1179 for 1 year of age, and 62 for 65 years of age and older. The incidence rate of IPD death per 100,000 population were 4.0 for younger than age 1 year, 0.9 for 1 year of age, and 11 for 65 years of age and older. PCV7 was licensed for children in 2000 and PCV13 was licensed for children in 2010. *(Modified from serial annual reports: Centers for Disease Control and Prevention. Active Bacterial Core surveillance (ABCs) Surveillance Reports, Emerging Infections Program Network, Streptococcus pneumoniae, 1997, 2001, 2005, 2009, and 2013. Available at: http://www.cdc.gov/abcs/reports-findings/surv-reports.hl.)*

An assessment of the clinical and economic burden of pneumococcal disease in older U.S. adults identified the burden of CAP (outpatient and hospitalized) in adults 50 years of age and older in the United States from published studies and then assumed that 30% of such cases were caused by *S. pneumoniae*. This study estimated that *S. pneumoniae* accounts for approximately 500,000 cases of nonbacteremic pneumococcal pneumonia (and an additional 29,500 cases of IPD) in adults 50 years of age and older in the United States each year, leading to US$3.7 billion in direct costs annually based on 2004–07 inputs.[106] Estimates included in this model are not consistent with the lower values for pneumococcal etiology of CAP found in several recent U.S. studies, thus limiting the validity of the model's output.[70,71,107] More recent data on the etiology of CAP in the United States and certain other developed countries suggest that pneumococcus may be responsible for 10% to 15% of cases.[70,71,107] Decreased cigarette smoking and greater use of PPSV23 in older adults and PCVs in children have contributed to this decline, but other unidentified factors also appear to play a role.

Risk Factors for Pneumococcal Disease

Those at the extremes of life in age are particularly susceptible to pneumococcal infection, as shown in Fig. 47.3.

Certain racial and ethnic groups are at increased risk. Several studies in the United States show substantially higher rates of IPD in blacks than in whites.[108–117] Even higher rates of invasive disease have been reported among the White Mountain Apaches in Arizona,[118,119] the Alaskan Native population,[120–123] and aboriginal populations in Australia.[124–127] The biologic explanation for these ethnic predispositions remains to be elucidated, but, in addition to genetically mediated susceptibility, environmental factors may play a role (e.g., crowding, exposure to respiratory irritants). Crowding, physical exhaustion, and stress contribute to well-documented outbreaks of pneumococcal pneumonia in adults.[66–69] These conditions, together with malnutrition and general poor health, help explain outbreaks among prisoners. Some of these, together with age, contribute to outbreaks of pneumococcal pneumonia in nursing homes.

Cigarette smoking is a strong and independent risk factor for IPD in adults. A case-control study of immunocompetent adults 18 to 64 years of age found a fourfold higher risk of IPD in current smokers and a 2.5-fold higher risk of IPD in those with passive exposure to smoke compared with adults who had never smoked and had no passive exposure.[114] Persons with chronic pulmonary diseases (such as chronic obstructive pulmonary disease, emphysema, and asthma) are at increased risk. By damaging pulmonary clearance (and perhaps by increasing bacterial adherence to epithelial cells), influenza virus infection strongly predisposes to pneumococcal pneumonia; other upper and lower respiratory viruses may also do so, albeit to a lesser degree.

Diseases that compromise the ability to generate antibody responses to new antigens render individuals especially susceptible to IPD. Best studied are patients with multiple myeloma, lymphoma, Hodgkin disease, and HIV infection, even before the onset of AIDS. In persons with HIV/AIDS in the pre–HIV-treatment era, the age-adjusted incidence of IPD was several hundred-fold greater than in the non–HIV-infected population, approaching 500 per 100,000 per year.[113,128] Transplant recipients are also at high risk of IPD, with an overall incidence of 146 cases of IPD per 100,000 per year.[128,129] The risk is highest in liver (354 cases per 100,000) and lung (239 cases per 100,000) transplant recipients.[130–133] A question of great relevance to the present discussion is whether such subjects would generate lasting antibody levels after receiving a pneumococcal vaccine (see below and Chapter 69).

Conditions that interfere with function of polymorphonuclear phagocytes are also associated with increased risk of IPD. Diabetes mellitus, chronic renal failure, and cirrhosis of the liver are good examples. Two specialized circumstances are also worth noting. Because the spleen plays a central role in removing nonopsonized bacteria from the bloodstream, persons who do not have a functioning spleen are at a high risk of overwhelming infection caused by *S. pneumoniae*. Persons with chronic CSF leak or cochlear implants have

a greatly increased risk of pneumococcal meningitis.[134–138] Ascites predisposes to pneumococcal peritonitis. Alcoholism and prior damage to heart valves predispose to pneumococcal endocarditis.

The degree of risk elevation is markedly greater for the immunocompromising conditions than for the other chronic diseases. For example, in 2000 the rates of IPD cases per 100,000 person-years were nine among healthy adults compared with 51 among adults with diabetes, 63 among adults with chronic lung disease, 94 among adults with chronic heart disease, and 100 among adults who abused alcohol. Among immunocompromised adults, disease rates are even higher, with rates of 300 among adults with solid cancer, 423 among adults with HIV/AIDS, and 503 among adults with hematologic cancer. Among the latter group, another study found that adults with multiple myeloma appear to be at particularly high risk.[116,128]

Reservoirs of Infection: The Carrier State

The human nasopharynx is the only natural reservoir for *S. pneumoniae.* Colonization of the nasopharyngeal epithelium represents the first step in the pathogenesis of pneumococcal infections.[139,140] Carriage studies provide an estimate of the baseline pneumococcal serotypes that are present in the nasopharynx and provide information for vaccine composition and to monitor changes induced by biologic pressure derived from antibiotic therapy or vaccinations. It is important to note that asymptomatic colonization is an active immunizing process leading to antibody production both in children and in adults.[141,142] If the host is relatively healthy and capable of producing antibody (or has antibody already circulating after vaccination), then disease is less likely to occur. In contrast, if the host is debilitated, has poor pulmonary clearance, or is unable to produce antibody, disease is far more likely to occur.

Colonization studies consistently demonstrate much higher rates of carriage among children than adults.[23,143–161] After acquisition, the duration of carriage is also longer in children compared with adults, and *S. pneumoniae* may be introduced into households by children and spread to other family members.[161] Among adults, carriage rates may be elevated among certain populations, including adults with AIDS, military trainees, cigarette smokers, and native Alaskans,[155–159] but lower in elderly persons.[162]

Although PPSVs are not widely regarded as effective in preventing colonization, rates of serotype-specific nasopharyngeal carriage among vaccine recipients in crowded adult settings significantly decreased in PPSV recipients. A study at a U.S. Army technical school found that PPSV4 reduced serotype-specific carriage by 55% (from 3.3% to 1.8%, $P < .01$).[163] Klugman and colleagues calculated carriage reductions among gold miners in South Africa of 70% for PPSV6 (from 23% to 7%, $P < .001$) and 65% for PPSV13 (from 51% to 18%, $P = .005$).[164]

Epidemiologic Impact of Pneumococcal Conjugate Vaccine Use in Children

Since PCV7 was widely adopted in the United States in 2000, IPD caused by those serotypes have nearly vanished in vaccine recipients. More importantly, as an indirect effect of vaccination, there has been a greater than 98% reduction in invasive disease resulting from vaccine serotypes in children and adults who have not received PCV7.[10–13,165–167] The reduction in unvaccinated adults lags behind that in vaccinated children by a few years, but ends up with similar declines. Reductions have been reported from other countries where PCVs are routinely used in infants, including Canada, England, Israel, Germany, and France.[62,168–171] By 2013, widespread adoption of PCV13 for American children since 2010 had already led to a 58% to 72% decline in IPD as a result of the inclusion of six new serotypes not previously included in PCV7.[13] The net effect is for the seven and then 13 serotypes to fall in incidence and proportion among adults, leaving the non-PCV13 serotypes to predominate in circulation.[168]

PASSIVE IMMUNIZATION

Fränkel demonstrated humoral immunity in 1886, by noting that rabbits that recovered from pneumococcal infection were immune to reinfection.[172] In 1891, Georg and Felix Klemperer showed that repeated vaccination of rabbits with killed pneumococci protected rabbits against challenge with *S. pneumoniae* and that transfer of serum (the humoral substance) to unvaccinated rabbits conferred immunity.[4–7,173] These early investigators also discovered that there were at least two different "types" of *S. pneumoniae* based on the absence of cross-protection. On this basis, they are considered the discoverers of serum therapy.

Cecil, Finland, and other investigators explored the value of type-specific serum therapy to treat pneumococcal infections in the late 1920s and 1930s.[7,174,175] These products were used widely in therapy until the advent of antibiotics.

Human intravenous immunoglobulin preparations commercially available in the United States contain varying amounts of antibody to pneumococcal capsular polysaccharides.[7,176–178] Chronic prophylaxis with intravenous immunoglobulin is used to prevent infections with encapsulated bacteria in patients with primary and certain other immunodeficiency disorders.

ACTIVE IMMUNIZATION

Early Developments

In the 1910s, Maynard,[179] Wright,[180] Cecil and Austin,[181] and Lister[182] undertook studies to show that African miners[179,180,182] or U.S. soldiers[181] were protected against pneumococcal pneumonia by repeated vaccination with killed pneumococci. In these studies, whole-cell pneumococcal vaccine appeared to reduce the occurrence of pneumococcal pneumonia by 50–80%,[179–182] although interpretation of some results is limited by methodologic issues, such as small sample size or lack of an appropriate comparison group.[163,183–186] Of interest, a recent reanalysis of data from trials of whole-cell killed bacterial vaccines given during the 1918 influenza pandemic suggests that whole-cell bacterial vaccines containing pneumococci may have reduced the risk of pneumonia and mortality among patients with pandemic influenza infection.[186]

In the 1920s, Heidelberger and Avery showed that humoral protection resulted specifically from antibody to the pneumococcal polysaccharide capsule.[187] In the 1930s, Felton isolated capsule for use as a vaccine.[188] Smillie used Felton's polysaccharide preparation to help abort an outbreak of pneumococcal pneumonia at a state hospital.[189]

Early Controlled Studies on Pneumococcal Polysaccharide Vaccines

Once antibody to capsular polysaccharide was shown to be protective, vaccination trials began in earnest. In two separate

studies beginning in 1937, Kaufman and coworkers randomized a total of 14,500 elderly persons, half of whom received polysaccharide vaccines against serotypes 1, 2, and 3 and half of whom received placebo.[190] They assessed all-cause pneumonia and death, at a time and place where 90% to 95% of all pneumonias were caused by *S. pneumoniae* and most of those were types 1, 2, or 3. They observed a 76% reduction in pneumonia in the first study (approximately 3500 subjects). In the second study (approximately 11,000 subjects), in which 47% of the patients were 70 years of age or older, the incidence of pneumonia was reduced 72% by vaccination.

In the 1930s, Felton and colleagues carried out controlled studies of PPSV among 18,000 U.S. Civilian Conservation Corps volunteers, documenting reductions in pneumonia incidence ranging from 59% to 89% (differences probably reflecting regional variation in prevalent serotypes).[191,192]

During World War II, the U.S. Armed Forces conducted a controlled study of a quadrivalent PPSV against serotypes 1, 2, 5, and 7 in 17,000 soldiers, documenting significant reductions in pneumonia caused by *S. pneumoniae* types 2 (93%) and 7 (100%), and a 75% reduction in pneumonia caused by type 5 (small number of cases, $P > .15$). There were not enough cases to show any decrease in disease caused by type 1. The investigators also monitored pneumonia caused by *S. pneumoniae* types 4 and 12 that were not included in the vaccine and there was no impact.[163] As a result of this body of work, two hexavalent vaccines were marketed in the United States in 1946, only to be voluntarily withdrawn within a few years, largely because physicians had little interest in a vaccine to prevent a disease they believed they now could treat successfully with penicillin.

In 1964, Austrian and Gold[193] showed conclusively that pneumococcal infections were still lethal, despite appropriate antimicrobial therapy. Austrian led efforts to develop a modern pneumococcal capsular polysaccharide vaccine once again. Within a decade, the type-specific efficacy of PPSV was demonstrated in clinical trials conducted among a subsequent cohort of gold miners in South Africa.[194,195] In 1977, a 14-valent capsular polysaccharide vaccine was licensed in the United States for persons 2 years of age and older, which was succeeded by the current 23-valent formulation in 1983.

Development of Contemporary Pneumococcal Polysaccharide Vaccines

Smit and colleagues undertook two vaccine trials in young adult gold miners in South Africa, one with a six-valent and one with a 12-valent PPSV (Table 47.1).[194,195] Vaccination was associated with a significant reduction in the risk of vaccine-type pneumococcal pneumonia; diagnoses were confirmed by radiography plus blood culture. Vaccine efficacy was estimated to be 76% with the six-valent vaccine and 92% with the 12-valent vaccine. The results of three other trials, evaluating either six-valent or 13-valent vaccines, in South African gold miners were reported by Austrian.[194] In an analysis of all three trials combined, PPSV was reported to reduce the risk of vaccine-type pneumococcal bacteremia by 82%.[195]

In the 1970s, a large randomized, double-blind, placebo-controlled clinical trial of a then-new 14-valent polysaccharide vaccine, was conducted in Papua New Guinea.[196] Study participants were rural highlanders who lived in poorly ventilated longhouses and had frequent respiratory infections. A total of 11,958 persons 10 years of age and older were enrolled in the trial and followed for 3 years. The risk of lower respiratory illness with vaccine-type pneumococci isolated from blood culture or lung aspirate was reduced by 86% (see Table 47.1).

The results of the South Africa trials provided the primary evidence supporting licensure of PPSV14 in the United States in 1977. Those trials provided consistent evidence of vaccine efficacy against pneumococcal pneumonia and pneumococcal bacteremia in adults who were at increased risk of pneumococcal infection. Since the mid-1970s, multiple randomized trials of PPSVs have been conducted among adults in the United States, Europe, and Japan. This product was then reformulated, broadening its spectrum of activity by including the most common adult serotypes, in the form of PPSV23 in 1983.

Interpretation of the results of pneumococcal vaccine trials must take into account the adequacy of the trial design, the study outcomes evaluated, the method of ascertaining the etiology of pneumonia, and whether the sample size was sufficient to detect a clinically meaningful reduction in risk of disease. Regarding sample size, Fedson and Liss[207] assessed each of the U.S. and European trials reported before 2004 for their statistical power to detect reductions in risk of all-cause pneumonia. None of the trials had sufficient person-years of observation to have 80% power to detect a 15% or greater reduction in risk of all-cause pneumonia in those trials.

23-Valent Pneumococcal Polysaccharide Vaccine Formulation

The currently available 23-valent vaccines (PPSV23) contain 25 μg of purified pneumococcal capsular polysaccharides from each of the following serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F.[14] Selection of the vaccine serotypes was based primarily on the distribution of strains causing IPD in various global case series in the late 1970s and early 1980s.[208,209] Before the introduction of PCV7 for children in the United States, these 23 serotypes accounted for more than 85% of strains that caused IPD in adults in the United States.[165,168] Since the introduction of PCV7 and then PCV13, and the resulting indirect protection, IPD caused by serotypes contained in PPSV23 has declined in older adults.[11,13,168] In 2013, pneumococcal serotypes contained in PPSV23 accounted for approximately 67% of IPD cases in adults 65 years of age and older in the United States, whereas PCV13 serotypes accounted for approximately 23% of adult IPD cases.[13,168]

Producers and Trade Names

Three vaccine manufacturers currently produce PPSV23. Pneumovax 23 (Merck) and Pneumo 23 (Sanofi Pasteur) formulations are available in Canada and Europe. An unbranded version is produced by Chengdu Institute of Biological Products for China. In the United States, only Pneumovax 23 is available. These vaccines consist of polysaccharides dissolved in isotonic saline, with either phenol (0.25%, Pneumovax 23) or thimerosal (0.01%, Pneumo 23) added as a preservative. They contain no adjuvants. Techniques that simultaneously measure the consistency of molecular size and the antigenicity of individual polysaccharides have helped to ensure uniformity between different lots of vaccine.[210]

Dosage and Route

PPSV23 should be administered as a single 0.5-mL dose intramuscularly or subcutaneously.[14] Intramuscular administration

TABLE 47.1 Randomized Clinical Trials of Pneumococcal Polysaccharide Vaccine Efficacy in Adults Reported Since 1970, Excluding Evaluations of HIV-Infected Persons

Reference, Vaccine Valency	Study Population	Outcome	VE[a] (%)	95% CI	No. Events/ No. Vaccinated	No. Events/ No. Unvaccinated
Smit[194] (1977), 6-valent	Gold miners in South Africa	Vaccine-type pneumococcal pneumonia	76	53–88	9/983	78/2036
		All-cause pneumonia	37	9–56	37/83	121/2036
		Bronchitis	8	–17 to 28	84/983	190/2036
Smit[194] (1977), 12-valent	Gold miners in South Africa	Vaccine-type pneumococcal pneumonia	92	38–99	1/540	25/1135
		All-cause pneumonia	32	–42 to 68	9/540	28/1135
		Bronchitis	42	–7 to 68	13/540	47/1135
Austrian[195] (1976), 6- or 13-valent	Gold miners in South Africa	Vaccine-type pneumonia and/or vaccine-type bacteremia	79	65–87	17/1493	160/3002
Riley[196] (1977), 14-valent	Papua New Guinea highlanders age ≥10 yr	All-cause mortality	21	1–37	133/5946	170/6012
		Respiratory mortality	27	0–46	68/5946	94/6012
		Death from pneumonia uncomplicated by chronic lung disease	43	6–66	23/5946	41/6012
		All-cause pneumonia	26	–13 to 52	36/2713	48/2660
		Cough illness	19	–3 to 36	114/2713	138/2660
		Cough illness with pneumococci isolated from blood culture or lung aspirate	86	48–97	2/2714	14/2660
Austrian[197](1980),[b] 12-valent	Inpatients at Dorothea Dix Psychiatric Hospital in Raleigh, NC	All-cause pneumonia	–22	–49 to 0	154/607	144/693
	Members age ≥45 yr of the Kaiser Permanente Health Plan in San Francisco	Vaccine-type bacteremia	100	*P* = .06	0/6782	4/6818
		Vaccine-type pneumonia	15	–47 to 51	24/6782	28/6818
		Non–vaccine-type infections	–15	–135 to 44	16/6782	14/6818
		All-cause pneumonia	2	–16 to 7	268/6782	274/6818
Gaillat[198] (1985),[c] 14-valent	Residents of 48 long-term care institutions in France	All-cause pneumonia	79	53–91	7/937	27/749
Simberkoff[199] (1986), 14-valent	U.S. Veterans, immunocompetent, and either age ≥55 yr or with renal, hepatic, cardiac or pulmonary disease, alcoholism, or diabetes mellitus	Pneumococcal pneumonia or bronchitis	–15	–48 to 11	43/1145	28/1150
		Pneumococcal pneumonia	–0.8	–29 to 43	17/1145	16/1150
		All-cause pneumonia	–39	–110 to 8	56/1145	41/1150
		All-cause mortality	–28	–57 to –5	211/1145	171/1150
Koivula[200]1(1997), 4-valent	Residents in a small town in Finland age ≥60 yr	All-cause pneumonia	–17	–66 to 17	69/1364	64/1473
		Pneumococcal pneumonia	15	–43 to 50	26/1364	33/1473
Örtqvist[201] (1998), 23-valent	Immunocompetent persons age 50–85 yr who had been previously discharged from a hospitalization for community-acquired pneumonia in Sweden	All-cause pneumonia	–20	–72 to 11	63/339	57/352
		Pneumococcal pneumonia	–28	–150 to 34	19/339	16/352
		All-cause mortality	–5	–88 to 38	29/339	28/352
		Vaccine-type bacteremic pneumonia	79	–77 to 98	1/339	5/352

TABLE 47.1 Randomized Clinical Trials of Pneumococcal Polysaccharide Vaccine Efficacy in Adults Reported Since 1970, Excluding Evaluations of HIV-Infected Persons *(Continued)*

Reference, Vaccine Valency	Study Population	Outcome	VE[a] (%)	95% CI	No. Events/ No. Vaccinated	No. Events/ No. Unvaccinated
Honkanen[202] (1999), 23-valent	Persons age ≥65 yr in northern Finland	All-cause pneumonia	–20	–50 to 10	145/13980	116/12945
		Pneumococcal pneumonia	–20	–90 to 20	52/13908	40/12945
		Vaccine-type bacteremia	60	–40 to 90	2/13980	5/12945
Alfageme[203] (2006), 23-valent	Immunocompetent COPD patients age 61–73 yr in Seville, Spain	Community-acquired pneumonia from pneumococcal infection or of unknown etiology	24	–24 to 54	25/298	33/298
		Pneumococcal pneumonia	100	*P* = .06	0/298	5/298
		Community- acquired pneumonia from organisms other than pneumococci	–7	–62 to –0.01	8/298	1/298
		All community-acquired pneumonia	3	–52 to 38	33/298	34/298
Furumoto[204] (2008), 23-valent	Persons age 40–80 yr with chronic lung disease in Japan	Pneumonia	0	–105 to 52	13/87	12/80
		Acute pulmonary disease exacerbation	30	9–46	42/87	55/80
Maruyama[205] (2010), 23-valent	Nursing home residents in Japan	All-cause pneumonia	45	22–61	63/502	104/04
		Pneumococcal pneumonia	64	32–81	14/502	37/504
Kawakami[206] (2010), 23-valent	Persons age ≥65 yr in Japan	All-cause pneumonia	25	–18 to 53	67/391	81/387
		All-cause pneumonia hospitalization	27	–16 to 56	60/391	76/391

[a]VE is calculated based on the formula VE = 1 – relative risk of disease in vaccinated versus unvaccinated. A negative VE estimate indicates a higher risk in vaccinated compared with unvaccinated persons; a zero VE estimate indicates no difference in risk between vaccinated and unvaccinated persons; and a positive VE indicates a lower risk in vaccinated compared with unvaccinated persons. If the confidence intervals for the VE estimate cross zero, that is, extend from negative to positive, or include zero, the VE estimate is not statistically significant.
[b]Study results obtained from Broome CV. Efficacy of pneumococcal polysaccharide vaccines. *Rev Infect Dis*. 1981;3(Suppl):S82–S96.
[c]Study results obtained from Fedson DS, Liss C. Precise answers to the wrong question: prospective clinical trials and the meta-analyses of pneumococcal vaccine in elderly and high-risk adults. *Vaccine*. 2004;22:927–946.
CI, confidence interval; COPD, chronic obstructive pulmonary disease; VE, vaccine effectiveness.

is considered less likely to cause injection-site reactions and, thus, is the commonly preferred route. The vaccine should not be administered intradermally. Influenza vaccine commonly is given simultaneously with PPSV23 at separate injection sites.[211] Administration of PPSV23 concomitantly with zoster vaccine (Zostavax) does not adversely affect the response to PPSV23 but, in a trial of concomitant administration, anti–varicella-zoster virus antibody levels were significantly lower after concomitant administration of PPSV23 and zoster vaccine live (Zostavax, Merck) compared with levels after administration of zoster vaccine alone, albeit the two study arms differed in their baseline values.[212] The U.S. Zostavax prescribing information advises that separating administration of the two vaccines by at least 4 weeks should be considered, although an observational study reported no difference in risk of medically attended herpes zoster after Zostavax in persons who received Zostavax concomitant with PPSV23, compared with persons who received Zostavax alone.[213]

If stored at 2°C to 8°C and not allowed to freeze, PPSV23 is stable for 24 months.

IMMUNOGENICITY OF 23-VALENT PNEUMOCOCCAL POLYSACCHARIDE VACCINE

Laboratory Methods to Assess Antibody Response

Two kinds of assays are used to assess antibody responses to pneumococcal vaccines. Enzyme-linked immunosorbent assays (ELISAs) quantify the amount, but not the function, of serum antibody to pneumococcal capsular polysaccharide. Opsonophagocytosis assays (OPAs) measure the degree to which serum antibody opsonizes pneumococci for uptake in vitro by human polymorphonuclear leukocytes and are, therefore, regarded as functional assays. Because ELISA measures total serotype-specific antipneumococcal antibody levels, including both functional and nonfunctional antibodies, the ELISA antibody concentrations may not necessarily correlate with functional antibody activity.[214] The capsular polysaccharide antigens used in assays contain contaminating cell-wall polysaccharide that is common to all pneumococci, and most persons, regardless of vaccination status, have antibodies to

cell-wall polysaccharide. Consequently, absorption of the serum specimen with cell-wall polysaccharide has been used in recent years to remove non–serotype-specific cross-reacting antibodies.[215] Even after removal of cell-wall polysaccharide antibodies, a variable amount of antibody to unrelated epitopes may still be measured by ELISA.[215–219] A heterologous type 22F pneumococcal polysaccharide can also be used to adsorb antibodies to these common epitopes.[220,221] Serotype 22F was chosen for this purpose because the capsular polysaccharide is readily available and at the time was thought not likely to be included in future conjugate vaccines. Other approaches include adsorption with serotypes 25 and 72, instead of 22F.[222,223] Such absorption has improved the correlation between immunoglobulin (Ig) G levels determined by ELISA and opsonophagocytic titers in most,[220,224–226] but not all,[216] studies.

Further insight into functional status can be obtained by ELISA if avidity studies are performed.[214] In these studies, a range of concentrations of sodium thiocyanate is added into the ELISA wells. If low concentrations reduce the amount of antibody measured by ELISA, the antibody is judged to be nonavid; low-avidity antibody correlates with low OPA activity and poor protection of mice following challenge with type-specific *S. pneumoniae*.

Correlates of Protection

The original studies that showed OPA to be a better correlate of protection than ELISA were carried out in mice that were pretreated with a range of concentrations of antibody and then challenged with *S. pneumoniae* of the relevant serotype.[214] There was considerable interest in establishing correlates of protection in children to facilitate evaluation and licensure of new pneumococcal vaccine formulations.

A World Health Organization (WHO) working group used data from three clinical efficacy trials of PCVs in infants to propose a protective threshold in infants of 0.35 μg/mL. The assays used in these trials included adsorption of sera with pneumococcal C polysaccharide, but not *S. pneumoniae* capsular type 22F.[227] It is important to note that the 0.35 μg/mL threshold was arbitrarily assigned to all capsular types, although it is likely that there is substantial variation from one serotype to another. It is unknown whether naturally acquired antibody or antibody that appears after PPSV23 would be protective at the same or different level.

It is important to remember that these pediatric correlates are based on aggregate performance, derived collectively from diverse serotypes; each serotype might be found to have its own pediatric protective value and confidence interval, if sufficient data were available. Furthermore, these data are derived from randomized vaccine trials in young children (who are typically naïve to pneumococci) and may not correlate well with protection for adults and seniors. Adults and seniors generally have baseline antibody levels that exceed the levels that are considered as protective thresholds in young children. There are no published data from randomized controlled trials demonstrating vaccine efficacy in adults to support identification of correlates of protection. Therefore, although the results of ELISA and OPAs can be used to identify the relative magnitude of the immune response to vaccination in adults, these markers cannot be used to define individuals as protected or susceptible to pneumococcal infection.

Immunogenicity in Adults

In immunocompetent adults, vaccination with PPSV23 induces significant increases in serotype-specific anticapsular antibody levels as measured by ELISA, as well as by functional activity as measured by OPA.[214,224–248] The magnitude of the immune response varies by serotype and varies widely from one individual to another. Some of this individual variation may be due to genetic factors.[249–253]

The magnitude of the immune response to vaccination diminishes with age. In general, most studies comparing ELISA IgG antibody responses between younger and older adults report lower postvaccination geometric mean titers (GMTs) in the older group, but these differences tend not to be substantial.[225,231,233,238,239] In contrast, OPA titers after vaccination are generally markedly lower in elderly adults compared with young adults (Fig. 47.4).[214,242,254,255] The lower OPA titers in elderly persons appear to be correlated with lower avidity of the IgG antibody for pneumococcal polysaccharide and lesser protection of mice that were given passive antibody from elderly humans and then challenged with pneumococci.[214] Lower postvaccination concentrations of serotype-specific IgM antibody in the elderly may be, in part, responsible.[255,256] After absorption of IgM antibody, the differences in OPA titers between the young adults and the elderly adult groups disappeared.[255] Older adults also exhibit a diminished clonal expansion of B cells in response to vaccination compared with younger adults.[256,257]

In a randomized clinical trial of hospitalized adults comparing PPSV23 to a regimen of PCV7 then PPSV23, no marked differences in antibody were noted in the two groups.[258] This study included stratification by a validated index of frailty, which found lower point estimates for antibody concentrations with higher levels of frailty, although wide confidence intervals led the differences to not be statistically significant.

In general, the immune response, as measured by ELISA, to PPSV23 in immunocompetent persons with chronic medical conditions, such as heart disease, lung disease, or diabetes, is comparable to that of age-matched healthy control subjects.[259–263] In patients with chronic lung disease, the antibody response to vaccination does not appear to be affected by concurrent treatment with oral or inhaled steroids.[262] Patients with chronic alcoholism and cirrhosis can usually mount an antibody response to PPSV23.[263,264] Older persons with low serum vitamin B_{12} levels may have reduced antibody responses to PPSV23.[265]

Immunogenicity in Children

Pneumococcal capsular polysaccharides are T-cell–independent antigens; consequently, most are traditionally considered poor immunogens in infants and young toddlers. Although highly immunogenic serotypes, such as type 3, can induce antibodies in infants as young as 3 months of age,[266] antibody responses to most other pneumococcal capsular polysaccharides are generally poor in children younger than 2 years of age.[208,266–272] A study of children in Fiji who received PPSV23 indicated that children who were 12 months of age are capable of mounting an adequate response to the polysaccharide antigens, with response varying substantially by serotype.[268] Children 2 years of age or older respond with good antibody production after vaccination with PPSV23,[270,273–276] although responses continue to improve until children are 5 to 8 years of age.[274] As discussed below, the primary vaccine for pediatric vaccination is PCV13; but it is recommended that children 2 years of age and older who are at elevated risk for IPD also receive a dose of PPSV23 for the benefit of its unique serotypes.

Immunogenicity in Immunocompromised Persons

The following section describes immune responses to PPSV23 in immunocompromised persons. The application of PPSV23

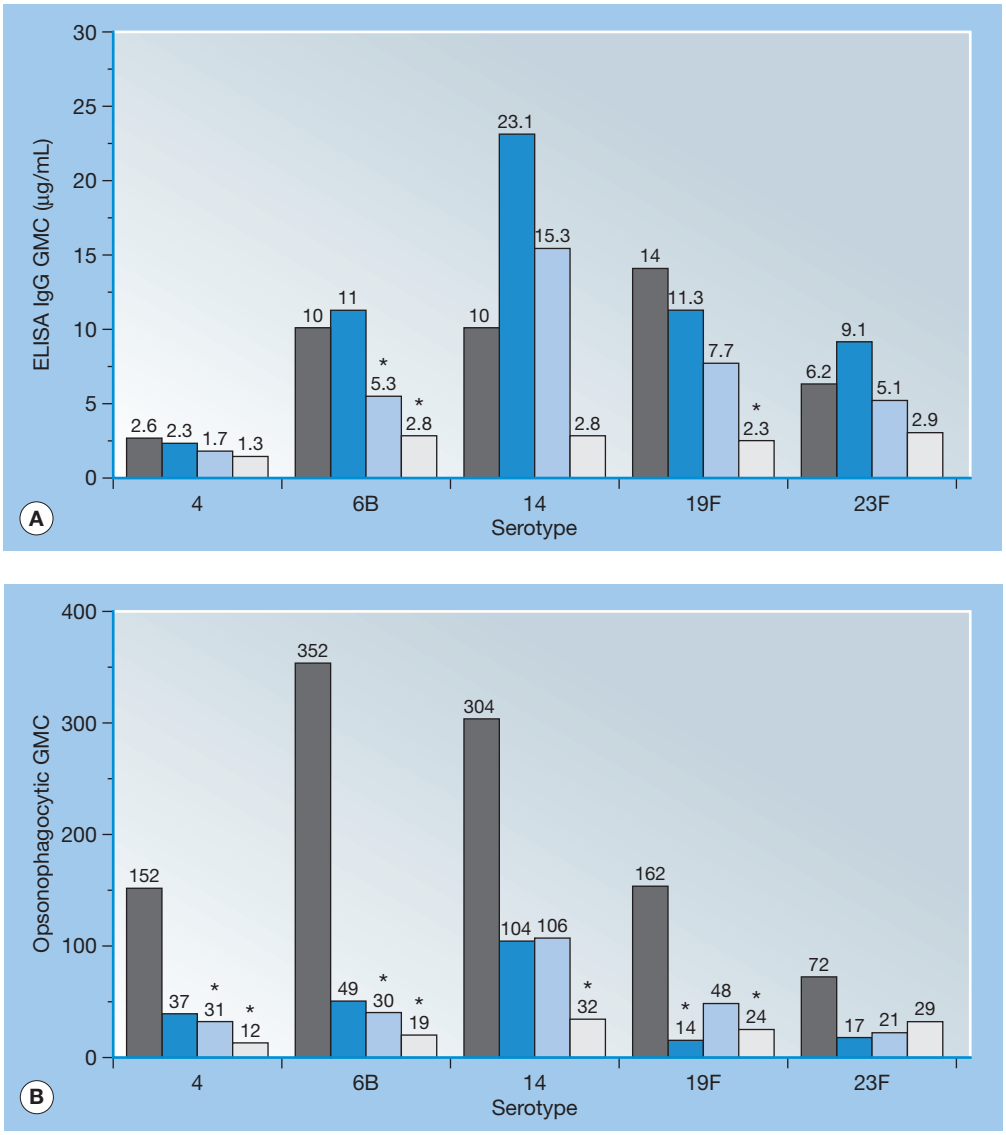


Figure 47.4. Comparison of serotype-specific enzyme-linked immunosorbent assay (ELISA) immunoglobulin (Ig) G geometric mean concentrations and opsonophagocytic antibody geometric mean titers in young adults and elderly adults. **A,** Geometric mean concentration (GMC, µg/mL) of IgG pneumococcal capsular polysaccharide-specific serum antibody, as determined by ELISA, for young control subjects (22–46 years of age; $n = 12$; *gray bars*) and for elderly age groups (63–79 years of age; $n = 10$, *dark blue bars*; 80–89 years of age; $n = 22$, *light blue bars*; and ≥90 years of age; $n = 14$, *white bars*) after vaccination with PPSV23. **B,** Geometric mean opsonophagocytic titer (GMT, reciprocal serum dilution) for the young control subjects and elderly age groups shown in panel **A** after vaccination. An *asterisk* designates a significant difference ($P < .05$) from values for the younger control subjects, per the Wilcoxon sample test. *(From Romero-Steiner S, Musher DM, Cetron MS, et al. Reduction in functional antibody activity against Streptococcus pneumoniae in vaccinated elderly individuals highly correlates with decreased IgG antibody avidity.* Clin Infect Dis. *1999;29:281–288.)*

in context with PCV13 in these patients is described in the later section on vaccination policy and recommendations.

HIV Infection

A large number of studies have evaluated the immunogenicity of PPSV23 in HIV-infected persons.[277–295] In HIV-infected persons who are not receiving highly active antiretroviral therapy (HAART), the response to PPSV23 is poor when compared with healthy control subjects, whether assayed by ELISA or OPA.[214,277–293] In these studies, postvaccination antibody levels in the HIV-infected groups have not consistently correlated with $CD4^+$ T-cell counts at time of vaccination.[283,284,286,288,289,291–293,296] Antibody levels after vaccination decline at about the same rate in HIV-infected and uninfected persons, but, because peak levels are lower in HIV-infected patients, a decline to undetectability could occur sooner in HIV-infected persons.

Preservation or reconstitution of immune function with antiretroviral therapy may be associated with an improved antibody response,[297] although conflicting results have been reported.[291,298,299]

Before the introduction of HAART, HIV-infected children demonstrated poor response to PPSV23.[300] A study in the post-HAART era documented an improved response, although it was still lower than in healthy control subjects.[277] In that study, a better antibody response in the HIV-infected children was correlated with a higher CD4 count at the time of vaccination.

Rodriguez-Barradas and colleagues found that in HIV-infected patients with CD4 cell counts of 200 or higher, delaying PPSV23 until the patient has received 6 or more months of HAART did not improve responses and may lead to missed opportunities for immunization.[301]

Hematopoietic Stem Cell Transplant

Among hematopoietic stem-cell transplant recipients, the antibody response to PPSV23 improves with increasing time after transplantation,[302–304] and so in the past, an initial PPSV23 vaccination was recommended at 12 months after hematopoietic stem-cell transplant, with a second vaccination 12 months later. However, the antibody response to PPSV23 vaccination is limited in this situation,[304–307] particularly in patients receiving corticosteroid treatment for graft-versus-host disease,[308] and is not improved with a second vaccination 12 months later.[309]

Solid-Organ Transplant

Solid-organ recipients can respond to PPSV23,[310–313] but the immune response is of lower magnitude[314] and of shorter duration[264] than in normal control subjects. Antibody levels decline rapidly after such vaccination.[315] Revaccination 2 years later leads to modest increases in antibody levels, but most do not reach levels seen initially.[316] In a randomized trial of adult renal transplant recipients, the opsonophagocytic antibody response to PCV was comparable to that of PPSV23.[313] At 3 years after vaccination, serotype-specific antibody titers had declined significantly compared with 8 weeks postvaccination for both the PCV7 and PPSV23 groups, with the two vaccine groups having comparable antibody concentrations.[317]

Renal Disease

Children with nephrotic syndrome[275,318] and children and adults with other chronic renal diseases and those on dialysis usually respond adequately to PPSV23.[319] However, in most of these patients, antibody levels rapidly decline.[318,320] It is uncertain to what extent this decline reflects poor antibody synthesis or loss of antibody in the urine. Revaccination 1 year later leads to good antibody responses in about one-half of renal disease patients, but antibody levels decline rapidly over the next few months.[319]

Hematologic Neoplasms

Several factors influence the immune responses to PPSV23 among patients with hematologic neoplasms, such as chronic lymphocytic leukemia, Hodgkin disease, and multiple myeloma. These factors include the stage of disease, whether or not a splenectomy has been performed, the extent of previous radiation therapy or chemotherapy, and the time between completion of treatment and vaccination.[321] See also Chapter 69.

Few patients with chronic lymphocytic leukemia respond to pneumococcal vaccine, even when they are not receiving chemotherapy.[322,323] Those who do respond have less-advanced disease and higher levels of IgG, IgG_2, and IgG_4.[323] Administration of granulocyte-macrophage colony-stimulating factor does not improve the immune response to PPSV23 in this population.[324] Children with acute leukemia who are on maintenance chemotherapy have poor antibody responses.[325] In contrast, a small study found that those in remission for 2 or more years responded well to PPSV23 vaccination.[326]

Patients with Hodgkin disease given PPSV23 before the onset of treatment have postvaccination antibody levels (as measured by ELISA) comparable to those of healthy vaccinated persons.[327,328] Once treatment begins, antibody responses to vaccination are reduced.[329] After completion of treatment, antibody responsiveness may be less than in untreated controls. Patients who do not respond to a first dose of PPSV23 tend not to respond to revaccination several months later.[330] These findings imply that Hodgkin disease patients should be vaccinated as soon as possible after the diagnosis is established and before treatment begins.[14,331–333]

Patients with multiple myeloma or Waldenström macroglobulinemia have low pneumococcal antibody levels before vaccination. After vaccination, antibody responses are poor, although some patients respond to a few serotypes.[331–336] The capacity to respond does not correlate with the severity of the disease or the timing of chemotherapy, and antibody levels are seldom sustained.

Functional or Anatomic Asplenia

Antibody responses to PPSV23 in postsplenectomy patients vary, largely because some patients are immunocompromised and others are not. After splenectomy for trauma, in presumably immunocompetent adults, antibody responses to PPSV23 are similar to those in normal control subjects.[337–340] In postsplenectomy trauma patients, opsonophagocytic antibody titers are higher in patients vaccinated 14 or more days after the splenectomy compared with those vaccinated on days 1 or 7 after splenectomy.[338] There is no difference in antibody titers in persons vaccinated at 28 days compared with 14 days.[337,341] In patients who undergo splenectomy for hematologic disease, responses are more variable,[275,327,328] antibody levels may decline rapidly after vaccination, and persons who do not respond to an initial vaccination may also have a poor response to revaccination.[342]

Other Conditions

In one small study, patients with solid tumors had an antibody response to vaccination that was similar to healthy control subjects.[343] The response to PPSV23 vaccination in persons with human T-cell lymphotropic virus II infection is comparable to the response in healthy adults in terms of magnitude, quality, and functional activity.[344]

Patients with systemic lupus erythematosus,[345–347] rheumatoid arthritis,[346] Felty syndrome,[348] and celiac sprue[349] are at increased risk for pneumococcal infections. Patients with systemic lupus erythematosus or rheumatoid arthritis may have a diminished response to PPSV23 compared with healthy persons.[346,350] Those with celiac sprue appear to respond normally.[349]

In patients with rheumatologic conditions, such as rheumatoid arthritis, ankylosing spondylitis, or psoriatic arthritis, treatment with anti–tumor necrosis factor (e.g., adalimumab, etanercept, infliximab) does not appear to impair the antibody response to PPSV23, although methotrexate therapy, given with or without anti–tumor necrosis factor therapy, reduces this response.[351–358] Other immunomodulatory agents used for the treatment of rheumatologic conditions may also influence the immune response to PPSV23. In healthy volunteers, a single dose of abatacept, a selective costimulatory modulator that inhibits CD28-dependent T-cell activation, reduced the immune response to PPSV23.[359] Similarly, rituximab, a monoclonal antibody that targets $CD20^+$ B cells, reduces the immune response to PPSV23 in patients with rheumatoid arthritis.[358,360] Together, these results suggest that, if possible, PPSV23 should be given to patients with rheumatologic disorders before initiation of treatment with agents

that diminish the immune response to vaccination (e.g., methotrexate, abatacept, rituximab).

In a prospective trial, patients with inflammatory bowel disease who were not receiving immunosuppressive therapy responded as well to PPSV23 as healthy control subjects.[361] The response to vaccination was lower in patients who were receiving anti–tumor necrosis factor therapy and concomitant immunomodulator therapy (e.g., 6-mercaptopurine, azathioprine, methotrexate). In one small study of adults with panhypopituitarism who were receiving glucocorticoid replacement as well as other conventional replacement therapy, a lower response to PPSV23 was found, compared with healthy control subjects of similar age.[362]

EFFICACY OF PNEUMOCOCCAL POLYSACCHARIDE VACCINES

Table 47.1[194,195,197–206] and Table 47.2[363–386] summarize key parameters of contemporary PPSV randomized clinical trials and observational studies of efficacy and effectiveness, respectively. Table 47.3 focuses on studies assessing pneumococcal pneumonia,[372,377,386,390–395] many of which were conducted before diagnostic tools to differentiate bacteremic (invasive) and nonbacteremic (noninvasive) pneumococcal pneumonia became available. The metaanalysis updated by the Cochrane Collaboration in 2013 included 25 randomized clinical trials (RCTs) and observational studies of various PPSVs that evaluated vaccine efficacy against pneumococcal outcomes.[396] Because *S. pneumoniae* was the principal cause of pneumonia in early studies, and bacteremia (which now represents approximately 25% of pneumococcal pneumonias) was often not sought by blood culture, some of these studies present data on all-cause pneumonia as well as on pneumococcal pneumonia or even vaccine-type–specific pneumococcal pneumonia. The vaccine-type analyses matched the valences included in the specific vaccines studied.

The primary outcomes of the most recent Cochrane Review included IPD and all-cause pneumonia.[396] The IPD analysis included 11 RCTs (with 18,634 vaccinees and 17,855 controls), yielding an odds ratio (OR) of 0.26 (95% confidence interval [CI], 0.14–0.45), corresponding to a protective vaccine effect of 74%. The corresponding values for seven observational studies were OR 0.48 (95% CI, 0.37–0.61), corresponding to efficacy of 52%. The all-cause pneumonia measure included 16 RCTs (with 22,643 vaccinees and 25,091 controls), yielding an OR of 0.72 (95% CI, 0.56–0.93), corresponding to a vaccine effect of 28%, albeit with statistical heterogeneity. Selected secondary outcomes include the following[396]:

- Vaccine-type IPD: five RCTs (13,889 vaccinees and 17,334 controls), with an OR of 0.18 (95% CI, 0.10–0.31).
- Definitive vaccine-type pneumococcal pneumonia (clinically and radiographically confirmed, with *S. pneumoniae* isolated from usually sterile site): four RCTs (15,583 vaccinees, 14,978 controls), with an OR of 0.13 (95% CI, 0.05–0.38).
- Presumptive vaccine-type pneumococcal pneumonia (clinically and radiographically confirmed, with *S. pneumoniae* isolated from culture of sputum or nasal swab): five RCTs (8,755 vaccinees, 9,813 controls), with an OR of 0.27 (95% CI, 0.08–0.87).

A few selected studies from the 2013 Cochrane metaanalysis are described further below, as examples of the range of published data considered.

In the Veterans Administration Cooperative Study conducted in the United States (see Table 47.1), 2295 immunocompetent veterans, who were defined as high-risk on the basis of age (≥55 years) and/or the presence of chronic cardiac, pulmonary, renal, or hepatic disease, and/or diabetes mellitus or alcoholism, were randomized to receive either PPSV14 or placebo.[199] Only one case of vaccine-type invasive pneumococcal infection occurred in each group. The frequency of probable pneumococcal pneumonia, defined as radiographically confirmed pneumonia with pneumococci isolated from sputum, was also balanced between the groups, although the cohorts were not balanced at baseline for history of prior pneumococcal infection.

Two clinical trials (see Table 47.1) conducted in Finland[202,203] and one conducted in Sweden[201] assessed PPSV23 efficacy against all-cause pneumonia and against an outcome of pneumococcal pneumonia that was largely defined on the basis of serologic testing for antibodies to pneumolysin in circulating immune complexes. These studies showed no protection from pneumococcal vaccine, but the number of disease outcomes was small, the overall rate of pneumonia was far greater than might have been expected for pneumococcal pneumonia, and the method for diagnosing pneumococcal pneumonia was subsequently shown to be invalid.[397] In one of these studies,[201] which was limited to people with a prior history of pneumonia, six cases of bacteremic pneumococcal pneumonia were identified, five of which were in the placebo group, for a 79% reduction in risk of this outcome ($P = .2$).

Most recently, a randomized, placebo-controlled, observer-blind trial was conducted among nursing home residents (mean age: 85 years) in Japan.[205] The study focused on pneumococcal pneumonia, defined by isolation of *S. pneumoniae* from blood, pleural fluid, or sputum, or by a positive BinaxNOW urine antigen test. The incidence of pneumococcal pneumonia per 1000 person years was 32 in the placebo group and 12 in the PPSV23 group ($P < .001$). Death from pneumococcal pneumonia was significantly higher in the placebo group than in the PPSV23 group (35.1% [13/37] vs 0% [0/14], $P < .01$). The Kaplan-Meier survival curves of participants without pneumococcal pneumonia in the placebo and PPSV23 groups generated a hazard ratio of 0.368 (95% CI, 0.199–0.680).

Because the incidence of *S. pneumoniae* as a cause of pneumonia has steadily declined over the past five decades, recent studies looking at all-cause pneumonia are becoming less likely to inform our understanding of pneumococcal-vaccine performance. For example, the U.S. Department of Defense recently conducted a RCT of PPSV23 in 153,000 military trainees, with all-cause pneumonia as one of the principal outcomes.[398] In that study, zero cases of pneumococcal pneumonia were identified in either the vaccine or the control group. The authors concluded that their study found a lack of PPSV23 effectiveness in that setting, but a more reasoned statement would be that PPSV23 was administered in a setting where *S. pneumoniae* disease was uncommon; hence no conclusion about PPSV23 effect could be reached. Their conclusion that PPSV23 is not appropriate for military recruits may be valid because of the apparent rarity of pneumococcal pneumonia in that population, but not because of inadequate vaccine effect.

Other Observational Evidence of Vaccine Effect

Case-control and other types of observational studies have generally shown that PPSVs effectively prevent pneumococcal pneumonia or IPD in immunocompetent adults (see Table 47.2), similar to the findings of the RCTs. In the 2013 iteration of the Cochrane metaanalysis, the OR for vaccine-type IPD in these studies was 0.45 (95% CI, 0.38–0.54).[396]

TABLE 47.2 Observational Studies of Pneumococcal Polysaccharide Vaccine Effectiveness Against Invasive Pneumococcal Disease in Non–HIV-Infected Populations

Reference	Design	Study Population	Subgroup	No. Cases of Invasive Pneumococcal Disease	VE (%)	95% CI
Shapiro[363] (1984)	Case control	Adults with an indication for pneumococcal vaccine admitted to Yale–New Haven hospital in Connecticut	All	90	67	13–87
			Immunocompromised	20	0	–1228 to 93
Bolan[364] (1986)	Indirect cohort	Isolates submitted to CDC	All	1887	64	47–76
			65+ with diabetes, heart disease, lung disease, or no underlying illness	199	61	53–80
Forrester[365] (1987)	Case control	Persons age ≥55 yr admitted to the Denver Veterans Administration Hospital	All	89	–34	–176 to 35
	Indirect cohort			89	–21	–221 to 55
Sims[366] (1988)	Case control	Immunocompetent persons age ≥55 yr admitted to 1 of 5 hospitals in Pennsylvania	All	122	70	36–86
Shapiro[367] (1991)	Case control	Adults with an indication for pneumococcal vaccine admitted to 1 of 11 hospitals in Connecticut	All	983	56	42–67
			Immunocompetent	808	61	47–72
			Immunocompromised	175	21	–55 to 60
	Indirect cohort		All	932	48	3–72
Butler[368] (1993)	Indirect cohort	Persons age ≥5 yr in the United States	All	2837	57	45–66
			Immunocompetent age ≥65 yr	525	75	57–85
			Anatomic asplenia, excluding persons with sickle-cell disease	166	77	14–95
Farr[369] (1995)	Case control	Persons age ≥2 yr with an indication for pneumococcal vaccination admitted to the University of Virginia Health Sciences Center in Virginia	All	85	81	34–94
Fiore[370] (1999)	Indirect cohort	Children age 2–5 yr with chronic illness[a] in the United States	All	173	63	8–85
			Sickle-cell disease	46	62	–294 to 98
			Other chronic illness	127	84	40–96
Benin[371] (2003)	Case control	Navajo adults age ≥18 yr with an indication for vaccination	All	108	26	–29 to 58
	Indirect cohort	Navajo adults age ≥18 yr	All	278	35	–33 to 69
Jackson[372] (2003)	Cohort	Community-dwelling members of a Washington State health plan age ≥65 yr	All	61	44	7–67
			Immunocompetent	39	54	13–76
			Immunocompromised	22	22	–87 to 68
Hedlund[373] (2003)	Cohort	Residents of Stockholm County, Sweden age ≥65 yr who were invited to receive influenza and pneumococcal vaccines in the fall of 1998	All	40	48	3–72
Adamkiewicz[374] (2003)	Indirect cohort	Children age <18 yr with sickle-cell disease at 8 centers in the United States	All	79	64	–6 to 88
Andrews[375] (2004)	Indirect cohort	Persons age ≥65 yr in Victoria, Australia	All	98	79	–14 to 96

TABLE 47.2 Observational Studies of Pneumococcal Polysaccharide Vaccine Effectiveness Against Invasive Pneumococcal Disease in Non–HIV-Infected Populations *(Continued)*

Reference	Design	Study Population	Subgroup	No. Cases of Invasive Pneumococcal Disease	VE (%)	95% CI
Dominguez[376] (2005)	Case control	Persons age ≥65 yr admitted to 12 hospitals in Catalonia, Spain	All	149	70	48–82
			Immunocompetent	103	76	51–88
			Immunocompromised	31	50	–44 to 82
Vila-Córcoles[377] (2006)	Cohort	Community-dwelling seniors age ≥65 yr in Catalonia, Spain	All	22	40	–165 to 78
Singleton[378] (2007)	Indirect cohort	Alaska Native adults	All	300	75	27–91
			20–39 yr	111	100	78–100
			40–54 yr	86	73	<0–96
			≥55 yr	103	<0	<0–78
Bliss[379] (2007)	Indirect cohort	White Mountain Apache adults ≥18 yr	All	92	68	3–90
			Persons without alcoholism	NR	96	47–100
			Persons with alcoholism	NR	25	–241 to 82
Mooney[380] (2008)	Screening method	Persons age ≥65 yr in Scotland[b]	All	135	62	45–73
Vila-Córcoles[381] (2009)	Case control	Community-dwelling seniors age ≥50 yr in Catalonia, Spain	All	94	66	34–83
Moberley[382] (2010)	Screening method	Indigenous adults age 15–49 yr in the Northern Territory of Australia	All	385	3	–43 to 35
	Indirect cohort		All	342	57	15–78
Vila-Córcoles[383] (2010)	Case control	Community-dwelling seniors age ≥60 yr in Catalonia, Spain	All	88	66	37–82
Rudnick[387] (2011)	Cohort	Population of metropolitan Toronto and regional municipality of Peel	All ≥65 yr old	2464	69	33–85
			Adults with chronic disease	NR	52	34–65
Andrews[388] (2012)	Screening method	Community-dwelling seniors age ≥65 yr in England and Wales	All, <2 yr since vaccination	47	48[c]	32–60
			All, 2–5 yr since vaccination	58	21[c]	3–36
Menzies[384] (2014)	Screening method	Community-dwelling seniors age ≥65 yr in Australia	All	1415	61	55–67
Gutiérrez Rodríguez[389] (2014)	Screening method	Community-dwelling seniors age ≥60 yr in Madrid, Spain	All	588	73	59–82
	Indirect cohort		All	588	45	23–60
Leventer-Roberts[385] (2015)	Case control	Beneficiaries of Clalit Health Services, Israel	All	212	42	19–59
Tsai[386] (2015)	Cohort	Taiwan National Health Insurance Research Database	All >75 yr	57	76	54–88

[a]Chronic illness included sickle cell disease, malignancy, leukemia, nephrotic syndrome, HIV infection, organ transplantation, CSF leak, congenital anomalies, asplenia, and immunosuppressive medications.
[b]Excluded persons with asplenia, hematologic malignancies, and nonhematologic malignancies on chemotherapy.
[c]Higher effectiveness values found for those age 65–74 yr. Other subgroup analyses reported in publication.
CI, Confidence interval; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; NR, not reported.

A case-control study from Spain identified adults with radiographically confirmed pneumococcal pneumonia, defined by isolation of pneumococcus from blood culture or by sputum culture, or by a positive BinaxNOW *S. pneumoniae* antigen test.[383] PPSV23 was associated with a significantly lower risk of pneumococcal bacteremia caused by vaccine serotypes (OR: 0.24; 95% CI, 0.09–0.66) (see Table 47.2).

Several studies have used an indirect-cohort method to assess vaccine effect.[364,378,379,382] In that design, only cases of IPD are evaluated; there is no comparison group of uninfected

TABLE 47.3 Cohort Studies of 23-Valent Pneumococcal Polysaccharide Vaccine Effectiveness Against Pneumonia in Noninstitutionalized Seniors

Reference	Senior Population	Person-Years of Follow-Up	Outcome	No. of Events	Association of Vaccination and Risk of the Outcome (Relative Risk, 95% CI)	
					Adjusted for Age and Sex	Adjusted for Age, Sex, and Participant Characteristics
Nichol[390] (1999)	1898 members of an HMO in Minnesota with a diagnosis code of COPD assigned during the baseline year	Not reported	Hospitalization assigned a pneumonia or influenza diagnosis code	174	NR	0.57 (0.38–0.84)
			All-cause mortality	275	NR	0.71 (0.56–0.91)
Jackson[372] (2003)	47,365 members of an HMO in Washington	127,180	Ambulatory and hospitalized community-acquired pneumonia	4489	NR	1.07 (0.99–1.14)
			Hospitalized community-acquired pneumonia	1428	1.21 (1.08–1.35)	1.14 (1.02–1.28)
			All-cause mortality	5690	0.88 (0.84–0.93)	0.96 (0.91–1.02)
Vila-Córcoles[377] (2006)	11,241 seniors assigned to 1 of 8 primary care centers in Tarragona, Catalonia, Spain	33,905	Ambulatory and hospitalized community-acquired pneumonia	473	1.03 (0.86–1.24)	0.79 (0.64–0.98)
			Hospitalized community-acquired pneumonia	355	0.96 (0.78–1.19)	0.74 (0.59–0.92)
			All-cause mortality	1497	0.83 (0.75–0.92)	0.97 (0.86–1.09)
			Pneumonia mortality	60	0.55 (0.33–0.91)	0.41 (0.23–0.72)
Skull[391] (2007)[a]	1952 cases and 2927 cohort members of hospitalized seniors in Victoria, Australia	NA	Hospitalization assigned a pneumonia diagnosis code	1952	NR	1.01 (0.87–1.16)
Rodriguez-Barradas[392] (2008)	934 participants in the Veterans Aging Cohort 5-Site Study	Not reported	Outpatient or inpatient encounter with a pneumonia or pneumococcal ICD9 code	12	NR	0.85 (0.25–2.95)
Ochoa-Gondar[393] (2008)	1298 seniors assigned to 1 of 8 primary care centers in Tarragona, Catalonia, Spain who had a diagnosis of chronic respiratory disease	3676	Ambulatory and hospitalized community-acquired pneumonia	171	0.93 (0.68–1.28)	0.77 (0.56–1.07)
			Hospitalized community-acquired pneumonia	137	0.87 (0.61–1.23)	0.70 (0.48–1.00)
			All-cause mortality	275	1.22 (0.92–1.62)	1.20 (0.91–1.59)
Gilbertson[394] (2011)	118,533 hemodialysis patients	Not reported	Hospitalization assigned a pneumonia diagnosis code	NR	NR	≈0.95 (not significant)[b]
			All-cause mortality	NR	NR	0.94 (0.90–0.98)
Ochoa-Gondar[395] (2014)	Community-dwelling seniors age ≥60 yr in Tarragona, Spain	76,033 person-years	Nonbacteremic pneumococcal community-acquired pneumonia	72	1.10 (0.69–1.77)	0.52 (0.29–0.92)
			Combined bacteremic and nonbacteremic pneumococcal community-acquired pneumonia	84	0.97 (0.63–1.50)	0.49 (0.29–0.84)
Tsai[386] (2015)	Taiwan National Health Insurance Research Database	Not reported	Pneumonia hospitalization, adults age ≥75 yr	57	60	59–61

[a]Case cohort study design.
[b]Extrapolated data.
COPD, Chronic obstructive pulmonary disease; HMO, health maintenance organization; ICD, International Classification of Diseases; NA, not applicable; NR, not reported.

persons. Vaccine effectiveness is estimated in patients with IPD by comparing the distribution of pneumococcal serotypes in vaccinated and unvaccinated subjects. A healthy-user effect should not influence the outcome; however, the method requires a similar proportion of vaccine-type and non–vaccine-type disease in unvaccinated populations.[399,400] For example, using this approach, Broome and colleagues found 64% efficacy in preventing IPD caused by vaccine types but no effect in nonvaccine types.[399] In three studies that used both the indirect-cohort and case-control methods,[365,367,371] the results of the two methods were similar.

A large indirect-cohort study included 2837 persons, 5 years of age and older, with pneumococcus isolated from blood or CSF at one of 58 U.S. hospitals that participated in a CDC surveillance system.[368] The overall vaccine effectiveness estimate was 57% (95% CI, 45–66%). Subgroup analyses showed similar levels of vaccine effectiveness for persons with diabetes mellitus, coronary vascular disease, congestive heart failure, chronic pulmonary disease, and anatomic asplenia, but not for persons with alcoholism/cirrhosis. Vaccination was associated with a significant reduction in risk of IPD in the smaller subgroups of persons with anatomic asplenia, but not for sickle cell disease, chronic renal failure, immunoglobulin deficiency, leukemia/lymphoma, or multiple myeloma, which raises the important issue of whether PPSV is effective in immunocompromised hosts.

In several studies, adults hospitalized with pneumonia have been evaluated to identify an association between prior PPSV23 and subsequent outcomes such as mortality.[394,401–404] Four studies reported that patients who had received PPSV23 were less likely to die, or be admitted to the intensive care unit, or to have a prolonged hospital stay, than unvaccinated patients,[394,401–403] although the possibility of confounding by underlying health status should be considered.[405] Two studies did not find an association between vaccination and length of survival after hospital discharge.[402,404]

In a retrospective cohort study of seniors enrolled in a health maintenance organization in western Washington State,[372] PPSV23 was associated with a 44% reduction in risk of pneumococcal bacteremia (see Table 47.2). Results of a large unrandomized cohort study from Tarragona, Spain, were published in 2013.[395] Cohort members were followed for 76,033 person-years, including 29,065 person-years for vaccinated subjects. After multivariable adjustment, vaccination with PPSV23 within 5 years was associated with reduced risks of bacteremic pneumococcal CAP (hazard ratio [HR], 0.38; 95% CI, 0.09–1.68), nonbacteremic pneumococcal CAP (HR, 0.52; 95% CI, 0.29–0.92), and overall pneumococcal CAP (HR, 0.49; 95% CI, 0.29–0.84).

Effectiveness of 23-Valent Pneumococcal Polysaccharide Vaccine in Immunocompromised Persons

Immunocompromised persons are at disproportionately high risk of IPD, in most instances because they have diminished capacity to make antibody to any vaccine. Not surprisingly, PPSVs are generally less effective or less immunogenic in immunocompromised persons than in relatively normal comparison groups. In most studies of immunocompromised persons, PPSV23 was not associated with a reduction in risk of IPD. The one study to find a significant reduction defined the immunocompromised subgroup as persons with immunodeficiency, asplenia, any solid-organ or hematologic cancer, chronic nephropathy, or long-term corticosteroid therapy.[384] Nearly all the IPD case patients defined as immunocompromised met the definition because of solid-organ cancers (stage not defined) or non–dialysis-dependent chronic renal failure. In the presumptively immunocompromised group (diagnosis not confirmed), PPSV23 vaccine effectiveness was estimated to be 71% (24–89%).

A nonrandomized, nonblinded study of persons 2 to 25 years of age with sickle cell disease evaluated 68 patients who were given a PPSV8 and a nonconcurrently enrolled comparison group of 106 unimmunized patients. During the 2-year follow-up period, eight of the unimmunized patients developed pneumococcal bacteremia compared with none of the immunized patients, which was interpreted as evidence for a protective effect of vaccination.[406]

Table 47.4 summarizes the results of published nonrandomized studies of PPSV23 effectiveness in HIV-infected persons.[392,407–419] Some were very small,[413,414] including only seven or 12 pneumococcal outcome events. The remaining studies evaluated a variety of outcomes, including IPD and noninvasive pneumococcal infection.

Four studies evaluated an outcome of IPD (or pneumococcal bacteremia) in HIV-infected people.[410,415,416,418] Two found no association of receipt of PPSV23 and risk of IPD,[416,418] one found a significant reduction in risk of pneumococcal bacteremia in vaccinated compared with unvaccinated persons,[415] and one found that vaccination was associated with a reduction in risk of IPD in whites but not blacks.[410]

Gebo and Dworkin found a beneficial effect of vaccination that was restricted to subgroups with a higher (≥200 or ≥500) CD4 count at the time of vaccination.[407,411] In contrast, a more recent study by Peñaranda found a significantly lower risk of pneumococcal infection in vaccinated persons, even when persons with a CD4 count of less than 200 were included.[417] That study was limited by definition of vaccination status in cases as vaccine received before the pneumococcal disease onset date, while vaccination status in the control group was defined as receipt at any time during the study period, which may have led to a bias toward a higher vaccination rate in the control group and thus an inflated estimate of vaccine effect. French and colleagues failed to show any benefit from PPSV23 in patients in Uganda who had advanced AIDS and were not being treated for HIV infection.[420,421]

On the one hand, these findings are discouraging: those persons who are at great need of pneumococcal vaccination are least likely to be protected by it. On the other hand, it is not surprising that persons who have conditions in which antibody responses are greatly diminished—for example, multiple myeloma, Hodgkin disease, lymphoma, and untreated AIDS—tend to not respond well to vaccination. The clinical point is that some such persons do respond, and there is no way of predicting who they will be; hence PPSV23 is recommended.[14,331–333]

Association of 23-Valent Pneumococcal Polysaccharide Vaccine and Risk of Myocardial Infarction or Stroke

Pneumococcal pneumonia can precipitate acute myocardial events.[422,423] Therefore, it is plausible that pneumococcal vaccination could indirectly reduce the incidence of myocardial infarction.

Studies evaluating the association of PPSV23 vaccination and risk of cardiovascular events such as myocardial infarction have yielded diverging results. Some studies found no association between PPSV23 and risk of myocardial infarction or stroke.[424–426] Others did find a protective effect.[427,428] A meta-analysis of 11 studies calculated a protective role for PPSV23 for total cardiovascular events (relative risk [RR], 0.86; 95% CI, 0.76–0.97), but not for specific events.[429] A protective effect was more prominent in older persons and those with higher cardiovascular risk in that analysis. Another metaanalysis by

TABLE 47.4 Observational Studies of 23-Valent Pneumococcal Polysaccharide Vaccine Effectiveness in HIV-Infected Adults

Reference	Study Population	Study Period	Design	Outcome	No. of Events[a]	Subgroup	Relative Risk in Vaccinated vs. Unvaccinated	95% CI
Gebo[407] (1996)	Cohort of outpatients in Baltimore, MD	1990–94	Case control	Invasive pneumococcal disease (42%) or pneumococci isolated from respiratory secretions	85	CD4 >200 at vaccination	0.22	0.05–0.98
Guerrero[408] (1999)	Outpatients in Los Angeles, CA	1990–98	Case control	All-cause pneumonia	127	All	0.31	0.16–0.62
						CD4 <100 at vaccination	0.36	0.16–0.84
						CD4 100–199 at vaccination	0.23	0.08–0.67
						CD4 >200 at vaccination	0.22	0.09–0.54
Navin[409] (2000)	Hospitalized patients in Atlanta, GA	1994–96	Case control	All-cause pneumonia	211	All	0.68	0.41–1.15
Breiman[410] (2000)	Hospitalized patients in San Francisco, CA and Atlanta, GA	1992–95	Case control	Invasive pneumococcal disease	176	White race	0.24	0.09–0.65
						Black race	0.76	0.39–1.50
Dworkin[411] (2001)	Adult and Adolescent Spectrum of Disease Project cohort in the U.S.	1990–98	Cohort	Pneumococcal infection	585	$CD4^+$ <200 at vaccination	1.0	0.7–1.4
						$CD4^+$ 200–500 at vaccination	1.0	0.8–1.4
						$CD4^+$ ≥500 at vaccination	0.5	0.3–0.9
Lindenburg[412] (2001)	48 Injection-drug users in Amsterdam who seroconverted to HIV during follow-up	1994–98	Cohort	All-cause pneumonia	65	All	1.01	0.53–1.91
Hung[413] (2004)	Prospectively identified group of 305 patients who consented to receive PPSV23 and 203 patients who did not in Taiwan	2000–02	Cohort	Pneumococcal infection	7	All	0.08	0.01–0.73
Lopez-Palomo[414] (2004)	Cohort of 300 outpatients in Cadiz, Spain	1997–2000	Cohort	Pneumococcal pneumonia	12	All	0.28[b]	0.07–1.06
Grau[415] (2005)	Cohort of outpatients in Barcelona, Spain	1997–2002	Case control	Pneumococcal bacteremia	122	All	0.39	0.15–0.97
Barry[416] (2006)	Cohort of outpatients in Baltimore, MD	1990–2003	Case control	Pneumococcal bacteremia	67	CD4 <300 at vaccination	0.7	0.4–1.3
						CD4 >300 at vaccination	0.8	0.4–1.7

TABLE 47.4 Observational Studies of 23-Valent Pneumococcal Polysaccharide Vaccine Effectiveness in HIV-Infected Adults *(Continued)*

Reference	Study Population	Study Period	Design	Outcome	No. of Events[a]	Subgroup	Relative Risk in Vaccinated vs. Unvaccinated	95% CI
Peñaranda[417] (2007)	Hospitalized patients in Spain	1995–2005	Case control	Pneumococcal infection	184	All	0.39	0.24–0.65
					69	CD4 <200	0.15	0.04–0.50[c]
					95	CD4 ≥200	0.55	0.31–0.99
Veras[418] (2007)	Inpatients and outpatient in Brazil	2000–04	Case control	Invasive pneumococcal disease	79		0.82	0.38–1.77
Rodriguez-Barradas[392] (2008)	692 participants in the Veterans Aging Cohort 5-Site Study	2001–04	Cohort	All-cause pneumonia	85	All	0.65	0.42–1.00
Teshale[419] (2008)	Adults in the Adult/ Adolescent Spectrum of HIV Disease (ASD) project	1996–2003	Cohort	All-cause pneumonia	4135	All	0.8	0.8–0.9

[a]Number of events = number of cases in a case-control study.
[b]At vaccination, 50 patients (31%) had a $CD4^+$ T-cell count <200/mL.
[c]These numbers represent a correction supplied by the author to the confidence interval reported in the publication.
CI, Confidence interval; PPSV, pneumococcal polysaccharide vaccine.

Ren and colleagues found a decreased risk of acute coronary syndrome (OR, 0.83; 95%CI, 0.71–0.97), but not for stroke (OR, 0.96; 95% CI, 0.87–1.05), in people 65 years or older.[430]

Vaccine Evaluations in Children

Clinical Trials in Immunocompetent Children

Several randomized controlled trials in the 1980s assessed the efficacy of eight-valent and 14-valent PPSVs in preventing otitis media in children younger than 6 years of age, an approach now superseded by the availability of PCVs for healthy children.[431–440] In Australia, a randomized, blinded trial showed no difference in the risk of acute otitis media in children younger than age 2 years (RR, 1.01; 95% CI, 0.81–1.25) and a possible trend toward a reduction in risk in children older than age 24 months (RR, 0.83; 95% CI, 0.69–1.01).[431] A metaanalysis of these trials found no evidence for an overall effect across all age groups or for a reduction in risk of otitis in children younger than 24 months of age who received PPSV, but the pooled analysis of children older than 24 months of age showed evidence of a significant vaccine effect (RR, 0.78; 95% CI, 0.63–0.97).[439]

PPSV is associated with a reduction in risk of death attributed solely to acute lower-respiratory infection in young children in Papua New Guinea.[441–443] A clinical trial of more than 7000 children, 4 months to 5 years of age, were randomized either individually or by hamlet cluster to receive PPSV14 or PPSV23 or placebo. Vaccination was reported to have reduced acute lower-respiratory infection mortality by 59% overall (95% CI, 19–79%) and by 50% (95% CI, 1–75%) in children vaccinated at 2 years of age or younger.

Clinical Trials in Children With Sickle Cell Disease

One randomized trial[444] evaluated the efficacy of pneumococcal polysaccharide vaccination for prevention of pneumococcal infection in children with sickle cell disease. No protective effect was demonstrated.[445] Most of the participants were younger than 2 years, so the results are not generalizable to older children, in whom the vaccine may be more immunogenic.

A nonrandomized, nonblinded study of persons with sickle cell disease, 2 to 25 years of age, evaluated 68 patients who were given a PPSV8 and a nonconcurrently enrolled comparison group of 106 unimmunized patients. During the 2-year follow-up period, eight unimmunized patients developed pneumococcal bacteremia compared with none of the immunized patients, which was interpreted as evidence for a protective effect of vaccination.[406]

Observational Studies of Vaccine Effectiveness in Children

Two observational studies of pneumococcal vaccine effectiveness against IPD included both children and adults,[368,369] but neither presented results of subgroup analyses of persons younger than 18 years of age. Two other indirect-cohort studies included only children and have yielded varying estimates of the effectiveness of 23-valent pneumococcal polysaccharide vaccination against invasive pneumococcal infection in children with sickle cell disease in the era before the availability of PCV (see Table 47.2).

The first study, an indirect-cohort analysis of data from a national surveillance system conducted by Fiore and colleagues,[370] included children 2 to 5 years of age with chronic illnesses considered to be a risk factor for pneumococcal infection who had pneumococcus isolated from blood or CSF during 1984 to 1996. Of the 173 children included in the study, 46 had sickle-cell disease, and vaccine effectiveness was not demonstrated in that subgroup. A significant 84% reduction in risk of IPD was, however, found in the subgroup of children without sickle cell disease, the majority (62%) of

whom had malignancy, nephrotic syndrome, HIV infection, or organ transplant.

The second study, conducted in conjunction with a multisite study of regional pediatric sickle-cell programs and which included only children with sickle-cell disease, reported no significant association of ever-receipt of pneumococcal vaccine with risk of IPD.[374] However, an alternate analysis that defined vaccinated children as only those who had received PPSV23 within the previous 3 years compared that group with a combined group of unvaccinated children and children vaccinated 3 or more years earlier and found an estimated effectiveness of 80% (95% CI, 40–94%).

Vaccine Evaluations of Persons With Asplenia

Evidence supporting PPSV efficacy in persons with anatomic asplenia has been provided by one observational study and one ecologic study. In the indirect-cohort study by Butler and colleagues,[368] vaccine efficacy was 77% (95% CI, 14–95%) in persons with asplenia. The characteristics of the patients, in terms of their indication for splenectomy, time since splenectomy, number of previous pneumococcal vaccinations, and time since last vaccination, were not reported. An assessment in Denmark found no cases of pneumococcal bacteremia in 292 children with splenectomies performed in 1979 to 1987, when PPSV was used routinely, compared with six cases per 1000 person-years in children splenectomized in 1969 to 1978, before the availability of PPSV.[446] The possible impact of the recommended use of antimicrobial prophylaxis on the Danish findings could not be determined.

Persistence of Vaccine Effect

In a case-control study involving all cases of IPD reported by 11 major hospitals in Connecticut, Shapiro and colleagues found 56% efficacy for PPSV23, including immunocompetent and immunocompromised adults of all ages. Among adults younger than age 55 years, vaccine efficacy was 93%, 89%, and 85% at less than 3, 3 to 5, and more than 5 years elapsed, respectively, but the initial efficacy declined with age and subsided more rapidly with time following vaccination (Table 47.5).[367] For persons 75 to 84 years old, vaccine was effective for the first 3 years after administration, and for older subjects protection was not statistically significant. This kind of study could be influenced by a so-called healthy-user effect, in which persons who were vaccinated were more likely to take better care of their health needs or have access to care than those who were not vaccinated.

Butler and colleagues conducted an indirect-cohort study that reached the opposite conclusion on persistence of effect (see Table 47.5).[368] In that study, among persons 5 years of age and older (median age, 50–57 years; see Table 47.2), in which the majority of subjects were older than 50 years, vaccine effectiveness 5 to 8 years after vaccination was 71% (95% CI, 24–89%), and 9 or more years after vaccination, it was 80% (95% CI, 16–95%). In a cohort study of persons 65 years of age and older,[372] vaccine effectiveness within 5 years of vaccination was 48% (95% CI, 6–71%); in the 5 to 7 years after vaccination, it was 36% (95% CI, −41% to 71%); and more than 7 years after vaccination, it was 42% (95% CI, −70% to 80%) (author's unpublished data). Bolan and colleagues reached conclusions similar to Butler's (see Table 47.5).[364]

Other studies offer differing findings on whether protection does or does not persist beyond 5 years.[378,395] Additional evidence is needed to describe the hazard (or survival) function for how long clinical protection from PPSV23 persists. Knowing that function would enable evidence-based policy recommendations for PPSV23 revaccination intervals.

Outcomes of Revaccination. Two published studies evaluated the effect of revaccination. In a study of Alaska Native adults, the point estimates of vaccine effectiveness, as determined by the indirect-cohort method, were 73% for the first dose of PPSV23 (95% CI, 19–92%) and 74% for revaccination (95% CI, <0–94%).[378] In a case-control study of Navajo adults, the risk of IPD in persons who had received two or more doses of PPSV23 was compared with that in unvaccinated persons.[371] In that analysis, revaccination reduced the risk of IPD by 40%, but the finding was not statistically significant (95% CI, −27% to 72%). There was no overall effectiveness of PPSV23 in this study, and the estimate of effectiveness after revaccination was not significantly different from that of cohort including people who received either primary or revaccination.

TABLE 47.5 Durability of Pneumococcal Polysaccharide Vaccine Effectiveness Against Invasive Pneumococcal Disease in Adults: Diverging Results From Three Studies With Two Distinct Designs

		Vaccine Effectiveness,% (95% CI) Time Since Vaccination			
Shapiro et al	**Age (yr)**		**<3 yr**	**3–5 yr**	**>5 yr**
	<55		93 (82–97)	89 (74–96)	85 (62–94)
	55–64		88 (70–95)	82 (57–93)	75 (38–90)
	65–74		80 (51–92)	71 (30–88)	58 (–2 to 83)
	75–84		67 (20–87)	53 (–15 to 81)	32 (–67 to 72)
	≥85		46 (–31 to 78)	22 (–90 to 68)	–13 (–174 to 54)
Butler et al		**<2 yr**	**2–4 yr**	**5–8 yr**	**≥9 yr**
		51 (22–69)	54 (28–70)	71 (24–89)	80 (16–95)
Bolan et al		**<1 yr**	**1 yr**	**2 yr**	**3–5 yr**
		60 (30–78)	53 (–4 to 79)	67 (14–87)	53 (–16 to 81)

CI, confidence interval.

Data from Shapiro ED, Berg AT, Austrian R, et al. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. N Engl J Med. *1991;325:1453–1460; Butler JC, Breiman RF, Campbell JF, et al. Pneumococcal polysaccharide vaccine efficacy. An evaluation of current recommendations.* JAMA. *1993;270:1826–1831; and Bolan G, Broome CV, Facklam RR, et al. Pneumococcal vaccine efficacy in selected populations in the United States.* Ann Intern Med. *1986;104(1):1–6.*

Antibody Persistence in Immunocompetent Adults. In older adults, anticapsular antibody levels induced by a first dose of PPSV23 decline substantially in the year after vaccination,[236,242,447] but mean levels remain above baseline levels through 5 or 10 years of observation (Fig. 47.5).[245] Similarly, functional antibody levels after PPSV23 in adults persist above concentrations in unvaccinated adults for this same time period.[247] The few studies with contrary findings involve populations of nonambulatory adults or those with confounding host-factor issues.[247] There is an inverse association between circulating antibody level just before primary vaccination or revaccination and the antibody increase post-vaccination.[247]

Immune Response to Revaccination in Immunocompetent Adults. PPSV23 induces a T-cell–independent response; as a result, there is no classical anamnestic, or booster, response to revaccination. In fact, in 1950 Heidelberger observed that repeated vaccination of healthy young adults at close intervals was followed by hyporesponsiveness—a lesser response, relative to earlier responses. At present, the precise mechanism for this hyporesponsiveness at short intervals remains unclear, although in mice, the lack of response following rechallenge with capsular polysaccharide from *S. pneumoniae* type 3 has been attributed to the activity of suppressor T cells that persist for varying intervals after the initial challenge.[448]

Older adults who receive a second dose of PPSV23 several years after initial vaccination demonstrate significant increases in anticapsular antibody levels.[225,241,244–246,447,449–454] The relative magnitude of the immune response to a second compared with a first pneumococcal polysaccharide vaccination has been evaluated in several ways. A review of all studies that compared the postvaccination antibody response in persons being revaccinated with a concurrent group of persons being vaccinated for the first time found consistent vigorous, statistically increased antibody responses at intervals of 5 years or more.[247]

In a group of 189 subjects, 60 to 64 years of age, who received a first dose of PPSV23 at study enrollment and a second dose of PPSV23 approximately 3.5 years later,[455] OPA GMTs to 12 of the PPSV23 serotypes were assessed at 1 month after each vaccination. The GMTs after the second PPSV23 were significantly lower than those after the first vaccination for eight of those 12 serotypes. In contrast, Musher and colleagues revaccinated a small group of patients who recovered from pneumococcal pneumonia,[454] showing that persons immunized within the previous year did not respond at all to revaccination. Those who had been vaccinated between 1 and 5 years previously had blunted responses, but those whose first dose of PPSV23 was 5 years or more previously had comparable antibody responses to those seen after the first vaccination.

One large study ($n = 981$) that followed adults 50 years of age and older after either a first or second PPSV23, found that IgG levels at 30 days postvaccination in persons who had been vaccinated 3 to 5 years previously tended to be lower than in those receiving a first dose of PPSV23, although the differences were modest and generally not statistically significant.[244] Antibody levels declined over time, and at 5 years after the study vaccination, geometric mean concentrations were similar in both the primary and revaccinated groups (with both concentrations still greater than at baseline). A substudy that tested samples by OPA, obtained from a subgroup of 120 subjects 65 years of age and older, reported similar findings.[225] When this study was extended to measure antibody responses 10 years after a first vaccination with PPSV23, no blunting of IgG responses was detected (see Fig. 47.4).[245]

A large study of primarily Alaska Native/Native American older adults found that antibody levels, measured by ELISA or an OPA assay, were similar in adults receiving their first PPSV23 ($n = 123$) to those receiving a second ($n = 121$) or a third or fourth PPSV23 ($n = 71$) (minimum interval of 6 years after a previous PPSV23).[246] Taken together, these studies suggest that revaccination with PPSV23 after an interval of 5 years or longer is effective in stimulating IgG responses similar to those observed after an initial vaccination.

Revaccination in HIV-Infected Persons

There are few studies evaluating the response to revaccination with PPSV23 in HIV-infected populations. Revaccination 3 to 8 years after first vaccination is associated with significant increases in serotype-specific IgG concentrations,[290,456] although antibody responses may be lower than those following a first vaccination.[290] In patients who do not respond to a first vaccination, revaccination 6 to 24 months later does not stimulate an antibody response.[457] In HIV-infected adults who received PPSV23, followed several years later by a series of three doses of PCV13, PCV13-type antibody concentrations after each of the PCV13 doses reached repeated peaks of similar magnitude—not a crescendo-like booster effect.[458]

Although two early studies in the pre-HAART era reported transient increases in viral load in HIV-infected children and adults after PPSV23,[459,460] these results were not confirmed. Numerous later studies show that there is no increased viral load and no adverse HIV consequences of HAART-treated HIV-infected patients after PPSV23.[290,291,456,461–464]

VACCINE SAFETY

Common Reactions

The most common adverse event in people vaccinated with PPSV23 in clinical trials were injection-site pain or soreness (60%), injection-site swelling or induration (20%), headache (18%), injection-site erythema (16%), asthenia or fatigue (13%), and myalgia (12%). The details reported in one study in adults appear in Table 47.6.[245] These reactions usually resolved within 2 to 3 days of vaccination and were typically mild. Fever occurred in less than 3% of PPSV23 recipients. Mild systemic adverse experiences (e.g., tiredness, muscle aches, chills) occurred in about one-third of vaccine recipients (compared to approximately 10% of placebo recipients). Such reactions occurred in approximately 20% of elderly adults. The incidence of severe vaccine-related systemic adverse events was less than 5%, including myalgia (2%), fatigue (1%), and headache (1%). Findings were similar in a separate study of adults receiving first, second, third, or fourth doses of PPSV23 (Table 47.7).[246] The safety profile of PPSV23 in children is similar to that in adults.[275,300]

Rare Reactions

There are two published reports of apparent anaphylactic reactions to PPSV23. The first was a 2-year-old nonatopic boy who had small bowel and liver disease and was awaiting liver transplantation and who developed bronchospasm and cutaneous and laryngeal edema immediately after vaccination, which resolved with appropriate treatment.[465] A diagnostic evaluation demonstrated IgE antibodies specific for pneumococcal polysaccharides. The second was a 3-year-old girl who had received three doses of seven-valent PCV before the age of 1 year, and who had a severe anaphylactic reaction within minutes of receipt of PPSV23 at age 3 years.[466]

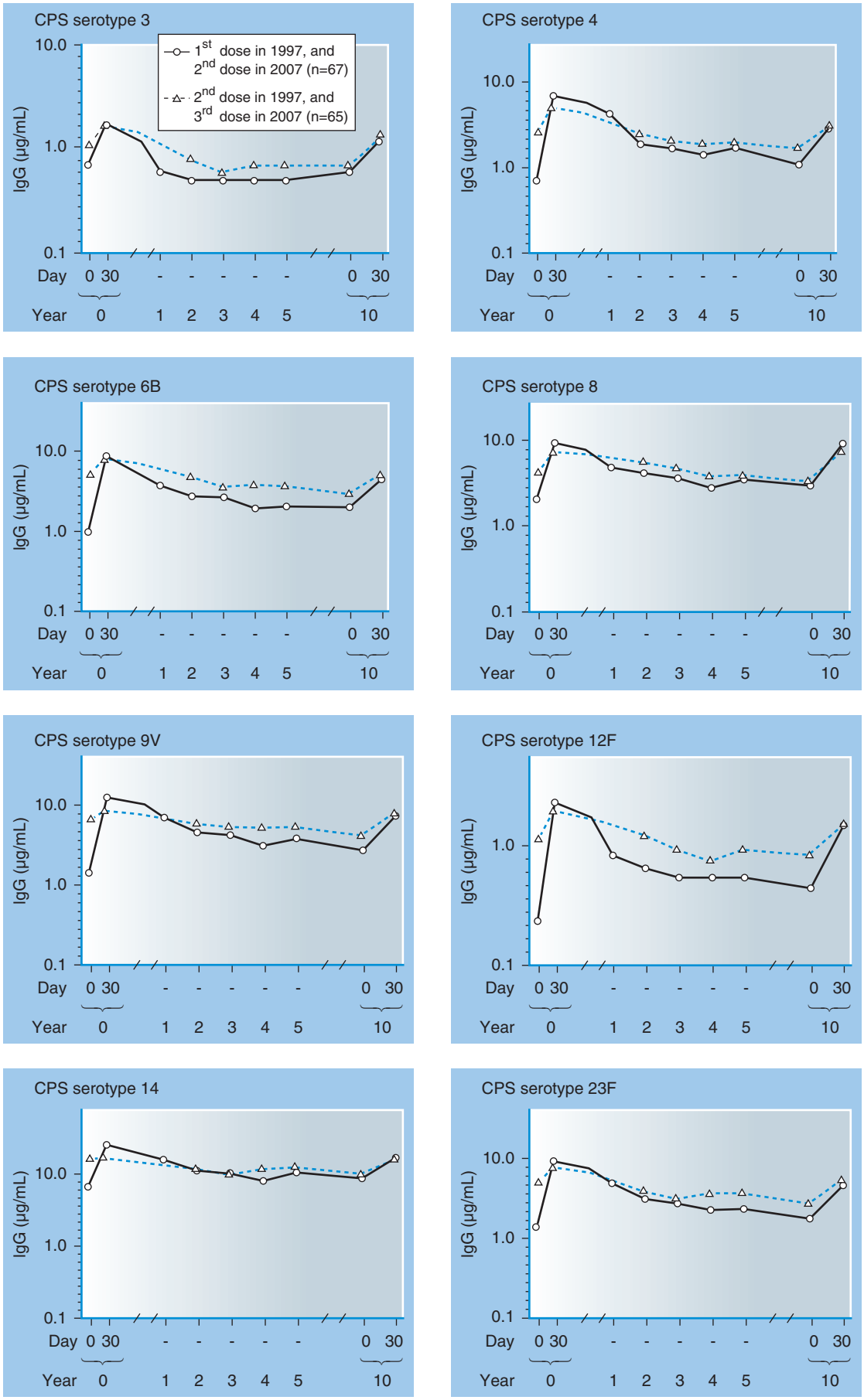


Figure 47.5. Immunoglobulin (Ig) G geometric mean concentrations (GMCs) to pneumococcal polysaccharides as measured by enzyme-linked immunosorbent assay (ELISA). The original protocol called for the annual collection of blood samples only from the first-dose recipients. A protocol amendment permitting the collection of annual blood samples from the second-dose recipients was implemented beginning in Year 2. CPS, capsular polysaccharide. *(From Musher DM, Manoff SB, McFetridge RD, et al. Antibody persistence 10 years after 1st and 2nd doses of 23-valent pneumococcal polysaccharide vaccine, and immunogenicity and safety of 2nd and 3rd doses in older adults.* Hum Vaccin. *2011;7:919–928.)*

TABLE 47.6 Proportion of PPSV23 Recipients With Injection-Site and Systemic Adverse Events by PPSV23 Dose Number

	Original Study		Extension Study	
Adverse Event	**1st PPSV23 Dose, % (N = 72)**	**2nd PPSV23 Dose, % (N = 71)**	**2nd PPSV23 Dose, % (N = 71)**	**3rd PPSV23 Dose, % (N = 71)**
ADVERSE EVENT AT INJECTION SITE				
Any	68	92	62	83
Pain	63	86	62	73
Swelling	19	56	20	38
Erythema	10	55	20	28
Severe pain	1	8	6	4
Severe swelling	1	20	4	4
Severe erythema	1	20	3	1
SYSTEMIC ADVERSE EVENTS				
Any	44	45	46	55
Fatigue	11	24	27	38
Myalgia	8	24	24	30
Headache	17	15	18	28
Chills	1	6	1	10
Temperature ≥37.8°C	0	1	0	6
Severe fatigue	1	4	0	3
Severe myalgia	1	4	0	1
Severe headache	0	4	0	3
Severe chills	0	0	0	0

Severe injection-site pain and severe systemic adverse events defined as incapacitating with inability to work or do usual activity.
Severe swelling or redness = maximum diameter ≥4 inches.
N, Number with follow-up; PPSV23, 23-valent pneumococcal polysaccharide vaccine.
From Musher DM, Manoff SB, McFetridge RD, et al. Antibody persistence 10 years after 1st and 2nd doses of 23-valent pneumococcal polysaccharide vaccine, and immunogenicity and safety of 2nd and 3rd doses in older adults. Hum Vaccin. *2011;7:919–928.*

TABLE 47.7 Reported Adverse Experiences Within 4 Days After Vaccination With PPSV23 in Subjects 55–74 Years of Age

Adverse Experience	Group 1 (1st dose of PPSV23), n (%) (N = 121)	Group 2 (2nd dose of PPSV23), n (%) (N = 119)	Group 3 (3rd or 4th dose of PPSV23), n (%) (N = 69)	*P* Value[a], Group 1 vs Other
Fever ≥37.5°C	4 (3)	7 (6)	3 (4)	.472 (.577)
Joint pain	24 (20)	45 (38)	23 (33)	.004 (.003)[b]
Chills	7 (6)	15 (13)	9 (13)	.099 (.072)
Fatigue	20 (17)	35 (29)	18 (26)	.019 (.027)[b]
Headache	11 (9)	30 (25)	9 (13)	.014 (.011)[b]
Redness, any	22 (18)	24 (20)	15 (22)	.433 (.685)
Redness, ≥4 cm	1 (1)	3 (3)	4 (6)	.133 (.155)
Swelling, any	21 (17)	36 (30)	23 (33)	.006 (.009)[b]
Swelling ≥4 cm	2 (2)	3 (3)	2 (3)	.701 (.709)
Severe arm soreness[c]	2 (2)	2 (2)	3 (4)	.829 (.709)
Moderate limitation in arm movement[d]	5 (4)	15 (13)	9 (13)	.025 (.015)[b]

[a]Mantel-Haenszel *P* value, adjusted for race (nonadjusted *P* value).
[b]Statistically significant differences.
[c]Soreness that completely prevents normal activities.
[d]Unable to raise arm above shoulder level.
N = number in each group who completed the reactogenicity questionnaire.
From Hammitt LL, Bulkow LR, Singleton RJ, et al. Repeat revaccination with 23-valent pneumococcal polysaccharide vaccine among adults aged 55–74 years living in Alaska: no evidence of hyporesponsiveness. Vaccine. *2011;29:2287–2295.*

Safety of Revaccination

In the early 1980s, revaccination was thought to be contraindicated because of concerns about injection-site reactions.[467] Those concerns were prompted by two studies conducted in the 1970s that reported a higher-than-expected frequency and severity of local injection-site reactions after revaccination of healthy adults within 2 years of their first vaccination.[468,469] Other reports documented limb swelling and other large local reactions in healthy adults and children vaccinated within 3 or 4 years of a first vaccination.[470,471]

Those initial policy restrictions have been lifted over time. Several more recent studies indicate that revaccination of older adults at intervals of 5 to 10 years tends to be associated with a higher frequency of injection-site reactions but is generally well tolerated (e.g., see Table 47.6).[245,447,449–451,472–474] These include both clinical trials and studies of large linked databases, summarized in a review article.[247] These injection-site reactions have been seen in individuals with preexisting antibody levels prior to the PPSV23 vaccination with higher preexisting antibody noted in those with shorter intervals between doses. Although injection-site reactions (e.g., pain, swelling, redness) were reported more commonly after PPSV23 revaccination than after primary vaccination in most studies, these reactions were typically mild to moderate in intensity and resolved within 5 days.[247]

The available data from two published retrospective studies indicate that a third dose of PPSV23 is not associated with an increased risk of medically attended adverse events in adults.[475,476]

INDICATIONS FOR 23-VALENT PNEUMOCOCCAL POLYSACCHARIDE VACCINE

In the United States and dozens of other countries, PPSV23 is indicated by the U.S. Food and Drug Administration (FDA) and other national regulators to help prevent pneumococcal disease in persons 50 years of age or older, as well as in persons older than 2 years of age who are at increased risk for pneumococcal disease (most often as a consequence of chronic health conditions).

General Recommendations in the United States

Adults

In the United States, the Advisory Committee on Immunization Practices (ACIP) recommends PPSV23 as the sole pneumococcal vaccine for immunocompetent adults 19 to 64 years of age who are at increased risk of IPD or its consequences (Table 47.8).[14,331–333] These subjects include persons with chronic heart disease (including congestive heart failure and cardiomyopathy, but excluding hypertension), chronic lung disease (including asthma, emphysema, and chronic obstructive pulmonary disease), diabetes mellitus, alcoholism, or chronic liver disease, and those who smoke cigarettes.

Currently,[491] the CDC recommends that immunocompromised adults and those adults 65 years or older receive a combined regimen of PCV13 followed by PPSV23.[14,331,492] A person is immunocompromised if the person has CSF leaks, cochlear implants, sickle cell disease, other hemoglobinopathies, congenital or acquired asplenia (i.e., functional or anatomic asplenia), congenital or acquired immunodeficiencies (including B- or T-lymphocyte deficiency, complement deficiencies [particularly C1, C2, C3, and C4 deficiencies], and phagocytic disorders [excluding chronic granulomatous disease]), HIV infection, chronic renal failure, nephrotic syndrome, leukemia, lymphoma, Hodgkin disease, generalized malignancy, iatrogenic immunosuppression (including glucocorticoids or radiation), solid-organ transplant, or multiple myeloma.

As of 2008, routine use of PPSV23 is no longer recommended for otherwise healthy Native American or Alaska Native persons younger than 65 years of age. This change from the prior recommendation for routine use of PPSV23 in Native American or Alaska Native persons 2 to 64 years of age was made in recognition of the fact that while those groups may have disproportionately higher rates of IPD, the bulk of the disease burden is among those with medical indications for PPSV23.[123,493] However, in special situations, public health authorities may recommend PPSV23 for Native American or Alaska Native adults 50 to 64 years of age who are living in areas where the risk of IPD is increased.

TABLE 47.8 Government-Funded Recommendations for Adult Pneumococcal Vaccination in Selected Countries

Country	People ≥60 or ≥65 Years	Adults With Specified Chronic Diseases, <65 Years	Adults With Immune Suppression	References (Year)
Australia	PPSV23	PPSV23	PPSV23	477 (2015)
Canada	PPSV23	PPSV23	PCV13 then PPSV23	478, 479 (2015)
Germany	PPSV23	PPSV23 or PCV13	PCV13 then PPSV23	480 (2014)
France	–	PPSV23	PCV13 then PPSV23	481 (2015)
Israel	PPSV23	PPSV23	PCV13 then PPSV23	482 (2015)
Japan	PPSV23	PPSV23 for 60–64	PPSV23 for 60–64	483 (2015)
Korea	PPSV23	PPSV23	PPSV23	484, 485 (2013)
Mexico	PPSV23	PPSV23	PPSV23	486 (2013)
Netherlands	–	PPSV23 in asplenia	–	487 (2012)
Sweden	PPSV23	PPSV23	PPSV23	488 (1994)
Taiwan	≥75 years old: PPSV23			489 (2013)
U.K.	PPSV23	PPSV23	PCV13 then PPSV23	490 (2014)
U.S.	PCV13 then PPSV23	PPSV23	PCV13 then PPSV23	14, 331–333 (2014)

PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.

Children and Adolescents

According to current recommendations of the ACIP and the American Academy of Pediatrics, U.S. children younger than 19 years of age with underlying medical conditions that are associated with an increased risk of IPD should receive PCV13 followed by PPSV23.[332,494] Underlying medical conditions that correspond with the recommendation for supplemental use of PPSV23 in children include chronic heart disease, chronic lung disease (including asthma), diabetes mellitus, CSF leaks, cochlear implant, alcoholism, sickle cell disease, other hemoglobinopathies, congenital or acquired asplenia, and immunocompromising conditions.[332,136,494] Receipt of PPSV23 is associated with a diminished immune response to PCV if given within a short interval[241,455]; therefore, all recommended doses of PCV13 should be given before the dose of PPSV23, at a minimum of 8 weeks after the last dose of PCV13.

Routine use of PPSV23 is not recommended for Native American or Alaska Native children. However, in special situations, public health authorities may recommend the use of PPSV23 after PCV13 for Native American or Alaska Native children 24 to 59 months of age who are living in areas in which risk of IPD is increased.[493]

The recommended immunization schedules for U.S. children, adolescents, and adults are updated annually and available at the CDC (http://www.cdc.gov/vaccines).

Revaccination

Revaccination with PPSV23 is recommended for certain subsets of primary vaccinees, such as certain subgroups of immunocompromised persons younger than 65 years. A single revaccination after 5 years is recommended for children and adults younger than 65 years who have chronic renal failure or nephrotic syndrome, functional or anatomic asplenia (e.g., sickle-cell disease or splenectomy), or another immunocompromising condition.[14,331,332,247] Some experts recommend revaccination of splenectomized patients every 6 to 7 years.

Vaccination of Specific Risk Groups

Persons With Functional or Anatomic Asplenia

PPSV23 is recommended for asplenic persons 2 years of age and older, after receiving PCV13. Patients scheduled for elective splenectomy should receive indicated vaccinations, which in children may include PCV13 and PPSV23, at least 2 weeks before the procedure. After emergent splenectomy, pneumococcal vaccination may begin as early as 1 day postoperatively, but there may be an advantage to delaying vaccination for 14 days postoperatively.

Persons With Cochlear Implants

In 2002, the FDA received reports of bacterial meningitis in persons with cochlear implants.[495] A subsequent investigation of 4264 children in the United States who received cochlear implants when they were younger than 6 years of age identified a rate of pneumococcal meningitis in the study population that was more than 30 times higher than that of children in the general U.S. population.[138] The risk of meningitis was significantly higher for patients with implants that included an electrode positioner, and those devices were estimated to account for approximately 50% of the cases of bacterial meningitis. A follow-up study found that children with implants with positioners remain at elevated risk of bacterial meningitis beyond 24 months after implantation, whereas a continued increase in risk was not found in children with implants without positioners.[496] Implant devices with electrode positioners were voluntarily recalled in 2002 and are no longer used.

Because of the increased risk of pneumococcal meningitis, all persons with cochlear implants should receive age-appropriate pneumococcal vaccination with both PCV13 and PPSV23, according to the schedules for persons at exceptionally high risk.[332,136] The dose of PPSV23 should follow at least 8 weeks after the last dose of PCV13. For maximal benefit, the doses of PCV13 and PPSV23 should be completed at least 2 weeks before implant surgery.

Persons Infected With HIV

In the United States, HIV-infected persons should receive PCV13 and then PPSV23 6 to 12 months later.[14,331,332] This approach is supported by the Infectious Diseases Society of America.[333] In patients whose CD4 cell count is greater than 200, there appears to be no benefit to waiting for the patient to receive 6 to 9 months of HAART before vaccinating, even if antiretroviral therapy has just been begun.[301] The Infectious Diseases Society of America recommends a second dose of PPSV23 5 years after the first.

Other Immunocompromised Persons

Pneumococcal vaccination is recommended for immunocompromised persons because of their increased risk of disease, although disease or therapy prevents many of these patients from deriving full benefit from any vaccination.[116,331–333] Persons being considered for immunosuppressive therapy should be vaccinated at least 2 weeks before treatment begins or, if this is not possible, 3 months after treatment stops because the antibody response to vaccination during or shortly after chemotherapy or radiation therapy is poor.

Recommendations in Other Countries

To date, most industrialized countries, including the United States, have retained PPSV23 as the sole recommended pneumococcal vaccine for adults 19 to 64 years old with health conditions predisposing to pneumococcal infection (see Table 47.8).[331–333,477–490] Several countries have adopted the combined PCV13-then-PPSV23 approach for immunocompromised adults, although in France and the Netherlands vaccination is recommended only for adults with high-risk conditions and not for healthy adults 65 years of age and older. In England, a risk group–only policy was implemented in 1992, with recommendations extended in 2003 to include all adults 65 years of age and older and, later, welders.[490] Table 47.8 shows that few countries have adopted government-sponsored recommendations for sequential use of PCV13-then-PPSV23 for people 65 years or older. While other national health authorities do not typically publish rationales for their decisions, the indirect effects among adults of pediatric PCV programs are typically cited as reducing the burden of pneumococcal disease sufficiently, such that they prefer to recommend PPSV23 solely, a product offering broader serotype coverage. Several health-economic models illustrate how this would be so.[497,498]

CONTRAINDICATIONS AND PRECAUTIONS

There are no contraindications to administering PPSV23 other than known hypersensitivity to a vaccine component or a previous severe reaction to the vaccine.

PUBLIC HEALTH CONSIDERATIONS

In the United States, achieving 90% coverage with pneumococcal vaccination of noninstitutionalized adults 65 years of age and older was a Healthy People 2000 and 2010 objective and is now a Healthy People 2020 objective (http://www.healthypeople.gov). Additional Healthy People 2020 objectives for pneumococcal vaccination include achieving 60% coverage for noninstitutionalized high-risk adults 19 to 64 years of age and achieving 90% coverage for institutionalized adults 18 years of age and older. In all recommended groups, actual vaccination levels remain far below target goals.

Among noninstitutionalized adults in the United States who are 65 years of age and older, PPSV23 coverage increased from 14% in 1989 to 60% in 2002 and has plateaued near that level ever since.[499] Vaccination rates among non-Hispanic blacks and Hispanics consistently have been substantially lower than among non-Hispanic whites. Racial disparities in PPSV23 coverage also exist among nursing-home residents.[500]

Coverage rates for PPSV23 are markedly lower among younger adults whose vaccination recommendation is based on underlying disease states. In 2012, the National Health Interview Survey estimated that only 20% of persons 19 to 64 years of age with one or more relevant health conditions had received PPSV23.[499]

Because increased PPSV23 coverage is an important public health goal, evidence-based evaluations of interventions to improve coverage are relevant.[501,502] Both the ACIP and the Task Force on Community Preventive Services recommend the use of standing order programs in both inpatient and outpatient settings to improve pneumococcal vaccination coverage. Standing orders programs, where allowed by state law, authorize nurses and pharmacists to administer vaccinations according to an institution- or physician-approved protocol without the need for a physician's examination or direct order.[503]

The Task Force found evidence that provider-reminder interventions, even when implemented alone, improved targeted vaccination coverage. Provider-reminder interventions are used to inform those who administer vaccinations that individual clients are due for specific vaccinations. The Task Force also recommended combinations of other interventions to improve vaccination rates. These include one or more interventions to enhance access to vaccination services (e.g., expand availability of vaccines in medical or other settings, or reduce out-of-pocket costs) with one or more provider- or system-based interventions and/or one or more interventions to increase client or community demand for targeted vaccination services. Examples include assessment and feedback for vaccination providers, provider education, client reminders, clinic-based education, communitywide education and policies or laws and regulations that require vaccination or other documentation of immunity as a condition of school attendance, residence, or employment.[501]

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48 Poliovirus Vaccine–Inactivated

Emmanuel Vidor

HISTORICAL INTRODUCTION

Not since the introduction of rabies vaccine by Louis Pasteur was public interest in vaccines stirred as much as by the development and testing of inactivated poliovirus vaccine (IPV), and not since Einstein had a scientist received the public adulation accorded to Jonas Salk, the vaccine's inventor. Contributing to this phenomenon were the rise of poliomyelitis as an epidemic disease, its notoriety with the public (augmented by the paralysis suffered by U.S. President Franklin Roosevelt), the publicity disseminated by the March of Dimes Foundation in its efforts to raise money for research, and the involvement of hundreds of thousands of U.S. children in the trial that demonstrated the efficacy of IPV. The trial was organized by Dr. Thomas Francis and sponsored by the National Foundation for Infantile Paralysis. It was a hallmark in vaccinology and the prototype for many later efficacy trials.[1–5] The trial began in April 1954, and the successful results were announced on April 12, 1955.[5] Licensure followed rapidly, with rapid and broad vaccine adoption in many countries.

Nevertheless, in the early 1960s, IPV was eclipsed by oral poliovirus vaccine (OPV), except in some northern European countries. Sixty years after its initial development IPV is renascent, owing to improvement in its manufacture and scaling up of capacities, its outstanding safety record, the major progress toward eradication of wild polioviruses, and the recognition that safety concerns associated with OPV (i.e., both sporadic vaccine-associated paralytic poliomyelitis [VAPP] cases and outbreaks of circulating vaccine-derived poliovirus [cVDPV]) require eventual cessation of OPV use. IPV, without those safety concerns, can provide a safety net to prevent reemergence of polio as OPV is removed from routine use.[6]

Disease caused by polioviruses is ancient. A famous Egyptian stele dating from 1403 to 1365 BC shows a man with flaccid paralysis of a leg. However, presumably owing to almost universal infection under the clinical protection of maternal antibodies, only sporadic cases were described until the 19th century. Early in that century, small outbreaks were noted in Europe, usually among infants living in rural areas. In 1870, Jean-Martin Charcot described the lesions in the gray matter of the spinal cord, and in 1890, Oscar Medin described a major outbreak in Sweden, where epidemics subsequently continued to occur. Epidemics were reported in the United States at the end of the 19th century, and in 1916 thousands of children were paralyzed during an epidemic in the northeastern United States. Fortunately, in 1908, Karl Landsteiner and Eric Popper isolated the virus of poliomyelitis, and studies of the agent began.[7]

The key discoveries that led to IPV development were as follows:

- Definition of the three serotypes of poliovirus by Bodian, Burnet, and colleagues.[8]
- Determination that poliovirus viremia precedes paralysis.[9]
- Confirmation that neutralizing antibodies protect against disease.[10]
- Demonstration by Enders and colleagues that the virus could be grown in cell culture.[11]

These discoveries permitted Salk, fresh from his success in developing an inactivated influenza vaccine as well as experienced in working with poliovirus, to start IPV development. Large quantities of virus were grown in roller tubes from monkey testicular and kidney cells and the kinetics of inactivation by formalin were studied. Salk concluded that if aggregates of virus were removed by filtration, poliovirus could be inactivated at a constant first-order rate, permitting complete killing if the process was of sufficient duration. Pools of trivalent vaccine were prepared at Connaught Laboratories in Toronto for use in the field trial of efficacy, which was conducted by Francis and associates in 1954. Although it did have some flaws,[12] the trial decisively demonstrated that IPV was protective, and in 1955 several U.S. manufacturers obtained licenses for their IPV and launched their products in the United States.[13] Very soon after, similar IPVs derived from the same concepts and developed by private or state-owned institutions were launched in different countries.[14–16] The Cutter incident (see "Adverse Events" later) in which recipients of IPV were paralyzed by residual live virus in the vaccine, underlined the necessity of removing viral aggregates to permit inactivation, but did not stop the use of IPV in many countries.

Years later, major developments improved the quality of IPV. The first, by van Wezel of Holland, was the development of techniques to select the best sources of monkey kidney cells, to grow the cells to high density on microbeads, and to concentrate the virus produced.[17] The second was the adaptation of the continuous African green monkey (*Cercopithecus aethiops*) kidney cell line (denominated Vero) to the production of poliovirus by Montagnon and colleagues at the Institut Mérieux (now Sanofi Pasteur) of Lyon, France.[18] The result of these improvements was the historically named enhanced-potency IPV (eIPV) which is the subject of this chapter, but because all IPVs now in use are of enhanced potency, hereafter the designation IPV will be used to refer to enhanced-potency IPV vaccines. (Later in the chapter, the cIPV will be used to distinguish the conventional IPV manufactured from wild-type poliovirus strains from sIPV, the Sabin IPV manufactured from the Sabin poliovirus strains. The IPV acronym will be used when referring to statements applicable to both types of IPV.)

The description of poliomyelitis as a disease, in addition to its virology, pathogenesis, and epidemiology, is discussed in Chapter 49. However, it is important to mention that the polioviruses are made of four capsid proteins, numbered VP1 to VP4 (Fig. 48.1). The first three are arranged on the surface with icosahedral symmetry whereas VP4 is an internal protein.[19] There are five epitopes present on VP1 to VP3 that are important to neutralization: sites 1, 2a, 2b, 3, and 4. These vary between serotypes and between strains, and precise mapping of their tridimensional structure helps identify their key immune targets.[20]

PASSIVE IMMUNIZATION

A field trial using human γ-globulin established the importance of viremia in the pathogenesis of the disease and the concept that antibodies were protective against paralysis. This trial, conducted in 1952 by Hammon and colleagues,[10]

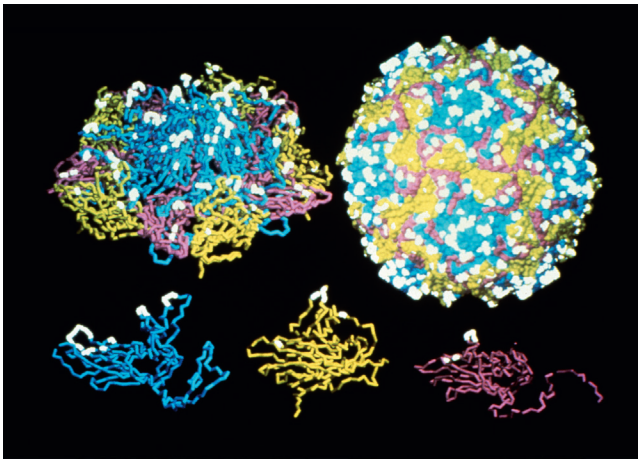


Figure 48.1. Antigenic sites of poliovirus are highlighted in white on the structure of the virus *(top right)*, a pentamer consisting of five copies of each of the capsid subunits *(top left)*, and the individual major capsid proteins VP1, VP2, and VP3 *(bottom left to right)*. VP1, blue; VP2, yellow; and VP3, red. *(Courtesy James Hogle and Arthur Olson.)*

involved more than 54,000 children, half of whom received γ-globulin and half of whom received gelatin. From the second to the eighth week after injection, paralytic poliomyelitis was reduced by 80%. Unfortunately, despite the large γ-globulin dose used in subjects (0.3 mL/kg), the protection proved temporary (8 weeks), rendering γ-globulin impractical as a public health strategy except in household contacts. Maternally produced antibodies transmitted via the placenta are also protective, and their half-life is only 28 days (see "Effect of Maternal Antibodies and of Neonatal Vaccination" later).

ACTIVE IMMUNIZATION

General Principles

IPV is a mixture of the three polioviruses made by harvesting cell culture supernatants and submitting them to purification and inactivation by formalin (one of the historical IPVs, developed by Lepine[21] at the Pasteur Institute in Paris, was inactivated by formalin and β-proprionolactone). The first IPV were produced from rhesus monkey (*Macaca mulatta*) primary kidney cell cultures, with all the problems of simian viruses that might be latent or replicating actively in cultured cells. The poliovirus strains used by Salk and by most manufacturers are wild-type polioviruses including Mahoney (type 1, the Brunhilde strain is still used by one manufacturer in Denmark), MEF-1 (type 2), and Saukett (type 3). During the past several years, IPV based on cultured and inactivated Sabin poliovirus strains (sIPV) has been developed and produced by a few manufacturers (see "Inactivated Polio Vaccine Manufactured from Sabin Strains" later).

Although the results of the historical Francis trial were positive (see "Efficacy of Inactivated Polio Vaccine Against Acute Flaccid Paralysis and Immune Correlates of Protection" later), the Cutter incident (see "Adverse Events" later) led to a change in manufacturing processes that lowered the immunogenicity of the early vaccine[22] and resulted in the resurgence of paralytic polio in vaccinated children during the late 1950s, which, in turn, weakened confidence in IPV.[23]

Fortunately, several technical advances during the 1970s permitted the rejuvenation of IPV, which, although based on principles similar to those of the first-generation vaccine, differs in three important aspects:

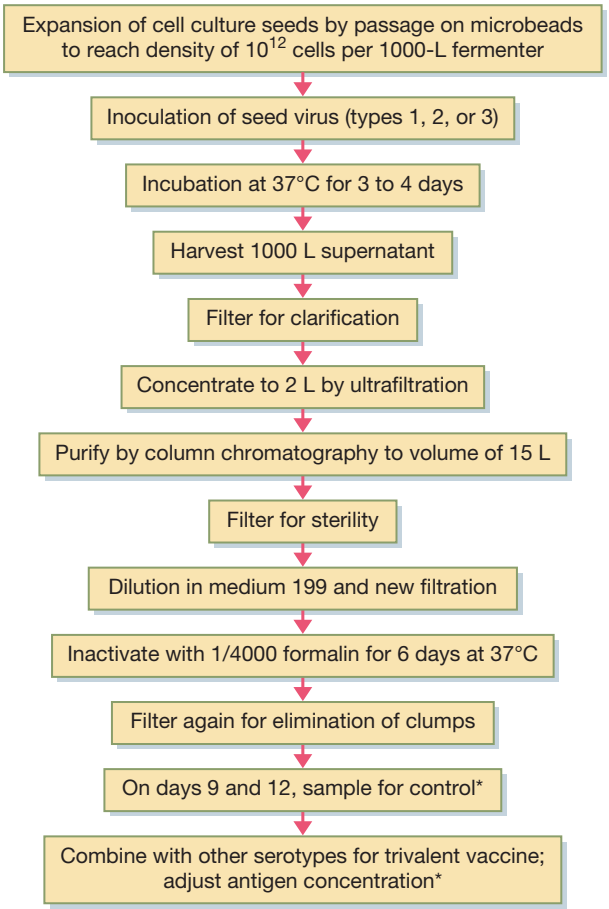


Figure 48.2. Production of enhanced-potency inactivated poliovirus vaccine. *, Sampling of an equivalent of at least 1500 human doses for control of effective inactivation.

1. The cell substrate on which the virus seeds are inoculated include continuous cultures of human diploid or simian cell lines, rather than primary, secondary, or tertiary cell cultures from captured or bred monkeys.
2. To increase density, cells are grown on microbeads dispensed in large bioreactors.
3. The virus harvest is concentrated before inactivation to increase the final antigen content.

Fig. 48.2 outlines the production steps of IPV.[24–30] The cells are expanded from a working cell bank adapted to grow on microbeads (Fig. 48.3) in large bioreactors (Fig. 48.4) until high cell density is reached. Growth medium is then removed, the cells are washed, and one of the three types of poliovirus is inoculated. By 72 to 96 hours of incubation at 37°C, the cells have been lysed by viral replication and the supernatants are collected. After clarification, the virus is concentrated 500-fold by ultrafiltration. To remove cellular proteins and DNA, the concentrated virus is passed through size exclusion and ion-exchange chromatography to yield purified material. At this point, there is less than 10 ng of DNA per human dose, a level considered to pose no hazard to recipients.[31] The concentrated purified virus is then inactivated by the addition of formalin and incubation at 37°C for 12 days. By day 4 viral inactivation should be almost complete, as confirmed by sampling for residual live virus. During inactivation of the virus, it is important to avoid viral clumping and to maintain a neutral pH. An extra filtration is included during inactivation

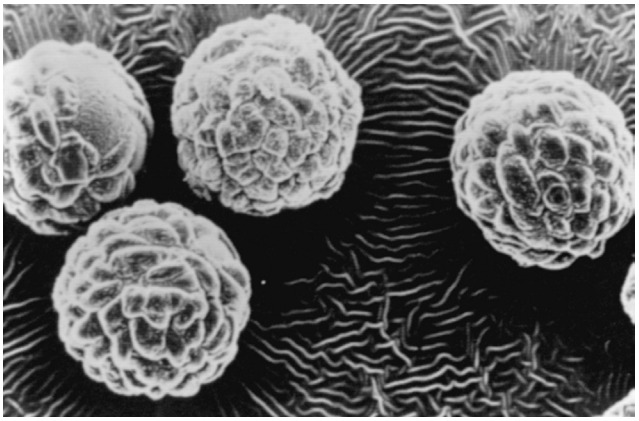


Figure 48.3. Kidney cells from African green monkeys (Vero cells) grown on microcarrier beads. *(Courtesy Dr. B. Montagnon, Institut Mérieux, Lyon.)*



Figure 48.4. Bioreactors of 1500 L capacity in which cells are grown for virus cultivation. *(Courtesy Sanofi Pasteur, Lyon.)*

to remove viral clumps.[32] Growth of polioviruses in Vero cells in serum-free conditions[33,34] or using other cell lines[35,36] improves yield with the hope of reducing costs of production. The final monovalent material from each serotype is subjected to tests for residual infectivity, which must be negative. The three monovalent inactivated viral pools are then mixed to form standardized for antigen content trivalent bulk antigen stored in concentrated form until formulation of the final vaccine is executed.

Content of the three poliovirus types in the final product, and also in intermediates, is expressed as D antigen (D-Ag) units (D-Ag are expressed only by intact virus particles). This determination was historically measured using gel diffusion (where 100 units were arbitrarily defined as the amount of D-Ag able to induce a 22-mm diameter precipitation circle in a gel diffusion assay[37] and shown to correlate with immune responses in the historical trials done with the enhanced potency IPV[38]) but is now assessed using enzyme-linked immunosorbent assays (ELISA). The measurement done on the trivalent bulk antigen constitutes the measure of in vitro potency of the product (antigenicity) and is the basis of the final vaccine formulation. All products target a specific amount of D-Ag content for poliovirus types 1, 2, and 3 in the final product, depending on the type of strain used for vaccine manufacture (conventional inactivated polio vaccine [cIPV] or sIPV), the reference material used in the assay, and the data treatment algorithm used to compute the optical density values measured in the ELISA (multiple or linear regression methods). Determination, standardization, and specifications for the D-Ag content of IPV are important issues and have been complicated with the emergence of the sIPV vaccines. Successive international reference vaccines have been characterized by the World Health Organization (WHO)[39,40] and suggestions have been made for the improvement of the ELISA that measures D-Ag content.[41] The key parameters in the performance of this assay include: (a) the nature (poly- or monoclonal and their specificity[ies]) of the antibodies used for antigen capture, (b) the nature (poly- or monoclonal and their specificity[ies]) of the antibodies used for antigen detection, (c) the method of calculation of results (multiple or linear regression methods), and (d) the nature of the reference antigen used in the assay. In addition, some reagents may detect the presence of polio C antigens (associated with noninfectious viruses). Consequently, using those reagents can provide a falsely high evaluation of the antigenic properties associated with D-Ag content of the vaccine (i.e., the final product may actually be subpotent resulting in decreased immunogenicity). Thus, given the variability in how D-Ag is measured, comparing the projected immunogenicity of various products based on the reported D-Ag content may not be always accurate (see the following section).[42,43]

The immunogenicity (in vivo potency) of IPV is measured by immune responses obtained in monkeys, rats, guinea pigs, mice, or chickens. The rat model is still used in some countries for in-process controls and/or release purposes. The variability of the methods of measuring poliovirus neutralizing antibodies in these assays complicates the comparison of potencies of different IPVs. Finally, prediction of immunogenicity in rats from antigen content as measured by ELISA has been uncertain[39] and the relationship between immunogenicity observed in animal models and in humans might not be straightforward.

All these parameters are surrogates for the clinical protective effect anticipated in humans vaccinated with IPV. To more closely approximate that effect, new potency assays have been evaluated like the use of transgenic mice, which express the CD-155 poliovirus receptor.[44,45] These mice can be immunized with IPV and then challenged with polioviruses to evaluate whether paralysis is prevented or not.

Inactivated Polio Vaccine Manufactured From Sabin Strains

Several studies have evaluated the biochemical differences between sIPV and cIPV, and particularly the effect of formaldehyde inactivation on the antigenic structures of polioviruses.[46,47] Using reactivity to monoclonal antibodies profiled by ELISA, it has been shown that inactivation with formaldehyde destroys some antigenic sites of the polioviruses.[48,49] Depending on the characteristics (reagents) of the D-Ag assay to measure the potency of resulting inactivated viruses, such alterations might not be measured.[50,51] Because of their genomic differences, the epitopes presented by the Sabin strains are different from the epitopes presented by the wild-type strains; therefore, they have a different sensitivity to formalin inactivation. A study[48] documented that the antigenic site 1 of the Sabin poliovirus 1 can be damaged by formalin, whereas the same antigenic site present at the surface of the wild-type Mahoney poliovirus 1 strain is not damaged by formalin. The consequence of this is that sIPV and cIPV do exhibit differences in their antigenicity profile,[48,52] with the result that the repertoire of antibodies induced by cIPV or sIPV might differ.[53,54] In a study done at the U.S. Food and Drug Administration (FDA) using the transgenic poliovirus receptor mice model, a type 2 sIPV prepared by the Japan Poliomyelitis

Research Institute (JPRI), elicited fivefold lower levels of MEF-1 neutralizing antibodies than Sabin 2 neutralizing antibodies and lower protection (20% to 100% paralysis, depending on dose and regimen) after virulent challenge than a type 2 cIPV prepared from MEF-1 strain (0 to 20% paralysis, depending on dose and regimen).[45] Similar findings were observed previously with a type 3 sIPV,[39] but not for a type 1 sIPV.[51] Whether these differences have any clinical implications with regard to protection in humans against paralysis induced by cIPV versus sIPV is not known.

Historically, the first attempts to develop sIPV occurred at the JPRI during the mid-1980s[55,56] and at the Lederle Laboratories in the United States[57] followed by the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) in The Netherlands.[50] In addition, since 2004 the WHO has been promoting the emergence of new players in the field of IPV vaccination by helping manufacturers develop sIPV. A major advantage of sIPV is the capability to open its manufacture to developing-country manufacturers. An escape of wild polio viruses from a manufacturing plant in an industrialized country with good hygiene and sanitation would not likely have the same consequences as escape in a developing country with crowding and poor hygiene and sanitation. That is why developing-country manufacturers cannot manufacture IPV made from wild polio viruses. However, escape of a Sabin strain does not have the same consequences as escape of a wild virus. Hence, developing-country manufacturers can produce vaccines in their own countries and offer the potential for reduced prices. The WHO's strategy is to help current OPV manufacturers to expand and transform their manufacturing facilities, allowing them to make sIPV. To accomplish this the WHO relies on Intravacc (ex-RIVM) for technology-transfer partnerships.[58]

Several groups have developed sIPVs independently from the WHO. The JPRI initiative, ongoing since the mid-1980s in Japan, has resulted in the licensure in Japan of two diphtheria, tetanus, and acellular pertussis (DTaP)-sIPV products by Biken and Kaketsuken[59,60] in 2012. In addition, JPRI is claiming to be engaged in technology transfer activities with Polyvac in Vietnam and with Biofarma in Indonesia. The Institute of Medical Biology, Chinese Academy of Medical Sciences (Kunming Institute) in China has developed a standalone sIPV product,[61,62] which is licensed in China, and is working on more complex combinations.[63] Finally, Intravacc (ex-RIVM) has developed a sIPV technology platform available for technology transfer[64–69] under the sponsorship of the WHO and work on optimization of the upstream (cell culture density, viral culture step, serum free media) and downstream (clarification, purification, inactivation) process to improve overall yields. Several companies have been awarded WHO support and are claiming to be engaged in such developments.[69a]

Inactivated Polio Vaccine Manufactured From Other Poliovirus Strains

Within the scope of developments that are undertaken toward lower-cost and/or safer (from a manufacturing perspective) IPV vaccines,[70] several groups are developing new poliovirus strain seeds that could be used for vaccine production.[71–73] Approaches are focused on developing more stable attenuated strains by manipulating the 5′ noncoding region of the viral genome, by increasing polymerase replication fidelity, or by codon deoptimization. Some groups are even trying to develop noninfectious seed strains.

Adjuvanted Inactivated Polio Vaccine

The positive role of adjuvants on the immunogenicity of IPV antigens has been known since the pioneering work done with mineral oil and oxide aluminum salts.[74,75] Later, through several IPV-containing whole-cell pertussis (wP)-based combinations, the positive role of calcium aluminum salts has been advocated.[76] Since then, it has been largely demonstrated through several randomized controlled trials (RCTs) that the immunogenicity of the IPV antigens contained in diphtheria, tetanus, and pertussis (DTP)-backboned combination vaccines (and therefore containing aluminum salts as adjuvant) is improved compared to standalone IPV vaccines.[77–83] This is particularly visible after an infant primary series where RCTs do consistently show geometric mean titers (GMTs) approximately twofold higher with combined vaccines. Therefore, reducing the amount of antigen in standalone IPV vaccines formulated with aluminum salt adjuvants might produce similar responses. This is an avenue followed by some groups[84] (two clinical trials sponsored by Statens Seruminstitut are ongoing in Denmark and in the Dominican Republic).

In addition, new adjuvants are being considered for IPV hoping that the reduction of antigen needs will allow more affordable and/or better mucosal immunity inducing IPVs.[85] Preclinical studies with cIPV or sIPV antigens show that several new adjuvants (squalene-based or oil-in-water emulsions supplemented or not with Toll-like receptor–agonists, polymers, chitosan, alphavirus, cytosine phosphate guanine oligodeoxynucleotides, cationic liposomes, saponin, lipopolysaccharide derivatives, double-mutant labile toxin [dmLT]) may decrease the amount of antigen needed to achieve the desired immune responses and/or skew the response toward higher secretory immunoglobulin A levels.[67,86–93] In a more futurist approach, IPV antigens incorporated into thermoresponsive gels with the dmLT adjuvant allowing sublingual immunization are being considered in some preclinical models.[94]

Vaccine Constituents Other Than Immunizing Antigens

Streptomycin, neomycin, and polymyxin B are used in the manufacturing steps to control bacterial bioburden, and they are largely eliminated during purification. Many of the manufacturers are engaged in removing these antibiotics during the viral culture step; nevertheless, the use of polymyxin B does have some effect on the quality of viral replication.[95] Trace amounts of these antibiotics may still be present in the final vaccine. When used as part of multivalent combination vaccines, the quality of the IPV bulk antigens and of their other constituents are of extreme importance in the behavior, potency, and stability of the formulated final drug product.

Thimerosal cannot be used to preserve multidose presentations of IPV-containing vaccines because it destroys the polio antigens,[96] but preservation of the final product is conferred by residual formalin and 2-phenoxyethanol. The recently updated WHO multidose vial policy[97] states that multidose (two-, five-, or 10-dose) vial presentations of IPV can be used up to 28 days after first use provided they are handled according to good vaccination practices and stored between usages at 2°C to 8°C. Incorporation of this policy into the licensure labeling of several IPV products will limit product wastage in the field compared to previous policy, which required disposal of vaccine after a single vaccination session.

Stability

IPV is stable for 3 years at 2°C to 8°C. Its thermoresistance varies, as expected, with levels and durations of high temperature excursions. At 37°C, loss of potency is observed in

a matter of days according to virus types. Most multidose products used in tender-driven markets are equipped with Vaccine Vial Monitors able to measure cumulated temperature exposure. Freezing diminishes the potency of IPV and should be avoided.

Producers of Inactivated Polio Vaccine Antigen and Available Vaccines

Table 48.1 lists the current manufacturers of IPV bulk antigen (drug substance) used in the licensed vaccines as of mid-2016. Eighteen to 36 months are needed from engaging working viral seed lots in the production of the vaccine antigens up to the packaged final IPV-containing batch release by all concerned (internal and external) control laboratories for a given market. During 2014, the global production capacity for IPV bulk antigen was estimated to be enough to allow 200 to 300 million doses of final product to be released in 2016. With the ongoing scale up of the historical manufacturers and the recent entry of new players, the global annual capacity for production of IPV vaccines will further increase.

All available IPVs contain inactivated viruses for serotypes 1, 2, and 3, and are available as standalone vaccines and as trivalent (Td-IPV), tetravalent (DTaP-IPV and Tdap-IPV), pentavalent (DTaP-IPV-Hib and DTaP-IPV-HB), and hexavalent (DTaP-IPV-HB-Hib) combination vaccines (see also Chapter 15). Those vaccines are used worldwide for infant/toddler primary immunization series and/or for booster immunization in children at the time of school entry, and for adolescents and adults. Some of these vaccines are also used for immunization in persons (most often adolescents or adults) not previously immunized or with unknown or incomplete immunization history. In some countries, standalone IPV vaccines formulated, filled, labeled, packaged, and released by a national manufacturer using IPV bulk antigens imported from another manufacturer are licensed or are under development.

TABLE 48.1 Manufacturers of Inactivated Polio Vaccine Bulk Antigen Used in Licensed Vaccines (as of Mid-2015)

Manufacturer	Where Made	Cell Substrate	Poliovirus Strains
Bilthoven Biologics (Serum Institute of India)	Netherlands	Vero	Mahoney, MEF-1, Saukett
GlaxoSmithKline	Belgium	Vero	Mahoney, MEF-1, Saukett
Japan Poliomyelitis Research Institute	Japan	Vero	Sabin 1, Sabin 2, Sabin 3
Kunming Institute	China	Vero	Sabin 1, Sabin 2, Sabin 3
Novartis (Glaxo SmithKline)	Italy	Vero	Mahoney, MEF-1, Saukett
Sanofi Pasteur	France	Vero	Mahoney, MEF-1, Saukett
Sanofi Pasteur	Canada	MRC-5	Mahoney, MEF-1, Saukett
Statens Serum Institute	Denmark	Vero	Brunhilde, MEF-1, Saukett

Most of the IPV standalone vaccines are WHO prequalified and all are compliant with the recently updated recommendations to assure the quality, safety, and efficacy of poliomyelitis vaccine (inactivated).[98] Some of them are manufactured in compliance with the third edition of the global action plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of routine OPV use (GAPIII).[99] The feasibility (and timing) of adoption by all manufacturers of these requirements is the subject of intense discussions, and the requirements imposed upon the manipulation of Sabin type 2 strain from April 2016 (which should be similar to those applicable for wild-type strains, which are mainly about manipulating viruses in BSL3+ environments) are adding complexity for Sabin IPV manufacturers or developers.

Historically, cIPV had been combined with diphtheria and tetanus toxoids combined with whole-cell pertussis (DTwP). The first combinations were licensed in 1964 in France and the United Kingdom, but today DTwP-cIPV–containing combinations no longer exist. Several manufacturers have embarked on the (re)development of wP-based hexavalent combination vaccines including IPV antigens.[100,101] One of the technical challenges is to manage the bidirectional deleterious effect on polio and pertussis potencies caused by residual thimerosal (associated with the non-IPV antigens) and residual formaldehyde and 2-phenoxy-ethanol (associated with the IPV antigens) present in the final vaccine formulation.

DOSE RESPONSES, DOSING, AND ROUTE OF ADMINISTRATION

Salk established that the immune response to cIPV is directly related to the dose of antigen.[102] The antigen content of all current cIPV proceeds from a series of dose–response clinical trials[38,103,104] performed in infants from 1977 to 1979, which were aimed at determining the optimal D-Ag content necessary for providing reliable protection after two doses of the cIPV antigens combined with other vaccine antigens (D, T, and wP, which were already routinely administered in infants at that age). This strategy was implemented by Jonas Salk and Charles Mérieux with the objective of developing and qualifying a cIPV formulation that was useful in Africa and that required two doses with a relatively long interval between them that could overcome the negative effect of circulating maternally transmitted poliovirus antibodies on the immune responses. These studies led to the current cIPV formulations with 40, 8, and 32 D-Ag units for poliovirus types 1, 2, and 3, respectively (corresponding to a formulation target resulting from measurements done by multiple-regression D-Ag ELISA on monovalent antigen bulks). These trials showed that the 40:8:32 D-Ag unit formulation was immunogenic in schedules consisting of two consecutive priming doses followed by a booster administered at least 6 months following the last infant priming dose to generate persistent immunity.[105] It should be noted that some of the historical studies documented the capacity of cIPV to induce antibody levels after the first dose,[38] which was confirmed later by a one-dose clinical efficacy of 36% (95% confidence limits, 0% to 67%) measured in Senegal during a WPV1 polio outbreak (see "Efficacy of Inactivated Polio Vaccine Against Acute Flaccid Paralysis and Immune Correlates of Protection" later).[106,107] Two clinical trials have been conducted with a high potency type 2 (monovalent) cIPV (32 D-Ag units compared to the standard 8 D-Ag units in trivalent IPV), exploring whether such vaccine will be more immunogenic should there be a need to quickly close the type 2 immunity gap that may exist in cohorts should

a poliovirus type 2 reemerge, particularly with plans to switch the OPV in routine use from trivalent oral poliovirus vaccine (tOPV), which contains type 2, to bivalent oral poliovirus vaccine 1 and 3 ($bOPV_{1\&3}$) which does not.[108,109]

Numerous infant/toddler/school-entry primary immunization regimens are currently used in many countries (Table 48.2). All IPV-only countries are in fact relying on IPV-containing combination vaccines. The subject of additional boosters after the school-entry age is discussed later (see "Duration of Immunity"). In some countries, along with routine pediatric IPV-only schedules, supplemental immunization activities with OPV are organized (see "Results of Vaccination Programs with Inactivated Polio Vaccine" later). Ideal dosage for cIPV in truly unvaccinated adolescents and adults is three doses. The first two doses can be given 1, or preferably 2, months apart, with the third dose given 6 to 12 months later. If there is urgency, the third dose can be given earlier, but the achieved antibody titers will not be as high. Adolescents or adults who are already primed and whose last polio vaccination occurred 10 to 20 years ago need only one booster dose to redevelop high titers.[110,111] In adult subjects with an unknown polio vaccination history, two doses of any kind of cIPV-containing combination vaccines given 1 month apart are sufficient to induce very high seroprotection rates and persisting circulating antibodies.[112,113]

IPV may be given subcutaneously or intramuscularly, and there is no published information from RCTs on the relative immunogenicity of IPV administered intramuscularly versus subcutaneously; however, as IPV is and will be administered increasingly frequently as part of IPV-containing DTP-backboned combination vaccines, and as the objective is to minimize local adverse events, IPV is now almost exclusively administered intramuscularly, even when used as a standalone vaccine.

TABLE 48.2 Schedules of IPV Administration for Primary Immunization in Infants, Toddlers, and School-Entry Children in Countries Recommending IPV-Only Schedules[a]

Schedule	Age	Countries
3+1+0	3, 4, 5, and 18 mo	Japan
	2, 4, 6, and 18 mo	Spain
	3, 4/5, 6, and 12/24 mo	Slovenia
	2, 4, 6, and 15 mo	Uruguay
	2, 3, 5, and 18 mo	Malaysia[b]
3+0+1	2, 4, 6 mo and 4 yr	Australia
	2, 4, 6 mo and 4/6 yr	Greece,[c] South Korea,[c] United States[c]
	2, 4, 6 mo and 4/5 yr	Ireland
	6 weeks, 3, 5 mo and 4 yr	New Zealand
	2, 4, 6 mo and 5/6 yr	Portugal
	2, 3, 4 mo and 3 yr	United Kingdom
2+1+1	2, 4, 12/18 mo and 4/6 yr	Greece,[c] South Korea,[c] United States[c]
	3, 5, 12 mo and 7/9 yr	Austria
	3, 5, 12 mo and 5 yr	Denmark
	3, 5, 12 mo and 4 yr	Finland
	2, 4, 11 mo and 6 yr	France
	3, 5, 12 mo and 14 yr	Iceland
	3, 5/6, 11/13 mo and 5/6 yr	Italy
	3, 5, 12 mo and 7 yr	Norway
	3, 5, 12 mo and 5/6 yr	Sweden
	2/3, 4/5, 10/11 mo and 5 yr	Slovakia
	2, 4, 11 mo and 6 yr	Romania
3+1+1	3, 4, 5, 18 mo and 5/7 yr	Belgium
	2, 3, 4, 16 mo and 6 yr	Bulgaria
	2, 4, 6, 18 mo and 4/6 yr	Canada
	2, 4, 6, 12/23 mo and 6/7 yr	Croatia
	2, 4, 6, 15/18 mo and 4/6 yr	Cyprus
	2, 3, 4, 10 mo and 10/11 yr	Czech Republic
	3, 4/5, 6, 24 mo and 6/7 yr	Estonia
	2, 3, 4, 11/14 mo and 9/17 yr	Germany
	2, 3, 4, 18 mo and 6 yr	Hungary
	2, 4, 6, 12 mo and 7 yr	Israel
	2, 4, 6, 12/15 mo and 7 yr	Latvia
	2, 4, 6, 15/23 mo and 4/7 yr	Liechtenstein
	2, 4, 6, 18 mo and 6/7 yr	Lithuania
	2, 3, 4, 13 mo and 5/6 yr	Luxemburg
	2, 3, 4, 18 mo and 16 yr	Malta
	2, 3, 4, 11 mo and 4 yr	Netherlands
	2, 4, 6, 15/24 mo and 4/7 yr	Switzerland

[a]As of June 2015, http://www.who.int/immunization/monitoring_surveillance/data/en/ and http://vaccine-schedule.ecdc.europa.eu/Pages/Scheduler.aspx

[b]OPV is recommended for the school-entry booster for children 7 years of age.

[c]Official recommendations are for the third dose to be given any time between 6 and 18 months of age, therefore falling in the "2+1+1" or the "3+0+1" schedule. Consider also the "3+1+1" schedule as acceptable.

IPV, inactivated polio vaccine; OPV, oral polio vaccine.

RESULTS OF VACCINATION

Measurement of Immune Responses

Although it is possible to measure serum antibodies to poliovirus by a variety of methods, the poliovirus-neutralizing antibody assay is the assay that best correlates with clinical protection.[114] Cell-mediated immunity responses have been explored in response to cIPV[115,116] but are not routinely performed nor they have been used by national regulatory agencies. They have shown the presence of specific T cells (by lymphoproliferation tests and flow cytometry) in the blood following vaccination. Measurement of mucosal immunity and protection induced by IPV is discussed later (see "Mucosal Immunity/Protection Induced by Inactivated Polio Vaccine").

IPV is a killed antigen; as a result, immune responses depend on the amount of antigens, the number of doses (when used for primary immunization), the interval between doses, the age at first dose (and, consequently, the level of maternally acquired poliovirus antibodies that can be present at time of vaccination, which can possibly diminish immune response), and the type of IPV-containing product used (standalone unadjuvanted vs aluminum-adjuvanted IPV-containing combinations). Several parameters are used to express the levels of neutralizing antibodies against polioviruses when assessing the overall responses obtained in a group of vaccinated individuals: the GMTs (sometimes median titers are used, which can lead to different results compared with GMTs); the percentage of subjects with neutralizing antibodies above the 1:8 threshold now considered as the serologic correlate of protection (historically the 1:4 threshold had been used) and very often referred to as the seroprotection rate; and the percentages of subjects presenting with a fourfold or greater rise in their neutralizing antibody titers between their prevaccination titers and postvaccination titers, adjusted or not for maternally derived antibody decay, and referred as to seroconversion. If maternal antibody decay is not factored in, seroconversion rates could be lower than the actual proportion of persons who make a significant immune response. It is known that maternal antibody does inhibit the immune response to IPV.[117] The percentage of subjects with neutralizing antibodies above the 1:8 threshold at a time when

maternally transmitted antibodies should have disappeared probably gives the best measure of the proportion of persons with protective immune responses to IPV.

The poliovirus neutralization antibody assay is used by the vast majority of laboratories assessing the immunogenicity of these vaccines.[118] The assay procedures are variable and have been shown to be sensitive to the nature of the cells used to grow the target virus (HEp-2 or Vero), the viral inoculum size, the duration and temperature of serum–virus interaction before viral growth, the number of serial dilutions of the tested sera, and the nature of the viral strains (Sabin or wild type) used in the test.[119,120] In addition, the type of assay readout (cytopathic effect or metabolic inhibition) has an influence. Attempts have been made to standardize this assay,[121,122] but there is no broad acceptance of international standards for its use.[123] Under some assay conditions, neutralizing titers are higher when sera from subjects vaccinated with cIPV are tested against wild-type strains compared to when tested against Sabin strains.[119,120] Lack of specific description of the serum panels used in standardization studies makes evaluation difficult. Some studies refer to the use of the trivalent reference serum lot IIA4 from the FDA that was prepared from vaccinated monkeys, whereas others refer to pools of sera obtained from humans in the United Kingdom, who were probably vaccinated with OPV or naturally infected. As described in "Inactivated Polio Vaccine Manufactured from Sabin Strains" earlier, it is conceivable that the paratopes (antigen-binding sites) of the neutralizing antibodies exhibited by sIPV-vaccinated subjects might differ from the paratopes exhibited by cIPV-vaccinated subjects, and that the overall levels of neutralizing antibodies measured in these subjects might be influenced by the nature of the viral strains used in the detection system. All the landmark historical studies, which led to the current cIPVs,[38,103,104] have based their neutralization assay on wild-type–derived poliovirus strains. The absence of indication that the type of strain used in the assay might be important, and the risks taken by laboratories manipulating wild-type–derived strains, particularly for those located in polio-free areas or in low- or middle-income countries, have induced many laboratories to switch to the use of the Sabin strains for this assay. This parameter should be considered when comparing data from clinical trials, particularly those done with sIPVs. The development of human or chimpanzee–human monoclonal antibodies that neutralize poliovirus strains, opening some therapeutic potential[124–127] and the three-dimensional mapping of the poliovirus epitopes antibody interactions[128] will provide additional information on this question.

Immunogenicity of Conventional Inactivated Polio Vaccine

An enormous number of studies and trials have been conducted over the last 35 years with cIPV-containing vaccines given in schedules of two or three doses during the first year of life. These studies used different formulations of cIPV, study designs, and schedules, and were conducted in a variety of countries with different ethno-ecological situations. A number of reviews of these studies have been published.[129–131] For example, Table 48.3 summarizes data collected from 30 study groups where cIPV-containing vaccines were administered to more than 4500 subjects in a two-dose primary series, usually at 2 and 4 months of age.[130] At completion of the two-dose immunization series, seroprotection rates ranged from 89% to 100% for poliovirus type 1, from 92% to 100% for poliovirus type 2, and from 70% to 100% for poliovirus type 3. Table 48.3 also summarizes responses after three doses. Seroprotection rates after three doses are clearly higher than after two, particularly when the schedule is 2-4-6 months. However, schedules of 3-4-5 and 2-3-4 months also give good responses, although lower than after 2-4-6 months, particularly with regard to GMTs. To achieve rapid protection in developing countries, vaccines are given on a 6-10-14–week schedule, which is not optimal for immune response against a variety of antigens because of the early age for starting immunization and the short interval between doses. Since the initial studies sponsored by the WHO,[132–134] many studies have been done in a wide range of epidemiological settings with different cIPV-containing vaccines. Many of these studies were conducted in countries where OPV was the routine vaccine used and therefore IPV vaccinees could have been exposed to Sabin polioviruses via direct contact with OPV or OPV-vaccinees (Table 48.4) (Sanofi Pasteur, studies HIT40498, EUV07199/HE9810, and A3R25 and A3R38; unpublished, data on file).[132–142] High seroprotection rates were achieved at completion of immunization in studies reporting this outcome. However, seroconversion rates, when reported, were more variable, presumably because of high maternal antibody levels observed in some studies. In one study in South Africa (Sanofi Pasteur, study A3R25 and A3R38; unpublished, data on file), antibodies were measured at 17 months of age after three doses of cIPV contained in a hexavalent combination vaccine given in infancy, and persistence of antibodies with good anamnestic response to a fourth dose were noted. At 17 months of age, 100%, 99.5%, and 97.8% of children still had neutralizing antibodies at titers greater than or equal to 1:8 against poliovirus types 1, 2, and 3, respectively, and a 40- to 56-fold increase in their GMT was observed from prebooster to

TABLE 48.3 Summary of Immunogenicity of cIPV After Two or Three Doses in the First Year of Life 1 Month After Last Vaccination

Schedule	Type 1		Type 2		Type 3		Study Groups	Approximate No. Subjects
	Seropositives	GMT	Seropositives	GMT	Seropositives	GMT		
2–4 mo	89–100%	17–355	92–100%	17–709	70–100%	50–1200	30	4500
2–4 and 12–18 mo	94–100%	495–2629	98–100%	1518–6637	97–100%	1256–4332	10	2000
2–4–6 mo	96–100%	143–2459	96–100%	78–2597	95–100%	187–3010	48	6000
3–4–5 mo	85–100%	110–475	98–100%	92–944	86–100%	89–1244	8	500
2–3–4 mo	93–100%	143–595	89–100%	91–561	95–100%	221–1493	18	2200

cIPV, conventional inactivated polio vaccine; GMT, geometric mean antibody titer.
Data from Vidor E, Meschievitz C, Plotkin SA. Fifteen years of experience with Vero-produced enhanced potency inactivated poliovirus vaccine. Pediatr Infect Dis J. *1997;16:312–322.*

TABLE 48.4 GMT and Percentages of Subjects Who Seroconverted (or With Poliovirus NA ≥1:8) After IPV-Containing Vaccines at 6, 10, and 14 Weeks of Age

Study	References	Post–Dose 3 Poliovirus Antibodies		
		Type 1	Type 2	Type 3
Oman, 1990–1992	132–134			
Product used		DTwP-IPV		
GMT		447	571	251
% seroconverted		88%	92%	91%
Gambia, 1990–1991	132–134			
Product used		DTwP-IPV		
GMT		79	144	241
% seroconverted		81%	82%	98%
Thailand, 1991–92	132–134			
Product used		DTwP-IPV		
GMT		49	68	136
% seroconverted		66%	63%	92%
South Africa, 1998[a]				
Product used		DTwP-IPV/Hib		
GMT		116	93	166
% with NA ≥1:8		99.2%	99.2%	99.2%
Philippines, 2000[b]				
Product used		DTaP-IPV-Hib		
GMT		863	768	901
% with NA ≥1:8		100%	100%	100%
Moldavia, 1998	135			
Product used		DTaP-IPV-HepB		
GMT		535	154	731
% with NA ≥1:8		98.7%	98%	98.7%
Moldavia, 1998	135			
Product used		DTwP-IPV/Hib		
GMT		170	88	544
% with NA ≥1:8		99.3%	97.2%	100%
Cuba, 2001	258			
Product used		DTwP-IPV/Hib		
GMT		304	304	858
% seroconverted		94%	83%	100%
South Africa, 2001[c]				
Product used		DTaP-IPV-HepB-Hib		
GMT		1226	661	1249
% with NA ≥1:4		100%	100%	100%
Philippines, 2003	137			
Product used		DTaP-IPV/Hib		
GMT		533	789	1968
% with NA ≥1:8		100%	100%	100%
Puerto Rico, 2003	138			
Product used		IPV		
GMT		222	147	724
% seroconverted		85.8%	86.2%	96.9%
South Africa, 2005	139			
Product used		DTaP-IPV/Hib		
GMT		1453	1699	2395
% with NA ≥1:8		100%	100%	100%
India, 2006	140			
Product used		DTaP-IPV/Hib		
GMT		440	458	1510
% with NA ≥1:8		100%	99.1%	100%
South Africa, 2006	141			
Product used		DTaP-IPV-HepB-Hib		
GMT		579	620	975
% with NA ≥1:8		100%	98.5%	100%
Philippines, 2008	142			
Product used		IPV		
GMT		585	795	774
% seroconverted		98.2%	98.2%	100%

[a]Sanofi Pasteur. Study HIT40498; unpublished, data on file.
[b]Sanofi Pasteur. Study EUV07199/HE9810; unpublished, data on file.
[c]Sanofi Pasteur. Study A3R25 and A3R38; unpublished, data on file.
DTaP, diphtheria, tetanus, and acellular pertussis vaccine; GMT, geometric mean antibody titer; HepB, hepatitis B vaccine; Hib, *Haemophilus influenzae* type b vaccine; IPV, inactivated poliovirus vaccine; NA, neutralizing antibodies.

postbooster. Similar observations were noted in another trial.[142] A direct comparison of the 2-4-6–month and the Expanded Program on Immunization (EPI) schedules was performed in Puerto Rico, where OPV was no longer used.[138] Seroconversion rates for types 1, 2, and 3, respectively, after three doses of the 2-4-6–month schedule were 100%, 100%, and 99%, whereas after the EPI schedule they were 85.8%, 86.2%, and 96.9%. Overall, the data clearly demonstrate that cIPV is immunogenic in an EPI schedule, although the titers achieved and the seroconversion rates may be lower compared with vaccination of infants at older ages. The humoral immunogenicity of cIPV in an EPI schedule appears to be superior to the use of OPV in such schedules in developing countries. After two or three doses in the first 6 months of life, antibody levels fall although the vaccinees usually retain seroprotective titers until the first booster is given during the second year of life, and this third or fourth injection gives a marked anamnestic response. Table 48.5 presents five studies conducted in the United States.[143–149] Nearly all infants were already seropositive after the second dose, although their antibody titers were generally below 1:100. Ethnicity background has never been observed to play a clinically relevant role in the immunogenicity profile of IPV.

Immune responses can be detected after the first dose of cIPV-containing vaccines[150,151,152] and depending on study designs, up to 50% seroconversion rates against the three poliovirus types can be observed (on average 33%, 41%, and 47% of vaccinees do seroconvert against types 1, 2, and 3, respectively, with better seroconversion at older ages). Persons who fail to seroconvert may be primed, and, when using as a definition of priming the detection of neutralizing antibody 7 days after vaccination in persons seronegative prior to that dose, one trial revealed that up to 90% of seronegative vaccinees were primed.[153] As summarized later under "Efficacy of Inactivated Polio Vaccine Against Acute Flaccid Paralysis and Immune Correlates of Protection," a one-dose schedule provides limited clinical efficacy (36% with 95% confidence limits ranging from 0% to 67%) against wild poliovirus type 1 in a case-control study in Senegal, suggesting seroconversion is the most appropriate correlate of protection, not priming.

TABLE 48.5 Studies of IPV Given Alone or in IPV Followed by OPV Sequential Schedules: % of Neutralizing Antibody to Indicated Strain ≥1:8 (Geometric Mean Titer)

	Vaccine Administered at Given Age (Month)					After Second Dose			After Third Dose[a]			Before Booster			After Booster[b]		
Reference	**No.[c]**	**2**	**4**	**6**	**12–18**	**Type 1**	**Type 2**	**Type 3**	**Type 1**	**Type 2**	**Type 3**	**Type 1**	**Type 2**	**Type 3**	**Type 1**	**Type 2**	**Type 3**
Faden et al[143,144]	116	IPV	IPV		IPV	96 (184)	100 (631)	96 (634)				90 (61)	96 (135)	92 (102)	96 (1954)	100 (5835)	100 (5187)
	34	IPV	IPV		OPV	100 (283)	100 (481)	100 (1132)				100 (128)	100 (334)	100 (151)	100 (3044)	100 (10,693)	100 (2347)
Blatter and Starr[145]	94	IPV	IPV		IPV	97 (44)	96 (105)	95 (83)				92 (22)	95 (42)	87 (23)	100 (2070)	100 (3419)	100 (1968)
	68	IPV	IPV		IPV	98 (88)	100 (256)	98 (162)				100 (41)	100 (71)	93 (35)	100 (2029)	100 (4388)	100 (2580)
Halsey et al[146]	75	IPV	IPV		OPV	94 (28)	98 (91)	96 (63)				85 (18)	96 (47)	81 (20)	100 (1568)	100 (7199)	96 (297)
	99	IPV	IPV		OPV	99 (90)	99 (120)	95 (126)				98 (47)	96 (61)	88 (29)	100 (1765)	100 (7516)	99 (709)
Onorato et al[147]	87	IPV	IPV	IPV	OPV	97 (74)	98 (82)	100 (110)	100 (463)	100 (652)	100 (605)	100 (72)	100 (98)	100 (91)	100 (2141)	100 (7169)	100 (1824)
	331	IPV	IPV		IPV	99	99	99							99	100	100
Modlin et al[148]	332	IPV	IPV		IPV	99	100	100							100	100	100
	101	IPV	IPV		IPV	97	92	78							100	100	100
Asturias E et al[149]	113	IPV	IPV	IPV		100 (1011)	99 (517)	99 (859)	100 (2542)	100 (2675)	100 (3248)						
	920	IPV	IPV	OPV		99 (1284)	100 (798)	99 (1089)	100 (1821)	100 (4294)	100 (2828)						
	101	OPV	OPV	OPV		97 (575)	100 (1641)	94 (350)	99 (685)	100 (1768)	98 (415)						

[a]In infancy.
[b]Booster is third or fourth dose, depending on the schedule.
[c]Number of enrolled subjects at beginning of study.
IPV, inactivated poliovirus vaccine; OPV, live oral poliovirus vaccine.

However, in contrast, Hungary, which had a major problem with VAPP, decided to institute a sequential schedule with one dose of IPV followed by OPV, and VAPP was eliminated, more compatible with priming offering clinical protection against paralyis.[154] These data are important in the context of current efforts by WHO toward development of affordable IPV solutions for the developing world (see "Rationale and Role of Inactivated Polio Vaccine in Poliomyelitis Eradication" later).

When considering all the factors, the data clearly indicate that the main drivers of the immunogenicity of IPV antigens, when used for the infant primary vaccination series, are the number of primary series injections, the age at first dose, the interval between doses, the presence of maternally transmitted antibodies, and the use of IPV in the form of an IPV-containing combination vaccine.

Immunogenicity of Sabin Inactivated Polio Vaccine

As a comparison of the relative D-Ag contents between sIPVs and cIPVs is not possible because of the different in vitro (D-Ag determination by ELISA) and in vivo (immunogenicity in animals) potency assays used to formulate and release these vaccines[39,40,42,43,50,155,156] the results of the clinical evaluations of sIPVs can only be summarized product by product.

Murph and colleagues[57] at Lederle in the United States manufactured a sIPV containing 20-12.5-35 D-Ag units (per 0.5 mL) of polioviruses types 1, 2, and 3, respectively, grown on primary monkey kidney cells. A study done in 1986 comprised 18 seropositive adults who received vaccine containing 10-6.25-17.5 of D-Ag units, 20-12.5-35 of D-Ag units, or 40-25-70 of D-Ag units per dose and nine seronegative adults who received one dose of a 20-12.5-35 of D-Ag units formulation. In the seropositive adults, the three vaccine formulations were able to boost serum neutralizing antibody levels with a noticeable dose–response effect. In seronegative adults, a response against the three polioviruses was observed. The drawback of this study was that no subject was naïve to polio antigens as they all had previously received OPV or cIPV during infancy. Infant studies with this vaccine were never reported.

Two early clinical trials have been done by the JPRI in the early nineties with a standalone sIPV vaccine. Phase I[156] was conducted in adults and in 108 infants 3 to 90 months of age. The D-Ag content of the vaccine was 30-30-50 D-Ag U/dose (types 1, 2, and 3, respectively). In the first trial, the vaccine was given in two subcutaneous doses to 10 seropositive adults at 4-week intervals. Safety was excellent. Antibody data obtained 2 weeks after the second dose (against Sabin and wild-type strains) showed high neutralizing responses in all volunteers. In the second trial, the vaccine was given by the same regimen to 108 infants (3 to 90 months old). Most infants were seronegative (titer <1:4) before immunization, except for type 2 where 40% were seropositive. Immunogenicity results 2 weeks after the second dose showed a high neutralizing response in all infants against types 1 and 3, but a low neutralizing response for type 2. Seroneutralizing GMTs against the homotypic Sabin strains were approximately 2000 for polio type 1, 300 for polio type 2, and 500 for polio type 3. Seronegative titers were fourfold to 1.3-fold lower against wild-type strains compared to the homotypic Sabin strains. Following this early development, the standalone JPRI sIPV development stopped in 2002, and JPRI initiated studies to reformulate the sIPV on the basis of new immunogenicity data obtained from rats and monkeys, resulting in modification of the D-Ag content to 1.5-50-50 D-Ag U/dose. JPRI embarked on an antigen supply agreement with several Japanese DTaP manufacturers, which allowed Biken and Kaketsuken to develop and license in Japan two similar DTaP-sIPV combination vaccines based on their Phase II and III trials in 2012.[157] Results showed 100% seroprevalence of Sabin strains neutralizing antibodies following a three-dose primary series in infants 3 to 8 months of age and with a 1-month interval between doses with durable antibody levels. When assessed against wild-type strains, 97.4% to 100% of sera exhibited levels above the 1:8 threshold, but their GMTs were lower (10- to 20-fold less for type 1, twofold less for type 2, and 1.5-fold less for type 3) than when tested against Sabin strains.[158,159]

The Kunming Institute has performed a multistep Phase I study in adults, children, and infants with several formulations of sIPV products containing from 15-16-22.5 to 45-64-67.5 D-Ag units for poliovirus types 1, 2, and 3, respectively, with good immunogenicity and a dose–response effect.[160] Following this study, a Phase II dose–response (three formulations) and comparative against OPV and cIPV study was done in infants.[161] The 30-32-45 content of D-Ag units for this Chinese vaccine was then selected for a Phase III comparative trial versus cIPV.[162] This vaccine was licensed in China in early 2015. Cross-neutralization evaluation of Phase II vaccinees' sera against a panel of strains (Mahoney, MEF-1, and Saukett, Sabin, recent wild-type isolates, and recent vaccine-derived polioviruses [VDPVs] Chinese isolates) have shown that the levels (titers) of antibodies able to neutralize the non-Sabin strains in sIPV vaccinees are lower compared to levels induced in cIPV vaccinees. Nevertheless, more than 95% of vaccinees are able to develop antibody levels above the clinically protective level (≥1:8) in both assays (against Sabin viruses and against wild type viruses) (Kunming Institute, personal communication, 2014).

Finally, Intravacc (former RIVM) has initiated a clinical program with a sIPV by exploring the dose–response effect of several unadjuvanted and aluminium-adjuvanted vaccine preparations. Phase I clinical trials have been conducted in adults in Poland[163] and Cuba[164] with a standalone sIPV product (20-32-64 D-Ag U/dose) versus an aluminium-adjuvanted standalone sIPV product (10-16-32 D-Ag U/dose) versus a cIPV. In addition, a Phase IIa dose-escalating trial has been completed in infants[165] in Poland, with three formulations of an aluminum-adjuvanted sIPV or three formulations of an unadjuvanted sIPV standalone product (six arms) versus a control standalone cIPV. All the results have shown the adequate safety of the products and the capacity of the different formulations to induce neutralizing antibody against Sabin strains (and against wild type strains with as expected lower levels) with a dose–response effect and a moderate adjuvant effect.

As noted earlier (see "Inactivated Polio Vaccine Manufactured from Sabin Strains"), sIPV differs from cIPV. Because of the biochemical differences between the poliovirus strains used for the manufacture of vaccine, sIPV does present to the immune system a collection of neutralizing epitopes different from the epitopes present in cIPV. Consequently this can lead to different populations of antibodies in the sera of vaccinees, which, in turn, could exhibit different neutralization profiles (more in terms of height of the titers than differences in proportion of seronegative to different wild poliovirus strains) vis-à-vis the diversity of viruses that might be encountered by vaccinees after vaccination (wild type, VDPV, and Sabin-like). It is not yet known whether by altering the amount of antigen contained in the sIPV, these differences can be overcome, or if these differences are clinically relevant or not. Some argue that as long as the early proportions of individuals able to

neutralize wild-type strains are high, the levels of antibodies achieved after vaccination do not matter. The polio outbreak in 2010 in the Congo-Brazzaville,[166] where a mutated type 1 poliovirus caused disease in OPV vaccinated persons, suggested poor "heterotypic immunity," which was contradicted by the fact that this outbreak had been cleared by subsequent OPV supplemental immunization activities. These observations are raising questions from a regulatory perspective.[167] It will be critical to continue to document the immune performance of the licensed sIPVs and to assure that antibody response in humans is broadly cross-reactive against all types of strains. In addition, the level of intestinal and oropharyngeal mucosal protection, relative to cIPV's protection, achievable with the licensed sIPV is not yet documented.

Effect of Maternal Antibodies and of Neonatal Vaccination

Studies document that maternally transmitted poliovirus antibodies present at the initiation of an infant primary series diminish the height of the antibody response to a cIPV primary series and can decrease seroconversion rates.[132,133,168–174] This is particularly evident on seroconversion rates but less so on seroprotection levels achieved at completion of immunization. This effect can be minimized by giving three doses of IPV during the first year of life with optimal spacing.

Neonatal vaccination with cIPV has been evaluated on several occasions.[175] Swartz and colleagues[176] showed that a single dose of cIPV at birth primed infants for a uniform response to a second dose given at age 6 months. Israeli infants immunized at birth with cIPV concomitantly with hepatitis B vaccine showed higher mean antibody levels for polio types 2 and 3 at 1 and 3 months of age than did infants who received cIPV at 2 months of age, but the difference disappeared at 7 months of age after both groups had received one additional dose of cIPV and two doses of OPV.[172] Hovi and colleagues[177] showed priming for higher titers in Pakistani infants whose three OPV doses at 8, 12, and 16 weeks of age were preceded by a birth dose of cIPV. Linder and colleagues[178] documented the immunogenicity of cIPV given at 2 months of age with or without a preceding cIPV given at 5 to 10 days of age in Israeli preterm infants. In infants who received cIPV within the first 2 weeks of life, 100%, 100%, and 97.9% had neutralizing antibodies at titers greater than or equal to 1:8 against poliovirus types 1, 2, and 3, respectively, 1 month after the dose given at 2 months of age versus 96%, 100%, and 71% of infants who had not received prior cIPV.

Jain and colleagues[179] documented the immunogenicity of a cIPV-only schedule given at 0, 6, and 10 weeks of age in Indian neonates and was able to demonstrate better seroconversion (80% seroconverted against all three poliovirus types) with this schedule than with an EPI schedule using OPV (at 6, 10, and 14 weeks of age) preceded by cIPV or OPV at birth (72% and 72% seroconverted against all three poliovirus types, respectively). Thus all these data do show that cIPV at birth appears to prime the immune system, and these data are aligned with results from cIPV-followed-by-OPV sequential schedule trials (see "Immunogenicity of Mixed/Sequential Schedules with Inactivated Polio Vaccine and Oral Polio Vaccine" later).

Immunogenicity of Inactivated Polio Vaccine When Injected Intradermally

The first reports of use of the intradermal route with IPV came from Salk.[180,181] These studies showed that the intradermal injection of 0.1 mL of aqueous formulation of the historical Salk IPV was immunogenic in children and adults. Soon after the launch of the first commercial historical IPVs in 1955, several countries obtained good results from vaccination programs with cIPV delivered intradermally (using the Mantoux technique) to maximize the use of the limited quantity available at that time.[182–188] Three proof-of-concept studies were conducted during the early 1990s by John's team in India using the modern cIPV. These studies demonstrated that one-fifth of the intramuscular dose is immunogenic in humans when delivered intradermally with needles, but none of these studies were randomized against a full intramuscular dose.[189–191]

In the context of WHO efforts to promote more affordable IPV, and supported by preclinical studies,[192] several trials have been done to document the performance of cIPV when intradermally delivered (under a volume of 0.1 mL corresponding to one-fifth of the regular volume intramuscularly delivered) either through needles or through disposable-cartridge jet injectors (DCJIs) under development, and used with different types of infant/toddler primary series immunization regimens (IPV-only or OPV-followed-by-IPV sequential regimen).

The WHO sponsored two RCT studies in Cuba[193,194] and in Oman[195] with two different cIPV preparations administered through two cIPV-only three-dose infant primary series schedules (6-10-14 weeks and 2-4-6 months). The vaccines were administered by the intradermal route with a DCJI (Biojector 2000 from Bioject, customized for intradermal administration) or by the intramuscular route with regular syringe and needle. The primary study objectives were to show noninferiority (20% clinical margin) of the seroconversion rate against poliovirus between the intradermal group versus the intramuscular group. Both studies demonstrated clinically relevant immunogenicity but lower responses with the 6-10-14–week schedule than with to the 2-4-6–month schedule. In the Oman study, infants were challenged at 7 months of age with monovalent oral poliovirus vaccine type 1 ($mOPV_1$), and 7 days later viral shedding prevalence was 74.8% in the intradermal group versus 63.1% in the intramuscular group. A third RCT done in the Philippines[142] compared the two routes of administration using the classical Mantoux technique for intradermal injection with a cIPV-only 6-10-14–week schedule, demonstrating noninferiority (5% clinical margin) of seroprotection post–dose 3 between the intradermal group versus the intramuscular group, but lower GMTs in the intradermal group.

A study from Cuba[153] evaluated whether a schedule of two consecutive 0.1-mL cIPV doses administered intradermally with a DCJI (Pharmajet) provided comparable seroconversion with a two-dose schedule of full 0.5-mL cIPV doses administered intramuscularly at 4 and 8 months of age. Seroconversion was assessed in each group after the first and second dose of study vaccines, and the proportion of subjects that responded with a priming immune response after the first dose of cIPV was determined. Results showed high seroconversion after the first dose of both fractional and full-dose cIPV, with significantly lower median titers for the intradermal arm, and priming evidence in more than 90% of the subjects who did not seroconvert after the first dose. The second cIPV dose, delivered by either route, closed the remaining seroconversion gaps and resulted in high antibody titers, although the titers were higher in children vaccinated by the intramuscular route.

A study conducted in India[196] (Moradabad district) reported inferior immune responses with cIPV administered by the intradermal route with another under-development DCJI (Pharmajet) customized for intradermal administration. The study included vaccination of several groups of

infants, 6 to 9 months of age, who had previously received multiple $mOPV_1$ and tOPV doses, randomized to receive two different cIPV vaccines delivered intramuscularly or one cIPV administered intradermally or two different $mOPV_1$ vaccines. Because of the very high levels of prevaccination antibodies against all three polioviruses and a suspected high rate of failure of the injection device to adequately deliver the full 0.1-mL volume of vaccine intradermally, the study failed to show that a fractional dose of cIPV was as immunogenic as full-dose cIPV intramuscularly, but did reveal an undisputable booster effect of the intradermal administration.

A study conducted in Cuba[197] evaluated one cIPV delivered intradermally (by needle or with two different underdevelopment DCJIs or with one established DCJI) versus intramuscularly in toddlers 12 to 20 months of age who had previously received two doses of tOPV through the routine Cuban campaign immunization program. Results showed similar and very high seroprotective rates in all groups 21 days after vaccination, and variable but lower median titers and booster responses in the four intradermal groups compared to the intramuscular group.

Finally, data have been generated in Dutch cIPV-only primed adults[198] ("3+1+1+1" regimen) with a cIPV delivered intradermally (DCJI) or intramuscularly. At day 28, the study showed lower mean antibody levels in the intradermal group versus the intramuscular full-dose group. In HIV-infected mostly OPV-primed adults with a cIPV delivered intradermally by a microneedle device versus intramuscularly, similar booster responses were obtained with a 40% intramuscular dose content delivered intradermally as a full-dose intramuscularly.[199]

Additional approaches, supported by the WHO, Programs for Appropriate Technology in Health (PATH), and the Bill and Melinda Gates Foundation, are aiming to develop hollow microneedle or dissolvable micro(bio)needle transdermal patches coated with lyophilized and stabilized IPV antigens.[200,201] Several groups have embarked on developmental work involving microneedle patches.[202–204]

In conclusion, as recently reviewed,[205] studies of intradermal administration of cIPV do provide evidence that this route is immunogenic but slightly less immunogenic than the administration of the regular intramuscular dose. In addition, as a consequence of the differences observed between needle intradermal and DCJI intradermal administration, further studies are required for optimization of dose and development of intradermal administration through industrialized DCJIs. A critical factor in making intradermal IPV use programmatically feasible is the development and licensure of ergonomic and high-injection-quality DCJIs.[206] This is because of concerns that intradermal delivery via needle and syringe can be difficult in the field; hence, having a device to assure appropriate intradermal administration would give greater confidence that the required antigen is being adequately delivered into the proper site. A clear licensing pathway, including what data on safety and immunogenicity will be required by national regulatory agencies, for intradermal administration is still not identified. In addition, this licensure pathway would imply a partnership between a given vaccine manufacturer and a given device manufacturer to assemble a specific application file supporting the use of a given IPV with a given intradermal device in a given regimen. Finally, the real affordability advantage of this approach still needs to be demonstrated.

Fractional Dosing and Supply Shortage

Due to the current insufficient supply of IPV (see "Producers of Inactivated Polio Vaccine Antigen and Available Vaccines" earlier and "Rationale and Role of Inactivated Polio Vaccine in Poliomyelitis Eradication" later), WHO has recently incorporated in their recommendations the use of fractional dosing through the intradermal (ID) route as an alternative to the intramuscular (IM) route.[206a,206b] This use is currently envisaged either in the context of the routine immunization activities or in campaign modes should VDPV2 be isolated in environmental samples or as cVDPV2,[206c] should the available quantities not be enough to ensure at least one IM dose of IPV in every child in all countries willing to introduce IPV.

Several trials have shown that two consecutive doses of fractional (ID) IPV compared well to one dose of full (IM) dose of IPV in infants regardless of whether they received tOPV or bOPV. Type 2 seroconversion, antibody levels, and priming were similar, if not better, after two fractional IPV doses (each one-fifth of a full dose).[153,193,195,206d,206e] These data will help vaccination recommending bodies to propose this alternate use of IPV as a way to maximize the available, but too limited, quantities of IPV.

Immunogenicity of Inactivated Polio Vaccine in Preterm Infants and in Immunocompromised Subjects

Prematurity does not appear to reduce the response to cIPV-containing vaccines when the vaccines are given at the usual postnatal age. Preterm infants all develop neutralizing antibodies after three doses of cIPV, although titers might be lower than in term infants,[116,178,207–210] particularly if the infants are chronically ill.[211]

Because IPV is the indicated polio vaccine for immunocompromised subjects even in countries that recommend OPV, the immunogenicity of IPV in these subjects is an important issue. Children infected with HIV who were given two doses of cIPV in early infancy responded reasonably well, probably because their immune systems were largely intact.[212] Similar observations were done in HIV-infected mostly OPV-primed adults following one booster dose.[199] In hemophiliac adults, however, HIV seropositivity had a negative effect on titer levels after cIPV, although all adults responded to some degree.[213] Chronic renal dialysis subjects also seroconverted in 90% or more of cases.[214] In patients who had undergone bone marrow transplantation and were reimmunized after transplantation, vaccination was usually successful in inducing antibodies, although at least two, and often three, doses were needed.[215,216] Follow-up of 134 stem cell transplant recipients who were given three doses of cIPV at 12 months after transplantation following a 0-2-6–month regimen found that 94%, 94%, and 90% were still seroprotected 10 years later for types 1, 2, and 3, respectively. Those patients who had chronic graft-versus-host disease had less-persistent antibody.[217] Taken together, these results demonstrate that cIPV-containing vaccines are immunogenic in many immunocompromised subjects.

Immunogenicity of Mixed/Sequential Schedules With Inactivated Polio Vaccine and Oral Polio Vaccine

When considering IPV/OPV mixed/sequential regimens, one must carefully distinguish the regimen under consideration: IPV-followed-by-OPV sequential schedule, OPV-followed-by-IPV sequential schedule, or mixed/combined IPV/OPV regimens where the two vaccines are administered at the same time for one or more occasions during the vaccination course. In addition, with the recent licensure of new OPVs ($bOPV_{1\&3}$,

$mOPV_1$, and $mOPV_3$), the type of OPV used in these trials has to be considered. Many trials have been performed to document these different types of IPV/OPV regimens, and key results are provided below, regimen by regimen. All these trials have been done with cIPV, as no data are yet available on the performance of sIPV when used in such schedules. In addition, many of these trials have investigated the prevalence, intensity, duration and genetics of poliovirus excretion after OPV vaccination/challenge administrations (see "Mucosal Immunity/Protection Induced By Inactivated Polio Vaccine" later).

cIPV-Followed-By-OPV Sequential Schedule Trial

cIPV-followed-by-tOPV sequential schedules consisting of one or two doses of cIPV followed by one or two doses of tOPV have been documented in many clinical trials with several cIPV-containing vaccines (standalone or wP/aP [acellular pertussis] combinations) in eight countries (Brazil, China, France, Guatemala, Mexico, Taiwan, United Kingdom, and United States) since 1986 (Sanofi Pasteur, studies HE9812 and IPV33-EXT, unpublished, data on file).[143,144,146,148,149,207,218–231] These trials have been either nonrandomized open studies or RCTs between cIPV-followed-by-tOPV sequential schedules versus cIPV-only and/or tOPV-only schedules. Results of these studies demonstrate that the introduction of at least one dose of cIPV at the start of the schedule increases post–primary series serum antibody levels compared to tOPV-only schedules, as well as induces a certain degree of mucosal intestinal protection (see "Mucosal Immunity/Protection Induced By Inactivated Polio Vaccine" later). From 1997 to 1999 the United States relied on a cIPV-followed-by-tOPV sequential schedule, in which two doses of cIPV were administered at 2 and 4 months of age, followed by two doses of tOPV administered at 6 to 18 months of age and again at school entry. Table 48.5 summarizes the excellent immunogenicity of this schedule.[143–149] Israel and Denmark have also used such sequential schedules for a while, with successful results. In Israel, two schedules have been used: cIPV at 2, 4, 6, and 12 months of age with tOPV administered at 7 and 13 months of age or cIPV at 2, 4, and 12 months of age plus tOPV at 4, 6, and 12 months of age. Persistent polio in the Gaza Strip despite extensive use of tOPV induced the authorities to change to a mixed sequential schedule, which caused a prompt drop in wild-strain isolations.[232] A study in the United Kingdom showed the advantages of a sequential schedule comprised of one dose of cIPV followed by two doses of tOPV in terms of immunogenicity.[222]

A particular use of this sequential schedule was also undertaken in Romania because of an unusually high rate of VAPP triggered by concurrent intramuscular injections.[233] For a limited time, infants in one province of Romania received cIPV at 2, 3, and 4 months of age together with tOPV at 4 and 9 months of age.[234] The schedule was well tolerated and highly immunogenic. No cases of polio occurred subsequently in this region, but too few children were involved to draw conclusions about the prevention of VAPP.

A completed trial in Chile[235] using $bOPV_{1\&3}$ compared humoral and intestinal immunity after three doses of cIPV against two sequential cIPV-followed-by-$bOPV_{1\&3}$ schedules (cIPV-cIPV-$bOPV_{1\&3}$ or cIPV-$bOPV_{1\&3}$-$bOPV_{1\&3}$) at 8, 16, and 24 weeks of age. All received oral $mOPV_2$ challenge at week 28. At week 28, more than 98% of all group subjects seroconverted against types 1 and 3, and type 2 seroconversion exceeded 96% after three or two cIPV doses. Interestingly, in the cIPV-$bOPV_{1\&3}$-$bOPV_{1\&3}$ group, 77.6% seroconversion to type 2 was achieved. Importantly, two-thirds of the type 2 nonresponders at 28 weeks in that group seroconverted within 1 week of $mOPV_2$ challenge, indicating priming against type 2, giving a combined 92% type 2 protection/priming rate for the cIPV-$bOPV_{1\&3}$-$bOPV_{1\&3}$ sequential regimen. As expected, maternally derived antibodies significantly interfered with immune responses. A key observation from that trial was that $bOPV_{1\&3}$ appeared to boost antibody levels (and also intestinal immunity, measured by type 2 shedding after $mOPV_2$ challenge) against type 2, suggesting some cross (heterotypic)-booster effect against type 2 (in the one-dose cIPV-primed subjects) by the two other serotypes included in $bOPV_{1\&3}$.

A key advantage of a sequential regimen of cIPV preceding OPV is to address the VAPP risk as it has been now well documented that starting polio immunization with cIPV can eliminate the occurrence of the VAPP associated with the first doses of OPV. This has proven to be a very successful strategy in the United States[236] and in Hungary.[154] From 1992 to 2006, Hungary switched from three annual mOPV campaigns to sequential schedule of one dose of cIPV-followed-by-tOPV and saw a complete cessation of VAPP.

With the recent data described above, this type of schedule might be advantageous in a context where $bOPV_{1\&3}$ will be the exclusive OPV vaccine used and where it will be crucial to guaranty that anti–polio type 2 immunity will be as high as possible in populations in case of reintroduction of poliovirus type 2 in those communities.

OPV-Followed-by-cIPV Sequential Schedule Trials

When first tested, this type of sequential schedule had the objective to close the immunity gap resulting from deficiencies in responses to tOPV use in tropical settings. Tested in the Ivory Coast,[237] a single dose of cIPV or tOPV was given after three doses of tOPV. Of those 9-month-old children who remained seronegative after the third dose of tOPV, 81%, 100%, and 67% seroconverted to types 1, 2, and 3 polio, respectively, after the single dose cIPV. The corresponding percentages for the tOPV administration were 14%, 27%, and 5%.

Another trial[238] evaluated the performance of cIPV versus three OPVs ($mOPV_3$ and tOPV from two manufacturers) used as a booster in 9-month-old Omani infants who had received five previous doses of tOPV. This supplemental dose of cIPV had excellent immunogenicity and led to higher increases in polio type 3 antibodies than did tOPV. In addition, intestinal mucosal immunity was assessed by administering a challenge dose of $mOPV_3$ at the 15-month visit. Overall, 13.2% of the infants excreted poliovirus type 3 and there were no significant differences in the rate of excretion of poliovirus type 3 among the study groups.

More recently, a study in India in 6- to 9-month-old infants who had previously received multiple doses of tOPV and $mOPV_1$ were given a single dose of cIPV. Nearly 100% of children who were seronegative to types 2 and 3 at the time of the dose seroconverted.[196] In addition, the dose of cIPV was associated with a marked boost in intestinal immunity as documented by decreased fecal shedding following an OPV challenge (see "Mucosal Immunity/Protection Induced By Inactivated Polio Vaccine" later).

A study in The Netherlands showed that cIPV vaccination was able to boost systemic and salivary immunoglobulin (Ig) A responses in previously tOPV-vaccinated people. In contrast, persons who received only past cIPV did not produce a salivary IgA response.[239]

John[240] evaluated the performance of cIPV to boost humoral (and also intestinal) immunity in 1- to 4-year-old

Indian infants who had received previous doses of tOPV. This supplemental dose of cIPV had excellent immunogenicity and led to higher increases in antibodies in all three polio types than did the $bOPV_{1\&3}$ challenge dose administered for intestinal immunity evaluation. Similar observations were obtained in parallel trials completed in tOPV- or $bOPV_{1\&3}$-primed infants in India,[241] Bangladesh,[242] Pakistan,[243] Nigeria,[243a] and Latin America.[244]

In conclusion, all studies do clearly show that one dose of cIPV administered in OPV-primed subjects is able to boost humoral (and, more importantly, intestinal) immunity (see "Mucosal Immunity/Protection Induced By Inactivated Polio Vaccine" later) much better than one additional OPV dose. Interestingly, results obtained with the most recent trials where OPV priming was done with $bOPV_{1\&3}$, seem to suggest that a cross(heterotypic)-priming is induced by $bOPV_{1\&3}$ and that a one-dose cIPV boost is able to achieve substantial humoral (and intestinal, see below) responses against type 2 poliovirus. These observations do confirm the wisdom of the current position recommended by the WHO in the scope of the polio endgame strategy.[6]

Mixed/Combined Inactivated Polio Vaccine/Oral Polio Vaccine Schedule Trials

The previously mentioned WHO study[132–134] compared four doses of tOPV, three doses of cIPV, and a schedule consisting of four doses of tOPV mixed with three doses of cIPV. Seroconversion rates and GMTs were highest in the mixed/combined OPV/IPV groups. In addition, when children of the three groups were challenged with tOPV, virus excretion was as low in the mixed/combined vaccine groups as in the tOPV group, confirming the presence of intestinal immunity.

In Pakistan, infants were randomly vaccinated with tOPV alone; tOPV and cIPV at 6, 10, and 14 weeks of age; or were given tOPV at those times plus a single dose of cIPV at 14 weeks. The immune responses (seropositive children at week 24) were better for types 1 and 3 when tOPV and cIPV were administered simultaneously at each visit (97% vs 89% and 98% vs 92% against types 1 and 3, respectively), but the single dose of cIPV at 14 weeks did not improve the tOPV-only responses (91% vs 89%, 98% vs 96%, and 95% vs 92% against types 1, 2, and 3, respectively).[245]

Mucosal Immunity/Protection Induced by Inactivated Polio Vaccine

When analyzing the level of mucosal immunity and protection afforded by IPV, it is important to distinguish the target body compartment (nasopharynx vs intestine), the type of immunization schedule (IPV-only, sequential, or mixed/combined) used, and the nature of the effect measured (presence of poliovirus neutralizing antibodies at the mucosal surfaces or prevalence, duration, and intensity of poliovirus shedding following a subsequent poliovirus exposure). In addition, as mentioned previously, all the data presented below have been done with cIPV, as no data are yet available on the performance of sIPV in terms of mucosal immunity/protection.

It was recognized early on that cIPV vaccinees could excrete poliovirus in stools and in nasopharyngeal secretions after challenge,[246–249] which was seen as an important disadvantage versus OPV. Time has tempered this vision, particularly because of the progressive observations that cIPV-induced nasopharyngeal immunity could limit the virus shedding from this site after challenge. Studies in monkeys demonstrated that pharyngeal excretion of poliovirus was inhibited in cIPV vaccinees equally or even more than in tOPV vaccinees.[250–252] Then, Marine,[253] who followed families exposed to natural wild-type 1 virus, found that pharyngeal infection was prevented by low levels of circulating neutralizing antibodies induced by cIPV; higher levels were associated with reduced intestinal infection (Table 48.6). Nasopharyngeal immunity induced by cIPV-only schedules, as measured by the levels of secretory IgA or IgG in secretions, may be less than the levels of such antibodies induced by OPV-only schedules. However, nasopharyngeal immunity as measured by resistance to a challenge by OPV viruses appears equivalent to such immunity induced by OPV. Many of the data come from Ogra who found that tOPV recipients developed nasopharyngeal, and sometimes duodenal, poliovirus-specific secretory IgA, whereas cIPV recipients produced lower quantities.[143,144,254,255] Nasopharyngeal and systemic antibody responses after three doses of cIPV, tOPV, or a cIPV-followed-by-tOPV sequential schedule have been observed (summarized in Table 48.7),

TABLE 48.6 Percent Excretion of Wild Poliovirus Type 1 in Children According to Level of Vaccine-Induced Serum Neutralizing Antibody

	% Excretion at Given Time After Infection			
	1–2 Wk		3–4 Wk	5–6 Wk
Antibody Titer	Pharynx	Stool	Stool	Stool
<8	75	93	82	60
8–64	38	97	81	54
>64	25	88	59	28

Data from Marine WM, Chin TDY, Gravelle CR. Limitation of fecal and pharyngeal poliovirus excretion in Salk-vaccinated children. Am J Hyg. *1962;76:173–195.*

TABLE 48.7 Levels of Serum-Neutralizing or Nasopharyngeal IgA Antibodies in Infants After Three Doses of IPV, OPV, or an IPV Followed by OPV Sequential Schedule

	OPV-OPV-OPV			IPV-IPV-IPV			IPV-IPV-OPV		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Serum neutralizing antibodies (%)	100	100	100	96	100	100	100	100	100
GMT	1470	3578	1522	1954	5835	5187	3044	10,693	2348
Nasopharyngeal sIgA antibodies (%)	100	100	100	89	91	89	75	81	81
GMT	69	97	129	24	25	31	19	22	23

GMT, geometric mean titer; IgA, immunoglobulin A; IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; sIgA, secretory immunoglobulin A.

From Faden H, Modlin JF, Thoms ML, et al. Comparative evaluation of immunization with live attenuated and enhanced-potency inactivated trivalent poliovirus vaccines in childhood: systemic and local immune responses. J Infect Dis. *1990;162:1291–1297.*

but nasopharyngeal antibody levels were the highest following the tOPV-only schedule. Nevertheless, another study showed equal secretory IgA levels in pharyngeal and stool samples of prior cIPV and tOPV vaccinees.[147] It has been shown on many occasions that both premature and full-term infants are able to develop nasopharyngeal IgA after immunization.[256] Thus, in epidemiological settings where the primary mode of inter-human transmission in affected communities is oral-to-oral (vs fecal-to-oral), cIPV-only schedules can effectively prevent transmission.

With regards to the question of intestinal immunity (and protection) induced by cIPV, the situation is more complex to analyze. It is true that cIPV-only schedules, whether measured by secretory IgA, by transuded IgG, or by resistance to a challenge (prevalence, duration, and intensity of viral shedding), appear to be inferior to such immunity generated by OPV-only schedules; nevertheless, the difference between cIPV- and tOPV-only schedules is variable and seems influenced by multiple factors. It is undisputed that cIPV vaccinees can excrete poliovirus following infection by wild-type or Sabin viruses. In one trial, 93% of cIPV-only vaccinated Finnish infants excreted type 3 virus in stools after a $mOPV_3$ challenge with 300,000 median tissue culture infective doses ($TCID_{50}$), but there was no control group.[257] The median length of poliovirus excretion was 35 to 42 days, and the excreted peak virus titers were $10^{5.6}$ $TCID_{50}$ per gram.[169] In another trial, children who had received three doses of cIPV or tOPV were challenged with two different potencies of $mOPV_1$.[147] The results (summarized in Table 48.8) revealed that, whereas few subjects in either group excreted virus from the pharynx, intestinal shedding was significantly lower in the tOPV group (82% vs 31% for the high-titer challenge and 46% vs 18% for the low-titer challenge). One study done in the United States had the advantage of a control group that was unvaccinated. In this study, two doses of cIPV or tOPV were followed by a dose of tOPV.[223] Viral excretion in stools was measured after that dose, in comparison to a group receiving tOPV for the first time. Table 48.9 summarizes the results, which show, in line with prior studies, that excretion of poliovirus in cIPV vaccinees is lower and shorter than in the unvaccinated, but higher and longer than in tOPV vaccinees. One Cuban study of cIPV given by the EPI schedule showed virus isolation prevalence 1 week after challenge with tOPV of 94%, and a mean log_{10} viral titer of any serotype of 3.46. The titers of virus shed were approximately 0.5 log lower in cIPV-vaccinated children postchallenge compared to unvaccinated children being vaccinated for the first time with tOPV.[258] Many additional studies have documented the lower prevalence, the possibly shorter duration, and the lower intensity of poliovirus shedding following cIPV schedules compared to unvaccinated control (first-time OPV recipients).[195,224,226]

In the two tOPV-followed-by-cIPV sequential schedule trials mentioned above,[240,241] a $bOPV_{1\&3}$ was used as the challenge vaccine to evaluate the quality of the intestinal immunity and protection induced by the cIPV boost done on a tOPV-priming. Results clearly indicated that the supplementary dose of cIPV boosted intestinal immunity against types 1 and 3, as evidenced by less shedding (in terms of prevalence, duration, and intensity) compared to tOPV-only vaccinated subjects. Similar observations were obtained in a parallel trial done in Bangladesh.[242]

With the recent introduction of $bOPV_{1\&3}$, new data have been generated on the quality of the intestinal immunity and protection against type 2 of cIPV-followed-by-$bOPV_{1\&3}$ sequential schedules. In the Chile study mentioned above,[235] the sequential regimen resulted in lower type 2 shedding incidence compared to the cIPV-only regimen, illustrating again the heterotypic (cross)-booster effect induced by $bOPV_{1\&3}$ against type 2 on the one- or two-dose cIPV-primed subjects mentioned before. In addition, several studies have assessed the effect of cIPV on boosting intestinal immunity against type 2 following a $bOPV_{1\&3}$-followed-by-cIPV sequential schedule.[244] Results confirmed that heterotypic (cross)-priming effect against type 2 on humoral and mucosal immunity can be induced by $bOPV_{1\&3}$, and that it can be boosted by cIPV.

Finally, studies have also examined the effect of prior cIPV-induced immunity on the mutation profile of tOPV viruses in the intestinal tract.[259–261] This phenomenon, referred to as reversion to virulence, is a regular feature of the replication of attenuated poliovirus strains, whereby the mutations in those strains responsible for attenuation in humans can revert to the virulent genotype. Although the suggestion has been made that prior cIPV immunization potentiates that reversion,[224,225] a relatively large study failed to show a significant difference in the mutations of excreted virus between cIPV- and tOPV-vaccinated subjects.[262] Analysis made on virus samples isolated during use of a tOPV-followed-by-cIPV sequential schedule in the United Kingdom[222] found that reversions occurred faster in vaccinees given cIPV or tOPV than in previously unvaccinated infants, suggesting that the virus attempts to increase its fitness in the presence of antibodies.[226,227]

What makes cIPV-only vaccinees excrete less poliovirus upon challenge? Hovi[169] studied IgA production in the intestine

TABLE 48.8 Isolation of Poliovirus From Stool or Pharynx of Prior Recipients of IPV or OPV After Challenge With Type 1 OPV

	No. of Pharyngeal Isolations (%)		No. of Stool Isolations (%)	
Challenge Dose	**IPV**	**OPV**	**IPV**	**OPV**
High (560,000–600,000 $TCID_{50}$)	1/45 (2)	3/45 (7)	37/45 (82)	14/45 (31)
Low (500–800 $TCID_{50}$)	0/48 (0)	0/34 (0)	22/48 (46)	6/34 (18)
Total	1/93 (1)	3/79 (4)	59/93 (63)	20/79 (25)

IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; $TCID_{50}$, median tissue culture infective dose.

From Onorato IM, Modlin JF, McBean AM, et al. Mucosal immunity induced by enhanced-potency inactivated and oral polio vaccines. J Infect Dis. *1991;163:1–6.*

TABLE 48.9 Viral Shedding in Stool of Any Type After Trivalent OPV Administration to IPV Vaccinees, OPV Vaccines, or Unvaccinated Infants

	1 Week Post OPV Challenge		3 Weeks Post OPV Challenge		
Prior Vaccination	**N**	**% PCR Positive (CI)**	**N**	**% PCR Positive (CI)**	**Geometric Mean Copy No. (CI)[a]**
None	48	92 (80–98)	48	81 (67–91)	627 (398–987)
tOPV × 2	41	22 (11–38)	42	5 (1–16)	NA
cIPV × 2	42	76 (61–88)	38	37 (22–54)	155 (53–456)

[a]Of positive stools.

CI, confidence interval; cIPV, conventional inactivated polio vaccine; IPV, inactivated polio vaccine; OPV, oral poliovirus vaccine; PCR, polymerase chain reaction; tOPV, trivalent oral poliovirus vaccine.

Adapted from Laassri M, Lottenbach K, Belshe R, et al. Effect of different vaccination schedules on excretion of oral poliovirus vaccine strains. J Infect Dis. *2005;192:2092–2098.*

of cIPV vaccinees and found little IgA until the vaccinees had been challenged with tOPV. A correlation was found between the detection of intestinal IgA and diminution of virus excretion, and between the height of the serum antibody titer and prevention of excretion in Israel.[263] A recent study did show that presence by day 7 of IgA in stool with neutralizing activities was associated with cessation of virus shedding and that previous cIPV administration was able to prime subjects for an early response to a poliovirus challenge.[264] In addition, some preliminary data on circulating antibody-producing B cells demonstrate that this might be a useful marker for prediction of mucosal immunity.[264a] In one trial where infants received $bOPV_{1\&3}$ at 6, 10, and 14 weeks plus IPV at 14 weeks,[244] an inverse correlation was noted between type 2 virus shedding following challenge and both serum type 2 neutralizing antibodies and mucosal type 2 neutralzing antibodies.[264b] Whether high serum-neutralizing antibodies are a correlate of intestinal protection is still debated and data are conflicting.

To summarize, the accumulated evidence on whether or not cIPV is able on its own to limit the prevalence of intestinal poliovirus excretion and/or its duration and/or its intensity following a challenge are not definitive.[265,266] Assuming it does, the key difficulty is to evaluate the epidemiological impact of such an attribute at the community level in situations where fecal-to-oral is the main mode of poliovirus transmission, and, consequently, to estimate the potential for herd protection with IPV.[267] In the city of Cordoba, Argentina, use of cIPV greatly reduced the circulation of OPV strains; although Sabin viruses were still occasionally detected in sewage, some of which had reverted to a more neurovirulent phenotype, no circulating VDPVs (cVDPVs) were detected.[268] A study showed that revertant and nonrevertant Sabin-strain viruses could be isolated from cIPV-vaccinated infants 10 weeks after the campaign and from sewage for at least 13 weeks.[269] The recent Israel episode[270–272] where wild type 1 polioviruses were regularly and widely isolated from environmental samples for 14 months, clearly indicated that an IPV-only schedule (with accumulation of nine consecutive annual birth cohorts from 2005 to 2013) could not prevent sustained wild poliovirus circulation, albeit silent without paralytic cases, in well-vaccinated Israeli communities that have been subject to poliovirus importations. While IPV may not be sufficient to prevent sustained transmission of wild poliovirus it may be adequate to prevent sustained transmission of Sabin viruses, which have a lower force of infection than wild viruses. Thus, IPV may reduce the potential for emergence of cVDPVs. The experience in Yogyakarta province of Indonesia, which switched to an all-cIPV schedule in 1997, and where during 5 years, despite being surrounded by OPV-using populations and intensive surveillance, no cVDPVs were detected, is interesting.[273]

Efficacy of Inactivated Polio Vaccine Against Acute Flaccid Paralysis and Immune Correlates of Protection

The clinical efficacy of cIPV in its original version was proved beyond a doubt in the original field trial conducted by Francis and colleagues.[1–5] In that trial, approximately 400,000 children randomly received vaccine or placebo, and another 200,000 were vaccinated and observed together with unvaccinated children. There were 71 cases of paralytic polio in vaccinees versus 445 in control subjects. In the placebo-controlled part of the study, 70 cases occurred in the placebo arm versus 11 in the vaccinated arm. The calculated efficacy of the vaccine was 80% to 90% against paralytic polio and 60% to 70% against all forms of polio. The efficacy of cIPV was later confirmed in several settings. Melnick[274] measured an efficacy of 96% through two polio seasons in Houston, Texas. In Senegal, two doses of a DTwP-cIPV vaccine were given in the Kolda area, which subsequently suffered an outbreak of type 1 polio. A case-control analysis revealed an efficacy for one dose of 36% (95% confidence limits, 0% to 67%) and for two doses of 89% (95% confidence limits, 62% to 97%).[106,107] In another study conducted in the North Arcot region of India, John[275] compared tOPV in one district with cIPV vaccination in two other districts. Vaccination coverage with three doses rose to 85% to 90% in the tOPV districts and 75% to 80% in the cIPV districts. Case-control analysis revealed an efficacy of 92% for cIPV and 66% for tOPV. During the introduction of cIPV into Canada, efficacy of the vaccine was calculated at greater than 90%.[276]

Poliovirus neutralizing antibody levels above the 1 : 8 dilution threshold are now well accepted by all national regulatory agencies as correlates of protection against clinical paralysis when reviewing license applications for cIPV-containing vaccines,[114,277] although a 1 : 4 dilution may also be protective. The question of whether the same threshold should be applied to sIPV-containing vaccines is still debatable (see "Inactivated Polio Vaccine Manufactured from Sabin Strains" and "Immunogenicity of Sabin Inactivated Polio Vaccine" earlier).

Community Protection

The best evidence for a community protection effect of cIPV is the experience in the United States where cIPV was introduced into routine use in 1955 and was replaced by OPV in 1962. A sharp drop in the numbers of cases of paralytic and nonparalytic polio was evident during the years 1955 to 1962 (Fig. 48.5). The apparent reduction in the number of cases observed exceeded the expectation based on the percentage of children vaccinated (Fig. 48.6).[278] More specific regional data were published that suggested a greater-than-expected reduction in polio cases.[279] A second example of community protection effect comes from The Netherlands where vaccination is refused by a religious community that is well dispersed throughout the country, although cIPV is routinely administered to the rest of the population. Two outbreaks of polio have occurred in this religious group, one caused by type 1 virus in 1978 (110 cases) and the second caused by type 3 virus from 1992 to 1993 (71 cases). Despite the wide circulation of the virus in this community, there was only one case of polio in other Dutch communities. Approximately 400,000 unvaccinated individuals not belonging to this religious community also remained unaffected.[280–285] The virulent viruses also spread to similar religious groups in North America, but cases only resulted from the 1978 outbreak.[286–288] Oostvogel and colleagues[289] did an analysis of the circulating viruses in schools affected by the outbreak in the Netherlands from 1992 to 1993. Proof of recent type 3 infection was found in 59.5% of the unvaccinated children and in 22.2% of the vaccinated children. The evidence for community protection comes from communities where oral-to-oral transmission was probably the dominant mode of interhuman poliovirus transmission. It is less clear if cIPV is able to induce community protection in communities where the fecal-to-oral route is thought to be the primary method of poliovirus transmission (see "Mucosal Immunity/Protection Induced By Inactivated Polio Vaccine" earlier).

Duration of Immunity

Studies have assessed the persistence of antibodies following different infant primary series regimens with cIPV-containing vaccines with or without a first booster administered during

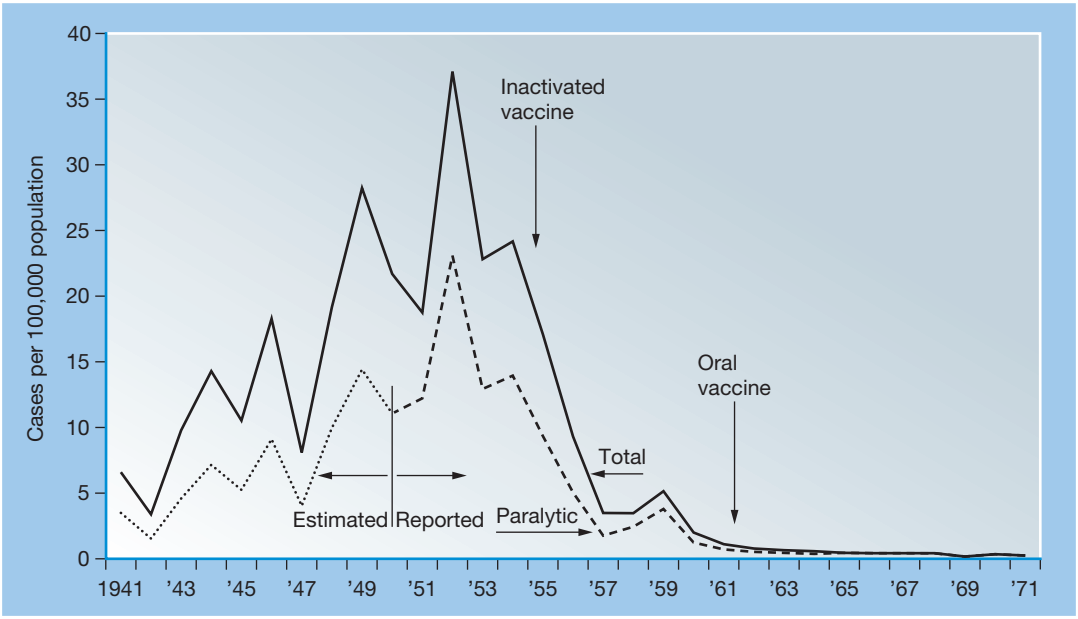


Figure 48.5. Incidence of polio in the United States. The oral polio vaccine was introduced from 1961 to 1962. The dashed line indicates the incidence of paralytic polio only; the solid line measures the incidence of both paralytic and nonparalytic polio. *(From Centers for Disease Control and Prevention (CDC).* Immunization Against Disease–1972*. Atlanta, GA: CDC; 1973.)*

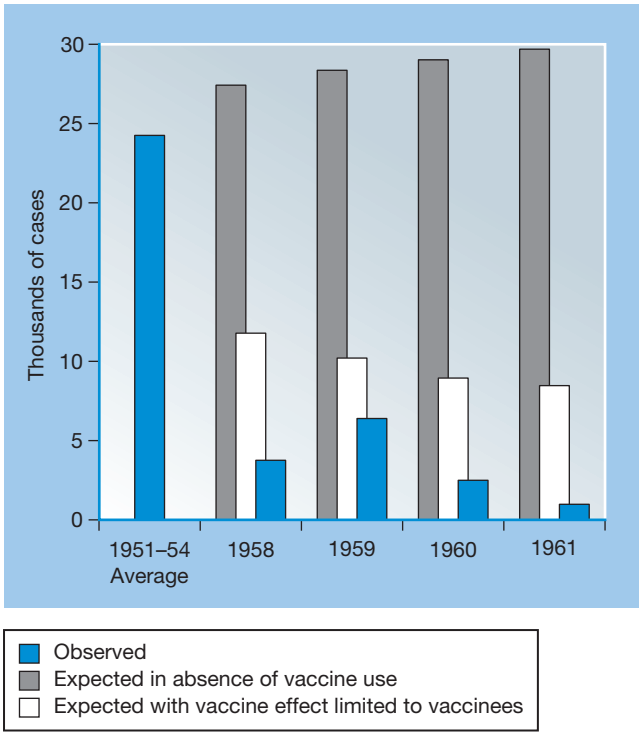


Figure 48.6. Herd effect induced by inactivated polio vaccine in the United States from 1958–1961. The number of observed cases of paralytic poliomyelitis was consistently lower than the number that would have been expected if vaccination had benefited only vaccinated individuals. *(From Stickle G. Observed and expected poliomyelitis in the United States, 1958–1961.* Am J Public Health*. 1964; 54:222–229.)*

the second year of life and/or at school-entry age. These studies have involved cohorts followed for varying periods of time vaccinated in trials aimed at documenting the early responses induced by cIPV-containing vaccines under development and/or licensure. The studies have been grouped by type of cIPV vaccine used first and then by type of primary series regimen for the trials done with the modern cIPV.

The first group of data includes studies done with the historical cIPV-containing vaccines. These low-potency vaccines contained quantities of antigen that were lower than the current formulations. Bottiger[290] evaluated 30 children given two doses of cIPV at approximately 9 and 10 months of age and a first booster dose at 2 years of age. When tested at 10 years of age (8 years after the last dose), 100% of the children still had seroprotective levels (≥1:4) of antibody to all three poliovirus types. In addition, in the same study, 220 Swedish children given the same low-dose vaccine with three doses at 9 months, 10 months, and 2 years of age, and a later second booster dose at 6 years of age were evaluated. At 18 years of age (12 years after the last dose), 100% of subjects were still seropositive (≥1:4) to all three serotypes. The decline of antibody titers was greater during the first 3 years after the 6-years-of-age booster (0.13–0.22 $\log_{10}$ fold-reduction per year) than during the next 9 years (0.05–0.10 $\log_{10}$ fold-reduction per year). In another study conducted in Sweden,[291] two primary series schedules were evaluated. All subjects were given a low-dose 20-4-16 D-Ag units cIPV concomitantly but at separate sites from DTaP and *Haemophilus influenzae* type b (Hib) vaccines. One group of 103 children were immunized using the standard Swedish schedule at 3, 5, and 12 months of age; the other group of 118 children were immunized at 2, 4, 6, and 15 months of age. At 4 years of age, 93% to 100% of children on the 3-5-12–month schedule and 96% to 100% of children on the 2-4-6-15–month schedule still had seroprotective titers (≥1:4) to the three poliovirus serotypes.

The second group of data includes studies done with modern cIPV-containing vaccines, and is further subgrouped according to the type of infant/toddler schedule used. That is,

two doses in infancy followed by one dose in the second year of life (2+1), three doses in infancy with no booster dose in the second year of life (3+0), or, finally, three doses in infancy followed by a booster in the second year of life (3+1). Table 48.10 summarizes the results (Sanofi Pasteur, studies A3R22 and TD517; unpublished, data on file).[229,262,292–301] Data show persistence of antibodies only up to the school-entry age, because all cohorts have received a school-entry booster with a cIPV-containing vaccine. No data are available in cohorts of subjects primed with these different infant/toddler schedules and having not received a school-entry booster with a cIPV-containing vaccine. Overall, these studies show that the persistence of poliovirus-neutralizing antibodies is well established at least until school-entry age by the 3+1, 2+1, and 3+0 schedules, with slightly higher titers in favor of the most complete schedule (3+1). In studies where evaluated, antibody titers declined more rapidly during the first 2 years after the last dose (0.45–0.60 $\log_{10}$ fold-reduction/year for each of the three serotypes) but remained more stable during the following years. When a school-entry booster is given following these different infant-toddler primary immunization schedules, there is evidence of a marked anamnestic response with very high titers when measured in the following month.[294,295,297–300] No data have yet been published on the persistence of poliovirus-neutralizing antibodies induced by sIPV.

Based on the historical Swedish data noted earlier, duration of protection is expected to be long term, and whether additional boosters after the toddler and/or the school-entry booster will induce lifelong protection is still debated.[302] No studies have yet evaluated persistence of poliovirus-neutralizing antibodies beyond childhood following complete primary immunization as the first countries who put in place routine

TABLE 48.10 Long-Term Persistence of Antibodies Up to School-Age Following Various Infant/Toddler Primary Series

Study	Ages of Immunization (Months)	Vaccine Used for Polio Primary Immunizations	Age at Serum Collection	SP Threshold	No. of Subjects	% Seropositive (GMT)		
						Type 1	Type 2	Type 3
2+1 Primary Series Infant/Toddler Schedule								
Murdin et al[262]	2, 4, and 18	IPV standalone	4–6 yr	≥1:8	147	97% (426)	99% (722)	94% (276)
Faden et al[229]	2, 4, and 12	IPV standalone	5 yr	≥1:10	27	100% (200)	100% (398)	100% (251)
Swartz[292]	2, 3.5, and 10	DTwP-IPV	4–5 yr	≥1:4	~50	100% (NA)	100% (NA)	100% (NA)
Carlsson et al[293]	3, 5, and 12	DTaP-IPV/Hib	5.5 yr	≥1:4	112	95% (72)	99% (133)	97% (98)
Black et al[294]	2, 4, and 6 2, 4, and 18	IPV standalone	4–6 yr	≥1:8	837–841 260–262	88% (32) 85% (31)	92% (40) 87% (35)	85% (38) 85% (38)
3+0 Primary Series Infant/Toddler Schedule								
Kitchin et al[295]	2, 3, and 4	DTaP-IPV-Hib	3.5–4.5 yr	≥1:8	77	89% (37)	85% (52)	92% (47)
Guerra et al[296]	2, 4, and 6	IPV standalone	4–5 yr	≥1:8	249	96% (63)	99% (84)	95% (61)
3+1 Primary Series Infant/Toddler Schedule								
Langue et al[297]	2, 3, 4, and 14–16	DTwP-IPV/Hib and DTaP-IPV/Hib	5–6 yr	≥1:5	162	94% (72)	96% (85)	99% (187)
Mallet et al[298]	2, 3, 4 or 2, 4, 6 and 12–16	DTaP-IPV/Hib	5–6 yr	≥1:5	234	94% (58)	96% (78)	96% (123)
Carlsson et al[293]	2, 4, 6, and 13	DTaP-IPV/Hib	5.5 yr	≥1:4	116	97% (92)	100% (125)	100% (202)
Danjou et al[299]	2, 3, 4, and 16–18 2, 3, 4, and 16–18	DTwP-IPV DTaP-IPV/Hib	4–7 yr 4–7 yr	≥1:5 ≥1:5	131 130	95% (88) 95% (86)	95% (72) 97% (94)	97% (121) 99% (109)
Gadjos et al[300]	2, 3, 4, and 16–18 2, 3, 4, and 16–18	DTaP-IPV/Hib DTaP-IPV/Hib	5.8–7 yr 5.8–7 yr	≥1:8 ≥1:8	383 375	92% (87) 88% (67)	96% (109) 93% (83)	96% (136) 92% (99)
SP study A3R22[a]	2, 3, 4 or 2, 4, 6, and 15–17	DTaP-IPV-HepB-Hib	5–6 yr	≥1:8	166	93% (91)	98% (115)	94% (138)
Guerra et al[296]	2, 4, 6, and 15	DTaP-IPV/Hib	4–5 yr	≥1:8	76–77	95% (172)	99% (265)	97% (284)
SP study TD517[b]	2, 4, 6, and 15 2, 4, and 6 or 2, 4, and 15	DTaP-IPV/Hib IPV standalone	4–5 yr	≥1:8	114 106 328 320	98% (157) 92% (120) 99% (130) 98% (134)	100% (226) 99% (242) 99% (151) 99% (152)	94% (170) 95% (143) 95% (122) 94% (111)
Zinke et al[301]	2, 3, 4 or 3, 4, 5 and 12–23	DTaP-IPV-HepB/Hib	4–6 yr 7–9 yr	≥1:8	174–185 144–148	>95% (87) 91% (52)	>95% (84) 91% (44)	97.2% (158) 97.2% (96)

[a]Sanofi Pasteur, study A3R22; unpublished, data on file.
[b]Sanofi Pasteur, study TD517; unpublished, data on file.
DTaP, diphtheria, tetanus, and acellular pertussis vaccine; GMT, geometric mean antibody titer; HepB, hepatitis B vaccine; Hib, *Haemophilus influenzae* type b vaccine, IPV, inactivated polio vaccine; NA, not available.

and exclusive use of modern cIPV-containing vaccines schedules started in the mid-1980s. Thus, the cohorts who received such schedules are in their mid-thirties. Nevertheless, some countries recommend post–school-entry age additional dose(s) (see Table 48.2). The availability of IPV-containing vaccines licensed for adolescents and adults and with boosting indications (IPV, Td-IPV, and Tdap-IPV) facilitates implementing such boosting strategies. Studies show that one (third) booster dose with a cIPV-containing vaccine in IPV-only 3+1+1 primed 11- to 14-year-old adolescents is able to induce persisting antibodies for at least 10 years.[303]

Salk argued that immune memory is established by primary vaccination with IPV and that no further immunizations are necessary.[304–306] He showed that, whereas unprimed subjects reacted with only low responses to a single dose of cIPV, primed individuals, even those originally given fractional doses, developed anamnestic responses. In his opinion, the vaccinees would respond similarly to an infection, thus preventing viremia and disease. Swartz[176] showed that newborns could be primed to develop an anamnestic response to cIPV at age 6 months if they were given a dose at birth. In contrast, from a group of elderly cIPV-vaccinated and seronegative who were challenged with tOPV, only a third had anamnestic serum antibody responses, suggesting immune memory.[307]

A minimal position would be to recommend four to five doses of an cIPV-containing vaccine with the last one administered at school-entry age, as, for example, the current U.S. schedule, which is ages 2, 4, and 6 to 18 months and 4 to 6 years (2+1+1 or 3+0+1); or in the United Kingdom, which is ages 2, 3, and 4 months and 3 to 5 years (3+0+1) (but in this case, the UK recommends a fifth dose at 13–18 years of age); or in some other European countries, which is ages 2, 4, 6, and 15 to 18 months and 4 to 6 years (3+1+1). No data are available on the long-term persistence of circulating antibodies and waning of intestinal immunity conferred by a single IPV dose to be administered per WHO recommendations (e.g., OPV at 6, 10, and 14 weeks along with IPV at 14 weeks) whereas it has been shown that intestinal immunity conferred by OPV can wane.[308] With the switch from tOPV to $bOPV_{1\&3}$, the single dose of IPV will be the only exposure children on this schedule have to the type 2 antigen.

ADVERSE EVENTS

The "Cutter incident" is probably a unique event in the field of vaccine safety and for sure in the history of IPV. Between April and June 1955, shortly after licensure in the United States, 204 acute flaccid paralysis (AFP) cases of type 1 polio were observed in association with use of vaccines manufactured by Cutter Laboratories.[309,310] After investigation, 60 cases in vaccinees and 89 in family contacts were judged to be caused by two lots of vaccine in which infectious virus had not been completely inactivated. Approximately 70,000 children received the two lots, and studies revealed infection in 10% to 25% of vaccinated children and found an incidence of one paralytic case in every 100 to 600 infections. The incriminated lots all had passed release tests, including monkey neurovirulence and tissue culture infectivity, but other lots similarly manufactured had failed. As a consequence, a requirement for all lots to pass safety tests was introduced at that time. The Cutter incident was the result of a lack of regulatory supervision of companies that had no experience in making IPVs, and served as a lesson in vaccine safety management.[311] Since the Cutter incident and the safety measures instituted as a result of that experience, there has been no evidence for defective manufacture of IPV, and since the initial launches of the modern IPVs, it can be estimated that more than 1 billion doses of cIPV-containing vaccines produced by the major manufacturers have been used without subsequent polio or other serious reaction.[130,262]

IPV is very well-tolerated. When infants are injected intramuscularly with cIPV standalone vaccine, injection-site erythema is seen in 0.5% to 1.5% of infants, induration in 3% to 11%, and tenderness in 14% to 29%.[312] The combination of cIPV with other vaccines, such as DTwP/DTaP (supplemented or not with Hib and hepatitis B), do not add to the reactions observed with those vaccines alone.[130,262] cIPV-containing vaccines are now licensed in almost all countries, and in 2014 it was estimated that 30 to 40 million newborn infants and 20 million school-entry children, adolescents, and adults had received at least one dose of a cIPV-containing vaccine. Overall, the numbers of adverse events reported to the manufacturers are low, and the types of reactions reported are within what is expected with this kind of unadjuvanted inactivated vaccine and without concentration in a single category. The data collected in the United States by the Centers for Disease Control and Prevention's (CDC's) Vaccine Adverse Event Reporting System database between 1991 and 1998 was reviewed for reactions attributed to cIPV[313] and there was no suggestion of causally related serious adverse events. In the cases when IPV is administered subcutaneously, as seen with other inactivated vaccines when injected subcutaneously,[314] the incidence of local reactions at the injection site are slightly higher compared to what is seen when using the intramuscular route.[315] Pharmacovigilance experience accumulated on IPV-containing vaccines have also not shown unexpected reactions attributable to IPV.

Finally, a safety concern had been raised because older versions of cIPV were contaminated by a simian virus 40 (SV40) brought by the primary rhesus monkey kidney cells used to grow polioviruses used in some historical IPVs and that such vaccines may have been involved in the late occurrence of mesothelial tumors, brain tumors, and non-Hodgkin lymphomas in individuals exposed to polio vaccines before the vaccines were cleared of the virus in 1963.[316–319] Similar concerns were raised regarding older OPVs. Although epidemiologic studies have been negative so far, some controversy still remains on the clinical implications of human exposure to SV40.[320,321]

Recommendations for Inactivated Polio Vaccine

Infants and Toddlers

All high-income and an increasing number of middle-income countries rely on IPV-only schedules in their routine public childhood vaccination programs (see Table 48.2). In addition a growing number of middle-income countries recommend either IPV-only schedules supplemented by National Immunization Days (NIDs) with OPV organized once or twice a year in all subjects younger than 5 years of age (e.g., Mexico), IPV-followed-by-OPV sequential regimens (e.g., Russia, Brazil, Malaysia, Israel), or mixed/combined IPV/OPV regimens where the two vaccines are administered at the same time for one or more occasions during the vaccination course (e.g., South Africa). It is anticipated that many more countries will soon adopt one of those schedules (India, China, Indonesia, etc). The multiplicity of the regimens currently in place in these countries is driven by potential differences in country-specific basic immunization programs and perceived risks from wild virus and vaccine poliovirus. Key factors considered are epidemiological (VAPP incidence, level of risk for poliovirus importations), sociological (lack of acceptance of VAPP risk), performance of the routine immunization program, presence of national manufacturer(s), vaccine procurement, and pharmacoeconomics.

With the recent adoption by the WHO and all Global Polio Eradication Initiative (GPEI) stakeholders of a revised strategy for the polio endgame strategy,[6] all remaining OPV-using countries (mainly low-income countries) are introducing IPV in their routine vaccination programs (as of the end of 2016, approximately half of them, the most at risk, have introduced at least one dose of IPV and the remaining may introduce IPV later with modalities to be defined depending on supply availability and on willingness to adopt the use of fractional dosing of IPV). The current recommendation is that at least one dose of IPV should be administered at the time of the third routine DTP visit where infants should simultaneously receive their third OPV infant series dose (the fourth OPV dose in those countries with a birth dose of OPV). The main rationale for this OPV-followed-by-IPV sequential regimen is to make sure that cohorts of infants acquire a minimal level of poliovirus type 2 immunity because tOPV has been replaced by $bOPV_{1\&3}$. Without IPV these cohorts could be vulnerable if poliovirus type 2 (wild type or VDPV) is reintroduced in their communities (see Chapter 49). In addition, as summarized above (see "Immunogenicity of Mixed/Sequential Schedules with Inactivated Polio Vaccine and Oral Polio Vaccine" and "Mucosal Immunity/Protection Induced by Inactivated Polio Vaccine") it is expected that this IPV dosing will boost poliovirus intestinal immunity induced by previous tOPV or $bOPV_{1\&3}$ administrations. Furthermore, potential heterotypic(cross)-priming effect against type 2 that has been observed in some $bOPV_{1\&3}$-followed-by-IPV sequential regimen studies[244] is particularly relevant in that context.

As of the end of 2016, IPV-containing vaccines are routinely recommended for infant vaccination against poliomyelitis in approximately 177 countries[322] (as of publication, the number of countries might be higher than this number) and it is hoped that by 2018, all countries will have achieved their IPV introduction. Several factors are driving the pace of this introduction (see "Public Health Considerations" later) and with the progressive ramp up of manufacturing capacities, the development of new IPV-containing products, and successful eradication of wild polioviruses, eventually the world will move to an all IPV schedule, once use of all OPV is stopped.

Children

In countries relying on IPV-only schedules in their routine public pediatric vaccination programs (see Table 48.2), a school-entry (or a later childhood, e.g., United Kingdom) booster is recommended as the last dose of the series. Several countries recommend subsequent boosters during adolescence (Austria, France, The Netherlands, Canada, United Kingdom, Greece).[323,324] For children who have not been vaccinated previously, or with unknown or incomplete vaccination history who need rapid protection because they are traveling to a zone where polioviruses are circulating, the recommended schedule is two doses 1 month apart followed by a booster 6 months later (or, if pressed for time, at least 1 month later).

Adults

In almost all countries relying on IPV-only schedules in their routine public childhood vaccination programs (see Table 48.2), routine (re)vaccination of adults or the elderly is not recommended. Very few countries recommend such routine boosting strategy (Austria, France, Greece).[323,324] Adults who have not been vaccinated previously, or with unknown or incomplete vaccination history, who need rapid protection because they are traveling to a zone where polioviruses are circulating, should always receive IPV, because VAPP after OPV appears to be more common in adulthood. In addition to the IPV standalone vaccine, IPV-containing combination vaccines including low-dose diphtheria and tetanus toxoids (some also including low-dose aP antigens) have been developed and licensed in many countries.[325–329] Similarly to children, in such situations the recommended schedule is two doses 1 month apart followed by a booster 6 months later (or, if pressed for time, at least 1 month later). Depending on the vaccination history, in some cases only one dose might be enough to boost adequately immune response. Many vaccination policy recommending bodies are regularly updating recommendations related to travel to polio-epidemic or polio-endemic area, and the WHO has acted similarly.[330] In principle, vaccination of previously unvaccinated adults is recommended when they are in contact with children excreting Sabin poliovirus[331] and adults who have been fully immunized but who are traveling to a wild poliovirus infected area should receive a one-time IPV booster.[332] In addition, the WHO recently amended its international travel recommendations stating that persons traveling from "recent polio exporting countries" should receive a dose of IPV or OPV no less than 4 weeks prior to departure and no more than 12 months prior to departure.[333]

High-Risk Groups

Personnel manipulating wild polioviruses should have previously completed vaccination. Healthcare workers should also be vaccinated because they may come into contact with wild poliovirus or reverted viruses excreted by vaccinees. IPV is universally recommended for subjects with known congenital or acquired immunodeficiency, in view of the VAPP risk in those subjects following contact (direct or indirect) with Sabin viruses even knowing their potential poor responses against vaccine antigens.[334] Family contacts of immunocompromised subjects should receive IPV instead of OPV to avoid being a source of vaccine viruses to their immunocompromised contacts. Those receiving systemic steroids or chemotherapy are included in this indication.

CONTRAINDICATIONS TO INACTIVATED POLIO VACCINE

Formal contraindications to IPV consist of a previous severe reaction to IPV vaccine or known or documented allergy to streptomycin, neomycin, or polymyxin B. In real life, these situations are extremely rare. Neither pregnancy nor breastfeeding is a contraindication.

SIMULTANEOUS USE WITH OTHER VACCINES

No clinically relevant interference effects have been described when IPV is used in association or in combination with licensed DTwP/DTaP, Hib, or hepatitis B vaccines. The largest experiences with IPV-containing combinations have been accumulated between the mid-1980s and the mid-1990s with DTwP-IPV and DTwP-IPV/Hib in France and Canada, and since the mid-1990s with DTaP-IPV–backboned combinations including hepatitis B and/or Hib in the United States, Canada, Europe, and elsewhere (see Chapter 15). In combination vaccines, IPV is compatible with DTaP, Hib, and hepatitis B, although generalization is difficult owing to the pharmaceutical specificities of all these drug substances, which are the key driver of whether these antigens can be combined. Extemporaneous mixing of two distinct liquid finished products should not be made by vaccinators. As mentioned earlier (see "Producers of Inactivated Polio Vaccine Antigen and Available Vaccines"), several IPV-containing DTwP-backboned hexavalent products are under development,[70,100,101] and in this case, the

key technical challenge is to manage the bidirectional interference between the wP and IPV antigens (caused by the presence of residual inactivating and bioburden control agents, e.g., thimerosal) on their respective potencies.

PUBLIC HEALTH CONSIDERATIONS

Results of Vaccination Programs With Inactivated Polio Vaccine

Experience with IPV in national public programs has been longest in Europe and in Canada. Some countries have used IPV exclusively since the mid-1950s, and some have used IPV as part of sequential or mixed/combined schedules with OPV.[131,262,335] Most of these experiences are reviewed in the following sections. It should be noted that in many countries listed below, a growing and significant proportion of immunization against polio is done in the private sector and that IPV-containing vaccines are the only products used in that sector (mainly driven by desire to avoid the risk of VAPP).

European Region

Many countries of this region have been early adopters of IPV.

Sweden has used IPV since 1957.[336] In 1989, modern IPV replaced the original vaccine and indigenous circulation of wild polioviruses was stopped by 1962,[337] although a subsequent outbreak occurred in an unvaccinated religious community without spread to the whole population.[285]

Similarly, IPV has been used in Finland for many years, starting with first-generation vaccine and changing to modern IPV in 1986. The only outbreak of polio reported since the introduction of IPV involved 10 cases in 1984–1985.[338–340] The type 3 component of the first-generation IPV was of low potency, with only 60% of three-dose vaccinees responding with neutralizing antibodies. Furthermore, vaccination coverage with the recommended six doses had slipped to 80% before onset of the outbreak. Investigations revealed that a type 3 wild-type virus was introduced, probably from Turkey, which was genetically distinct from the Saukett virus strain used to manufacture the vaccine and, because of that it escaped neutralization. A trypsin-treated vaccine was developed in an attempt to correct the specificity of the type 3 response,[257] but tests showed that it did not improve the immunogenicity. tOPV was brought in to stop the outbreak, after which the Finns returned to using IPV, but in the form of the modern vaccine. The modern IPV did induce neutralizing antibodies to the Finnish mutant virus.[337]

Denmark started with a sequential schedule in 1968. Starting in 1970, Danish infants received cIPV at 5, 6, and 15 months of age, followed by OPV at 2, 3, and 4 years of age. Single polio cases were diagnosed in 1969, 1976, 1980, and 1986, and the last two were imported. No wild-type virus has been identified in sewage samples since 1968. Not surprisingly, seroimmunity has been virtually 100% at all ages of the Danish population.[341] No VAPP has occurred among 1.5 million Danes who have received two or more doses of IPV, and in 1997 Denmark changed to an IPV-only schedule.

In The Netherlands where modern cIPV had its roots in the late 1970s,[342] polio has been prevented in the population by the use of a DTwP-cIPV combination vaccine and, more recently, a DTaP-cIPV vaccine. Regular serosurveys revealed high seroprevalence rates for polioviruses types 1, 2, and 3.[343,344] As described earlier, however, two outbreaks have occurred in Protestant religious communities refusing vaccination without spread to others (see "Community Protection").

Polio disappeared from Iceland in 1960 after the introduction of cIPV in 1956.[337]

Norway started vaccination with cIPV in the late 1950s but switched to tOPV in 1965. After that switch, there were six cases of VAPP, of which five were in unvaccinated people and the sixth in an individual given cIPV 10 years earlier (L. Flagstrud and H. Nokleby, personal communication). Because most of the population had received cIPV previously, it appears that VAPP had been almost completely prevented by prior IPV vaccination. Norway switched back to cIPV in 1979, and since then the only reported poliomyelitis has been imported from abroad.[337]

France is a good example of a country exposed to regular poliovirus importations that has kept the disease at bay with cIPV vaccination only. France started vaccination with cIPV in 1956, but in 1965 tOPV became the recommended vaccine. Both tOPV and cIPV were in use until 1983, when modern cIPV was exclusively recommended. VAPP occurred sporadically during the use of tOPV but ceased to be seen after 1986. The last wild poliovirus AFP case was reported in 1989, and attempts to find indigenous wild-type viruses in sewage have not been successful since 1989, whereas imported Sabin-like virus have been sporadically detected from 2000 to 2010.[345]

The United Kingdom switched from tOPV use to exclusive use of cIPV in 2004 when a pentavalent DTaP-IPV/Hib combination became licensed and was then recommended.

Russia adopted IPV in 2009 with initially cIPV at 3, 4.5, and 6 months of age followed by OPV at 18 and 20 months of age and 14 years. In 2011, following the 2010 wild poliovirus type 1 outbreak in Uzbekistan,[346] Russia decided to replace the 6 months of age cIPV dose with OPV.

All other European countries use cIPV-only schedules, based on the use of DTaP-backboned pentavalent and hexavalent combination vaccines.

Americas Region

All Canadian provinces have used cIPV-only, OPV-only, or cIPV-followed-by-OPV sequential schedules since the inception of vaccination in 1955, and the experience has been particularly large in Ontario, Canada's most populous province. Since 1997, all provinces are using cIPV-only schedules through the use of cIPV-containing DTaP-backboned pentavalent and hexavalent combination vaccines. The last indigenous case of polio occurred in 1988, and was related to an imported virus. Introductions of poliovirus from The Netherlands in 1979 and 1992 and from the Indian subcontinent in 1996 failed to spread to the general population.[276,347] Ontario, the province with the largest immigrant population, has had the most introductions, but other provinces using tOPV have also had wild poliovirus introduced. In every case, the wild virus was confined to unvaccinated immigrant groups or unvaccinated religious communities, while the general population was unscathed.

The early use of first-generation IPV in the United States was discussed earlier (see "Community Protection"). Despite incomplete application of the vaccine, polio incidence fell 95% between the introduction of the vaccine in 1955 and its abandonment in 1961 (see Fig. 48.5). The remaining cases, many of them in IPV vaccinees, sapped confidence in the vaccine and caused its replacement by tOPV.[262] The last cases of indigenously acquired wild-type paralytic poliomyelitis in the United States occurred in 1979 among unvaccinated Amish children in Pennsylvania, Missouri, Iowa, and Wisconsin. Table 48.11 summarizes the evolution in polio vaccination policy in the United States. Opinion gradually shifted away from tOPV and toward cIPV, but with the preference for a combination vaccine. Although a combination vaccine containing cIPV was not licensed at that time, cIPV

TABLE 48.11 Steps in U.S. Decisions Regarding Polio Vaccination

1955	Historical IPV licensed
1961	Polio outbreak shows partial effectiveness of IPV; epidemiologic data show that IPV does not completely prevent poliovirus circulation
1963	tOPV licensed, replaces IPV as recommended vaccine
1964	Surgeon General's committee concludes that 57 cases of VAPP occurred between 1961 and 1964 (reversion to virulence first recognized in 1955)
1970s	Van Wezel and Cohen at RIVM improve manufacturing processes of IPV
1977	IOM report recommends OPV for children, IPV for adults
1978	Institut Mérieux develops technologies for manufacturing IPV in Vero cells; monkey cells no longer needed
1988	IOM report recommends staying with OPV until IPV combination available or 90% coverage reached
1995	IOM workshop recommends moving toward IPV
1997	Sequential schedule adopted by ACIP
1999	ACIP recommends sequential schedule or all IPV; OPV alone not recommended
2000	IPV only schedule recommended

ACIP, Advisory Committee on Immunization Practices; IOM, Institute of Medicine; IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; tOPV, trivalent oral poliovirus vaccine; RIVM, Rijksinstituut Voor Volksgezondheid en Milien; VAPP, vaccine-associated paralytic poliomyelitis.

was reintroduced into recommended use in the United States (in its enhanced-potency form) in 1997 as part of a sequential schedule consisting of two doses of cIPV at 2 and 4 months of age, followed by two doses of tOPV at 12 to 18 months and 4 to 6 years of age.[348] VAPP cases immediately decreased to three in 1997 and one in 1998. All these cases occurred in children whose physicians had elected to start immunization with tOPV rather than the recommended cIPV.[236] Three years later, in 2000, the United States chose an cIPV-only regimen.[331] The primary reason for the reintroduction of cIPV was the perception that polio was vanishing from the world, whereas paralysis associated with tOPV was exacting an average yearly toll of 8 cases.[334,349,350] A study done in 2006 in contiguous Mexico border towns showed that IPV-vaccinated U.S. infants did not become infected with Sabin virus despite high-level poliovirus importation.[351] Before the switch occurred, concern was expressed that the use of standalone cIPV vaccine might decrease immunization rates because of the necessity of additional injections. However, this was not the case, and immunization rates were unaffected by the change to sequential and then cIPV-only schedules.[352–354]

Mexico (since 2007) and Costa Rica (since 2010) rely on an cIPV-only schedule, in the form of a pentavalent DTaP-IPV/Hib combination vaccine, with continuation of NIDs with OPV twice a year in Mexico in all children 6 months to 5 years of age (and who have received two or more IPV vaccinations). In Mexico, studies monitoring circulating Sabin viruses and VDPVs after the May 2009 biannual OPV NID revealed that Sabin viruses were isolated for 5 to 8 weeks after the NID (as expected) and that VDPV types 1 and 2 (which could have emerged from previous NIDs) could be isolated.[355] The suboptimal coverage reached both for the 2009 routine immunization program (71%, 73%, 78%, and 95% for the four cities sampled) and for the 2009–10 OPV NIDs (57%, 65%, 93%, and 80% for the four cities sampled) were probable factors favoring emergence of these VDPVs.

Brazil adopted OPV in 1960 and progressively deployed routine and high coverage programs. In addition, biannual OPV NIDs were instituted at the beginning of the 1980s. This led to termination of wild poliovirus transmission with the last case of wild-type AFP detected in 1989. In 2012, Brazil switched to cIPV in the form of a cIPV-followed-by-OPV sequential regimen (IPV at 2 and 4 months followed by OPV at 6 and 15 months), supplemented by an annual OPV NID for all children 6 months to 5 years of age.[356] This move was mainly driven by the objective to control the VAPP risk.

Eastern Mediterranean Region

Israel has used both polio vaccines in an attempt to prevent polio. After a brief experience with the original low-potency IPV,[16] Israel started routine tOPV vaccination in 1960. Vaccination coverage reached high levels among both Jewish and Arab children. Nevertheless, sporadic poliomyelitis continued among Jews, and small epidemics continued to occur in the West Bank and Gaza.[357–359] In view of the failure of tOPV to control polio, in 1978 Israel introduced a mixed/combined schedule where tOPV was administered at 1, 2.5, 4, 5.5, and 12 months of age, and cIPV (as a DTwP-cIPV vaccine) was given at 2.5 and 4 months of age. For a time in the 1980s, there were no cases in Israel proper and only sporadic cases in the Palestinian areas.[357,358] All went well until 1988, when an epidemic of 15 cases of type 1 polio occurred in Israel,[359] localized in one of two districts that had adopted cIPV-only vaccination of infants. Although the analysis of this epidemic is controversial, it is clear that antibody responses to tOPV were suboptimal among Israeli young adults, resulting in a low level of resistance. Conversely, the wild virus may have circulated among infants immunized with cIPV only, allowing spread to their parents. The response to the epidemic included mass vaccination with tOPV and the institution of three doses of DTwP-cIPV in the routine vaccination scheme together with four simultaneous doses of OPV.[359] Since 1988, no cases of polio have been reported in Israel or its territories, despite an outbreak in neighboring Jordan from 1991 to 1992 that caused wild virus circulation in Gaza.[360] In 2005, Israel adopted a cIPV-only (3+1+1) regimen. More recently, the episode of silent circulation of a wild-type 1 poliovirus from February 2013 to March 2014[270–272] illustrates the limits of cIPV-only schedules when deployed in communities with high poliovirus transmissibility potential[361] (see "Mucosal Immunity/Protection Induced By Inactivated Polio Vaccine" earlier). To extinguish this circulation, Israel modified its immunization regimen by adding $bOPV_{1\&3}$ at the 6- and 18-months-of-age vaccination visits, after having conducted IPV and $bOPV_{1\&3}$ supplemental immunization activities.

Western Pacific Region

Malaysia switched to cIPV in eight provinces in 2008 and then nationwide in 2010. The regimen is a 3+1 cIPV-only regimen followed by an OPV boost at school-entry age (7 years). As of the end of 2012, no polioviruses have been isolated from AFP cases, and a few Sabin-like polioviruses (and no VDPVs) have been isolated from environmental samples.[362]

Australia decided to switch to a cIPV-only regimen in 2005 when a cost-effective cIPV-containing aP combination became available.[363,364]

New Zealand switched to a cIPV-only regime in 2002 and documented a very rapid elimination of the infants excreting Sabin-like viruses and a disappearance of these viruses within

4 months in environmental samples. In addition, despite recurrent events of isolations of Sabin-like viruses indicative of importation events from OPV-using countries, no VDPVs emerged.[365]

After a very short and limited usage of the historical IPV, Japan relied on OPV from 1961 until September 2012. AFP cases caused by wild polioviruses disappeared in 1980; all remaining cases were VAPP, which continued to occur with an incidence of 1 per 1.4 million recipients of any OPV doses. This situation progressively undermined public acceptance and vaccination coverage for the biannual two-dose infant tOPV schedule dropped to a low of 67.2% during the spring 2012 round. In late 2012 two DTaP-sIPV products were licensed (see "Inactivated Polio Vaccine Manufactured from Sabin Strains" earlier), and with the already licensed cIPV (early 2012), Japan switched to an IPV-only (3+1) schedule in September 2012. Since then environmental surveillance has confirmed the disappearance of Sabin viruses.[366–368]

Southeast Asia Region

In Yogyakarta province of Indonesia, a cIPV-only schedule was adopted starting in September 2007, with administration at 2, 3, 4, and 9 months of age. Surveys showed no drop in vaccination coverage from the preexisting greater than 95% level. Seroprevalence surveys revealed 100% of recipients of three doses or more of cIPV had high seroprotective levels. Finally, Sabin-like polioviruses were only isolated a few times in sewage samples after the switch to cIPV (5% of samples compared to 58% of samples before the cIPV introduction) and no VDPVs were detected.[273] As expected this demonstration project showed the rapid elimination in the environment of the Sabin-like viruses with the only source being surrounding regions still using tOPV. During the 5-year surveillance period, no VDPV emerged illustrating the extreme importance of high vaccination coverage. As a result this province has adopted a cIPV-only schedule and Indonesia is on the verge deploying this schedule nationwide.

Africa Region

The Republic of South Africa introduced cIPV in their routine immunization program in 2009,[369] and is following a unique regimen consisting of tOPV at birth followed by tOPV and cIPV at 6 weeks of age, cIPV at 10 and 14 weeks of age and at 18 months of age. In addition, cIPV is administered in the form of a DTaP-IPV/Hib pentavalent combination vaccine. In addition, tOPV NIDs are conducted every 3 years, targeting vaccination of all children younger than 5 years of age, regardless of prior immunization status.

The 2013 wild poliovirus type 1 outbreak which occurred in the Horn of Africa, originating from Nigeria, prompted Kenya to use cIPV in a campaign together with tOPV as an outbreak response in more than 100,000 refugees younger than 5 years of age. Surveys indicated that greater than 90% coverage could be achieved with both vaccines. This program showed that both IPV and OPV could be used in mass campaigns with high coverage obtained for both vaccines in an actual field setting.[370]

Rationale and Role of Inactivated Polio Vaccine in Poliomyelitis Eradication

Since the early 1960s, there has been controversy regarding which polio vaccine should be chosen for routine immunization of infants—IPV or OPV. This led to considerable debate.[335,371–375] However, it is now recognized that both vaccines are necessary to achieve and sustain the eradication.[6,376–379] The data and experience to date show that IPV may be used in a variety of ways to support eradication, which is influenced by a multitude of specific factors that are reviewed below.[380,381] WHO even before the recently amended strategy[6] stated that "The national choice of vaccines and vaccination schedules during the preeradication period must include OPV or IPV, or a combination of both, and should be based on assessments of the probabilities and consequences of wild poliovirus importation."[382] Nevertheless, it is clear that after eradication of the circulation of polioviruses, the use of OPV will have to stop.

For the three countries still endemic for wild poliovirus and the more numerous countries at high risk of importation and reestablishment of transmission, especially countries with crowding and poor hygiene and sanitation, which would facilitate the fecal–oral spread of virus, OPV is critical. This is because OPV induces higher levels of intestinal immunity than IPV. However, IPV can have an important role because it induces high levels of individual protection even in developing countries with fewer doses than OPV and overcomes OPV deficiencies by bypassing the intestines, which can impede OPV seroconversion in developing countries. IPV also boosts intestinal and humoral immunity in prior OPV vaccinees who have not seroconverted, particularly against type 2 after $bOPV_{1\&3}$ administration. Thus, IPV following OPV can improve protection against the current circulating wild virus types because it improves on both the systemic and mucosal immunity induced by OPV.

IPV also has a major role to play in preventing VAPP and emergence and transmission of VDPVs. As cVDPV can be considered a complication of a suboptimal usage of OPV, then only two possibilities exist to alleviate this risk completely where OPV is used: either total elimination of the use of any form of OPV or achievement of extremely high vaccination coverage in all populations, subpopulations, and geographies. This may be an unreasonable expectation; as long as OPV is used, there is potential for generation of more cVDPVs. Furthermore, even with good and universal coverage with OPV, VAPP will occur. The elimination of VAPP is a key driver for many of the countries that have adopted a regimen in which polio immunization starts with IPV (one or two doses), as this is the only way to eliminate the risk of VAPP when continuing to use OPV in the schedule.[154,236] The prevalence of poliovirus antibodies in pregnant mothers (reflecting the past dynamic of the circulation of the poliovirus in the communities) is also a factor that has been taken into consideration to determine the best timing for using IPV, and to maximize its immunogenicity benefits should only a limited number of doses (e.g., one dose) be used in the schedule. The nature of the IPV-containing vaccine desired to be used in the program is also an important element as some countries may decide to switch toward aP-containing vaccines, and therefore may decide to rely on DTaP-IPV combination vaccines to simplify the logistics and programmatic aspects of their routine national immunization program. The existence of a national manufacturer, engaged or not in the manufacturing of OPV and/or of an IPV-containing product (particularly the Sabin IPV), continues to be an important driver in the choice of the products to be used. Finally, pharmacoeconomic considerations based on cost-to-benefit evaluation of several potential strategies have been major drivers in many countries. A dose of OPV costs approximately $0.15 for UNICEF or for other types of high-volume public markets. The cost of one dose of IPV standalone varies largely depending on the type of market (UNICEF procurement, Global Alliance for Vaccines and Immunization (GAVI)-funded; direct procurement, nationally funded; private) and the type (and presentation) of vaccine used, so no unique number can be provided as the financial conditions

of these markets are extremely diverse. Several funding mechanisms and tiered pricing approaches have been put in place to make IPV affordable and introducible in all the countries that have not been able to use it previously because of price considerations. As a general rule, product cost drives the decision as to the type of IPV-containing vaccine considered (standalone or aP combinations), the type of presentation (syringe, 1-, 2-, 5-, or 10-dose vials), the country(ies) where manufacturing operations are performed (distinguishing antigen, product formulation, filling of the finished product, and packaging operations), and the volumes considered. Although the price per dose of any IPV-containing vaccine is greater than the price of OPV, the human and financial costs of VAPP and VDPV, the high amount of wastage with OPV, and the cost of keeping the oral vaccine frozen also must be taken into account when deciding which vaccine to use. Several evaluations have been done on the evolution of the overall supply and its effect on price dynamic and several pharmacoeconomic models have been developed.[383–391] As mentioned in previous sections, in the scope of its GPEI Strategic Plan 2013–18,[392,393] WHO and all its stakeholders are engaged in promoting research and development activities toward the emergence of more affordable IPV solutions. As of today, the only concrete and available option is the Sabin IPV that has been developed by two manufacturers but because of their scale and location of production whether they are truly making IPV more "affordable" is not fully clear (see "Inactivated Polio Vaccine Manufactured from Sabin Strains" earlier).

Table 48.12 summarizes the advantages and disadvantages of each of the key regimens listed above. In essence, the key arguments for IPV usage are safety (no VAPP if starting first and no risk of inducing VDPVs if used alone), predictable and consistent humoral responses, and the possibility of its inclusion in combination vaccines. The key arguments for OPV usage are induction of better mucosal intestinal immunity, ease of administration to large populations, and possibility of indirect vaccination of contacts not reached by the routine immunization program. The arguments for using the two products in the schedule (whatever sequence) are to fuse the immunogenicity advantages of each vaccine with less or null

TABLE 48.12 Advantages and Disadvantages of OPV-Only, IPV-Only, Sequential, and Mixed/Combined Vaccination Schedules

Feature	OPV Only	IPV Only	IPV Followed by OPV	OPV Followed by IPV	Mixed/Combined
VAPP	1 case per 250,000 to 800,000 first vaccinations	No cases	VAPP cases eliminated when regimen starts with IPV (birth dose OPV not being a risk factor)	Same as with OPV only	VAPP risk linked to the timing of IPV use in the regimen
VDPV	Risk associated with performance of routine program	No risk	Risk associated with performance of routine program	Risk associated with performance of routine program	Risk associated with performance of routine program
Safety (other than VAPP and VDPV)	Excellent	Excellent	Excellent	Excellent	Excellent
Systemic immunity	Variable by-dose responses in some settings	Good	Good	Good	Good
Intestinal immunity	The gold standard	Much lower than with OPV, but shorter excretion and reduced titers in excreta after infection compared to unvaccinated Transmission impact variable	The gold standard	Ability of IPV to boost OPV-induced intestinal immunity	The gold standard and ability of IPV to boost OPV-induced intestinal immunity
Oropharyngeal immunity	Excellent	Excellent	Excellent	Excellent	Excellent
Prevention of AFP	Excellent	Excellent	Excellent	Excellent	Excellent
Transmission to contacts and secondary vaccination	Yes	No	Yes	Yes	Yes
Extra injections	No	Yes if IPV used as a standalone vaccine	Yes if IPV used as a standalone vaccine	Yes if IPV used as a standalone vaccine	Yes if IPV used as a standalone vaccine
Reduced compliance	No	Possible if standalone vaccine. Limited if used as an IPV-containing vaccine	Possible if standalone vaccine. Limited if used as an IPV-containing vaccine	Possible if standalone vaccine. Limited if used as an IPV-containing vaccine	Possible if standalone vaccine. Limited if used as an IPV-containing vaccine

AFP, acute flaccid paralysis; IPV, inactivated polio vaccine; OPV, oral poliovirus vaccine; VAPP, vaccine-associated paralytic polio; VDPV, vaccine-derived poliovirus.

risk of VAPP when IPV is started first (depending on the first OPV dosing timing) (discussed in detail in Chapter 48).

Endemic wild poliovirus circulation has been stopped in four of the six WHO regions, and wild poliovirus type 1 seems to be the only one serotype still circulating in three remaining endemic countries (Nigeria, Pakistan, and Afghanistan). The data as of the end of 2016 do clearly show that the circulation of wild polioviruses is at its lowest ever level.[394] It is undisputed that OPV has been the main driver in the successful eradication efforts in much of the developing world and has brought the progress of a greater than 99% reduction in cases since the GPEI began in 1988. It is also true and undisputed that the difficulties and the limits of OPV-only programs will never be able to achieve total and sustained eradication of disease caused by polioviruses because OPV itself can cause disease, either as VAPP or cVDPVs. This has been recognized by the WHO and its stakeholders after years of debate. As long as OPV is used, VAPP will remain a burden,[395] and VDPV a real threat to eradication.[396] The stated strategy of the WHO is to stop using any form of OPV once global eradication of wild polioviruses has been certified. The successful switch from tOPV to $bOPV_{1\&3}$ is a first step toward this OPV use eradication. After that milestone, it is likely that IPV will continue to be used in countries that manufacture IPV, and it is also likely that IPV will be made available to the other countries that wish to use it. The risk for countries that will stop using IPV is the potential for late recognition of a return of polioviruses.[397,398] Because so many infections are subclinical, by the time AFP cases are recognized, they are very difficult to contain, particularly if the underlying population immunity is low among cohorts born following the stoppage of all polio immunization. This strategy of not relying on IPV involves simply observing for poliovirus circulation in the environment and for cases of poliovirus-induced paralysis in the absence of vaccination and then use outbreak-specific monovalent OPV to contain the viruses when they are detected.[399] In this context, the well-known existence of immunocompromised individuals excreting immunodeficiency-related VDPVs for long periods of time[400] raises concern that in future years they could reseed the population. In addition, the theoretical risk of deliberate use of infectious virus derived from synthetic viral genomes as a biologic weapon also has to be considered.[401–403] Therefore, it is not possible to say today if (and possibly when and why) IPV usage will cease. The last GPEI Strategic Plan 2013–18 stated that "Recognizing that the risks associated with eventual bOPV cessation may be similar to those associated with OPV2 cessation, it is recommended that countries plan to continue administering at least one dose of IPV in their immunization programmes for at least 5 years after bOPV cessation."[392] With the new WHO strategy, the world has engaged in a larger use of IPV,[404–406] and the challenges are high.[407] Companies have multiple incentives to produce more IPV and develop new approaches to immunization against poliomyelitis. The more cogent response to cost is that IPV should not be used as a standalone vaccine with the attendant expenses of separate administration, but as part of combination vaccines.[408] Combinations containing IPV based on DTaP backbones are readily available, but one missing product is an affordable pentavalent or hexavalent combination made on a DTP backbone for low-income countries. As mentioned previously (see "Producers of Inactivated Polio Vaccine Antigen and Available Vaccines" earlier) several developments are ongoing. Sixty years after the birth of IPV[409] a very diverse experience has been accumulated with several IPV-containing vaccines. The appropriate IPV policy for a given country and the world should be driven by country and global goals, as well as societal values, epidemiologic characteristics, and other considerations.[410] In that context, many countries have adopted IPV but with different approaches in terms of immunization regimen, each approach adapted to the adopting country's specificities and objectives. It is undisputed that IPV will remain a tool in the fight against poliovirus and poliovirus-induced paralysis.

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49 Poliovirus Vaccine–Live

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The written history of poliomyelitis can be traced back more than 200 years to the first description of the disease as a separate clinical entity by Michael Underwood in 1789.[1] Since then, many important scientific discoveries and public health milestones have been associated with poliomyelitis. Undoubtedly, the most consequential of these was the development of effective poliovirus vaccines,[2] which paved the way for the implementation of control programs, and a resolution by the World Health Assembly that established the goal of global eradication of poliomyelitis by the year 2000.[3]

Implementation of the eradication strategies led to elimination of poliomyelitis caused by wild poliovirus in the World Health Organization (WHO) Regions of the Americas, the Western Pacific, Europe, and South-East Asia, comprising more than 80% of world's population, and to substantial decreases in polio incidence in the rest of the world. Indigenous wild poliovirus type 2 was last detected in India in 1999, and indigenous type 3 was last detected in Nigeria in November 2012. By mid-2016, only three countries (Afghanistan, Pakistan, and Nigeria) have never interrupted indigenous wild poliovirus type 1 transmission, and the introduction of innovative new tools, such as monovalent oral poliovirus vaccines (mOPVs) in 2005 and bivalent OPV (bOPV) in 2009, and the greater use of inactivated poliovirus vaccine (IPV) since 2014 have facilitated progress toward eradication.

Although the written history of poliomyelitis is relatively succinct, an Egyptian stele from the 18th dynasty (dated between 1403 and 1365 BCE) depicts a "crippled young man, apparently a priest, with a withered and shortened right leg, and with his foot held in a typical equinus position characteristic of flaccid paralysis."[4] This stele demonstrates that poliomyelitis probably has affected humankind since ancient times. Underwood[1] introduced the term *debility of the lower extremities* in 1789; other researchers suggested a series of alternative terms, including *Lähmungszustände der unteren Extremitäten* in 1840,[5] *morning paralysis* in 1843,[6] *paralysie essentielle chez les enfants* in 1851,[7] *paralysie atrophiques graisseues de l'enfance* in 1855,[8] *spinale Kinderlähmung* in 1860,[9] *tephromyelitis anterior acuta parenchymatose* in 1872,[10] and *poliomyelitis anterior acuta* in 1874.[11] *Poliomyelitis anterior acuta* is based on anatomic location of lesions within the spinal cord—which was discovered in the early 1870s—and constructed from the Greek words *polios* (i.e., gray) and *myelos* (i.e., marrow, the gray matter of the spinal cord) with the ending "-itis" to imply inflammation. Although the terms *Heine-Medin disease* in 1907[12] and *infantile paralysis* were proposed subsequently, *poliomyelitis* (and the shortened term, *polio*) prevailed and became the standard designation for the disease.

In the late 19th and early 20th centuries, a change in the epidemiology of poliomyelitis from a predominantly endemic to an epidemic form was observed in Sweden and Norway,[12–14] heralding similar changes in other industrialized countries. Our understanding of these changes in the epidemiology was greatly aided by groundbreaking investigations of the three largest poliomyelitis outbreaks of the time: (a) 132 cases in Rutland County, Vermont, in 1894 by Charles Caverly[15]; (b) 1031 cases in Sweden in 1905 by Ivar Wickman[12,14]; and (c) more than 9000 cases in New York in 1916.[16] Wickman[14] was the first to recognize that abortive cases might equal or outnumber cases with paralytic manifestations and that these cases may be significant in the propagation of the infection. These outbreaks were caused by an accumulation of a sufficient number of susceptible children to sustain epidemic transmission of poliovirus, presumably because improvements in hygiene and sanitation had delayed poliovirus exposure from infancy to later in life.

Landsteiner and Popper[17] reported in 1908, on the basis of microscopic examination of spinal cords from two monkeys that had been injected intraperitoneally with a suspension of ground-up cord from a fatal human case, that a "filtrable agent" (i.e., virus) was the cause of poliomyelitis. In 1931, Burnet and Macnamara[18] determined that more than one serotype of virus could cause poliomyelitis and that immunity to one serotype did not confer immunity to another serotype. These investigators based their findings on cross-immunity and serologic tests; most importantly, they showed that three monkeys that had recovered from one serotype (and might have been expected to be immune) developed paralytic disease after injection of another serotype. This report had profound implications, although not immediately appreciated at the time, in terms of redefining the epidemiology of the disease and with respect to directing the subsequent development of vaccines. In 1948, an effort was launched to determine the number of distinct poliovirus strains. This effort was coordinated by the Committee on Typing of the National Foundation for Infantile Paralysis, which reported in 1951 that three and only three serotypes of poliovirus, designated types 1, 2, and 3, were the cause of poliomyelitis.[19] In 1949, Enders and colleagues[20] demonstrated that poliovirus could be grown in nonnervous, human embryonic tissue, work that was later honored with the Nobel Prize. Thus, the determination of the number of poliovirus serotypes, the ability for large-scale growth of the virus, and the finding that circulating antibody had a protective effect against poliomyelitis[21–26] were essential preconditions for the development of effective poliovirus vaccines.

Two approaches for vaccine development pursued at the time were successful: inactivation of poliovirus by formalin,[27] fully developed by Jonas Salk and colleagues, licensed as IPV in 1955 after one of the largest controlled field trials ever conducted[2]; and the attenuation of the three serotypes of poliovirus by Albert Sabin, licensed in 1961 as mOPV and in 1963 as trivalent OPV (tOPV).[28] The widespread use of IPV and OPV rapidly controlled poliomyelitis in industrialized countries.

The invention of the Drinker respirator (i.e., the "iron lung") beginning in 1928 and its widespread use in the 1930s and 1940s rapidly decreased the case-fatality rate of bulbar forms of poliomyelitis.[29] Epidemic poliomyelitis in the early part of the 20th century was associated with a high case-fatality rate (27.1% during the New York epidemic of 1916).[16,30,31] Further improvements in hygiene and sanitation delayed the median age of poliovirus infection from younger than 5 years in the 1910s to 5 to 9 years in the 1940s,[32,33] and allowed the accumulation of large numbers of people susceptible to poliomyelitis. Epidemics of ever-increasing magnitude began to occur in the United States and Europe until the mid to late 1950s, when vaccines became available

and changed the epidemiology anew. Because increasing age seemed to be the primary risk factor for bulbar paralysis—the basis for the "central dogma" of the epidemiology of poliomyelitis[34]—an increasing proportion of cases required respiratory support, and whole wards of iron lungs were devoted to caring for bulbar poliomyelitis victims in the 1940s and 1950s.

Poliomyelitis is intimately linked with some of the greatest triumphs in medicine, including scientific breakthroughs, public health achievements, and advancements in social justice. The concept of social justice, the indiscriminate benefit of scientific discoveries or access to care and rehabilitation, was pioneered by the National Foundation for Infantile Paralysis, which raised funds through annual March of Dimes campaigns that covered treatment and rehabilitation costs of poliomyelitis victims.[35] The Omnibus Budget Reconciliation Act (1993), which established the Vaccines for Children Program, ensures that poliovirus vaccines are available for poor children in the United States. Ultimately, the successful conclusion of the Global Polio Eradication Initiative (GPEI) will benefit all children equally.

The history of poliomyelitis has been reviewed in detail by Paul[4] and Eggers.[36]

WHY IS THE DISEASE IMPORTANT?

Poliomyelitis was the leading cause of permanent disability in children in the prevaccine era.[32] Besides the considerable disease burden, poliomyelitis was much feared in the prevaccine era because it could strike anybody, no means existed of protecting oneself or one's children, and, unlike the situation with other diseases such as measles, from which most children recover or die rapidly, society was reminded every day of the devastating effects of this crippling disease.

Disease control programs using poliovirus vaccines have prevented and continue to prevent millions of children from becoming paralyzed. In 1988, when the global eradication target was adopted, the WHO estimated that approximately 350,000 cases of paralytic poliomyelitis were occurring annually,[37] despite availability of effective vaccines during the prior 3 decades. Poliomyelitis has gained renewed attention in recent years because the feasibility of its eradication has been demonstrated[37,38] and because of the visibility of the ongoing global effort to eradicate poliovirus.[39–41]

BACKGROUND

Clinical Description

Poliomyelitis is an acute infectious disease caused by any of three serotypes of poliovirus that replicate initially in the gastrointestinal (pharyngeal and intestinal) tract and, rarely, in the motor neurons of the anterior horn cells in the spinal cord, where replication of virus results in cell destruction and flaccid paralysis of the muscles the cells innervate (i.e., spinal poliomyelitis). On occasion, brainstem cells innervating respiratory muscles can be affected, resulting in difficulties in breathing (i.e., bulbar paralysis). In addition to the acute paralysis, late manifestations with exacerbation of weakness or new paralysis (i.e., postpolio syndrome) can be observed in a significant proportion of patients decades after the acute paralytic episode.

Poliovirus exposure in a person susceptible to poliomyelitis results in one of the following consequences: (a) inapparent infection without symptoms, (b) minor illness, (c) nonparalytic poliomyelitis (aseptic meningitis), or (d) paralytic poliomyelitis.[32,42–44] Inapparent infection without symptoms is the most frequent outcome (72%) after poliovirus exposure in susceptible people.[45] Minor illness is the most frequent form (24%) of the disease, characterized by transient illness associated with a few days of fever, malaise, drowsiness, headache, nausea, vomiting, constipation, or sore throat, in various combinations.[45] Nonparalytic poliomyelitis (aseptic meningitis) is a relatively rare outcome (4%) of poliovirus infection. It begins usually as a minor illness characterized by fever, sore throat, vomiting, and malaise, and 1 to 2 days later, signs of meningeal irritation become apparent, including stiffness of the neck or back; vomiting; severe headache; and pain in the limbs, back, and neck.[42] This form of the disease lasts 2 to 10 days, and recovery is usually rapid and complete. In a small proportion of these cases, the disease advances to transient mild muscle weakness or paralysis.

Paralytic poliomyelitis is a rare outcome (usually <1%) of poliovirus infections among susceptible persons. Its clinical course is characterized by a minor illness of several days and a symptom-free period of 1 to 3 days, followed by rapid onset of flaccid paralysis with fever and progression to the maximum extent of paralysis within a few days. This characteristic clinical course of a minor illness followed by the major illness with paralysis has been likened to the two humps of the dromedary.[46,47] (In actuality, this is a misnomer, because the dromedary is a one-humped camel.) Among adolescent and adult cases of poliomyelitis, the minor illness is often absent, and these groups also seem to experience more severe pain in the affected extremities. After the resolution of fever, usually there is no further progression of paralysis. If paralysis of an extremity is not complete, it is more pronounced proximally. Paralysis is usually asymmetric, associated with diminished or complete loss of deep tendon reflexes and an intact sensory system. Paralytic manifestations in extremities begin proximally and progress to involve distal muscle groups (i.e., descending paralysis). Depending on the anatomic location of motor neuron damage in the spinal cord or in the brainstem, spinal, mixed spinal-bulbar, or bulbar paralysis involving primarily respiratory muscles may be observed. The anterior horn cells (and brainstem cells), just like other nerve cells of the central nervous system (CNS), cannot be regenerated or replaced and paralysis is permanent. Nevertheless, because of compensation of other, still functioning muscles, partial or total recovery can be achieved, usually within the first 6 months after onset of disease. Detailed clinical descriptions may be found in a number of reviews and books.[48–51]

Postpolio syndrome, a term invented in the early 1980s, refers to a disease entity that encompasses the late manifestations of acute paralytic poliomyelitis.[52] Aside from previously published case reports and case series, the first systematic investigation of postpolio syndrome was published in 1984.[53] After an interval of 15 to 40 years, many people (25% to 40%) who contracted paralytic poliomyelitis in their childhood may experience muscle pain and exacerbation of existing weakness or may develop new weakness or paralysis. Factors that enhance the risk of postpolio syndrome include: (a) increasing time since acute poliovirus infection, (b) presence of permanent residual impairment after recovery from the acute illness, and (c) female sex. The exact cause of these late effects is unknown, although it is not a consequence of persistent infection. The pathogenesis of postpolio syndrome is thought to involve late attrition of oversized motor units that developed during the recovery process of paralytic poliomyelitis.[52] Postpolio syndrome has been described in people infected during the era of wild poliovirus circulation. An excellent summary of the current scientific knowledge of postpolio syndrome has been published.[54]

Virology

General Properties of Polioviruses. Polioviruses, as members of species-C of the *Enterovirus* genus of the family *Picornaviridae* (*piccolo*, Italian, small; *rna*, RNA genome), share most properties with other members of that species and genus.[55–57] Polioviruses are small (~30 nm in diameter[58]), nonenveloped viruses with capsids of icosahedral symmetry enclosing a single-stranded, positive-sense RNA genome. Poliovirus capsids contain no essential lipids, and infectivity is insensitive to inactivation by detergents and lipid solvents such as ether, chloroform, and alcohol.[59] The viruses are stable at pH 3 to 5 for 1 to 3 hours, and can therefore pass through the stomach without inactivation. Exposure to 0.3% formaldehyde, pH of less than 1, pH of greater than 9, or free residual chlorine at 0.3 to 0.5 parts per million causes rapid inactivation. Infectivity is stable indefinitely at −20°C or lower, and stable for weeks at 4°C, but is rapidly inactivated at temperatures above 50°C.[60] Molar concentrations of $MgCl_2$ significantly increase the thermal stability of poliovirions,[61] both at elevated and ambient temperatures, and $MgCl_2$ is added to many OPV preparations to preserve potency.[62,63]

The genome is approximately 7500 nucleotides long, has a small (22-amino acid) basic protein, VPg, covalently linked to the 5′ end, and is polyadenylated at the 3′ end. The single open reading frame is flanked by a long (~740 nucleotides) 5′-untranslated region (5′-UTR) and a short (~70 nucleotides) 3′-UTR. Complete genomic sequences have been determined for numerous representatives of each the three serotypes, including those of the three Sabin OPV strains.[64] Only the sequences encoding the capsid proteins are unique to polioviruses, as the flanking sequences are frequently exchanged by recombination with the closely related species-C enteroviruses during circulation.[65–69] The poliovirion consists of 60 copies each of four capsid proteins (VP1–VP4) that form a highly structured capsid shell.[58] The three major proteins (VP1, VP2, VP3) share a similar basic architecture and were probably derived from a common ancestral protein.[58] The smallest protein, VP4, internalized in the native virion, is formed by the cleavage of the precursor VP0 (VP4 + VP2) during final maturation of the virion. The external surface of the poliovirion is decorated by peptide loops extending from VP1, VP2, and VP3, which form the neutralizing antigenic sites.[70,71] Polioviruses attach to and enter cells via the specific poliovirus receptor (PVR) on the cytoplasmic membrane; the PVR was later identified as CD155 (cluster of differentiation 155) or Necl5 (nectin-like molecule 5), a transmembrane glycoprotein with three extracellular immunoglobulin-like domains.[72–75] CD155 normally functions as a cell adhesion molecule that controls cell movement and proliferation, especially during embryonic development,[75] a function that is "misused" by poliovirus to gain entry into human cells.[76] The key distinguishing properties of poliovirus capsids are their antigenic surfaces and their abilities to specifically bind to CD155, as the sequences and structures of the internal capsid domains are largely conserved among species-C enteroviruses.

Antigenic Properties. There are three poliovirus serotypes (Table 49.1).[19,77] The three-dimensional X-ray crystallographic structures of representatives of all three serotypes have been determined.[58,79,80] Three (or four) neutralizing antigenic sites have been identified by patterns of reactivity with neutralizing murine monoclonal antibodies,[70] and the assignments have been confirmed by high-resolution X-ray crystallography.[79–81] Neutralizing antigenic site 1 is continuous and formed by a loop in VP1; sites 2 and 3 are discontinuous and formed from loops contributed by different capsid proteins. The major type-specific differences in the capsid polypeptides primarily reside on the most surface-accessible peptide loops, which represent less than 4% of the total capsid protein.[64] Although the neutralizing antigenic sites vary within each serotype,[82–90] the range of variability is constrained, possibly because of steric requirements for interaction with CD155,[91,92] such that all polioviruses within a serotype can be neutralized by type-specific antisera and poliovirus vaccines (both IPV and OPV) can induce protective immunity to all known antigenic variants. Poliovirus antigenic evolution differs importantly from that of influenza virus in that there is no cumulative antigenic divergence from ancestral viruses during person-to-person transmission, and genetically unrelated viruses may have similar antigenic properties and shared epitopes.

Limited cross-neutralization has been observed for all three poliovirus serotypes,[95] and a shared epitope between types 1 and 2 has been identified by mapping escape mutants to cross-reactive neutralizing monoclonal antibody.[96] Recently, chimeric chimpanzee–human monoclonal antibodies have been produced showing patterns of strong cross-neutralization.[97] Epitope mapping with these primate monoclonal antibodies have identified shared determinants not previously recognized by studies using murine monoclonal antibodies,[97] suggesting that the poliovirus antigenic surface may be more complex than previously thought.

Poliovirus antigenic properties have been reviewed by Minor.[70]

Poliovirus Replication Cycle. Poliovirus (and picornavirus) replication has been reviewed in depth.[55,98,99] Virus attaches to cells through specific interactions between the aminoterminal variable domain 1 of CD155 and a "canyon" that surrounds the fivefold axis of the virion.[92,100–104] After endocytosis, viral RNA is uncoated and released into the cytoplasm,[104] VPg is cleaved from 5′ end of the RNA, and the RNA is translated. Translation is under the control of the internal ribosome entry site (IRES), an element (nucleotides ~130–600) within the 5′-UTR that has a highly conserved stem–loop structure.[99,105] The translation product is a single polypeptide, the polyprotein, which is cleaved by virus-encoded proteinases, $2A^{pro}$ and (primarily) $3C^{pro}$, into mature viral proteins.[106] Host protein synthesis is rapidly inhibited by the cleavage by $2A^{pro}$ of the translation initiation factor eIF4G, which is required for initiation of translation of capped host messenger RNA but not for the internal initiation of translation from the poliovirus IRES.[99,107] One cleavage product is $3D^{pol}$, an RNA-dependent

TABLE 49.1 Polioviruses

Poliovirus Serotype	Prototype[a] Strain Designation	Geographic Origin	Illness in Person Yielding Virus	Reference
1	Brunhilde	Maryland	Paralytic poliomyelitis[a]	Bodian et al[77]
2	Lansing	Michigan	Fatal paralytic poliomyelitis[b]	Armstrong[93]
3	Leon	California	Fatal paralytic poliomyelitis[b]	Kessel and Pait[94]

[a]Virus recovered from feces.
[b]Virus recovered from spinal cord.

RNA polymerase, that catalyzes the synthesis of negative-polarity (−) RNA strands from the genomic and messenger RNA–polarity (+) strands forming a duplex called the replicative form.[108,109] Multiple copies of positive RNA strands are produced from negative-strand templates in replicative intermediates arrayed in intracellular membrane complexes.[108,109] VPg is cleaved from some newly synthesized positive RNA strands for programming as messenger RNA and further translation.[106] Other positive strands are encapsidated during the maturation step in which the VP0 precursor to VP4 and VP2 is cleaved followed by release of infectious virions from the infected cell. The entire replication cycle takes place within the cytoplasm, and poliovirus can replicate in anucleate cells. Infected cells show cytopathic effects within 6 hours, and can release up to 10,000 infectious virus particles upon cell lysis and death. This rapid rate of cellular destruction accounts for the rapid progression of paralysis when poliovirus infects motor neurons.[110]

PATHOGENESIS AS IT RELATES TO PREVENTION

The pathogenesis of poliovirus infection indicates that prevention through immunization can be accomplished by inhibiting replication at and dissemination from the gastrointestinal tract, by inhibiting the viremia that follows, or by doing both. After a person is exposed to poliovirus by way of the oral cavity, the virus attaches and enters specific cells that express the PVR.[72] The virus replicates locally at the sites of virus implantation (e.g., tonsils, intestinal M cells, and Peyer patches of the ileum) or at the lymph nodes that drain these tissues. The first approach to prevention requires the presence of local secretory immunoglobulin (Ig) A antibody. The second approach, because spread occurs primarily by way of the bloodstream to other susceptible tissues (i.e., other lymph nodes, brown fat, and the CNS) or by way of retrograde axonal transport to the CNS, requires the presence of circulating neutralizing antibody.

The host range of poliovirus and tissue tropism is determined by the expression of the PVR.[72] Tissue tropism refers to the ability of poliovirus to replicate in specific cells.[111] Early studies on poliovirus pathogenesis were conducted in primates, but the development of PVR transgenic mice has led to a renaissance in pathogenesis studies.[112,113] Most of the earlier observations with primates[48,49,51] have been confirmed in the PVR transgenic mouse model,[113] and new tools have been used to investigate important unresolved questions about poliovirus pathogenesis. For example, in situ hybridization with nucleic acid probes of the PVR in transgenic mice suggested PVR expression was limited to the CNS, thymus, lung, kidney, and adrenal glands, and, more recently, to monocytes (mononuclear phagocytes).[114] Replication of poliovirus in motor neurons results in cell destruction and paralysis. The chief limitation of the mouse model is the low level of expression of the PVR in mouse intestinal tissue, resulting in inefficient replication of virus at the primary site of natural infection.[113] The transgenic mouse model has facilitated neurovirulence studies but has limited usefulness in assessing the infectivity or transmissibility of the different poliovirus strains because of the difficulties in orally infecting the mice. Thus, the lack of an animal model to study infectivity and transmissibility of poliovirus remains a major obstacle in learning more about these aspects of the poliovirus.

Poliovirus may be found in the blood of patients with the abortive form ("minor illness"), and it can be detected several days before onset of clinical signs of CNS involvement in patients in whom nonparalytic or paralytic poliomyelitis develops.[24,115] The virus is regularly present in the throat and in the stools before the onset of illness. In persons who have clinical or subclinical infection, virus is excreted in the feces for several weeks[45] and in saliva for 1 to 2 weeks. The mean duration of wild poliovirus type 1 excretion in fecal specimens is 24 days (median, 20–29 days), with an observed range of 1 to 114 days.[45]

For further details of pathogenesis and pathology, see Bodian,[48] Bodian and Horstmann,[49] Sabin,[51] Racaniello and Ren,[113] Mueller, Wimmer, and Cello,[116] Nathanson,[117] and Koike and Nomoto.[118]

DIAGNOSIS

Paralytic poliomyelitis caused by imported wild poliovirus or by vaccine-related poliovirus has become a rare disease in the United States and other industrialized countries. Therefore, physicians may not be familiar with the disease or consider the diagnosis of poliomyelitis until other more frequent causes of acute flaccid paralysis (AFP) have been ruled out. The diagnosis of paralytic poliomyelitis is dependent on the following: (a) clinical course, (b) virologic testing, (c) special studies, and (d) residual neurologic deficit 60 days after onset of symptoms. For surveillance purposes, any case with physician-diagnosed suspected poliomyelitis is investigated in the United States, and a case is confirmed if a panel of independent experts determines that the case definition* for paralytic poliomyelitis has been met. The WHO is using a sensitive screening case definition: any case of AFP in a person younger than 15 years of age or a case in a person of any age in whom poliomyelitis is suspected. This definition attempts to capture all cases of paralysis that might be caused by poliovirus, including cases erroneously diagnosed as other clinical syndromes such as Guillain-Barré syndrome. This sensitive screening definition is balanced by a specific case classification system (i.e., virologic case classification scheme) that relies on isolation of poliovirus from stool specimens to confirm cases of AFP as poliomyelitis. As countries improve the quality and timeliness of AFP surveillance, the virologic case classification has replaced the clinical case classification scheme in all endemic and recently endemic countries (see "Disease Control Strategies" later).

Clinical Course

The clinical course (see "Clinical Description" earlier) is helpful in ruling in or ruling out paralytic poliomyelitis. Several studies in the developing world have attempted to assess the sensitivity and specificity of different clinical case definitions for paralytic poliomyelitis and compared these with the "gold standard" of virologically confirmed poliomyelitis based on poliovirus isolation from stool specimens. These studies reported similar findings.[119–121] The largest study reported a sensitivity of 64% and a specificity of 82% for a case definition that included age younger than 6 years, fever at onset, and rapid progress to maximum extent of paralysis (≤4 days).[119] The addition of a specific pattern of paralysis (proximal, unilateral, or absence of paralysis in all four extremities) increased the specificity with varying degrees of loss in sensitivity. The case definitions and case classification schemes have been reviewed.[122] Data from India suggest that residual paralysis 60 days after onset of paralysis is the strongest predictor for confirmed poliomyelitis (i.e., isolating wild

*"A patient must have had paralysis clinically and epidemiologically compatible with poliomyelitis and, at 60 days after onset of symptoms, had residual neurologic deficit, had died, or had no information available on neurologic residua." This case definition was formerly known as best available paralytic poliomyelitis case count.

poliovirus in stool samples during the initial examination). These findings highlight the importance of a 60-day follow-up examination to assess whether areas with wild poliovirus circulation may have been missed.

Virologic Testing

Because AFP has many etiologies, including Guillain-Barré syndrome, transverse myelitis, and infection with nonpolio enteroviruses (see "Differential Diagnosis" later), laboratory confirmation is critical to establishing the diagnosis of poliomyelitis. The basic approach is to attempt to isolate poliovirus from the stools of patients with AFP, and to characterize any poliovirus isolates to determine whether they are vaccine-related or wild. Detailed descriptions of standard laboratory principles and procedures for investigation of enterovirus infections are available.[123] The WHO has published a manual for the virologic investigation of poliomyelitis cases that includes protocols for the isolation of poliovirus.[124] This manual has become the standard guide for the isolation and characterization of polioviruses for laboratories in the WHO's Global Polio Laboratory Network (GPLN, see "Surveillance" later) for poliomyelitis eradication and is widely used in other diagnostic laboratories.

Poliovirus Isolation

Polioviruses can be grown in a wide range of human and simian cells (see http://www.atcc.org/),[123] but two cell lines are routinely used in combination by the GPLN for virus isolation[124]: (a) RD cells (a continuous line from human rhabdomyosarcoma[125]), which are highly sensitive to poliovirus infection and yield virus at high titers,[59] and (b) L20B cells (a derivative of the mouse L cell line engineered to express the human PVR, CD155), which are highly selective for growth of poliovirus.[124,126] RD cells have the advantage of being very sensitive to poliovirus infection and yielding high poliovirus titers in culture. RD cells will support the replication of other human enteroviruses, but not Coxsackie B viruses. L20B cells will support the replication of polioviruses, but, like their parenteral mouse L cells, are resistant to infection by most nonpolio enteroviruses. Thus L20B cells are used for the selective cultivation of polioviruses.

Poliovirus may be recovered from stool, throat swabs, or cerebrospinal fluid taken soon after the onset of illness and from stool specimens collected over longer periods of time (usually up to 8 weeks after the start of infection).[127] Poliovirus isolation rates from cerebrospinal fluid are generally low; however, when virus is found, a causal relationship between a poliovirus serotype and paralytic disease is strongly suggested. The WHO recommends that two stool samples be collected at least 24 hours apart to confirm the diagnosis, because excretion of virus can be intermittent and the sensitivity of isolation is less than 100%. Wild poliovirus has been found in stool samples of 63% to 93% of patients during the first 2 weeks of illness, in 35% to 75% during the third and fourth weeks, and in less than 50% during the fifth and sixth weeks.[127] The duration of viral shedding is reduced among children who were previously vaccinated, had preexisting homologous antibody induced by live poliovirus, or had a previous intestinal infection with homologous poliovirus.[127]

Clinical specimens are processed to produce a virus suspension largely free of bacteria and other debris, to which antibiotics are added to inhibit the growth of residual bacteria, and the suspension is inoculated onto cell cultures. Cells are monitored daily for cytopathic effects, which appear typically within 3 to 6 days of incubation.

Molecular Identification Methods

Intratypic differentiation (ITD) of poliovirus isolates (testing whether they are vaccine-related or wild) is performed throughout the GPLN using real-time reverse transcriptase-polymerase chain reaction (rRT-PCR).[124] A series of primer pairs and specific fluorescent probes have been developed that identify isolates hierarchically: (a) as enteroviruses (panEV), (b) as polioviruses (panPV), (c) by poliovirus serotype (Sero1, Sero2, Sero3), and (d) whether vaccine-related (Sab1, Sab2, Sab3).[128–130] The sets of rRT-PCR reagents are deployed as kits for routine use by the GPLN.[131] Molecular reagents and technical conditions for rRT-PCR are regularly updated to improve diagnostic sensitivities and meet changing GPEI needs. For example, current ITD kits no longer use the Sero1, Sero2, Sero3 reagents and new rRT-PCR reagents have been introduced that identify wild polioviruses by genotype and facilitate screening for genetically divergent vaccine-derived polioviruses (VDPVs) (VDPV1, VDPV2, VDPV3).[132,133] The rapid evolution and high genetic diversity within and across poliovirus serotypes presented special challenges to development of the panPV and serotype-specific primer and probe sets, as it was necessary to use degenerate and inosine-containing oligonucleotides to base-pair with the appropriate specificities at positions of codon degeneracy.[128,129] Although the nondegenerate Sabin vaccine strain-specific rRT-PCR reagents can be used in a multiplex format, the complexity of the degenerate reagents limits the number of reactions that can be combined in multiplex.

ITD screens for wild polioviruses and VDPVs and screens out OPV-like polioviruses that are unlikely to be of current epidemiologic importance. Since 2001, all wild poliovirus and VDPV isolates are sequenced by GPLN laboratories following standardized procedures and using standardized sequencing primer sets. The approximately 900-nucleotide interval (representing ~15% of the total genome) encoding the major capsid protein, VP1, is routinely sequenced. VP1 sequences are used for routine comparisons because they encode several serotype-specific antigenic sites[70] and evolve primarily by successive fixation of nucleotide substitutions rather than by recombination.[88,134] Wider genomic intervals, up to the complete genome, may be sequenced to obtain higher epidemiologic resolution or to address specific virologic questions.[66,85,135,136] Serotype- and genotype-specific sequencing primers have been developed to specifically amplify components of heterotypic and homotypic poliovirus mixtures, bypassing selective cultivation in the presence of neutralizing antibody or incubation at supraoptimal temperatures.[137] Sequence relationships among poliovirus isolates are summarized in phylogenetic trees and genotypic maps that are distributed monthly by GPLN laboratories to Ministries of Health, WHO Country and Regional Offices, WHO-Geneva, and other GPLN laboratories. The use of genomic sequencing has given rise to a new discipline that combines the tools and concepts of classical epidemiology with those of microbiology, biochemistry, genetics and evolutionary biology (see "Molecular Epidemiology of Poliovirus" later).[138]

Environmental Surveillance

Environmental surveillance by sampling of sewage is an important supplement to AFP surveillance that can increase the sensitivity for detecting poliovirus circulation and excretion because most poliovirus infections are inapparent.[139] Environmental surveillance is widely implemented in Europe (conducted by 20 countries, including Israel[140–144]) and Japan[145] as a component of enterovirus (and poliovirus) surveillance. In 2013–14, sustained circulation in Israel of wild poliovirus

type 1 originating from Pakistan was detected by routine environmental surveillance in the absence of polio cases.[146,147] Israel responded by conducting nationwide vaccination campaigns with IPV and bOPV whose impact on wild poliovirus circulation was monitored by environmental surveillance.[146,147] Routine environmental surveillance has been conducted in Egypt since 2000,[148–150] and has been extended to India,[151] Pakistan,[152] Nigeria,[153] and Afghanistan,[139] with plans for further expansion in countries at high risk of reestablishment of poliovirus circulation either by importation or by the emergence of circulating vaccine-derived polioviruses (cVDPVs).[139] Environmental surveillance has also provided supportive evidence for the eradication of wild poliovirus type 3.[154]

Environmental surveillance has also helped inform the endgame strategy for the GPEI. A key question is the persistence of vaccine-related viruses in the population and environment following cessation of OPV use. In Cuba, where OPV is delivered only in mass campaigns in the form of two rounds of National Immunization Days (NIDs), vaccine-related viruses were detected in stool surveys for up to 8 weeks and in the environment for up to 15 weeks after the second NID round.[155] New Zealand shifted from OPV to IPV in February, 2002, and vaccine-related viruses were regularly detected in sewage samples until May, 2002. Sporadic vaccine-related isolates detected subsequently showed very limited sequence divergence from the parental OPV strains, indicating that they were recent imports from OPV-using countries rather than persistence of vaccine-related viruses in the community.[156] The pilot IPV project in Yogjakarta Province in Indonesia has by-and-large confirmed these findings.[157]

An important difference between environmental sampling and AFP surveillance is that environmental sampling is most sensitive in locations with developed sewage systems or open sewage canals in large slums, and therefore is usually established in more urbanized settings. Sewage sampling sites in urban centers are selected to include communities of migrant populations from rural areas. Environmental surveillance is necessarily localized, targeted, and intermittent, in contrast to a well-functioning AFP surveillance system that monitors the entire population on a continuous basis. Consequently, poliovirus isolates obtained by the two surveillance approaches may yield different kinds of public health information. For example, a wild poliovirus isolate from an AFP case is directly linked to a specific patient from a specific locale, and usually signals many other inapparent infections in the community. In contrast, the high sensitivity of environmental sampling can result in multiple poliovirus isolations from a single infected person, but does not yield further information about the specific source of infection. However, some information about the extent of poliovirus circulation can be obtained from the extent of genetic diversity of polioviruses obtained at a sampling site.[150,158]

Serologic Testing

Serologic testing may be helpful in establishing the diagnosis but often does not contribute, and sometimes may cause confusion, because (a) antibody rises have already occurred by the time the first specimen has been collected, (b) antibody may be present to one or more serotypes because of previous or recent vaccination, and (c) heterotypic responses may be observed to one serotype after exposure to another serotype. There are no reliable means of distinguishing antibody induced by vaccine-related versus wild poliovirus. Standard protocols for neutralization assays to determine levels of antibody to poliovirus are available.[124,159] Paired serum specimens are required to demonstrate a fourfold or greater rise in antibody titer between acute and convalescent sera. The first serum specimen should be collected as soon as possible after onset of paralytic manifestations, and the second specimen should be collected 2 to 3 weeks later. Neutralizing antibodies appear early and are usually already detectable at the time of onset of paralysis. However, if the first specimen is taken early enough, a rise in titer may be demonstrated during the course of the disease. In a study from Louisiana, specimens were collected as soon as possible after hospital admission from poliomyelitis patients and approximately 6 weeks later; in 36% of patients, a fourfold rise in poliovirus antibody titer could be demonstrated; in 61% of patients, reciprocal titers were above 320 and did not change; and in 3% of patients, reciprocal titers were below 320 and remained unchanged.[160]

Neutralizing assays using Sabin strains continue to be the gold-standard method for the detection of type-specific antibody in sera.[22,160a] Neutralization antibody induced by a single serotype may not be completely serotype specific. In practical terms, this seldom constitutes a problem because the heterotypic response results in low levels of neutralizing antibody. Because of the limitations described, serology may be more important in excluding (e.g., no detectable antibody) the diagnosis of poliomyelitis than in confirming it. However, intrathecal immune responses can be measured and offer the advantage of attributing a causal relationship between a poliovirus serotype and paralytic disease.[161] The GPLN has developed standardized tests for poliovirus IgA and IgM antibody in serum.

Special Studies

Nerve conduction and electromyography studies can point to the anatomic location of the paralysis[162]—destruction of anterior horn cells in the spinal cord versus a demyelinating or axonal degenerative process in the peripheral nerves—helping to exclude the most frequent cause of nonpolio AFP, Guillain-Barré syndrome. Magnetic resonance imaging has been used infrequently, but, in at least one patient with poliomyelitis, it highlighted the anterior column of the spinal cord.[163] Analysis of spinal fluid may be helpful in ruling out other causes. In paralytic poliomyelitis, the cerebrospinal fluid contains an increased number of leukocytes, usually 10 to 200/mL, and seldom more than 500/mL.[164,165] At the onset of signs of CNS involvement, the ratio of polymorphonuclear cells to lymphocytes is high, but within a few days, the ratio is reversed. The total white blood cell count slowly subsides to normal levels. The protein content of the cerebrospinal fluid initially is elevated only slightly (average, in nonparalytic cases, is approximately 46 mg/100 mL [range: 15–165 mg/100 mL]; in paralytic cases, the average is approximately 68 mg/100 mL [range: 25–250 mg/100 mL]), but it rises gradually in paralytic cases until the third week, generally returning to normal by the sixth week.[164] Glucose levels are usually within the normal range. In fatal cases, spinal cord and brainstem tissue samples should be examined for the typical lesions caused by viral replication and destruction of the motor neuron cells.

Residual Neurologic Deficit

The clinical case definition for paralytic poliomyelitis requires a residual neurologic deficit at 60 days after onset of paralysis. Such a neurologic deficit may be apparent as complete flaccid paralysis of one or more extremities or partial paralysis or weakness of muscles or muscle groups. In the latter instance, because of functional recovery (intact muscles may compensate for muscles that are not innervated), it may be more difficult to establish a neurologic deficit. The most severe cases of poliomyelitis in terms of complications and fatal outcomes occur in persons with underlying immunodeficiency disorders.

Differential Diagnosis

The differential diagnoses of AFP have been reviewed.[166] The list of underlying causes of AFP is extensive (Table 49.2). The causes can be classified according to the pathophysiologic mechanisms and anatomic sites of the etiologic factors. For example, poliovirus damages primarily the anterior horn cells of the spinal cord. This damage leads secondarily to paralysis of extremity muscles (Fig. 49.1). The distinguishing features of poliomyelitis—Guillain-Barré syndrome, transverse myelitis, and traumatic neuritis (neuritis secondary to the trauma of injections)—are given in Table 49.3.

In general, in the absence of wild virus-induced poliomyelitis, Guillain-Barré syndrome accounts for 50% or more of the cases of AFP in industrialized countries such as the United Kingdom and Australia, and in developing countries in Latin America.[167–169] At times, nonpolio enteroviruses have been associated with cases of polio-like paralytic disease, but this has been uncommon. Coxsackie virus A7 has been associated with outbreaks of paralytic disease,[170,171] and enterovirus 71 has been involved in several outbreaks of CNS disease, including polio-like paralysis, resulting in fatal cases,[172] with recent large outbreaks in East Asia.[173,174] More recently, a novel enterovirus D68 strain was associated with AFP in the United States.[175,175a] Two motor neuron diseases in childhood are Werdnig-Hoffmann disease, a rapidly progressing, often fatal disorder of early childhood, and Wohlfart-Kugelberg-Welander disease, a more benign disorder with a generally later onset.[176] Electromyographic findings are useful in establishing the diagnosis of these disorders.[177] China paralytic syndrome, a distinct disease entity that appears different from Guillain-Barré syndrome and poliomyelitis, has been described among children and adults in northern China.[178] Early symptoms of this disease include leg weakness and resistance to neck flexion. The weakness ascends rapidly, affects symmetrically the arms and respiratory muscles, and progresses to a maximum extent of weakness within 6 days on average. Electromyography indicates denervation potentials in weak muscles and suggests

TABLE 49.2 Causes and Differential Diagnosis of Acute Flaccid Paralysis

INFECTIOUS	
VIRAL	
Enteroviruses	Poliomyelitis, Coxsackie A (A7, A9, A4, A5, A10) and Coxsackie B (B1–B5), echoviruses (6, 9; 1–4, 7, 11, 14, 16–18, 30) enterovirus D68, enterovirus 70, enterovirus 71
Other viruses	Myxoviruses (mumps virus), togaviruses, and arborviruses, Epstein–Barr virus; HIV, Japanese B encephalitis virus, West Nile virus
Bacterial	*Campylobacter jejuni* (leading cause of Guillain-Barré syndrome)
METABOLIC: TRANSIENT AND PERIODIC PARALYSES	
Hypokalemic	Familial, Sjögren syndrome, hyperthyroidism, gossypol-induced (toxic phenolic pigment in cottonseed), association with barium poisoning, association with hyperaldosteronism
Normokalemic or hyperkalemic	Familial, adynamia episodica hereditaria of Gamstorp
Hypophosphatemia	
DRUG INDUCED	
Heroin	
Antibiotics	Aminoglycosides, polymyxin B, tetracyclines
ORGANIC	
Volatile hydrocarbons	Hexane, methyl butyl ketone, carbon disulfide
Tricresyl phosphate	Jamaican ginger tonic; contamination of cooking oil, mustard oil, or flour
Other	Cantharidin, diethyltoluamide (DEET), dithiobiuret (rat poison), triethyldodecyl ammonium bromide (mouse poison)
TOXIN	
Bacterial	Botulinum, diphtheria, tetanus (cephalic form), *Moraxella*
Fungal: mycotoxins	*Penicillium citreoviridae, Penicillium islandicum, Penicillium citrinum*
Insect	Tick paralysis; spider venom; cockroach, beetle; wasp venom; Lepidoptera larvae
Parasite, protozoa, dinoflagellate	Paralytic shellfish poisoning–saxitoxin, ichthyotoxism (sardines)
Reptiles: snake venom	Cobra, Australian elapid, krait, mamba, sea snake
Plants and plant toxin	*Gloriosa superba* (daisy [root]), *Lathyrus* species (sweet pea), *Aconitum* (monkshood), hemlock (parsley), *Karwinskia humboldtiana* (*Coyotillo*; buckthorn [berries]), *Calliopsis* species (daisy), *Cassia* (bean), *Cycas* (evergreen [seeds]), *Gelsemium* (blossoms), *Heliotropium* (bush tea shrub), *Melochia* species (stems), *Oenanthe* species (parsnips)
Metals	Organic tin compounds, lead
Pesticides	EPN, trichlorfon (Dipterex), dichlorvos (DDVP), DEF, isofenphos (Oftanol), leptophos (Phosvel)
Inherited, congenital, acquired	Werdnig-Hoffmann disease, Wohlfart-Kugelberg-Welander disease, porphyric polyneuropathy
Unknown, multiple causes	Guillain-Barré syndrome, China paralytic syndrome, Bell palsy, transverse myelitis
Asthma	Polio-like Hopkins syndrome

EPN, ethyl *p*-nitrophenyl thionobenzene phosphonate.

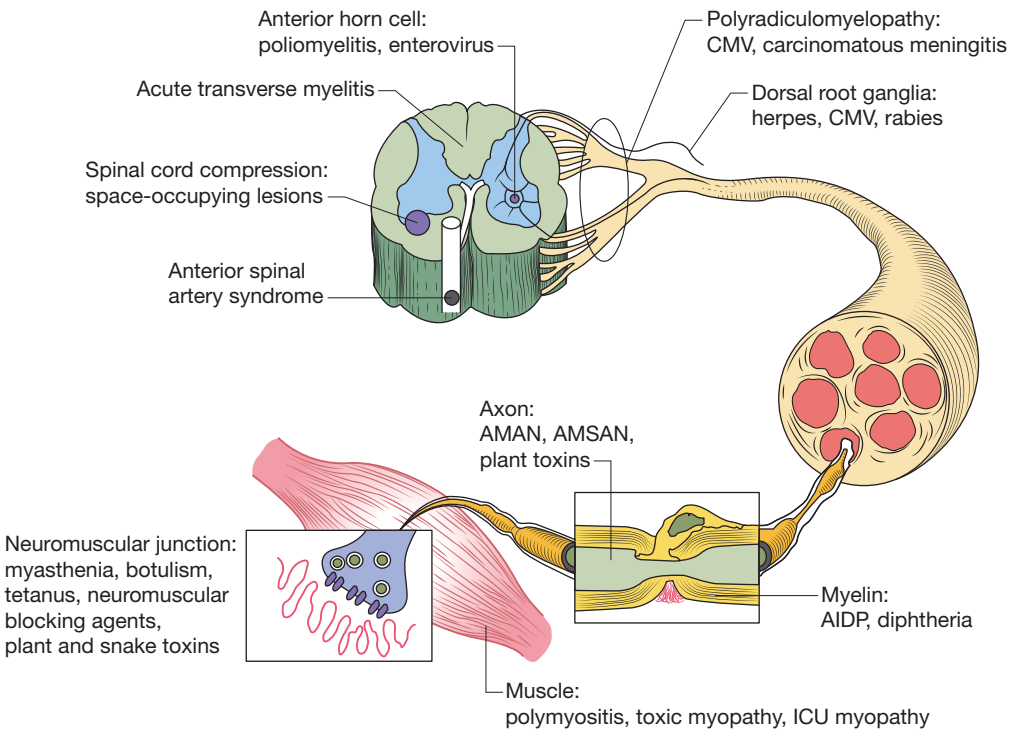


Figure 49.1. Pathophysiologic mechanisms and anatomic sites of etiologic factors for acute flaccid paralysis. AIDP, acute inflammatory demyelinating polyneuropathy; AMAN, acute motor axonal neuropathy; AMSAN, acute motor-sensory axonal neuropathy; CMV, cytomegalovirus; ICU, intensive care. *(Modified from Marx A, Glass J, Sutter RW. Differential diagnoses of acute flaccid paralysis and its role in poliomyelitis surveillance.* Epidemiol Rev. *2000;22:298–31; and Ho TW, McKhann GM, Griffin JW. Human autoimmune neuropathies.* Ann Rev Neurosci. *1998;21:187–226, copyright Annual Reviews, www.annualreviews.org.)*

that this entity may be a reversible distal motor nerve terminal or anterior horn lesion. Tick-bite paralysis occurs infrequently and is manifested by flaccid ascending paralysis that usually resolves rapidly after tick removal. Botulism toxins can also cause descending paralysis—characterized by symmetric impairment of cranial nerves, followed by a descending pattern of weakness or paralysis of the extremities and trunk.[179] A relatively frequent complication among approximately 10% to 15% of patients with diphtheria is paralysis of the soft palate and peripheral nerves resulting from diphtheria toxin[180]; tetanus toxin can cause flaccid paralysis of the muscles innervated by the affected cranial nerves (i.e., cephalic tetanus).[181] Case reports have linked AFP and West Nile virus (a flavivirus) infection among elderly adults in Mississippi and Louisiana, suggesting that West Nile virus may damage the anterior horn cells of the spinal cord to cause paralytic disease.[182–184] Japanese encephalitis infection seems to be an important contributing cause of AFP in areas where this virus is endemic.[185]

The following signs and symptoms help in distinguishing poliomyelitis from other causes of AFP: (a) fever present at onset of paralysis, (b) rapid progression to maximum paralysis, (c) usually asymmetric paralysis, and (d) more pronounced paralysis proximally than distally (i.e., descending paralysis). However, as poliomyelitis becomes an increasingly rare disease, unusual clinical manifestations, such as symmetric paralysis of the lower extremities (many cases caused by intramuscular injections, which increase the risk of paralytic manifestations in the extremity that, in the absence of intramuscular injections, would not have become paralyzed)[186] or mild paralysis or weakness in partially immune children, may be seen.

EPIDEMIOLOGY

General Epidemiology

Poliomyelitis was once a ubiquitous, highly contagious, seasonal viral disease (more pronounced in moderate-climate countries) caused by three serotypes of poliovirus (types 1, 2, and 3) that infected nearly every person in a given population in the absence of vaccination.[32] Paralytic manifestations are a rare outcome (<1%) of poliovirus infections. Important exceptions are island or isolated populations (e.g., Eskimo), which can remain unaffected by the virus for varying periods and, after reintroduction, can experience outbreaks of poliomyelitis that affect all age groups that were not affected by the previous wave of infection.[187] Poliovirus type 1 seems to be the most neurovirulent of the three serotypes.[188] Most epidemic and endemic cases of poliomyelitis are caused by poliovirus type 1, followed by type 3. The last naturally acquired cases of wild virus type 2 were detected in India in 1999 and the wild type 2 virus was declared eradicated in 2015,[46,189,190] and the last case of poliovirus type 3 was reported in November 2012 in Nigeria (as of January 3, 2017).[191] Peak transmission occurs among infants and young children (tropical areas) and school-age children (temperate zones).

Poliomyelitis is transmitted by person-to-person spread through fecal–oral and oral–oral routes or, less frequently, by a common vehicle (e.g., water, milk).[192,193] People remain most infectious immediately before and 1 to 2 weeks after onset of paralytic disease, although poliovirus replicates for substantially longer periods and is excreted for 3 to 6 weeks in feces and approximately 2 weeks in saliva.[45] Thus, the period of communicability may be 4 to 8 weeks. Secondary infection rates of susceptible household or institutional contacts,

TABLE 49.3 Distinguishing Features of Four Common Diagnoses of Acute Flaccid Paralysis

Feature	Poliomyelitis	Guillain-Barré Syndrome	Traumatic Neuritis (After Injection)	Transverse Myelitis
Development of paralysis	24- to 48-h onset to full paralysis	From hours to 10 days	From hours to 4 days	From hours to 4 days
Fever at onset	High; always present at onset of flaccid paralysis; gone when progression of paralysis stops	Not common	Commonly present before, during, and after flaccid paralysis	Rarely present
Flaccid paralysis	Descending	Ascending		
Muscle tone	Reduced or absent in affected limbs	Global hypotonia	Reduced or absent in affected limb	Hypotonia in affected limbs
Deep tendon reflexes	Decreased or absent	Globally absent	Decreased or absent	Absent in lower limbs early; hyperreflexia late
Sensation	Severe myalgia; backache; no sensory changes	Cramps; tingling; hypoesthesia of palms and soles	Pain in gluteus; hypothermia	Anesthesia of lower limbs with sensory level
Cranial nerve involvement	Only when bulbar involvement present	Often present, affecting nerves VII, IX, X, XI, XII	Absent	Absent
Respiratory insufficiency	Only when bulbar involvement present	In severe cases, enhanced by bacterial pneumonia	Absent	Sometimes
Autonomic signs and symptoms	Rare	Frequent blood pressure alterations; sweating; blushing; body temperature fluctuations	Hypothermia in affected limb	Present
Cerebrospinal fluid	Inflammatory	Albumin-cytologic dissociation	Normal	Normal or mild elevation in cells
Bladder dysfunction	Rare	Transient	Never	Present
Nerve conduction velocity, third wk	Abnormal: anterior horn cell disease (normal during first 2 wk)	Abnormal: slowed conduction; decreased motor amplitudes	Abnormal: axonal damage	Normal or abnormal; no diagnostic value
Electromyography at 3 wk	Abnormal	Normal	Normal	Normal
Sequelae at 3 mo and up to 1 y	Severe, asymmetric atrophy; skeletal deformities developing later	Symmetric atrophy of distal muscles	Moderate atrophy; only in affected limbs	Flaccid diplegia; atrophy after years

Modified from Global Programme for Vaccines and Immunization. Field Guide for Supplementary Activities Aimed at Achieving Polio Eradication. *Geneva, Switzerland: World Health Organization; 1996.*

probably mediated by fecal–oral spread, are high—greater than 90%.[45] The incubation period between infection and first symptoms (minor illness) is 3 to 6 days and from infection to onset of paralytic disease is usually 7 to 21 days, with a range of 3 to 35 days.[194] Most exposures to polioviruses result in inapparent infections.[32,42,43] On the basis of serologic surveys in the prevaccine era[195,196] and lameness surveys in developing countries, it seems that, in the absence of a control program with vaccines, paralytic disease will develop in approximately 1 in 200 children (0.5%).[197]

Until recently, excretion of poliovirus was thought to be limited to 4 to 6 weeks among immunocompetent persons and those younger than 3 years of age among immunodeficient persons. In 1997, a case report suggested that an immunodeficient patient with common variable immunodeficiency disorder (CVID) who acquired vaccine-associated paralytic poliomyelitis (VAPP) in 1981 may have excreted VDPV for approximately 7 years before onset of paralysis.[198,199] The WHO maintains a registry for such cases (prolonged, poliovirus excretion among immunodeficient individuals). The data in the registry (data as of July 2016) contain 94 persons with CVID, agammaglobulinemia, severe combined immunodeficiency disorder, or other immunodeficiency disorders, who have excreted poliovirus for prolonged periods, most for 6 months or longer (prolonged excretion), including seven persons who excreted poliovirus for 5 years or longer (chronic excreters), based on epidemiologic data and molecular sequencing information[200] (see "Molecular Epidemiology of Poliovirus" later) and WHO unpublished data.[85,198,199,201–208] Table 49.4 displays an analysis of the cases contained in the registry. Many of the long-term carriers stopped excreting poliovirus spontaneously. As of mid-2016, only one of the seven chronic excreters is known to continue to excrete polioviruses (probably >25 years),[209] one has stopped excretion spontaneously, four have died, and for one, the excretion status is unknown. None of the viruses examined from these carriers showed recombination with other nonpolio enteroviruses. The absence of recombination may suggest that the poliovirus had replicated in a single person rather than through person-to-person transmission in the community (see "Vaccine-Derived Polioviruses" later).[85,198–206] A recent literature review summarized the epidemiology of 69 immunodeficiency-related vaccine-derived poliovirus (iVDPV) cases.[210] To mitigate the risks of iVDPV cases, an ambitious antiviral development effort is underway and has identified promising capsid-binding inhibitors and protease inhibitors that may be effective to eliminate

TABLE 49.4 Baseline Characteristics of Immunodeficiency-Related Vaccine-Derived Poliovirus Patients (N = 94)[a]

Year Diagnosed	No. of Cases
1961–1965	2
1966–1970	0
1971–1975	0
1976–1980	2
1981–1985	1
1986–1990	6
1991–1995	4
1996–2000	4
2001–2005	12
2006–2010	19
2011–2015	44
World Bank Income Group (Cases in OECD Countries)	
High income	26 (22)
Upper middle	8 (2)
Lower middle	53
Low income	7
Age at diagnosis	
Under 6m	16
6 mo–1 y	30
1–3 y	30
3–5 y	0
5–10 y	6
10–20 y	6
20 y +	4
Immunodeficiency Disorder	
Antibody disorder	32
SCID	24
CVID	15
Other	23
Length of excretion	
<6 mo	13
Prolonged (6 mo–5 y)	56
Chronic (>5 y)	7
Unknown	18
Outcome	
Alive	21
Alive and stopped excreting	20
Dead	44
Unknown	9

[a]World Health Organization (WHO) data as of July 2016.
CVID, common variable immunodeficiency disorder; OECD, Organisation for Economic Co-operation and Development; SCID, severe combined immunodeficiency.

prolonged or chronic excretion of poliovirus.[211] Because of the likelihood of resistance, a combination therapy is likely needed. Furthermore, work continues to identify neutralizing monoclonal antibodies that could be used to clear these poliovirus infections, either as standalone or in combination with antiviral agents.

Between 1976 and 1995, 48 outbreaks involving approximately 17,000 cases of paralytic poliomyelitis were reported in the literature.[212] These outbreaks involved primarily unvaccinated or inadequately vaccinated subgroups and were caused predominantly by poliovirus type 1 (74%). On the basis of this review, cases in developing countries occurred mostly among children younger than 2 years of age, whereas cases in industrialized countries tended to occur in older people who had remained susceptible to poliomyelitis. As an expression of the changing epidemiology resulting from the progress of polio eradication efforts, outbreaks in Albania (1996),[213] Namibia (2006),[214] Cape Verde (2000),[215] Tajikistan (2010),[216] and Congo-Brazzaville (2010)[216,217] have involved a substantial proportion of cases in adolescents and young adults associated with a high case-fatality rate. The changing epidemiology of poliomyelitis has also been documented by analyzing outbreaks occurring between 1996 and 2012.[218]

Besides age and being unvaccinated or inadequately vaccinated, several factors have been shown to increase the risk of acquiring paralytic manifestations, including intramuscular injections with diphtheria and tetanus toxoids and pertussis vaccine (DTP)[219,220] or antibiotics,[221,222] strenuous exercise,[223–225] injury such as fractures, and pregnancy.[226] Provocation poliomyelitis describes the enhanced risk of paralytic manifestations that follows injection in the 30 days preceding paralysis onset. More than 40 years ago, Nathanson and Bodian[227,228] demonstrated that retrograde axonal transport is responsible for the poliovirus invasion of the CNS in provocation poliomyelitis. More recently, Gromeier and Wimmer[112] and Gromeier and colleagues[229] suggested that the temporary expression of the human PVR on peripheral neurons during the repair process of injured nerves may enhance poliovirus access into peripheral neurons. Retrograde transport in the axon via the fast system seems to shorten further the period from initial access of the poliovirus to the peripheral neuron to the virus reaching the motor neuron cells of the CNS.[230] This limits the time during which the immune system could develop an effective response. Aggravation poliomyelitis describes the elevated risk of paralytic disease that follows strenuous exercise shortly before paralysis onset (in the preceding 24 to 48 hours).

Removal of tonsils and adenoids predisposes to bulbar poliomyelitis.[231] Clinical observations on this fact were reported in the early 20th century.[231,232] Rhesus monkeys, when inoculated with poliovirus in the tonsillopharyngeal region, developed poliomyelitis with greater frequency than when they were inoculated by other routes.[4] Later, von Magnus and Melnick[233] demonstrated that, if cynomolgus monkeys were given poliovirus by the oral route, their susceptibility was greatly enhanced in animals that had recently removed tonsils. Ogra[234] and Ogra and Karzon[235] studied 40 children before and after removal of tonsils and adenoids. The children ranged from 3 to 11 years old and had been immunized with live attenuated poliovirus vaccine 6 months to 6 years previously. Before tonsillectomy, IgA poliovirus antibody was present in appreciable titers in the nasopharynx of all children, but no IgM or IgG antibody was detectable. Significantly, however, after tonsillectomy, the preexisting IgA poliovirus antibody level in the nasopharynx sharply declined in all children studied. Mean antibody titers decreased threefold to fourfold. Thus, removal of tonsils may eliminate a valuable source of

immunocompetent tissue particularly important in conferring resistance to poliovirus.

Lower socioeconomic status is a risk for paralytic poliomyelitis in developing countries,[236] probably because children belonging to the lower socioeconomic group experience more intense exposure to poliovirus (i.e., a higher virus inoculum, which has been shown in experimental studies to be a risk factor for paralytic disease[30]). In addition, the children are also at higher risk for primary vaccine failure after OPV because of more frequent concurrent enterovirus infections.[236–239]

In a study of twins, concordance with regard to paralytic poliomyelitis was found in 36% of monozygous pairs compared with 6% of dizygous pairs.[240] The authors concluded that the data were consistent with "the theory that susceptibility may be conditioned by the homozygous state of a recessive gene."[240] A human leukocyte antigen complex study suggested that human leukocyte antigen–encoded genetic factors control resistance to the paralytic form of poliomyelitis.[241] Data on genetic susceptibility to poliomyelitis were reviewed by Wyatt,[242] who proposed that multiple-linked genes determine whether an infection with poliovirus results in paralytic disease.

The case-fatality rate is variable and depends primarily on the age groups affected. The highest case-fatality rates have been reported from epidemic cases in the early 20th century[16,30,32] and among adolescents and young adults, but are commonly between 5% and 10%.[30,32] Even in the 1990s, the case-fatality rate could be high, as occurred in a large outbreak of poliomyelitis in Albania in 1996, which reported a case-fatality rate of 10%.[213] More recently, an outbreak of poliomyelitis occurred on the Cape Verde Islands in 2000. Of the 33 reported cases, seven people died, for a case-fatality rate of 21%. The case-fatality rates were 0%, 20%, and 57% among cases in person younger than age 5 years, 5 to 14 years of age, and 15 years of age or older, respectively.[215] In 2011, a large outbreak of poliomyelitis caused by poliovirus type 1 centered in Pointe Noir, western part of Congo-Brazzaville, involved a substantial proportion of young adult men. As might be expected with this age distribution, the corresponding case-fatality rate was high (>30%).[217,243–245] Apes, such as chimpanzees, gorillas, and orangutans, are susceptible to poliovirus and can experience paralytic disease after poliovirus infection; outbreaks of poliomyelitis in apes have been reported in captivity and in the wild.[246–248] It is unlikely that apes have any role in the sustained transmission of this virus[249] because of the limited size of the ape populations. Most monkeys cannot be infected by oral administration of poliovirus and would not be expected to participate in the chain of transmission. In short, there is no significant animal reservoir for poliovirus.[249]

Results from mathematical modeling suggest that the force of poliovirus infection, measured primarily by the average age at infection among populations in the prevaccine era, is substantially higher in developing countries compared with industrialized countries. For example, the basic reproductive number (a measure of infectivity) of wild poliovirus is between 3 and 5 in the United States, which means that, on average, an infected person introduced into a fully susceptible population would transmit the poliovirus to three to five contacts. In contrast, the average infected person in a developing tropical setting would be expected to have transmitted the infection to 10 to 12 contacts.[250,251] As population immunity increases and many of the contacts of an infected person are no longer susceptible, the number of transmissions decreases. When the reproductive rate is less than 1 because of high population immunity, transmission will eventually cease. The herd immunity threshold is the level of immunity in the population (e.g., in the "herd") at which an infected person, on average, would transmit the infection to less than one susceptible contact.

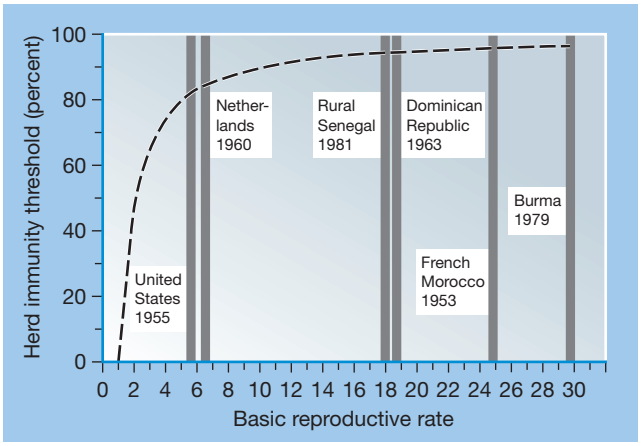


Figure 49.2. Herd immunity threshold levels for selected industrialized and developing countries, based on basic reproductive rate, or R_0. Threshold values for herd immunity were calculated using $1 \approx (1/R_0)$, where R_0 is 1 (life expectancy/average age at infection with poliovirus). Herd immunity threshold values are shown by the dashed line. The solid bars are the basic reproductive rate in a given population. *(From Patriarca PA, Sutter RW, Oostvogel PM. Outbreaks of paralytic poliomyelitis, 1976–1995.* J Infect Dis*. 1997;175[Suppl 1]: S165–S172.)*

Whereas poliomyelitis outbreaks in industrialized countries can be prevented with overall population immunity levels of approximately 66% to 80%, outbreaks in developing countries with inadequate sanitation and hygiene could still occur with immunity levels as high as 94% to 97% (Fig. 49.2). These findings may be helpful in explaining why there was no spread to the general population after the outbreaks in The Netherlands, Canada, and the United States[252,253] and why widespread transmission among well-vaccinated populations occurred in many outbreaks in developing countries.[254,255] A seroprevalence survey of poliovirus antibodies during the final stage of polio eradication in Egypt suggested that between 97% and 100% immunity against poliovirus type 1 may have been needed to interrupt type 1 transmission in a country with almost ideal conditions for poliovirus circulation.[256] Recent seroprevalence surveys in Northern India, conducted in the 2009–11 period, after introduction and widespread use of bOPV (types 1 and 3), have demonstrated high seroprevalence levels of antibodies against poliovirus type 1 (98% to 99%) among infants 6 to 7 months old, but considerably lower levels against poliovirus types 2 and 3.[257]

Epidemiologic Patterns and Incidence of Poliomyelitis

The epidemiology of poliomyelitis changed substantially during the last century. Three epidemiologic patterns have been observed: (a) endemic, (b) epidemic (prevaccine), and (c) vaccine era. Polioviruses probably circulated in an uninterrupted endemic manner for many centuries, infecting new cohorts of susceptible infants continuously, almost all early in life, when maternally derived antibody transferred from mother to newborn still provided some protection against paralysis.

A change from endemic transmission to periodic epidemics was first observed in some temperate-climate countries (e.g., Norway, Sweden, the United States) late in the 19th century and at the beginning of the 20th century.[12–15] The delay in median age of poliovirus exposure permitted the accumulation of sufficient children susceptible to poliomyelitis to

permit periodic outbreaks. In the United States, the median age of poliovirus infection increased from younger than 5 years of age at the beginning of the 20th century to 5 to 9 years of age in the 1940s, before poliovirus vaccine licensure.[32] In contrast, approximately 80% of the cases were in children younger than 5 years of age during the large epidemic in New York in 1916.[16] The generally accepted explanation, supported by numerous studies, is that, in a temperate-zone climate with increased economic development and correspondingly improved resources for community sanitation and household hygiene, exposure to polioviruses was postponed to later in life. Epidemic transmission became the primary epidemiologic pattern in temperate-climate countries, such as the industrialized countries in Europe and North America, until poliomyelitis was brought under control after introduction of effective vaccines (Fig. 49.3).

In developing countries, particularly tropical areas, an endemic epidemiologic pattern predominated until recently. Poliovirus exposure occurred early in life. Although earlier theories suggested that paralytic poliomyelitis was not a health burden in tropical countries because of early exposure of infants to virus at a time when levels of maternally derived antibodies protected them from paralytic disease, more recent studies have disproved these theories. The history and scientific evidence for this misconception—that poliomyelitis was not a significant public health problem in developing countries—was reviewed in detail in the corresponding chapter of the second edition of *Vaccines*.[258] In the last three decades, a series of lameness surveys were conducted in many developing countries that reported between five and 10 lameness cases per 1000 children in the age group studied,[197] suggesting that approximately one in 100 to one in 200 children acquire paralytic disease attributable to poliovirus. The WHO estimates that, in the absence of vaccination, at least one of every 200 children would become paralyzed by poliovirus and there would be approximately 650,000 cases of paralytic disease annually, the great majority of which would occur in children from developing countries. With improving vaccination coverage, a shift from an endemic to an epidemic pattern of poliovirus transmission has been observed in some developing countries that experienced large epidemics.[212,255,259–261]

The vaccine era began in the United States and in many European countries, Canada, Australia, New Zealand, and Japan after introduction of IPV in 1955.[2] The incidence of paralytic poliomyelitis decreased rapidly from 18,308 reported cases in the United States in 1954, the year immediately preceding IPV licensure, to 2499 cases in 1957, a decline of 86% only 3 years after the availability and widespread use of IPV. The relative upswing in reported cases in 1959 (6289 cases), with many cases having a history of receiving several prior doses of IPV, raised concerns regarding the clinical efficacy of IPV in preventing paralytic disease.[262] Nevertheless, continued and accelerated IPV use decreased the incidence of paralytic poliomyelitis to nearly record low levels (2525 cases) in the United States by 1960. Widespread use of IPV in other countries was followed by substantial decreases in the incidence of poliomyelitis and, in some European countries, including Finland, Iceland, The Netherlands, and Sweden, resulted in the apparent elimination of indigenous wild poliovirus transmission.[263,264]

The OPV era started in the United States with licensure of mOPV in 1961, followed by licensure of tOPV in 1963.[28] Although live attenuated OPV was developed in the United States, the first large-scale production and the large field trials that proved the safety and efficacy of the vaccine took place in the former Soviet Union. A mass immunization program was initiated in the Soviet Union in 1959 and completed in 1960, covering 77.5 million people, or 36.7% of the entire population. The immunization campaign was followed by a sharp decrease in the incidence of poliomyelitis, from 10.6 per 100,000 population in 1958 to 0.43 per 100,000 population in 1963. Between 1964 and 1979, the incidence remained at a level of 0.01 to 0.1 per 100,000 population.[265] Similar declines in the incidence of poliomyelitis were observed in other European countries, Australia, New Zealand, Canada, and the United States after the introduction of OPV. In the United States, mOPV was administered initially in mass vaccination campaigns in 1962, called Sabin Oral Saturdays/Sundays, followed by a routine vaccination program that administered vaccine to infants year-round.[28,266,267] The impact of administering OPV to a population that already had high immunity levels generated by previous natural infection or vaccination with IPV was impressive. Substantial reductions in the reported number of poliomyelitis cases were observed from 988 cases in 1961 to 61 cases in 1965. In 1973, only seven cases of poliomyelitis were reported. Epidemic poliomyelitis

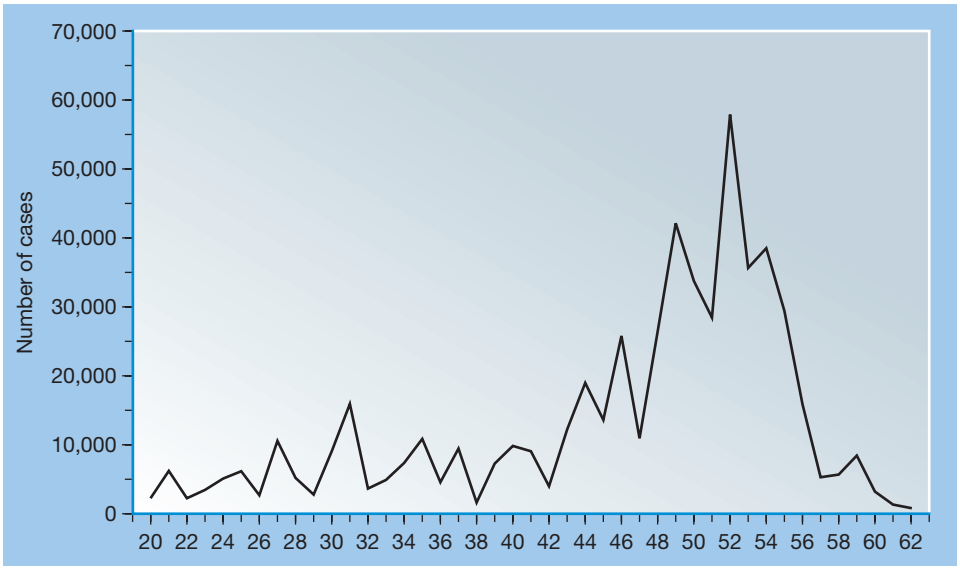


Figure 49.3. Reported cases of poliomyelitis, United States, 1920–1962. *(From Centers for Disease Control and Prevention.)*

also was brought under control, with the last outbreak in the general population occurring in Texas along the U.S.–Mexico border in 1970, followed by small outbreaks in 1972 and 1979 among religious groups whose members object to vaccination.[253] The last indigenously acquired case of poliomyelitis caused by wild poliovirus was detected in 1979. Between 1985 and 2000, aside from three imported cases of poliomyelitis (the most recent was reported in 1993), all cases have been vaccine-associated.[268] Rarely has a serious disease been controlled as rapidly and dramatically as has poliomyelitis in the United States and other industrialized countries.

The history of controlling poliomyelitis in many developing, particularly tropical, countries has been more recent. There is a notable exception: Cuba seems to have interrupted wild poliovirus after two rounds of mass vaccination campaigns in 1962.[269] In many other developing countries, however, national vaccination programs were not operational until the late 1970s and early 1980s, and global OPV coverage with three doses among children 1 year of age only reached 80% by 1990.[270] Wherever moderately high levels of OPV coverage were achieved, the incidence of poliomyelitis decreased by more than would be expected,[271] but endemic transmission of polioviruses continued, and cases of poliomyelitis continued to be reported. In addition to achieving high routine coverage with three doses of OPV, control of poliomyelitis required additional supplemental doses of OPV that were incorporated into the routine vaccination schedule in some countries; other countries needed to administer supplemental doses of OPV in mass campaigns. For example, in Brazil, control of poliomyelitis could not be accomplished until mass vaccination campaigns were initiated in 1980 (Fig. 49.4). The impact of these mass campaigns on poliomyelitis incidence was dramatic; the number of reported cases decreased from 1290 in 1980 to 122 in 1981, a decrease of more than 90%.[272] Based on the experience of the GPEI, once a poliovirus serotype has not been detected for 6 months or longer, it seems that circulation has been interrupted in that geographic location. However, while this may be valid in general, the length of "silent circulation" (without detection of overt cases of paralytic disease) is very much dependent on surveillance sensitivity and serotype. For example, for type 3, because of the lower case-to-infection ratio, a minimum period of at least 12 months may be needed, even under conditions of adequate surveillance. For certification purposes, a minimum period of 3 years or longer is anticipated to ensure confidence.[273]

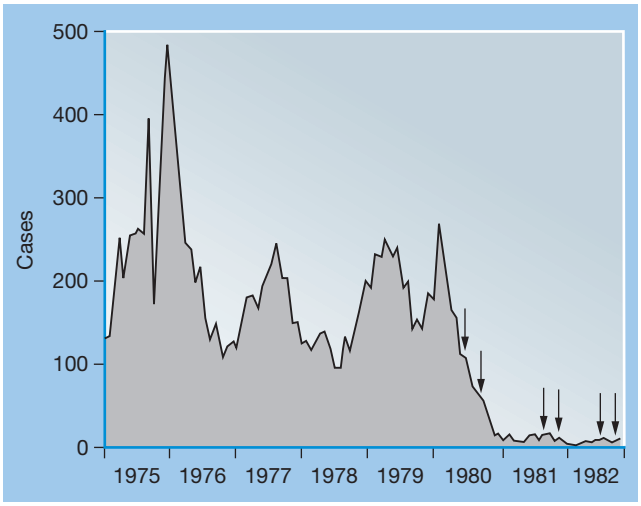


Figure 49.4. Cases of poliomyelitis by 4-week periods in Brazil, 1975–1982. Arrows indicate national immunization days. *(From Risi JB. The control of poliomyelitis in Brazil.* Rev Infect Dis. *1984;6[Suppl 2]:S400–S403, with permission.)*

Significance as a Public Health Problem

In the absence of effective control programs with poliovirus vaccine, paralysis develops in approximately one of every 200 children (see "General Epidemiology" earlier) after exposure to polioviruses,[197] followed in most cases by permanent disability; 5% to 10% of patients with paralytic disease have a fatal outcome.[30,31] Thus, with a global birth cohort of approximately 130 million surviving infants in 2015, approximately 650,000 children would be expected to acquire paralytic poliomyelitis resulting in permanent disability each year, and between 32,500 and 65,000 of the cases would result in poliomyelitis-associated deaths. In the United States, a report estimated that, in the absence of a control program, more than $3 billion ($926 million in direct costs and $2.1 billion in indirect costs) would be required each year to cover the treatment and other related costs of patients with poliomyelitis.[274] A recent study modeling different control scenarios suggested these scenarios will be more costly in the long run than finishing the eradication effort.[275] In addition to the acute manifestations of poliomyelitis, patients may experience postpolio syndrome decades after the acute episode; postpolio syndrome is associated with new muscle pain, exacerbation of existing muscle weakness, or the development of new weakness or paralysis[52] that may require additional therapy, rehabilitation, and respiratory support.

Despite the availability of two highly effective vaccines, poliomyelitis still exerts a significant public health impact in the world. In the United States in the prevaccine era, the peak incidence year of poliomyelitis was 1952, when 57,879 cases of poliomyelitis were reported (including 21,269 cases of paralytic disease).[276] After the availability and widespread use of poliovirus vaccines beginning in 1955, poliomyelitis was rapidly controlled in industrialized countries and in other areas where vaccines were used effectively. Globally, the Expanded Programme on Immunization, a program of the WHO established in 1974, provided leadership and technical guidance to national programs in most developing countries to improve vaccination coverage. Coverage levels apparently reached 80% with three doses of OPV among children 1 year of age for the first time in 1990,[270] resulting in substantial decreases in the global morbidity and mortality burden of poliomyelitis. Despite this success, the WHO estimated that approximately 350,000 cases of paralytic poliomyelitis associated with permanent disability occurred in 1988, the year the global polio eradication target was adopted.[37] Because of rapid progress toward polio eradication, the worldwide reported incidence of poliomyelitis was only 494 cases in 2001[39] (and the number of cases associated with wild poliovirus isolation was 483). Between 2005 and 2010, the number of poliomyelitis cases globally has fluctuated between 1293 and 1997, and the number of countries reporting any wild poliovirus has varied between 20 and 23. During this same period, the reporting completeness has further improved[39,277]; thus, it is unlikely that many cases of poliomyelitis remained undetected in 2010 (and even more unlikely that significant areas of poliovirus circulation were missed, but there are areas not accessible owing to conflict and populations that are difficult to survey, such as nomads). As of November 29, 2016, 74 virologically confirmed poliomyelitis cases were reported to WHO for 2015, and 34 virologically confirmed cases had been reported thus far to the WHO from Afghanistan, Pakistan, and Nigeria.[39,278]

Paralytic poliomyelitis continues to be a serious, albeit declining, threat to children in polio-endemic countries and

occasionally to people residing in industrialized countries that have primarily achieved good control of poliomyelitis for many years.[279,280] Even in countries with well-vaccinated populations that have eliminated indigenous wild poliovirus circulation for decades, gaps in population immunity may persist, particularly in groups objecting to vaccination (e.g., religious groups such as the Amish in the United States, The Netherlands Reformatory Church, and related groups in Canada) or groups that are not reached effectively by national vaccination programs (e.g., Roma, previously referred to as "gypsies").[252,281–283] In the United States, the last two outbreaks of poliomyelitis occurred in 1972 and 1979 among members of religious groups objecting to vaccination.[253] The 1979 outbreak was an extension of an outbreak affecting first The Netherlands in 1978 and then Canada.[252,282] An outbreak of poliomyelitis affecting the same religious group in The Netherlands also occurred in 1992 to 1993.[280] On the basis of genomic sequence,[284] the poliovirus type 1 strain causing the epidemic in The Netherlands in 1978 had its origin in Turkey. The recent outbreak was caused by poliovirus type 3, which was most likely imported from the Indian subcontinent.[285] In Spain, the last cases of poliomyelitis in 1980 to 1981 were detected among Roma children.[281] In both outbreaks in Bulgaria in 1990–91 and in 2000,[286,287] as well as in the Romanian outbreak in 1991–92,[288] Roma children were exclusively affected or constituted a substantial proportion of poliomyelitis cases. Although no cases of paralytic poliomyelitis were detected in the outbreaks in the United States (1979), Canada (1978), and The Netherlands (1978 and 1992–93) beyond the affected unvaccinated or inadequately vaccinated subpopulations, wild poliovirus exposure of people in the general population and the establishment of subsequent endemic and epidemic transmission remain a concern.

Molecular Epidemiology of Poliovirus

The application of genomic sequencing of poliovirus isolates has added a new dimension to the understanding of the epidemiology of poliomyelitis.[289] Because fewer than 1% of susceptible children infected with wild poliovirus show signs of paralysis,[46] AFP surveillance alone has limited ability to track the patterns of poliovirus spread, especially over wide geographic areas. However, the combined genetic and surveillance data can be used to detect links between poliomyelitis cases, reconstruct chains of transmission at high-resolution, and unequivocally establish the sources and timing of importations from the remaining poliovirus reservoirs.[8,136]

The high resolution of poliovirus molecular epidemiology follows from the very high rate of evolution of the poliovirus RNA genome.[88,290–292] Most of the nucleotide substitutions generate synonymous codons[88] and the basic biological properties of wild polioviruses remain unchanged. Estimates of the rates of total nucleotide substitution into poliovirus capsid regions average approximately 10^{-2} substitutions per site per year (Table 49.5).[66,85,88,290,291,293,294] The rates appear to be similar across the three poliovirus serotypes and for both circulating polioviruses and polioviruses associated with chronic infections, and constitute a robust poliovirus molecular clock. The clock is stochastic, and estimates of dates of infections obtained over short time periods may have wide confidence intervals such that wider sequence windows may be needed to improve estimates.[85,136,294] In addition, the Sabin strains are subject to selection against the attenuating substitutions during the early phases of replication in the human intestine,[293] which, when coupled with active recombination[294] and incorporation of "hitchhiker" mutations, can accelerate the initial rates of substitution. Consequently, estimates from the molecular clock of the duration of shedding of newly emergent VDPVs can be longer than those inferred from the vaccination records,[63,293,295] even as molecular clock estimates for the duration of more prolonged infections may closely match the clinical data.[90,205,294] After approximately 10 years of divergence, variable nucleotide sites become increasingly saturated, yielding underestimates of the total time of virus replication, and requiring special analytic methods to obtain more robust estimates.[88,296]

Sequence analyses offer an additional tool to monitor the progress of the GPEI, and has shown that poliovirus genotypes (viruses within a genotype differ by <15% in their nucleotide sequences) and genetic clusters within genotypes (viruses within a cluster differ by <5% in their nucleotide sequences) disappear sequentially through intensive immunization efforts (Fig. 49.5).[152,153,297] Experience shows that in settings of sensitive surveillance a genotype that is not detected for more than a year has probably become extinct.[289,299] Molecular epidemiology has established the existence of numerous poliovirus genotypes that had been endemic to different regions of the world (Fig. 49.5).[297,289] The last wild poliovirus type 2 isolate (detected in India 1999) was a representative of the South Asian type 2 genotype, the last of many wild type 2 genotypes in circulation during the prevaccine era (Fig. 49.5).[69] Similarly, representatives of the last two wild poliovirus type 3 genotypes were most recently detected in Pakistan in April 2012 and in Nigeria in November 2012.[154] The most recent detection of wild poliovirus type 1 in Borneo State, Nigeria, in August 2016 was a genotype indigenous to northern Nigeria[299a]; thus only the wild poliovirus type 1 genotypes indigenous to Afghanistan, Nigeria, and Pakistan were detected in 2016 (Fig. 49.5).

TABLE 49.5 Poliovirus Molecular Clock Rate Estimates

Poliovirus	Location	Interval (y)	VP1 Substitution Rate (nt s/s/y)	Reference
Wild poliovirus type 1	Andean countries	10	1.03×10^{-2}	Jorba et al[88]
Wild poliovirus type 1	Central Asia	4.1	1.19×10^{-2}	Gavrilin et al[290]
Wild poliovirus type 1	China	2.5	1.15×10^{-2}	Liu et al[291]
iVDPV type 1	Taiwan	2.5–3.0	1.14×10^{-2}	Yang et al[294]
iVDPV type 1	United Kingdom	1.8	1.24×10^{-2}	Odoom et al[89]
cVDPV type 2a	Nigeria	6.2	1.12×10^{-2}	Burns et al[136]
Wild poliovirus type 3	Egypt	6.4	1.19×10^{-2}	CDC, unpublished
iVDPV type 3	United Kingdom	1.7	1.28×10^{-2}	Martín et al[85]

CDC, Centers for Disease Control and Prevention; cVDPV, circulating vaccine-derived poliovirus; iVDPV, immunodeficiency-associated vaccine-derived poliovirus; nt s/s/y, total nucleotide substitutions per site per year.

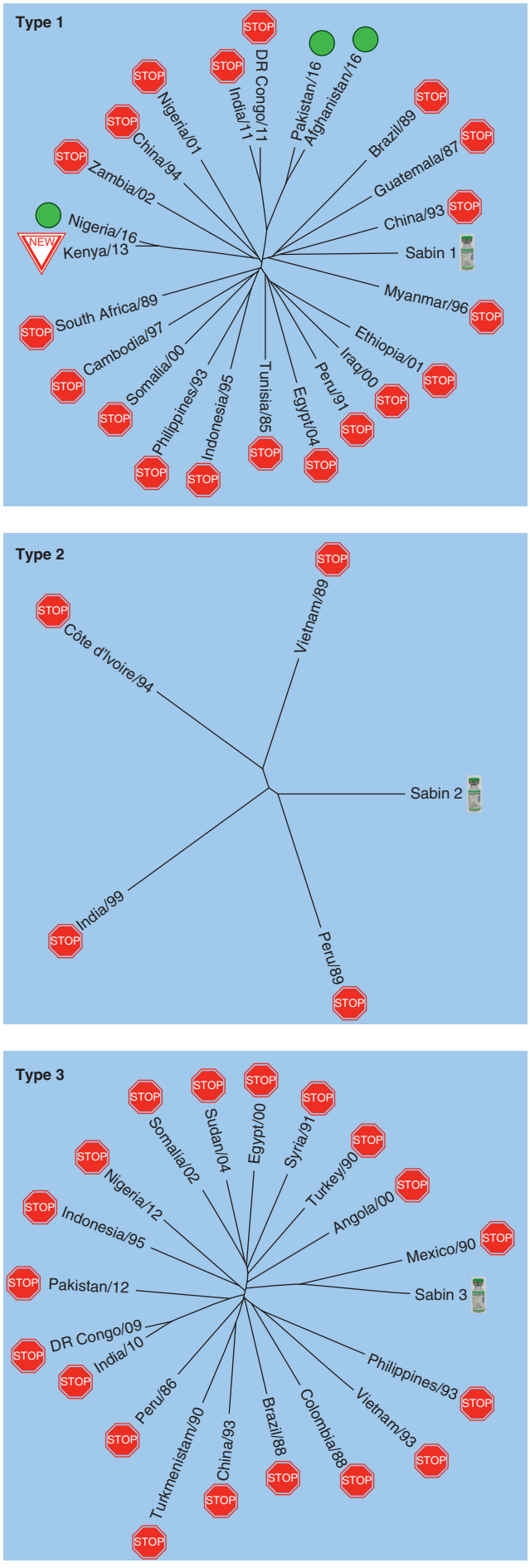


Figure 49.5. Progressive eradication of wild poliovirus (WPV) genotypes,* 1986–2015. Radial phylogenetic trees summarize the VP1 sequence relationships among representatives of each WPV genotype (a genotype shares less than 85% nucleotide sequence identity in the VP1 region with another genotype of the same serotype) detected since the launch of polio eradication activities in the Americas in 1985. Genotypes believed to be extinct are represented by stop signs; genotypes where evidence is accumulating that circulation has stopped (but less than 3 years since the last isolate) are represented by yield signs; the WPV1 genotype circulating in Pakistan and Afghanistan is indicated by the green circles. Isolates (identified by country and year of isolation) of the extinct genotypes are usually the last known representative of that genotype (exception: Cambodia/97 is represented by a closely related WPV1 isolate from Vietnam). *(VP1 sequences of isolates from Pakistan and Afghanistan courtesy Salmaan Sharif and Sohail Zaidi of the National Institutes of Health, Islamabad, Pakistan; isolate sequences from India courtesy Jagadish Deshpande of the Enterovirus Research Centre, Mumbai, India.)*

Molecular epidemiologic analyses show that poliovirus type 3 appears to circulate more locally than type 1,[299] and that poliovirus type 1 appears to be most commonly associated with importations from neighboring countries and with intercontinental or global spread of the virus.[134,218,298–304] In some settings, different genotypes of poliovirus type 1 have been found to have cocirculated in a geographically limited area.[134,260,300,305]

Molecular epidemiologic methods are routinely used to help identify reservoir communities with low population immunity, and where demographic and environmental conditions favor poliovirus circulation. During the peak months of poliovirus circulation, virus spreads from the reservoir communities to adjacent nonreservoir indicator communities (where the density of nonimmune, susceptible children can support some poliovirus circulation during the peak transmission season). This has led to a refinement in the concept of virus importation, which, in previous usage, referred to virus transmission across national boundaries. Although many importations over long distances have been documented,[212,299] reservoir communities and their associated indicator communities frequently overlap international borders,[299,306] underscoring the importance of regional synchronization of NIDs and Subnational Immunization Days. Equally important are the patterns of importation from reservoir communities to indicator communities within a country.[136,306,307] High vaccine coverage in the reservoir communities, especially in mass campaigns conducted during the low transmission season, prevents the subsequent spread to indicator communities.

Sequence analysis led to the recognition of highly divergent iVDPVs[85,199] and cVDPVs[66,69,308] (see "Vaccine-Derived Polioviruses" later), and it has been used to resolve, at high resolution, chains of cVDPV transmission[69,309–311] and separate iVDPV lineages in individual immunodeficient patients with prolonged infections.[85,199,293–295]

Molecular epidemiologic methods have also opened a new avenue for detecting gaps in polio surveillance. In areas with good surveillance, poliovirus isolates representing frequent sampling of a single chain of transmission are typically closely related (usually >99.5% VP1 sequence identity among the closest relatives). These closely related viruses are routinely visualized as sequences connected by short branches on phylogenetic trees. Long-branch connections between isolate sequences indicate missing information. If the virus is imported, the missing information may be recovered from the

sequence relationships to viruses from the source reservoir.[298] However, in many other circumstances, no closely related viruses can be found, and the recent virologic history of the isolate lineage is indeterminate. For example, gaps in AFP surveillance in southern Egypt were inferred from the sequence data, because indigenous type 3 isolates in 1999 appeared as "orphan viruses" at the tips of long branches on phylogenetic trees (orphan virus is an isolate that at the time of its detection had no other virus more closely related than 1.5% difference in the VP1 region), and the closest relatives were isolated nearly 3 years earlier,[312] observations that highlighted the importance of environmental surveillance to improve sensitivity. Orphan viruses had been repeatedly found in areas with insensitive surveillance. The GPLN regularly monitors for the appearance of poliovirus orphan lineages as a means to assess surveillance sensitivity.

Contamination of cultures was once a potentially serious challenge to the integrity of poliovirus surveillance data. Improved procedures and facilities in GPLN laboratories have all but eliminated contamination, despite high workloads. Moreover, sequence analysis can distinguish contaminants from true clinical isolates. Contaminants are easily recognized when they are standard wild reference strains, but are more difficult to recognize when they are the wild polioviruses indigenous to a country or community. However, when wild polioviruses isolated at different times and locations have identical VP1 sequences, contamination is suspected, because such sequence identities are inconsistent with the rapid rate of evolution of the poliovirus genomes. Contamination can be definitively confirmed (or ruled out) by genomic sequencing of complete genomes.

PASSIVE IMMUNIZATION

Therapeutic use of convalescent serum for poliomyelitis was first suggested as early as 1915.[313] Several trials using convalescent serum, administered by a single intrathecal injection, intraspinally, intravenously, or subcutaneously, reported conflicting results.[314] However, convalescent serum use was advocated by some until a well-controlled trial during a poliomyelitis outbreak in 1931 provided no statistical evidence that the therapy was of value.[315]

Administration of antibody was pursued again in the late 1940s and early 1950s as a means of preventing poliomyelitis by passive immunization. It had been demonstrated previously that low levels of circulating neutralizing antibody were protective against the paralytic manifestations of poliomyelitis in experimental animal models and in humans.[315] In addition, infants in the first few months of life rarely acquired paralytic poliomyelitis, presumably because maternally derived, type-specific poliovirus antibodies provided some protection. However, this protective effect is relatively short-lived. Maternally derived antibody declines with a half-life of approximately 28 days and can rarely be detected in infants older than 6 months.[316]

A large field trial funded by the National Foundation for Infantile Paralysis demonstrated in 1952 that immunoglobulin (i.e., gamma globulin) was effective in preventing paralytic disease if it was administered before the presumed exposure to poliovirus, but that the protective effect was relatively short-lived, approximately 5 to 8 weeks.[317] In this study, a single dose of 0.14 mL/kg body weight was administered intramuscularly.[317] However, evaluation of large-scale use of gamma globulin in 1953 by the National Advisory Committee for the Evaluation of Gamma Globulin in the Prophylaxis of Poliomyelitis concluded that "its preventive effect in community prophylaxis during 1953 has not been demonstrated." Also, no modification of the severity of paralysis was shown. Nevertheless, the committee could not say that the use of gamma globulin by mass inoculation produced no effect.[318] Although these conclusions were modified somewhat by Hammon and colleagues,[317] who pointed out that this evaluation had serious, perhaps fatal flaws—the gamma globulin was given far too late to be expected to have much or any effect and this was not a controlled experiment with appropriate controls—the committee report, nevertheless, dampened enthusiasm for this approach. In addition, in view of the progress toward the subsequent development of effective poliovirus vaccines that induced active immunity, presumably for life, gamma globulin as a tool to prevent poliomyelitis was not further pursued.

Passive immunity to poliomyelitis (and other diseases) among immunodeficient people, particularly people with agammaglobulinemia or hypogammaglobulinemia, is achieved through substitution therapy, namely, the regular administration (monthly) of intravenous immunoglobulin. Doses of 100 to 400 mg/kg body weight are used.[319] Intramuscular immunoglobulin also may be used, but it seems less effective. Immunoglobulin, whether it is formulated to be administered intramuscularly or intravenously, must pass the requirements of the U.S. Food and Drug Administration, which include a minimum titer for antibody to poliovirus type 1. In addition, immunoglobulin or hyperimmune sera may be used to eliminate chronic enterovirus infections,[320] including chronic infections with poliovirus,[321] and the oral route of administration sometimes has been used.[320] High-titer monoclonal antibody preparations are being evaluated for prevention of paralytic poliomyelitis as postexposure prophylaxis or to clear prolonged chronic poliovirus excretion among immunodeficient persons.[97]

ACTIVE IMMUNITY

Early Approaches

Research on inducing active immunity in monkeys by administering ground-up spinal cord and observing whether the animals died of poliomyelitis began as early as 1910.[315] Inactivation of poliovirus by formalin was first reported in 1911.[322] Brodie and Goldblum[323] and Brodie[324] first used subinfective doses of live poliovirus and later mixed live poliovirus with hyperimmune serum before using phenol and formalin as inactivating substances. After further small-scale experiments to optimize formalin inactivation and administration of the inactivated vaccine to small numbers of rhesus monkeys, adult volunteers, and children, the vaccine was administered to approximately 3000 children.[325,326] However, this approach was controversial and not pursued further because of concerns about efficacy and safety. In 1935, Kolmer[327] pioneered the use of attenuated live poliovirus that had been passaged continually in monkeys and prepared a suspension of monkey spinal cord in 1% sodium ricinoleate. The vaccine was administered to approximately 10,000 children, at least 10 of whom acquired paralytic poliomyelitis shortly after vaccination, for a rate of approximately one case per 1000 children vaccinated.[327,328] In short, both approaches—inactivation by formalin and attenuation of live poliovirus—that were successfully used later by Salk and Sabin had been used during these trials. In retrospect, it is clear that, without knowledge of the number of poliovirus serotypes and a better understanding of the differences between attenuation and chemical inactivation, these early vaccine development efforts were doomed to failure.

Advances in tissue culture growth of poliovirus in the late 1940s[20] renewed interest in poliovirus vaccines, and some of the first reports on attenuation of wild poliovirus for vaccine purposes were published in the early 1950s.[329–332] Essentially,

attenuated strains were developed by several passages of virus at high concentration in cell cultures of rodent CNS tissue or non–nervous system tissue from monkeys, followed by selection of attenuated variants at limiting dilutions or from single plaques.

Description of Vaccine

How Strains Were Developed

The history of early developments of oral vaccines can be reviewed in detail in reports of meetings published between 1958 and 1961[333–336] and in the corresponding chapter of the second edition of *Vaccines*.[258] As with most major scientific achievements, crucial contributions came from a number of investigative teams.[28,337–339] Koprowski and colleagues[329] reported the successful immunization of humans against poliomyelitis with a live poliovirus vaccine as early as 1950. Although these attenuated strains were not selected for licensing, this work meant that, by 1960, when not only their strains, but also several other candidate vaccines, were well into their testing and field trial use, a 10-year record of evidence was available on patterns of response in humans who had received the type 2 strain in 1950.[340]

Continued development and testing of candidate strains were conducted in three institutions: the Children's Hospital Research Foundation, Cincinnati, Ohio (A. B. Sabin); Lederle Laboratories, Wayne, NJ (Herald Cox and colleagues); and the Wistar Institute, Philadelphia (Koprowski and colleagues).[335,336] The work with attenuated polioviruses was advanced toward practical usefulness, particularly by Sabin, who meticulously studied a number of progeny of single virus particles for neurotropism in monkeys and finally selected three for small experimental trials in humans.[341] Much of the early efforts in the development of candidate strains were devoted to (a) maintaining high degrees of infectivity in cell culture and the human intestinal tract, (b) inducing detectable levels of neutralizing antibody in a high proportion of susceptible (seronegative) recipients, (c) displaying low neurovirulence in monkeys, (d) demonstrating a lack of association with paralytic disease in humans, and (e) maintaining genetic stability after replication in the human host.[254] The efforts of a number of investigators to develop and test suitable attenuated poliovirus strains came to fruition during 1955 to 1959, and large-scale field trials were held in many countries under a variety of conditions. Many of these trials in humans involved the sequential administration of monovalent formulations of poliovirus types 1, 2, and 3. A number of investigators around the world participated in studies in which these candidate strains were fed to millions of people. At the two conferences held by the Pan American Health Organization (1959 and 1960), these investigators joined in assessing the results.[335,336]

The field trials[335,336] and subsequent studies focused not only on the human populations fed candidate virus, but also on the virus populations recovered from stool samples of vaccinees. Because the attenuated polioviruses are living systems that must multiply to immunize, it was essential to know as much as possible about the progeny viruses let loose in nature—agents that still retain their property of infecting humans. All poliovirus strains, regardless of how highly attenuated, retain the property of multiplying and destroying cells in the monkey spinal cord. The degree to which this property is retained, however, varies over an enormous range as one progresses from the virulent strains to the highly attenuated ones suitable for vaccine use. The laboratory techniques are such that different degrees of neurotropism, even among attenuated strains, can be detected.

In 1958, a detailed comparison of candidate strains was conducted.[342,343] The attenuated strains that had been developed at the Yale Poliomyelitis Study Unit had already been shown to have too high a degree of reversion in chimpanzees and were dropped from consideration.[331,344] At Baylor College of Medicine, Houston, Texas, extensive comparison was made of the Sabin strains and the Lederle-Cox strains for neurovirulence in hundreds of monkeys inoculated by the intracerebral and intraspinal routes.[342–345] At the Division of Biologics Standards of the National Institutes of Health, Murray and colleagues[346] compared three sets of candidate strains as follows: Lederle-Cox, Sabin, and Koprowski-Wistar. Although the studies were done in two different laboratories and with some variations in methods, the overall findings were essentially in agreement. It was clear that the Lederle and Wistar strains were more neurotropic for monkeys than the Sabin strains. Because the results favored the Sabin strains, these strains are the ones that have been licensed, manufactured, and used almost universally since then.

Tables 49.6 through 49.9 show the passage histories of the three Sabin vaccine seeds now in use.[347] For example, the type 1 strain was derived from the Mahoney strain, initially isolated by Francis and Mack in 1941. Salk made additional monkey

TABLE 49.6 Poliovirus Type 1, Sabin Strain, Passage History[a]

Year	Manipulation	Designation
1941	Frances and Mack, isolation of Mahoney strain Salk, 14 MKTC and 2 monkey testicular cell passages	Mahoney strain
1953	Li and Schaefer, 11 MKTC passages; additional tissue culture passages in monkey kidney and skin	LS strain; LS-a; LS-b; LS-c
1954	Sabin, 5 passages in cynomolgus MKTC (3 terminal dilutions); 3 single-plaque passage; selection by neurovirulence testing	LS-c; 2ab
1956	Sabin, 2 passages in cynomolgus MKTC	LS-c; 2ab/KP_2 = SO
1956	MSD, 1 passage in rhesus MKTC	LS-c, 2ab/KP_3

[a]Data from the World Health Organization Consultative Group on Poliomyelitis Vaccines, 1985.
MKTC, monkey kidney tissue culture; MSD, Merck Sharp & Dohme.

TABLE 49.7 Poliovirus Type 2, Sabin Strain, Passage History[a]

Year	Manipulation	Designation
—	Fox and Gelfand, P712 strain isolated	P712
1954	Sabin, 4 passages (3 terminal dilutions) in cynomolgus MKTC; 3 serial passages of plaque isolates; selection by neurovirulence testing: fed to chimpanzees; 3 single-plaque passages	P712, Ch; P712, Ch, 2ab
1956	Sabin, 2 passages in cynomolgus MKTC	P 712, Ch, 2ab/KP_2 = SO
1956	MSD, 1 passage in rhesus MKTC	P 712, Ch, 2ab/KP_3

[a]Data from World Health Organization Consultative Group on Poliomyelitis Vaccines, 1985.
MKTC, monkey kidney tissue culture; MSD, Merck Sharp & Dohme.

TABLE 49.8 Poliovirus Type 3, Sabin Strain, Passage History[a]

Year	Manipulation	Designation
1937	Kessel and Stimpert, Leon strain isolated, 20 intracerebral passages in rhesus monkeys	Leon strain
1952	Melnick, 8 passages in rhesus testicular tissue culture	
1953	Sabin, 3 passages in cynomolgus MKTC; 30 rapid passages at low dilution in cynomolgus MKTC; 3 terminal dilution passages; 1 low-dilution pass; 9 plaques isolated, single-plaques passed 3 times; selection by neurovirulence testing	Leon 12a,b
1956	Sabin, 3 passages in cynomolgus MKTC	Leon 12a,b/KP_3 = SO
1956	MSD, 1 passage in rhesus MKTC	Leon 12a,b/KP_4

[a]Data from World Health Organization Consultative Group on Poliomyelitis Vaccines, 1985.
MKTC, monkey kidney tissue culture; MSD, Merck Sharp & Dohme.

TABLE 49.9 Poliovirus Type 3, Sabin Strain, RNA-Derived, Passage History[a]

Year	Manipulation	Designation
1959	Pfizer, 1 passage of SOM in cercopithecoid MKTC with SV40 antiserum	SO + 2 = 127-B-111
1962	Pfizer, RNA extraction and plaque cloning, selection by rct40 marker test, 2 plaque purifications	SO + 3 = 457-111
1978	Seed stocks acquired by Institute Mérieux and distributed to others when Pfizer ceased operations	

[a]Data from World Health Organization Consultative Group on Poliomyelitis Vaccines, 1985.
MKTC, monkey kidney tissue culture. SO, Sabin original; SOM, SO + 1.

kidney and monkey testicular passages. In 1953, Li and Schaeffer made 11 monkey kidney tissue culture passages, yielding the partially attenuated LS strain, and additional passages in monkey kidney and skin to yield the further attenuated strain LS-c. Then Sabin, in 1954, carried the LS-c strain through terminal dilutions and single-plaque passages, carefully selecting by neurovirulence testing, finally obtaining strain LS-c, 2ab. Two further passes, in cynomolgus kidney, yielded LS-c, 2ab/KP_2, designated SO (Sabin original). Bettylee Hampil, at Merck Sharp & Dohme, made one additional passage in rhesus monkey kidney tissue culture to derive LS-c, 2ab/KP_3, designated SO + 1, or SOM. The current vaccine is SO + 4, four tissue culture passages beyond the SO. A maximum of five passages is permitted, after which earlier (grandmother) seeds must be thawed and used to prepare new mother seeds. Because of inherent difficulties in maintaining the genetic stability of the Sabin type 3 seed stock, manufacturers have turned to an RNA-derived passage and clone of the strain, labeled SOR. This seed has yielded a vaccine of greater consistency and stability than the original Sabin seed. Grandmother seeds are potentially available through the WHO.

Genetic Determinants of Attenuation of the Sabin Oral Polio Vaccine Strains

Identification of the genetic determinants of attenuation of the Sabin OPV strains has been comprehensively reviewed.[71,73,113,348] Two basic approaches have been taken: (a) sequence comparisons between the Sabin strains and their neurovirulent wild parents (types 1 and 3) or neurovirulent revertants (types 2 and 3) obtained from patients with VAPP; and (b) investigation of the contribution of specific nucleotide substitutions to attenuation using infectious complementary DNA (cDNA) clones (see "Genetic Stability of Vaccine Seed Strains" later). A common feature of the Sabin strains is the presence of nucleotide substitutions in the IRES in the 5′-UTR, which have been clearly shown to be critical attenuating mutations (Fig. 49.6). Additional mutations encoding amino acid substitutions in the capsid region contribute to and stabilize the attenuated phenotype.

Sabin 1. The 57 nucleotide substitutions distinguishing the Sabin 1 strain from its neurovirulent parent, Mahoney/USA41, are scattered throughout the genome.[353] Six map to the 5′-UTR, 49 map to the coding region (21 of which encode amino acid substitutions), and two map to the 3′-UTR. Infectious cDNA constructs containing different combinations of blocks of Sabin 1 and Mahoney sequences were tested for neurovirulence in monkeys or transgenic mice expressing the CD155 receptor, for temperature sensitivity, and for other phenotypic properties distinguishing the two strains.[349,354] The single most important determinant of the attenuated phenotype of Sabin 1 was the A→G substitution at position 480 (abbreviated A480G) in the IRES.[355] Four other substitutions contributing to the attenuated phenotype mapped to the capsid region (one in VP4, one in VP3, and two in VP1), and a substitution contributing to the temperature-sensitive phenotype (but not to the attenuated phenotype) mapped to the $3D^{pol}$ region encoding the RNA-dependent RNA polymerase (see Fig. 49.6).[349,354,356] The relatively high stability of the attenuated phenotype of Sabin 1 is attributed to multiple attenuating substitutions and their relative contributions to the phenotype. However, neurovirulent revertants have been selected in the laboratory that have only two (U525C, a suppressor that restores base pairing at 480G [see below] and C6203U, a histidine→tyrosine back-mutation in the 3D-polymerase) or three mutations.[357]

Sabin 2. Only two nucleotide substitutions (G481A in the IRES, and C2909U encoding a threonine→isoleucine substitution at position 143 of VP1) appear to be main determinants of the attenuated phenotype of Sabin 2 (see Fig. 49.6).[350,358] Because P712 has inherently low neurovirulence,[359] identification of critical attenuating sites in Sabin 2 involved determination of the effects of introduction of sequences derived from a minimally divergent neurovirulent revertant of Sabin 2 (obtained from a case of VAPP) into infectious cDNA constructs derived from Sabin 2.[350,358] Weakly attenuating substitutions mapped to nucleotide positions 437 in the 5′-UTR, and/or 868 (VP4 region), and/or 4076 (2B region).[358]

Sabin 3. Detailed analysis of the attenuated phenotype of Sabin 3 has been possible because the neurovirulent parental strain, Leon/USA37, differs from Sabin 3 by only 10 nucleotide substitutions.[360] In addition, numerous neurovirulent revertants of the Sabin 3 strain have been isolated from patients with VAPP[347] and from healthy OPV recipients.[351] Only two substitutions—C472U in the IRES and C2034U encoding a serine→phenylalanine substitution at position 91

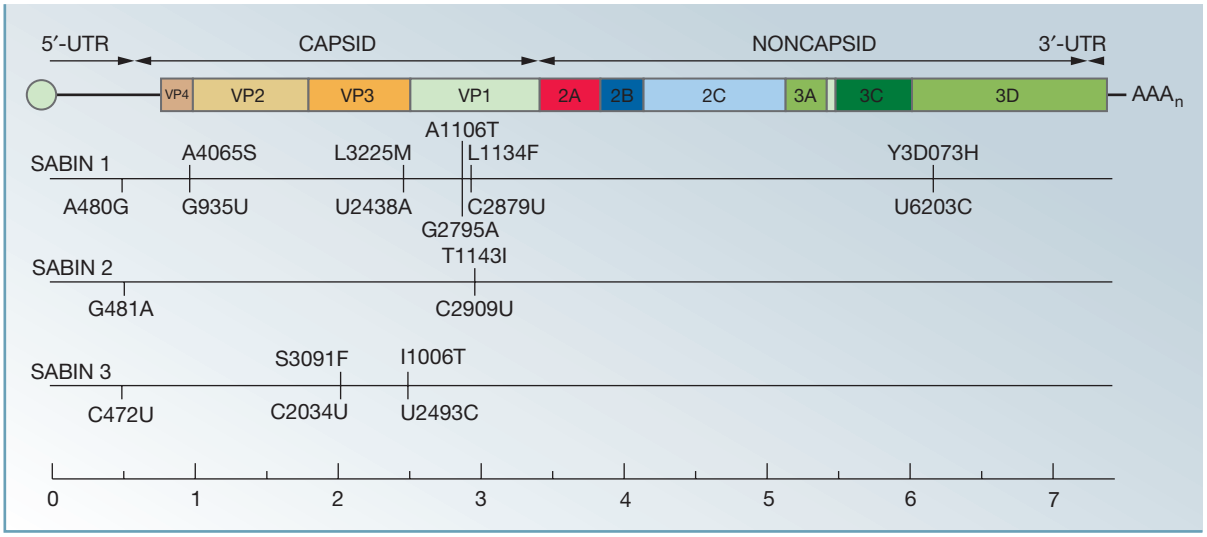


Figure 49.6. Location of principal attenuating nucleotide (lower bars) and amino acid (upper bars) substitutions in each of the three Sabin oral polio vaccine (OPV) strains. Abbreviations of nucleotide residues: A, adenine; C, cytosine; G, guanine; U, uracil. Abbreviations for amino acid residues: A, alanine; C, cysteine; F, phenylalanine; H, histidine; I, isoleucine; L, leucine; M, methionine; S, serine; T, threonine; Y, tyrosine. Substitutions are shown as nonattenuated parent-position–Sabin strain; nucleotide positions are numbered consecutively from residue 1 of the RNA genome; and amino acid positions are indicated by the abbreviated name for viral protein (4, VP4; 2, VP2; 3, VP3; 1, VP1; 3D, 3D-polymerase) and numbered consecutively from residue 1 of each protein. For example, a guanine (Mahoney) → uracil (Sabin 1) substitution at RNA position 935 (G935U) encodes an alanine (Mahoney) → serine (Sabin 1) replacement at residue 65 of VP4 (A4065S). The Y3D073H substitution in Sabin 1 and the S3091F substitution in Sabin 3 are important determinants of temperature sensitivity. UTR, untranslated region. *(Data from references 347, 349, 350, 351, and 352.)*

of VP3—appear to be the main determinants of the attenuated phenotype (see Fig. 49.6).[71,347,351] A third substitution, U2493C encoding an isoleucine→threonine substitution at position 6 of VP1, appears to be a minor determinant of attenuation that also reduces the replicative fitness of Sabin 3.[352] The U2493C substitution is strongly selected against during cell culture passage and during replication in the human intestine.[361]

Quantitative determination of the contributions of each substitution is complicated by several factors: (a) the role of minor determinants of attenuation is difficult to measure, (b) some substitutions have pleiotropic effects on phenotype, (c) some Sabin strain phenotypes require a combination of substitutions, (d) second-site mutations can suppress the attenuated phenotype in various ways, and (e) the outcome of experimental neurovirulence tests may vary with the choice of experimental animals (monkeys vs transgenic mice) or the route of injection (intraspinal vs intracerebral).[358,361,362] Examples of substitutions with pleiotropic effects are: (a) the serine→phenyalanine substitution at position 91 of VP3 which confers both attenuation and temperature sensitivity to Sabin 3[363] and (b) the tyrosine→histidine substitution at position 73 of the 3D polymerase of Sabin 1, which is an important determinant of temperature sensitivity[349,364] and a minor contributor to attenuation.[364] The relationship between poliovirus neurovirulence in experimental animals (where virus is introduced directly into the CNS) and pathogenicity for humans (where virus is introduced by ingestion) cannot be measured in controlled experiments, and remains ambiguous. Clearly the Sabin OPV strains are several orders of magnitude less neurovirulent than wild polioviruses, as indicated by the very low incidence of VAPP[365] compared with the high incidence of paralytic poliomyelitis in areas where wild polioviruses are circulating.

The attenuating substitutions of the Sabin strains are sometimes described as mutations impairing the specific determinants of poliovirus neurovirulence. However, attenuation is a specific phenotype of each Sabin strain. Poliovirus neurovirulence, by contrast, is a much more complex property. Expression of a neurovirulent phenotype requires the efficient function of numerous steps in the natural life cycle of the virus and, thus, the efficient expression of several viral genes.[55] Impairment in the expression of any of these poliovirus genes can reduce replicative fitness and confer a more attenuated phenotype. However, such mutants may not be suitable candidates for live-virus vaccines. The Sabin strains contain specific genetic defects that are not found among the highly diverse population of circulating wild polioviruses. These exceptional defects are unstable to replication in the human intestine, and variants with higher replicative fitness are regularly selected. Other combinations of substitutions can produce highly attenuated polioviruses potentially suitable for vaccine use.[366] However, the attenuating substitutions of the Sabin strains were shown to confer highly favorable biological properties, making these strains the best available candidates in the early 1960s for licensure as OPVs (see "How Strains Were Developed" earlier).

Functional Basis for Attenuation of the Sabin Oral Polio Vaccine Strains

The determination in the early 1980s of the complete genomes of the Sabin strains and their neurovirulent parents (in the case of types 1 and 3),[64,353,360,367,368] and the availability of infectious cDNA poliovirus clones[347,354,369] opened the way for detailed analysis of the biological mechanisms for the attenuated phenotype of the Sabin OPV strains.[71] The most detailed analyses have been of the substitutions in the IRES (A480G in Sabin 1, G481A in Sabin 2, and C472U in Sabin 3) that are the principal attenuating mutations of the respective Sabin strains. These substitutions map to a specific domain of RNA

secondary structure (stem–loop region V) within the IRES of the 5′-UTR which is highly conserved among polioviruses and related enteroviruses. The attenuating substitutions in the IRES alter stem–loop structures[71,370–372] and are not found in natural wild poliovirus isolates. Mutations restoring the original stem–loop structure in the IRES (Sabin 1: G480A [back-mutation] or U525C [suppressor]; Sabin 2: A481G [back-mutation]; Sabin 3: U472C [back-mutation]) are frequently found in vaccine-related isolates from healthy OPV recipients[370,373] and patients with VAPP,[71] as well as from the environment.[145] The localization of these important determinants to the IRES,[374] the initiation site for translation of the poliovirus polyprotein, suggested that an important aspect of attenuation may involve a deficiency in translation for the Sabin strains. Indeed, in vitro translation experiments demonstrated that decreased efficiency in the initiation of translation is associated with the U472 substitution in Sabin 3[375,376] and the G480 substitution in Sabin 1.[370,371] This view is further supported by the finding that replication of the Sabin strains is similar to that of neurovirulent polioviruses in HeLa (Henrietta Lacks) cells, but is differentially reduced in neuroblastoma cells.[377,378] The reduced Sabin strain yields are associated with lower efficiencies of translation in neuroblastoma cells.[371,377,378] A possible molecular mechanism for the translational deficit involves the polypyrimidine tract–binding protein (PTB), a cellular protein that interacts with the IRES and which facilitates poliovirus translation.[379] The attenuating mutations weaken the interaction of PTB with the IRES in neuroblastoma cells, but not in HeLa cells.[379] Moreover, the translational deficit for Sabin 3 is moderate in intestinal cells, where PTB levels are high, but severe in neurons, where PTB levels are low. Intact spinal cords from chick embryos have reduced levels of PTB and translation of Sabin 3 in the neuronal cells is less efficient than that of neurovirulent parental Leon strain.[380]

Despite the strong experimental support for the hypothesis that a preferential defect in translation in motor neurons is a major contributor to the attenuated phenotype of the Sabin strains, this view has been challenged by newer studies in mice in which translation from the Sabin 3 IRES was equivalently reduced in cells of neuronal and nonneuronal origin.[381] It was suggested that attenuation is determined after internal ribosome entry, that the Sabin strains have a reduced fitness for replication in all cells, and that reduced efficiency of replication in intestinal cells may permit the development of an immune response before sufficient numbers of virus reach the spinal cord and brain.[381]

The major attenuating determinants in the IRES are the ones that have been most intensively studied, particularly that of Sabin 3 because substitutions at position 472 have more pronounced effects on the attenuated phenotype than do substitutions at position 480 in Sabin 1 or 481 in Sabin 2. Results of similar studies with Sabin 1 and Sabin 2 are usually in agreement with the Sabin 3 findings.[349,350,358] The capsid determinants may affect the attenuated phenotype in various ways such as by interfering with virus assembly,[363] by reduced efficiency of binding to the CD155 PVR,[349] or by reducing capsid stabilities.[81] It has been emphasized that the attenuated phenotype is composite in nature involving the complex interaction of multiple determinants.[73]

Sabin and other developers of OPV strains struck a balance between low neuropathogenicity, good immunogenicity, and acceptable levels of genetic stability.[382] The high genetic stability of the Sabin 1 vaccine strain is especially important, because wild type 1 polioviruses typically have high paralytic attack rates and can spread over wide geographic areas in explosive outbreaks.[212,289,299,301–303,383] Sabin 2 may revert more rapidly, but its immunogenicity is very high[63,382] and the paralytic attack rates of wild type 2 polioviruses are low.[46,384] Sabin 3 is associated with the highest rates of VAPP, which is probably a consequence of low genetic stability of the critical attenuating substitution,[385] relatively low immunogenicity,[386] and an intermediate paralytic attack rate for type 3 polioviruses.[46,384] Nonetheless, all three Sabin strains normally have very low pathogenic potentials, and incidence of VAPP in countries with high rates of OPV coverage[268] are several orders of magnitude lower than the incidence of paralytic poliomyelitis in areas with circulating wild polioviruses.

How Trivalent Vaccine Was Developed

Viral replication in the gastrointestinal tract and seroconversion were usually demonstrated in 80% to 100% of seronegative recipients with a single dose of monovalent vaccines at dosage levels of 10^5 median tissue culture infective doses ($TCID_{50}$).[387–391] However, when doses of 10^5 $TCID_{50}$ of each poliovirus serotype were mixed and administered as trivalent preparations, the replication and antibody production were consistently lower for some types compared with the sequential administration of monovalent vaccines.[392–398] This effect could be modified somewhat by increasing the doses of each type ($\geq 10^7$ $TCID_{50}$).[399,400] In addition, these studies showed that tOPV of similar potency for each serotype was associated with a predominance of poliovirus type 2 excretion and significantly higher type 2 antibody titers than for poliovirus types 1 and 3. These early trials did not evaluate the impact of increasing the quantity of one serotype or reducing the quantity of another on seroconversion to all three serotypes because the interference effect of type 2 often could be overcome by administering three or more doses of the trivalent vaccine.

In 1961, a large study in Canada tested a "balanced" formulation of tOPV (10^6 $TCID_{50}$ for Sabin type 1, 10^5 $TCID_{50}$ for Sabin type 2, and $10^{5.5}$ $TCID_{50}$ for Sabin type 3).[401] A single dose of this balanced (10:1:3) vaccine was administered to nearly 24,000 people, including 106 previously seronegative subjects, 103 (97%) of whom seroconverted to all three serotypes. Although one could conclude from this study that a single dose of OPV may be sufficiently immunogenic for a routine program, only triple-seronegative infants were included in this analysis of the Canadian trial, so the results represent the best possible scenario for inducing optimal levels of seroconversion because the infants lacked maternally derived antibody that can interfere with seroconversion. On the basis of these findings and an unpublished study from Guam, the balanced formulation of OPV was licensed in Canada in 1962 and in the United States in 1963.

Dosage and Route

In the United States, before discontinuation of OPV in 2000, the trivalent live attenuated OPV was formulated to contain at least $10^{5.5}$ $TCID_{50}$ for poliovirus type 1, $10^{4.5}$ $TCID_{50}$ for type 2, and $10^{5.2}$ $TCID_{50}$ for type 3.[402,403] However, the U.S. manufacturer routinely exceeded these minimum requirements, and an evaluation by a WHO reference laboratory found potency levels of $10^{6.5}$, $10^{5.4}$, and $10^{6.3}$ $TCID_{50}$ per dose of types 1, 2, and 3, respectively (WHO unpublished data). The WHO requires the following minimum $TCID_{50}$ values for each vaccine poliovirus serotype: $10^{5.9} \pm 0.5$ $TCID_{50}$ for type 1, $10^{5.0} \pm 0.5$ $TCID_{50}$ for type 2, and $10^{5.7} \pm 0.5$ $TCID_{50}$ for type 3. Because of evidence from an evaluation in Brazil that poliovirus type 3 immunogenicity is not satisfactory in a trivalent formulation with $10^{5.5}$ (300,000) $TCID_{50}$, particularly in tropical countries,[404] the WHO's Global Advisory Group recommended in 1990 that the type 3 component of OPV should

be increased to $10^{5.8}$ (600,000) $TCID_{50}$.[405] The OPV purchased by UNICEF beginning with the 1992 to 1993 tender period included this recommendation.

The recommended route of administration for OPV is oral, by releasing the vaccine volume (0.5 mL) contained in single-dose droppers into the oral cavity[406] for vaccine manufactured in the United States (before 2000, when OPV production was discontinued) or by providing 2 drops (≈0.1 mL) of OPV contained in multidose vials produced by many non–U.S. manufacturers.[407] Although in the early 1960s some manufacturers put OPV into dragées, at present, only OPV produced in China is in dragée form; before administration, the dragée must be ground up and mixed with water.

Producers

At least 10 manufacturers around the world are producing OPV using the Sabin vaccine seeds (Table 49.10). The list includes producers in Belgium, China (two), France, Indonesia, Iran, Japan, Mexico, Russia, and Vietnam. In addition, manufacturers in Brazil, Egypt, India (several companies), Pakistan, and Thailand have the capacity for filling and finishing bulk OPV produced by one of the primary manufacturers.

Most of these manufacturers use seed strains of types 1 and 2 no more than two passages away from the WHO master seed (SO + 1, i.e., Sabin original plus one passage). There is more

TABLE 49.10 Manufacturers and Fillers of Oral Poliovirus Vaccine, 2016[a]

			Licensed Product (Prequalified)				
	Location	**Cell Substrate[b]**	**tOPV[d]**	**mOPV1**	**mOPV2**	**mOPV3**	**bOPV**
MANUFACTURER							
Sanofi Pasteur	Lyon, France	Vero	**Yes (yes)**	**Yes (yes)**	**Yes (yes)**	**Yes (yes)**	**Yes (yes)**
Beijing Institute of Biological Products	Beijing, China	Human diploid 2BS	Yes	No	No	No	Yes
Bio Farma	Bandung, Indonesia	PMK	**Yes (yes)**	**Yes (yes)**	No	No	**Yes (yes)**
BIRMEX (Instituto Nacional de Virologia)	Mexico City, Mexico	Vero	Yes	No	No	No	No
Chumakov Institute (Institute of Poliomyelitis and Viral Encephalitides)	Moscow, Russian Federation	PMK	Yes	No	No	No	Yes
GlaxoSmithKline	Rixensart, Belgium	MRC-5	**Yes (yes)**	**Yes (yes)**	**Yes (yes)**	**Yes (yes)**	**Yes (yes)**
POLYVAC (Poliomyelitis Vaccine Research and Production Center)	Hanoi, Vietnam	PMK	Yes	No	No	No	No
JPRI (Japan Poliomyelitis Research Institute)	Tokyo, Japan	PMK	Yes	No	No	No	No
Kunming Institute of Medical Sciences	Kunming, China	PMK	Yes	No	No	No	No
Razi Institute (Vaccine & Serum Institute)	Hessarak, Iran	MRC-5	Yes	No	No	No	Yes
FILLER (FROM IMPORTED BULK)							
Bio-Manguinhos	Brazil	—	Yes	No	No	No	No
VACSERA	Cairo, Egypt	—	Yes	No	No	No	No
Bharat Biotech	Hyderabad, India	—	**Yes (yes)**	**Yes (yes)**	No	**Yes (yes)**	**Yes (yes)**
BIBCOL	Uttar Pradesh, India	—	Yes	No	No	No	No
BIO-MED	New Dehli, India	—	Yes	No	No	No	No
HBPLC-Haffkine	Mumbai, India	—	**Yes (yes)**	**Yes (yes)**	No	No	**Yes (yes)**
Panacea Biotech+	New Delhi, India	—	Yes	Yes	Yes	Yes	Yes
Serum Institute of India	Pune, India	—	**Yes (yes)**[c]	No	No	No	**Yes (yes)**[c]
National Institute of Health	Islamabad, Pakistan	—	Yes	No	No	No	No
Government Pharmaceutical Organization	Bangkok, Thailand	—	Yes	No	No	No	No

[a]Since 2007, five manufacturers have discontinued production: Novartis (ex Chiron), Siena, Italy; Chiron Behringwerke, Marburg, Germany; Evans Medical Ltd, Medeva-Speke, Liverpool, England; TORLAK, Belgrade, Serbia; and Wyeth-Lederle Vaccines and Pediatrics, Pearl River, NY. **In bold**, products that are currently World Health Organization prequalified for UNICEF procurement on behalf of the Global Polio Eradication Initiative.
[b]For the fillers, the cell substrate depends on the bulk vaccine supplier.
[c]License for export only.
[d]None of these manufacturers is currently producing or distributing tOPV.
bOPV, bivalent oral poliovirus vaccine; mOPV, monovalent oral poliovirus vaccine; tOPV, trivalent oral poliovirus vaccine.

variation in the type 3 seed used; most manufacturers are now using the Pfizer RNA-derived seed (SOR + 1). The type 3 seed used by one manufacturer in China, the Zhong III strain, has been shown by oligonucleotide fingerprinting to be indirectly derived from Sabin type 3.[408] Vero cells and human diploid cells are used by at least one manufacturer each for growing their vaccine viruses; the others continue to use primary monkey kidney cells. It is recommended that the cells for cultivation be taken from monkeys bred in captivity. Like the cell cultures used, the monkey colony should be shown to be free of extraneous viruses and other pathogens.

Preparations Available (Including Combinations)

Although mOPV formulations of the three poliovirus serotypes were used widely in the early 1960s, they were replaced by tOPV starting in 1963 in the United States and other countries. Exceptions include Hungary, which used all three types of mOPV sequentially until the early 1990s,[409] and South Africa, which routinely used monovalent type 1 OPV (mOPV1) in its routine immunization program until the early 1990s.[410] The mOPV formulations, although used extensively in the early 1960s, were no longer licensed in 2004. To improve the immunogenicity of OPV in developing countries, the Advisory Committee for Poliomyelitis Eradication, the principal technical oversight committee for the GPEI at the time, recommended in 2004 the rapid development and large-scale use of mOPV1.[411] Currently, six manufacturers and fillers (fillers procure bulk OPV from an established manufacturer with bulk production and then blend, fill, and release the product) have developed and received regulatory approval for use of mOPV1 (Bharat Biotech International Ltd, Bio Farma, GlaxoSmithKline, Haffkine, Panacea Biotec Ltd, and Sanofi Pasteur). Three manufacturers and fillers developed and received regulatory approval for mOPV3 (Bharat Biotech International Ltd, GlaxoSmithKline, Sanofi Pasteur, and Panacea Biotec Ltd). Three companies (Panacea Biotec Ltd, GlaxoSmithKline, and Sanofi Pasteur) have licensed mOPV2. In addition to accelerating eradication, these mOPVs will also be used for a stockpile of vaccine available for potential outbreak control during the posteradication era.[391] The use of bOPV (types 1 and 3) was first proposed in 1997 coincident with the impending eradication of wild type 2 polioviruses, which occurred in 1999.[412–414] However, also in 1997, an unfavorable regulatory opinion highlighted the obstacles to proceeding with bOPV licensure and delayed further consideration until after mOPV formulations were licensed in 2005. A total of 10 manufacturers and fillers are producing bOPV (Table 49.10). bOPV has received regulatory approval or has been used in 48 countries for supplementary immunization activities, during which more than 5 billion doses have been administered between 2009 and 2014. After mOPV withdrawal in April 2016, bOPV became the vaccine of choice for routine immunization together with IPV (see "Routine Immunization" later). mOPV, bOPV, and tOPV are available in multidose vials (10-, 20-, and 50-dose vials); tOPV is also available in single-dose preparations. No combination products with OPV as one of the components have been licensed.

Constituents (Including Antibiotics and Preservatives)

The growth medium for the cells consists of Eagle basal medium, the components of which include Earle balanced salt solution, amino acids, antibiotics, and calf serum. After the cells have grown out, the medium is removed and replaced with fresh medium that contains the inoculating virus but no calf serum. The final vaccine is diluted with a modified cell culture maintenance medium that usually contains stabilizer. Each dose of OPV may contain trace amounts of penicillin, neomycin, polymyxin, and streptomycin (see "Contraindications and Precautions" later) and magnesium chloride or sugar (sucrose and sorbitol) as stabilizers. The vaccine contains phenol red as an indicator of pH.

Vaccine Stability

Before any vaccine lot, whether tOPV, mOPV, or bOPV, can be released, it must be tested and meet the requirements of the national control authorities. If the vaccine is purchased by UNICEF, it also must meet the WHO requirements for manufacture, safety, and potency. Because OPV is a live viral vaccine, it is labile unless it is stored at low temperatures (frozen). Thermostability requirements were defined by the WHO as OPV that loses less than 0.5 $\log_{10}$ of titer of each of the three vaccine strains after exposure to 37°C for 2 days.[415] In addition, current regulations require that, for maintenance of potency, the vaccine must be stored and shipped frozen, and that, after thawing, it must be held in the refrigerator at no more than 10°C for a period not to exceed 30 days, after which time it must be discarded.

In the early 1960s, it was found that the infectivity of the enteroviruses could be preserved even when they were heated at 50°C if molar magnesium chloride was added,[416] a property that is still used in their identification and characterization. This discovery was applied rapidly to live vaccines[94] not only in the laboratory,[417] but also in the field, where stabilized vaccines were used effectively to halt type 1 and type 3 outbreaks.[418] In laboratory studies with OPV containing magnesium chloride as a stabilizer,[419] the vaccine showed so little loss in virus titer after long-term storage at −20°C that the predicted half-life was calculated as 92 years. It also was noted that vaccine stabilized with magnesium chloride had no significant loss of potency after as many as nine cycles of alternate warm and cold conditions. Sucrose and sorbitol have also been used as stabilizers, but they have been less effective, particularly at high temperatures.[406] One manufacturer has introduced a concentrated phosphate buffer—lactalbumin hydrolysate—as a stabilizer; it works well at 4°C, but more data are needed to learn how effective it would be in the field at higher temperatures. It has been shown that deuterium oxide with magnesium chloride improves the thermal stability[420]; however, no OPV vaccines in use today contain deuterium oxide.

Individual vaccine vial monitors were introduced in 1996 for all OPV procured by UNICEF.[421] The vaccine vial monitors respond to heat exposure with a change in color. The potential benefits of vaccine vial monitors include (a) the ability to keep opened vials without having to discard partially used vials at the end of the day, (b) a decrease of at least 30% in vaccine wastage rates, (c) flexibility to take the vaccine beyond the cold chain to reach remote locations, and, most important, (d) reassurance for the vaccinator at peripheral vaccination sites that the vaccine is potent.[421]

Regulatory Requirements for Oral Polio Vaccine Licensure

National control or regulatory agencies, such as the U.S. Food and Drug Administration, the UK National Institute for Biological Standards and Control, and similar national regulatory authorities in other countries, provide guidance to manufacturers for the production and licensing of OPV.[402] In addition, the WHO provides regulations that manufacturers must follow to be eligible to sell vaccine through the UNICEF tender. The WHO regulations contain three sections:

(a) manufacturing requirements, (b) national control requirements, and (c) requirements for poliomyelitis vaccine (oral) prepared in primary cultures of monkey kidney cells. In addition, a summary protocol for poliomyelitis vaccine (oral) production is provided in Appendix 7 of that document.[415,422]

The WHO recommends that all candidate vaccine strains be evaluated in large-scale field trials before licensure because of the possibility that neurovirulence testing in monkeys may not always predict the actual behavior of vaccine strains (i.e., reversion to neurovirulence and potential for epidemic spread) in humans under field conditions.[423] USOL-D bac, a new poliovirus type 3 vaccine strain, was developed by the Institute of Sera and Vaccines in Prague, Czechoslovakia, in 1962.[424] Virus isolated in stool after administration of Sabin type 3 had a higher degree of neurovirulence than the corresponding mutants of USOL-D bac. In addition, this vaccine strain had passed all neurovirulence tests as determined in monkeys and seemed to be safer than the Sabin type 3 strain. In 1968, an extensive outbreak of poliomyelitis caused by poliovirus type 3 occurred in Poland 4 months after a small vaccine trial with Sabin type 3 and USOL-D bac vaccine strains had been carried out.[425] Subsequent molecular investigations indicated that USOL-D bac had been responsible for the epidemic.[408,426,427] This experience in humans indicates that monkey neurovirulence testing alone is insufficient to ensure vaccine safety. This conclusion is supported by data from the United States. Neurovirulence information, based on monkey neurovirulence tests, from more than 80 individual vaccine lots of Sabin type 3 virus produced between 1964 and 1983 was reviewed.[428] The authors of this study concluded that type 3 OPV is, if anything, less neurovirulent than is type 1 OPV. However, in field use, type 3 OPV is associated with vaccine-related poliomyelitis more frequently than is type 1 OPV.[428] The molecular mechanisms for poliovirus attenuation and experiences with neurovirulence testing during the prior 40 years were reviewed in a meeting in 1991.[429]

Genetic Stability of Vaccine Seed Strains

Live virus vaccines for RNA viruses present special challenges for development, manufacture, and administration because the mutation rates of RNA genomes are many orders of magnitude higher than those of DNA genomes.[430,431] The primary cause of this high mutability is that the viral RNA-dependent RNA polymerases lack the proofreading mechanisms associated with DNA polymerases of DNA viruses and cellular organisms.[432,433] The absence of proofreading mechanisms in most RNA polymerases limits the size of RNA virus genomes because the likelihood of incorporation of deleterious mutations increases with genome length.[431] RNA virus populations exist as "quasispecies," that is, as a heterogeneous collection of sequence variants distributed about an average "master" sequence.[434,435] Quasispecies are capable of rapid evolution under challenging environmental conditions because variants with higher genetic fitness for the new conditions may be preexistent in the populations.[434]

The above considerations are of relevance to poliovirus populations in general[435,436] and to OPV preparations in particular.[385,437] OPV preparations have been shown to be quasispecies, with revertant variants of the critical attenuating 5′-UTR mutations representing approximately 0.1% of the population.[385,437] The biologic pressures applied in the selection for the attenuated OPV strains[359] are partially reversed when the strains are propagated at large scale for OPV production.[438] The stringent growth and postproduction testing requirements for OPV are designed to limit the enrichment for variants with increased neurovirulence and to assure a product of uniformly high safety. Several key measures were implemented to limit the enrichment for variants: (a) use of cell substrates favoring maintenance of the attenuated variants, (b) limitation of the number of production passages from the original OPV seed strains, (c) reselection from the OPV seed stocks of clones with low neurovirulence, and (d) incubation of OPV production cultures at temperatures (~34°C) favoring the maintenance of the temperature-sensitive phenotypes of the OPV strains.

The master sequences of each Sabin OPV seed strain have been maintained indefinitely as infectious cDNA plasmids in the bacterium *Escherichia coli*,[439] and the quasispecies populations of OPV preparations have been preserved by polymerase chain reaction engineering.[440] Poliovirus sequences contained within the recombinant plasmids are genetically stable because the bacterial DNA polymerases replicating the plasmid sequences have high-fidelity proofreading mechanisms. However, RNA transcripts produced in vitro from recombinant DNA plasmids are not genetically homogeneous, because the RNA polymerases catalyzing the transcription lack proofreading mechanisms. Because there is no biologic selection during the transcription process for variant genomes with reduced attenuation, the RNA used to transfect cultured cells has more uniform genetic and biologic properties. Nonetheless, deep sequencing has shown that the distribution of minor sequence variants within the virus quasispecies population may fluctuate stochastically when OPV strains are again propagated in cell culture.[436,437] Choice of cell substrate and production conditions can minimize the selection of neurovirulent variants.[441] However, the most intense selection occurs when OPV is fed to a child, and both rapid selection of mutational variants and recombination can result in the excretion of virus with increased potentials for neurovirulence and transmissibility (see "Vaccine-Derived Polioviruses" later).[71,294] Deeper understanding of the genetic lability of OPV and other live RNA virus vaccines has prompted the recent development of more genetically stable OPV strains (see "New Oral Polio Vaccine Strains").[435,441–444]

New Oral Polio Vaccine Strains

Recognition of the appearance of VAPP soon after licensure of OPV[445] prompted efforts to develop more genetically stable OPV strains, especially to type 3.[424] However, a large poliomyelitis outbreak associated with an inadequately quarantined field trial of an experimental type 3 OPV strain discouraged further efforts to develop new OPV strains by traditional methods.[427] Advances in the understanding of the genetic basis for attenuation of the Sabin OPV strains[361] generated renewed interest in developing OPV strains with improved genetic stabilities. Nomoto and colleagues constructed chimeras with the capsid regions of Sabin 2 and Sabin 3 inserted into the genetic background of the more highly substituted and stable Sabin 1 strain,[446] and increased the attenuation of Sabin 1 by introducing deletions into the IRES in the 5′-UTR.[447] Agol and associates also constructed more highly attenuated strains by modification of IRES sequences,[448] while Gromeier and associates attenuated the neurovirulent Mahoney strain by replacement of its IRES with homologous IRES sequences from human rhinovirus type 2.[374] Macadam and associates introduced several mutations into the IRES of Sabin 3 that conferred high genetic and phenotypic stabilities to the type 3 strain and to type 1 and 2 strains in which the original Sabin 5′-UTR sequences are replaced by the engineered homologues.[449]

Heightened interest in more stable OPV strains followed the rising incidence of cVDPV outbreaks and the recognition of prolonged iVDPV infections (see "Vaccine-Derived Polioviruses" later).[136,450] However, the observation that the critical

determinants of attenuation in the 5′-UTR are frequently lost in cVDPVs by recombination with closely related non-polio enteroviruses[136,450] called for new approaches to reduce the frequency of cVDPV emergence, especially to type 2. A consortium of five research laboratories, supported by the Bill & Melinda Gates Foundation, has sought to develop a new, genetically stable type 2 OPV strain for possible use in emergency OPV stockpiles (see "New Polio Endgame"). Several approaches have been taken by the consortium: (a) further engineering of the IRES to stabilize the attenuated phenotype[441]; (b) relocation of a *cis*-acting replication element (*cre*) from the 2C noncapsid region to the 5′-UTR (to reduce recombination)[451]; (c) incorporation of additional CpG and UpA dinucleotides within and across synonymous capsid region codons (codon deoptimization or CpG/UpA desuppression; targeting the capsid region sequences limits the phenotypic effects of recombination)[442,443,452,453]; and (d) incorporation of amino acid substitutions into the 3D polymerase to increase replicative fidelity[435,454] and reduce recombination frequency.[444] The modular nature of the poliovirus genome, as illustrated by the properties of both natural recombinants[66,136] and engineered chimeras,[374,441,446] permits construction of strains that are both highly attenuated, genetically stable, and poised for clinical evaluation.

RESULTS OF VACCINATION

Immune Response

Administration of OPV, similar to natural exposure to polioviruses, initiates a complex process that eventually results in humoral (systemic) and mucosal (local) immunity. The kinetics of this immune response have been reviewed in detail by Ogra and Karzon.[235] Production of IgM antibody predominates initially, can be detected as early as 1 to 3 days after infection, and disappears after 2 to 3 months. IgG antibody increases during this same period, eventually constitutes the predominant class of persistent antibody, and may last for life.[187] Fig. 49.7 shows the development of serum and secretory antibody responses to OPV compared with IPV.[235] The broader response of nasal and duodenal IgA associated with OPV administration is apparent. The humoral immune response is not completely serotype-specific,[188,455] and some degree of cross-protection (heterotypic cross-reaction) has been observed. Preexisting antibody to poliovirus type 2 may modify the risk of paralytic poliomyelitis after exposure to poliovirus type 1.[456] The significance of cell-mediated immunity to poliovirus exposure remains to be shown,[457] although cytotoxic T-cell responses may contribute to the inflammation and cell necrosis that characterize poliovirus infections of the CNS.

Viremia has been demonstrated, commonly after ingestion of type 2 OPV. Free virus is present in serum between days 2 and 5 after vaccination, and virus, bound to antibody, can be detected for a few additional days.[458,459] Bound virus in serum is detected by acid treatment, which inactivates the antibody and liberates active virus.

The majority of infants (70% to 90%) who are susceptible to poliomyelitis excrete poliovirus after administration of OPV. A study conducted during the winter of 1960 in Houston reported that 72%, 88%, and 75% of infants 0 to 6 months old excreted homologous poliovirus types 1, 2, and 3, respectively, after vaccination with monovalent poliovirus vaccines (Fig. 49.8).[460] The same study showed that familial and extrafamilial contacts of vaccinated children are exposed secondarily to excreted poliovirus and, in turn, also excrete poliovirus in feces. The highest proportion of type 2 mOPV-vaccinated infants excreted homologous virus in the first week after vaccination, the highest proportion of familial contacts excreted virus during the second week, and the highest proportion of

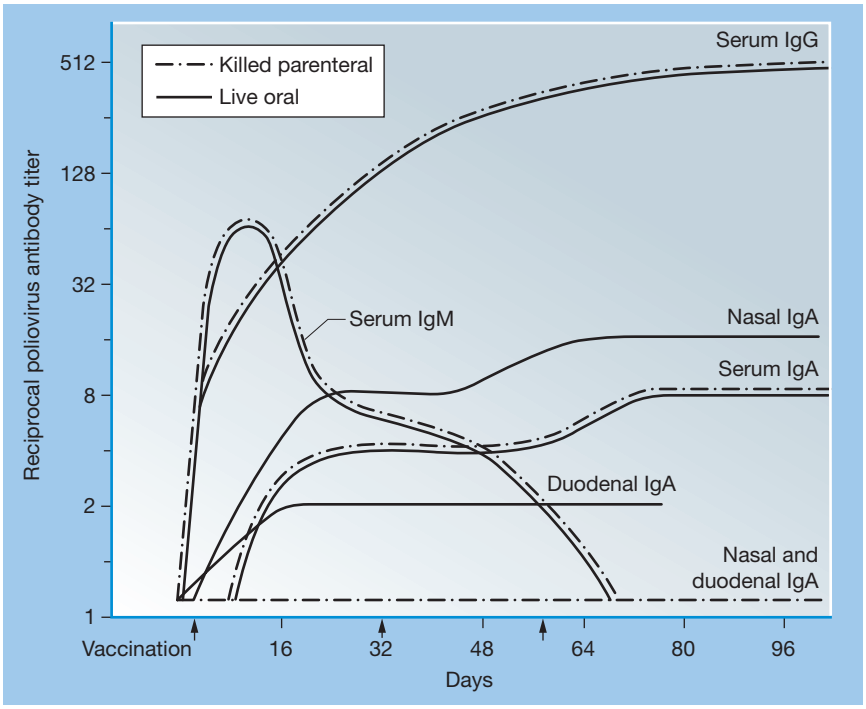


Figure 49.7. Serum and secretory antibody responses to orally administered, live attenuated oral poliovirus vaccine (OPV) and to intramuscularly administered inactivated poliovirus vaccine (IPV). Ig, immunoglobulin. *(From Ogra PL, Fishaut M, Gallagher MR. Viral vaccination via the mucosal routes.* Rev Infect Dis. *1980;2:352–369.)*

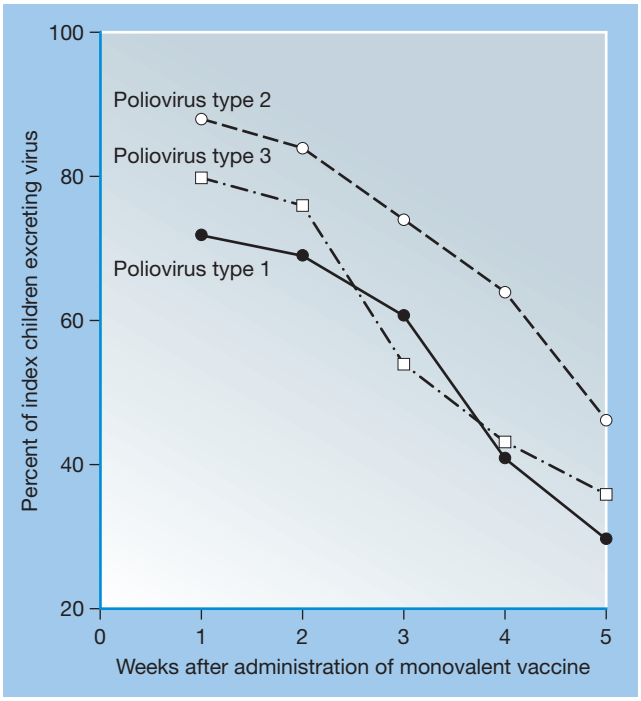


Figure 49.8. Weekly excretion rates of homotypic virus by index children after administration of monovalent poliovaccines. *(From Benyesh-Melnick M, Melnick JL, Rawls WE, et al. Studies on the immunogenicity, communicability, and genetic stability of oral poliovaccine administered during the winter.* Am J Epidemiol. *1967; 86:112–136.)*

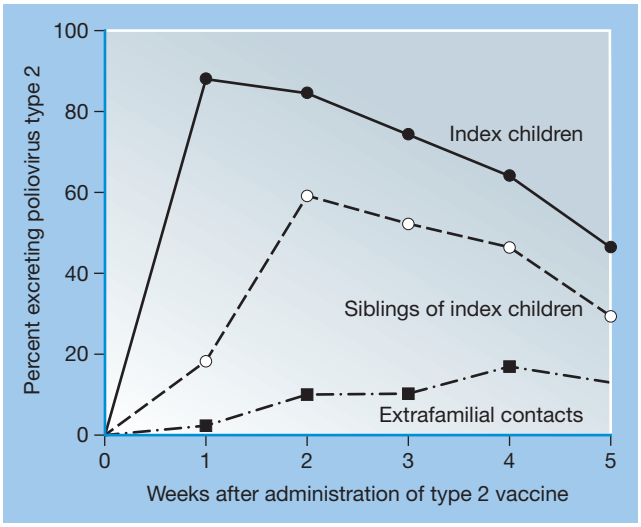


Figure 49.9. Weekly excretion rates of homotypic virus following administration of type 2 monovalent vaccine for index children, their siblings younger than 5 years of age, and extrafamilial contacts. *(From Benyesh-Melnick M, Melnick JL, Rawls WE, et al. Studies on the immunogenicity, communicability, and genetic stability of oral poliovaccine administered during the winter.* Am J Epidemiol. *1967;86:112–136.)*

nonfamilial contacts excreted virus during the fourth week (Fig. 49.9).

In industrialized countries, after complete primary vaccination with three doses of OPV, 95% or more of recipients seroconvert and develop long-lasting immunity to all three poliovirus serotypes. In a trial in the United States, 39% of vaccinees seroconverted to poliovirus type 1, 84% to poliovirus type 2, and 71% to poliovirus type 3 after a single dose of OPV.[461] After receipt of two doses of OPV, seroprevalence was 92% to poliovirus type 1, 100% to poliovirus type 2, and 96% to poliovirus type 3; and after three doses of OPV, 97% had antibodies to poliovirus type 1, 100% to poliovirus type 2, and 100% to poliovirus type 3.[461] This study used a formulation of at least $10^{5.5}$, $10^{4.5}$, and $10^{5.2}$ $TCID_{50}$ of types 1, 2, and 3, respectively, of tOPV vaccine produced in the United States and confirmed earlier studies conducted between 1959 and 1962, which administered OPV to infants and children who were initially seronegative. These earlier studies reported that after two doses, the proportion of recipients seroconverting was 90% to 93% for type 1, 99% to 100% for type 2, and 76% to 98% for type 3; after a third dose, 100% converted to types 1 and 2, and 87% to 100% seroconverted to type 3.[316,334–336,462]

However, OPV seems to be considerably less immunogenic in developing countries. A comprehensive review of the immunogenicity of OPV in developing countries reported that a weighted average of only 73% (range: 36% to 99%), 90% (range: 77% to 100%), and 70% (range: 40% to 99%) of children participating in these studies have detectable antibody to poliovirus types 1, 2, and 3, respectively, after three OPV doses (Table 49.11).[254] Results of controlled trials conducted after the review[254] have confirmed the lower immunogenicity of OPV, particularly to poliovirus type 3, in developing countries.[463–465] A number of hypotheses attempt to explain the differences in OPV performance between industrialized and developing countries. These include interference from concurrent infections with other enteroviruses or diarrhea, both of which are more prevalent in developing countries.[463–466] Recently, two hypotheses for the low immunogenicity were tested and could not be confirmed: (a) zinc deficiency in Pakistan,[467] and (b) insufficient buffering to counter stomach acidity in Bangladesh.[468] Furthermore, another study in Pakistan demonstrated that a dose of IPV is more efficient than a dose of OPV in closing the immunogenicity gaps among chronically malnourished infants.[469] In addition, there may be nonspecific factors not yet known; and colostrum contains secretory IgA, which can influence seroconversion.[470–472] Children with higher levels of maternal antibody have lower seroconversion rates. Maternally derived antibodies are higher in newborns in developing countries compared with newborns in the industrialized world,[461,463] and OPV is administered at an earlier age (birth, 6, 10, and 14 weeks) when the influence of maternally derived antibody on vaccine virus replication and, hence, seroconversion is more distinct.[254] Increasing the potency of OPV can correct some of these limitations of the currently formulated OPV[465,473]; this also can be achieved by administering additional doses of OPV in the routine program or through mass campaigns.[122]

mOPV or bOPV can overcome some of the limitations of tOPV, including the interference among the three Sabin strains. Because the type 2 component of the tOPV is considerably more immunogenic than the other components, vaccinees usually seroconvert to type 2 first (because type 2 interferes with seroconversion to types 1 and 3). In contrast, with monovalent vaccines, a type-specific immune response can be targeted according to programmatic needs. mOPV is more immunogenic than tOPV in inducing a type-specific immune response in industrialized and developing countries. A comprehensive review of experiences with mOPV demonstrated that following a single dose, median seroconversion rates in industrialized countries of 95% (range: 90% to 100%) to poliovirus type 1, 98% (range: 83% to 100%) to poliovirus

TABLE 49.11 Seroconversion/Seroprevalence After Receipt of Three Doses of Oral Poliovirus Vaccine in the Developing World

Country	Percentage With Neutralizing Antibody to Indicated Serotype			Age at First OPV Dose (mo)	Interval Between Doses (mo)	No. Studied	Lowest Dilution Tested[a]	Vaccine Formulation[b]
	Type 1	Type 2	Type 3					
Thailand	69	95	69	3	1.5	92	1:4	2:2:2
Thailand	90	100	100	3	1.5	82	1:4	5:1:2
Mexico	75	93	59	6	1	75	NA	3:3:3
India	61	77	40	3	1	74	1:10	4:4:4
South Africa	85	96	90	2	1.5	956	1:10	4:2:2
Iran	77	77	60	2	1	354	1:10	5:1:3
Iran	91	92	77	2	1	595	1:10	5:1:3
South Africa	86	96	75	0	1.5	176	1:8	6:2:3
China	99	98	99	0	3	100	1:4	10:1:3
Sri Lanka	97	98	98	3	2	65	1:8	10:1:3
Israel	95	98	93	2	1.5	121	1:10	10:1:3
Kenya	92	98	90	2	2	45	1:8	10:1:3
Brazil	91	98	94	2	2	75	1:5	10:1:3
South Africa	87	95	90	3	1	77	1:10	10:1:3
India	86	84	62	3	1.5	158	1:4	10:1:3
Mali	82	90	76	2	2	118	1:8	10:1:3
The Gambia	81	95	56	2	1	182	1:10	10:1:3
Brazil	80	95	56	2	>2	161	1:8	10:1:3
India	73	87	63	0	1	139	1:8	10:1:3
India	72	88	79	1.5	1	86	1:8	10:1:3
Sri Lanka	69	91	78	2	2	68	1:4	10:1:3
India	66	95	72	1.5	1	61	1:8	10:1:3
Kenya	63	92	60	6	2	65	1:8	10:1:3
India	63	75	68	2	1	71	1:10	10:1:3
India	58	92	83	3	1	78	1:10	10:1:3
Brazil	55	71	65	3	1.5	30	1:8	10:1:3
Nigeria	48	92	52	2	1.5	56	1:8	10:1:3
India	47	75	59	3	1.5	50	1:16	10:1:3
Kenya	44	77	60	6	2	31	1:8	10:1:3
India	40	75	51	2	2	87	1:8	10:1:3
Ghana	36	73	61	3	6	75	1:8	10:1:3
Morocco	89	95	74	2	1	122	1:10	20:2:13
Weighted average	73	90	70					

[a]Lowest antibody dilution tested.
[b]The ratio of potencies of type 1 to type 2 to type 3.
Adapted from Patriarca PA, Wright PF, John TF. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. Rev Infect Dis. *1991;13:926–939.*

type 2, and 94% (range: 70% to 100%) to poliovirus type 3 were reported. In developing countries, the median seroconversion rates following a single dose of mOPV were lower, 81% (range: 53% to 89%) to poliovirus type 1, 89% (range: 77% to 93%) to poliovirus type 2, and 72% (range: 52% to 80%) to poliovirus type 3.[391] Considerable variation in immunogenicity of OPV has been documented in developing tropical countries, ranging from poor (e.g., Northern India) to excellent (e.g., Indonesia) immunogenicity.[474,475]

Monovalent OPV became the vaccine of choice for supplemental immunization campaigns between 2005 and 2009 in polio-endemic or polio-epidemic areas, including in previously polio-free countries with recent importation of wild poliovirus. The vast majority of these vaccines are formulated as mOPV1 corresponding to the programmatic need to control wild poliovirus type 1. A total of more than 1 billion doses of mOPV1 were administered during this period. A case-control study has reported that the efficacy of mOPV1 was

approximately threefold higher than that of the type 1 component of tOPV.[474] Massive use of mOPV1 resulted in the virtual elimination of poliovirus type 1 in the most difficult to control districts in western Uttar Pradesh, India. The mOPV3 has been used selectively in areas with imported or endemic circulation of poliovirus type 3. Finally, type 2 immunogenicity is high in tOPV,[476] but mOPV2 may be needed for potential applications in controlling an outbreak of type 2 cVDPV before and after cessation of OPV. A clinical trial in India confirmed that the immunogenicity of bOPV was superior to types 1 and 3 compared with tOPV, and not inferior to the respective mOPVs. The cumulative two-dose seroconversion for poliovirus type 1 was 90% for mOPV1 and 86% for bOPV compared with 63% with tOPV; for type 3 poliovirus, it was 84% for mOPV3 and 74% for bOPV compared with 52% for tOPV.[476] In 2010, more than a decade since wild poliovirus type 2 was last detected,[477] bOPV (types 1 and 3) became the vaccine of choice to eradicate the remaining chains of poliovirus transmission.[478] A recent study with bOPV confirmed these findings and furthermore reported similar immunogenicity whether the doses were given at shorter intervals (2 weeks) compared to standard intervals.[479] The main advantage of bOPV is that it enhances individual and population immunity simultaneously for poliovirus types 1 and 3, without any serious loss in immunogenicity compared with the mOPVs. Fig. 49.10 highlights the countries that have used bOPV between 2009 and 2014. Between 2009 and 2014, more than 5 billion doses of bOPV were used in campaigns by the GPEI.

Mucosal Immunity

Intestinal mucosal immunity, primarily mediated by locally produced secretory IgA after live poliovirus exposure, is measured primarily by resistance to poliovirus replication and excretion in the pharynx and intestine after challenge with mOPV or tOPV[335,336] or by measuring directly the IgA in stool or pharyngeal specimens.[480,481] After challenge, lower titers of virus are excreted for significantly shorter periods among vaccinees compared with unvaccinated children (Table 49.12).[482] Secretory IgA can be measured directly in stool, saliva, and

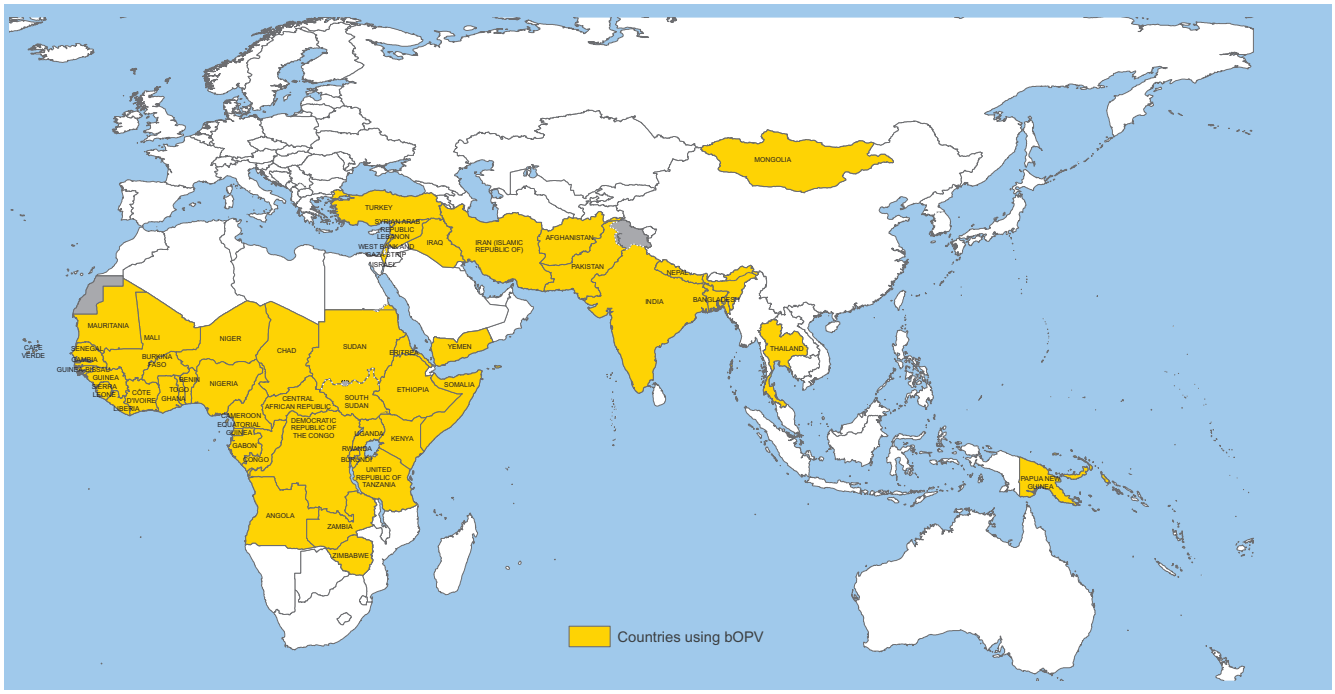


Figure 49.10. Countries using bivalent types 1 and 3 oral poliovirus vaccine (bOPV) between 2009 and 2014. Yellow-shaded countries used bOPV in supplemental immunization activities.

TABLE 49.12 Intestinal Immunity in Vaccinated (OPV or IPV) and Naturally Immune and Susceptible Children

Study Group	Proportion Excreting	Mean Duration of Excretion (d)	Mean Titer of Virus Excreted (log $TCID_{50}$)	Excretion Index (million)[a]	Reference
Susceptible control subjects	0.80	20.4	5.15	2.305	95
IPV vaccinated	0.74	12.3	4.11	0.1173	99
OPV vaccinated	0.37	4.6	2.18	0.00022	99
Naturally immune	0.37	5.4	2.03	0.00022	99

[a]Excretion index is the proportion of children excreting challenge type 1 virus times the mean duration of excretion days times the titer of virus excreted.
IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; $TCID_{50}$, median tissue culture infective dose.
Data from Fine PE, Carneiro IA. Transmissibility and persistence of oral polio vaccine viruses: implications for the global poliomyelitis initiative. Am J Epidemiol. *1999;150:1001–1021; Global Programme for Vaccines and Immunization.* Report of the Second Meeting of the Global Commission for the Certification of the Eradication of Poliomyelitis, Geneva, 1 May 1997. *Geneva, Switzerland: World Health Organization; 1998; and Ghendon YU, Sanakoyeva II. Comparison of resistance of the intestinal tract to poliomyelitis vaccine (Sabin's strains) in persons after naturally and experimentally acquired immunity.* Acta Virol. *1961;5:265–273.*

breast milk to assess the degree of mucosal immunity. These methods are difficult to perform, require tedious standardization, and are rarely used. Mucosal immunity may exist even when levels of serum antibody are negligible,[337,483] although the degree of mucosal immunity seems most closely correlated with the titer of homologous humoral antibody[484]: the lower the titer, the more likely excretion of challenge virus can be demonstrated. One study reported that mucosal immunity may be strain-specific and that mucosal immunity induced by one vaccine poliovirus strain may not induce mucosal immunity against another strain.[485] Intestinal mucosal immunity induced by IPV is less effective against infection than that induced by OPV, as measured by the proportion of vaccinees excreting virus or the duration of excretion. However, the clinical importance of these differences in reducing wild virus spread in highly immunized populations in industrialized countries is not clear because IPV seems to reduce shedding compared with no vaccine and pharyngeal spread may be important in industrialized countries, and IPV induces pharyngeal immunity.[486,487] IPV and OPV induce equivalent pharyngeal mucosal immunity based on studies in industrialized countries that rely on vaccine virus challenge to assess mucosal immunity.[488] In contrast, in developing countries with inadequate hygiene and great potential for fecal–oral spread of enteric viruses, the clear increase in mucosal (intestinal) immunity induced by OPV over IPV would seem to offer a major advantage to OPV in reducing the circulation of polioviruses. A recent study in India indicated that IPV compared to OPV can more effectively boost mucosal immunity in infants and children with a history of multiple doses of OPV.[489] Secretory IgA has an important role in defense against poliovirus infections,[235,488,490] and all available evidence indicates that the immune response after vaccination with OPV (or boosted in previously OPV-vaccinated infants by IPV) is similar to that after infection with wild poliovirus.

Few studies have provided data on the persistence of mucosal immunity. No data are available from developing countries about the duration of mucosal immunity for polioviruses. Several studies have assessed resistance to oral challenge by vaccine viruses years after the initial administration of OPV. One study reported that children were completely resistant to intestinal infection 10 years after vaccination, unless prechallenge serum antibodies were 1:8 or lower.[484] Another study reported similar findings on the relationship of humoral antibody and resistance to excretion.[491] A study in India reported that healthy contacts of poliomyelitis cases, despite multiple doses of previous OPV, may still be infected and excrete wild-type poliovirus, albeit in relatively low frequency (<1%).[481] If exposure is known, such as in vaccine trials, as many as 50% of 10-year old children may excrete poliovirus after bOPV challenge.[489] In addition, another study from India suggests that intestinal mucosal immunity is relatively short-lived (as low as ~6 months).[492] No data are available on the long-term persistence of secretory IgA for polioviruses. Recently, a study by Wright et al demonstrated a correlation between neutralizing antibody in stool and excretion of poliovirus following challenge.[492a] This offers a potential avenue for assessing mucosal immunity without reliance on challenge poliovirus. However, looking at the mucosal immunity induced by another enterovirus, echovirus type 6, might provide insights. A study assessing the falloff in secretory IgA titer to echovirus type 6 in the pharynx and intestine reported no declines during a 4-year follow-up period,[493] although the possibility of boosting with echovirus 6 or other enteroviruses in the follow-up period cannot be excluded.

When poliovirus is ingested, the virus has contact with proteolytic enzymes such as trypsin, which may alter viral antigens.[490] Secretory and humoral antibody responses after OPV include those against the new antigens associated with trypsin-cleaved virus. Such antigens are not accessible in IPV, and, consequently, the immune response after IPV is more limited.[494]

Evidence of Oral Polio Vaccine Effectiveness

A large body of empiric and scientific evidence has accumulated since the late 1950s that demonstrates the effectiveness of OPV in preventing paralytic disease. The use of OPV was pioneered in the former Soviet Union,[495–497] and the approaches developed in the Soviet Union led to rapid control or elimination of poliomyelitis in many countries. The most prominent example of the effectiveness of OPV is the success of the GPEI,[39] including the Western Hemisphere, and the WHO's Western Pacific, European, and South-East Asian Regions, which were certified free of wild poliovirus by Regional Certification Commissions in 1994, 2000, 2002, and 2014, respectively.[498–501]

OPV has curtailed epidemics and has greatly reduced the incidence of poliomyelitis, often eliminating the pattern of expected seasonal increase in poliomyelitis cases.[49,267,271,418,497,502–510] Two vaccine effectiveness studies were conducted in the 1990s.[255,511] The study in Oman estimated that the effectiveness of three doses of OPV in preventing paralytic disease was approximately 90%.[255] Much of the earlier evidence of small and large trials is contained in meeting reports.[335,336]

The ability of OPV to infect contacts of vaccine recipients (i.e., "contact spread") and "indirectly vaccinate" these contacts against poliomyelitis is considered by many to be another advantage of OPV compared with IPV. OPV vaccine virus spread has been demonstrated by prospective virologic studies and by serologic studies in industrialized and developing countries.[460,464,512–515] Serologic surveys have shown that the proportion of people who have antibodies is considerably greater than would be expected by vaccination or by the circulation of wild polioviruses. After OPV introduction in Yaoundé, Cameroon, the incidence of paralytic poliomyelitis decreased by 85%, although only 35% of children 12 to 13 months old received three doses of OPV.[271] Among infants who received IPV in Oman, the seroconversion rates were significantly higher among infants whose study period coincided with a mass OPV campaign that was conducted elsewhere in the country (Fig. 49.11).[464] A serologic survey among unvaccinated inner-city children in the United States also demonstrated that a substantial proportion of the children are exposed secondarily to vaccine viruses (Fig. 49.12).[514] In a study conducted in the United Kingdom, infants received a dose of IPV at 2 months of age. In the ensuing 1 month period and before any OPV was administered, 11% of infants excreted poliovirus type 1 and 4% excreted poliovirus type 2 in stool specimens.[515] These data indicate that vaccine virus spreads easily from OPV recipients to contacts in industrialized and in developing countries.

Correlate of Protection

A comprehensive review of the correlate of protection against poliovirus was published in 1995.[22] Briefly, the data suggest that any titer of homologous neutralizing antibody is protective against paralytic consequences of poliomyelitis. Furthermore, a large body of data suggests that immunity, once induced by wild poliovirus or the vaccine virus, is lifelong in immunocompetent individuals regardless of waning of circulating antibody.

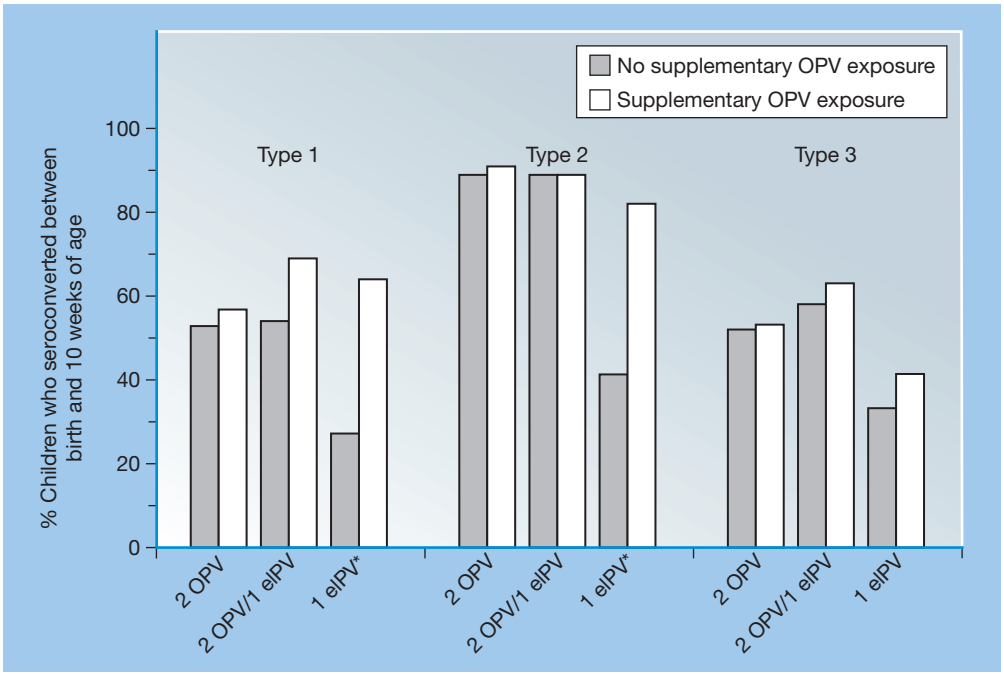


Figure 49.11. Seroconversion to poliovirus types 1, 2, and 3 between birth and 10 weeks of age among children not exposed compared with children exposed secondarily to oral polio vaccine (OPV) mass campaigns, by vaccine group, Oman. eIPV, enhanced-potency inactivated polio vaccine. *$P < .05$. *(From World Health Organization Collaborative Study Group on Oral and Inactivated Poliovirus Vaccines. Combined immunization of infants with oral and inactivated poliovirus vaccines: results of a randomized trial in The Gambia, Oman, and Thailand.* J Infect Dis. *1997;175[Suppl 1]:S215–S227.)*

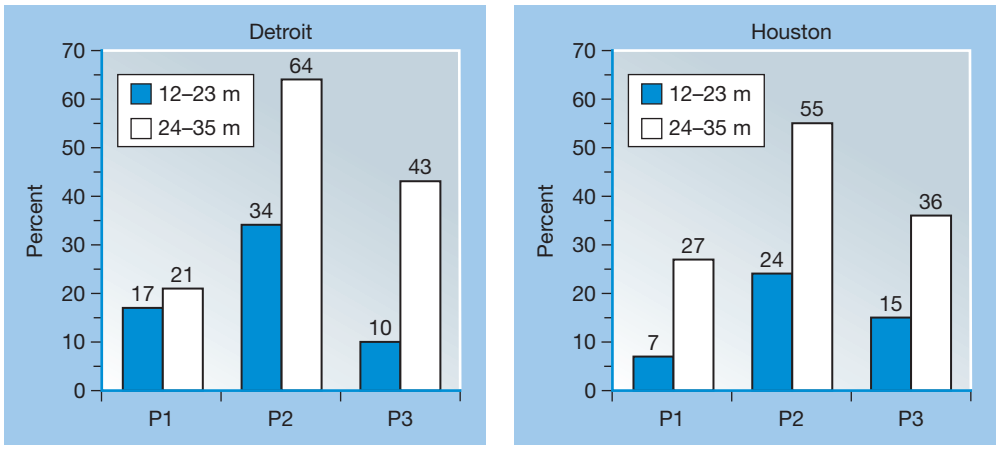


Figure 49.12. Poliovirus antibody seroprevalence among unvaccinated inner-city preschool children, by age groups, Detroit and Houston, 1990–1991. P1, poliovirus type 1; P2, poliovirus type 2; P3, poliovirus type 3; 12–23 m, 12 to 23 months of age; 24–35 m, 24 to 35 months of age. *(Data from Chen RT, Hausinger S, Dajani AS, et al. Seroprevalence of antibody against poliovirus in inner-city preschool children: implications for vaccination policy in the United States.* JAMA. *1996;275:1639–1645.)*

Duration of Immunity

The immune response following vaccination or infection with wild poliovirus is similar, and thus the data on persistence of immunity following wild poliovirus infection can be generalized to vaccine viruses, and support that the related immunity is lifelong. In an isolated Eskimo population, immunity induced by wild poliovirus was shown to have persisted for at least 40 years in the absence of any further exposure during the intervening period.[187] The best evidence of the persistence of vaccine-induced immunity is the absence of disease in adolescents and adults who had been vaccinated previously with OPV and the persistence of type-specific antibody assessed in population-based surveys.[516,517] However, interpretation of these data is difficult because of potential repeated exposures to shed virus. In prospective studies in which the same vaccinees were observed for several years, antibodies to types 1 and 2 were found in more than 90% of children and to type 3 in 83% to 95%.[28,518–523] Data from population-based studies of antibody seroprevalence conducted among Army recruits in the United States in 1989 (>95% to poliovirus types 1 and 2 and >85% to poliovirus type 3),[517] among school-age children in Massachusetts in 1981 (>99% to poliovirus type 1, >99% to poliovirus type 2, and >99% to poliovirus type 3),[516] and in The Gambia (88.1% to poliovirus type 1 and 89.3% to poliovirus type 3 among 3- to 4-year-old children)[524]

demonstrate that poliovirus antibodies induced by OPV persist for many years. Similar data are available from Sri Lanka where the prevalence of detectable antibody reached a peak in 9- to 11-month-old infants (≥95% to all three serotypes), remained stable at this level for serotypes 1 and 3, but declined to ~75% for serotype 3.[525]

Of interest, the median titer against poliovirus type 3 was substantially lower compared with those against types 1 and 2 among 9- to 11-month-old infants.[525]

Adverse Events

Vaccine-Associated Paralytic Poliomyelitis

In the early 1990s, the Institute of Medicine reviewed adverse events associated with childhood vaccines, including poliovirus vaccines.[526] The major adverse event associated with OPV is VAPP. Shortly after licensure and widespread use of mOPV, cases with paralytic manifestations followed vaccination with monovalent type 3 vaccines. These cases were considered clinically consistent with poliomyelitis and were supported by laboratory findings that did not exclude a possible causal relationship to the administration of oral vaccine. The report by the Surgeon General describes the earliest cases of VAPP.[527]

In 1969, the WHO coordinated a collaborative study to obtain data on the potential risks associated with OPV use. The findings of the first 5- and 10-year follow-up studies were published.[528,529] During the 5-year period from 1980 to 1984, 395 cases of acute persisting spinal paralysis were reported from 13 countries with a total population of 547 million.[530] The risk of VAPP (in recipients or contacts of recipients) was less than 0.3 cases per million doses of OPV distributed (or <1 case per 3.3 million doses), and the average annual incidence of VAPP was 0.14 per 1 million people (range: 0.0–0.33), excluding Romania. Romania reported an average annual incidence of 2.7 per 1 million people.

Although some have challenged the existence of VAPP,[28,531,532] believing that the cases of paralysis have different etiologic factors, the following evidence supports vaccine viruses as causative:

- Clinical syndromes are typical of poliomyelitis.
- Vaccine virus is frequently isolated from cases.
- History of exposure to vaccine is often obtained.
- Recipient and contact cases cluster after receipt of the first dose of OPV. (One would expect virtually equal numbers of cases after each dose if there were other etiologic agents causing the illnesses.)
- Shed viruses have mutated toward neurovirulence.
- The incidence of VAPP is highest in immunodeficient people with B-cell deficiencies, a group that is also at higher risk of poliomyelitis from wild poliovirus.[533] The completeness of reporting of VAPP cases to the Centers for Disease Control and Prevention (CDC) was estimated to be 81%.[534]

Between 1990 and 2003, a total of 61 cases classified as VAPP were reported in the United States, including 27 (44%) among immunologically normal vaccine recipients, 10 (16%) among immunologically normal contacts of vaccine recipients, six (10%) among immunologically normal nonhousehold contacts, 16 (26%) among immunologically compromised OPV recipients or contacts of OPV recipients, one indeterminate case (2%), and one imported case (2%).[534,535] Fig. 49.13 shows VAPP by year from 1964 to 2003; no cases occurred after the United States implemented an all-IPV policy in 2000. Since then, no VAPP has been reported in the United States, with two exceptions: In 2005, the first case of imported VAPP in an immunologically compromised person was reported.

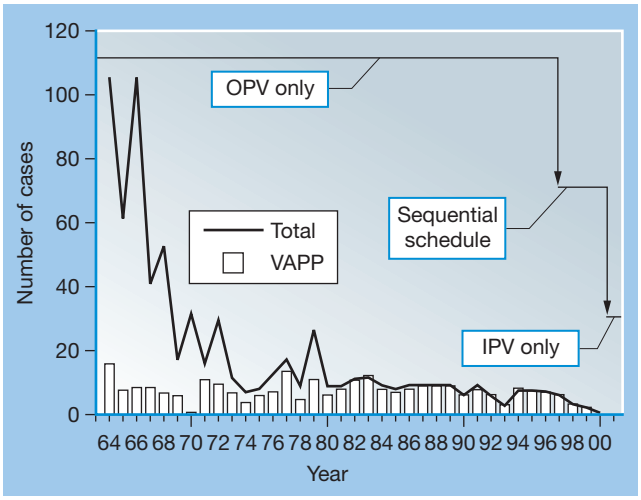


Figure 49.13. Total reported paralytic poliomyelitis cases and vaccine-associated paralytic poliomyelitis (VAPP), United States, 1964–2000. IPV, inactivated polio vaccine; OPV, oral polio vaccine. *(From Centers for Disease Control and Prevention.)*

TABLE 49.13 Ratio of Number of Cases of Vaccine-Associated Paralytic Poliomyelitis per Million Doses of Oral Poliovirus Vaccine Distributed, United States, 1980–1999

Case Category	Ratio of Number of Cases per Million Doses of OPV Distributed: Overall	First Dose	Subsequent Doses	Relative Risk[a]
Recipient	1:6.4	1:1.4	1:35.4	25.3
Contact	1:13.3	1:4.5	1:23.6	5.2
Community-acquired	1:57.7	NA	1:47.2	NA
Immunologically abnormal	1:10.8	1:6.3	1:12.9	2.0
Total[b]	1:2.9	1:0.9	1:5.9	6.6

[a]First dose ratio to subsequent dose ratio.
[b]Includes normal and immunologically abnormal cases.
NA, not available; OPV, oral polio vaccine.
Adapted from Alexander LN, Seward JF, Santibanez TA, et al. Vaccine policy changes and epidemiology of poliomyelitis in the United States. JAMA. 2004;292:1696–1701.

The person had never been vaccinated because of a religious exemption and acquired the poliovirus infection during travel to an OPV-using country in Central or South America.[536] The second case occurred in 2009 in an adult with longstanding common variable immunodeficiency whose infection probably occurred through household contact with her child who had received OPV 12 years earlier.[90]

For the period 1990 to 1999, the risk of VAPP in the United States was estimated as one case per 2.9 million doses of OPV distributed; for children receiving the first doses of OPV, the risk was estimated as one case per 1.4 million children vaccinated (Table 49.13).[535,537] The risk of VAPP is highest after the first dose of OPV. Recipients of a first dose and their contacts had a 6.6-fold higher risk of VAPP than did recipients of subsequent doses and their contacts. People with immunodeficiency disorders are at highest risk for VAPP. The risk of VAPP among immunocompromised people is elevated to more than 3200 times the risk for immunocompetent people.[538] Almost

all cases occurred in people with congenital or acquired immunodeficiency. Immunodeficient people with VAPP primarily had abnormalities affecting the B-cell system (humoral immunity), with agammaglobulinemia or hypogammaglobulinemia most frequently associated with VAPP.[538] Except for one VAPP case with immunodeficiency disorder, in all other cases, the precipitating event for the diagnosis of immunodeficiency was the onset of paralytic disease. Poliovirus type 3 is the virus most frequently isolated from immunocompetent people with VAPP. In contrast, poliovirus type 2 is the most common virus detected in immunodeficient people with VAPP. Poliovirus type 1 is rarely isolated from cases with VAPP.[268]

The epidemiology of VAPP was summarized in the United States for the 1990 to 2003 period. During this period, three different vaccination schedules were in use: (a) almost exclusive use of OPV for primary prevention of poliomyelitis, 1990–96; (b) use of a sequential schedule of first IPV then OPV, 1997–99; and (c) the introduction of an exclusive IPV schedule in 2000. Overall, the VAPP estimates for the first period were consistent with earlier estimates (1 case per 2.9 million doses distributed). Although 13 VAPP cases occurred during the 1997–99 period, none occurred in recipients of the sequential IPV-OPV schedule. Finally, as expected, no VAPP cases were reported following the introduction of an all-IPV schedule, resulting in the final step toward polio control, the elimination of VAPP.[535]

Romania and Hungary have consistently reported higher rates of VAPP than other countries with well-developed surveillance systems. Until recently, Romania and Hungary used tOPV and mOPV, respectively, solely in campaigns.[288,409] The high risk of VAPP in Hungary was associated primarily with the administration of mOPV3.[409] The high risk of VAPP in Romania can be attributed to provocation poliomyelitis (i.e., multiple intramuscular injections in the 30 days before paralytic manifestations).[539] People with VAPP in Romania had received, on average, 16.8 intramuscular injections, primarily for antibiotics, in the 30 days before onset of paralysis. Analysis of VAPP cases in the United States between 1980 and 1993 suggested that cases with a history of intramuscular injections received an average of only 1.5 intramuscular injections in the 45 days before onset of paralysis; no clustering of injections during the 45-day period was observed.[540] In contrast, in Romania, most injections were received in the periods from 0 to 7 days and from 8 to 14 days before onset. It seems unlikely that intramuscular injections contribute substantially to the VAPP burden in the United States. The risk of VAPP in the Americas, where Latin America administered large quantities of OPV in mass campaigns (NIDs) to eradicate poliomyelitis, was similar to the VAPP risk reported in the United States and other countries.[541] An analysis in India further refined the risk estimates for one large developing country and demonstrated that the risk of VAPP is substantially lower in this country despite large quantities of OPV administered in mass vaccination campaigns and through routine immunization programs (>700 million doses of OPV in 1999 alone).[542] The risk among first-dose recipients was estimated as one case per 2.8 million children (compared with a first-dose recipient risk of one case per 1.4 million children in the United States). The lower risk estimates in India may be explained in part by the following: (a) The proportion with and the titers of maternally derived antibody are high among newborns because wild poliovirus circulated widely until recently and may have led to frequent virus exposure and boosting of titers in the general population. (b) A birth dose of OPV is recommended for institutional births (thus inducing immunity when the newborn is still under protection against poliomyelitis from maternally derived antibody). (c) The routine vaccination schedule calls for OPV doses at 6, 10, and 14 weeks of age (again inducing immunity at earlier ages than in industrialized countries). Thus, high levels of maternal antibody and OPV vaccination at an age when maternal antibody would be expected to protect against VAPP seem to be the main reasons why the observed risk of VAPP in India (and probably in other countries under similar circumstances) seems to be lower than that in industrialized countries.[542]

The global burden of VAPP has been estimated by the WHO as between 250 and 500 cases globally each year, based on a total incidence of two to four cases per million birth cohort.[543] A recent review of VAPP updates the epidemiologic information and provides more current the risk estimates.[365] The review reported that the majority of recipient VAPP cases were associated with type 3 poliovirus (42%), followed by type 2 (26%), type 1 (20%), and mixtures of more than one virus (15%). Immunodeficient children are subject to infection, frequently fatal, by a wide variety of normally benign or avirulent agents. Nonpolio enteroviruses may cause serious or fatal illnesses in immunocompromised people.[544,545] A prominent feature of such infections is the patient's difficulty or inability to eradicate the virus from the CNS; some patients continue to yield virus from cerebrospinal fluid for up to 3 years.[546] As of the end of 2015, 94 persons with immunodeficiency disorders have been shown to excrete poliovirus for prolonged periods of approximately 6 months or longer (see "General Epidemiology" earlier) since the early 1960s. Two cases in this series deserve special attention. A patient with VAPP in the United States may have excreted virus for approximately 7 years before the onset of paralytic disease in 1981,[198,199] and a person from the United Kingdom who does not have paralytic disease has excreted poliovirus for more than 25 years (as of 2015).[200] In immunodeficient persons, poliovirus infection, by a wild virus or a vaccine strain, may develop in an atypical manner, with an incubation period longer than 28 days, a high mortality rate after a long chronic illness, and unusual lesions in the CNS.[22,321,538,547]

Vaccine-Derived Polioviruses

In principle, all clinical and environmental vaccine-related poliovirus isolates are VDPVs. However, for the purposes of poliovirus surveillance vaccine-related isolates have been classified into two broad categories: (a) OPV-like isolates, which have limited divergence from their parental OPV strains and are ubiquitous wherever OPV is used, and (b) VDPV isolates, whose higher level of VP1 sequence divergence from their parental OPV strains (>1% [types 1 and 3] or >0.6% [type 2]) indicates prolonged replication (or transmission) of the vaccine virus. VDPVs are identified by the laboratories of the GPLN and reported for further epidemiologic and clinical investigations and classification into either of two well-defined categories: (a) cVDPVs and (b) iVDPVs. If the VDPVs cannot be definitively categorized, they are classified as ambiguous VDPVs (aVDPVs).[548]

Although the vast majority of vaccine-related isolates are OPV-like, VDPVs are of particular interest because of their implications for current and future strategies for global polio eradication.[251,450,548–550] VDPVs can cause paralytic polio in humans and have the potential for sustained circulation. The clinical signs and severity of paralysis associated with VDPV and wild poliovirus infections are indistinguishable. VDPVs resemble wild polioviruses phenotypically[450] and differ from the majority of vaccine-related poliovirus isolates by having genetic properties consistent with prolonged replication or transmission. Because poliovirus genomes evolve at a rate of approximately 1% per year (see Table 49.5), vaccine-related viruses that differ from the corresponding OPV strain by more

than 1% of VP1 nucleotide positions are estimated to have replicated for at least 1 year in one or more persons after administration of an OPV dose. This is substantially longer than the normal period of 4 to 6 weeks of vaccine virus replication in an OPV recipient.[127] The demarcation for type 2 VDPVs was lowered to 0.6% to increase sensitivity for early detection of type 2 cVDPV outbreaks.[133,136,551]

It seems likely that many OPV-like isolates have recovered the capacity for higher neurovirulence and possibly increased transmissibility. The small number of substitutions controlling neurovirulence (see Fig. 49.6) was found to have reverted among many OPV-like isolates, especially among isolates of types 2 and 3.[351,358,373] Because the critical attenuating mutations in the IRES of the Sabin strains also affect fitness for virus replication in the human intestine,[373] it appears possible that revertants at these sites would have a higher fitness for person-to-person spread. However, spread is normally limited by high OPV coverage, and the VDPVs represent viruses whose potentials for prolonged replication or transmission have been clearly demonstrated.

The three categories of VDPVs differ in their public health importance. cVDPVs pose the same public health threat as wild polioviruses because they have recovered the biological properties of wild polioviruses, have the potential to circulate for years in settings where poliovirus vaccination coverage to prevent that particular type is low, and require the same control measures. iVDPVs may be excreted by persons with certain primary immunodeficiencies for many (>10) years with no apparent paralytic signs.[90,209] Persons infected with iVDPVs without paralysis are at risk of developing paralytic poliomyelitis[90,199,205] and may infect others with poliovirus, presenting the potential risk of outbreaks in areas with low poliovirus vaccine coverage.[310] aVDPVs are heterogeneous: some represent the initial isolates from cVDPV outbreaks, whereas others, such as highly divergent aVDPVs detected in sewage, are probably iVDPVs from unrecognized chronic infections.

Circulating Vaccine-Derived Polioviruses. Among the two well-defined categories of VDPVs, cVDPVs are of the greatest current public health concern.[450,550] Traditionally, the cVDPVs definition required evidence of transmission (i.e., more than one case). In 2015, the definition was refined to increase the sensitivity for initiating an outbreak response action in preparation for the switch from tOPV to bOPV (i.e., the removal of Sabin type 2 poliovirus from the OPV). In short, a VDPV case can now be classified as cVDPV outbreak if the molecular findings suggest 15 or more nucleotide changes in the VP1 region of the genome. All cVDPVs, implying that transmission is ongoing, require programmatic action for outbreak response. Since 2000, cVDPV outbreaks have occurred in 23 countries, with the large majority (87%) of reported cases associated with type 2 (Table 49.14). Type 1 cVDPVs were associated with outbreaks in seven countries with the largest number of cases (n = 46) reported from Indonesia in 2005.[66,305] In contrast, type 3 cVDPV outbreaks are rare, accounting for only 1.5% of known cVDPV cases, an unexpected finding because the type 3 OPV strain is a major contributor to VAPP in OPV recipients.[549] Because the case-to-infection ratio for poliovirus type 2 infections is low, the number of type 2 cVDPV infections worldwide since 2000 is estimated at approximately 1 million.[136,552]

During 2005–15, a polio outbreak of 417 cases associated with type 2 cVDPV was reported from 11 northern and three north-central states of Nigeria (see Fig. 49.13 and Table 49.14).[136,556,557] The outbreak peaked at 153 cases in 2009, but 11 indigenous cases were reported in 2014 and a type 2 cVDPV of the major lineage group[136] was detected in sewage in 2015.[548] Genetic analysis resolved the outbreak into more than 20 independent type 2 VDPV emergences that occurred during 2004–12, at least seven of which established circulating lineages.[136,557] The outbreak occurred in states where routine immunization coverage with tOPV was low and NIDs and Subnational Immunization Days were infrequent and often did not include type 2–containing vaccine.[556,557] Spread of the type 2 cVDPV from northern Nigeria has been limited, with only six cases in Niger from multiple importations and one case in Chad from an importation in 2010 (see Table 49.14). The cVDPV lineage originating from Chad was most recently detected in a contact (of a wild poliovirus type 1 case) in Borneo State, Nigeria, in August 2016. The limited spread may indicate that surrounding areas have higher levels of population immunity to poliovirus type 2 (because of the high immunogenicity of Sabin 2 and its higher tendency to infect OPV contacts).[299]

Since 2006, 96% of cVDPV cases have also been associated with type 2 cVDPVs (see Fig. 49.13), several of which were associated with multiple independent emergences (see Table 49.14).[133] Other type 2 cVDPVs have emerged successively in the same geographic area.[554] In several settings, this reflects the continued weakness in routine immunization with tOPV and the extensive use of mOPV1 and bOPV in mass campaigns, which probably allowed substantial accumulation of susceptible persons to type 2 in the population allowing the type 2 virus in children who received tOPV in routine immunization to spread and mutate. In addition, it also probably reflects the greater tendency of the Sabin 2 OPV strain to revert and spread to contacts.[549]

Key risk factors for cVDPV emergence and spread are: (a) development of immunity gaps arising from low OPV coverage, (b) prior elimination of the corresponding wild poliovirus serotype (except Indonesia[305]), (c) emphasis on use of mOPV and bOPV in NIDs and Subnational Immunization Days,[133,556] leading to increasing susceptibility to type 2 in the population, and (d) insensitive AFP surveillance. Many of these factors exist in areas of insecurity and where rates of routine tOPV coverage remain low, such as in parts of Afghanistan, the Central African Republic, northern Nigeria, Pakistan, Somalia, and Yemen. In this context, type 2 VDPVs present the greatest threat for emergence,[133] and routine immunization was strengthened and, up to April 2016 (see "Laboratory Containment of Polioviruses"), regular immunization campaigns with tOPV were conducted to close any type 2 population immunity gaps.

The large majority of cVDPVs shown in Table 49.14 have vaccine/nonvaccine recombinant genomes, which are rare among OPV-like and iVDPV isolates.[549] Recombination with other species-C enteroviruses frequently occurs during wild virus circulation, and may be interpreted as an indication of person-to-person transmission. Whether recombination facilitates cVDPV emergence is unclear, because type 1 cVDPVs from China had nonrecombinant genomes[553] and recombination continues after emergence, similar to what is observed with wild polioviruses.[136]

Immunodeficiency-Associated Vaccine-Derived Polioviruses. It has long been recognized that patients with primary immunodeficiencies (defects in antibody production) could become chronically infected when exposed to OPV.[561] Unambiguous demonstration that vaccine-related poliovirus isolates from immunodeficient patients had unusual sequence properties awaited the application of molecular tools, such as oligonucleotide fingerprinting[199] and genomic sequencing,[85,199,205] to poliovirus diagnostics. The extent of sequence divergence is correlated with the duration of the prolonged infection.[85,90,199,205,294] Not all isolates from immunodeficient patients are classified as iVDPVs. Some isolates are from

TABLE 49.14 Outbreaks of Circulating Vaccine-Derived Poliovirus, 1988–2015[a,450]

Country	Year	No. of Reported cVDPV Cases	Sero-Type	VP1 nt Divergence (%)	Estimated Independent Emergences	Routine Immunization Coverage With Three Doses of OPV (%)	Estimated Duration of Circulation
Egypt[69]	~1984–1993[b]	30	2	4.0–7.0	>1	Reported high	~10 yr
Dominican Republic[66]	2000–2001	13[c]	1	~1.9	1	<30 in most affected areas	>6 mo
Haiti[66]	2000–2001	8	1	~2.6	1	<30 nationwide	~2 yr
Philippines[309]	2001	3	1	3.1–3.5	1	OPV shortage over the preceding 2 y	~2 yr
Madagascar[308]	2001–2002	5	2	2.5–3.0	1	<50 nationwide	~2.5 yr
China[553]	2004	2	1	1.0–1.2	1	<50 around case	~1 yr
Madagascar[554]	2005	3	2	1.1–1.8	1	<50 nationwide	~1 yr
Indonesia[305]	2005	46	1	1.1 to ~3.0	1	<40 in Madura	~2 yr
Cambodia[555]	2005–2006	3	3	1.9–2.4	1	<50 around case	>1 yr
Nigeria[136,548,556–558]	2005–2015	417[d]	2	0.7–8.4	>20	<40 in northern states	~10 yr
D.R Congo[559,560]	2005–2012	64	2	0.7–3.5	>10	68 nationwide (low locally)	~5 yr
Myanmar[555]	2006–2007	5	1	1.5–2.2	1	82 nationwide	~2 yr
Niger[133,558]	2006–2013	7[e]	2	0.7–5.2	—	44	—
Ethiopia[559]	2008–2009	4	2	0.8–1.2	2	~60 in affected areas	~1 yr
Somalia[133,560]	2008–2013	19	2	0.7–4.0	4	26 nationwide	~4 yr
India[560]	2009–2010	17	2	1.0–1.6	5	~50 in Uttar Pradesh and Bihar	~1.5 yr
Ethiopia[560]	2009–2010	7	3	1.3–3.1	1	~60 in affected areas	~2.5 yr
Afghanistan[558,560]	2010–2013	25[f]	2	0.9–5.5	2	13 in south	~3 yr
Chad[560]	2010	1[g]	2	5.3	—	56	—
Mozambique[133]	2011	2	1	3.0–4.3	1	73	~4 yr
Yemen[133]	2011	9	2	0.7–1.6	4	89	~1.5 yr
Yemen[558]	2011–2013	4	3	2.0–3.0	1	89	~2.5 yr
China[558]	2011–2012	3	2	0.7–1.8	1	99	~1.5 yr
Chad[558]	2012–2013	16	2	0.7–2.1	2	56	~2 yr
Pakistan[548,558]	2012–2015	82	2	0.7–3.7	2	73	~3.5 yr
Cameroon[558]	2013	4[h]	2	1.2–2.0	—	85	—
Kenya[558]	2013–2014	3[i]	2	4.3–4.9	—	82	—
South Sudan[548]	2014	2	2	1.0	1	50	~1 yr
Madagascar[548]	2014–2015	9	1	2.2	1	73 (low locally)	~3 yr

[a]Data as of July 7, 2015 (http://polioeradication.org/wp-content/uploads/2016/12/WPV_2011-2016_29NOV.pdf).
[b]Start of outbreak estimated from the molecular clock.[69]
[c]cVDPV1 was imported from Haiti into the Dominican Republic.[66]
[d]Includes 22 cases in 2013 and 2014 associated with cVDPV2 imported from Chad.[548]
[e]Importations from Nigeria (2006, 2; 2009, 2; 2010, 1; 2011, 1; 2013, 1).[558,560]
[f]Includes 3 cases in 2012 and 1 case in 2013 associated with cVDPV2 imported from Pakistan.[548]
[g]Importation from Nigeria.[560]
[h]Importation from Chad.[558]
[i]Importation from Somalia.[558]
cVDPV, circulating vaccine-derived poliovirus; nt, nucleotide; OPV, oral polio vaccine.

specimens taken early in the prolonged infection and no subsequent specimens were taken. In other situations, either the prolonged infections had resolved spontaneously or the patient died from complications of the immunodeficiency (including fatal poliomyelitis).[562] Prolonged iVDPV infections are independent events, and the isolates obtained from such infection trace separate pathways of divergence from the original OPV strains.

Since the introduction of OPV in 1961 (data as of mid-2016), 94 persons with primary immunodeficiencies have been found worldwide to be excreting iVDPVs (indicating prolonged infections); the majority of these immunodeficiencies were detected only after onset of AFP.[548] After implementation of intensified surveillance for VDPVs and special studies of iVDPV excretion among persons with primary immune deficiency syndromes in developing and middle-income

countries,[563] detection of new iVDPV infections increased.[548] The extent of sequence divergence is correlated with the duration of the prolonged infection (≤5 years).[85,90,199,205,294] Four of the iVDPV isolates are highly divergent (>10% VP1 sequence divergence from the parental OPV strain), suggesting that the chronic poliovirus infections (>5 years) had persisted for approximately 10 years.[450] Patients with the most divergent isolates and the most prolonged (chronic) infections have CVID, a group of late-onset immunodeficiencies that have multiple etiologies.[564] CVID is the most prevalent (~1 in 50,000)[564] of the primary immunodeficiencies, but only a small proportion of CVID patients exposed to OPV become chronically infected with iVDPVs.

Unlike cVDPV outbreaks, prolonged iVDPV infections cannot be prevented by high OPV coverage and can be prevented only by cessation of OPV use. Persons with prolonged iVDPV infections can transmit poliovirus to others, raising the risk for VDPV circulation in settings of low population immunity to the corresponding poliovirus serotype. So far, nearly all reports of persistent iVDPV infections have been from countries with high to intermediate levels of development, where the rates of vaccine coverage are high and where the survival times of immunodeficient patients may be extended by treatment with intravenous immunoglobulin. The survival rates for persons with primary immunodeficiencies are probably very low in developing countries at highest risk for poliovirus spread. The population of prolonged iVDPV excretors is declining in developed countries because some patients have died, some have cleared their infections, and no new iVDPV infections have been found in countries that have shifted to IPV. However, the prevalence of long-term iVDPV excretors might be higher than suggested by existing surveillance of persons with primary immunodeficiencies, and several aVDPVs have properties closely resembling iVDPVs (see below). The development of treatment for prolonged iVDPV infections might facilitate detection of and access to those with prolonged infections.[211]

Type 2 iVDPVs are the most prevalent (65%), followed by types 1 (18%) and 3 (17%).[548] Some patients have heterotypic iVDPV infections (i.e., types 1 and 2 or types 2 and 3), with the isolates having similar degrees of divergence from the parental OPV strains, consistent with a common OPV dose initiating the infections. In addition to the occasional heterotypic infections, iVDPV isolates generally have distinguishing genetic properties, including heterogeneity at sites of nucleotide variability within serotypes (indicative of mixed virus populations), extensive antigenic variability, and either nonrecombinant or vaccine/vaccine recombinant genomes.[450,548] The underlying reason for this pattern of recombination may be that individuals with prolonged iVDPV infections may be resistant to superinfection by other enteroviruses.

Ambiguous Vaccine-Derived Polioviruses. Unlike the well-defined cVDPV and iVDPV categories, aVDPVs are a heterogeneous collection of isolates. Some aVDPVs have diverged by just over 1% from the parental OPV strains, have no detected progeny, and may reflect the extremes of the usual distribution of vaccine-related variants in countries using OPV.[450] Detection of other aVDPVs have foreshadowed subsequent cVDPV outbreaks[133,565]; others indicate limited person-to-person spread of OPV virus in small communities with gaps in OPV coverage,[310,311,549,566,567] whereas others appear to indicate more prolonged circulation. Most of these latter aVDPVs have vaccine/nonvaccine recombinant genomes typical of cVDPVs.

Many of the aVDPVs are isolated from the environment, and some have genetic properties (mixed VDPV populations, extensive antigenic changes, and nonrecombinant or vaccine/vaccine recombinant genomes) typical of iVDPVs. Some highly divergent aVDPVs have been detected in Israel,[548,568] Estonia,[296] Slovakia,[569] and Finland,[570] countries with high rates of polio vaccination coverage that are unlikely to have extensive VDPV transmission. The sequence divergence of some of these isolates is consistent with more than 15 years of replication from the original initiating OPV dose. It is likely that the sources of these viruses are immunodeficient persons with asymptomatic VDPV infections, some of whom may be at risk for subsequent paralytic poliomyelitis, although the infected persons remain to be identified.[450]

Simian Virus 40

During the early years of OPV production, some cell cultures were contaminated with simian virus 40 (SV40), a virus that causes cancer in rodents.[571] Although concerns were raised about the carcinogenic potential of SV40 in vaccinees and their offspring, long-term follow-up studies do not support such an association.[572,573] A meeting convened at the National Institutes of Health in 1997 reexamined the available evidence and concluded that "no measurable increase in neoplastic diseases has occurred in humans exposed to SV40 contaminated polio vaccines."[574] Subsequently, a report evaluated the cancer risks of birth cohorts potentially exposed to SV40 and concluded that these cohorts did not experience a significantly increased risk for the cancer outcomes studied.[575] Nevertheless, the question of whether SV40 exposure can increase cancer risk continues to be debated. [576,577] A review by the Immunization Safety Committee (under the auspices of the Institute of Medicine) concluded in 2002 that because the epidemiologic studies were sufficiently flawed, the evidence was inadequate to conclude whether SV40-contaminated polio vaccine caused cancer.[578] Several subsequent cohort and case-control studies using more scientifically rigorous methods failed to support a relationship between SV40 and cancer.[579–582] Cell lines currently used for OPV production come from monkeys raised in colonies free of SV40 or from well-characterized continuous cell lines (Vero cells). In addition, OPV must be screened for known viruses; consequently, SV40 is not present in current lots of OPV vaccine.

Guillain-Barré Syndrome

A review by the Institute of Medicine in 1992 suggested that "the evidence favors acceptance of a causal relationship between OPV and Guillain-Barré syndrome."[526] This conclusion was based primarily on data from Finland, where an OPV mass campaign was conducted to control an outbreak of poliomyelitis and Guillain-Barré syndrome developed in 27 patients within 10 weeks after initiation of the campaign.[583,584] However, after the Institute of Medicine review was completed, the Finnish data were reanalyzed and an observational study was completed in the United States.[585,586] The observational study in the United States showed that rates of Guillain-Barré syndrome after OPV were similar to rates that would have been expected in the absence of vaccination. The reanalysis in Finland suggested that the increase in risk for Guillain-Barré syndrome began before the mass vaccination campaign had been initiated and noted that an influenza epidemic occurred concurrently with the mass campaign. Thus, the available data do not support a causal relationship between OPV and Guillain-Barré syndrome.

Contraindications and Precautions

Contraindications for OPV administration differ somewhat depending on where the vaccine recipient resides (Table 49.15). The risk-to-benefit ratio of OPV versus no vaccine

TABLE 49.15 Contraindications and Precautions for Use of Oral Poliovirus Vaccine in the United Kingdom,[a] United States,[a] and Developing Countries

Type[587,588]		United Kingdom[589]	United States[537]	Developing Country (WHO)[270]
Contraindications	Known or suspected immunodeficiency disorder or immunocompromised	Yes	Yes	Yes[b]
	Family member known to have or suspected of having immunodeficiency disorder or being immunocompromised	Yes	Yes	Yes[c]
	HIV infection	No[c]	Yes	No[d]
	Age ≥18 y	No	Yes (use IPV)	NA
	Allergic reaction to previous doses of OPV or one of its components	No	Yes	NA
Precautions	Pregnancy	Yes	Yes[e]	NA
	Diarrhea	Yes (postpone)	No	Yes (repeated doses)

[a]Recommendations formulated when OPV was vaccine of choice. United States switched in 2000, and the UK in 2004 to an all-IPV schedule.
[b]If immunodeficiency is known.
[c]For HIV-positive symptomatic individuals, IPV may be used instead of OPV at the discretion of the clinician.
[d]Yes, if clinical disease.
[e]If immediate protection against poliomyelitis is necessary, IPV or OPV can be used.
IPV, inactivated poliovirus vaccine; NA, not applicable; OPV, oral poliovirus vaccine; WHO, World Health Organization.
From World Health Organization. Polio vaccines: WHO position paper, January 2014. Wkly Epidemiol Rec. *2014;89:73–92.*

among children residing in countries with endemic poliomyelitis or recent endemic poliomyelitis favors OPV administration in almost all circumstances. Because of the availability of IPV in a number of industrialized countries and because the need for mucosal (intestinal) immunity is not as important compared with polio-endemic countries, industrialized countries may have more contraindications to OPV use than developing countries. However, because IPV is or soon will be available in most countries, some countries may revise the list of contraindications and precautions to ensure that known immunodeficient individuals and their family may only receive IPV. In general, OPV is contraindicated in people with known immunodeficiency disorders and people receiving cancer chemotherapy. Age older than 18 years and pregnancy were precautions in the United States, and OPV doses should be repeated in children who vomit within 30 minutes of OPV receipt. In developing countries, OPV doses administered to children with diarrhea also should be repeated. More details on contraindications and precautions are discussed in the following sections.

Altered Immune States

In industrialized countries, people with known or suspected immunodeficiency disorders, such as severe combined immunodeficiency, CVID, agammaglobulinemia, and hypogammaglobulinemia, should not receive OPV.[537,538,587,589] Similarly, in people with altered immune states resulting from diseases such as leukemia, lymphoma, or generalized malignant disease or with immune systems compromised by therapy with corticosteroids, alkylating drugs, antimetabolites, or radiation, OPV is contraindicated. Because of the potential for vaccine spread, OPV should not be given to a child who is a member of a family in which there are immunocompromised persons. Also, where there has been a family member with immunodeficiency in the past, OPV should not be given to another child unless that child is known to be immunocompetent.

Human Immunodeficiency Virus Infection

In many industrialized countries, OPV in people infected with HIV is considered a precaution or a contraindication. OPV also should not be used for immunizing household contacts of patients with the immunodeficiency disorder; instead, IPV is recommended.

In developing countries, the HIV status of infants is often not known and IPV is not available as an alternative vaccine. In these settings, OPV is administered early in life (i.e., 6, 10, and 14 weeks of age) on the basis of WHO recommendations; this is an age when HIV infection would not be expected to have caused immunodeficiency. Several studies showed that OPV induces antibody to polioviruses in a similar proportion of HIV-infected infants compared with the response in non–HIV-infected infants.[590,591] HIV infection does not seem to be a risk factor for paralytic poliomyelitis caused by wild poliovirus[592] or for VAPP. There are only two case reports in the literature, one from Romania and one from Zimbabwe, that link HIV infection and VAPP.[593,594] Studies in Kenya and from Côte d'Ivoire evaluated HIV-infected children and adults with AIDS for long-term excretion of poliovirus. These studies did not detect long-term excreters and suggest that the risk of long-term carriers of poliovirus among persons with acquired immunodeficiency syndrome may be low.[595] A study in South Africa demonstrated divergent vaccine virus in some HIV-infected persons; however, it is not possible to ascertain whether this reflects extended replication in an individual or recent exposure.[596]

Previous Allergic Reaction

The OPV may contain trace amounts of penicillin, neomycin, polymyxin, and streptomycin, and a previous allergic reaction to OPV or a similar reaction to these antibiotics constitutes a contraindication to further OPV receipt.[537,589]

Adult Use

The recommendations for use of OPV among unvaccinated adults 18 years or older in industrialized countries is not uniform. In the United States, adults should not receive OPV because of concerns about a slightly elevated risk of VAPP in adults.[527] In the United Kingdom, no such restrictions were recommended.[589] Nevertheless, at-risk adults in the United States may receive OPV if they had been primed by OPV previously.[537] However, to control wild poliovirus circulation among older age groups, such as in outbreaks in Israel,[597] Albania,[213] Cape Verde,[215] Namibia,[220] Tajikistan,[216] and most

recently in Congo-Brazzaville,[217,243] and the Democratic Republic of the Congo,[243] it has been necessary to extend the target age group for control measures with OPV and, in some instances, to target the entire population.

Pregnancy

In most industrialized countries, pregnancy is a contraindication or a precaution to OPV receipt.[589] Two evaluations have found no link between adverse outcomes and administration of OPV to pregnant women.[598,599] Nevertheless, most immunization authorities recommend that immunization during pregnancy generally should be avoided for reasons of theoretical risk. However, if immediate protection against poliomyelitis is needed, IPV or OPV can be used.[537,587]

Diarrhea

In developing countries, OPV may be given to a child with diarrhea; however, because of the lower immunogenicity of OPV in these countries, the dose should not be counted as a valid dose toward completing the routine schedule and should be repeated 4 weeks later.[463,464,537,587,600]

Simultaneous Administration With Other Vaccines

In industrialized countries and in developing countries, OPV is usually administered with other vaccines, including (where appropriate) bacille Calmette-Guérin (BCG), DTP, hepatitis B, measles, *Haemophilus influenzae* type b, and other vaccines used routinely,[537,600] because no interference between these vaccines and OPV has been observed. However, newer studies conducted in Guinea-Bissau suggest that simultaneous administration of BCG and OPV at birth could decrease immune responses to BCG.[601,602] In addition, with the licensure of rotavirus vaccines, the issue of potential interference between these vaccines has been raised. At this point, the evidence suggests that simultaneous administration of rotavirus with OPV will not decrease the immunogenicity of OPV.[603,604]

Intussusception

Following withdrawal of a specific rotavirus vaccine associated with intussusception in the United States in the early 2000s, the issue of OPV and intussusception gained renewed interest. Several studies in the United Kingdom and Cuba show that there is no evidence for an association between OPV and intussusception.[605,606]

Sequential and Combined Schedules

As of early 2014, the WHO does not recommend an all-OPV schedule for poliomyelitis prevention through routine immunization.[607] Thus, all countries should use schedules that are based either on IPV-only or sequential schedules using both IPV and OPV. Over time many countries have adjusted their routine immunization schedules. For example, the United States shifted from an exclusive OPV schedule to a preferred sequential schedule of two doses of IPV followed by two doses of OPV in 1997 and to an IPV-only schedule in 2000.[588,608] Many countries in Central and Eastern Europe and elsewhere have examined the advantages and disadvantages of sequential vaccination schedules for adoption into national vaccination policies. Sequential schedules offer the following scientific and programmatic advantages:

- The schedule should reduce the number of VAPP cases among recipients by at least 95% and to some degree among contacts because the oral mucosal immunity induced by two doses of IPV should reduce spread of vaccine virus.
- Continued use of OPV will induce effective intestinal immunity,[609] thereby enhancing community resistance to transmission of imported wild poliovirus.
- There is opportunity for spread of vaccine virus, thus immunizing people missed by routine immunization. (This becomes less important as immunization coverage reaches high levels.)
- The number of injections in the second year of life is reduced from what would be required with use of an IPV-only schedule, making compliance with the overall schedule easier.
- The schedule will lead to stocking of both poliovirus vaccines by healthcare providers, facilitating choice by parents and providers.

In April 2016, all previous OPV-using countries adapted a new routine immunization schedule for poliomyelitis prevention that relies on three or more doses of bOPV (given at [birth] 6, 10, and 14 weeks) supplemented by a dose of IPV at 14 weeks given concurrently with bOPV.[607] The global switch was implemented very efficiently.[609a] The immunogenicity of the new sequential schedules was assessed by reviewing data on the vaccines used, the age at administration, the number of doses, and the interval between doses. In addition to the results of the immunogenicity studies, there is a growing body of evidence suggesting the effectiveness of this approach in controlling VAPP and, by extension, poliomyelitis caused by wild poliovirus.[610]

In the United States, a small trial first demonstrated the immunogenicity of two sequential schedules of IPV and OPV in inducing antibody.[611] Subsequently, a series of studies were conducted in the United States to assess the immunogenicity of sequential schedules of IPV followed by OPV. In general, these studies showed that a series of at least three doses of either vaccine—IPV or OPV or with sequential or combined use—was necessary to induce antibody to all three poliovirus serotypes in more than 90% of vaccinees.[612–614]

To assess the effectiveness of three sequential schedules in inducing mucosal immunity in the United States, vaccinees received a dose of OPV at age 18 months (3 months after the last dose of poliovirus vaccine), and stool specimens were obtained before challenge (day 0) and 3, 7, and 21 days thereafter. One dose of OPV at 15 months after two doses of IPV at 2 and 4 months reduced shedding of poliovirus types 2 and 3 (after challenge with OPV at 18 months), but did not decrease poliovirus type 1 shedding. Two doses of OPV after IPV decreased shedding of poliovirus type 1 and further decreased shedding of poliovirus type 3. The study, conducted by The Johns Hopkins University, demonstrated that at least two doses of OPV were needed to induce mucosal immunity sufficient to significantly decrease the proportion of vaccinees excreting poliovirus.[609] The proportion of study subjects who had received prior IPV and excreted type 1 (18%) was much lower than expected based on other challenge studies with OPV. The reasons for this discrepancy are not known because other challenge studies of IPV vaccinees with tOPV led to 80% shedding of poliovirus type 1[615] and with mOPV led to 70% excretion.[490] We believe the most likely explanation is that the study duration (16 months) may have allowed participants (including participants receiving only IPV) to be exposed to vaccine virus excreted from other OPV-vaccinated infants, masking differences that may have existed shortly after the completion of the primary series.

No combined schedules of IPV and OPV have been evaluated in the United States, except for one study arm, which was part of a larger study of sequential schedules, that used one dose of OPV and IPV simultaneously at 4 months of age

(IPV at 2 months, IPV/OPV at 4 months, OPV at 6 and 15 months).[609] In this trial, two doses of IPV and three doses of OPV resulted in seroprevalence levels of 99% to 100% against poliovirus types 1, 2, and 3 compared with 99% to 100% after three doses of IPV and 96% to 100% after three doses of OPV.

In developing countries, the major issue is how to improve the immunogenicity of OPV with IPV use. In these settings, schedules using OPV followed by IPV were evaluated. These studies demonstrated that IPV after a primary series of OPV can correct the low immunogenicity of OPV alone that was commonly reported from tropical developing countries. A clinical trial conducted in India confirmed the excellent immunogenicity of bOPV at birth, 6, and 10 weeks, followed by bOPV and IPV at 14 weeks.[616] One month after the administration of the last doses of polio vaccines, 99% of participants seroconverted to poliovirus type 1, between 69% and 77% to poliovirus type 2, and 99% to poliovirus type 3. Similarly, a study conducted in Chile demonstrated an equally excellent immunogenicity of a schedule of one or two doses of IPV followed by one or two doses of bOPV.[617] In the Côte d'Ivoire, schedules that added a dose of IPV administered simultaneously with measles vaccine, after a course of three doses of OPV administered by the routine program, resulted in a significantly higher proportion of children with antibodies against poliovirus types 1 and 3 compared with a control group that received an additional dose of OPV. Administration of a dose of IPV increased seroprevalence from 85% to 97% for poliovirus type 1 and from 76% to 92% for poliovirus type 3.[618] The experiences in the Gaza Strip are of particular interest. In this area, a new combined and sequential schedule of OPV and IPV (mOPV1 during the first month, simultaneous administration of OPV and IPV at 2 to 3 and 3 to 4 months, and OPV at 5 to 6 and 12 to 14 months) reduced the incidence of poliomyelitis during the first 3 years after the change in vaccination schedule from an annual incidence of 10 per 100,000 population to 2.2 per 100,000 population.[610,619,620] In Israel, after the outbreak in 1988,[597] a sequential schedule of IPV and OPV was used for routine vaccination[621] until 2005, when Israel adopted an all-IPV schedule.[622] However, Israel modified the routine immunization schedule to include bOPV after the extensive "silent" type 1 circulation in 2013–14.

In the developing world, however, a large randomized trial in The Gambia, Oman, and Thailand compared the immunogenicity of the following: (a) a combined schedule of four doses of OPV administered at birth, 6, 10, and 14 weeks and IPV administered simultaneously with OPV at 6, 10, and 14 weeks; (b) four doses of OPV administered at birth, 6, 10, and 14 weeks; and (c) three doses of IPV administered at 6, 10, and 14 weeks. The combined schedule with seven doses of poliovirus vaccines performed significantly better (95% to 99% for poliovirus type 1, 99% seroconversion to 100% for poliovirus type 2, and 97% to 100% for poliovirus type 3) compared with four doses of OPV or three doses of IPV (Table 49.16).[464] In addition, the combined schedule was not affected by socioeconomic status or level of maternal antibody, in contrast with the comparison groups that received OPV or IPV, respectively. This study demonstrated that a combined schedule could correct the lower immunogenicity of OPV in developing countries, but additional doses of vaccine are required.[254] Intestinal mucosal immunity induced by the combined schedule in the IPV/OPV group was similar to that of the OPV group and significantly better than that of the IPV group.[464] A study in Guatemala comparing schedules of IPV, OPV, and IPV/OPV combined demonstrated high seroprevalence and found no significant differences between the groups after three doses.[623]

One study reported no benefit of one dose of OPV after three doses of IPV in terms of type 3 seroprevalence and geometric mean antibody titer[624]; however, a larger study in Oman demonstrated that additional doses of OPV significantly increased seroprevalence and geometric mean antibody titer to polioviruses types 1 and 3.[625] This study confirmed earlier observations about the incremental benefit of adding additional doses of OPV in terms of seroconversion and geometric mean antibody titer.[122,254,473,625] There is renewed interest in adding IPV to OPV for campaign use in limited places in the most difficult to control areas for poliomyelitis to boost both humoral and mucosal immunity and facilitate the interruption of poliovirus transmission.

Limited data are available on the persistence of poliovirus antibody induced by combined or sequential schedules of poliovirus vaccines. A single study in the United States evaluated the persistence of antibody 4 years after a primary series of sequential schedules.[626] Antibody persistence was excellent, but titers decreased by 10- to 100-fold during the first 2 years of follow-up, and thereafter the titers remained relatively stable.

Results of Controlled Trials of Protection Against Disease

There are no data from controlled trials of sequential schedules that were used as the outcome measure of the prevention of paralytic disease. Because detectable antibody to polioviruses provides an excellent correlate for protection, results of controlled trials are redundant and would be unethical to conduct.[22]

TABLE 49.16 Proportion Seropositive for Neutralizing Antibody to Poliovirus Types 1, 2, and 3 in Infants Immunized With OPV Alone, IPV Alone, or OPV and IPV Simultaneously in The Gambia, Oman, and Thailand

Age and Type of Vaccine Administered					Proportion Seropositive (%)		
Birth	6 wk	10 wk	14 wk	Serotype	The Gambia (N = 118)	Oman (N = 183)	Thailand (N = 145)
OPV	OPV	OPV	OPV	1	85	90	98
				2	97	98	100
				3	72	73	100
	IPV	IPV	IPV	1	81	88	66
				2	82	92	63
				3	92	91	92
OPV	OPV/IPV	OPV/IPV	OPV/IPV	1	97	95	99
				2	100	99	100
				3	99	97	100

IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine.

Data from World Health Organization Collaborative Study Group on Oral and Inactivated Poliovirus Vaccines. Combined immunization of infants with oral and inactivated poliovirus vaccines: results of a randomized trial in The Gambia, Oman and Thailand. J Infect Dis. *1997;175(Suppl 1):S215–S227.*

Potential Adverse Reactions With Sequential Schedules

Genetic sequencing studies suggest that reversion of Sabin strains to potentially more neurovirulent phenotypes occurs commonly after OPV administration.[330–347,349–351,627–633] Two relatively small studies[634,635] indicated that the use of a sequential schedule may not reduce the frequency of such mutations. However, one larger study suggests that the use of a dose of IPV before two or more doses of OPV may reduce the amount of type 3 virus shed, the most common cause of VAPP, but probably will not influence the shedding of type 1 or type 2 viruses or the extent of reversion.[636] VAPP has been rarely reported after sequential schedules using at least one dose of IPV before multiple doses OPV. In Hungary, a country that had a high risk of VAPP during decades, the switch to a sequential schedule (with one dose of IPV) was associated with the apparent elimination of VAPP. However, there is debate whether one dose of IPV is adequate to prevent VAPP.

Recommendations for Vaccine Use

Two major objectives of vaccination, protection at the youngest possible age and minimum rates of attrition (i.e., dropout) between OPV doses, govern the development of routine vaccination schedules in industrialized and developing countries. In each of these settings, an optimal balance must be found between these objectives.[122]

Industrialized Countries

Most industrialized countries, including many Western European countries, have recommended schedules in the past that relied exclusively on OPV for the prevention of poliomyelitis. More recently, encouraged by progress of the GPEI and by the desire to reduce or eliminate the burden of VAPP, many of the high- and middle-income countries are reevaluating their vaccination policy options. Since April 2016, all countries are using schedules that include at least one dose of IPV (Fig. 49.14). The world is now transitioning to introduce IPV. However, until all OPV is stopped, these countries rely primarily on tOPV, and after OPV2 withdrawal (see "New Polio Endgame" later), on bOPV for routine immunization. The major differences in the recommended schedules between industrialized and developing countries include (a) age at first dose, (b) vaccines used for each dose, and (c) interval between doses. The recommendations for poliomyelitis prevention in the United States were revised most recently in 2000.[588] A schedule relying only on IPV is now recommended by the CDC and the American Academy of Pediatrics for primary poliovirus vaccination of children in the United States. The schedule calls for three doses of IPV administered at 2 and 4 months and between 6 and 18 months. A preschool booster dose of IPV is recommended after age 4 years. Similarly, in the United Kingdom, an IPV-only schedule was introduced in 2004; doses are provided at 2, 3, and 4 months of age, and booster doses at pre–school-age (3 years and 4 months), and in adolescence (13 to 18 years) (Table 49.17).[589] The minimum recommended interval between doses of OPV for routine vaccination varies; in the United States, 6 to 8 weeks were recommended. For the United Kingdom, intervals between OPV doses of 4 weeks were preferred.[589] Table 49.18 shows the major advantages and disadvantages of the three poliovirus vaccination schedules.

Routine immunization for adults residing in the continental United States and many other industrialized countries is not believed to be necessary because of the small risk of exposure to wild poliovirus.[537,587,589] However, adults who are at increased risk because of contact with a patient infected with wild poliovirus or who are working with polioviruses and adults planning travel to an epidemic or endemic area should be immunized.

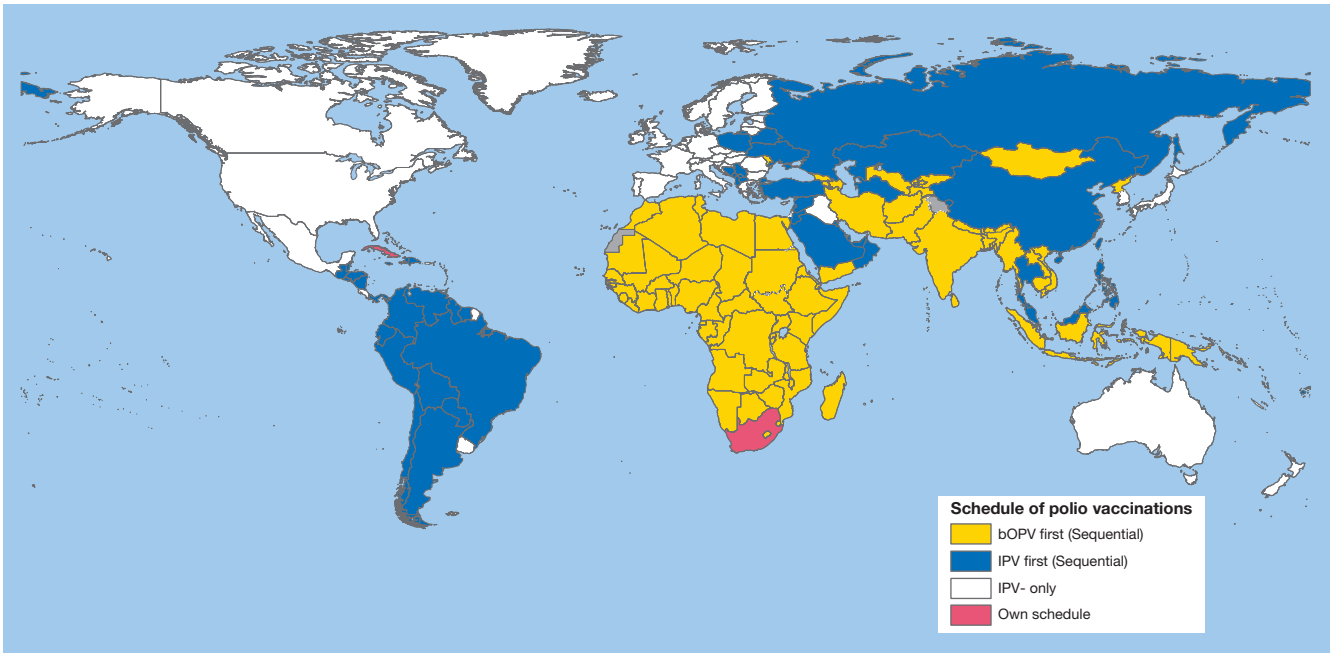


Figure 49.14. Countries using inactivated polio vaccine (IPV) only, a sequential schedule of IPV followed by bivalent oral poliovirus vaccine (bOPV), or a sequential schedule of bOPV followed by IPV in their national routine immunization program for the prevention of poliomyelitis following the withdrawal of Sabin type 2 from the oral polio vaccine (OPV) (implemented in April 2016). White, IPV only; blue, sequential schedule of IPV followed by bOPV; yellow, sequential schedule of bOPV and IPV; green, own schedule.

TABLE 49.17 Poliovirus Vaccination Schedules in the United States and the United Kingdom, 2000 to 2006, and WHO in 2013

Country	Vaccine	Age at Vaccine Administration				Recommendation Valid
United States	OPV IPV	2 mo 2 mo	4 mo 4 mo	6–18 mo 6–18 mo	4–6 yr 4–6 yr	Until 1999 From 2000
United Kingdom	OPV IPV	2 mo 2 mo	3 mo 3 mo	4 mo 4 mo	School entry[a] 3–5 yr; 13–18 yr	Until 2004 From 2005
WHO	OPV[b] IPV	6 wk	10 wk	14 wk 14 wk		From 2013

[a]At age 3 years and 4 months; in addition, an adolescence booster is provided at age 13–18 years.
[b]bOPV after April 2016 (OPV2 withdrawal).
IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; WHO, World Health Organization.
From World Health Organization. Polio vaccines: WHO position paper, January 2014. Wkly Epidemiol Rec. *2014;89:73–92.*

TABLE 49.18 Advantages and Disadvantages of the Three Poliovirus Vaccination Schedules

Attribute	OPV Only	IPV Only	IPV/OPV Sequential
VAPP	2–4 cases per million birth cohort[a]	None	≥95% reduction from OPV-only schedule
Other serious adverse events	None known	None known	None known
Systemic immunity	High	High	High
Mucosal immunity	High	Lower	High
Secondary transmission of vaccine virus	Yes	No	Some
Emergence of circulating vaccine-derived poliovirus	Yes	No	Probably reduced
Extra injections or visits needed	No	Yes	Yes
Compliance with immunization schedule	High	Possibly reduced	Possibly reduced
Future combination vaccines	Unlikely	Likely	Likely (IPV)
Current cost	Low	Higher	Intermediate

[a]First-dose recipient risk.
IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine; VAPP, vaccine-associated paralytic poliomyelitis.
Modified from Centers for Disease Control and Prevention. Poliomyelitis prevention in the United States: introduction of a sequential vaccination schedule of inactivated poliovirus vaccine followed by oral poliovirus vaccine: recommendations of the Advisory Committee on Immunization Practices. MMWR. *1997;46(RR-3):1–25.*

Developing Countries

Until 2013, the WHO-recommended schedule called for the administration of four doses of OPV at birth, 6, 10, and 14 weeks of age for polio-endemic or recently polio-endemic countries. This is particularly important in areas in which frequent importation or endemic circulation of wild polioviruses takes place and in which a majority of infants are exposed to all three poliovirus types early in life.[212,254,610,619] For these areas, the primary immunization schedules not only should begin early, with the first dose being given to newborns, but also—and most important—should be completed as early as possible. Many countries also recommend a dose of OPV in the second year of life, usually at age 18 months given simultaneously with DTP.

This four-dose WHO-recommended schedule is supported by data from China,[637] where a schedule that included a birth dose of OPV performed considerably better than a schedule without a birth dose, particularly for type 3 (97% vs 74% seroprevalence). These recommendations have been further supported and extended by a review of the available literature on the efficacy of early immunization with DTP and OPV.[238,638–640] In 2010, the WHO issued a position paper on using polio vaccine in the preeradication era of poliomyelitis. This paper offers guidance to countries that are considering changes in routine immunization schedules for the preeradication era of polio eradication.[641] The new WHO recommendations for the choice of vaccine (and schedule) are determined by the potential for importation of poliovirus and the risk for transmission (coverage, socioeconomic status, hygiene) following importation. OPV and at least one dose of IPV are the vaccines recommended.[607]

Short-Interval Additional Dose Strategy

The short-interval additional dose strategy was developed to take advantage of limited access to high-risk, particularly inaccessible areas in order to provide multiple doses of OPV in a very short period, and was first piloted in parts of northwestern Kenya. By 2013–14, the strategy was widely used in polio-endemic countries, especially Pakistan, primarily in national and subnational vaccination campaigns to eliminate the remaining chains of poliovirus transmission. Two clinical studies have confirmed that the decrease from a 30-day interval, to a 14-day interval (for bOPV), or a 7-day interval (for mOPV1) is not associated with decreased immunogenicity.[479,642] These scientific data support the continued, and possibly expanded, use of this strategy.

Future Use of Poliovirus: The Role of New Oncolytic Poliovirus Immunotherapy of Cancer

The hope that oncolytic viruses could serve as effective antitumor agents predates the discovery of viruses and is rooted in 19th century clinical observations that cancer patients experienced (usually transient) remission following naturally acquired infectious diseases such as influenza, viral hepatitis, and chickenpox.[643] Clinical trials in the 1950s to 1970s demonstrated tumor regression in some patients following treatment with diverse viral agents, but serious side effects and the risk of secondary infections from the use of natural infectious agents appeared to dim the prospects for virotherapy.[643] Prospects again brightened in the 1990s with the availability of

genetically engineered attenuated oncolytic viruses and virotherapeutic application in animals of an engineered herpes simplex virus.[644] Currently, clinical trials of oncolytic viruses include representatives of at least 10 diverse virus families,[645] including the picornaviruses coxsackievirus A21[646] and poliovirus.[647] Prominent among these trials has been the complete radiographic and clinical responses of several patients with glioblastoma treated by direct injection of tumors with a highly attenuated derivative of Sabin 1, PVS-RIPO, engineered by exchange of the Sabin 1 IRES with that of a distant picornavirus relative, human rhinovirus type 2 (the rhinovirus IRES does not support replication in neurons).[648,649] Key to therapy with PVS-RIPO is its capacity to specifically target cells expressing the PVR, CD155, present at high levels on the cells of embryonic tissues and several tumors, including colorectal carcinoma, cutaneous melanoma, and glioblastoma.[650–652] Oncolytic activity is hypothesized to be mediated by a combination of direct and rapid lytic infection of some tumor cells, innate antiviral activation, and stimulation of an adaptive antitumor cytotoxic T-cell response.[652] The benefits of PVS-RIPO are potentially large for certain kinds of cancers whereas the containment risks (see "Laboratory Containment of Polioviruses") associated with its continued use appear to be very small because (a) PVS-RIPO is highly attenuated and genetically stable, (b) all patients must have high immune titers to type 1 poliovirus before treatment, (c) PVS-RIPO is injected directly into the tumor rather than systemically, (d) virotherapy induces an extremely strong neutralizing antibody response, (e) patients are regularly monitored for shedding of poliovirus in body fluids and none has been found, and (f) strict measures are implemented to prevent inadvertent exposure to poliovirus.[649]

PUBLIC HEALTH CONSIDERATIONS

Epidemiologic Results of Vaccination

Surveillance data since 1980 suggest that continuing transmission of indigenous wild poliovirus has been interrupted in the United States, during a period when the country relied on OPV for immunization.[268,533] As part of the certification of the Western Hemisphere as polio-free, all countries in the Americas, including the United States, were certified free of indigenous wild poliovirus in 1994 by an International Commission convened by the Pan American Health Organization on the basis of a detailed review of available data by national committees.[498] Fig. 49.15 shows data on poliovirus circulation during 1988 to 2015 by WHO region, a period during which the GPEI had been implemented in all WHO regions. Progress has been impressive. In 1988, 125 countries were polio-endemic; by the end of 2016 only three countries were considered polio endemic (i.e., had never interrupted indigenous virus transmission: Afghanistan, Pakistan, and Nigeria; importation with limited transmission in time and space does not constitute reestablishment of endemicity). Egypt was removed from the list of endemic countries at the end of January 2006 following a 12-month period without detection of poliovirus despite excellent surveillance performance from AFP surveillance and from environmental surveillance. Globally, cases of poliomyelitis decreased more than 99% from an estimated 350,000 cases in 1988 to 1349 in 2010, 359 in 2014, 74 in 2015, and 35 in 2016 (as of January 3, 2017). Wild poliovirus type 2 was last detected in October 1999 in Uttar Pradesh, India, and has been officially declared as eradicated.[39,190] Wild poliovirus type 3 was detected in seven countries in 2010 (Afghanistan, Chad, India, Nigeria, Niger, Mali, and Pakistan), but stopped in 2012, with the last case reported from Nigeria in November 2012. Wild poliovirus type 1 was detected globally in 2016, only in Afghanistan and Pakistan (a shared reservoir) and Nigeria. Outside the three polio-endemic countries, no importation events were detected in 2015–16 (data as of November 2016) (Fig. 49.16). The latest information on the status of the polio eradication initiative can be found at the GPEI (http://www.polioeradication.org).

Between 1988 and 2016, the GPEI prevented more than 15 million children from the crippling consequences of poliomyelitis and averted more than 1.5 million deaths, some as a result of poliomyelitis prevention but most because vitamin A supplements were administered to the same target children in many polio-endemic countries during national and subnational immunization campaigns.[658,659] Outside these three polio-endemic countries, no important events were detected in 2015–16 (as of November 29, 2016).

Disease Control Strategies

In 1988, the World Health Assembly, the governing body of the WHO, resolved to eradicate polio globally by the year 2000.[3] The global resolution followed the 1990 regional elimination goal established in 1985 by the countries of the Western Hemisphere. The last case of poliomyelitis associated with wild poliovirus isolation in the Americas was reported from Peru in 1991, and the entire hemisphere was certified free of indigenous wild poliovirus by an International Certification Commission in 1994.[498]

The following strategies to achieve polio eradication, developed in the Western Hemisphere, were adopted by the WHO for worldwide implementation in all polio-endemic countries[37]:

- Achieving and maintaining high routine coverage in infants younger than 1 year with at least three doses of OPV3.
- Administering supplemental doses of OPV to all young children (usually children younger than 5 years of age) during NIDs to rapidly interrupt poliovirus transmission.
- Conducting "mopping-up" vaccination campaigns—localized campaigns targeting high-risk areas where poliovirus transmission is most likely to persist at low levels.
- Developing sensitive systems of epidemiologic and laboratory surveillance, including establishing surveillance of cases of AFP.

Routine Immunization

Control of poliomyelitis and the global eradication initiative are greatly aided by well-functioning routine immunization programs that deliver potent OPV to a high proportion of infants in the first year of life. Indeed, there is a clear association between routine coverage and length of transmission of poliovirus following an importation into a previously polio-free country. The estimated global coverage with three doses of diphtheria-tetanus-pertussis (DTP3) vaccine (assumed to reflect the routine OPV3 coverage) among infants younger than 1 year of age was 86% in 2014. All WHO regions reported a coverage of more than 80% except for the African Region, where coverage improved from 57% in 1990 to 77% in 2014, but continues to be less than the coverage achieved in the other WHO regions.[270,660] However, these global and regional figures may mask substantial variation in coverage reported among and within individual countries.

National Immunization Days

Mass campaigns with OPV—administered during NIDs—are the only proven strategy to reduce widespread transmission of

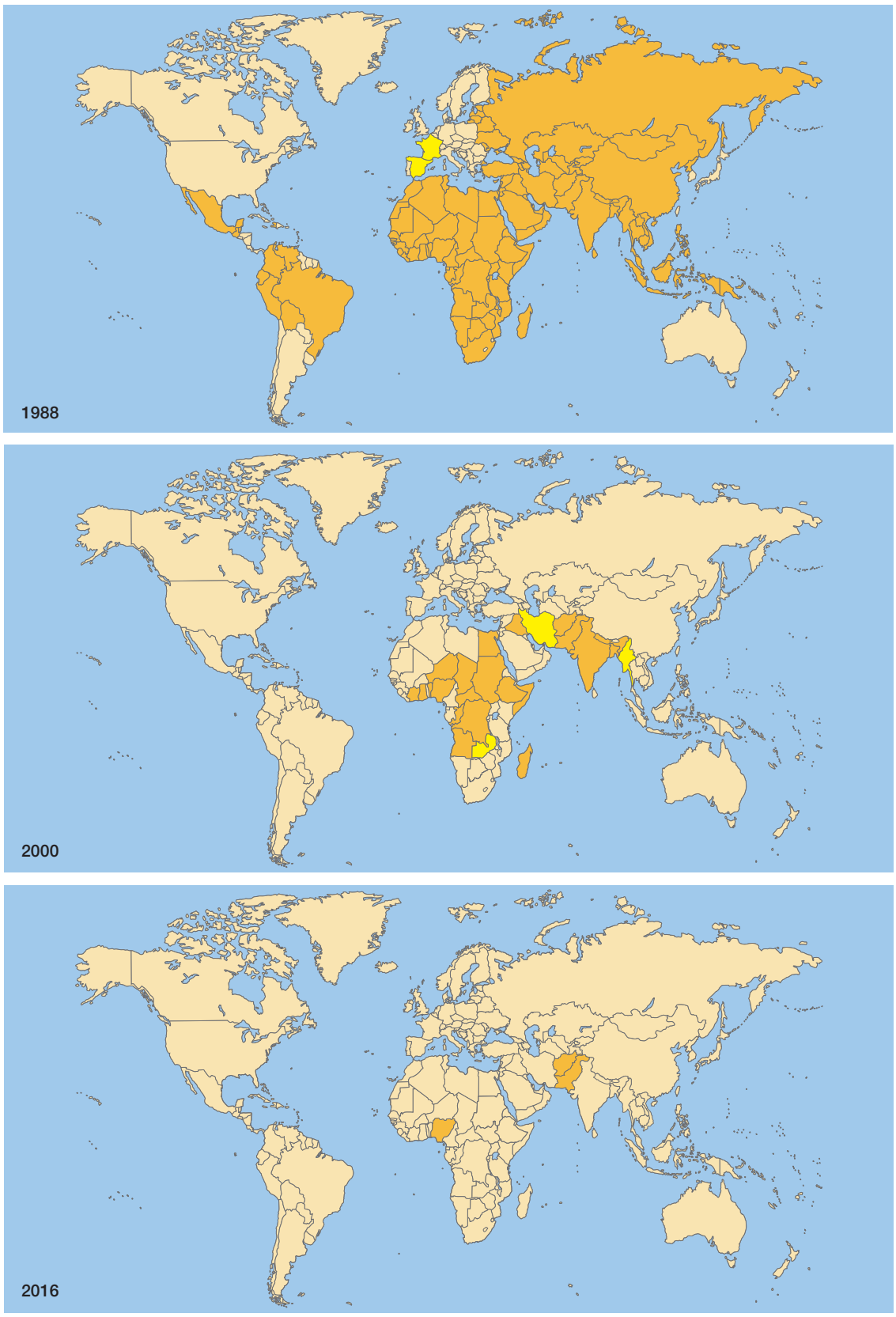


Figure 49.15. Progress toward global polio eradication, 1988–2016. Orange, countries with indigenous wild polioviruses (WPVs); yellow, countries with one or more cases associated with imported WPV; light brown, polio-free countries. Countries of the former Soviet Union were grouped together in the1988 map, reflecting endemicity in the Caucasus and Central Asian republics.

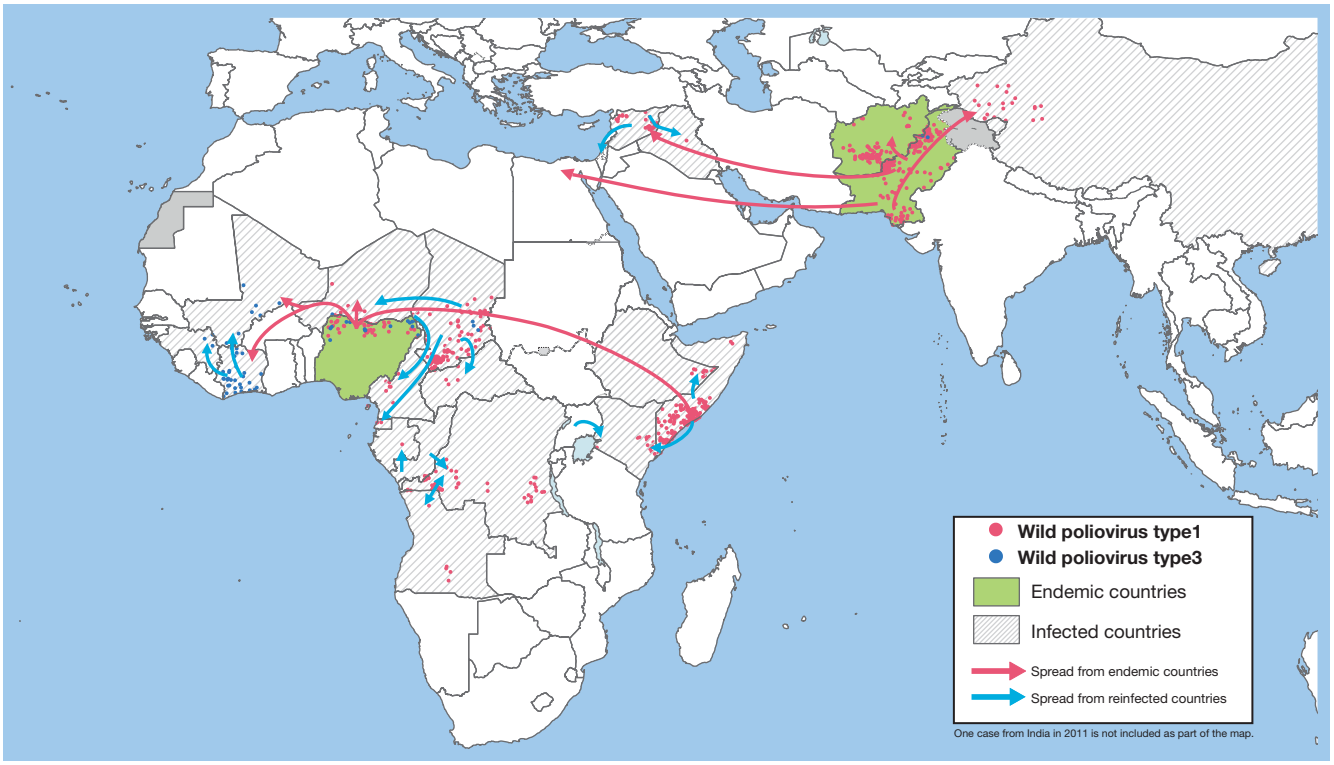


Figure 49.16. Wild poliovirus cases and importation routes, worldwide 2011–2014. Excludes virus detected from environmental surveillance and vaccine-derived polioviruses. *(Data from references 653 to 657.)*

wild poliovirus in endemic countries.[661,662] The NIDs are conducted twice annually for a short period (1 to 3 days) during which one dose of OPV is administered to all children in the target age group, usually children younger than 5 years of age, regardless of prior vaccination history. A second dose is administered in the same way after an interval of 4 to 6 weeks. The NIDs usually take place during the low transmission season when conditions are optimal to interrupt the few remaining chains of poliovirus transmission. Most countries provide OPV during NIDs, relying primarily on fixed sites, including vaccination clinics supplemented by a large number of temporary vaccination sites. With the acceleration of eradication activities in 1999, many of the remaining polio-endemic countries began using a strategy relying on house-to-house administration of OPV during NIDs.

The NIDs are necessary in developing countries to rapidly increase immunity levels in the population to achieve and surpass herd immunity threshold levels for poliomyelitis and, hence, rapidly interrupt the transmission of poliovirus. OPV administered in campaigns also seems to be more immunogenic compared with OPV administered in the routine program,[663,664] probably for the following reasons: (a) NIDs are conducted during the low poliovirus transmission season because this is the period when the fewest chains of poliovirus transmission are maintained. (b) NIDs are conducted during the low transmission season for other enteroviruses that may interfere with poliovirus seroconversion.[665] (c) The cold chain can be better maintained for these short campaigns. (d) Massive use of OPV probably also results in intensive secondary spread of shed virus.[464] Children residing in polio-endemic countries using NIDs may receive 13 to 14 doses of OPV by the time they reach their fifth birthday.[122,473] These OPV doses are administered by the routine program (three or four doses) and through NIDs (two doses annually during the first 5 years of life). These additional doses of OPV, administered during NIDs, should correct the lower immunogenicity of OPV commonly observed in tropical areas.

In 2014, 332 supplementary immunization activities with OPV were conducted in 42 countries, reaching more than 1.8 billion people, the vast majority children younger than 5 years, administering approximately 2.2 billion doses of OPV. Each of the endemic countries conducted at least six large-scale supplemental immunization campaigns in 2014, while each of the reinfected countries with transmission conducted a minimum of four large-scale supplemental immunization campaigns in 2014. All countries used well-supervised, house-to-house vaccination in part or all of the target area for supplementary immunization activities to further increase the quality and reach the highest possible coverage among children younger than 5 years of age. The NIDs in India—vaccinating as many as 157 million children in a single round—represent the largest mass campaigns ever conducted.

Mopping-Up Campaigns

To eliminate the last potential or known reservoirs of wild poliovirus circulation, mopping-up vaccination campaigns are conducted. These mopping-up campaigns usually target children younger than 5 years of age with two doses of OPV separated by an interval of 4 to 6 weeks. These campaigns include house-to-house administration of OPV to reach any children who may have been missed during NIDs. Mopping up is a critical component to achieve interruption of the final chains of poliovirus transmission in all polio-endemic countries. Risk areas (often targeting more than 1 million children

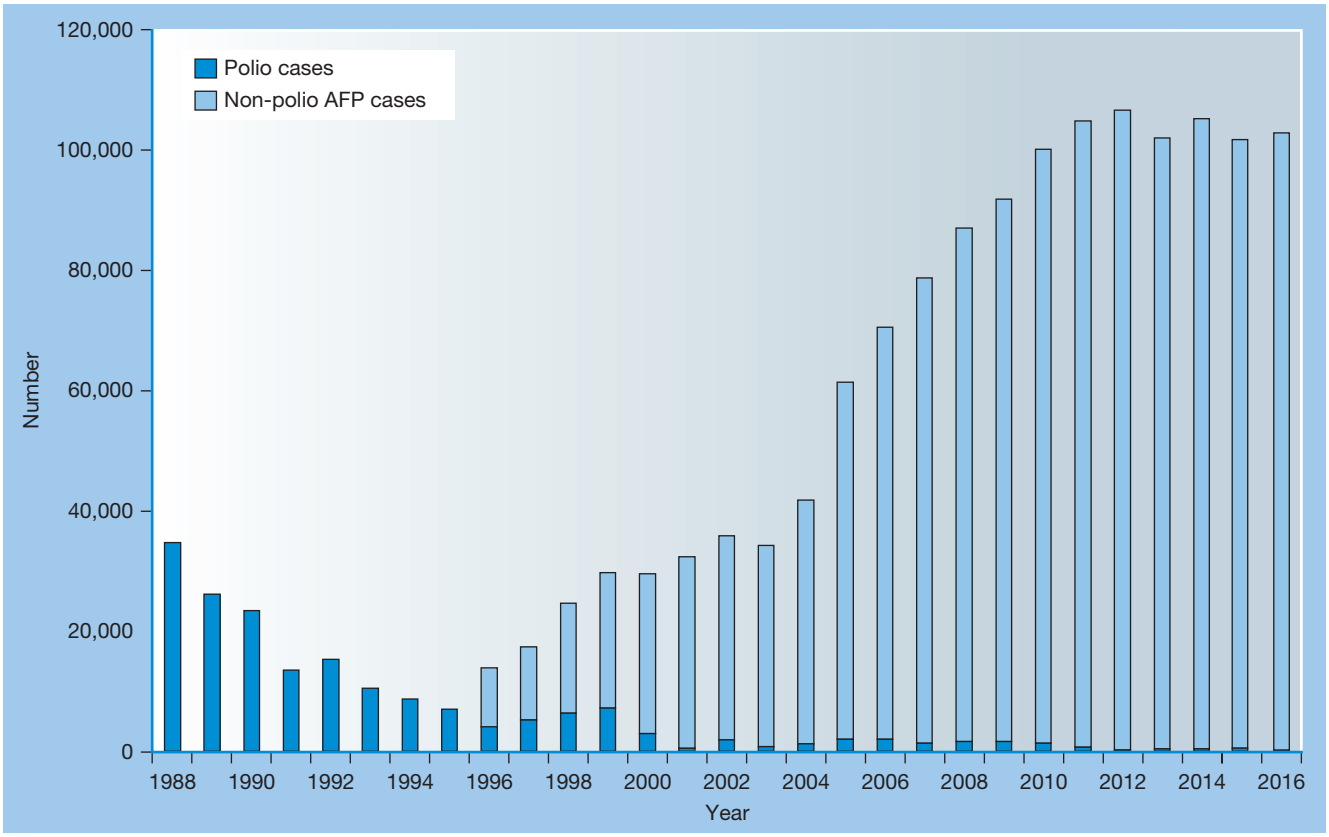


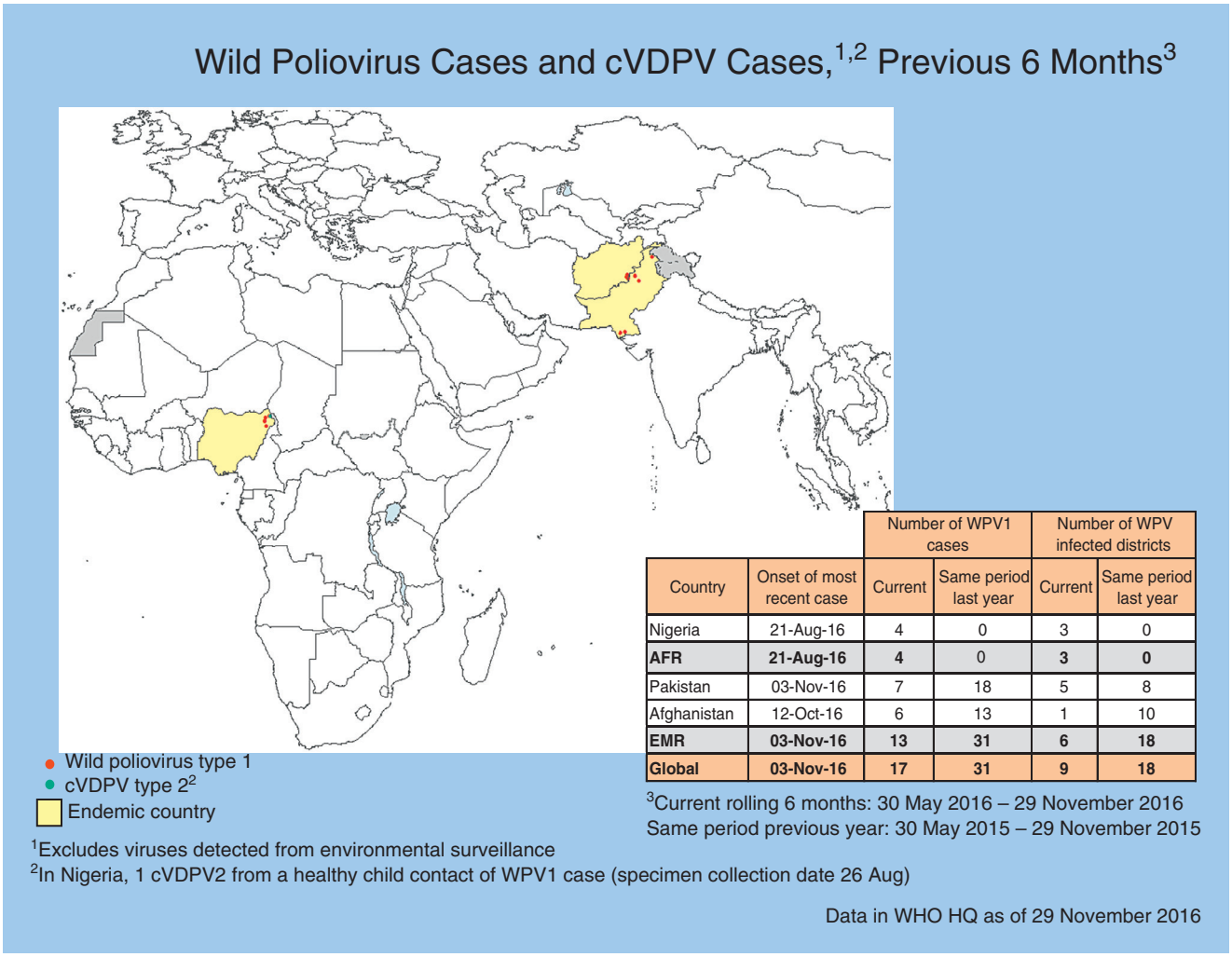
Figure 49.17. Total reported acute flaccid paralysis (AFP) cases and confirmed poliomyelitis cases, 1988–2016. *(From the World Health Organization.)*

younger than 5 years of age to have the optimal impact), usually defined at county or district levels, to be included in mopping up include those with recent circulation of wild poliovirus (usually within the last 3 years), low vaccination coverage, suboptimal surveillance, large migrant or refugee populations, and common borders with known poliovirus-endemic areas.

These supplemental immunization activities have been successful in decreasing the number of reported poliomyelitis cases globally from 35,251 (estimated to be approximately 350,000 cases) in 1988 (when the polio eradication target was adopted) to 359 in 2014, a decrease of more than 99% (Fig. 49.17).[39] Fig. 49.18 displays the remaining AFP cases associated with wild poliovirus isolation during a 12-month period in 2014. A detailed review of the current status of the GPEI was published in 1997,[669] with annual progress reports, the most recent in 2015.[39,667]

Surveillance

Surveillance for cases of AFP and for wild poliovirus is critical for guiding programmatic activities and for contributing to the eventual certification of polio-free status. Systems for AFP surveillance have been established in all polio-endemic or recently endemic countries. Surveillance relies on two complementary and mutually reinforcing components: (a) AFP case investigations and (b) virologic studies of polioviruses obtained from clinical specimens. The major reason for using a symptom (e.g., AFP) rather than a diagnosis (e.g., poliomyelitis) is to ensure that the sensitivity of the surveillance system can be maximized; all possible cases of poliomyelitis, including those with atypical manifestations, will be included in the surveillance system. In addition, AFP surveillance helps monitor the quality of surveillance even in the absence of cases of poliomyelitis. In the last stages of the eradication program, no cases of poliomyelitis (except for rare cases of VAPP) would be expected to be detected. Thus, it would be impossible to determine whether the absence of poliomyelitis cases represents "true" absence or deficiencies in surveillance. On the basis of the experience in the Americas, in each population, a rate of at least one case of nonpolio AFP per 100,000 population younger than 15 years of age would be expected annually (this rate was increased to at least two cases of nonpolio AFP for polio-endemic and recently infected countries), and achievement of such a rate would indicate adequate surveillance, defined as the ability of the surveillance system to detect wild poliovirus circulation resulting from indigenous transmission or virus importation, should it occur. A global network of 145 formally accredited laboratories has been established to process all stool specimens collected from AFP cases worldwide for virologic investigations (Fig. 49.19). The formal annual accreditation process relies on six criteria (Table 49.19). Standard methods and reagents are used to isolate virus in tissue cultures and perform ITD as wild, Sabin-derived (<1% genomic sequencing divergence from Sabin parent strains), or vaccine-derived (>1% genomic sequencing divergence from Sabin parent strains) poliovirus. For poliovirus type 2, 0.6% (six nucleotide changes) or greater qualify isolate to be included as cVDPV. All wild poliovirus strains and all VDPVs are sequenced in specialized network laboratories to guide programmatic action. Based on additional virologic and epidemiologic data, VDPVs can further be classified as



Country	Onset of most recent case	Number of WPV1 cases		Number of WPV infected districts	
		Current	Same period last year	Current	Same period last year
Nigeria	21-Aug-16	4	0	3	0
AFR	**21-Aug-16**	**4**	0	**3**	**0**
Pakistan	03-Nov-16	7	18	5	8
Afghanistan	12-Oct-16	6	13	1	10
EMR	**03-Nov-16**	**13**	**31**	**6**	**18**
Global	**03-Nov-16**	**17**	**31**	**9**	**18**

Figure 49.18. Acute flaccid paralysis cases with wild poliovirus (WPV) isolation, previous 6 months (February 5 to August 4, 2015). Excludes viruses detected from environmental surveillance. AFR, African Region; EMR, Eastern Mediterranean Region.

circulating (cVDPV) or associated with prolonged replication in an immunodeficient person (iVDPV) (see "Vaccine-Derived Polioviruses" earlier).

All six regions of the WHO have achieved a rate of slightly more than one nonpolio AFP case per 100,000 population younger than 15 years of age in 2001 and 2015. Table 49.20 shows the AFP cases and wild poliovirus cases for the WHO regions in 2001 and 2015.[39,668] In 2005, the Advisory Committee on Poliomyelitis Eradication recommended that the nonpolio AFP rate in polio-endemic countries should be at least 2/100,000 persons younger than 15 years of age. All three polio-endemic WHO regions reported a nonpolio AFP rate of more than 3/100,000 persons younger than 15 years of age in 2015 (African Region, 5.1; Eastern Mediterranean Region, 5.2; and South-East Asia Region, 7.3 (data as of June 16, 2015), greatly increasing the sensitivity of poliomyelitis detection. All WHO regions also surpassed the second most important quality indicator for adequate surveillance (i.e., the proportion of AFP cases from which two stool samples had been obtained within 14 days of paralysis onset). This indicator was 89% in 2015 globally. Table 49.21 provides a comprehensive list of performance indicators that are used to monitor the quality of AFP surveillance.

Remaining Obstacles to Eradication

The major obstacle to achieve eradication is the failure to implement adequately the current eradication strategies. The eradication strategies have been successful in almost all countries and regions within countries. However, the three remaining polio-endemic countries (i.e., those that never eliminated indigenous wild poliovirus transmission) pose special challenges. In Afghanistan, access in the southeastern part of the country is limited owing to security concerns. In Pakistan, access in the tribal territories (Federally Administered Tribal Areas and Khyber Pakhtunkhwa) along the border with Afghanistan remains a problem, and coverage in some accessible areas remains suboptimal. The killing of vaccinators further complicates the issue, requiring new security strategies to ensure the safety of these health workers. In Nigeria, access to areas not controlled by the government will be critical to wiping this reservoir in Borneo State. Innovative strategies and implementation of new tools must be tailored for each of these polio-endemic areas to achieve eradication. In addition, access to and reaching high immunization coverage among migrant and nomad populations is an issue for all these polio-endemic countries.

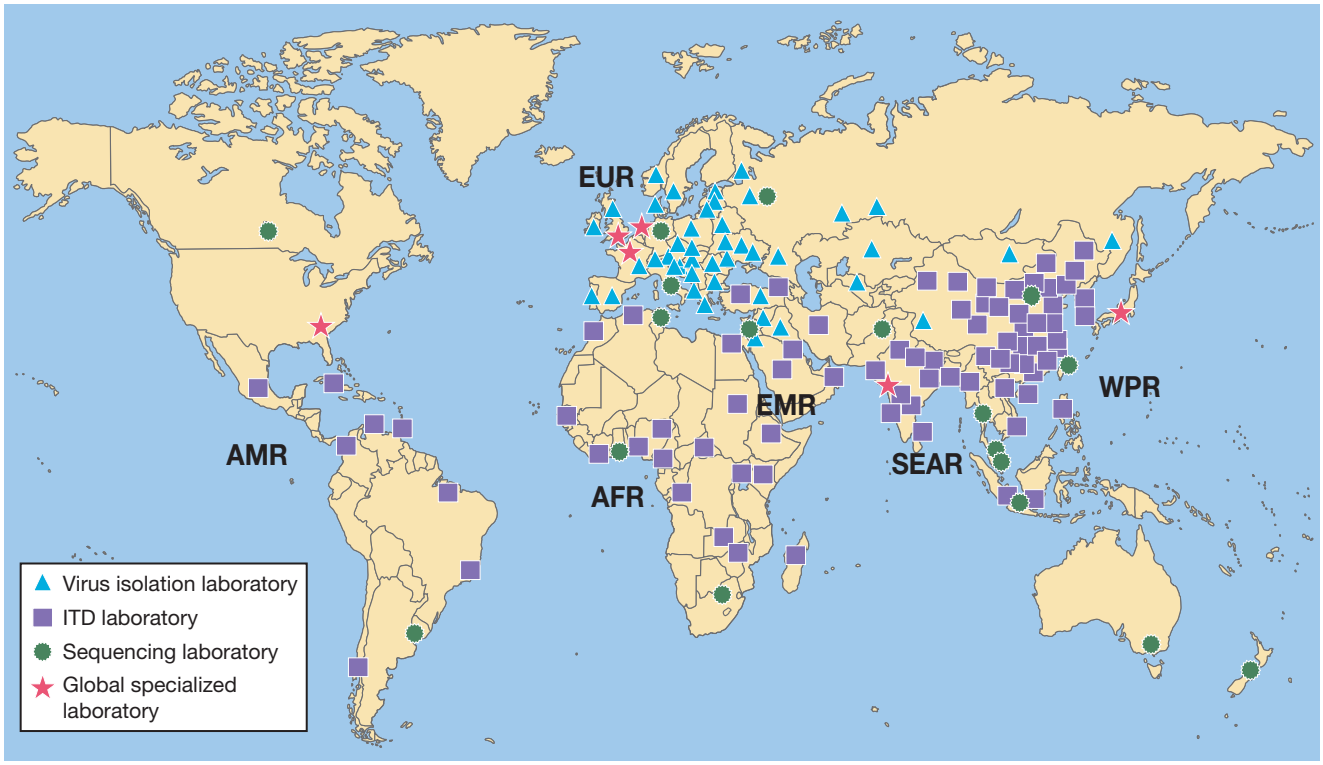


Figure 49.19. Laboratories of the Global Polio Laboratory Network (GPLN), organized by diagnostic function. Viral isolation laboratory *(blue triangle)*, ITD (intratypic differentiation) laboratory *(purple square)*, sequencing laboratory *(green circle)*, global specialized laboratory *(red star)*. WHO Regions: AFR, African Region; AMR, American Region; EMR, Eastern Mediterranean Region; EUR, European Region; SEAR, South-East Asia Region; WPR, Western Pacific Region.

TABLE 49.19 Indicators for Laboratories Included in the Global Polio Laboratory Network (GPLN) by Functionality Status (Virus Isolation, Intratypic Differentiation, and Sequencing)

VIRUS ISOLATION LABORATORIES	
1.	Tests are performed on at least 150 stool specimens annually.
2.	Results are reported on at least 80% of all acute flaccid paralysis (AFP) specimens within 14 days.
3.	At least 80% of suspected poliovirus isolates from AFP cases and contacts are forwarded for intratypic differentiation (ITD) within 7 days, if applicable.
4.	Accuracy of identification of polioviruses among all isolates obtained in L20B cells is at least 90%.
5.	Score on most recent virus isolation proficiency testing is at least 90%.
6.	Score on annual on-site review is at least 80%.
INTRATYPIC DIFFERENTIATION (AS WILD OR VACCINE-DERIVED) LABORATORIES	
1.	ITD test results on ≥80% of poliovirus isolates are reported within 7 days.
2.	Non–Sabin-like poliovirus and ITD-discordant isolates from ≥80% of AFP cases, contacts, and other sources are referred for sequencing within 7 days of detection, if applicable.
3.	Score on most recent intratypic differentiation PT is at least 90%. • Score for rRT-PCR ITD • Score for rRT-PCR VDPV screening
4.	Accuracy of poliovirus serotype and intratype is at least 90%.
5.	Score on annual on-site review is at least 90%.
SEQUENCING LABORATORIES	
1.	Nucleotide sequences are generated and analyzed on site for at least 25 polio or non-polio enterovirus isolates annually.
2.	Complete viral protein 1 (VP)1 nucleotide sequence results on ≥80% of poliovirus isolates are reported within 7 days.
3.	The score is ≥90% on the most recent WHO poliovirus sequence proficiency test.
4.	The score on annual on-site review is at least 90%.

TABLE 49.20 Acute Flaccid Paralysis and Wild Poliovirus Cases by WHO Region and Globally, 2001–2016[a]

	AFP Cases (Virologically Confirmed Wild Poliovirus Cases) Reported[b]						
	WHO Region[c]						
Year	AFR	AMR	EMR	EUR	SEAR	WPR	Global
2001	8542 (69)	207 (0)	365 (143)	164 (3)[d]	1012 (268)	629 (0)	3319 (483)
2002	8587 (208)	268 (0)	4625 (110)	117 (0)	1200 (100)	635 (0)	3632 (118)
2003	8181 (446)	229 (0)	5290 (113)	129 (0)	1189 (225)	697 (0)	3415 (784)
2004	9719 (934)	209 (0)	6176 (187)	116 (0)	1670 (134)	621 (0)	4211 (155)
2005	1183 (879)	213 (0)	8849 (727)	179 (0)	3130 (373)	680 (0)	6234 (179)
2006	1272 (189)	251 (0)	8739 (107)	181 (0)	3665 (701)	711 (0)	6819 (197)
2007	1280 (367)	211 (0)	9394 (58)	149 (0)	4624 (894)	637 (0)	7795 (115)
2008	1456 (912)	263 (0)	1099 (174)	160 (0)	5009 (565)	617 (0)	8504 (151)
2009	1527 (691)	173 (0)	1011 (176)	163 (0)	5462 (741)	691 (0)	9027 (104)
2010	1600 (657)	206 (0)	1138 (169)	287 (478)[e]	6056 (48)	601 (0)	9888 (152)
2011	1636 (350)	104 (0)	1142 (278)	144 (0)	6531 (1)	703 (21)[f]	10460 (650)
2012	1810 (128)	237 (0)	1100 (95)	129 (0)	6676 (0)	785 (0)	10637 (223)
2013	2053 (80)	169 (0)	1140 (336)	100 (0)	5956 (0)	647 (0)	10165 (416)
2014	2248 (17)	215 (0)	1225 (342)	191 (0)	5936 (0)	673 (0)	10493 (359)
2015	26,057 (0)	1949 (0)	13196 (74)	1617 (0)	50,910 (0)	6525 (0)	100,254 (74)
2016[g]	30,610 (4)	2037 (0)	14,147 (30)	1657 (0)	48,046 (0)	6236 (0)	102,733 (35)

[a]Starting in 2001, all wild poliovirus case counts were based on virologic confirmation by the GPLN.
[b]Circulating vaccine-derived poliovirus outbreaks not included.
[c]WHO Regions: AFR, Africa; AMR, Americas; EMR, Eastern Mediterranean; EUR, Europe; SEAR, Southeast Asia; WPR, Western Pacific.
[d]Importations of wild poliovirus type 1 into Bulgaria (from Pakistan) and Georgia (from India).[298]
[e]Outbreaks in Tajikistan, Turkmenistan, Kazakhstan, and Russian Federation following importation of wild poliovirus type 1 from India.[669]
[f]Outbreak in Xinjiang, China following importation of wild poliovirus type 1 from Pakistan.[670]
[g]Data as of December 28, 2016.
AFP, acute flaccid paralysis; GPLN, Global Polio Laboratory Network; WHO, World Health Organization.
From WHO (see https://extranet.who.int/polis/public/CaseCount.aspx).

TABLE 49.21 Indicators of Acute Flaccid Paralysis Disease Surveillance and Laboratory Performance

Indicator	Target
Nonpolio AFP rate in children <15 y in endemic countries	≥2/10000
Nonpolio AFP rate in children <15 y in nonendemic countries	≥1/10000
Completeness of monthly reporting	≥90%
Timeliness of monthly reporting	≥80%
Reported AFP cases investigated ≤48 h after report	≥80%
Reported AFP cases with 2 stool specimens collected ≤14 d since onset	≥80%
Reported AFP cases with a follow-up examination at least 60 d after paralysis onset to verify the presence or absence of residual paralysis	≥80%
Specimens arriving at national laboratory within 3 d of being sent	≥80%
Specimens arriving at laboratory in good condition[a]	≥80%
Specimens with a turnaround time of ≤28 d between receipt and reporting of results	≥80%

[a]*Good condition* means that, on arrival (a) there is ice or frozen icepacks or a temperature indicator (showing <8°C) in the container, (b) the specimen volume is adequate (>5 g), (c) there is no evidence of leakage or desiccation, and (d) appropriate documentation (laboratory request/reporting form) is completed.
AFP, acute flaccid paralysis.

Historically, most importation into polio-free countries has originated in India or Nigeria. However, given the progress toward eradication in these countries, Pakistan is associated with the most recent exportations of virus.

New Polio Endgame

Completion of polio eradication requires the elimination of all polioviruses from human populations, whether wild poliovirus (WPV) or those originating from the OPV.[278,477,672] While it was apparent for longer than a decade that the continued use of OPV was not compatible with polio eradication, only the *Polio Eradication & Endgame Strategic Plan 2013–2018* provides the detailed framework for interruption of WPV transmission in remaining reservoirs and lays out the plan for the new polio endgame, which includes the sequential withdrawal of Sabin strains, starting with type 2, and the concurrent (or earlier) introduction of IPV, for risk mitigation purposes.

The objectives and supporting data that form the backbone for the policy decision to switch from tOPV to bOPV (i.e., OPV2 withdrawal), to introduce one dose of IPV into routine immunization schedules, and describe the proposed implementation of this policy in tOPV-using countries are summarized below.

Although most infections with OPV are self-limited, in immunodeficient individuals such viruses may replicate for prolonged or chronic periods. Similarly, prolonged person-to-person transmission is possible in communities with low immunity against poliovirus. Both of these mechanisms could

potentially lead to the reestablishment of endemicity and negate the polio eradication efforts.[155,156] Therefore, strategies for the elimination of all polioviruses, including the attenuated Sabin vaccine viruses originating from the OPV, needed to be developed and implemented successfully to achieve polio eradication.

The Strategic Advisory Group of Experts (SAGE) on Vaccines and Immunization, a committee established to advise the WHO on global policies and strategies for vaccines and immunization, established a working group in August 2008 to advise the GPEI on two major programmatic issues: (a) the use of IPV in low- and middle-income settings; and (b) on the polio endgame strategy to reduce long-term risks associated with OPV.[673,674]

This SAGE process has led to the development of a comprehensive plan entitled the *Polio Eradication & Endgame Strategic Plan 2013–2018* for the interruption of WPV transmission and to achieve the long-term goals of the posteradication period.[550,675] The plan contains four objectives: (a) to interrupt WPV transmission; (b) to strengthen immunization systems and the withdrawal of OPV; (c) to implement containment of polioviruses and to certify the world as polio-free; and (d) to plan the legacy of polio eradication.

The plan addresses the cessation of the type 2 component of OPV (switch from tOPV to bOPV), and the global introduction of at least one dose of IPV into routine immunization schedules of all countries relying currently only on OPV for poliomyelitis prevention.

Switch From Trivalent Oral Poliovirus Vaccine to Bivalent Oral Poliovirus Vaccine and Inactivated Polio Vaccine Introduction Into Routine Immunization Schedules. Of the three serotypes of WPVs: types 1, 2, and 3 (WPV 1, WPV 2 and WPV 3), WPV 2 was successfully eradicated in 1999.[477,677] However, at the end of 2013, 144 countries relied on tOPV, containing Sabin type 2 live vaccine virus, for routine immunizations against poliomyelitis; 124 of these, including all Global Alliance for Vaccines and Immunization (GAVI)-eligible countries (except Ukraine), used exclusively tOPV, and 20 countries used both tOPV and IPV in sequential immunization schedules. The remaining 50 WHO member states use IPV exclusively; these are primarily industrialized countries (see Fig. 49.14).

Polioviruses contained in OPV are live-attenuated polioviruses (see "Virology" earlier), which, in rare circumstances, can revert to neurovirulent forms and cause VAPP, clinically indistinguishable from paralytic poliomyelitis caused by WPVs.[542,678–680] It is estimated that each year there are between 250 and 500 cases of VAPP worldwide (WHO, unpublished data).

However, more important and a potential obstacle to polio eradication, Sabin viruses may mutate and subsequently gain the ability to circulate in communities though person-to-person transmission. These polioviruses, referred to as VDPVs, also have lost their attenuating mutations, and therefore reacquired the neurovirulence and transmission characteristics of WPVs. In very rare occasions, VDPVs could potentially reestablish endemic and epidemic transmission, and are, therefore, incompatible with polio eradication (see "Vaccine-Derived Polioviruses" earlier).[549,681] Since WPV2 eradication in 1999, VDPVs caused by type 2 Sabin strains (VDPV2) have been reported from 17 countries causing 599 reported paralytic cases (data as of December 28, 2016).

OPV vaccine is needed to achieve eradication, but it also poses a grave risk to eradication. This paradox needs to be addressed proactively with the strategy of a phased withdrawal of all Sabin strains from OPV, starting with type 2: switching tOPV to bOPV.

Thus, OPV2 withdrawal will stop the introduction of Sabin type 2. Before the withdrawal of type 2 in April 2016, more than 2 billion doses were administered annually. Therefore, the withdrawal should eliminate the VAPP caused by Sabin 2 and the cVDPV2 outbreaks attributed to the type 2 Sabin virus. bOPV developed and licensed in 2009 for campaign use achieves higher seroconversion rates compared to tOPV for types 1 and 3.[476] In the Indian trial, after two doses of OPV vaccines, seroconversion to poliovirus type 1 was 86% for bOPV compared with 63% for tOPV ($P < .0001$), and to poliovirus type 3 it was 74% for bOPV compared with 52% for tOPV ($P < .0001$). Thus, the use of bOPV will likely increase population immunity against polioviruses (except for poliovirus type 2). Introduction of bOPV into campaign use in India has likely facilitated the elimination of polio in that country.[682]

As a consequence, and to eliminate the risks associated with continued use of Sabin type 2–containing vaccines, SAGE recommended the withdrawal all OPV vaccines containing Sabin type 2 poliovirus, adhering to the following three principles: (a) the switch must be universal; (b) the switch must be synchronized; and (c) IPV must be introduced in every country, ideally 6 months prior to the switch. The switch must be universal and synchronized because following the switch, the population immunity to type 2 polioviruses will decrease, increasing over time the risk for paralytic disease among individuals and the possibility of outbreaks. As a result, bOPV-vaccinated populations would become vulnerable to type 2 importations from areas where tOPV would still be in use, leading to circulation and mutation of those viruses resulting in the emergence of new cVDPV2 outbreaks. And potentially cVDPV2, if occurring in tOPV-using areas, could lead to exportations from those areas into bOPV-only using countries, leading to significant type 2 outbreaks.

Modeling data suggest that this potential risk (silent circulation leading to epidemic transmission) will be highest in the immediate post-OPV2 withdrawal period, when these viruses are still replicating and mutating in the intestinal tract of millions of recently OPV-vaccinated infants. This risk will likely rapidly decline as illustrated by the detection of OPV virus in environmental samples for only limited periods (i.e., 3 months) following cessation of OPV use.[155,156,683,684] To mitigate the consequences of type 2 cVDPV outbreaks (in terms of paralytic disease) in the immediate post–OPV2-cessation era and for the longer term, SAGE recommended the universal introduction of at least one dose of IPV into routine immunization schedules in OPV-only using countries. Data suggest that one dose of IPV will provide an immunity base that can be rapidly boosted in case of cVDPV2 emergence.[685] A priming immune response, as measured by induction of antibody within 7 days after vaccination in a child previously seronegative, was observed to poliovirus types 1, 2, and 3 in 97.6%, 98.3%, and 98.1% of infants who were revaccinated after failing to seroconvert after receiving a single dose of IPV at 4 months of age in Cuba.[685]

In addition to providing protection against cVDPV2, IPV boosts mucosal immunity to polioviruses in previously OPV-vaccinated recipients.[489] IPV also helps close the population humoral immunity gap in children who had not seroconverted following OPV vaccinations.[618,686] Overall, the bOPV and IPV schedule is expected to provide higher levels of protection against poliomyelitis than a schedule with tOPV alone.

Decision Points for Oral Polio Vaccine Type 2 Withdrawal. To make the decision when to switch from tOPV to bOPV (implemented in April 2016), several readiness criteria were assessed, including that all persistent cVDPV2 outbreaks (documented circulation for more than 6 months after

initially detected [i.e., failure of programmatic efforts to contain the outbreak]) be stopped. In 2013–14, three persistent outbreaks with cVDPV2 were ongoing: (a) the longstanding cVDPV outbreak in Nigeria[136,556,557]; (b) an outbreak with cases covering Chad, Cameroon, Niger, and Nigeria; and (c) an outbreak with cases detected in Afghanistan and Pakistan. While these persistent outbreaks appeared to have been stopped (data as of October 28, 2015), two new persistent outbreaks evolved in both Pakistan and Nigeria. Other readiness criteria included (a) the absence of WPV2 which was validated by the Global Certification Commission[190]; (b) a mOPV2 stockpile must be established and available for outbreak control following a possible postswitch poliovirus type 2 emergence; (c) the laboratory containment standards for type 2 polioviruses must be implemented (Phase I and Phase II); (d) sufficient vaccine supplies of bOPV must be available and licensed in all countries for routine immunization use; and (e) sufficient supply of affordable IPV must be available to meet the global demand.

The Strategic Plan (2013–18) for polio eradication includes as a key objective the sequential cessation of vaccination with Sabin strains (starting with poliovirus type 2). This objective makes it necessary to replace tOPV with bOPV for routine and supplemental immunization activities, and to add IPV into routine immunization schedules for risk mitigation.[686a] The withdrawal of Sabin type 2 from the tOPV is necessary to secure polio eradication for the future, since the live attenuated Sabin strains may mutate or recombine to reacquire greater neurovirulence and transmissibility. The resultant VDPVs are phenotypically indistinguishable from wild polioviruses and have the potential to reestablish sustained endemic and epidemic transmission.

The criteria for and the timing of the switch were monitored by the principal technical oversight committee for immunization (i.e., the SAGE).[686b] The programmatic responsibility for implementing the switch was with the GPEI. Within WHO, the Immunization, Vaccines and Biologicals Department (IVB) department, together with the regional and country offices, provided guidance to member countries. Soon after endorsement of the Strategic Plan by SAGE and the World Health Assembly, a cascading political and technical advocacy effort was implemented, starting with reviews of the switch objectives and endorsement by Regional Committees, Regional Technical Advisory Groups (TAGs), and National Immunization Technical Advisory Groups (NITAGs). Countries were encouraged to prepare comprehensive national switch action plans based on a standard global template.

Of great importance was the regulatory approval of bOPV in the 155 countries that needed to introduce this vaccine, and through using different regulatory approval channels (e.g., national registration, WHO-prequalification or emergency approval) it was possible that bOPV could be used in all countries that needed it. Furthermore, the same regulatory channels were used for the 125 countries needing to introduce IPV. In 2014, bOPV was the primary regulatory activity focus, and this shifted to IPV in 2015.[686c]

In addition, finding regulatory solutions for domestic producers of tOPV was another priority. Domestic producers in China, India, Indonesia, Iran, Mexico, Russian Federation, and Vietnam needed to license nationally produced bOPV. By implementing an intensive advocacy effort, and conducting the relevant clinical trial work, where required, all of these countries were able to license bOPV nationally or obtain approval for importation of WHO-prequalified bOPV.

Later, a growing and unexpected challenge emerged with the rapidly deteriorating IPV supply situation before and after April 2016. This has delayed IPV introduction (20 countries) and delayed re-supply of IPV (31 countries). IPV supply is prioritized by country risk category, with tier 1 countries (those with outbreaks of cVDPV2, or poliovirus type 1-endemic) receiving the highest priority.[686d] On the other hand, tier 3 and 4 countries, those assumed to be at lowest risk, constitute the majority of countries that currently do not receive IPV. In these countries new birth cohorts are growing up without any immunity to poliovirus type 2. It is anticipated that in 2018, the supply situation will return to normal (i.e., all countries should receive sufficient doses of IPV).

To respond to the IPV supply situation, some countries have introduced a schedule of two fractional doses of IPV, with doses administered at 6 and 14 weeks.[686e,686f] This schedule is both dose sparing and more immunogenic than the current single full dose IPV recommendation.

Once the timing for the switch was finalized by SAGE in October 2015 for April 2016,[686b] the implementation efforts were augmented with provision of funding, where needed, and consultants, where requested. Each country had to decide on a 2-week switch period during April 2016 in which tOPV was withdrawn and replaced bOPV. tOPV was then discarded according to national preference of an appropriate method. At the end of the switch period, an extensive monitoring phase was implemented that included national and international monitors to verify that tOPV had been discarded (i.e., could not be found in national, regional, and health center vaccine stores).

The switch was completed in virtually all countries within the selected 2-week period, and the monitors found very few vials of tOPV missed by the national implementation staff. This globally synchronized effort is a great example of success of what can be achieved if the global public health community unites behind an important public health goal and is willing to set in motion the necessary action.

Implementation of Inactivated Polio Vaccine Into Routine Immunization Schedules. In terms of progress toward IPV introduction, 124 countries have introduced IPV, 29 countries have introduced IPV but have not been resupplied (and are in stock-out), and 21 countries did not introduce IPV in 2016 because of IPV supply constraints. This progress is the result of an unprecedented effort by all immunization partners (Expanded Programme on Immunization, GAVI, GPEI) to facilitate and fund, by-and-large, the introduction efforts. At present, it is planned that the complete withdrawal of OPV vaccines (including bOPV) will be implemented 3 years after the last WPV is detected.

Major Risks Associated With Oral Polio Vaccine Cessation. While there are compelling benefits to eventually stop OPV, these must be weighed against the associated risks. The risks can be considered in three categories: the live attenuated poliovirus strains used for OPV production (e.g., Sabin strains), VDPVs, and WPVs. In each case, the risk is of human infection and subsequent transmission following ingestion of the virus. Poliovirus survival in the environment is finite; infectivity is lost during a period of days, under hot dry conditions, to weeks or several months under cool, moist conditions.[249]

The term *Sabin strain* poliovirus denotes one of the three live attenuated viruses that Albert Sabin developed for the OPV (see "Description of Vaccine" earlier). Sabin strain polioviruses are ubiquitous because of routine immunization programs that currently use approximately 2 billion doses of OPV each year globally to vaccinate children in more than 150 countries. Such live attenuated polioviruses are present in a variety of other settings owing to their use as seed viruses for OPV production, as reference standards in vaccine quality assurance and control testing, as controls for some polio

diagnostic tests, and for basic research in a number of laboratories and for teaching purposes in some academic centers. While the vast majority of OPV recipients will lead to time-limited (i.e., 3 to 4 weeks) intestinal replication and shedding of the virus, Sabin viruses rarely can give rise to cVDPVs or generate an iVDPV. Of particular importance for risk management planning is the danger posed by the emergence of one or more cVDPVs immediately after countries stop using OPV, when population immunity will already be starting to decline. Mathematical modeling suggests that even with simultaneous cessation of OPV use worldwide, a 60% to 95% chance exists of at least one such outbreak occurring somewhere in the world during the 12 months immediately after cessation, with that risk declining to 1% to 6% at 36 months and much lower thereafter.[687] To decrease the risks of cVDPV emergence, simultaneous type-specific OPV cessation worldwide is of critical importance. Should cVDPV outbreaks occur, mOPV will be used for rapid type-specific control and elimination. However, the use of mOPV itself may be associated with generating new cVDPVs. To minimize this risk, high coverage with mOPV is essential, and other strategies such as supplemental IPV or use of antiviral compounds may be needed in a ring campaign around the initial mOPV-targeted areas.[688] After global cessation of OPV, cohorts susceptible to poliomyelitis will accumulate rapidly, and the risk assessment would likely change.[689]

Retaining WPVs in laboratories represents another concern. These are currently used as seed viruses for the production of IPV, in vaccine quality control and assurance testing, as controls for some diagnostic tests, and in research laboratories. Although WPVs are no longer nearly as ubiquitous as Sabin polioviruses, the consequences of an inadvertent or intentional release in the post-OPV era pose a potentially far greater threat. A recent consequence assessment suggests that whereas transmission of a wild or Sabin strain virus may be self-limiting if released into an IPV-vaccinated population in a moderate climate zone in the post-OPV era, a WPV released into an unvaccinated population in a tropical developing country would almost certainly result in a large-scale outbreak with a real risk of eventually reestablishing endemic transmission globally.[689]

As a final concern, full-length poliovirus cDNA has been synthesized by assembling oligonucleotides of plus and minus strand polarity. The synthetic poliovirus cDNA was transcribed by RNA polymerase into viral RNA that translated and replicated in a cell-free extract, resulting in de novo synthesis of infectious poliovirus,[690] demonstrating that, even if poliovirus has become extinct (complete destruction of all virus globally), laboratories could create fully infectious polioviruses.

Fig. 49.20 summarizes, based on current knowledge, the expected evolution of these risks in low- and high-income countries during a probable 3- to 5-year "OPV cessation" phase following confirmation of global interruption of WPV transmission and appropriate biocontainment of all poliovirus stocks globally.

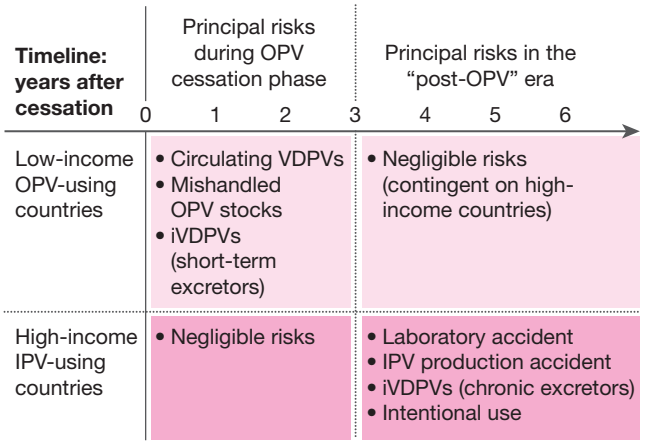


Figure 49.20. Evolution of risk of poliovirus following discontinuation of oral poliovirus vaccine (OPV) among high- and low-income countries. IPV, inactivated polio vaccine; iVDPV, immunodeficiency-related vaccine-derived poliovirus; VDPV, vaccine-derived poliovirus.

Risk Reduction and Management. A comprehensive approach must be taken to optimize the management of the risks associated with OPV cessation. The core principles forming such an approach must be to (a) simultaneously stop the routine use of OPV worldwide, while population immunity is high, with the subsequent recall and destruction of remaining OPV stocks; (b) reduce the number of procedures performed with polioviruses to those that are essential in the post-OPV era; (c) replace, where possible, WPVs with Sabin strain viruses for any procedure that must continue to be conducted in the post-OPV era; (d) minimize the number of sites handling or storing *any* polioviruses or potentially poliovirus-infectious materials (wild viruses before OPV cessation; Sabin strains immediately thereafter), and limit these sites to areas where the consequences of an inadvertent release could be minimized; (e) institute processes to ensure that residual poliovirus-containing sites fully implement appropriate biocontainment and biosafety procedures; (f) maintain high-level surveillance to identify and monitor iVDPVs and to detect an inadvertent or intentional release of any poliovirus into the human population; and (g) establish a stockpile of mOPVs with internationally agreed-on criteria for their use in mounting type-specific outbreak responses to circulating polioviruses in the post-OPV era.

Minimizing the risks associated with OPV cessation requires the endorsement of the international community. Additional risk reduction or risk management strategies may be required in areas that pose particular risks during or after OPV cessation. For example, OPV-using areas with large, high-density populations and low routine immunization coverage may be at high risk for generating cVDPVs and need special attention, such as maximizing the population immunity before stopping OPV. Countries or communities with facilities that continue to store or handle polioviruses in the post-OPV era for IPV production and quality assurance purposes, to provide international diagnostic services, or to conduct specialized research may constitute an international biohazard and require extraordinary measures to prevent or minimize the consequences of inadvertent virus release.

Interruption of Wild Transmission and Containment of Wild Poliovirus. The first and most critical prerequisite before OPV can be discontinued is to ensure that WPV has been eradicated globally. The processes for certification are well-established (see "Certification of Polio Eradication" later), and containment of WPV is an integral part of certification. The objective of containment is to minimize the possibility that poliovirus will be reintroduced into the community. Laboratories holding poliovirus or potentially infectious materials need to ensure safe handling and ultimately appropriate laboratory containment. At present, containment activities focus on a survey of laboratories and inventory for WPV and potentially infectious materials. Eventually, appropriate containment will be needed for all polioviruses (Sabin, VDPVs, and WPV), whether in laboratories or in vaccine production facilities.

Global Surveillance and Notification Capacity. Surveillance strategies relying on AFP to detect all paralytic cases will need to continue for at least 5 years after interruption of WPV circulation (2 to 3 years before OPV cessation and 3 years after detection of the last cVDPVs). At that point, the risk of undetected cVDPV should be negligible. Long-term surveillance strategies would then likely focus on the detection of events (i.e., clusters of paralytic cases), the prompt epidemiologic and virologic investigation of such events, and the institution of effective control measures. Future surveillance efforts will also focus on identifying persons with iVDPVs. The expanded use of environmental surveillance (i.e., sewage sampling) in selected areas to supplement AFP surveillance has been endorsed, particularly in the highest-risk areas. The study in New Zealand conducted before and after the switch to universal IPV provides useful information about the kinetics of vaccine virus disappearance.[156] The New Zealand study relied on three monitoring systems—pediatric inpatient surveillance, AFP surveillance, and environmental surveillance—to detect Sabin-like viruses before, during, and after the switch from OPV to IPV. One month after the switch, no Sabin viruses were detected from pediatric inpatient and AFP surveillance, but environmental surveillance detected Sabin-like viruses until May 2002 (4 months after the switch). After May 2002, Sabin-like viruses were isolated infrequently, and genetic studies showed that these isolates represented recent importations rather than continued circulation of Sabin-like viruses. Thus, in a developed, temperate-climate country, Sabin-like viruses did not circulate for more than 4 months after the switch from OPV to IPV.

Vaccine Stockpile and Response Capacity. This area of work has two major objectives: (a) short-term, to rapidly control outbreaks of cVDPVs, should these occur during a 3- to 5-year transition period after OPV cessation, and (b) long-term, to minimize the consequences of poliovirus reintroduction into the community until polio immunization can be reestablished globally. Therefore, in these countries, infants will no longer be vaccinated against polio, and population immunity to polioviruses will decrease over time. If poliovirus is reintroduced into these countries, rapid control efforts using large quantities of mOPV will be required for outbreak control.

The WHO has established a vaccine stockpile relying on mOPV to allow type-specific responses to the reintroduction of one or more poliovirus serotypes, maximize the immune response, and avoid introduction of unnecessary vaccine poliovirus serotypes. Since 2004–05, mOPV1 and mOPV3 have been licensed in several countries and widely used (see the discussion of mOPV1 development). In 2009, mOPV2 was licensed by a manufacturer and a filler. The WHO is also assessing the role of IPV in such settings, particularly to boost population immunity in high-risk areas not yet affected by such an outbreak.

A stockpile of polio vaccine is necessary after OPV cessation. Many of the basic stockpile issues, such as the size, the vaccine (mOPV) and the composition (filled vaccine vials, bulk, or a combination), number of storage sites, and release protocols (e.g., who retains the authority to release vaccine) have been refined.[691] The primary purpose of a stockpile-and-response capacity is to ensure that control efforts can be instituted immediately after detection of a cVDPV at any time during the 3- to 5-year period following OPV cessation. For the longer term after OPV cessation, other options need to be explored, such as restarting the production of polio vaccines. The immunogenicity and the kinetics of the immune response of stockpile vaccines become critical decision determinants. Monovalent (i.e., type-specific) OPVs are the vaccine-of-choice for the stockpile[391] for the following reasons: (a) immunogenicity per dose is substantially higher compared with tOPV or IPV (one dose of mOPV may provide an immune response to the specific serotype that is equivalent to three doses of tOPV); (b) mOPVs are faster in inducing a type-specific immune response (one would not have to wait for administration of several doses); and (c) their use would be directed against a specific serotype of poliovirus, without introducing unnecessary serotypes of vaccine virus. Since December 2004, regulatory approval for mOPV1, mOPV2, and mOPV3 has been obtained (see Table 49.10).

Inactivated Polio Vaccine Vaccination Requirements. The WHO has prepared position statements on IPV for the pre-eradication and posteradication eras that provide guidance.[641,692,693] In addition, a decision was made to introduce one dose (or more) of IPV in all OPV-using countries.[607] Recent work suggests that a one-dose fractional IPV given in the second half of the first year (to reduce the influence of maternally derived antibody on IPV immunogenicity) may provide a low-cost option to induce and maintain a basic immunity against all three poliovirus serotypes. The use of fractional-dose IPV has recently been endorsed for outbreak response by the GPEI to counter the current IPV supply limitations.

The introduction of IPV is funded in GAVI-eligible and graduated countries for at least 5 years.

Coordinated Oral Polio Vaccine Cessation. To minimize the risk of emergence of and exposure to cVDPVs during the period following OPV cessation, all countries will need to stop the use of type-specific OPV Sabin strains during a relatively short period (ideally, a few weeks) everywhere, and all must institute mechanisms to ensure that OPV from throughout the health system has been recalled and destroyed. There must be agreement that no country will again use OPV, unless specifically endorsed by the international community for control of an outbreak.

Sabin Poliovirus Containment. Shortly after global OPV cessation, Sabin polioviruses must be contained. The WHO has prepared a *Global Action Plan to Minimize Facility-Associated Risk in the Post-Eradication/OPV Era*, which was updated in 2014.[694,695] This update is responsive to the new endgame strategic plan which calls for the sequential cessation of Sabin strains (starting with Sabin type 2), and the introduction of IPV in all OPV-using countries for risk mitigation purposes.

The communication of the endgame issues is another very important objective of the GPEI. It is important to understand the current thinking and to appreciate that it is a work in progress, and that new scientific data, programmatic experiences, and expert advice all will feed into a program of work that allows the adoption of policies that minimize the paralytic burden of poliomyelitis to current and future generations of children.[696,697]

There have been voices that have questioned whether eradication is feasible.[698,699] However, if the program learned anything during the past few years, it is that if eradication efforts are stopped, the world will have to live with hundreds of thousands of cases of paralytic disease again because routine immunization coverage in many parts of the world has not improved substantially since 1988. Hence, susceptible populations will accumulate, leading to large polio outbreaks. Only the aggressive use of mass vaccination campaigns has led to control and elimination of polio transmission in many parts of the world. The experiences from outbreaks, particularly in Yemen, where imported poliovirus caused 479 cases in a country with suboptimal routine immunization, and interruption of mass vaccination campaigns for more than 1 year,

present an example of how polioviruses can exploit the immunity gaps in the population. Similar large-scale outbreaks that occurred in Tajikistan[216] and Congo-Brazzaville[217,243] in 2010 were the result of critical immunity gaps against polioviruses. To achieve eradication, such importations need to be controlled rapidly and, still better, prevented by eliminating the reservoirs in polio-endemic countries that export viruses into countries currently polio free.

Certification and Verification of Polio Eradication

The WHO has established a formal process for the certification of polio eradication to ensure that future immunization policy decisions are based on the best available evidence.[700] The certification process for polio is modeled on the International Smallpox Certification Commission, which certified in December 1979 that smallpox had been eradicated.[701] However, polio certification differs in important ways from the smallpox model. The Pan American Health Organization established its Regional Certification Commission (RCC) in 1990, and certified regional eradication of indigenous WPVs in 1994,[702,703] setting the standard for certification by individual regions and implicitly considering genetic evidence to distinguish indigenous WPVs from imported virus. Because of the high proportion of inapparent WPV infections, regional certification requires a period of at least 3 years of sensitive surveillance since the last reported case associated with indigenous WPV. Small outbreaks from imported virus occurred on the eve of certification in the Western Pacific (in China in 1999[704,705]) and in Europe (in Bulgaria[706] and Georgia[298,500] in 2001), but they were quickly controlled and followed by intensive surveillance. Certification of eradication of indigenous WPVs by four WHO Regions (comprising >80% of the world's population)—the Americas in 1994,[702,703] the Western Pacific in 2000,[707] Europe in 2002,[500] and South-East Asia in 2014[501,708]—has stood the test of time. No indigenous WPV circulation has been detected in these regions postcertification.[709,710] Cases and outbreaks associated with imported WPVs and cVDPVs (in addition to cases of VAPP) have occurred subsequently, but circulation has been promptly stopped by effective outbreak control measures.[66,309,311,553,669,670,711] Similarly, documentation in all countries except Pakistan and Afghanistan has been accepted by the Eastern Mediterranean RCC. Several countries in the African Region have also prepared detailed documentation of polio-free status.[550]

Review of documentation to assess polio-free status is organized into three tiers.[700] National Certification Committees (NCCs) meet to critically assess national data and prepare detailed reports to RCCs, which, in turn, report to the Global Certification Commission (GCC). The NCCs, RCCs, and GCC meet regularly, and in the regions already certified as free of poliovirus circulation, the RCCs, working with NCCs, are primarily responsible to oversee and support activities to maintain the polio-free status. The NCC reports contain current information on (a) national demographic data, including high-risk populations; (b) poliovirus vaccine coverage and any changes in vaccine policy; (c) surveillance data and indicators of surveillance sensitivity; (d) descriptions of any poliomyelitis cases; (e) information on the performance of laboratories serving each country; and (f) progress toward poliovirus containment. NCC, RCC, and GCC members are charged to serve as independent experts. Following certification of all WHO regions, the GCC reviews all RCC reports and other relevant information to determine when the world can be declared free of all poliovirus circulation.[700]

Before type 2 OPV cessation (the tOPV-to-bOPV switch) in April 2016, the GCC reviewed all available evidence that WPV type 2 circulation (see Fig. 49.5) has ceased (confirmation of eradication of wild type 2 poliovirus).[550,712] The evidence was overwhelming that all WPV type 2 circulation stopped after 1999. Although low-level type 2 cVDPV circulation continued into 2015 (see "Vaccine-Derived Polioviruses" earlier), aggressive tOPV campaigns were scheduled with the aim of interrupting all type 2 cVDPV transmission.

Laboratory Containment of Polioviruses

Containment is integral to poliovirus eradication.[713–715] Unlike smallpox, where very few facilities stored materials containing (virus stocks) or potentially containing (clinical specimens) smallpox virus (variola), many facilities worldwide have stored materials containing polioviruses. The purpose of containment is to minimize, as much as possible, the risk of reintroduction into the community of polioviruses stored in laboratories and vaccine production facilities. In 1995, the GCC approved the first *WHO Global Action Plan for Laboratory Containment of Wild Polioviruses*,[716] and established satisfactory completion of containment activities as a requirement for regional certification.[717] The third edition of the global action plan (GAPIII), endorsed by the World Health Assembly in May 2015, links containment to national and international milestones in polio eradication.[695] Under GAPIII, containment will be implemented by countries in three phases, starting with poliovirus type 2. Key activities of Phase I include (a) conducting national surveys of all biomedical laboratories, establishment of type 2 inventories, and reporting to the RCCs; (b) destroying all unneeded poliovirus type 2 materials (risk elimination); (c) transferring needed poliovirus type 2 materials to essential poliovirus facilities; and (d) certifying essential poliovirus facilities for containment (biorisk management). Phase II has started and includes two parts: (a) containment of WPV type 2 and type 2 VDPVs (began at the start of 2016), and (b) containment of type 2 OPV-related polioviruses (begins within 3 months of the bOPV-to-tOPV switch). Phase III begins 3 years after the last detection of any WPV and just before certification of global polio eradication, and also has two parts: (a) containment of all WPVs, and (b) containment of OPV-related polioviruses. The timetable for completion of each phase of containment is based on progress toward type-specific cessation of poliovirus transmission. Nationally certified essential facilities—which will be limited to the absolute minimum needed for vaccine production, quality control, reference, and research—must meet strict, standardized criteria for biorisk management and be located in areas of lowest population risk; performance may be verified by the WHO.[719]

In accordance with the original GCC requirement, Phase I containment of all WPV was completed in 154 of 194 WHO member states, in three WHO Regions certified polio-free, is nearing completion in the South-East Asia and Eastern Mediterranean Regions, and is in progress in the African Region.[715] Worldwide more than 225,000 facilities were included in national laboratory surveys (some facilities housed more than one laboratory storing WPV infectious or potentially infectious materials) and 532 facilities in 45 countries reported storing WPV infectious materials or potentially infectious materials.[715] For example, in the United States, among the 105,356 laboratories surveyed, 180 laboratories in 122 institutions were found to be storing materials containing or potentially containing WPV; 87 held WPV stocks, 56 stored materials potentially containing WPVs, and 37 stored both.[719] Europe completed national surveys in 2006, and the Western Pacific Region in 2009.[720]

A particular challenge will be the appropriate handling and containment of clinical materials (e.g., stool specimens or throat swabs) collected and stored for purposes other than

polio diagnostics,[721] especially specimens collected during the weeks following the last campaigns using type 2–containing OPV and, later, all OPV. The WHO and national health governments must continue to alert the broader health community of the need for containment of all poliovirus infectious or potentially infectious materials.

CONCLUSION

Poliomyelitis has affected humankind since ancient times. It appeared as an epidemic disease in industrialized countries in the 19th century and the beginning of the 20th century. Development and widespread use of poliovirus vaccines have effectively controlled poliomyelitis in all but two countries globally. Type 1 is the only circulating WPV serotype detected since 2012. The new endgame strategy with the objective to remove all polioviruses from populations will likely facilitate eradication, relegating this once much-feared crippling disease to one that future generations will know only by history.[41]

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50 Rabies Vaccines

Charles E. Rupprecht, Thirumeni Nagarajan, and Hildegund Ertl

> *"I have seen agony in death only once, in a patient with rabies; he remained acutely aware of every stage in the process of his own disintegration over a twenty four-hour period, right up to his final moment."*
>
> **Lewis Thomas, *The Lives of a Cell*. New York, NY: Bantam Books; 1974.**

Rabies is an acute, progressive viral encephalitis transmitted from animal to animal or from animal to human by exposure to saliva via bites or other sources of infectious virus. After a bite, virus in saliva attaches to peripheral nerve endings and travels to the brain. Although all warm-blooded vertebrates are believed susceptible to experimental infection, in nature, rabies is a predominant disease of mammals, involving primarily the Canidae (e.g., dogs, wolves, foxes, coyotes, and jackals), Procyonidae (e.g., raccoons), Viverridae (e.g., mongooses), Mephitidae (e.g., skunks), Mustelidae (e.g., ferrets, badgers), and Chiroptera (bats) as reservoirs or vectors.

Human infection is nearly always secondary to animal bite (or very rarely by scratch or other routes), although exposures through the inhalation of virus, inoculation with improperly inactivated vaccine, or transplantation of infected corneas, tissues, and organs have occurred. In most of the world, the major reservoir for human rabies is the dog, responsible for an enormous incidence of bites. Although not functioning as actual reservoirs, per se, cats are known vectors of the disease.

HISTORICAL PERSPECTIVE

Besides focal communications on rabies vaccinology, tens of thousands of highly relevant scientific papers have been written on the diagnosis, epidemiology, immunology, molecular biology, pathogenesis, prevention, treatment, and control of this malady during the late 19th through early 21st centuries, of which only a very small fraction are cited in this chapter. Moreover, considering that rabies is one of the oldest infectious diseases, the following brief summary cannot do justice to the rich history accumulated over the past four millennia. Interested readers are directed to G.M. Baer's "Rabbis and Rabies: A Pictorial History of Rabies Throughout the Ages" (Tlaxcala, Mexico: Laboratorios Baer; 1996) for a more engaging and erudite introduction to this fascinating and overlooked topic.

It has been suggested that rabies existed before 2300 BC, because of a description in the Mesopotamian Laws of Eshnunna.[1] Ancient texts from Egypt, Persia, Palestine, India, and China also seem to contain allusions to this disease.[2] The first certain references to rabies appear in Greek literature. Homer, Democritus, and Aristotle referred to lyssa (related to a mythological Greek goddess of rage and fury) in their writings, and in the 1st century AD, the Roman scholar Aulus Cornelius Celsus provided an accurate description of the disease and the wide range of species susceptible to infection. Rabies is mentioned frequently in Arabic and Western writings during the Middle Ages. In the 15th century AD, the Italian savant Girolamo Fracastoro suggested the concept of the "incurable wound," that is, the disease was always considered lethal and not treatable. In the same vein, the Talmud also mentions that one should not believe people who say that they were bitten by a rabid animal, yet survived.

By the 1500s, shortly after Cortez's exploration of the Americas, the first Bishop of Oceania described "small animals" that bit the toes of Spanish soldiers during the night. The soldiers later died of a disease that may have been rabies. Other Spanish sources describe vampire bats that even now have a major role in the perpetuation of rabies throughout Latin America. As mentioned later, current virologic data suggest that carnivore rabies may have been absent in the New World until introduced with rabid dogs within the 16th century.[2]

Early in the 19th century, one school of thought held that rabies was purely a psychiatric disease. Nevertheless, the dramatic symptoms and nearly 100% fatality rate of rabies attracted the scientific curiosity of many of the first modern microbiologists.[1,3]

The German scientist, Gottfried Zinke, demonstrated experimental transmission of rabies virus in 1804 by inoculation of human saliva into animals. In 1879, in Lyon, France, Pierre-Victor Galtier transmitted rabies virus from dog to rabbit and from rabbit to rabbit and used intravenous injections of infected material to immunize sheep and goats. However, Galtier's work was overshadowed by that of his famous contemporary, Louis Pasteur. Much of the credit for applied rabies research belongs to Pasteur, but one must not overlook the contributions of his other collaborators, Roux, Chamberland, and Thuillier. These investigators conducted most of his critical laboratory manipulations because Pasteur was partially disabled by a stroke at the time.

In 1881, the Pasteur group established that the central nervous system (CNS) was the principal site of rabies virus replication. They transmitted the disease by submeningeal inoculation of infected material into rabbits and were able to maintain the virus in this host for more than 100 passages. Roux noticed that the virulence of infected rabbit spinal cords decreased rapidly when they were suspended in dry air and was extinguished almost completely in 15 days. From this observation, Pasteur developed a practical method of vaccination. Dogs injected subcutaneously with serial suspensions of fragments of infected spinal cords, beginning with cord dried long enough to be avirulent and using successively less-dried cords, resisted rabies when they were injected intracerebrally with a virulent virus.

At least 50 dogs were protected by Pasteur's protocol. Although experiments performed to demonstrate that animals could be made refractory to rabies by vaccination after bites by "mad" dogs were inconclusive, successful inoculation of one person, Joseph Meister, was attempted on July 6, 1885, after other unsuccessful and unpublicized attempts at human vaccination. Meister, who had been bitten 14 times by a rabid dog some 60 hours previously, received a subcutaneous inoculation of spinal cord suspension derived from rabid rabbits and preserved in a flask of potassium hydroxide for 15 days. Twelve successive inoculations were made with cords of increasing virulence, for a total of 13 inoculations during an approximately 10-day period. The boy not only resisted the original animal source of rabies virus infection, but also

survived large quantities of highly virulent virus that were contained in the final doses of vaccine.

This Pasteur method, as the first generation of rabies vaccination, aroused great interest in medical circles and, despite some major disagreements, was rapidly accepted. Thereafter, the Pasteur Institute of Paris was founded in 1888, and within a decade, there were Pasteur Institutes throughout the world providing rabies vaccination, and setting a precedent for vaccination well into the 20th century.

Criticism, however, was forthcoming. Occasional failures raised questions about the safety of the vaccine, especially because virulent material was inoculated into the patients by the end of the vaccine series. Moreover, there were no controls for comparison to confirm the efficacy of the method. Much later, one tragic accident in Fortaleza, Brazil, in which children died after vaccination, showed that the so-called fixed virus could be virulent if not prepared properly, which led to a century of focus upon the improvement of rabies vaccine purity, potency, safety and efficacy, which prevails to this day.

Understandably, few diseases have carried the historical blend of loathing and superstition attributed to this zoonosis, bridging the gap from scientific fact to folklore, including partial responsibility for the legends of vampires and werewolves. Curiously, in an interesting twist on the macabre, some believe Edgar Allan Poe may have died of rabies in Baltimore, which is not surprising given the variety of ways the disease may present clinically.[4]

CLINICAL PRESENTATION

Rabies vaccination is not successful once a patient presents with signs and symptoms of encephalitis. Such comprehensive clinical descriptions of human rabies have been published by many authors for centuries.[4–18]

While the specific disease may be considered a continuum because of a diversity of variables, in general, the clinical syndrome in humans consists of the following overlapping stages: incubation period, prodrome, acute neurologic phase, coma, and death.[9] Very rarely does recovery occur, even after heroic measures. Once clinical signs occur, death is nearly inevitable.

During the incubation period, there are no symptoms and no means of diagnosis. After exposure, the incubation time of rabies is highly variable in humans, ranging in extreme cases from approximately 5 to 6 days to several years.[5–7] Typically, humans develop symptoms after 20 to 60 days. The length of the incubation time is in part influenced by the site of exposure and tends to be shorter when the virus enters locations closer to the brain. Very long incubation periods in excess of several years have been reported.[8,19,20] Initially, these were viewed with doubt. Nevertheless, such lengthy periods have been substantiated by genetic analysis of the infecting virus, which can pinpoint where and how the infection was acquired initially. For example, immigrants or travelers became infected with a viral variant from a region they had not visited for many months to years and did not occur in the region where the patient eventually succumbed.[7,21,21] However, the specific anatomical location where lyssaviruses remain in vivo, during such long incubation periods, is unclear. Opinions differ if virus remains in the periphery, close to the site of infection, or becomes dormant after entry to the nervous system. After breaching the skin or mucosae, the virus may exist in a stable form within a cellular compartment, relatively shielded from proteolytic enzymes or detection by the immune system.

Most human rabies cases are caused by dog bites. Approximately 37% to 57% of unvaccinated individuals with known exposure to a rabid dog develop rabies. The risk of disease is linked presumably to the amount of virus present in saliva as well as the location and the severity of exposure.[9] The latter is supported by an increased (~80%) risk of disease, following attacks by rabid wolves. The incidence of symptomatic rabies in unvaccinated persons after exposure to other rabid animals, such as bats, is unknown, but may be lower, as suggested by documentation of viral seropositivity, in healthy humans residing in areas of Amazonia, where vampire bat rabies is common.[22]

The initial prodromal symptoms of rabies consist of general malaise, anorexia, fatigue, headache, vomiting, sore throat, fever, and in 50% to 80% of cases, pain or paresthesia close to the bite site, potentially indicative of neuronal damage. Some patients display psychological symptoms, such as anxiety, agitation, irritability, nervousness, insomnia, or depression. The prodromal phase, lasting for 2 to 10 days, is followed by neurological symptoms of rabies, which in humans can present as a furious or paralytic form. The furious form, generally associated with dog bites, is more common and afflicts approximately 65 to 70% of patients. Furious rabies is characterized by periods of hyperactivity, aggressive behavior, agitation, hallucinations, delusions, hypersalivation, muscle fasciculations, seizures, fever, excessive sweating, and priapism. Patients develop difficulties swallowing and hydrophobia (so-called fear of water) as a consequence of painful spasms of their throat muscles, which can be triggered by the mere sight, sound, or even mention of water. Patients may also develop fear of light (photophobia) and air currents (aerophobia). Hyperactive periods in humans last approximately 1 to 5 minutes. These periods may occur spontaneously or are triggered by a variety of stimuli. Between hyperactive periods, the patients are alert and able to communicate, although over time their mental faculties deteriorate.[10]

Paralytic, or "dumb," rabies seems more common after exposure to rabid bats. Patients develop ascending muscle weakness, loss of sensation, and paralysis starting in the extremities and then spreading upwards. Eventually, with either form of rabies, patients fall into a coma and die of cardiac or respiratory arrest. Without supportive care, death occurs within approximately 5 days of furious rabies and approximately 13 days of paralytic rabies. Intensive care can prolong life for a few days or even months, but in general does not prevent death.

In countries where canine rabies is enzootic, this zoonosis is fairly easy to diagnose, with the onset of classical symptoms in an encephalitic patient with a history of suspicious animal bite. However, rabies may not be suspected or easily diagnosed, especially in countries where human disease is rare.[13] This, in turn, is one explanation for the occurrence of human transplantation of tissues and organs, from individuals who succumbed to an undiagnosed illness, to recipients who later developed rabies, and thereby inadvertently allowing for a postmortem diagnosis in the donors, as to cause of death.[23,24]

Rabies may be confused with encephalitis caused by other infectious agents, such as herpes simplex virus, enteroviruses, arboviruses, toxoplasma, or prions, or toxins, such as tetanus toxin. Rabies may mimic atropine poisoning, intracranial malignancies, acute cerebral injuries (e.g., a stroke), or transverse myelitis caused by infections with measles virus or mycoplasma. Some individuals may exhibit rabies symptoms that result from a hysterical reaction to an animal bite. For a thorough diagnosis of rabies, careful history about contacts with animals, unprovoked animal attacks, availability of the animal for testing, vaccination status of the animal, camping with potential exposure to bats, and the like is essential. Physical examination should reveal autonomic instability in furious rabies and symmetric paralysis that may be mistaken for Guillain-Barré syndrome in paralytic rabies. Laboratory testing can be used to rule out other infectious diseases. Magnetic resonance imaging and computed tomography (CT) scanning of

the brain may show some abnormalities, but neither is specific for a diagnosis of rabies.[25–27]

Electroencephalography may show sudden drops in amplitude as a result of vasospasms of cerebral vessels, but, again, findings are not specific to rabies.[14] Cerebrospinal fluid (CSF) commonly shows lymphocytic pleocytosis, but few to no changes in glucose or protein content. Positive antemortem diagnosis requires several tests, performed by a qualified reference laboratory. Skin biopsy specimens can be tested with fluorescent-labeled antibodies for detection of viral antigen present in the cutaneous nerves at the base of hair follicles. Impressions of corneal epithelia can be analyzed using the same method. Harvesting of either type of sample should be conducted with topical anesthesia by a dermatologist or an ophthalmologist. Saliva, CSF, urine sediment, and pulmonary secretions may allow for isolation of virus within approximately 2 weeks after onset of symptoms or such samples can be tested for viral products upon reverse transcription and amplification by polymerase chain reaction (PCR). Antibodies to rabies virus can be detected from serum or CSF via neutralization by 1 to 2 weeks after onset of symptoms.

Clinical signs of rabies in animals are compatible with an acute, progressive encephalitis. An initial prodromal phase, often characterized by changes in behavior and loss of appetite, is followed by a phase where the animal becomes aggressive and may bite without provocation. Other animals may become somnolent. Animals may develop paralysis and quickly die thereafter. As a rule, rabies in animals is diagnosed postmortem from brain impressions using the direct fluorescent antibody test. Microscopic examination of stained sections demonstrates inclusion bodies in the cytoplasm of neurons, especially in the brainstem and Ammon's horn of the hippocampus. Brain impressions can be tested by a fluorescent-conjugated antibody for detection of viral antigens. Alternative methods, such as those based on biotinylated virus-specific antibodies that allow for easier and faster onsite diagnosis by routine light microscopy, rather than immunofluorescence, are being developed.[28]

Historically, isolation of rabies virus had been reported from as much as 0.5% of so-called normal dogs in Ethiopia and elsewhere, presumably in the late incubation stage or early prodromal phase, but the significance of this finding to human rabies occurrence is unknown.[29] The ultimate documentation of a true "carrier state" in rabies for any species awaits further rigorous, objective corroboration, given several alternative explanations.

Attempted Treatment of Symptomatic Rabies

Prevention by vaccination, which is highly effective, needs to be distinguished from medical treatment after clinical onset, which is highly problematic. Rabies has the highest case fatality of any infectious disease. During the 20th century, there were fewer than 10 cases of documented survival among humans who showed signs of disease, most of whom recovered with sequelae.[30–35] Until recently, there had been no serious attempts at therapy, and all putative survivors had received some form of preexposure or postexposure rabies vaccination before illness.[36]

Attempted treatment in the vast majority of cases tended to be palliative, rather than curative. The nearly 100% mortality rate, combined with agonizing symptoms, limited more aggressive treatment, which in most cases merely prolonged suffering for very few individuals that may have had a very low realistic chance of recovery. Routine treatment of human cases was restricted to sedatives, narcotic analgesics such as morphine or its analogues, anticonvulsants, and neuromuscular blockers.[37]

Although transmission of virus from patients to healthcare providers has not been documented, exposure should be minimized by appropriate containment and personal protective devices, as used through barrier nursing procedures. In some cases, more aggressive treatment could be attempted, but with a clear understanding of the patient or the patient's family that death may not be prevented and that in case the patient survives, the patient may suffer lifelong debilitating neurological symptoms. Historically, numerous treatments have been tried, but all ultimately failed. For example, hyperimmune serum, even if administered in high doses, rabies vaccination, treatment with interferon, interferon-inducers, or antiviral drugs, have not been effective.[38,39,40]

During 2004, however, a 15-year-old girl who developed rabies after a bat bite was treated with ketamine and midazolam to induce coma and the antiviral drugs ribavirin and amantadine. She survived with no major cognitive impairment and minimal neurological sequelae.[41] This so-called Milwaukee protocol was tested in its original form or in a revised version without ribavirin in other patients.[42–45] Of the more than 40 patients treated to date, at least five were reported to have survived, implying that aggressive treatment may have some benefits. Patients who survived tended to be young and developed robust immune responses to rabies virus before or during treatment. However, it has been suggested that treatment success was linked not only to superb intensive care, but also to genetic components of the survivors that enabled them to develop an unusually potent virus-neutralizing antibody (VNA) response early after infection. One could offer a counterargument that because passive transfer of rabies hyperimmune serum offers no long-term clinical benefit, that an antiviral B-cell response by itself does not suffice to clear the virus. Nevertheless, the kinetics of the endogenous immune response, potential secretion of antibodies by B cells that migrate into the CNS, as well as added drugs such as ketamine an *N*-methyl-D-aspartate acid receptor antagonists may have contributed to survival.[46,47,48] Another possibility is that positive treatment outcome is linked to peculiarities of the infecting virus. For example, rabies virus was never isolated from the majority of patients who had received experimental treatment protocols. This, in turn, could suggest that the infecting dose was small in the periphery or the virus was somewhat more "temperate" than other street virus variants. In summary, there is no reliable proven therapy for symptomatic rabies.[49] Given the renewed interest after initiation of the Milwaukee protocol, variations thereof might be tried in selected patients, especially if they had previously been vaccinated or presented with an already detectable immune response to virus. Additional research is needed on pathobiological insights, antiviral drugs and development of improved treatments using relevant animal models that will be practical for resource-poor countries, where rabies is most common.[50]

VIROLOGY

An introduction to applied virology yields important insights as to the basic immune mechanisms utilized during rabies vaccination. Rabies virus belongs to the family Rhabdoviridae, genus *Lyssavirus*, consisting of genetically related enveloped viruses with a single, nonsegmented, negative-stranded RNA. The virion is shaped like a bullet, approximately 200 nm long and 75 nm wide. The virus contains multiple copies of the following five structural proteins: virion transcriptase (L), glycoprotein (G), nucleoprotein (N), phosphoprotein (P), and matrix protein (M). The L, N, and P proteins are bound noncovalently to the virion RNA, and the resulting ribonucleoprotein (RNP) complex forms a helically coiled nucleocapsid

structure within the virion. The crystal structure of the RNP has been resolved.[51] The nucleocapsid sequesters the RNA and shields it from the cellular environment. This RNP complex is surrounded by a lipoprotein envelope consisting of the M protein and the surface projection of the trimeric G protein extends to the exterior of the virus.[52]

Like other RNA viruses, rabies viruses have been proposed by some to exist as quasispecies.[53,54] Phylogenetic analyses suggest rabies originated in bats.[55–57] Transfer of rabies viruses from the Old to the New World may have occurred at the time of colonization of the Americas, where it was already established in bats, and subsequently in carnivorous mammals, such as dogs, but the evidence is incomplete.[55,56,58]

The rabies virus G protein, which is a trimer of approximately 67 kDa, is the major antigen responsible for inducing production of VNAs and for conferring immunity against lethal infection with rabies virus.[59,60] The G protein also contains determinants of virulence.[61,62] The G gene was the first rabies virus gene to be cloned and sequenced.[63] From the nucleotide sequence, a polypeptide 524 amino acids long was deduced, which included a signal sequence of 19 amino acids.[52] An arginine at position 333 seems important as one virulence factor, expressed as an ability to spread faster throughout the nervous system, related to properties of neuroinvasiveness and transynaptic spread.[64,65]

Despite the importance of the G gene to virulence and attenuation, critical features related to viral pathogenesis are diverse.[66] For example, the P protein interferes with host interferon production.[67,68] The development of a reverse genetics system for application to rabies virus offers a more detailed analysis of virulence factors.[69] Attenuation may be appreciated as a mirror image to virulence and includes additional elements, such as inhibition of apoptosis of the infected nerve cell. The G and M proteins are responsible largely for blocking apoptosis after infection by virulent viruses, which is, in effect, a protective mechanism for the host. Highly attenuated strains have alterations in those proteins, rendering them proapoptotic.[70–72] Rabies virus genomic RNA is also protected from generating innate immune responses by its tight physical association with the N protein.[73] The immunogenic activity of purified native and smaller fragments of G protein, both naturally occurring and derived by chemical cleavage of the rabies virus G protein, has been compared in a variety of ways to determine the structural basis of VNA production after immunization.[52] Although the definitive role of the humoral immune response to rabies may be debated,[74–76] evidence for the importance of VNA in prevention of viral infection is convincing in humans and other mammals. With regard to a cellular response, T-helper cells are necessary for antibody induction, whereas cytolytic T cells directed against the N protein induced by vaccination may be important for destroying infected extraneural cells before entry of virus into the CNS. Interestingly, the cytolytic T-cell response may be suppressed in natural infection.

Routine sequence analysis of rabies virus variants has not revealed substantive differences accounting for the clinical appearance of furious or paralytic rabies.[77]

"Rabies-Related" Viruses

Rabies is not caused by rabies virus alone. Since the 1950s, viruses have been isolated from animals and humans that were serologically related to rabies virus. Although some members of this group showed a degree of immunologic cross-reactivity with rabies virus, they were sufficiently different to be originally classified as "rabies-related" viruses.[78] The *Lyssavirus* genus is now considered to contain at least 15 members: rabies virus, as the type species of the genus; Lagos bat virus, originally isolated from bats in Nigeria[79]; Mokola virus, isolated from a shrew in Nigeria and suspected in at least one human case[80]; Duvenhage virus, isolated from African bats and humans bitten by African bats[80]; European bat virus 1, isolated from European bats and a human case in Russia; European bat virus 2, isolated from European bats and a human case in Finland[81]; and Australian Bat *Lyssavirus*, isolated from Australian bats, horses, and persons exposed to these reservoirs.[82,83] By the end of the 20th century, in contrast with these six other genotypes, no human cases had been associated with Lagos bat virus. Additionally, Mokola virus was found to cause lethal infection in rabies-vaccinated dogs and cats and was one of the few lyssaviruses not proven to be harbored by bats.[84,85] European Bat *Lyssavirus* I was isolated from bats captured in Poland, Denmark, Finland, and northern provinces of Germany.[80] Both European Bat lyssaviruses are apparently widely distributed in European bats, and despite the belief that the United Kingdom was "rabies-free," European Bat *Lyssavirus*, transmitted by bat bite, was documented as the cause of a human case of rabies in Scotland.[8,86] Previously, four deaths from bat lyssaviruses had been reported in Europe.[87] Although tests using experimentally inoculated mice showed that human diploid cell rabies vaccine could protect against European bat viruses,[88] and vaccination of exposed humans has so far been uniformly successful,[86] only 73% of vaccinated patients developed VNA to these viruses, usually when they had mounted a strong antibody response to the rabies vaccine virus.[89] Both Lagos and Mokola are genotypically distant from typical rabies viruses and have been placed in a separate group, phylogroup 2. Experimental vaccines against Mokola do not protect against Lagos.[90] By the 21st century, other newly discovered agents were characterized and accepted as additional *Lyssavirus* species in Africa and Eurasia.[91] One example, West Caucasian bat virus, was described as one of the most phylogenetically disparate members of the genus. Notwithstanding the lack of vaccine cross-reactivity of these agents, the ultimate public health significance of such emerging lyssaviruses remains to be determined.[92] In many parts of the developing world, where these viruses reside, surveillance is much less than ideal. Renewed pathogen discovery, new agent characterization, and related epidemiologic investigations are desirable to ensure adequate public education and preparedness, especially as new lyssaviruses are described, because all have the ability to cause rabies and an ecological perspective on predicting the emergence of viral pathogens among the *Chiroptera* is poor, in developed and developing countries alike. For example, during 2010, Kuzmin and colleagues[93] reported the discovery of "Shimoni bat virus," isolated from a bat in Kenya. By 2011, they reported a likely reservoir of the Shimoni bat virus.[94] Concomitantly, during 2011, "Bokeloh bat virus," detected in Lower Saxony, Germany, and later in France, was characterized as a new *Lyssavirus*.[95] Clearly, others await characterization throughout this century.

PATHOGENESIS AS IT RELATES TO PREVENTION

Appreciation of viral pathogenesis is critical as a background as to how, when, and why vaccination may be used appropriately to prevent this fatal zoonosis. After inoculation in a wound, rabies virus may take days or weeks to reach the CNS. It is this fact, at least in part, which makes postexposure prophylaxis (PEP) possible. During this early period, the virus is susceptible to VNA, and even to mechanical removal by washing. There is some evidence that initial replication may occur in muscle cells surrounding the wound, providing an amplification of the original inoculum. However, experimental data show that CNS entry can occur without any prior

replication in muscle tissue.[46,96] Another site proposed for possible harborage of rabies virus before entry into the CNS is the macrophage, from which the virus could reactivate to cause disease[97]; but the importance of replication in nonneural cells to the pathogenesis of rabies remains highly controversial.[98] In any event, at some point the virus enters nerve cells through nerve spindles of sensory nerves or neuromuscular junctions of motor nerves.[99] Rabies virus G protein has sequences similar to certain neurotoxins,[100–102] and there are many viral receptors, including the α subunit of the nicotinic acetylcholine receptors of the neuromuscular junction,[100–104] the neural cell adhesion molecule, the neurotrophin receptor, p75NTR, and, perhaps, certain lipoproteins on the cell membrane. Thereafter, the virus enters the neuronal cytosol, where it replicates and spreads within the CNS.[105,106] Dietzschold and colleagues demonstrated that the action of rabies VNA was not exerted solely outside the cell.[107] In one animal model, the effectiveness of antibody was associated with entry into the cell by endocytosis and inhibition of viral transcription. Whether antibody may act directly or by signal transduction to inhibit viral protein synthesis is unclear.[108]

Once in a neuron, rabies virus travels rapidly within the axons at a rate of approximately 8 to 20 mm/day in rodents, probably faster in humans (15–100 mm/day). Experimental studies in rodents showed that virus can reach the CNS in 3 to 5 days, where it causes a widespread encephalitis.[109]

After establishment in the neurons of the CNS, the virus moves in the opposite direction, down the axons to replicate in peripheral tissues, most notably in the nerve plexus and acinar cells of the salivary glands, from which excretion in saliva permits viral transmission by bite to maintain the circuit of infection.[78] However, at the end stage of infection, other extraneural tissues also are affected, including the heart, pancreas, adrenal glands, and gastrointestinal tract.[110,111]

In addition, the finding of virus disseminated throughout the body, in patients who acquired rabies from virus-infected organ transplants, raises questions about the possibility of altered pathogenesis through such unusual routes. The presence of virus in nonneuronal cells may also explain cases with long incubation periods.

The pathophysiology of the fatal outcome is not completely understood.[112] Although encephalitis is widespread, neuronal destruction is not.[113] Death may result from the involvement and dysfunction of brain centers controlling the cardiorespiratory system. In general, the histologic presence of inclusions (i.e., Negri bodies) parallels that of rabies virus antigens, although many infected cells do not have these inclusions. Rabies virus antigens are most prevalent in the periaqueductal gray matter and the Purkinje cells of the cerebellum, but the quantity of rabies virus does not correlate with severity of symptoms.[114,115] Virulent rabies virus variants may be more capable of evading host innate immunity and of destroying neuronal processes.[116,117] The production of nitric oxide within the brain and general downregulation of host genes have been advanced as possible explanations for brain dysfunction.[46] Although the fatality rate in rabies is extremely elevated, recovery has been documented in some animals, such as the dog.[118]

Clinically, the neural mechanisms that distinguish furious from paralytic rabies are not well understood, but electrophysiologic studies suggest that denervation and anterior horn cell dysfunction are prominent in the former, whereas in paralytic rabies, there is inflammation and demyelination of peripheral nerves.[119,120] The mechanism causing demyelination could involve autoimmunity or a bystander effect of immune response to virus in the axons.[5] Hemachudha and colleagues emphasized the role of cellular immunity in the course of rabies.[5] They correlated strong T-cell responses and cytokine secretion (in particular, interleukin-6) with early death and the encephalitic form of the disease, and weak T-cell responses with paralysis and longer survival. Whereas it is certain that lack of an immune response to rabies virus, particularly antibodies, leads to fatal disease, there is thus some evidence that, on the contrary, some cellular responses are responsible for neural pathology.[121,122]

Evidence of a serologic response to rabies virus can be demonstrated by a variety of laboratory techniques, including mouse neutralization, fluorescent focus inhibition, indirect fluorescent antibody, plaque neutralization, immunolysis of rabies virus–infected cells, and binding techniques using radioimmunoassay or enzyme-linked immunosorbent assay procedures. Serum antibodies develop relatively late after natural infection in humans. In persons without a history of vaccination, serum antibodies may be first detected on or about the 6th to 10th day of illness, and thereafter can rise rapidly to high levels. Antibodies are also present in CSF later in the clinical course. Antibody titers of CSF are higher than would be expected from seepage into CSF from circulating blood, indicating local production. Because vaccination does not ordinarily induce CSF antibodies, the presence of CSF antibody titers supports the diagnosis of clinical rabies.[30,31]

The absence of most detectable serum antibodies until around the second week of illness (if at all) and of CSF antibodies until approximately the third week of illness (when significant systemic and neurologic problems occur) raises the possibility that some of the clinical symptoms result from the interaction of host antibodies with rabies virus–infected cells.[105] In mouse experiments, neutralizing antibodies and infiltration of cellular inflammatory cells were necessary to clear infection with an attenuated rabies from the CNS, but this process occurs too late in the usual clinical situation.[123]

Most persons who die of rabies develop specific antibodies to the virus, particularly if they received PEP. However, that response does not protect against a lethal outcome once illness is present, and might even contribute to the disease. Experimental findings suggest that the inflammatory response is associated with opening the blood–brain barrier, delivering antibody-producing B cells to the CNS. Nevertheless, this response usually fails or occurs too late to be therapeutic.

Immune Responses to Rabies Virus and Vaccines

Both innate and adaptive immune responses are operative during viral infection and vaccination. Innate responses to rabies virus are triggered by the intracellular RNA sensor retinoic acid–inducible gene 1 (RIG-I) and lead to secretion of proinflammatory cytokines.[124] At least in mice, one study showed that the innate response does not promote viral clearance.[125] Another study, also conducted in mice, showed that an attenuated rabies virus interacts with the Toll-like receptor (TLR) 7, which uses myeloid differentiation 88 (MyD88) as an adaptor molecule. Mice lacking MyD88 or TLR7 showed increased mortality, suggesting that in this model, innate responses provide some degree of protection.[126] Innate responses were also investigated in raccoons, which, unlike rodents, are a natural reservoir for rabies virus. In this species, the innate response upon rabies virus infection was not detected early at the site of inoculation but rather at late time points within the spinal cord, the brain, and the salivary glands.[127]

Adaptive immune responses also develop with a delay upon infection. Most humans lack neutralizing antibodies by the time their symptoms first develop. In general, such antibodies, once they become detectable, are only found in serum but not, or only at low titers, in CSF.[128,129] This very late and commonly weak antibody response may reflect that the dose

of the initial virus inoculum is too small to trigger T- and B-cell activation at the site of infection and that virus, once it replicates within the CNS, is relatively shielded from immune surveillance.

Humoral responses may also be impaired by viral immune-evasion mechanisms. In mice, rabies virus causes lymphopenia, presumably by causing lymphocyte apoptosis.[130,131] In addition, the P protein of rabies virus, upon interaction with STAT1, inhibits interferon signaling, which may prevent B-cell maturation.[68] Nevertheless, attenuated recombinant rabies viruses induce excellent B-cell responses, not only to transgene products but also to the viral G protein, in mice as well as nonhuman primates, indicating that low antibody responses in human rabies victims is unlikely to be linked only to active immunosuppression by viral antigens.[132]

Curiously, some humans as well as other mammals, without any evidence of an active rabies virus infection or a history of rabies vaccination exhibit rabies VNA, suggesting that they had been exposed, but then underwent an abortive infection and managed to clear the virus without progressing toward fatal or serious disease.[22] The presence of antibodies indicates that virus must have replicated to levels that sufficed to cause B-cell activation and expansion. As antibodies in patients with disease only become detectable several days after onset of neurological symptoms, this phenomenon may also suggest infection with a somewhat "attenuated" variant that replicates in the periphery. Knowledge on rabies virus–specific $CD4^+$ or $CD8^+$ T-cell responses in infected humans is virtually absent, limiting insights for therapy targeting such effectors.

Protection after vaccination is provided significantly by the induction of rabies VNA. Induction of such antibodies requires help from $CD4^+$ T cells, which are induced by inactivated rabies vaccine.[132–134] Experiments in mice showed initial responses by T-helper cell-independent short-lived plasma cells, that develop outside germinal centers and produce immunoglobulin (Ig) M antibodies for early protection.[135] This is followed by a wave of long-lived plasma cells that develop within germinal centers and produce switched, affinity-matured antibodies.[136] In one trial, upon booster immunization after a year, rather than the more typical schedule of shorter intervals between vaccine doses, antibody titers to rabies virus were sustained.[137,138] The vaccine also induces memory B cells that appear to persist for the life span of an individual, as they can be recalled 10 years or more later.[139] Apparently, $CD8^+$ T cells, which, as studies in mice indicate, do not contribute to protection against rabies virus, are not induced by current human rabies vaccines.[76,140]

DIAGNOSIS

Vaccination against rabies is recommended for persons at known or potential risk of exposure or those who have already been exposed. Persons exposed to virus do not have rabies and prophylaxis is intended to prevent such an occurrence. The history of an associated bite from a known or suspected rabid animal, coupled with the striking clinical manifestations, should provide a reasonably simple diagnosis of rabies. However, such straightforward attributes are not always present. Especially in the absence of a documented exposure source, clinical diagnosis of rabies requires differentiation from a wide variety of diseases that can cause neurologic symptoms. Because laboratory diagnosis may not be possible during the first week of illness, presumptive diagnosis based on clinical symptoms is important.

Definitive diagnosis of rabies virus infection of humans and suspected animal vectors depends on the detection and identification in infected brain tissue of rabies virus antigens or intracytoplasmic neuronal inclusions (Negri bodies); viral nucleic acid by reverse transcription–polymerase chain reaction (RT-PCR)[141]; the presence of rabies virus-specific antibodies in the CSF, or in the serum of unvaccinated patients; and on the isolation and identification of the virus from brain tissue, saliva, or other infected substances. The standard diagnostic technique is to search for rabies virus antigens in brain tissue by fluorescent antibody staining. The brainstem, cerebellum, and thalamus provide some of the best samples.[142] Identification of viral nucleic acid by RT-PCR is useful, particularly if specimens are in poor condition. Demonstration of Negri bodies has a variable sensitivity and is of only historical interest as a primary method, notwithstanding their detection microscopically upon postmortem examination of brain tissue, particularly when rabies is not suspected. Virus isolation is a procedure used for confirmation of other positive test results. Isolation can be accomplished in mouse neuroblastoma cell culture or by intracerebral inoculation of suckling mice.[143] Although diagnostic procedures generally are initiated in tissue specimens obtained postmortem, rabies virus infection also can be identified in during the extended course of the disease. In addition to its usefulness for brain biopsy specimens, the fluorescent antibody staining technique enables detection of viral antigens in impressions of infected cornea or in cryoscopic sections of skin biopsy samples from the hairline of the neck, where antigens can be detected in the nerves surrounding the hair follicles.[144]

In addition, as shown in Table 50.1, RT-PCR on saliva provides a rapid and sensitive test for rabies as early as 5 days after onset of symptoms.[145,146] Improved sensitivity was obtained when an RNA polymerase was included in the reaction and electrochemiluminescence was used for detection of product. This technique, called nucleic acid-based sequence

TABLE 50.1 Examples of Antemortem Diagnostic Test Results for 20 Human Patients With Rabies in the United States, 1980–1996

Test	No. of Patients Positive for Rabies Virus/Total Tested (%)	Earliest Positive Result, Day of Illness
RT-PCR of saliva for rabies virus RNA	10/10 (100)	5
Rabies virus antigen in brain biopsy	3/3 (100)	8
Rabies virus antigen in nuchal skin	10/15[a] (67)	5
Virus isolation from saliva	9/15[b] (60)	5
Serum antibody to rabies virus	10/18 (56)	5[c]
Rabies virus antigen in cornea	2/8 (25)	14
CSF antibody to rabies virus	2/13 (15)	15[d]

[a]Two patients had earlier skin biopsies that were negative but became positive on subsequent biopsy.
[b]One patient had an earlier test that was negative.
[c]Latest negative result on day 24; median to positive result was 10 days.
[d]Latest negative result on day 24.
CSF, cerebrospinal fluid; RT-PCR, reverse transcription–polymerase chain reaction.
Data from Noah DL, Drenzek CL, Smith JS, et al. Epidemiology of human rabies in the United States, 1980 to 1996. Ann Intern Med. *1998;128:922–930.*

amplification, detected the rabies virus genome in saliva, concentrated urine sediment, and CSF as early as 2 or 3 days after symptoms.[147,148] Serial tests should be performed to increase sensitivity.[148] One study in Thailand showed high sensitivity (91%) of rabies virus genome detection on the first day of hospitalization, particularly in saliva.[149] Both CSF and concentrated urine sediment specimens may also be useful. However, the genome was not detected in all patients before death. A latex agglutination test for viral antigen in dog saliva also has been developed.[150] One report found rabies virus–specific immune complexes in the CSF of 77% of patients with rabies 5 to 7 days after onset of disease.[151]

As an example of human rabies diagnosis in a developed country, between 1980 and 2005, more than 65 cases of human rabies were diagnosed in the United States, but not all were diagnosed before death, probably because a minority had a known history of exposure to potentially rabid animals. This problem arises in large part because many of the patients had been infected with rabies virus variants from bats, possibly as a result of undocumented or ignored bat bites.[152]

EPIZOOTIOLOGY AND EPIDEMIOLOGY

A thorough understanding of rabies as a fundamental disease of nature, affecting many other species, is essential, to properly conduct risk assessments for both human and domestic animal pre- and postexposure applications.

Mammals

Rabies is a disease of domestic and wild mammals, particularly dogs and related canid species, as well as raccoons, mongooses, skunks, and bats. Although birds are susceptible under experimental conditions, natural cases are very rare.[153] In areas in which domestic animal control programs are not extensively developed, dogs and cats account for most of the rabid animals reported and cause the majority (>90%) of human rabies exposures and deaths. After effective domestic animal rabies control programs in these areas, the numbers of rabid dogs and cats markedly decrease, as illustrated in the United States from the 1940s to the 1960s. Wildlife is then recognized as the main reservoir of rabies virus. In the United States since 1960, the majority of cases of animal rabies have been in wildlife species, and most human rabies cases have been secondary to bites by rabid wildlife, especially bats.[11,154] Luckily, bats do not often transmit virus to dogs or cats.[155,156]

Illustrating the relationship among different variants and hosts, Fig. 50.1 is a composite map of the United States showing the major carnivore reservoirs in each region.[157,158] The salient features shown are that skunks are the important vectors for rabies in the western and central United States, whereas raccoon rabies dominates in the east. The original focus of raccoon rabies was in the southeast, but a second front developed in the mid-Atlantic and northeast owing to translocation of infected animals, and now raccoon rabies is contiguous from Maine to Florida and west to Ohio.[159] Foci of red fox rabies are evident in Canada and Alaska. Rabies in Puerto Rico is caused by the mongoose, which was introduced as an invasive species. Rabies control is costly: in New York State alone, the cost of rabies prevention in humans was calculated to be $2.3 million per year.[160]

Table 50.2 delineates rabies cases by species for the United States in 2014.[158] Table 50.3 lists the principal animal vectors of rabies throughout the world.[146] In Western Europe, before oral vaccination foxes accounted for up to 80% of rabid animals. Infection in raccoon dogs is becoming more common in eastern Europe.[161,162] Molecular analysis of European viruses suggests that rabies originated in dogs, but now affects red foxes and raccoon dogs.[163] Infection in red foxes and raccoon dogs has gradually spread from east to west. Canine rabies is still widespread in Asia, Africa, and parts of Latin America. Between 1993 and 2009, there was a significant drop in canine and human cases in Latin America.[164] However, dogs remained the predominant vector for humans (65%), followed by bats (15%) and cats (3%). Vaccination of pet dogs is an effective strategy for protection of humans and has eliminated carnivore rabies in Great Britain, Iceland, Japan, and many other islands.[105,165] An immunization coverage of 60% to 70% is estimated to prevent canine rabies outbreaks.[166]

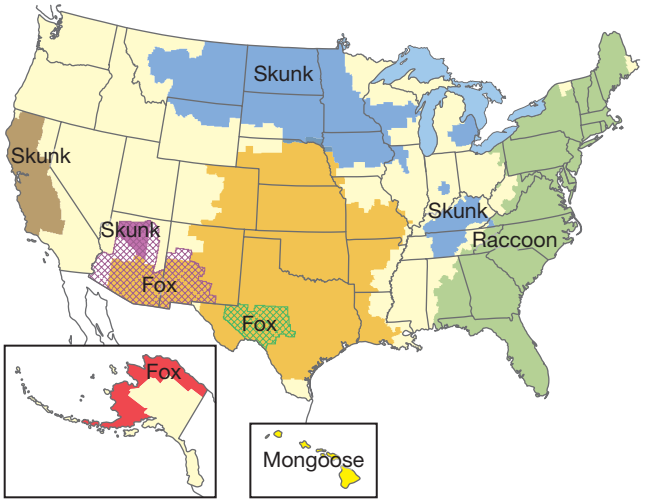


Figure 50.1. Recent historical distribution of major carnivore reservoirs of rabies viruses in the United States. *(Modified from Blanton JD, Palmer D, Rupprecht CE. Rabies surveillance in the United States during 2009.* J Am Vet Med Assoc. *2010;237:646–657.)*

TABLE 50.2 Cases of Rabies in the United States, 2014[a]

	Number	Percentage
WILDLIFE		
Raccoons	1822	30.2
Bats	1756	29.1
Skunks	1588	26.3
Foxes	311	5.2
Rodents/lagomorphs	45	0.7
Other	66	1.1
Subtotal	**5588**	**92.6**
DOMESTIC ANIMALS		
Cats	272	4.5
Cattle	78	1.3
Dogs	59	1.0
Other	36	0.6
Subtotal	**445**	**7.4**
Humans	**1**	
Total	**6034**	**100%**

[a]During 2014, more than 100,000 samples were submitted for consideration in laboratory diagnosis.

From Monroe BP, Yager P, Blanton J, et al. Rabies surveillance in the United States during 2014. J Am Vet Med Assoc. *2016;248(7):777–788.*

TABLE 50.3 Principal Global Animal Reservoirs/Vectors of Lyssaviruses[a]

Location	Reservoirs/Vectors
North America	Raccoons, skunks, foxes, bats, and mongooses
Western Europe	Bats and foxes
Eastern Europe	Foxes, dogs, raccoon dogs, wolves, and bats
Latin America	Dogs, bats, foxes, skunks, and marmosets
Caribbean	Mongooses, dogs, cats, and bats
Australia	Bats
Africa	Dogs, jackals, mongooses, foxes, and bats
Asia	Dogs, cats, ferrets, badgers, monkeys, mongooses, bats, and arctic foxes

[a]None listed in Antarctica, Oceania, and a few other, mostly insular locations, reported as presumably "rabies free" (even though laboratory-based surveillance may be entirely lacking or wholly inadequate by current global epidemiological standards).

In support of historical and epidemiologic observations in developed countries, one Thai study confirmed that rabid dogs and cats uniformly die within 10 days of the onset of illness.[167]

Foxes are important vectors in Canada, Alaska, and Russia. Mongooses imported into the Caribbean Islands now form a reservoir for rabies, and mongooses serve the same role in southern Africa and parts of Asia. The vampire bat is a major threat to livestock in Latin America and has been involved in many biting incidents in humans. Rabid cattle also may excrete rabies virus in saliva.[168] Like all mammals, rodents are susceptible to infection[105] but are infrequently rabid, and human transmission of disease by these animals has not occurred. In Thailand, 95% of the animals involved in biting incidents are dogs, often younger than 6 months,[169] with cats accounting for another 3%. The remaining 2% include monkeys, civets, tigers, and other animals, which testifies to the wide host range of rabies virus. Dogs randomly captured in Thailand developed rabies within 1 month in 3% to 4% of cases, and, interestingly, there was serologic evidence suggestive of prior rabies virus exposure or prior vaccination in approximately 15% to 20% of dogs.[170]

Rabies in animals (excluding bats) is absent in many islands such as the United Kingdom, Australia, and Japan. Much of Western Europe is also now "rabies free" in carnivores, although rabies still occurs in Central and Eastern Europe. For example, from 2000 to 2005, there were 45 non-imported cases of human rabies in Europe, but 44 occurred in Eastern Europe, and the remaining case was caused by a bat *Lyssavirus*.[171] Many of the large Asian countries (such as China), most of Africa, Russia and the republics from the former Soviet Union, and some parts of South America still report considerable rabies in domestic animals.

Travelers are at risk, as confirmed by the report of more than 17 human cases of rabies diagnosed in the United States but acquired abroad between 1990 and 2015.[158] The risk of rabies exposure in Nepal was calculated to be 5.7 per 1000 person-years for expatriates and 1.9 per 1000 person-years for tourists.[172] The epidemiology of rabies has been revolutionized by viral typing, initially with monoclonal antibodies since the late 1970s.[173] Panels of these antibodies, directed against epitopes specific to isolates from different animal species and from different geographic locations, are used to identify viruses. Thus, it is now possible to identify the source of an isolate from humans or animals and to demonstrate that the infection was transmitted far away in time and place. Significantly, genetic sequencing of the viral genome after RT-PCR amplification has supplemented greatly our knowledge of viral variability.[174] Laboratory "fixed virus" strains differ in sequence from wild "street viruses" by as much as 10% to 15% of nucleotides. Sequence data suggest that many viruses in the Western Hemisphere and in South Africa, and elsewhere, were imported from Europe.[175,176]

Bats

Bats are not incidental vectors of rabies virus, but more likely the original hosts of *Lyssavirus*, which in some distant past developed epizootiologic cycles in other mammals, such as dogs and other carnivores by spillover infections.[55] More genomic studies support this idea. The widespread infection of insectivorous bats throughout the United States was first documented in the 1950s. However, the importance of bat rabies, particularly from the silver-haired bat *Lasionycteris noctivagans*, to human transmission has become more evident (see "Human Rabies" later). The eastern tricolored bat *(Perimyotis subflavus)* is important in the East and Midwest.[177] Although most rabid bats appear ill,[178] an infected bat may act normally.[179,180] The rabies virus recovered from the silver-haired bat is one variant that seems to be able to replicate better in epidermal tissues.[181]

Experimental data also suggest that virus from silver-haired and possibly tricolored bats is more pathogenic for mice than other bat rabies viruses.[182] Analysis of natural transmission to other mammals supports the idea that rabies viruses associated with these two species are more infectious than viruses found in other bats.[177] The virulence of the viruses from silver-haired bats may relate to blockage of the passage of immune cells to the CNS.[183]

Bats are also important reservoirs of rabies for wild animals in the United States, as are vampire bats in South America.[184,185] Although neutralizing antibody titers are not always raised in humans against other rabies-related viruses maintained by bats,[89] there is good cross-neutralization of bat rabies virus variants by standard cell culture vaccines (CCVs).[186] Beyond the New World, bat lyssaviruses have been responsible for fatal rabies cases in humans and other animals in Africa, Australia, and Eurasia.[187–190]

NONBITE TRANSMISSION

Nonbite transmission has been reviewed by Gibbons,[191] who found at least 27 well-documented cases of transmission by means other than bites and 17 other less-well-documented cases. Of the total 44 cases, 18 were caused by improperly inactivated vaccine in Brazil, eight were from corneal transplants, eight from contamination of skin whose integrity had been impaired, four by aerosols created in laboratory or bat-infested caves, and six from human-to-human transmission. Among the alleged historical causes of human-to-human transmission were transplacental passage, lactation, kissing, intercourse, and providing healthcare. Gibbons[191] also found three reports of transmission by human bite.

Transmission to transplant recipients from organ donors is particularly worrisome. In one case, transmission occurred to four patients who received kidneys, liver, and an artery segment from a donor with undiagnosed rabies.[23] A similar scenario was reported for German transplant recipients in 2005. Additional cases were reported again recently in the United States from a donor infected with raccoon rabies virus.[24] Thus, these cases demonstrate that rabies virus is disseminated throughout the body, at least late in the disease, and that direct implantation of infected tissue may transmit rabies to humans. Deaths of suspected encephalitis should be investigated for

rabies or other etiologic agents before tissues from the donor are used.

Infection by aerosol has been suspected in unique cave habitats inhabited by millions of bats and implicated under certain laboratory conditions.[11,192] Other lyssaviruses potentially could also be transmitted by aerosol.[193] Unwitting corneal transplantation from patients who died of rabies also has resulted in transmission.[11,194] As mentioned, human-to-human transmission by bite is possible but extremely rare.[191,195,196]

HUMAN RABIES

The epidemiology of human rabies follows closely the epizootiology of animal rabies as regards exposures and case causality. The dog is the major global reservoir of rabies. In the United States alone, more than 1 million dog bites occur each year,[197] and the situation is worse in many other parts of the world.[198] Human rabies has been reported from all continents except Antarctica, but the majority of cases occur in countries where canine rabies is not well controlled. The World Health Organization (WHO) estimate for humans vaccinated due to exposure to rabies exceeds 15 million annually.[199] The WHO also estimated more than 34,000 human rabies cases during 2015, most of them in India.[199a] More recent global estimates suggest more than 70,000 human rabies cases annually.[200]

The annual incidence of rabies deaths per 100,000 population has been calculated as approximately 2 in India, 0.01 to 0.2 in Latin America, and an uncertain 0.0001 to 13 in Africa.[195,201] Human rabies is most common in people younger than 15 years of age, with approximately 40% of cases found in children 5 to 14 years of age; however, all age groups are susceptible. The majority of rabies victims are male. In one study in the United States, the highest incidence of human rabies PEP occurred among rural boys, primarily during the summer months.[202]

In the United States, bats have emerged as the leading transmitter of rabies virus to humans. Excluding transplant recipients infected by a common donor, between 1990 and 2015 bats were associated in most diagnosed human rabies cases of presumed domestic origin.[158,177] There were also other cases associated with nonindigenous canine rabies viruses during the same period.[203] No definitive contact with bats could be elicited in the majority of cases, and only a handful of patients had a documented history of a bite reported before diagnosis, suggesting that memory lapses, neural impairment, and, rarely, ignored or unperceived bites in some persons may have been responsible.[204–206] A similar situation exists in Canada, and this phenomenon should be operative in any areas in which bats serve as reservoirs.[196]

VACCINATION

Passive Immunization

Antiserum alone may not prevent rabies and is not recommended, except in combination with vaccine (see "Serum and Vaccine Prophylaxis" later). When antiserum is provided in advance of vaccine, it may interfere with active immunization.

Active Immunization

Vaccines

Typically, vaccines are administered before exposure to an infectious agent and are otherwise not very useful after exposure has occurred. Historically, rabies is an exception, in comparison to most other vaccine-preventable infectious diseases, because immunization proceeds as a critical intervention not only before but also after exposure to virus. Over the past century, a variety of substrates, such as whole animal tissues, primary cell cultures, diploid cells, and continuous cell lines, have been used widely for virus propagation. Interestingly, all these systems remain in use today for human rabies vaccine production, which is probably not the case with other human viral vaccines that remain in widespread use. The field of rabies vaccinology has progressed enormously, especially during the latter part of the last century, with regard to authentication of cell substrates and virus strains, a better understanding of the risks of using animal origin materials, design of commercially viable serum-free medium, improvement in downstream processes, knowledge of problematic impurities, and strategies to mitigate these concerns.

Prior Approaches

Table 50.4 summarizes the history of the development of rabies vaccines and lists major currently available vaccines. Many laboratory "fixed rabies virus" strains have been used for preparation of rabies vaccines. Fig. 50.2 gives the presumed history of some of these strains.

For more than 70 years after Pasteur's original work, only vaccines containing nerve tissue were available. Major modifications in nerve tissue vaccine preparation were introduced by Fermi[207] and by Semple,[208] who used phenol to partially or completely inactivate virus. Adverse reactions to rabies vaccines containing brain tissue have been recognized since the time of Pasteur. In addition to neurologic complications attributed to the presence of myelinated tissue in the vaccine, "fixed virus" may be pathogenic for humans, contrary to the "Pasteurian dogma," although it took 75 years before it was proved that some cases of paralysis after vaccination were caused by imperfectly inactivated vaccine virus. More than a century after development, adult animal neural tissue vaccines are highly discouraged but still used in some developing countries, such as Ethiopia.[209]

Myelin-free vaccines prepared from neonatal mouse brains were introduced by Fuenzalida and colleagues[210] in 1956 and are still used in parts of the world, such as in Latin America. Introduction of the duck embryo vaccine (DEV), prepared from virus propagated in embryonated duck eggs,[211] greatly reduced the number and severity of postvaccinal reactions, but DEV was less immunogenic than the brain tissue vaccine. For mouse brain and DEV vaccines, 14 to 23 daily inoculations were recommended, but even this "heroic" dosage did not always protect against rabies after severe exposure. Thus, there had long been a pressing need for a highly immunogenic antirabies vaccine that could be used safely and effectively at low doses for primary immunization and for prevention after viral exposure.

CELL CULTURE RABIES VACCINES

The solution to the problem of safety of rabies vaccines lay in the development of vaccines prepared from rabies virus grown in tissue culture free of neuronal tissue. The first attempts to develop a tissue culture vaccine were made by Kissling[212] in 1958 and by Fenje[213] in 1960. Both investigators used the primary hamster kidney (PHK) cell for rabies virus production.

All modern CCVs must contain rabies virus antigen with a potency of at least 2.5 IU per intramuscular dose. However, a metaanalysis of immunogenicity data revealed that higher concentrations of antigen did not translate into higher immune responses.[214] Historical fixed rabies virus strains for

TABLE 50.4 Important Past and Present Rabies Vaccines for Humans

Vaccine Name	Manufacturer	Type	Substrate	Remarks	Where Used
NERVE TISSUE					
Pasteur	None	Inactivated by drying	Rabbit spinal cord	Residual live virus	NLU
Fermi	None	Phenolized live virus	Sheep, goat, or rabbit brains	Contained nerve tissue, residual live virus?	NLU
Semple	Many	Phenol inactivated	Sheep, goat, or rabbit brains	Contains nerve tissue	Asia, Africa
Fuenzalida	Many	Inactivated	Suckling mouse brain	Decreased myelin	South America
AVIAN					
PDEV	Zydus Cadila	β-Propiolactone inactivated	Duck embryo	Purified by ultracentrifugation	Europe, worldwide
DEV	None	Inactivated	Duck embryo	Allergy to avian antigens	NLU
CELL CULTURE					
HDCV	Sanofi Pasteur	β-Propiolactone inactivated	Human cultured fibroblasts	Expensive, standard for rabies vaccine	United States, Europe, worldwide
RVA	Bioport (NLU)	β-Propiolactone inactivated	Fetal rhesus cell culture	Fewer allergic reactions	United States
PHKCV	Local	Formalin inactivated	Primary Syrian hamster kidney cell culture	Used historically in People's Republic of China	China, Russia
PCECV	Novartis (Chiron Behring)	β-Propiolactone inactivated	Chick embryo cell culture	Purified by ultracentrifugation	United States, Europe, worldwide
PVRV	Sanofi Pasteur	β-Propiolactone inactivated	Vero cell line	Purified by ultracentrifugation	Europe, worldwide

DEV, duck embryo vaccine; HDCV, human diploid cell vaccine; NLU, no longer used; PCECV, primary chick embryo cell vaccine; PDEV, purified DEV; PHKCV, primary hamster kidney cell vaccine; PVRV, purified Vero rabies vaccine; RVA, Rhesus cell rabies vaccine.

vaccine production include Pasteur, Pitman-Moore (PM), challenge virus standard, Flury low egg passage (LEP), Flury high egg passage (HEP), Kelev, and ERA/Street Alabama Dufferin (SAD).[215]

Human Diploid Cell Vaccine

In the early 1960s, workers at the Wistar Institute, Philadelphia, Pennsylvania, selected the human diploid cell strain WI-38 for virus propagation to avoid the difficulties inherent in the use of primary tissue cultures, such as induction of allergy to animal proteins.[216,217] The vaccine thus developed, the human diploid cell vaccine (HDCV), containing concentrated and purified virus, evoked much better immune responses in experimental animals and in humans than did DEV, suckling mouse brain, or adult brain tissue vaccines.[218,219] The technical advances leading to the development of the vaccine included the adaptation of the PM strain of virus to WI-38 cells,[219] the inactivation of cell-free virus by β-propiolactone, and the concentration of virus by ultrafiltration.[217]

Currently, HDCV is produced in MRC-5 human fibroblasts that are inoculated with the PM L503 3 M strain. Virus-containing supernatants are harvested and concentrated 10 to 20 times by ultrafiltration or ultracentrifugation, reaching a titer of approximately 10^7 median lethal doses per milliliter before inactivation, which is done with 1:4000 β-propiolactone. Potency is assessed by the National Institutes of Health test in mice (although new improved potency tests are under development) and is at least 2.5 IU/dose.

After 4 years of clinical studies in volunteers,[220] the vaccine was used in humans exposed to severe wounds by rabid dogs and wolves in Iran. All vaccinated people developed VNA, survived, and remained free of rabies.[221] The HDCV was first licensed in Europe for preexposure and postexposure immunization of humans in 1976 and was licensed in the United States in June 1980. Winkler[222] summarized the results of 5 years of clinical experience in the United States with no failures to prevent rabies. The vaccine is used for preexposure immunization and booster doses for maintenance of antibody and for postexposure immunization. It is estimated that more than 1.5 million people have been vaccinated with HDCV throughout the world.

In essence, HDCV is the concentrated supernatant of MRC-5 human embryo fibroblast cell cultures infected with rabies virus. Each dose of the vaccine sold in the United States contains rabies virus inactivated by β-propiolactone, 5% human albumin, phenolsulfonphthalein, and neomycin sulfate (<150 μg) as an antibiotic. The vaccine is lyophilized to a powder form and reconstituted in sterile water. The vaccine contains no preservative or stabilizer.

A HDCV was produced previously in the United States by Wyeth Laboratories, with use of N-tributylphosphate as the inactivating agent. As of 1984, all HDCV sold in the United States is manufactured by Sanofi Pasteur (Lyon, France) according to the method above. In Europe, Novartis Vaccines (formerly Chiron Behring) produced HDCV from the PM strain until 2004 before switching to Flury LEP. Neither of the two HDCVs contain human albumin or phenolsulfonphthalein (see "Allergic Reactions" later).

The Serum Institute of India also produced a β-propiolactone–inactivated rabies vaccine made in human diploid cells. The vaccine contains aluminum phosphate as an adjuvant and is presented in liquid form with thimerosal as a preservative.

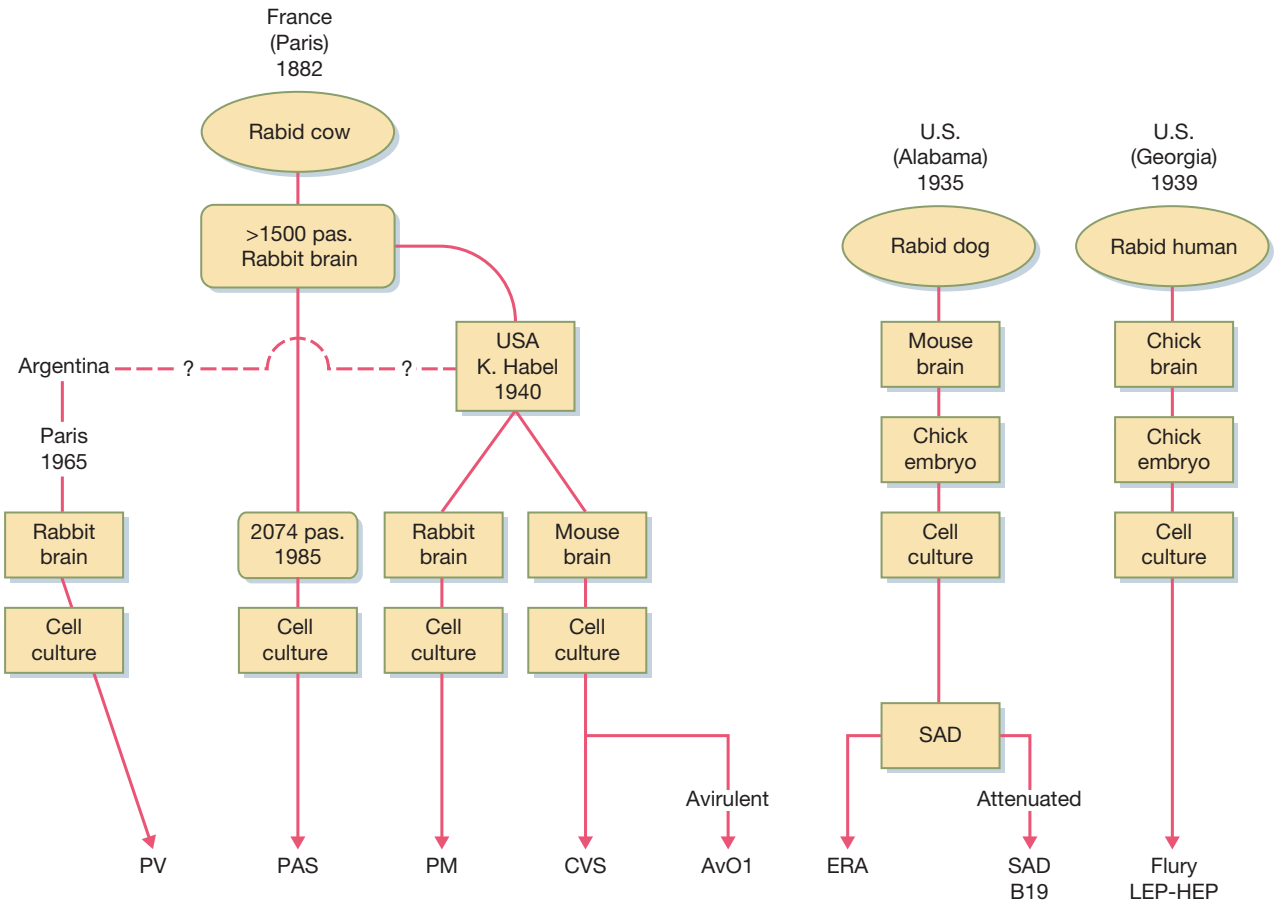


Figure 50.2. History of strains of rabies viruses used as vaccine seeds. HEP, high egg passage; LEP, low egg passage; pas, passages; PAS, Pasteur; PM, Pitman-Moore; PV, Pasteur virus; SAD, Street Alabama Dufferin. *(From Sacramento D, Badrane H, Bourhy H, et al. Molecular epidemiology of rabies virus in France: comparison with vaccine strains.* J Gen Virol. *1992;73:1149–1158.)*

Ideal storage conditions are 2°C to 8°C, at which temperature the vaccine is stable for at least 3.5 years.[223] However, vaccine stored for 1 month at 37°C was still potent.[224,225]

Vero Cell Vaccine

There have been intense efforts worldwide to produce vaccines at lower cost that meet or improve on the levels of safety and efficacy achieved originally with HDCV. Table 50.4 lists several of these new tissue culture vaccines. All modern CCVs must have a potency of at least 2.5 IU/dose as measured by the National Institutes of Health test.

A continuous African green monkey kidney cell line, termed Vero (after an Esperanto abbreviation of "verda reno," meaning green kidney), was developed for use as a cell substrate for viral vaccines, and a purified Vero-cell culture rabies vaccine (PVRV) based on growth of virus in that cell line has been developed and licensed by Sanofi Pasteur in Europe and in many countries in the developing world.[226] For example, other countries producing a Vero-cell rabies vaccine include India (e.g., Bharat Biotech, Wockhardt, the Human Biologicals Institute) and China (e.g., Chengdu Biotechnology, Wuhan Institute, Liaoning Yisheng, Changsheng Life Sciences). An advantage of the Vero cell is that it can be grown and infected on microcarrier beads and cultivated in fermenters to produce large volumes of tissue culture fluid containing rabies virus.

The rabies virus strain in PVRV is the same as that used for HDCV production. The cells are grown in bioreactors in which titers of at least 10^7 median tissue culture infective dose ($TCID_{50}$) are obtained routinely. The harvested virus is concentrated and then purified by zonal centrifugation and ultrafiltration, followed by inactivation with β-propiolactone. The final product contains human albumin. Clinical studies have shown that the VNA responses after primary and booster injections of PVRV are equivalent to those seen after preexposure or postexposure vaccination with HDCV.[227] Postexposure protection after severe exposures has been demonstrated in Thailand[228] and China,[229] without unusual adverse reactions.

A chromatographically purified Vero-cell culture rabies vaccine (CPRV) has been developed to reduce cellular DNA and foreign protein content. This vaccine is well tolerated and highly immunogenic, although slightly less so than HDCV, as shown in Table 50.5.[230–233] CPRV was used successfully in the Philippines to protect against severe rabies exposures.[234]

Vero cell rabies vaccine has also been produced without the presence of serum in the cell culture medium.[235]

Purified Chick Embryo Cell Culture Vaccine

Purified chick embryo cell culture vaccine (PCECV), prepared by Novartis Vaccines (formerly Chiron Behring), has been evaluated together with HDCV in postexposure protection of animals and humans.[236–239] No significant differences in protection between the two vaccines were observed. To prepare PCECV, the Flury LEP-C25 strain is grown in primary chick embryo fibroblasts. The virus is inactivated by 0.025% β-propiolactone and then concentrated and purified by density gradient centrifugation. The lyophilized vaccine contains

TABLE 50.5 GMT of RFFIT and Seroconversion After Primary Human Immunization With a Five-Dose Series of HDCV or CPRV and HRIG Administered on Day 0

	CPRV (n = 18)		HDCV (n = 124)	
Days After First Dose	GMT (IU/mL)	% Seropositive	GMT (IU/mL)	% Seropositive
0	0	0.025	0	0.025
7	1.7	0.17	4.03	0.18
14	100	6.9	100	10.3
28	100	14.6	100	20.5
42	100	16.9	100	19.4
90	100	7.8	100	15.4
180	98.3	3.4	99.2	7.2
365	92.2	1.6	98.3	3.7

CPRV, chromatographically purified Vero cell culture rabies vaccine; GMT, geometric mean titer; HDCV, human diploid cell vaccine; HRIG, human rabies immunoglobulin; RFFIT, rapid fluorescent focus inhibition test.

From Jones RL, Froeschle JE, Atmar RL, et al. Immunogenicity, safety and lot consistency in adults of a chromatographically purified Vero-cell rabies vaccine: a randomized, double-blind trial with human diploid cell rabies vaccine. Vaccine. *2001;19:4635–4643.*

TABLE 50.6 Rabies Geometric Mean Titer in Humans After Three Doses of Intramuscular Human Diploid Cell Vaccine or Purified Chick Embryo Cell Vaccine

Day	HDCV	PCECV
0	<0.05 (n = 79)	<0.05 (n = 82)
28	12.0 (n = 79)	9.3 (n = 82)
49	25.8 (n = 79)	25.3 (n = 82)
387	1.49 (n = 69)	2.92 (n = 67)

HDCV, human diploid cell vaccine; PCECV, primary chick embryo cell vaccine.

From Briggs DJ, Dreeson DW, Nicolay U, et al. Purified chick embryo cell culture rabies vaccine: interchangeability with human diploid cell culture rabies vaccine and comparison of one- versus two-dose post-exposure booster regimen for previously immunized persons. Vaccine. *2000;19:1055–1060.*

processed gelatin as a stabilizer. The PCECV is now registered worldwide, including in the United States, and more than 80 million doses have been used. Reconstitution studies have shown that the rehydrated vaccine is stable for at least 1 week in the refrigerator.[240]

PCECV has been tested extensively in the United States, Germany, the United Kingdom, Thailand, Croatia,[241] and India.[242] During a 10-year experience with PCECV in India, the vaccine was well tolerated, with only 55 (4%) of 1375 people reporting reactions, although some urticarial reactions have been seen.[36] Immunogenicity was also good, with geometric mean concentrations of neutralizing antibodies after PEP of approximately 4 IU/mL and of less than 1 IU/mL in only 0.9% of patients. Table 50.6 shows a comparative study with HDCV.[243]

PCECV is licensed in the United States under the trade name RabAvert. Although not licensed for intradermal use in the United States, it is used by that route elsewhere (see "Schedules Using the Intradermal Route" later). A similar type of vaccine (Flury HEP strain) is produced in Japan with limited distribution.[244] PCECV vaccinees develop a positive skin test against yellow fever vaccine, probably because of chicken protein common to both vaccines, and the two should be administered with caution in egg-allergic patients.[245]

Hamster Kidney

Primary hamster kidney cell culture vaccine (PHKCV) was produced by the Institute of Poliomyelitis and Virus Encephalitides, Moscow, and by several institutes in China, including the Wuhan Institute of Biological Products. PHKCV was approved for use in China in 1980, where it completely replaced the Semple-type rabies vaccine. The Chinese vaccine contained the Beijing strain, which is inactivated by formalin. The final material contains 0.01% thimerosal, 10 mg human albumin, and aluminum hydroxide. Two types of PHKCV (adjuvant and freeze-dried concentrate) are available. The PHKCV with adjuvant is liquid and without adjuvant is freeze dried. The vaccine is supplied as a freeze-dried concentrate and is administered on a five- or six-dose schedule in China.[246] PHKCV was reportedly well tolerated and protective against proven rabies virus exposures.[247]

For development of an even less expensive rabies vaccine, a baby hamster kidney cell line (BHK-21) was considered as a vaccine substrate.[248]

Duck Embryo

The Swiss Serum and Vaccine Institute (Bern, Switzerland) introduced a purified duck embryo vaccine (PDEV) that used the PM strain. No duck protein was detectable by the immunodiffusion test. Concentration was achieved by density gradient ultracentrifugation. The virus was inactivated by β-propiolactone and thimerosal was added as a preservative. Antigenic potency and antibody responses evoked in humans were comparable to those of HDCV.[249] Mild local reactions were noted that occurred only slightly more frequently than with HDCV.

Production of PDEV was transferred to Zydus Cadila Health Care, Ltd, an Indian manufacturer.[250] This modern PDEV is registered and marketed in more than 25 countries; is highly immunogenic, safe, and well tolerated; and is similar to HDCV, PCECV, and PVRV.

Fetal Rhesus Lung

To provide an alternative to vaccine made in human cells, the Michigan Department of Health and Human Services (and later a commercial entity, Bioport) prepared a rhesus diploid cell culture vaccine. The Kissling–challenge virus standard rabies strain was adapted to DBS-FRHL-2 cell cultures (a fetal rhesus monkey lung fibroblast), inactivated with β-propiolactone, and adjuvanted with aluminum phosphate.[251,252] It was given on the same preexposure schedules as HDCV or PCECV and was considered equally safe and effective.[253] The rhesus diploid cell culture vaccine was licensed in the United States by Bioport, but is not currently available commercially.

IMMUNIZATION SCHEDULES

Table 50.7 shows the current immunization schedules and Warrell has published a review of the subject.[254,255]

Preexposure

The current recommended preexposure immunization regimen in the United States is three doses of HDCV or PCECV on days 0, 7, and 21 or 28. The dose is 1 mL administered

TABLE 50.7 Regimens for Human Preexposure and Postexposure Vaccination With Rabies Vaccines

Vaccination	Route	Days Doses Are Given	Remarks
Preexposure	IM[a]	0, 7, 21, or 28	Standard U.S. 3-dose regimen
	ID[b]	0, 7, 21, or 28	Economical, but not to be used in persons taking antimalarial medications; unlicensed route in U.S.
Postexposure[c]	IM[a]	0, 3, 7, 14	U.S. and WHO 4-dose recommendation
	IM[a]	0 (2 doses), 7, 21	Used in some countries, so-called 2-1-1 schedule
	ID[b]	0, 3, 7, 28 (2 doses each)	Used in Asia with PVRV, PCECV, so-called 2-2-2-0-2 schedule
	ID[b,d]	0 (8 doses), 7 (4 doses), 28, 90	Used in developing countries with HDCV, PCECV, or PVRV cell culture vaccines, so-called 8-0-4-0-1-1
	ID	0 (4 doses), 7 (2 doses), 28, 90	Simpler ID schedule
Booster	IM[a]	0, 3	Only after documented vaccination with cell culture vaccine[c,e]
(for preexposure)	ID[b]	0, 3	Only after documented vaccination with cell culture vaccine[c,e]

[a]Give 0.5 mL (PVRV) or 1.0 mL, depending on the vaccine, into the deltoid.
[b]Give 0.1 mL over the deltoids.
[c]Together with rabies immunoglobulin.
[d]Give 0.1 mL at multiple sites (see text).
[e]Or demonstrated presence of virus neutralizing antibodies after other vaccines.
HDCV, human diploid cell vaccine; ID, intradermal; IM, intramuscular; PCECV, primary chick embryo cell vaccine; PVRV, purified Vero-cell rabies vaccine; WHO, World Health Organization.

intramuscularly (see "Schedules Using the Intradermal Route" later). More than 60,000 people have received rabies preexposure immunization with HDCV in the United States and there have been no rabies cases in any of these persons. Recently, Khawplod and colleagues proposed a 1-day preexposure schedule, in which a single full dose of vaccine administered intramuscularly or two 0.2 mL doses administered intradermally were able to prime the immune system for a rapid anamnestic response to booster doses given 1 year later on days 0 and 3.[256]

Bat handlers in the United Kingdom received three doses of HDCV or PCECV. All seroconverted, but titers diminished with age.[257] An Australian group immunized more than 1500 travelers by the intradermal route, and only 0.46% failed to reach a level of 0.5 IU/mL.[258] On the other hand, a Japanese-made PCECV given by the same route raised only low titers of antibodies.[259]

An attempt was made to use suckling mouse brain vaccine in a preexposure schedule of 0, 2, 4, and 30 days, but the results were unsatisfactory.[260]

Postexposure

The postexposure regimen recommended in the United States and by the WHO from 1999 to 2009 was rabies immune globulin (RIG) on day 0 and a CCV on each of days 0, 3, 7, 14, and 28.[261] In Europe, a sixth dose had been previously recommended at 90 days, but this dose did not substantially raise titers beyond those obtained after five doses.[262] However, in part because of a vaccine supply limitation in the United States, during 2009 a four-dose schedule (0, 3, 7, and 14 days) was adopted after an analysis of epidemiological, clinical, and experimental effectiveness data.[263] Fig. 50.3 illustrates the immunogenicity of rabies vaccines, showing that a fifth dose does not increase titers substantially beyond those achieved by four doses. The WHO suggests a choice between a four- and five-dose schedule.[264] The dose inoculated intramuscularly is 0.5 or 1 mL depending on the manufacturer.

Exposed persons who have received prior preexposure or postexposure vaccination with a CCV or who have proven rabies VNA after other vaccines should receive an intramuscular injection on each of days 0 and 3, without RIG. Anamnestic responses are excellent.[260] Persons who have received a nerve tissue vaccine without a documented VNA response should undergo the full postexposure regimen.

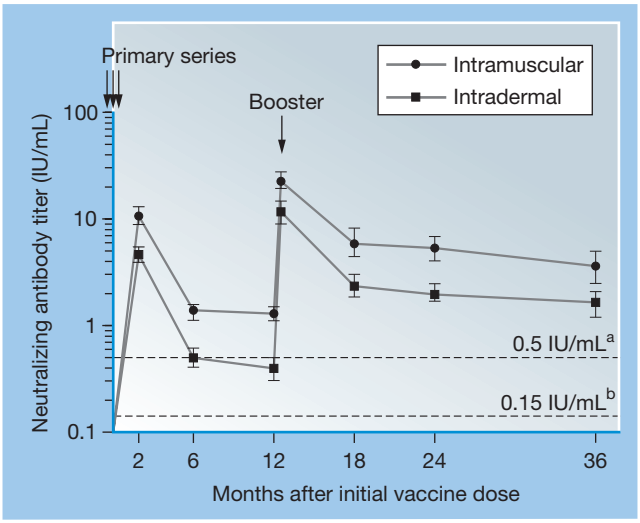


Figure 50.3. Kinetics of neutralizing antibody response in humans to purified Vero-cell rabies vaccine after two regimens of primary and booster preexposure immunization given intradermally or intramuscularly. [a]Minimally adequate titers defined by the World Health Organization. [b]Minimally adequate titers defined by the U.S. Advisory Committee on Immunization Practices and Centers for Disease Control and Prevention.

Alternative Vaccine Routes

The application of rabies vaccination faces several practical problems, particularly in developing countries. These include the expense of the vaccine, the cost and the unavailability of antiserum, and the difficulty for patients to return enough times to complete the series of injections. Therefore, alternative schedules have been suggested to decrease the number of visits, and the intradermal route, rather than the intramuscular route of injection, has been used to decrease the volume of vaccine and the cost.

Schedules With Fewer Visits

An alternative schedule developed in Yugoslavia and also extensively used in France was designated 2-1-1.[265,266] It consists of two 1.0-mL injections intramuscularly on day 0 and one each on days 7 and 21. Suckling mouse brain vaccine also has been successfully used according to this schedule.[267] However, when passive immunization is simultaneously given, some reports[268–270] showed that the average antibody response and the persistence of antibody are compromised, whereas other reports[271,272] show no interference. Immunosuppression by human rabies immune globulin (HRIG) seems to be absent when HDCV is the vaccine used in the 2-1-1 schedule. However, a failure of the 2-1-1 schedule after a bite on the lip has been reported,[273] and perhaps the use of the 2-1-1 schedule is best restricted to nonbite exposures and situations where patient compliance is very poor. Given the high potency of most modern CCV today, the immunological utility of two simultaneous doses administered on day 0 may be questionable. Moreover, if supply is restricted, two vaccine doses may not be adequate for such basic consideration, as well as the limitation in available anatomic sites for combined immunoglobulin and vaccine, particularly in large-bodied persons.

Schedules Using the Intradermal Route

The expense of a full regimen of intramuscular HDCV (more than US$1000 in the United States for a five-dose regimen) led to attempts to reduce the cost by taking advantage of the intradermal route. For example, Aoki and colleagues,[274] Turner and colleagues,[275] and Nicholson and associates[276] demonstrated that rapid antibody responses could be induced by various intradermal regimens, including multisite postexposure inoculations.

Intradermal regimens have now undergone extensive evaluations and are indicated for postexposure vaccination in the circumstances of inadequate funds and the availability of staff experienced in intradermal injection technique.[277,278] The vaccines that may be used by this route include HDCV, PVRV, and PCECV. The antigenic content of the rehydrated vaccine should always be at least 0.25 IU/0.1 mL. The injection should be given with a 1.0-mL syringe and a 25- or 27-gauge needle, with the needle introduced parallel to the skin into the epidermal layer. A papule should always be produced at the site of the injection.[279]

For preexposure immunization, three doses are ideal (0, 7, and 21 or 28 days) although even one dose has been claimed to induce B-cell memory that responds to boosting.[225,256] Intradermal injection has been widely used to immunize veterinary students and others at high risk of rabies virus exposure. Antibody titers may be lower after intradermal than after intramuscular inoculation but still adequate (see Fig. 50.3). For example, an Australian study using HDCV demonstrated seroconversion in 98% of travelers.[280] Local reactions are annoying but tolerable after intradermal vaccination, and systemic reactions are virtually absent. However, in 1983, the death in Kenya from rabies of a Peace Corps volunteer who had been preimmunized with HDCV by the intradermal regimen (but not boosted after a rabid dog bite), raised questions regarding the efficacy of this route.[281] Intensive review of immunization records revealed that people vaccinated overseas, whether intramuscularly or intradermally, often had lower and shorter-lived antibody responses than expected from observations made in people immunized in the United States. One factor shown to be significantly associated with lower immunologic responses was the concurrent administration of chloroquine for antimalarial chemoprophylaxis.[227] People receiving concurrent antimalarial chemoprophylaxis or other immunogens are now recommended to receive the intramuscular and not the intradermal regimen for antirabies preexposure immunization to maximize their antibody responses, given the immunomodulatory effects that chloroquine may have upon dendritic cells.[282] The situation in some countries, such as the United States, is now complicated by the removal of the intradermal formulation from the market. Whereas multiple 0.1-mL intradermal doses can be withdrawn from vials containing an intramuscular dose, this practice is usually considered off-label use. However, postexposure intradermal immunization using vaccine from intramuscular vials is licensed in Thailand and other countries to make CCVs more readily available for rabies virus exposures.

Warrell and colleagues[283,284] developed a concept of multiple intradermal vaccinations. According to one regimen, HDCV or PCECV was given intradermally at eight different sites (deltoid, suprascapular, thigh, and abdominal wall on day 0; four 0.1-mL doses over the deltoid and thigh on day 7; and single doses over the deltoid on days 28 and 91).[285] However, antibodies were not induced any more rapidly by this regimen than by others, and concomitant antiserum is still indicated for bite and mucosal exposures.[286] Thereafter, a four-site intradermal postexposure regimen was proposed by the same authors, consisting of four inoculations of 0.1 mL on day 0, followed by two inoculations of 0.1 mL on day 7 and one inoculation on each of days 28 and 90.[287] When compared with the intramuscular, eight-site intradermal and two-site intradermal regimens, the four-site regimen proved to be equal or superior in immunogenicity (Table 50.8). Preliminary data have also been published on an accelerated four-site schedule administered on days 0, 3, and 7,[288] showing complete seroconversion.

Phanuphak and associates[289] developed another intradermal schedule that became a standard regimen used in Thailand. PVRV or PCECV was given over both deltoids in a volume of 0.1 mL on days 0, 3, and 7, followed by single injections on days 28 and 90. However, because many patients did not return for the 90-day dose, the Thai regimen was changed to give two injections on day 28, but none subsequently. Antibody titers with the new regimen were equivalent at 90 days.[290] The two intradermal regimens were compared in India, where the eight-site regimen induced higher antibody titers, particularly at 7 days.[285] Intradermal vaccination is considerably less expensive than the full intramuscular use of CCV.[170,289,291,292] Although not recommended, in practice, the vaccine is sometimes reconstituted and stored for several days under refrigeration to use the same vial for the first three injections of vaccine. So far, this specific practice has not resulted in known vaccination failures. Nevertheless, as these are single use vials intended to be used within a few hours, caution is urged to maintain the cold chain and to avoid contamination.[293]

Many clinical studies have occurred on the use of intradermal vaccination. For example, rabies virus VNA responses after intradermal vaccination with HDCV or PVRV were compared in Thailand.[291] All vaccinees in both groups were seropositive at day 14, whereas at day 90, adequate VNA levels were found in 95% of PVRV recipients and 96% of HDCV recipients. A comparison of intradermal PVRV or PCECV with intramuscular PCECV showed no important differences in antibody responses.[294] However, the intradermal regimen used by the Thai Red Cross, even with double doses, did not induce VNA faster than classical regimens, and administration of passive antibody is still necessary to provide immunity during the first week after exposure.[286] In addition, Thai workers simulated two-dose postexposure booster vaccination in subjects previously vaccinated intradermally or intramuscularly with PVRV. The subjects vaccinated and boosted intradermally all developed anamnestic responses, although these were slower and

TABLE 50.8 Current Human Rabies Postexposure Prophylaxis Schedule, United States

Vaccination Status	Intervention	Regimen[a]
Not previously vaccinated	Wound cleansing	All PEP should begin with immediate thorough cleansing of any wounds with soap and water. If available, a virucidal agent (e.g., povidone-iodine solution) should be used to irrigate the wounds.
	HRIG	Administer 20 IU/kg body weight. If anatomically feasible, the full dose should be infiltrated around and into the wound(s), and any remaining volume should be administered at an anatomical site (IM) distant from vaccine administration. Also, RIG should not be administered in the same syringe as vaccine. Because RIG might partially suppress active production of rabies virus antibody, no more than the recommended dose should be administered.
	Vaccine	HDCV or PCECV, 1.0 mL, IM (deltoid area[b]); 1 each on days 0,[c] 3, 7, and 14.[d]
Previously vaccinated[e]	Wound cleansing	All PEP should begin with immediate, thorough cleansing of all wounds with soap and water. If available, a virucidal agent such as povidone-iodine solution should be used to irrigate the wounds.
	HRIG	HRIG should not be administered.
	Vaccine	HDCV or PCECV, 1.0 mL, IM (deltoid area[b]), 1 each on days 0[c] and 3.

[a]These regimens are applicable for persons in all age groups, including children.
[b]The deltoid area is the only acceptable site of vaccination for adults and older children. For younger children, the outer aspect of the thigh may be used.
[c]Day 0 is the day dose 1 of vaccine is administered.
[d]For persons with immunosuppression, rabies PEP should be administered using all five doses of vaccine on days 0, 3, 7, 14, and 28.
[e]Any person with a history of preexposure vaccination with HDCV, PCECV, or RVA; prior PEP with HDCV, PCECV, or RVA; or previous vaccination with any other type of rabies vaccine and a documented history of antibody response to the prior vaccination.
HDCV, human diploid cell vaccine; HRIG, human rabies immunoglobulin; IM, intramuscular; PCECV, purified chick embryo cell vaccine; PEP, postexposure prophylaxis; RVA, rabies vaccine adsorbed.
From Rupprecht CE, Briggs D, Brown CM, et al. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies: recommendations of the advisory committee on immunization practices. MMWR Recomm Rep. *2010;59(RR-2):6. Erratum in* MMWR Recomm Rep. *2010;59:493.*

TABLE 50.9 Rabies Virus Neutralization Antibody Titers After Human Intradermal Vaccination on Days 0, 3, 7, and 30 or Intramuscular Vaccination on Days 0, 3, 7, and 14

Day	No. of Patients/GMT	Intradermal PCECV	Intradermal PVRV	Intramuscular PCECV
7	n	58	59	37
	GMT[a]	0.34 (0.05–19.1)	0.32 (0.1–2.2)	0.29 (<0.05–19.1)
14	n	59	59	37
	GMT	28.5 (1.1–1318.0)	28.9 (1.6–350.0)	12.3 (0.4–301.0)
30	n	55	57	36
	GMT	10.9 (1.5–171.0)	10.9 (0.6–157.0)	18.5 (0.5–217.0)
90	n	53	58	36
	GMT	3.0 (0.4–59.1)	2.7 (0.5–47.0)	4.7 (0.5–60.9)

[a]Values in parentheses are the GMT range. The GMT is given as IU/mL.
GMT, geometric mean titer of rabies virus neutralizing antibodies; n, number of patients per protocol; PCECV, primary chick embryo cell vaccine; PVRV, purified Vero-cell rabies vaccine.
From Briggs DJ, Banzhoff A, Nicolay U, et al. Antibody response of patients after postexposure rabies vaccination with small intradermal doses of purified chick embryo cell vaccine or purified Vero cell rabies vaccine. Bull World Health Organ. *2000;78:696.*

of lower magnitude than in the all-intramuscular group.[295] Conversely, a booster study done with a four-site intradermal regimen showed higher titers than after intramuscular immunization.[296] Using the Thai regimen, PVRV and PCECV were both shown to be immunogenic by the intradermal route if the intradermal dose was 0.1 mL and given on days 0, 3, 7, and 30, as shown in Table 50.9.[294,297,298]

Intradermal PCECV was also studied in Thailand[299] with and without concomitant administration of HRIG. Neutralizing antibodies peaked at a geometric mean titer (GMT) of approximately 10 IU in both groups, and there were no known failures of protection. A newer study in healthy volunteers showed that even at a dose of 0.032 antigen units, all subjects given the Thai Red Cross regimen (2-2-2-0-2) developed VNA by 14 days.[300,301] The same regimen, together with antiserum, was given to 113 patients severely exposed to rabid animals, of whom 112 developed titers higher than 0.5 IU/mL by day 14.[302] One current Thai Red Cross schedule used routinely consists of two intradermal injections of 0.1 mL given on each of days 0, 3, 7, and 28 and is referred to as the 2-2-2-0-2 schedule.[303] In India, an intradermal regimen of PCECV using two 0.1-mL doses on days 0, 3, 7, 14, and 28 resulted in all subjects having adequate levels of antibody by day 14, but titers were lower subsequently than in a parallel group given PCECV intramuscularly.[304]

In India, intradermal vaccination is practiced with WHO preapproved vaccines, enabling a reduction in cost from US$81 for the intramuscular regimen to approximately US$13 for an intradermal regimen.[305]

One microneedle device for intradermal injection has been studied. A four-dose regimen gave titers somewhat lower than by intramuscular administration, but all subjects seroconverted.[306]

In summary, intradermal regimens work as well as intramuscular regimens, except in some circumstances in which the host is likely to mount a lower antibody response from

immunosuppression. Although not now licensed in developed countries, such as the United States, intradermal vaccination is widely and successfully practiced in developing countries where cost of vaccine is comparatively high.

SERUM AND VACCINE PROPHYLAXIS

The proper postexposure prevention of rabies always includes combined vaccination and passive antibody administration in naïve patients, even when nerve tissue vaccine is used.[307] The use of antirabies serum in PEP goes back many years. During 1891, Babes and Cerchez[308] treated people severely bitten by rabid wolves with whole blood from vaccinated humans and dogs. In Iran, Baltazard and Ghodssi[309] observed an overall mortality of 25% in patients bitten by confirmed rabid wolves and treated with nerve tissue vaccine, regardless of the severity and site of the wounds. Among persons wounded in the head and face, mortality was 42%. When these results were compared with mortality rates observed in persons exposed to rabid wolves but untreated for various reasons, it seemed that the postexposure vaccination of severely exposed persons with vaccine alone conferred insignificant protection.

The efficacy of the combined use of vaccine and rabies serum to improve these results was established through collaborative studies proposed by a WHO Expert Committee on Rabies. The superior results obtained experimentally by Habel and Koprowski[310] were confirmed in a field study in Iran during 1954. Of five patients severely bitten by rabid wolves and treated with vaccine alone, three contracted rabies and died, whereas of 13 patients similarly bitten by the same wolf and treated with vaccine and serum, only one died. Thereafter, Cho and Lawson[311] performed an experiment on postexposure rabies vaccination in an animal model. When dogs were inoculated in the femoral muscle, vaccine alone was not protective because of the short incubation period in this experimental system, whereas serum alone protected approximately 50% and serum together with vaccine protected 100%. Similar results were reported by Hanlon and colleagues,[312] who used monoclonal antibodies against rabies virus as a successful alternative to RIG. Baer and Cleary[313] showed that HRIG had synergistic activity with HDCV in a mouse challenge model. Thus, these investigations demonstrated again the importance of rapid protection with antibodies and the synergy of serum with postexposure vaccination.

The intended purpose of RIG is to provide rabies VNA before an active response to the vaccine occurs. Isotyping of antibody after vaccination suggests that most IgG antibody may not appear for 14 days.[249] Evidence that the need for simultaneous RIG and HDCV is not merely theoretical is provided by reports of rabies that occurred after administration of HDCV without RIG.[314,315]

The first commercially available rabies antisera were produced in horses, but approximately 40% of adult recipients developed serum sickness.[316,317] A purified and pepsin-treated equine RIG (ERIG) was developed by the Swiss Serum Institute and Sanofi Pasteur and was widely used in Asia, but not in the United States. It was associated with only a 1% rate of serum sickness reactions,[318] after a recommended dose of 40 IU/kg.

Wilde and coworkers[318] and Tantawichien and colleagues[319] in Thailand carefully studied the use of ERIG. In their experience, the risk of anaphylaxis was only one in 35,000 people, and only 1% to 1.6% of recipients developed serum sickness. Moreover, although a skin test with 0.02 mL of a 1 : 100 dilution of ERIG is recommended before use, and the response is positive in 5% to 10% of patients, a positive test response was not predictive of serum sickness. They considered a risk of anaphylaxis to be present only if there is a wheal of 10 mm or greater in diameter or if a wheal of 5 to 10 mm is accompanied by a flare of 20 mm or more. A study of 286 Indian patients treated with ERIG showed no cases of anaphylaxis.[320] To ensure safety from contaminating equine viruses, a new heat-treated and more purified ERIG has been developed, which should give fewer reactions.[321,322] Both the older and new ERIG contain F(ab′)2 fragments rather than the whole equine globulin. The kinetics of ERIG fractions vary substantially between intact and fragmented immunoglobulin molecules, with obvious differences in experimental PEP studies of animals. Moreover, in the process of substitution of new for old, cost has increased and ERIG supplies have diminished, creating shortages in some areas.[323]

A recent review of PEP in the Philippines, in which purified ERIG was used, followed up 144 patients exposed to laboratory-confirmed rabid animals. There were two deaths due to rabies: one child with multiple deep wounds on the neck and another who did not also undergo vaccination.[324]

A HRIG was prepared originally by Bayer (formerly Cutter, Talecris, and now Grifols) and Sanofi Pasteur from the plasma of volunteers immunized with HDCV to provide an antibody preparation that would not produce serum sickness. The products sold in the United States currently are Imogam Rabies-HT (Sanofi Pasteur) and Bayrab (Grifols Biological Products). Administration of HRIG has not been associated with reactions other than occasional local pain and low-grade fever.[325] The dose is 20 IU/kg, with as much as possible of the volume instilled at the site of the bite, and the remainder, if any, given into the muscle at a distant site. The WHO recommends that HRIG be used in preference to ERIG for all severe multiple bites.[215] The recommended dose should not be exceeded, because too much passive antibody may have a dampening effect on the active antibody responses.[326] For the same reason, the dose should not be repeated. However, if the HRIG (or ERIG) was not given immediately with vaccine, it should still be given up to the seventh day after exposure, by which time an active response to vaccination should begin. After administration of HRIG, serum neutralizing antibodies can be detected within 24 hours, reach a level of about 0.1 IU at 3 days, and decay with a half-life of about 21 days.[327] In Thailand, patients started receiving vaccine up to 5 days before coming for RIG treatment, but this delay was not accompanied by suppression of the active immune response.[321]

An interesting case report describes a patient with furious rabies who became paralyzed after receiving intravenous HRIG.[77] An autoimmune mechanism involving deposition of immune complexes on axons has been suggested but not proven.

Local infiltration of antibodies is crucial because serum levels of VNA achieved after intramuscular administration alone may be low or negligible for several days.[321,322] Local infiltration provides additional VNA at the site of contamination with the infecting virus. To have a sufficient volume for infiltration in the case of multiple bites, particularly in children, the RIG may be diluted in normal saline.[328,329] Rabies developed in five children in whom local infiltration was not performed as recommended.[330]

Current recommendations of the U.S. Advisory Committee on Immunization Practices call for the local infiltration of the entire HRIG dose, if feasible. If not, the remainder should be given intramuscularly at a site distant from the vaccine injection. Where HRIG is not available, ERIG, such as in its new chromatographically purified and heat-treated form, should be used.[323]

For a discussion of antirabies monoclonal antibodies as an alternative for RIG, see "Monoclonal Antibodies" later.

RESULTS OF HISTORICAL IMMUNIZATION

Immune Responses

Nerve tissue vaccines are not optimal with regard to safety or efficacy.[331]

Extensive studies have been conducted of antibody responses to CCVs.[a] Several key findings of those studies may be generalized, as follows:

1. The most important immune response to rabies vaccines is production of relevant antibodies to the G protein of the viral envelope.[56,336] Antibody is normally measured by neutralization, such as the inhibition of fluorescent foci. However, the choice of the virus used in the neutralization test is important because the use of a virus homologous to the vaccine strain may yield approximately 30% higher titers than do heterologous viruses.[346]
2. Typically, IgM antibodies appear after approximately 4 days and IgG antibodies by 7 to 14 days after the first dose of vaccine.[347]
3. Vaccine given during the first 14 days prime the immune system, but for preexposure vaccination, at least one booster dose at 21 to 30 days or later seems necessary for high and persistent titers.
4. Three doses of CCV delivered over 21 to 28 days induce antibodies in virtually100% of healthy persons and can be used as a preexposure regimen.
5. With intramuscular vaccination, the primary regimen for postexposure use is four doses given during the first 2 weeks in healthy persons.
6. Intradermal regimens using multiple sites (two to four or more) administered on four to five occasions between days 0 and 30 also induce adequate antibody responses and use much lower volumes of vaccine, which is an economic advantage.
7. Antibody titers after vaccination with CCV are usually greater than 10 IU/mL, significantly higher than obtained in persons given nerve tissue vaccine. Antibody induction exceeding 0.5 IU/mL is nearly always achieved in healthy persons, and this level is used as a benchmark for a successful immune response after vaccination.
8. Given ideal immunization responses observed to date, it is not necessary to check rabies VNA titers after routine preexposure or postexposure immunization with CCV, unless the vaccinee is immunosuppressed (see later), has received chloroquine with intradermal vaccination, or has undergone a significant deviation from standard recommendations.[348] HIV-infected patients may respond poorly to rabies vaccines[349] and should be monitored serologically.
9. Age is a factor in response, as persons older than 50 years of age may respond less well than younger persons, but all should seroconvert.[350,351]
10. The place of cell-mediated immunity in routine prophylaxis remains uncertain. Clearly, although cellular responses of the T-helper and the human leukocyte antigen (HLA)-restricted cytotoxic T-cell types are produced, direct proof of a useful function of the latter is lacking, whereas VNA are demonstrably effective.[65,352–354]
11. Certain host response genes are important, as vaccinees of HLA groups B7 and DR2 show early and higher antibody responses, whereas those of HLA group DR3 respond later and with lower levels.[283]
12. There is apparent "molecular mimicry" of the nicotine receptor–binding motif between rabies virus G protein and HIV glycoprotein 120, which may induce antibodies to HIV in some rabies vaccinees.[355,356] False-positive tests for antibodies to HIV have been reported after rabies vaccination,[357,358] but the phenomenon seems to be quite uncommon.[359]
13. Seroconversion with VNA titers greater than 0.5 IU/mL was achieved in 33% of people receiving two doses of vaccine but in 100% of recipients of three or more doses.[360]
14. A preliminary study showed that vaccination elicited a range of cytokine and chemokine responses but failed to distinguish between intramuscular and intradermal vaccination.[361]
15. Modern CCV have been shown to protect against all known rabies viruses, but cross reactivity against other diverse lyssaviruses associated with bat reservoirs in Africa and Asia is much less than ideal.[362]

Tables 50.5 and 50.9 show the typical evolution of VNA after vaccination.[363]

Effectiveness

True efficacy trials for human rabies vaccines are difficult to undertake on the basis of both epidemiological and ethical grounds. Nevertheless, there are a variety of comparative data from various clinical trials conducted throughout the world that demonstrate the utility of vaccination. The effectiveness of Semple-type nerve tissue vaccine has been estimated to be approximately 84% in India,[364] although protection may be lower after severe exposures. For CCVs, Nicholson[365] estimated a failure rate of one in 80,000 in developed countries and between one in 12,000 and one in 30,000 in developing countries.

During the development of HDCV, comparative effectiveness studies were carried out in Iran, Germany, and the United States. In Iran, 45 people exposed in eight different incidents to eight proven rabid wolves or dogs were given six doses of HDCV after exposure. Antirabies serum also was given with the first vaccine doses. The presence of rabies virus in the brain was confirmed for all eight animals. In four animals tested, high virus titers also were found in the salivary glands. All human vaccinees developed antibodies, and none developed rabies.[221] In Germany, 63 persons bitten by rabid animals were uniformly protected. The accumulated experience in the United States was summarized in 1980, at which time 90 people exposed to rabid animals had all survived after HDCV vaccination.[366] Similar inferences can be made for PCECV, especially in the United States. Between a recall of HDCV in 2004 and its reintroduction during 2006, PCECV was the only human rabies vaccine available for routine PEP. These epidemiologic data are supported by multiple studies that demonstrate the effectiveness of PCECV in preexposure prophylaxis or PEP scenarios in developing countries.

The HDCV produced by Berna was subjected to trial in 100 Thai patients exposed to proven rabid animals. All patients were protected by the standard five-dose regimen, although the GMT of rabies VNA at 90 days after the first vaccination was relatively low, at 2.57 IU.[367]

Understandably, there have been no placebo-controlled studies of the efficacy of human rabies vaccines. The CCVs, including PVRV, PCECV, PDEV, and PHKCV, have been accepted based on the induction of VNA and lack of failures after postexposure vaccination.[a]

[a]References 36, 147, 169, 178–180, 186, 193–197, 199, 202, 204, 206, 209–214, 218, 221, 226–228, 231, 236, 237, 239, 242, 244, 246, 252, 253, 267, 276–279, 280–285, 332–345

[a]References 36, 180, 193–196, 199, 202, 204, 206, 209, 210, 212, 213, 228, 236–239, 242, 244, 246, 251, 252, 332, 333

An analysis of rabies postexposure vaccination in the United States showed an average of more than 23,000 courses given per year, and although the decision to vaccinate was often made without consulting public health subject matter experts in rabies, no failures were seen.[368] A review of 20 years of use of PVRV revealed only one failure after a standard regimen, occurring in a child with multiple bites on the face.[369] Postmarketing surveillance of an Indian-made HDCV also showed broad protection.[370]

In addition, the efficacy of rabies vaccination has been well supported using a variety of animal species as human surrogates. For example, protection studies in mice showed that VNA induced by the G protein contained in HDCV neutralized 17 different street rabies viruses.[355]

PERSISTENCE OF IMMUNITY AND BOOSTER DOSES

Rabies VNA do not always remain at elevated levels for long periods after vaccination with the usual preexposure schedules. By 1 year, VNA may fall to geometric mean levels between 1 and 3.5 IU,[217,371,372] and by 2 years may fall below the minimum acceptable level of 0.5 IU in approximately 15% to 20% of subjects.[373] However, B-cell memory is prolonged,[139] and Thraenhart and colleagues[374] reported the presence of VNA in the serum of 18 people vaccinated 2 to 14 years earlier. Antibodies reacting with many virus proteins were still present, in addition to lymphocyte proliferation responses to the same proteins. A survey of travelers to Nepal vaccinated against rabies during the preceding 5 years showed antibodies in 37 of 38 people.[375]

Booster doses of vaccine are efficient in restoring VNA, with 100% of subjects showing a fivefold rise by day 7.[371] Apparently, two booster doses may enhance the speed of the booster response.[376] Therefore, previously immunized persons who are again exposed to rabies should receive two booster doses 3 days apart, without RIG. Boosters can be given intramuscularly or intradermally, although no preparation approved for intradermal use is available in the United States. Studies[240,377–379] have compared four-site intradermal inoculations with the standard two-dose intramuscular inoculations in previously vaccinated volunteers undergoing simulated reexposure to rabies and found that the intradermal group had better booster responses. Vietnamese children vaccinated 5 years earlier were easily boosted.[380] When maintenance of VNA is desired after preexposure vaccination, as in laboratory workers, a booster may be given at 1 year after primary vaccination, which invariably produces an anamnestic response (see further discussion in "Indications for Vaccination" later). However, because of allergic reactions to the HDCV used in the United States (see "Reactions to Cell Culture Vaccines" later), routine boosters are not recommended but rather serological surveillance should be performed to monitor VNA levels. Laboratory workers who have continuous exposure to rabies virus should have antibody levels checked every 6 months and should receive a single booster immunization if the titer falls below complete virus neutralization at a serum dilution of 1:5 (or approximately 0.1 IU/mL).

VACCINATION OF IMMUNOSUPPRESSED PERSONS

Briggs and Schwenke[381] compared the persistence of antibodies in civilians and in Peace Corps volunteers who receive chloroquine for prophylaxis of malaria. At 1.5 to 2 years after primary vaccination, adequate titers were found in 99% of civilians and 88% of Peace Corps volunteers who received the vaccine intramuscularly and in 93% of civilians and 64% of Peace Corps volunteers who received the vaccine intradermally, suggesting that chloroquine was immunosuppressive.

Numerous studies on HIV-infected patients have shown that, although rabies vaccination is safe and generally effective,[382,383] persons with $CD4^+$ cell counts less than $300/mm^3$ or 15% of lymphocytes are less likely to respond with VNA.[349,382,384–386] Patients receiving immunosuppressive drugs and persons with diabetes also should be monitored for proper VNA responses.[387,388] In contrast, pregnant women respond normally to rabies vaccine, and there is no suggestion of fetal risk.[389,390]

Malnourished children respond to PCECV rabies vaccine with satisfactory antibody titers.[391]

A study of intradermal rabies vaccination in patients undergoing hemodialysis showed adequate immune responses,[392] as did intramuscular vaccination in pediatric solid-organ transplant recipients.[393]

Replication-defective *Vaccinia* viruses carrying rabies G and N genes have been shown to protect B-cell–deficient mice, and future development of such biologics might be of value in B-cell–deficient persons exposed to rabies.[394]

PROTECTION AGAINST LYSSAVIRUSES OTHER THAN GENOTYPE I

The ability of vaccines formulated with classical rabies virus to protect against other rhabdoviruses in the *Lyssavirus* genus remains questionable. However, it seems that some antibodies are cross-neutralizing and that cross-protection is good in animals challenged with some species, such as the European bat and Australian lyssaviruses by parenteral routes, although not after intracerebral inoculation.[395] This is particularly comforting for prevention of rabies caused by European bat lyssaviruses, although titers may be more variable.[396] However, the results for more distant Asian and African lyssaviruses depend on relative genetic distance, with lower protection for the most distantly related lyssaviruses.[312]

CORRELATES OF IMMUNOPROTECTION

Preexposure vaccination with potent rabies CCV leads to the development of antibodies. Vaccination also induces production of T cells, which protect vaccinated mice in the absence of neutralizing antibodies. A high level of cell-mediated cytotoxic activity can be maintained by repeated inoculations of vaccine, and the presence of antibodies does not interfere with the secondary stimulation of sensitized lymphocytes.

Although the exact mechanism of protection of humans through postexposure vaccination is unknown, neutralizing antibodies have a major role.[397] The fact that only monoclonal antibodies that interact with macrophages are effective in protection of mice against disease may indicate that a complex mechanism is involved in antibody protection after challenge.

Concentrated and inactivated rabies vaccine of tissue culture origin is able to induce high levels of circulating interferon a few hours after its administration and can protect animals from rabies infection if it is given shortly before or after challenge with virus. This interferon-induced protection, however, is not specific because similar protection can be obtained with concentrated vaccines produced from unrelated viruses, such as influenza and Kern Canyon. However, only rabies vaccine can protect when it is given several days before challenge with rabies virus. The combined treatment with interferon or interferon inducers in addition to rabies vaccine is more efficacious than vaccine alone in experimental animals, when treatment is initiated several hours after challenge.[398]

Considering the critical usefulness of antibodies, the additional role of interferon and other cytokines in human prophylaxis is likely important, but less defined.

In reality, when humans are bitten by a rabid animal, the viral dose, route, and severity cannot be controlled in nature. As such, there is no distinct seroprotective antibody level defined for humans or other animals as an absolute predictor of outcome. The WHO international standard of 0.5 IU/mL is merely one arbitrary level chosen as a representative value that defines a given amount of VNA that can be measured in a serum neutralization test by reference laboratories. However, no vaccine is 100% efficacious, and no set level of VNA is acceptable as a facsimile of direct outcome. In practice, after viral exposure, previously vaccinated persons should receive immediate wound care and booster doses of vaccine to induce a rapid anamnestic response—and not rely on routine laboratory antibody testing, or a predetermined titer, to decide upon a course of immunization by medical providers. Similarly, in experimental testing of vaccine efficacy, animals having no detectable VNA may be protected against viral challenge, and animals with a titer even greater than 0.5 IU/mL can develop rabies. Clearly, the immune response during rabies prophylaxis after viral infection is complex, and VNA are obviously important, but understanding the basis for this repertoire should lead to knowledgeable intervention and the more strategic development of improved future biologics.[399]

VACCINATION FAILURES

Most human cases of rabies occur because no prophylaxis was given. Clearly, modern rabies postexposure vaccination is highly effective, but failures can occur when recommendations are not followed. As rabies in the biting animal is frequently untested and thus unknown, it is impossible to give a figure for the true rate of failure, but it is probably less than 1%. Most failures involve inadequate PEP, such as delays between exposure and initiation of care, and no administration of RIG, and often involve severe exposures, such as multiple bites on the head and neck.

For example, Thraenhart and colleagues[332] reviewed 28 cases of rabies that developed despite PEP with modern vaccines. In 90% of cases, RIG had not been administered or had been administered incorrectly. Other errors included passive immunization more than 24 hours before vaccine administration (which may have interfered with the active induction of immunity), incorrect local wound cleansing, injection of vaccine into the buttocks instead of the deltoid muscles, and late initiation of immunization. Only two patients, both of whom had severe facial injuries, were considered to be true prophylaxis failures. Wilde[400] described 15 cases of apparent vaccine failure in detail, and others have been described by Shantavasinkul and colleagues[401] and Hemachudha and colleagues.[402]

An early death phenomenon has been described in animals exposed to rabies and then vaccinated. In other words, some animals that are vaccinated but nevertheless develop rabies die sooner than challenged unvaccinated animals. It is unclear whether this phenomenon is mediated by antibodies, cytotoxic T cells, or a combination of the two. Willoughby[38] reviewed this subject and concluded that early death has not been convincingly demonstrated in humans, although there was a hint of it in patients bitten by rabid bats.

A follow-up study of effectiveness after 15 million doses of PCECV reported 47 suspected failures. All occurred in India and Thailand, and in no case were WHO guidelines followed completely.[403] Other failures also may relate to deviations from standard prophylaxis.[273,404,405] However, a bite on the face with a large inoculum of rabies virus may overwhelm PEP, especially if delays or perceived deviations from protocol occur.[362]

ADVERSE REACTIONS

Historically, it is not uncommon to encounter adverse reactions after human or veterinary vaccination. Today, products have improved greatly regarding safety, but there is no vaccine known that is completely free from all adverse events. However, vaccines differ specifically from each other in terms of the incidence and degree of adverse reactions. Moreover, although several adverse reactions are common to all vaccines, there also are some vaccine-specific adverse reactions. Vaccine components that may elicit an allergic response include active immunizing antigens, conjugating agents, preservatives, stabilizers, antimicrobial agents, adjuvants, and culture media used in the preparation of vaccines.[406] Components often implicated in vaccine allergies include egg and gelatin proteins. In general, vaccines contain both active and inactive ingredients that can cause adverse reactions, including anaphylaxis and allergy. Such ingredients include foreign proteins (e.g., albumin) used during manufacture, viral inactivants (e.g., β-propiolactone), preservatives (e.g., thimerosal), stabilizers (e.g., serum), adjuvants (e.g., alum), antibiotics (e.g., neomycin), and the antigen itself, especially when insufficiently purified.

Reactions to Rabies Vaccines Containing Animal Brain Tissues

There are several types of reactions to rabies vaccines containing animal brain tissue. These reactions include general systemic, local, and severe and fatal reactions.[219]

General Systemic Reactions

The various minor disorders that may develop during or after a course of antirabies vaccination include fever, headache, insomnia, palpitations, and diarrhea. Sensitization to proteins contained in older nerve tissue vaccines can cause a sudden shock-like collapse, usually toward the end of the course of treatment.

Local Reactions

Erythematous patches may develop approximately 7 to 10 days after the beginning of antirabies vaccination. Local erythema and swelling might also appear a few hours after vaccine injection and fade in 6 to 8 hours.

Severe and Fatal Reactions

A patient may have serious and often fatal illness after nerve tissue vaccine. These accidents are of two types: (a) *rage de laboratoire* (laboratory rabies infection), a disease induced by the non-inactivated fixed virus present in the vaccine, and (b) neuroparalytic accidents, which present the greatest prevalence of danger from rabies vaccination. All types of vaccine containing adult mammalian nervous tissues exhibit similar capacities for inducing neuroparalytic reactions. The neuroparalytic accident usually develops between the 13th and 15th days of vaccination and may assume one of the following forms:

- *Landry type*—In this type of accident, the patient rapidly develops fever and has pain in the back. Flaccid paralysis of the legs begins, and within 1 day the arms become

paralyzed. Later the paralysis spreads to the face, tongue, and other muscles. The fatality rate is approximately 30%; in the remaining 70%, recovery usually occurs rapidly. The incidence varies between one in 7000 and one in 42,000 recipients.[407]

- *Dorsolumbar type*—Less severe than the Landry type, this is the most common form of neuroparalytic accident. Clinical features are explicable by the presence of dorsolumbar myelitis. The patient may be febrile and feel weak, with paralysis of the lower limbs, diminished sensation, and sphincter disturbances. Typically, the fatality rate does not exceed 5%.
- *Neuritic type*—In this type of accident, the patient may be febrile and usually shows a temporary paralysis of the facial, oculomotor, glossopharyngeal, or vagus nerve.
- Optic neuritis has also been reported.[408]

Neuroparalytic accidents are caused by allergic "encephalomyelitis," attributable specifically to sensitization to adult nerve tissue antigen (i.e., myelin basic protein).[409] High-titered antibodies to human myelin proteins and to gangliosides often are detected in patients who are affected.[410] The incidence of these reactions to nerve tissue vaccine varies widely from 0.017% (1:6000) to 0.44% (1:230) and is definitely lower in people who received DEV (1:32,000) and in people receiving properly manufactured vaccine of newborn rodent brain (1:8000).

One study observed 1392 Tunisian adults who were given a Semple-type vaccine prepared by phenol inactivation of rabies virus–infected lamb brains.[411] In seven patients, neurologic complications developed, including paralysis or paresis in five. Most of the patients had elevated cell counts in their CSF. A rate of one neurologic complication in 200 vaccinations is unacceptable and illustrates the desirability of replacing nerve tissue vaccines with CCVs. Progress is ongoing, as demonstrated by the major progress in India, which switched to CCVs.

Reactions to Cell Culture Vaccines

General Reactions

The CCVs are widely accepted as well-tolerated rabies vaccines, although reported reaction rates to primary immunization have varied with the monitoring system. In large-scale testing of the safety and immunogenicity of HDCV performed on American veterinary students, adverse reaction rates observed in more than 1770 volunteers were as follows: significantly sore arm, 15% to 25%; headache, 5% to 8%; malaise, nausea, or both, 2% to 5%; and allergic edema, 0.1%.[217] In another study of postexposure vaccination, 21.0% had local reactions, 3.6% had fever, 7.0% had headache, and 5.0% had nausea.[412] The most common local reactions are erythema, pain, and induration. These results have been confirmed by newer studies.[230] When HDCV is administered to children, in whom psychological overlay is presumably less than in adults, there are fewer complaints.

An observational study of 290 healthcare workers given PCECV showed 53% had systemic reactions, including fatigue, malaise, headache, chills, and fever, and 5% of subjects discontinued vaccination. However, there was no control group.[413] Data concerning the same vaccine were collected by the VAERS (Vaccine Adverse Event Reporting System) passive reporting system. Headache was the most prominent symptom reported. Neurologic events did not seem to be related to the vaccine, and other reported reactions were usually nonserious or temporary.[414,415] A study comparing PDEV with PVRV and PCECV showed no differences in reactions.[416] PCECV was safely coadministered to Thai children along with Japanese encephalitis vaccine.[417]

Allergic Reactions

After licensure of HDCV in the United States and its widespread use, allergic reactions began to be reported, principally after booster doses.[418,419] The overall incidence of reactions was 11 in 10,000 (0.11%) vaccinees, but, after boosters, the incidence rose to 6%.[420] Anaphylactic type 1 (IgE) reactions occurred in approximately 10% of the reported cases, all during the primary series (one in 10,000 vaccinations), but the majority seemed to be type 3 hypersensitivity (IgG–IgM) reactions occurring 2 to 21 days after booster doses (Table 50.10). These reactions have been attributed to antigenicity conferred on human albumin used as stabilizer in the vaccine by the β-propiolactone used to inactivate the virus, which increases the capacity of the albumin to form immune complexes.[421–423] Fortunately, respiratory symptoms are mild, and there have been no deaths. Antihistamines, epinephrine, and, occasionally, steroids have been used in successful treatment of the reactions, which resolved in 2 to 3 days.

Newer vaccines are produced by use of additional purification steps to remove human albumin. Systemic reactions to booster doses are uncommon with these vaccines and allergies appear comparatively rare.[406,424,425]

Twenty cases of possible anaphylaxis after PCECV were reported to VAERS.[414]

Neurologic Reactions

Although several cases of CNS disease, including transient neuroparalytic illness of the Guillain-Barré type, have been reported among the millions of persons given HDCV,[426–431] this rate is considered too low to be causally related to vaccination because the background incidence of such diseases is approximately one in 100,000 per year. The low incidence after HDCV compares with a neurologic complication rate of one in 1600 people for nerve tissue vaccine, one in 8000 people for suckling mouse brain vaccine, and one in 32,000 people for DEV. A review of Guillain-Barré syndrome after vaccination described several cases after rabies vaccination, but at rates consistent with coincidence.[432]

TABLE 50.10 Signs and Symptoms in Three Cohorts Containing 255 Human Subjects Reporting Presumed Immune Complex–Type Hypersensitivity Reactions[a] After Booster Immunization With Human Diploid Cell Rabies Vaccine Given Intradermally or Intramuscularly

Number with any sign or symptom[b]	29 (11.4)
Pruritic rash	17 (59)
Urticaria	24 (83)
Edema	14 (48)
Joint	4 (14)
Fever	1 (3)
Difficulty breathing	1 (3)
Mean delay after booster before reaction	9.6 d
Range, intradermally	3–13 d
Range, intramuscularly	8–11 d

[a]Coombs and Gell type III.
[b]Data are given as number (percentage).
From Centers for Disease Control and Prevention (CDC). Systemic allergic reactions following immunization with human diploid cell rabies vaccine. MMWR Morb Mortal Wkly Rep. *1984;33:185–188.*

In Thailand, a switch from Semple-type vaccine to HDCV resulted in a drop in the rate of neurologic complications from one in 155 to less than one in 50,000 treatments. At the same time, the failure rate dropped from one in 2000 to one in 25,000 treatments without the use of RIG.[433] If reactions occur after one CCV, a switch can be made to another produced in a different cell substrate without major concerns.

A case of bilateral neuroretinitis has been reported after PCECV.[434]

INDICATIONS FOR VACCINATION

Preexposure Vaccination

All high-risk professionals, such as veterinarians, hunters, trappers, animal control personnel, mail carriers, cavers, and laboratory workers contemplating working with rabies virus, should be prophylactically immunized against rabies. Table 50.7 gives the recommended regimens. After receiving a three-dose preexposure regimen, persons who are continuously exposed to high concentrations of rabies virus in the laboratory should have VNA levels checked every 6 months and should be given a booster dose intramuscularly or intradermally if the titer is less than adequate (complete virus neutralization at a serum dilution of 1:5 [~0.1 IU/mL], or 0.5 IU). Veterinarians and others exposed regularly to rabid animals or rabies virus should be similarly checked every 2 years.

Peace Corps workers, missionaries, or others who remain for long periods in rabies-enzootic countries certainly deserve consideration for preexposure vaccination.[435] A survey of travelers revealed that, after an average of 17 days in Thailand, 1.3% and 8.9% had been bitten or licked by a dog, respectively, and 0.5% had required rabies vaccination.[436] Moreover, postexposure vaccination in developing countries is often complicated by problems of availability of potent vaccine and RIG.[437] However, a decision analysis concluded that routine preexposure vaccination would cost US$275,000 per case averted and that it should be individualized according to the traveler's situation.[438] One recommendation would be to vaccinate the travelers who will be staying in remote areas of hyperendemic dog rabies for more than a few days, particularly children. Travelers should be warned about the dangers of rabies and other zoonotic diseases and educated about animal bite prevention strategies.[439]

In countries where rabies is endemic and children are frequently exposed to rabid animals (such as residents in Amazonia because of nightly feeding of blood meals by vampire bats), one might contemplate prophylactic vaccination against rabies as part of future pediatric immunization. Dog bites are a considerable problem in many areas of the world and accounted for more than 5% of visits to the emergency department of a Bangkok hospital, of which 55% were by children.[440] So far, prophylaxis has been restricted primarily to the children of Westerners going to live in areas enzootic for rabies,[441] but clinical trials in children have been performed in developing countries.[442] A preliminary study was done with the PVRV vaccine in Thailand, where two doses were given intramuscularly in association with routine pediatric immunizations at 2 and 4 months of age.[443] Seroconversion to rabies occurred in 100% of infants, without significant interference with the other vaccines. A similar study was done in Vietnam with three doses of intradermal rabies vaccine at 2, 3, and 4 months of age, with an adequate immune response (GMT, 12 IU/mL).[444] An economic analysis concluded that preexposure vaccination becomes cost-effective when the local suspect dog-bite incidence is between 2% and 30%.[445]

Preexposure Booster Vaccination

After preexposure vaccination, antibodies may decline sharply within the first year of vaccination. If a booster dose is given 1 year later, subjects segregate into two groups: "ideal" responders, who develop a titer greater than 30 IU by 14 days after booster, and "poor" responders, whose titers are much lower. The former, who represent 75% of subjects, may not need further booster vaccination for 10 years or more, whereas the latter may require more frequent boosters as long as they remain at risk.[137] This strategy may reduce costs.[446,447]

Primary vaccination by the intradermal route may provide less-sustained immunity, but the intradermal route is an effective means of administering a routine booster.[225]

Postexposure Prophylaxis

Guidelines and schedules for PEP are given in Tables 50.11 through 50.13.

The essential triad of PEP is local wound care, vaccination, and antiserum administration. Local first aid of bites and

TABLE 50.11 Current Human Rabies Postexposure Prophylaxis Guide for the United States

Animal Type	Evaluation and Disposition of Animal	Postexposure Prophylaxis Recommendations
Dogs, cats, and ferrets	Healthy and available for 10 days of observation Rabid or suspected rabid Unknown (e.g., escaped)	Persons should not begin prophylaxis unless animal develops clinical signs of rabies[a] Immediately vaccinate Consult public health officials
Skunks, raccoons, foxes, and most other carnivores; bats	Regarded as rabid unless animal proven negative by laboratory tests[b]	Consider immediate vaccination
Livestock, small rodents, lagomorphs (rabbits and hares)	Consider individually	Consult public health officials
Bites of squirrels, hamsters, guinea pigs, gerbils, large rodents (woodchucks and chipmunks, rats, mice and other small rodents, rabbits, and hares, almost never beavers), and other mammals		Require antirabies postexposure prophylaxis

[a]During the 10-day observation period, begin postexposure prophylaxis at the first sign of rabies in a dog, cat, or ferret that has bitten someone. If the animal exhibits clinical signs of rabies, it should be humanely killed immediately and tested.

[b]The animal should be humanely killed and tested as soon as possible. Holding for observation is not recommended. Discontinue vaccine if immunofluorescence test results of the animal are negative.

From Rupprecht CE, Briggs D, Brown CM, et al. Centers for Disease Control and Prevention (CDC). Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies: recommendations of the advisory committee on immunization practices. MMWR Recomm Rep. *2010;59(RR-2):1–9.*

scratches consists of vigorous washing with soap and water, ideally for at least 15 minutes. Surgical suturing should be avoided for approximately 7 days, if possible, but in any case, RIG (see later) should always be administered before suturing.[448] Antibiotics and tetanus toxoid may be indicated to prevent other infections.[449]

A decision to give PEP should be based on a risk assessment, in part on consideration of the following points[450,451]:

1. Was the patient's skin broken by a bite or scratch, or were mucous membranes contaminated? If not, no true exposure has occurred. Seeing or touching a rabid animal is not an exposure. The risk of rabies after a bite by a rabid animal has been estimated at 5% to 80%, whereas the risk after scratches is much less (0.1% to 1%). The risk after mucous membrane contact is extremely low.[443]
2. If the bite was by a dog or cat, is domestic animal rabies found in the particular geographic area? Many areas of the world, such as Oceania, Antarctica, and the United Kingdom, are free of rabies in carnivores. In many cities of the United States, even stray animals are unlikely to be rabid.
3. Was the dog or cat vaccinated against rabies? Vaccination diminishes the risk, but not completely. Proper vaccination of pets should not be accepted at face value. Documentation should be required to show at least two vaccinations with a potent vaccine, as one dose of inactivated vaccine is insufficient to always warrant efficacy, especially if administered in within the last 30 days.[448,452]
4. Is the biting animal a healthy domestic dog or cat and available for observation? If yes, PEP may be postponed. However, in rabies-enzootic areas, vaccination is advisable, even if the domestic animal appears normal, because of short incubation periods in humans and the frequent difficulty in being certain of the identity of the biting animal.[453]
5. If the bite was inflicted by a wild animal, was it a species likely (e.g., skunk or raccoon) or unlikely (e.g., squirrel or rat) to be rabid? Local conditions must be taken into account. For example, in the United States, rabies has been reported occasionally in larger bodied rodents, such as woodchucks or beavers, a very unlikely finding elsewhere. Rapid diagnostics, performed within 24 to 48 hours of exposure, will assist in PEP decisions.
6. Was the bite provoked or unprovoked? This criterion is useful only in areas of low incidence and not where the incidence of rabies in dogs is elevated.[448] In Thailand, even when the animal's behavior appeared normal, an assessment of provocation did not correlate with the presence of rabies at necropsy of the animal.[454] Attempts to play with wild animals should be considered provocative.
7. Was there exposure to a rabid human? Under these circumstances, use the same criteria for vaccination as with a biting animal. Only persons who were bitten or scratched, who had mucosal exposure, such as with mouth-to-mouth resuscitation, or who were otherwise exposed to infectious saliva or nerve tissues need to be vaccinated.[452]
8. Should RIG be administered? In naïve, previously unvaccinated persons, RIG should always be given in combination with vaccine for transdermal or mucous membrane exposure (see "Serum and Vaccine Prophylaxis" earlier). The dose of HRIG is 20 IU/kg and that of ERIG, 40 IU/kg. As much as possible of the RIG should be inoculated locally, diluted if necessary in saline to provide sufficient volume. If necessary, local anesthesia can be provided with procaine-type compounds.[455] The remainder of the RIG, if any, should be given in the deltoid or other muscles.

TABLE 50.12 Comparative Immunogenicity of Different Human Rabies Vaccination Schedules[a]

Route	Day 7	Day 14	Day 90	Day 365
IM	0.36	228	6.2	1.3
ID (2 sites)	0.88	364	9.1	4.6
ID (4 sites)	0.44	335	7.2	2.5
ID (8 sites)	0.67	308	9.8	3.2

[a]Data are given as geometric mean titer.
ID, intradermally; IM, intramuscularly.
Data from Warrell MJ, Riddell A, Yu LM, et al. A simplified 4-site economical intradermal post-exposure rabies vaccine regimen: a randomised controlled comparison with standard methods. PLoS Negl Trop Dis. *2008;2:e224.*

TABLE 50.13 World Health Organization Recommendations for Human Rabies Prophylaxis

Category	Type of Contact With a Suspected or Confirmed Rabid Domestic or Wild[a] Animal or an Animal Unavailable for Testing	Type of Exposure	Recommended Prophylaxis
I	Touching or feeding of animals; licks on intact skin	None	None, if reliable case history is available
II	Nibbling of uncovered skin; minor scratches or abrasions without bleeding		Administer vaccine immediately.[b] Stop vaccination if animal remains healthy throughout an observation period of 10 days[c] or if animal is proven to be negative for rabies by a reliable laboratory using appropriate diagnostic techniques.
III	Single or multiple transdermal bites or scratches, licks on broken skin; contamination of mucous membrane with saliva (i.e., licks); exposure to bats[d]	Severe	Administer rabies immunoglobulin and vaccine immediately. Stop vaccination if animal remains healthy throughout an observation period of 10 days or if animal is found to be negative for rabies by reliable laboratory using appropriate diagnostic techniques.

[a]Exposure to rodents, rabbits, and hares seldom, if ever, requires specific antirabies postexposure prophylaxis.
[b]If an apparently healthy dog or cat in or from a low-risk area is placed under observation, the situation may warrant delaying initiation of vaccination.
[c]This observation period applies only to dogs and cats. Except in the case of threatened or endangered species, other domestic and wild animals suspected as rabid should be euthanized and their tissues examined for the presence of rabies antigen using appropriate laboratory techniques.
[d]Postexposure prophylaxis should be considered when contact between a human and a bat has occurred unless the exposed person can rule out a bite or scratch or exposure to a mucous membrane.
From World Health Organization Expert Consultation on Rabies. Second Report. 2013. WHO Technical Report Series. *Geneva, Switzerland: World Health Organization; 2013:1–139.*

9. Is patient age important? The vaccine dose is the same regardless of age. Children tolerate vaccination well and demonstrate excellent antibody response, as demonstrated in different ethnic groups.[344,456–458]
10. What about bats? Rabies PEP is recommended for all people with bite, scratch, or mucous membrane exposure to a bat, unless the bat is available for diagnostic testing and is negative for evidence of rabies virus infection. If a report on the presence of rabies by laboratory diagnosis is not available rapidly, such as within approximately 3 days, PEP should be started if an exposure cannot be excluded reasonably after thorough investigation. The inability of care providers to elicit information surrounding potential exposures may be influenced by the sometimes tiny injury inflicted by a bat bite (in comparison with lesions inflicted by larger carnivores) or by circumstances that hinder accurate recall of events. Consequently, PEP is also appropriate even in the absence of a demonstrable bite or scratch when there is reasonable probability that such contact occurred under certain circumstances (e.g., a sleeping person awakens to find a bat in the room, an adult witnesses a bat in the room with a previously unattended child, a mentally deficient person, or an intoxicated person). Fortunately, tests for VNA obtained from humans after vaccination with HDCV or PCECV show ideal neutralization of rabies viruses associated with bats.[459]

Unnecessary PEP can be avoided if circumstances are found that make the chances of rabies virus exposure remote. When exposures occur to animals in which rabies is not suspected, large numbers of people often receive vaccine.[460] A survey of practice in 11 university-associated urban emergency departments revealed that rabies prophylaxis was administered inappropriately in approximately 40% of cases.[461] Exposure to bats in the absence of known bites is a complicated situation, and consultation with experienced public health providers is warranted. Tables 50.11 through 50.13 should be consulted as a guide for the selection of PEP in the United States and elsewhere in the world.[452,462] Table 50.7 describes both preexposure and PEP regimens.

Postexposure Prophylaxis Booster Vaccination

If a person has been vaccinated with a CCV and is later reexposed to rabies virus, two booster injections of a vaccine are recommended, because one dose may not be sufficient.[463] Persons who received vaccine intradermally for their primary series may be particularly at risk for a slower response to booster.[464] Another suggestion from Thailand is to give four simultaneous intradermal vaccinations as a single booster.[465] However, subjects who previously received CCV uniformly have long-lasting immunologic memory,[466] and there is no evidence that more than two boosters are necessary. Persons who gave a history of vaccination with nerve tissue vaccines responded poorly to boosters in 18% of cases[467] and should, therefore, receive a full primary regimen unless antibodies were previously shown to be present.

CONTRAINDICATIONS TO VACCINATION

In reality, when the disease is present, all individuals appear vulnerable for exposure to, for example, lyssaviruses, irrespective of age, nutritional status, immune competence, physiological status, when they visit or live in rabies endemic regions. However, not everyone is equal in response to active immunity, because vaccination necessitates immunocompetent subjects with a mature immune system. In contrast, this is not the case to conferring passive immunity, which involves transfer of preformed antibodies, which can mediate the desired pharmacological action independent of the recipient's immune system.

Obviously, there are no true contraindications to rabies vaccination, when viral exposure is documented. Because rabies is a highly lethal zoonosis, any theoretical contraindication should be considered very carefully before disqualifying a person for PEP.

Persons with histories of severe allergies are more prone to having allergic reactions to rabies vaccine. When persons with such allergies are vaccinated, prophylactic antihistamines should be given and epinephrine should be available. If an allergic reaction occurs, one may give an alternative vaccine of different tissue origin (e.g., PVRV or PCECV in the case of a reaction to HDCV). A similar strategy was applied in allergic persons in whom brain tissue vaccine caused symptoms of CNS involvement during the course of injections. Administration of nerve tissue vaccine was interrupted immediately, and the series was completed with vaccine produced in tissue other than brain.

Only severe reactions not controlled with premedication may be grounds for interruption of rabies vaccination. Treatment with steroids may control allergic reactions but may also inhibit VNA responses. Accordingly, VNA titers should be determined after the last dose if steroids have been used. Similarly, patients receiving immunosuppressive medications for other diseases should have VNA levels checked after immunization to verify an adequate response to the vaccine.[462] If titers are inadequate, a booster dose should be administered.

Pregnancy is not a contraindication to rabies vaccination.[468,469] For example, follow-up of 202 Thai women vaccinated during pregnancy revealed no excess of medical complications or abnormal births.[470] Under no circumstances should a pregnancy be terminated because of a theoretical concern of transplacental transmission of rabies virus. Vaccination of the newborn is probably unnecessary, but has been carried out successfully.[471]

PUBLIC HEALTH CONSIDERATIONS

Rabies Vaccination of Animals

From the beginning of his involvement with rabies, Pasteur recognized that indirect protection of humans could be achieved effectively through the vaccination of dogs. Although dogs were used to obtain most of the experimental data on protection from 1884 to 1885, it was not until the early 1920s that a practical and successful canine vaccine was developed.

The first use of mass vaccination of dogs was a modified Semple type prepared by Umeno and Doi[472] in Tokyo in 1921. It proved effective in controlling rabies in dogs in Japan and in other countries that produced and used this type of vaccine. The quality of vaccine improved greatly with the introduction by Habel[412] of a standard mouse potency test for the Semple-type vaccine, which ensured the potency of the vaccines in mass vaccination programs.

In 1945, Johnson[473] demonstrated that a single dose of a potent, phenol-inactivated vaccine protected dogs against a challenge with street rabies virus for a period of more than 1 year. From 1945 on, this was virtually the only type of vaccine used for control of rabies in dogs, cats, and other domestic animals.

A modified live virus vaccine was introduced by Koprowski[474] in 1948. Successive passages of a strain of virus of human origin, first in 1-day-old chicks and later in embryonated hens' eggs, resulted in the loss of pathogenicity for dogs, providing an attenuated virus strain safe for dogs, called Flury LEP. Further passages of Flury LEP virus in embryonated hens' eggs

resulted in a vaccine that was no longer virulent for adult laboratory animals yet was still lethal for newborn mice, called Flury HEP. Both Flury LEP and Flury HEP were given to many types of domestic animals in different parts of the world. However, the live attenuated Flury virus vaccine was discontinued in the late 1970s in the United States.

Another attenuated strain of rabies virus, ERA, was introduced by Canadian workers in 1964.[475] The ERA vaccine was shown to provide excellent immunity, lasting for at least 3 years. However, several vaccine-induced cases of rabies in cats and other animals resulted in the cessation of its routine use.

Inactivated rabies vaccines for animals prepared from brains of newborn mice or from virus of cell culture origin were introduced in the 1980s and are now in general use in Europe and the Americas. However, only cell culture inactivated virus vaccines and live recombinant vaccines are licensed for domestic animals in the United States.

Elimination of human rabies by control of canine rabies through vaccination, population management, and education about responsible pet ownership has been a successful strategy increasingly in developing countries, as demonstrated in Latin America and in parts of Asia, such as in Thailand.[476,477] In areas where canine rabies has been eliminated and the dog can be observed for 10 days by qualified persons, immediate vaccination may not be necessary and should be started only if the animal develops signs of rabies.

Oral vaccination of wildlife to prevent the spread of rabies in terrestrial animals such as foxes, raccoons, coyotes, and raccoon dogs has become possible. The SAD B19 attenuated strain has been placed into baits for the vaccination of foxes.[431,478] The virus is grown on a baby hamster kidney cell line and is stable even at high environmental temperatures. A dose of approximately 10^6 infectious units immunizes approximately 99% of foxes, which has allowed its wide application in Germany and elsewhere in Europe.[479,480] However, because the SAD B19 strain retains residual pathogenicity for rodents, a more attenuated strain, called SAG2, was developed successfully.[481,482]

Genetic engineering has been applied to rabies immunization by the construction of a *Vaccinia* virus recombinant containing the gene for the G protein (V-RG).[483] The recombinant is placed in baits, and, following ingestion by animals, multiplies only in the tonsils and the buccal area. Extensive tests conducted in many species have confirmed the safety and efficacy of vaccination with this construct, and field tests in Europe, Canada, and the United States have confirmed the promising laboratory results.[484–488] At least two human infections with the *Vaccinia* recombinant produced local lesions but no lasting effect.[489] Widespread application of V-RG in Belgium starting in 1989 reduced fox rabies from 841 cases in that year to 2 cases in 1993. Concomitantly, a marked drop occurred in human exposures requiring vaccination.[490] The widespread use of these oral vaccines has eliminated red fox rabies in Western Europe. In North America, more than 100 million doses of V-RG delivered by air have blocked the spread of raccoon rabies from the eastern seaboard, controlled gray fox rabies in Texas, eliminated coyote rabies from the U.S.–Mexican border, and suppressed red fox rabies in Southern Ontario.[491–493]

In the United States, two types of rabies vaccines are available for domestic animals: inactivated virus vaccines and recombinant vaccines. Both types require boosters, usually after 1 or 3 years.[493]

New rabies vaccines given orally have been shown to protect dogs from challenge with rabies virus, equivalent to other rabies vaccines.[494] This methodology may be of particular interest for developing countries. Recombinant adenoviruses also have shown usefulness in the laboratory and in the field as oral or parenteral vaccines.[495,496]

Utility of Rabies Vaccination of Humans

More than 15 million people are vaccinated each year after presumed exposure to lyssaviruses, particularly in Asia.[497] It is difficult to define precisely the effect of rabies vaccination on the incidence of human rabies because the risk of the disease is so variable. Nevertheless, when untreated persons who were bitten by proven rabid animals are followed up, disease rates of between 3% and 80% are observed,[6,498] depending on the location and severity of bites.[499] In the United States, relatively few of the 30,000 to 40,000 people vaccinated annually are actually at risk for rabies. However, the paucity of cases of rabies in persons given potent vaccines together with antiserum argues that countless cases of rabies are being prevented. In Texas in 1989, 34% of the skunks, 19% of the foxes, and 15% of the bats involved in biting incidents were rabid; the risk to humans is obvious.[500]

Control of dog rabies is jointly responsible with vaccination of exposed humans for the current rarity of human rabies in North America and Europe. In developing countries, even nerve tissue vaccine reduces the incidence of rabies in vaccinees,[498] but large numbers of exposed persons turn to indigenous methods of rabies control and never receive vaccine.[501] Thus, a great need exists for inexpensive rabies vaccines and for health education to aid in appropriate vaccine use.

THE "NEXT-GENERATION" RABIES VACCINES

Several vaccines can be considered next- or fourth-generation vaccines subsequent to the first three major contributions: post–19th century from the pasteurian, nerve tissue, and CCV products, listed in Table 50.4.[502,503]

Vaccinia and canarypox vectors containing the rabies G protein have been prepared and tested in humans.[504–506] With both vectors, two injections at 1-month intervals raised levels of rabies VNA, although less than two injections of HDCV were given at the same interval. A third dose of the vectors gave striking booster effects to persons who had received the vectors previously and to persons who had received the HDCV previously. Other new vectors that have been constructed include other poxviruses; human, canine, and chimpanzee adenoviruses; and nonpathogenic rhabdoviruses.[507,508] A rabies virus was deleted for the *M* gene and, although unable to replicate, was immunogenic and efficacious.[509,510] Another rabies virus with two attenuating mutations and two genes for the glycoprotein has been developed and is avirulent and protective in animals.[511] Plasmid vaccines containing the complementary DNA of the gene for the rabies virus G glycoprotein have been constructed and tested in animals.[512,513] Protection following challenge with rabies virus was demonstrated in mice, dogs, cats, and monkeys.[514–518] In one study, a single injection of 100 μg of a DNA expressing the rabies virus G protein protected dogs challenged 1 year later.[519] Similarly, DNA-vaccinated monkeys were also protected against challenge 1 year after a single intramuscular injection.[520] An intranasal DNA vaccine was also protective in animals. All the aforementioned experimental vaccines protected mice against rabies virus challenge, but, unfortunately, none except the canarypox-rabies has been tested in humans, which did induce antibodies, as mentioned.[505]

Besides the G protein, the rabies virus RNP protein also may be important in protection.[521,522] Although the G protein alone is protective in experimental animals, so is the N protein, but without the induction of VNA. After vaccination with CCV, antibodies appear to N and G proteins.[523] The function of

antibodies against N protein may be to help induce cellular immune responses, but also seem to enhance antibody responses to G protein.[74,524] The N protein might be used to sensitize hosts to viral proteins so that subsequent injection of CCV would result in prompt development of higher titers of VNA. However, the N protein does not seem to broaden the neutralizing responses against rabies-related lyssaviruses.[525] The N protein can be produced in a baculovirus vector, which is a potential source of antigen for large-scale preexposure immunization of humans and animals.[526] Large amounts of G protein also can be produced by baculovirus vectors.[527] Synthetic peptides have been constructed that produce antibodies in laboratory animals, but none of the peptides has so far induced development of VNAs or protection against rabies virus challenge. Antiidiotypic antibodies as vaccines are still experimental.[528] It is possible to genetically engineer attenuated rabies virus strains and to have them serve as vectors of foreign genes.[54] Engineering involving reverse genetics also may allow for the construction of species-specific rabies virus vaccines.

Two potentially inexpensive technologies for rabies vaccine production are expression of the G protein in genetically modified plants and the generation of recombinant plant viruses, both of which might be administered orally to humans. Genetically modified plants could also be the substrate for production of parenterally administered rabies virus antigen production.[529–531] However, the concentrations of antigen in plants may not be adequate for protective and long-lasting immunity to rabies for all species.

MONOCLONAL ANTIBODIES

The limited availability of HRIG or ERIG in developing countries remains a major problem.[532] Safety concerns on the use of human RIG plus its limited availability have generated attempts to find other means of providing rabies virus antibodies. Although ERIG has been a useful substitute, it too has safety issues and is not always available. Moreover, consistency of production has been a problem.[533] Monoclonal antibodies that neutralize rabies virus have been produced and might be an alternative to HRIG.[534–537] The first monoclonal antibodies were generated in mice at the Wistar Institute during the late 1970s, but more recently, cocktails of antibodies generated in human cells have been produced.[538–540]

For example, a cocktail of three monoclonal antibodies was developed by investigators in Philadelphia and at the Centers for Disease Control and Prevention.[541,542] Similarly, Crucell Holland developed two antibodies that were complementary to each other, because they are directed against two different epitopes on the G protein.[543] These two antibodies, called CR57 and CR4098, have been combined and tested against rabies in Syrian hamsters, in comparison with HRIG. The monoclonal antibodies efficiently neutralized 26 different street rabies viruses, did not block significantly the immunogenicity of concomitant vaccination, and protected hamsters against a challenge with a virulent rabies virus.[544]

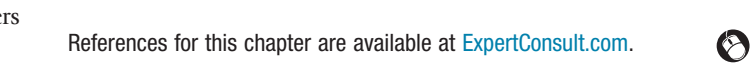
Escape mutants were also neutralized by the combination. Such monoclonal antibodies may soon come into commercial use. The expression of monoclonal antibodies as single or double-chain antibodies in plants could make production less expensive.[545,546]

CONCLUSIONS

The majority of human and animal rabies cases could be prevented by control focused upon canine populations through more responsible pet ownership, surgical or chemical contraception, improved management of community dogs, and most importantly, mass canine vaccination. The last is a widely effective technique when the cost is acceptable, where booster vaccination of pets can be required, and where there is an adequate medical and veterinary infrastructure. In other areas, with different socioeconomic conditions, only the first three methods may be feasible, often with difficulty. An inexpensive, safe, and effective oral vaccine for dogs may provide a great advance in the control of rabies, and, as stressed earlier, oral vaccines for major wildlife species already have had a major impact on rabies epizootiology. Lethal control of animal reservoirs is not recommended and unlike the situation for wild carnivores, management of rabies in bats, such as by vaccination, is not presently feasible.

Preexposure vaccination with CCVs also could reduce the incidence of human rabies in high-risk areas and professions, as has been achieved among veterinarians and Peace Corps workers. Routine preexposure vaccination of children in areas where animal rabies is prevalent is under consideration as a paradigm shift to reduce health disparities, such as in Amazonia. Elimination of rabies in dogs should receive major emphasis, which will serve to markedly reduce vaccination of humans. Improving laboratory-based surveillance to document the burden of disease, applying dose-saving regimens of existing vaccines, understanding the fundamental immunological basis for host responses to emerging lyssaviruses for new biologics, appreciating the basic health economics of prophylaxis, and providing global outreach around World Rabies Day are major One Health opportunities today that were not appreciated during the early 20th century.[153,187–190,288,399,547–553] Within the next several years, the application of monoclonal antibodies and production of recombinant rabies virus antigen vaccines, perhaps produced in genetically modified plants, are considerations for renewed rabies prevention and control.[554] Comparative applied clinical trials with existing vaccines and basic scientific insights into the holistic immunology of human responses to rabies virus antigens will lead to the selection, development and use of purer, more potent, safer, less expensive, and more effective biologics from an objective, evidence-based perspective and more rational administration routes and schedules for specific populations at risk in the decades ahead.[555–570]

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51 Respiratory Syncytial Virus Vaccines

Ruth A. Karron

Respiratory syncytial virus (RSV) is the most important cause of viral acute lower respiratory tract illness in infants and children worldwide.[1–3] In the United States, it is estimated that approximately 150,000 infants are hospitalized annually with RSV pneumonia or bronchiolitis.[4] A recent review of national databases in the United States estimated RSV mortality rates in children at approximately three to four per 10,000 admissions, or approximately 50 to 160 deaths per year,[5] although historic estimates were severalfold higher for premature infants[6] and for those with chronic lung disease,[7] congenital heart disease,[8] or primary immunodeficiency disorders.[9,10] RSV is the leading viral cause of hospitalization and reduction in pulmonary function for children with cystic fibrosis.[11–13] In Native American and Alaska Native children, the risk of hospitalization for RSV is three to five times greater than that of the general U.S. pediatric population.[14,15] RSV also accounts for a substantial proportion of outpatient visits in U.S. children younger than 5 years of age.[3] Globally, RSV was estimated to have caused 33.8 million (95% confidence interval, 19.3–46.2) cases of acute lower respiratory tract illness in children younger than 5 years of age in 2005.[16] The global burden of RSV-associated mortality has been estimated using various methodologies; values range from between 66,000 and 199,000 in 2005[16] to 234,000 in 2010.[17] Additional efforts are underway to provide more precise global estimates of RSV morbidity and mortality in children.

Although traditionally regarded as a pediatric pathogen, RSV also can cause life-threatening pulmonary disease in hematopoietic stem cell transplant (HSCT) recipients. The elderly are also at risk for severe RSV disease,[18–22] and nearly 180,000 RSV-associated hospitalizations of the elderly are estimated to occur annually in the United States.[20]

RSV was discovered in 1956 and its importance as a respiratory pathogen in infants was documented shortly thereafter, but a number of obstacles have impeded vaccine development. The peak of severe disease occurs in very young infants, who may not respond adequately to vaccination because of immunologic immaturity, suppression of the immune response by maternally derived antibody, or both[1,23] Because serious RSV disease can occur in high-risk persons who have experienced previous RSV infection as well as in RSV-naïve infants, it is likely that more than one type of RSV vaccine is needed to immunize all those who would benefit from vaccination. In addition, an RSV vaccine must not potentiate naturally occurring RSV disease, as was observed with the formalin-inactivated RSV (FI-RSV) vaccine (see "Past Experience: Formalin-Inactivated Respiratory Syncytial Virus Vaccine" later).[24–26]

Despite these challenges, there has been a tremendous surge in RSV vaccine research in the past 5 years, with more than 60 vaccine candidates in clinical development and more than 20 vaccines in clinical trials as of December 2016 (Fig. 51.1). This chapter describes recent efforts to develop safe and effective RSV vaccines for populations at risk, with a primary focus on vaccine candidates currently being evaluated in clinical trials.

BACKGROUND

Clinical Disease

Reinfection with RSV occurs throughout life, although disease manifestations differ. In young children, RSV infections are associated with a spectrum of respiratory illness, ranging from mild upper respiratory illness to life-threatening bronchiolitis and pneumonia. In infants younger than 6 weeks old, poor feeding and lethargy may predominate, and apnea may occur in the absence of other respiratory signs or symptoms.[27] Otitis media occurs frequently; RSV is the principal cause of viral otitis media in children.[28]

The nature of the relationship between RSV infection in early childhood and subsequent development of reactive airway disease remains an open question. Infants hospitalized with RSV infection frequently have demonstrable abnormalities in pulmonary function for years after the initial event (reviewed by Wu and Hartert[29]). However, it is not clear whether RSV causes abnormal airway function through lung injury and immunomodulation, whether RSV is only one of many triggers in susceptible persons, or whether both are true: genetic factors influence the immune response to RSV, and RSV elicits particular immune responses that may injure the developing lung.[29] Intervention studies may help resolve this issue. A recent placebo-controlled trial of the RSV monoclonal antibody (mAb) palivizumab in late preterm infants demonstrated that the infants who received palivizumab had a significant reduction in total wheezing days compared with those who received placebo.[30] Conversely, a placebo-controlled trial of the RSV mAb motavizumab in full-term Native American infants failed to show an effect on the incidence of medically attended wheezing through 3 years of age.[31] The planned RSV vaccine and mAb efficacy trials described later will provide important opportunities for further assessment of potential causal relationships between RSV and asthma.

In healthy young adults, RSV infections are typically associated with mild upper respiratory illness.[32] Elderly adults attending adult daycare or in long-term care facilities and who are infected with RSV may have a combination of symptoms that include rhinorrhea (67% to 92%), cough (90% to 97%), fever (20% to 56%), and wheezing (6% to 35%).[33] Pneumonia occurs in up to 10% of these persons.[19,34,35]

Immunocompromised patients, particularly those with hematologic malignancies and those undergoing HSCT or lung transplant, are at high risk for severe RSV disease.[36] In these patients, upper respiratory illness precedes lower respiratory illness, and the presence of rhinorrhea, sinusitis, or otalgia are clinical features that may help distinguish between RSV and cytomegalovirus pneumonia.[37–40] The severity of disease depends on the type and magnitude of immunosuppression, with rates of RSV pneumonia of up to 75% in leukemic patients[39] and of RSV pneumonia in 24% to 79% of HSCT patients.[41] For HSCT recipients, infection that occurs preengraftment is associated with the highest risk of pneumonia and death, but mortality is still high in those who develop pneumonia postengraftment.[41] In the United States, prior infection with HIV does not appear to increase the morbidity

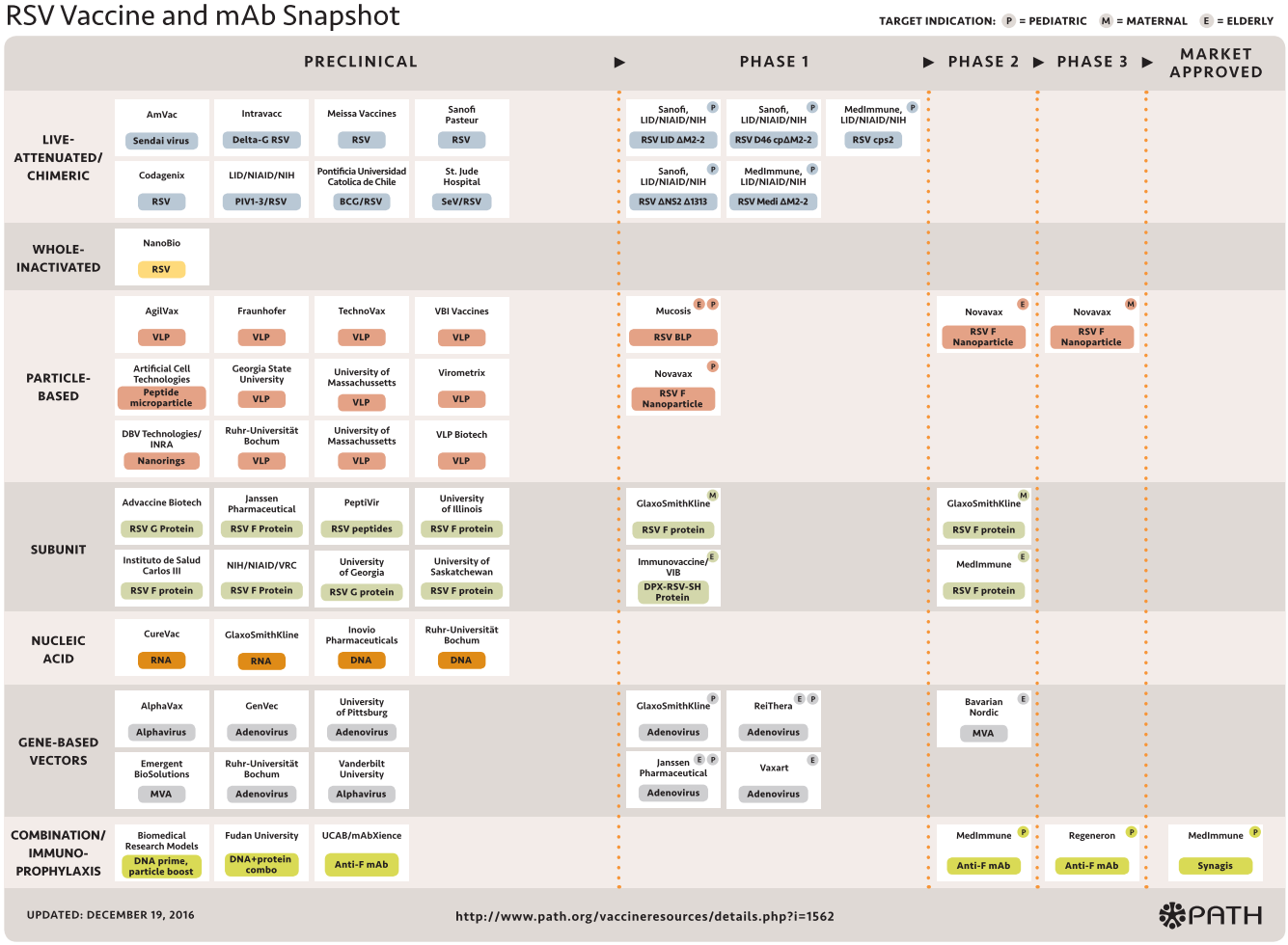


Figure 51.1. "Snapshot" of respiratory syncytial virus (RSV) vaccines in preclinical and clinical development. mAb, monoclonal antibody. *(Courtesy Carrie Trujillo and Deborah Higgins and PATH. Available at http://www.path.org/vaccineresources/details.php?i=1562.)*

and mortality associated with RSV infections in children, although prolonged viral excretion has been reported.[42] However, in South Africa, RSV is associated with greater morbidity and mortality in HIV-infected than in HIV-noninfected children, although the contribution of other illnesses cannot be excluded.[43]

Virus

RSV is a member of the family Paramyxoviridae. The viral genome is composed of a single strand of negative-sense RNA that is tightly associated with viral protein to form the nucleocapsid, and the viral envelope is a plasma membrane-derived lipid bilayer that contains virally encoded transmembrane proteins. A viral polymerase that transcribes genomic RNA into messenger RNA is packaged within the virion.

RSV is a member of the genus *Pneumovirus* and is composed of 15,222 nucleotides that encode three transmembrane surface proteins (F, G, and SH), two matrix proteins (M and M2), three nucleocapsid proteins (N, P, and L), and two nonstructural proteins (NS1 and NS2). The surface fusion (F) and attachment (G) glycoproteins are the only viral components that induce RSV neutralizing antibody and therefore are important targets for vaccine development. The F protein, in combination with proteins G and SH, is responsible for fusion of the viral envelope with the host cell membranes and for the characteristic syncytium formation in cell culture. Its genome is highly conserved between RSV groups. Along with the F protein, the G protein mediates attachment to the host cell surface and is largely responsible for the antigenic diversity observed between RSV groups (see below). A secreted form of the G protein that lacks the N-terminal signal/anchor region also is produced, and it is reported that up to 80% of the G glycoprotein released from cells 24 hours after infection is in this secreted form.[44] The function of the secreted G protein is not known, although it may serve as a decoy for RSV neutralizing antibody[1] or as an inhibitor of innate immune responses.[45] The NS1 and NS2 proteins suppress a key component of host innate immune defense by blocking induction (NS1) or signaling (NS2) of type I and type III interferons (IFNs).[46] The NS2 protein may also contribute to airway obstruction by promoting shedding of infected epithelial cells.[47]

Cross-neutralization studies show that RSV isolates can be classified into two groups, designated A and B.[48] Although RSV A and B strains differ in all 10 viral proteins, the G glycoprotein shows the greatest divergence between groups, with only 53% amino acid homology between prototype RSV A and B viruses.[49] Group A RSV infection may cause more-severe disease than group B RSV, although this has not been

definitively established.[50,51] The impact of this antigenic dimorphism is not completely understood, but young children experiencing their second RSV infection frequently are reinfected with virus from the same group.[52]

EPIDEMIOLOGY

By 2 years of age, almost all children will have been infected with RSV, and approximately 50% will have been infected twice.[53] Reinfection with RSV can occur throughout life and is often symptomatic; however, RSV generally does not cause lower respiratory illness in immunocompetent adults and healthy older children.[32]

RSV epidemics occur yearly during late fall, winter, and early spring in temperate climates and in the rainy season in some, but not all, tropical climates.[54] Group A and group B viruses cocirculate during epidemics, although one may predominate.[55–57]

Humans are the only known reservoir for RSV. Spread of RSV from contaminated nasal secretions occurs via large droplets rather than small-particle aerosols, so close contact with an infected person or contaminated environmental surface is required for transmission.[58,59] RSV can persist as a fomite on hard surfaces for several hours,[60,61] and for this reason is an important cause of nosocomial respiratory illness, particularly on pediatric wards.[61]

MECHANISMS OF IMMUNITY AND CORRELATES OF PROTECTION

Virus-specific immune responses are largely responsible for protection against RSV-associated lower respiratory illness and recovery from RSV infection. Immunity to RSV is mediated via humoral and cellular effectors, including serum antibody (acquired as a result of infection or maternally derived in young infants), secretory antibody, and major histocompatibility complex class I–restricted cytotoxic T lymphocytes. The RSV F glycoprotein also may elicit innate immune responses via Toll-like receptors and CD14.[62,63] Natural immunity to RSV is incomplete, and reinfection occurs throughout life, as has been demonstrated by epidemiologic[53,64] and challenge studies in healthy young adults.[65] Healthy older children and adults, however, usually are protected against RSV-associated lower respiratory illness. In general, humoral immune responses (secretory and serum antibodies) appear to protect against infection of the upper and lower respiratory tract, respectively, while cell-mediated responses directed against internal proteins appear to terminate infection and may contribute to viral clearance from the lungs.[66] RSV-specific cytotoxic T lymphocytes have been detected in infants recovering from RSV infection[67] and may contribute to short-term protection, such as reinfection during the same RSV season. Although the adoptive transfer of primed T cells will halt RSV replication in immunodeficient mice, the adoptive transfer of RSV-specific cytotoxic T lymphocytes also may potentiate disease,[68] suggesting that there may be an immune component to RSV illness. CD4 T-helper type 2 (Th2)-associated responses have been associated with eosinophilia, increased mucus production, and delayed viral clearance in some small animal models and may also contribute to the pathogenesis of RSV disease.[69–72]

The role of local immunity in the protection of the upper respiratory tract against RSV is suggested by experimental data from studies in cotton rats[73–75] and adult volunteers,[76] and by observational data from infants. In adults, the presence of secretory neutralizing antibody, but not serum antibody, correlated with protection of the upper respiratory tract against RSV infection.[76] In infants, the development of immunoglobulin A in nasal secretions correlated temporally with viral clearance following natural infection.[77]

RSV replicates exclusively in the respiratory epithelium. For this reason, serum neutralizing antibody does not prevent clinically apparent infection, as it does for pathogens that produce viremia, such as measles and varicella. However, high titers of RSV serum neutralizing antibody protect the lower respiratory tract against RSV infection or disease, as has been demonstrated by animal studies,[73–75] epidemiologic observations in infants and young children,[78–80] and clinical trials of RSV hyperimmunoglobulin and mAbs in high-risk infants (see "Passive Immunization Against Respiratory Syncytial Virus" later).

Primary infection with RSV does not always elicit an immune response that will protect the lower respiratory tract because RSV-associated lower respiratory illness can occur in young children experiencing their second episode of RSV.[53,79] Young infants often develop levels of neutralizing antibody and F and G glycoprotein antibodies to RSV that are only 15% to 25% of those observed in older children.[81] However, some of this reduction in antibody response may be because of suppression from maternally derived antibody rather than immunologic immaturity, because very young infants with low levels of maternally derived antibody can develop high titers of serum neutralizing antibody in response to RSV infection.[82] Young infants may also have suboptimal innate immune responses and dysregulated T-cell responses to RSV, although available data are limited.[83] The suboptimal response of some young infants to primary infection with RSV has important implications for vaccine development because it suggests that more than one dose of vaccine may be needed for active immunization of infants, particularly as these infants may have high levels of neutralizing antibody acquired from maternal immunization or from an extended half-life mAb (see "Vaccine Development" later).

The immunologic requirements for protection against severe RSV disease are not clearly defined. Although it is known that a high level of serum neutralizing antibody (titers >1 : 200, as measured in a 60% complement-enhanced plaque-reduction neutralization assay) is sufficient for protection of the lower respiratory tract,[84,85] it is not known whether it is necessary in the case of vaccines that induce a broad range of systemic and mucosal humoral and cellular immune responses. In adults and older children who have been previously infected with RSV, it is reasonable to expect that an effective RSV vaccine will boost levels of serum neutralizing antibodies. However, it is conceivable that vaccination may protect young RSV-naïve infants against severe RSV even if high titers of neutralizing antibodies are not induced. In these infants, "challenge" with a second dose of a live attenuated vaccine may help predict whether some protection has been conferred by the first dose (see "Biologically Derived Live Attenuated Vaccines" later).[86] Palivizumab-competing antibody is also being measured in the context of RSV F nanoparticle vaccine trials (see "Vaccine Development" later) and may be evaluated in clinical trials of other RSV F subunit vaccines. Binding and competition assays for other epitopes on the prefusion and postfusion forms of RSV F (see later) are also being developed and may be evaluated in the context of clinical trials. Ultimately, correlates of protection against severe disease in young infants may differ by type of vaccine used and will need to be evaluated in the context of efficacy trials. It will also be important to be able to compare the antibody generated in various trials, either through use of standardized neutralizing antibody assays or through development of a common biological standard so that results obtained using different assays can be directly compared. Work on standardization is being undertaken by

Programs for Appropriate Technology in Health (PATH) and the World Health Organization (WHO).

PASSIVE IMMUNIZATION AGAINST RESPIRATORY SYNCYTIAL VIRUS

Based on experimental data in animals[73–75] and epidemiologic data in humans[79] suggesting that RSV-neutralizing antibody protected against lower respiratory illness, two products containing high titers of RSV neutralizing antibody were licensed for clinical administration. The first of these, RSV Immune Globulin Intravenous (RSV-IGIV; RespiGam [MedImmune]), led to significant reductions in the rate and severity of lower respiratory illness when administered to high-risk infants at doses of 750 mg/kg.[84] The titer of RSV neutralizing antibody achieved in infants who received this dose of RSV-IGIV was comparable to that demonstrated to protect the lungs of cotton rats against RSV infection in earlier studies.[84,85] The protective effect of RSV-IGIV in these young infants was confirmed by a subsequent placebo-controlled trial.[87] RSV-IGIV was replaced by a monoclonal neutralizing antibody directed against antigenic site II on the RSV F glycoprotein (palivizumab/Synagis; [MedImmune]), which also reduces the risk of hospitalization for RSV disease in premature infants and infants with chronic lung disease.[88] Because palivizumab is 50- to 100-fold more potent than RSV-IGIV, it can be administered intramuscularly in monthly doses. In infants with cyanotic heart disease, RSV-IGIV was associated with an increased incidence of adverse events,[84] but a Phase III study demonstrated that palivizumab was safe and effective in preventing hospitalizations for RSV in children with congenital heart disease.[89] In July 2014, the American Academy of Pediatrics released revised guidelines for the use of palivizumab in high-risk infants and children in the United States, based on expected burden of disease and cost-effectiveness considerations. In otherwise healthy preterm infants, these new guidelines further restrict the use of palivizumab during the first year of life to those born at less than 29 weeks of gestation. Infants born between 29 and 32 weeks of gestation who have had a supplemental oxygen requirement, as well as infants with chronic lung disease of prematurity, hemodynamically significant congenital heart disease, neuromuscular disorders and certain immunodeficiencies, as well as Alaska Native and Native American children, may also be considered for prophylaxis during the first year of life. Children with an ongoing oxygen requirement or who are severely immunocompromised may receive palivizumab prophylaxis during a second RSV season (until 24 months of age). Detailed recommendations are provided in the American Academy of Pediatrics policy statement.[90] Phase I trials have also shown that palivizumab was well tolerated in a small number of HSCT recipients.[91] Although the prophylactic efficacy of RSV-IGIV and palivizumab have been established, neither appears to ameliorate RSV disease when given therapeutically.[92,93]

A more potent RSV mAb, designated motavizumab (MEDI-524), was also developed by MedImmune-AstraZeneca. Compared with palivizumab, motavizumab increases neutralizing activity in vitro and in vivo, and decreases RSV replication in both the upper and lower respiratory tracts in preclinical studies.[94,95] Two Phase III efficacy trials of motavizumab were conducted. In a noninferiority trial, 6635 preterm infants were randomized to receive motavizumab or palivizumab. Infants and children who received motavizumab had a 26% relative reduction in RSV hospitalization and a 50% relative reduction in medically attended lower respiratory illnesses compared with those who received palivizumab.[96] Motavizumab was also evaluated in a randomized, double-blind, placebo-controlled trial in more than 2000 full-term healthy Native American infants (NCT00121108). In this study, motavizumab was found to reduce RSV hospitalizations by 87% and outpatient RSV-associated medically attended lower respiratory illnesses by 71%.[31,97] Motavizumab was not licensed by the U.S. Food and Drug Administration (FDA) in 2010 because superiority to palivizumab was not clearly demonstrated and it is no longer in clinical development.

MedImmune is currently developing an extended half-life mAb directed against the site Ø neutralizing epitope on RSV F (see "Respiratory Syncytial Virus Subunit and Particle Vaccines" below). This mAb contains a YTE mutation in the Fc portion of the immunoglobulin. YTE mutations lead to increased binding to the neonatal Fc receptor and prolonged half-life.[98] This product, designated MEDI8897 (see Fig. 51.1), is intended for universal use in infants, to be administered seasonally or at birth depending on geographic location. As of this writing, MEDI8897 is being evaluated in healthy preterm infants (NCT02290340).

VACCINE DEVELOPMENT

Successful RSV vaccines should prevent serious RSV-associated lower respiratory illness in those at risk. The primary target populations for amelioration of disease through RSV vaccination are young infants and the elderly; however, toddlers and preschool children might also benefit from RSV immunization. Although the majority of RSV-associated deaths and hospitalizations occur in the first few months, there is a substantial burden of serious RSV lower respiratory illness throughout the first year of life,[3,99] as well as medically attended outpatient RSV illness throughout the first 5 years of life.[3] It is unlikely that a single RSV vaccine or type of vaccine will be able to address the total pediatric burden. For this reason, efforts are underway to develop nonreplicating vaccines for passive protection of the very youngest infants through maternal immunization, and replicating vaccines (live-attenuated RSV or vectored vaccines) for active immunization of slightly older infants and young children.[100] Nonreplicating vaccines might also be used to boost immunity in non–RSV-naïve toddlers, and are being combined with novel adjuvants to boost preexisting immunity in the elderly.

RSV is recognized as an important global pediatric pathogen and the stated goal of vaccine developers, regulators, funding organizations, and researchers is to evaluate and license RSV vaccines to protect children in low-, middle-, and high-income countries. As of this writing, global vaccine efficacy studies are being designed, with ongoing discussions of clinical end points that would be relevant in a variety of settings.[100]

Past Experience: Formalin-Inactivated Respiratory Syncytial Virus Vaccine

In the early 1960s, a FI-RSV was prepared and tested in infants and children. This vaccine, designated Lot 100, was administered as two or three intramuscular doses, separated by 1 to 3 months, to infants and children between 2 months and 7 years of age.[24–26,101] Lot 100 not only failed to protect against wild-type (wt) RSV disease, but induced an exaggerated clinical response to wt RSV infection in infants who were RSV naïve before vaccination. Many vaccinees were hospitalized with lower respiratory illness; in one study, the hospitalization rate of vaccinees approached 80% compared with 5% in control vaccinees.[24,25] Tragically, two infants who received Lot 100 died following wt RSV infection, one at 14 months and the second at 16 months of age.[24,25] RSV was readily isolated from the lower respiratory tracts of these infants.

The mechanisms responsible for the FI-RSV vaccine-associated disease enhancement are still not completely understood. However, data obtained from Lot 100 recipients[101–103] and from studies in rodent models[104,105] have led to the hypothesis that children vaccinated with FI-RSV remained susceptible to infection with wt RSV because vaccination produced inadequate levels of serum neutralizing antibodies and did not induce local immunity. Once infected with wt RSV, virus was not readily cleared because FI-RSV did not prime for a $CD8^+$ cytotoxic T-cell response, and the viral infection produced a direct cytopathic effect in the lower respiratory tracts of these infants. In addition, immunization with FI-RSV primed for a Th2-cell–like response, with increased local production of interleukins 4, 5, and 10; an influx of lymphocytes and eosinophils; the release of additional mediators, and resultant inflammation and bronchoconstriction.[106–108] Immunization with FI-RSV may have also induced low-avidity antibody, leading to immune complex deposition.[109,110]

The clinical experience with FI-RSV and the information gleaned from animal models of disease enhancement suggest key features of an RSV vaccine to be administered to RSV-naïve infants. The vaccine should induce protective levels of neutralizing antibody, as well as CD8 RSV-specific cytotoxic T cells, and a pattern of CD4 response similar to that evoked by wt RSV. A live attenuated native RSV or vectored vaccine is most likely to exhibit these characteristics.[1] Nonreplicating vaccines could be used to boost immunity in older children and adults at risk, or to provide passive protection to the infant through maternal immunization.[100,111]

Respiratory Syncytial Virus Subunit and Particle Vaccines

Considerable progress has been made in the development of RSV subunit and nanoparticle vaccines, with 28 vaccines in preclinical development and 8 vaccines in clinical development as of December 2016 (see Fig. 51.1). The primary target populations for these vaccines are the elderly and pregnant women, who would be immunized to prevent severe disease in their infants through passive transfer of antibody. Most of these vaccines contain the RSV fusion (F) glycoprotein. RSV F is required for viral infection, is the locus for most of the neutralizing activity detected in human sera, and is largely conserved between RSV A and B strains. In addition, palivizumab, which effectively protects high-risk infants against severe RSV disease, is directed against the site II epitope on the RSV F glycoprotein.

Development of RSV F vaccines has been greatly enhanced by recent progress in elucidating the antigenic structure of the RSV F glycoprotein.[112–116] RSV F is a class I fusion glycoprotein that requires proteolytic cleavage for activation. The mature F glycoprotein consists of two subunits (F1 and F2) that are linked by disulfide bonds and assemble as trimers. RSV F is present on the viral surface in both a prefusion and a postfusion conformation (Fig. 51.2). The prefusion conformation is metastable, and triggering of prefusion F leads to fusion of the viral and cell membranes and a change in conformation such that RSV F assumes the stable postfusion form.[116] Much of the neutralizing activity in human adult sera is directed against the prefusion form of RSV F.[117]

The crystalline structures of both prefusion[114] and postfusion F[112,113] have been solved, leading to the localization of neutralizing epitopes on both structures (see Fig. 51.2). The mAbs directed against each of these epitopes do not prevent virus binding but prevent fusion of the virus and cell membranes. Site II and site IV epitopes are present on both prefusion and postfusion F.[116,118] The site Ø epitope is unique to prefusion F (see Fig. 51.2), and antibodies directed against site Ø are highly neutralizing.[116] Recently, the mAb AM14 was found to recognize a novel potent

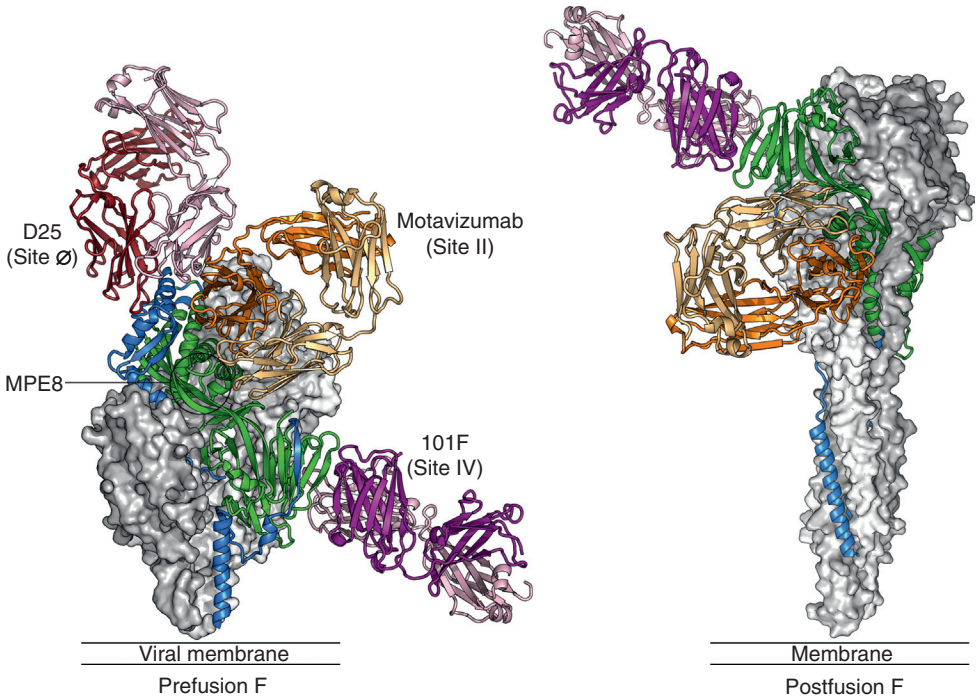


Figure 51.2. Molecular structure of the prefusion *(left)* and postfusion *(right)* conformations of the respiratory syncytial virus (RSV) F glycoprotein. The site II epitope (the palivizumab and motavizumab binding site) and the site IV epitope are present on the prefusion and postfusion conformations of RSV F, whereas site Ø (bound by D25, the monoclonal antibody used to develop MEDI8897) is only present on the prefusion molecule. *(Courtesy Jason McLellan. Modified from McLellan JS. Neutralizing epitopes on the respiratory syncytial virus fusion glycoprotein.* Curr Opin Virol. *2015;11:70–75.)*

neutralizing quaternary epitope that spans the RSV F prefusion trimer.[119]

Elucidation of the structures of RSV prefusion and postfusion F has enhanced the development and characterization of RSV F subunit vaccine candidates. The candidate furthest advanced in clinical trials is a baculovirus-expressed uncleaved RSV F in the postfusion conformation that forms rosettes (nanoparticles) and is manufactured by Novavax. In a Phase I dose-ranging study in healthy adults (NCT01290419), the RSV F nanoparticle vaccine was well-tolerated and a 60 µg dose with alum induced twofold increases in neutralizing antibodies as measured by microneutralization assay, although greater increases were observed in the subpopulation with lower baseline microneutralization titers.[120] In contrast, the vaccine elicited approximately 10-fold increases in immunoglobulin G antibody that bound to postfusion F, and similar increases in palivizumab-competing antibody immunoglobulin G that bound to antigenic site II and competed with palivizumab for binding to postfusion F. These data suggest that the RSV F nanoparticle vaccine induces higher levels of nonneutralizing antibody than neutralizing antibody. Similar results were obtained when the nanoparticle vaccine was evaluated in a Phase II study in women of childbearing age.[121] A Phase II study comparing an unadjuvanted 135 µg dose of this vaccine to placebo was completed in the elderly; 44% efficacy against all RSV disease and 46% efficacy against RSV lower respiratory illness were reported (unpublished data; Novavax press release, August 10, 2015, available at http://www.multivu.com/players/English/7590851-novavax-rsv/). In November 2015, Novavax initiated a Phase III efficacy trial of the RSV F nanoparticle vaccine (135 µg, unadjuvanted) in the elderly (NCT02608502) but failed to demonstrate efficacy.[121a] Additional studies with an adjuvanted formulation are being planned. In December 2015, Novavax initiated a Phase III efficacy trial of the RSV F nanoparticle vaccine (120 µg with alum) in women in the third trimester of pregnancy to prevent RSV illness in their infants (NCT02624947). Additional placebo-controlled trials of the alum-adjuvanted RSV F nanoparticle vaccine are ongoing in RSV-seropositive children (NCT02296463).

As of this writing, GlaxoSmithKline and MedImmune also have RSV F vaccines in clinical trials (see Fig. 51.1). The GlaxoSmithKline RSV prefusion F vaccine was well-tolerated in healthy men and elicited neutralizing antibody responses in most vaccinees (NCT01905215; RSV 2014, Stellenbosch, South Africa, abstract #114). Evaluation of this vaccine in healthy women is ongoing (NCT02360475), with plans for evaluation in pregnant women, the target population. An RSV postfusion F vaccine is also in development by GlaxoSmithKline (formerly by Novartis) and has been evaluated in healthy adults with and without the MF59 adjuvant (NCT02298179). MedImmune has developed a soluble RSV F (sF) vaccine adjuvanted with glucopyranosyl lipid A in a squalene-based oil-in-water stable emulsion (GLA-SE) for use in the elderly. RSV sF is currently being evaluated with different dosages of GLA-SE in adults older than age 60 years alone or in combination with inactivated influenza vaccine (NCT02289820). A number of additional RSV F subunit vaccines are in preclinical development (see Fig. 51.1), including DS-Cav1, a stabilized preF construct. In nonhuman primates, DS-Cav1 elicited neutralizing antibody responses that were approximately 10-fold greater than those elicited by postfusion F.[116,122]

Subunit vaccines targeting RSV G, the viral surface glycoprotein that mediates attachment, are also being developed (see Fig. 51.1). In addition, the RSV SH gene, which encodes a viroporin, has also been targeted for vaccine development[123] and a vaccine containing the extracellular domain of SH (SHe) and the DepoVax adjuvant (DPX-RSV) is currently being evaluated in healthy middle-aged adults (NCT 02472548; see Fig. 51.1).

Live Attenuated Respiratory Syncytial Virus Vaccines

Live attenuated vaccines may offer several advantages over nonreplicating vaccines, especially for RSV-naïve infants and young children. Intranasal immunization with a live attenuated vaccine should induce both systemic and local immunity and may therefore protect against both upper respiratory and lower respiratory illnesses.[124] Also, the immune response to a live vaccine should closely resemble the response to natural infection and therefore not produce enhanced disease on exposure to wild-type virus.[1] Similar to other live attenuated intranasal respiratory virus candidate vaccines,[125,126] live intranasal RSV candidate vaccines have been shown to replicate in young infants in the presence of maternally acquired antibody.[86,127] This feature will be critical for the success of live attenuated RSV vaccines in young infants. Multiple doses will probably be required for young infants.

Biologically Derived Live Attenuated Respiratory Syncytial Virus Vaccines

Several strategies for the development of a live attenuated RSV vaccine were originally explored, including the creation of host-range mutants, cold-passaged (*cp*) mutants, and temperature-sensitive (*ts*) mutants (which are unable to grow at high temperatures). In brief, these candidate vaccines were either underattenuated (*cp*RSV and RSV *ts*-1) and transmissible[1,127,128] or overattenuated (RSV *ts*-2).[1,129] Of importance, enhanced disease was not observed when infants who received RSV *ts*-1 or *cp*RSV were naturally infected with wt RSV.[128,130] A series of live attenuated RSV A *cpts* candidate vaccines were derived from further attenuation of *cp*RSV through chemical mutagenesis. Several of these candidate intranasally administered vaccines were evaluated in Phase I clinical trials in adults and children,[86,131] and one (*cpts*248/404) was evaluated in infants as young as 1 month old.[86] The *cpts*248/404 vaccine was highly attenuated in these infants but caused nasal congestion that in some instances interfered with feeding and sleeping.[86] Although the *cpts*248/404 vaccine was not sufficiently attenuated for young infants, evaluation of this candidate vaccine provided important information regarding replication and immunogenicity of a live attenuated RSV vaccine in the presence of maternal antibodies, phenotypic stability of a *cpts* vaccine, and preliminary evidence of protection against illness following wt RSV infection.[86]

Genetically Engineered (Complementary DNA–Derived) Live Attenuated Respiratory Syncytial Virus Vaccines

The ability to recover infectious virus from complementary DNA clones of RSV[132] has provided insight into the genetic basis of attenuation of biologically derived vaccines and hastened the development of additional live attenuated RSV candidate vaccines through the use of recombinant technology.[1,23,133] Mutations present in *cp*RSV and six of its *ts* derivatives were inserted into wt RSV singly and in combination, and the majority of attenuating mutations were found to occur in the polymerase gene, with a notable exception being the 404 mutation in the *M* gene start signal.[1,23,133–135] Using this information, attenuating mutations from biologically derived vaccines have been combined to produce further attenuated candidate vaccines.[1,133] In addition, deletion (Δ) of a nonessential gene (*SH*, *NS1*, *NS2*, or *M2-2*)[136] in combination with known attenuating *cp* and *ts* mutations has also been used to produce

highly attenuated vaccines.[137,138] Two of these candidates, designated rA2cp248/404ΔSH and rA2cp248/404/1030ΔSH, were evaluated in young children.[139] rA2cp248/404ΔSH was not more attenuated than its biologic parent, *cpts*248/404. However, addition of the 1030-amino-acid–point mutation to rA2cp248/404ΔSH yielded rA2cp248/404/1030ΔSH, which was highly attenuated in infants as young as 1 to 2 months and moderately immunogenic.[139] This candidate vaccine, now designated MEDI-559, was also evaluated in a Phase I/IIa trials in infants and children ages 5 to 24 months (NCT00767416). Vaccine "take," as measured by seroresponse or vaccine virus shedding was observed in 95% of vaccinees; 59% had an antibody response as measured by microneutralization assay. In the first 28 days postvaccination, a higher incidence of medically attended lower respiratory illness was observed in vaccinees compared to placebo recipients (9% vs 2%, not statistically significant). None of these episodes was associated with shedding of vaccine virus and the incidence of medically attended lower respiratory illness did not differ between groups after 28 days. The authors concluded that further safety evaluations would be needed prior to initiation of trials in younger infants.[140]

Rational vaccine design based on an understanding of RSV gene function has led to the design of deletion mutant vaccines. One such vaccine candidate, designated RSVΔM2–2, lacks the RSV *M2-2* gene. Compared with wt RSV, RSVΔM2-2 has increased accumulation of intracellular viral messenger RNA and increased expression of RSV proteins, which could enhance vaccine-induced immunity per infectious unit of virus.[141] This vaccine virus was attenuated in nonhuman primates.[142] RSV MEDI ΔM2-2 was evaluated in a Phase I clinical trial (NCT01459198, see Fig. 51.1) and was found to be highly restricted in replication yet more immunogenic than rA2 cp248/404/1030/ΔSH, the previous lead live attenuated RSV vaccine candidate. RSV MEDI ΔM2-2 also primed for a potent anamnestic response following natural exposure to wt RSV.[143] This apparent "delinking" of vaccine virus replication and immunogenicity is an important advance in the development of live-attenuated RSV vaccines. Further development of vaccine candidates containing the ΔM2-2 mutation is in progress.

An additional set of deletion mutant vaccines, rA2cpΔNS2, rA2cp248/404ΔNS2, and rA2cp530/1009ΔNS2, were also evaluated in clinical trials.[144] These vaccines all had deletion of the interferon antagonist *NS2* gene in common, which could, theoretically, enhance innate and adaptive immune responses.[145] rA2cpΔNS2 was overattenuated for adults, and rA2cp248/404ΔNS2 and rA2cp530/1009ΔNS2 were overattenuated for children at the doses studied.[144] Nevertheless, these studies demonstrated that deletion of *NS2* attenuates RSV infection. An alternative *NS2* deletion mutant, designated ΔNS2Δ1313I1314L,[146] is currently being evaluated in young children (NCT01893554, see Fig. 51.1). Similarly, deletion of the *NS1* gene, which also inhibits IFN-α and IFN-β induction,[145] may yield an attenuated recombinant RSV candidate vaccine.[142]

Vectored Respiratory Syncytial Virus Vaccines

Recombinant technology also provides the opportunity for creation of chimeric viruses containing the RSV F and G surface glycoprotein, with one or more internal genes provided by related respiratory viruses. A recombinant chimeric vaccine containing the bovine PIV3 backbone, human PIV3 surface glycoproteins (rB/HPIV3), and RSV F glycoproteins, designated MEDI-534, was evaluated in a Phase I trial in 49 RSV and HPIV3 doubly seronegative children ages 6 to 24 months. The children were randomized to receive three doses of vaccine or placebo. All children who received the highest dose of vaccine (10^6 median tissue culture infectious dose) were infected with vaccine virus, and seroconversion to RSV and HPIV3 occurred in 50% and 100%, respectively, of recipients of this vaccine dose.[147] Evaluation of the vaccine virus shed by study participants revealed nucleotide changes that were expected to reduce RSV F expression,[148] which might explain the suboptimal antibody response to RSV. No further evaluation of this vaccine candidate is planned. The National Institutes of Health has developed a new rB/HPIV3 vector containing the RSV prefusion F protein. In a hamster model, this vaccine induced high titers of RSV neutralizing antibody, protected against challenge with wt RSV, and was genetically stable.[149] Clinical evaluation of this vectored vaccine is anticipated.

Gene-based replication deficient vectors represent an additional approach to the development of RSV vaccines. GlaxoSmithKline (Okairos) completed the Phase I evaluation in adults of immunization with vectors expressing the RSV F, N, and M2-1 genes[150]: the chimpanzee adenovirus vector PanAd3-RSV and the modified *Vaccinia* virus Ankara (MVA)-RSV were tested in several prime-boost combinations, with some doses of the chimpanzee adenovirus vaccine administered intranasally (NCT01805921; see Fig. 51.1). RSV neutralizing antibody titers were boosted after the PanAd3-RSV prime, and RSV T-cell responses were greatest after the MVA-RSV boost.[151] Janssen (Crucell) is currently evaluating prime/boost combinations of adenovirus 26 and adenovirus 35 vectors expressing RSV F (Ad26.RSV.FA2 and Ad35.RSV.FA2) in adults (NCT02440035 and NCT02561871; see Fig. 51.1).

CONCLUSIONS

Over the past several years, increasing recognition of the global burden of RSV disease[16,17] has reinvigorated interest in the development of RSV vaccines. A large number of RSV vaccines and a new RSV mAb are in late preclinical or clinical development. Standardized methodologies for the assessment of clinical end points and for measuring antibody responses will be important for efficacy trials of lead candidates, which are anticipated to start soon. Development of safe and effective RSV vaccines for protection of vulnerable infant and elderly populations is more likely now than at any time since the discovery of this important respiratory pathogen.

References for this chapter are available at ExpertConsult.com.

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52 Rotavirus Vaccines

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Rotaviruses were the leading cause of severe dehydrating diarrhea in infants and young children throughout the world before rotavirus vaccines became available. Most children were infected by the time they reached 2 to 3 years of age.[1,2] Even in developed nations, where standards of hygiene are high, rotavirus was the most common cause of severe infant diarrhea.[3]

In the United States, before the introduction of a vaccine, rotavirus accounted for approximately 2.7 million illness episodes, 500,000 physician visits, 55,000 to 70,000 hospitalizations, and 20 to 60 deaths each year, and the economic burden of disease was estimated at approximately US$1 billion each year in medical and indirect costs.[4–7] In less-developed countries, rotaviruses ranked highest among the multiple microbial causes of severe gastroenteritis in children and contributed disproportionately to the mortality associated with the disease.[8–10a] Worldwide, rotavirus was estimated to cause approximately 200,000 deaths in children in 2013, with the highest mortality rates occurring in countries of sub-Saharan Africa.[11–16] Extensive global efforts to disseminate oral rehydration therapy in the developing world have contributed to a decline in diarrhea deaths.[17] However, the continuing mortality associated with rotavirus, 500 to 600 deaths per day, suggests that prevention through universal application of a safe, economical, and efficacious vaccine is the best means of drastically decreasing the morbidity and mortality from rotavirus.

The first rotavirus vaccine to be licensed (RotaShield, Wyeth Lederle) was introduced into the U.S. immunization program in 1998, but was withdrawn a year later when it was associated with intussusception. Two other rotavirus vaccines, RotaTeq (Merck) and Rotarix (GlaxoSmithKline) were introduced into the United States 7 and 9 years later, respectively. Following studies of more than 70,000 infants, the Advisory Committee on Immunization Practices (ACIP) recommended RotaTeq for routine use in the United States in February 2006. Following studies of more than 60,000 infants, Rotarix was licensed for use in the European Union and Latin America in 2006, and for use in the United States in April 2008. Globally, as of May 2016, a total of 81 countries, including 38 low-income countries that are eligible for support for vaccine purchase from the Vaccine Alliance, Gavi, have implemented rotavirus vaccines in their national immunization programs (Fig. 52.1).

This chapter describes the globally licensed RotaTeq and Rotarix vaccines and other rotavirus vaccines.

CLINICAL DISEASE

Evaluation of children admitted to the hospital with rotavirus infection reveals a consistent pattern. The disease is characterized by the sudden onset of watery diarrhea, fever, and vomiting.[18–21] Most disease is mild, but a small proportion (approximately <1%) of children infected with rotavirus[4,5] will develop dehydration associated with severe loss of sodium and chloride in the stools and a compensated metabolic acidosis. In children admitted to the hospital with dehydration, fever and vomiting usually persist for 2 to 3 days and diarrhea for 4 to 5 days.[18–21] Detection of rotavirus antigen in the plasma of infected children is common[22] and may be associated with disease severity.[23] Disease severity is also predicted by elevated hepatic transaminases and prolonged viral excretion in the feces.[24,25]

VIRUS

Rotavirus infection has been detected in most common species of domestic animals and in many species of wild mammals and birds. Eight groups (species) of rotavirus have been described (A to H) based on genetic and antigenic differences in the VP6 protein—only viruses in groups A, B, and C are known to cause disease in humans. Whereas the great majority of human and animal rotaviruses share one set of common group antigens (group A rotaviruses),[26] animal rotaviruses within group A can be distinguished from human strains on the basis of type-specific surface antigens. Animals appear to be neither a reservoir for human strains nor a common source for direct transmission of rotaviruses to humans.

Certain rotaviruses of bovine and simian origin were propagated in cell culture to high titer before development of methods to propagate strains from humans.[27,28] Therefore, the molecular structure of rotavirus was largely determined by studies of the bovine strain, Nebraska Calf Diarrhea Virus,[29] and the simian strain, SA11.[30]

Rotaviruses are 70-nm icosahedral viruses that constitute a distinct genus of the family Reoviridae.[26] The virus is composed of three layers (an outer and inner capsid and a core) that encase the genome of 11 segments of double-stranded RNA.[26] All but segment 11, which contains information for two proteins (NSP5 and NSP6), encode a single protein. Gene segments can be separated on the basis of their molecular weight by polyacrylamide gel electrophoresis.[26] When mixed infections with distinct rotavirus strains occur under experimental conditions or in nature, the gene segments may reassort independently, producing progeny viruses of mixed parentage. Analysis of reassortant rotaviruses has led to identification of the gene segments encoding each of the structural polypeptides. This identification, in turn, has made possible techniques to intentionally reassort rotavirus strains and prepare candidate vaccine strains that incorporate desirable phenotypic characteristics of different parental viruses.[31–33]

Three major structural and one nonstructural protein of rotavirus are of interest in vaccine development (Table 52.1). The outer layer of rotavirus contains two distinct proteins: VP4 and VP7 (Fig. 52.2).[26] Each of these proteins bears type-specific antigenic determinants, elicits serotype-specific neutralizing antibodies, and induces serotype-specific protective immune responses in vivo.[34–37] Protein VP7 is encoded by gene segments 7, 8, or 9 in different rotavirus strains; protein VP4 is encoded by gene segment 4.[26] The most highly represented viral structural protein is VP6, which is found in the internal capsid and bears group-specific antigenic determinants.[38] Three structural proteins (VP1, VP2, and VP3) form the viral core and six other nonstructural proteins (NSP1, NSP2, NSP3, NSP4, NSP5, and NSP6) are also made during infection.[26] NSP4 is an enterotoxin.[39]

Both of the surface proteins, VP4 and VP7, elicit distinct serotype-specific neutralizing antibodies as well as, in certain

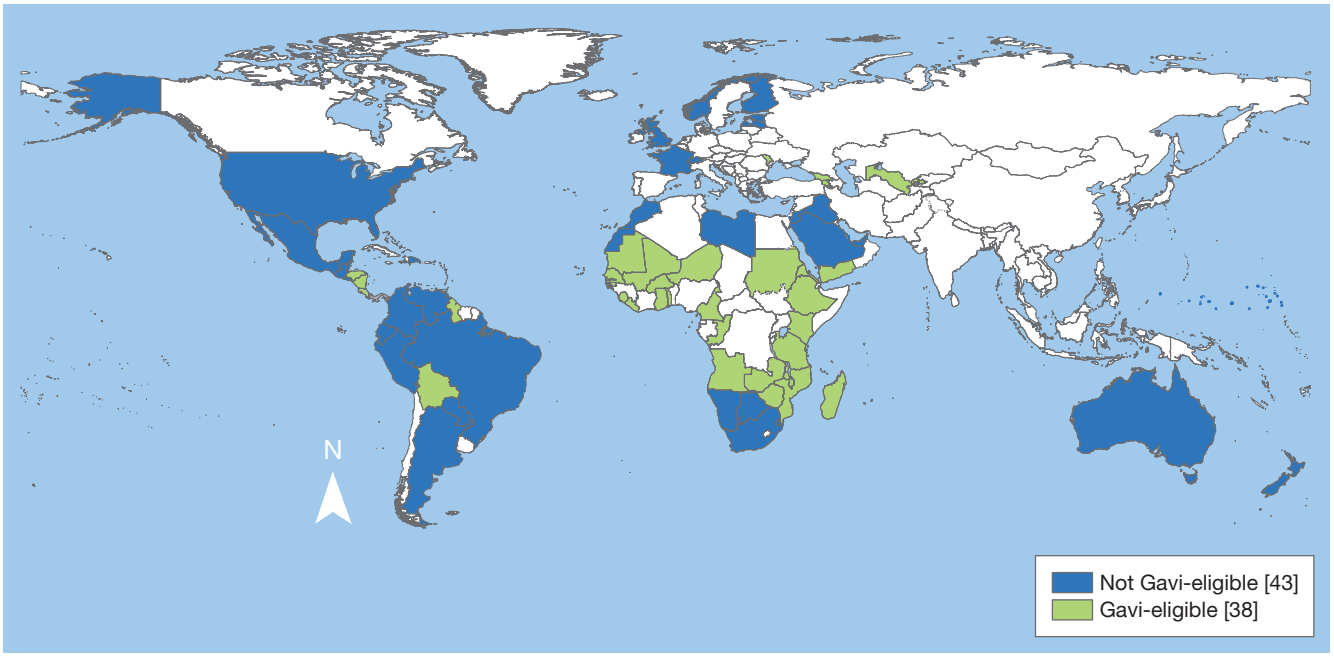


Figure 52.1. Countries that have included rotavirus vaccines in their national immunization programs, as of May 2016.

TABLE 52.1 Biologically Significant Structural and Nonstructural Rotavirus Proteins

Designation	Product of Gene Segment	Approximate Molecular Weight (kDa)	Virion Localization	Biological Significance
VP4[a]	4	88,000	Outer capsid	Type-specific antigen, hemagglutinin
VP6	6	44,000	Inner capsid	Major subgroup antigen
VP7	7, 8, or 9	38,000	Outer capsid	Type-specific antigen
NSP4	10	28,000	Nonstructural	Enterotoxin[a]

[a]Trypsin cleaves VP4 yielding VP5 (MW 60,000) and VP8 (MW 27,000).

Data from Estes MK, Cohen J. Rotavirus gene structure and function. Microbiol Rev. *1989;53:410–449; Bellamy AR, Both GW. Molecular biology of rotaviruses.* Adv Virus Res. *1990;38:1–43; and Ball JM, Tian P, Zeng CQY, et al. Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein.* Science. *1996;272:101–104.*

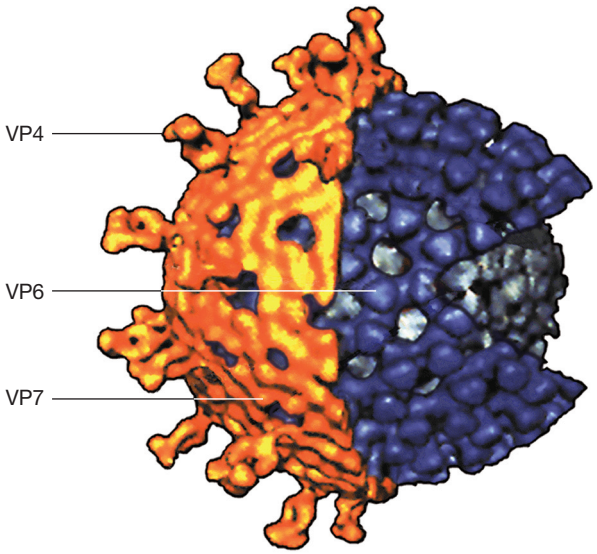


Figure 52.2. Three layers of a rotavirus particle: inner core, inner capsid, and outer capsid. Two surface proteins (VP4 and VP7) and one inner capsid protein (VP6) are shown.

circumstances, cross-reactive neutralizing antibodies.[40–46] The VP7 protein is glycosylated and serotypes determined by this protein are termed *G types*[26]; 27 rotavirus G serotypes have been identified. The VP4 protein is cleaved by the protease trypsin into VP5 and VP8; serotypes determined by the latter protein are termed *P types*.[26] The P protein presents in the form of 60 protein dimer spikes (the hemagglutinin) that extend beyond the VP7 surface of the virion while also penetrating through the outer capsid to interact with VP6 on the inner capsid.[47] Because of extensive cross-reactivity among different P types, it has not been possible to classify all P types by use of polyclonal hyperimmune antisera or monoclonal antibodies alone, although the major P serotypes have been established. This led to development of an additional system of P typing into genotypes identified by nucleic acid hybridization and nucleic acid sequencing studies.[48] Thirty-seven P genotypes have been identified.

Because of the above phenomena, a complex nomenclature has been developed. A complete rotavirus serotype is described using a number (or a number and letter) for the P serotype, followed by a number in brackets that represents the P genotype. This is followed or preceded by the G type designation (e.g., P1A[8]G1). Table 52.2 lists the most common human serotypes.

TABLE 52.2 Common Human Group A Rotavirus Serotypes Worldwide

VP4 Serotype (Genotypes)	Associated VP7 Types
P1A[8]	G1, G3, G4, G9, G12
P1B[4]	G2
P2[6]	G9, G12

PATHOGENESIS

Studies of rotavirus pathology and pathophysiology in animals and humans focus on four important questions:

1. In which intestinal and nonintestinal tissues do rotaviruses replicate and induce disease?
2. By what mechanism does rotavirus induce gastroenteritis?
3. Why is rotavirus-induced gastroenteritis primarily a disease of the young?
4. What factors determine why rotavirus disease is more severe in developing than in developed countries?

Studies of natural rotavirus infection indicate that rotavirus replication is restricted to mature villous epithelial cells in the mucosal surface of the small intestine.[49–54] Replication progresses from the proximal to the distal small intestine.[52,54] Rotaviruses do not appear to replicate in immature epithelial cells of the villous crypt or in M cells overlying Peyer patches.[55] Despite epidemiologic support for respiratory spread, there is no clear evidence that rotaviruses productively replicate in organs or tissues other than the intestine. Rotavirus antigen, RNA, and live virus have been identified in blood during rotavirus infection, and RNA has been identified in cerebrospinal fluid, but the significance of these observations is unknown[14,22,23,55a,55b,55c]; rotaviruses have never been shown to replicate productively in humans in sites distant to the intestine. Although simian rotaviruses have been shown to replicate in hepatocytes of inbred mice, often leading to biliary atresia,[56,57] the relevance of these findings to human infection remains unclear.

Rotavirus replication in intestinal epithelial cells causes several physiologic and morphologic changes. Infected animals have a decreased capacity to absorb sodium, glucose, and water and have decreased levels of intestinal lactase, alkaline phosphatase, and sucrase.[58,59] These findings are consistent with an absorptive abnormality associated with an accelerated migration of immature epithelial cells toward the villous tip. Because no inflammatory changes occur in the lamina propria, in Peyer patches, or at the intestinal mucosal surface,[49–54] it is unlikely that intestinal epithelial cell damage is mediated by the host immune response.

One of the rotavirus nonstructural proteins, NSP4, acts as a viral enterotoxin.[39] Exposure of intestinal epithelial cells to NSP4 induces diarrhea in suckling mice in an age-dependent, dose-dependent, and specific manner. Disease is caused by excess chloride secretion by a calcium-dependent signaling pathway that can be blocked by oral administration of human immunoglobulin.[60,61] This finding is consistent with animal studies using reassortant rotaviruses generated from pathogenic and nonpathogenic strains,[62] indicating that the genes that encode pathogenic VP3, VP4, VP7, and NSP4 of the pathogenic strain were all required to reconstitute virulence. Because virus replication is required for generation of nonstructural proteins, it is not surprising that attenuation of rotavirus virulence can occur without attenuation of NSP4.[63]

Rotavirus infections are more likely to be severe in children 3 to 24 months of age than in younger infants or older children and adults.[64–67] Several studies have offered possible explanations for these differences in age susceptibility. First, children of increasing age may be protected by a virus-specific immune response generated by repeated natural infections.[68] Protection of young infants may be mediated by passively transferred, transplacental, maternal antibodies. Breastfeeding clearly protects against rotavirus disease.[69] Second, infant mice have a larger percentage of intestinal epithelial cells with putative rotavirus-binding proteins on the surface than do older mice[70]—an observation that correlates directly with the age susceptibility to disease. Last, rotavirus entry into target cells is facilitated by cleavage of VP4,[71,72] which occurs in the presence of trypsin, elastase, or pancreatin.[73] Quantities of these exopeptidases are decreased in intestinal fluid secretions of newborn infants compared with older infants and young children.[74]

Children in developing countries are more susceptible to severe rotavirus disease than those in developed countries.[75] This is probably a consequence of poor access to hydration therapy and medical care, poor nutrition, concomitant infections with other viruses and enteropathogenic and toxin-producing bacteria, and possible differences in the gut microbiome.[76,77] Several studies in animals support the hypotheses that poor nutrition,[78,79] or associated bacterial infections, may enhance the severity of rotavirus-induced enteritis.[55,79,80]

IMMUNOLOGIC FACTORS ASSOCIATED WITH PROTECTION AGAINST DISEASE

Protection against reinfection, which has been reviewed,[81,82] focuses on two important questions:

1. Which effector function or functions of the immune response mediate protection?
2. What is the importance of including different rotavirus serotypes in an optimal vaccine?

In 1983, the role of immunity to rotavirus was demonstrated by studies of natural infection in neonates.[83] Neonates infected in the first month of life were not protected against rotavirus reinfection but were protected against moderate to severe disease on reinfection. Conversely, neonates not infected with rotavirus during the first month of life were fully susceptible to diarrheal disease associated with the first rotavirus infection. Since then, studies of natural infection in infants and young children indicate that prior infections protect against severe disease upon reinfection.[84,85] In birth cohort studies from Mexico,[68] Guinea Bissau,[86] and India,[86a] a primary rotavirus infection conferred 77%, 70%, and 43% protection, respectively, against subsequent rotavirus disease (any severity). In Mexico, two previous infections provided 100% protection against subsequent moderate or severe rotavirus disease, but in India, three previous infections provided only 79% protection against this outcome. Such cohort studies from various geographic areas have examined preexisting antirotavirus IgG and IgA levels and subsequent rotavirus infections and disease episodes, with differing estimates of association.[84,85,86b,86c] Protection is associated with the presence of virus-specific immunoglobulin (Ig) A at the intestinal mucosal surface[87,88] and therefore serum IgA responses are often measured to evaluate immune responses to vaccines. However serum IgA antibodies do not accurately predict rotavirus-specific IgA antibody levels at the intestinal surface of infants.

While IgG- or IgA-binding antibody responses to vaccine have not consistently correlated with protective efficacy,[88a,88b] a recent analysis found serum antirotavirus IgA titers, in

aggregate, correlated with vaccine efficacy based on clinical trials of the two vaccines currently in wide use.[88c] Serotype-specific serum neutralizing antibody responses are often also measured. This is based on the knowledge that maternal protection following rotavirus infection is at least partially serotype-specific and virus-neutralizing antibodies have most often correlated with protection in many other infectious diseases.

Although natural rotavirus infection protects against moderate to severe disease caused by reinfection, some children experience repeated episodes of diarrhea with the same serotype during the following rotavirus season,[83,89–99] and a small number of children develop symptomatic rotavirus infection twice within the same season.[87] These observations are consistent with the fact that effector functions at mucosal surfaces, such as production of virus-specific secretory IgA (sIgA), are usually short-lived and that rotavirus-specific sIgA often is not detected at the intestinal mucosal surface within 1 year of symptomatic infection.[83,87–100] Modification of the severity of rotavirus disease caused by reinfection is most likely mediated by production of virus-specific sIgA by memory rotavirus-specific B cells located in the intestinal lamina propria.[101]

The presence of rotavirus-specific sIgA at the intestinal mucosal surface (as reflected in the feces) and in serum is predictive of protection against disease in studies of natural infection but not in vaccine trials. Virus-specific IgA in feces or serum did not predict protection against disease after immunization of infants with simian-human reassortant rotaviruses.[102–104] Several explanations for this phenomenon have been posited. First, the absence of rotavirus-specific sIgA in feces does not necessarily predict the absence of rotavirus-specific memory B cells in the intestinal lamina propria.[101] The presence of rotavirus-specific memory B cells in the lamina propria can only be determined by intestinal biopsy. Second, protection against rotavirus disease after immunization with animal rotaviruses might be mediated in part by virus-specific cytotoxic T lymphocytes. Whereas the role of rotavirus-specific cytotoxic T lymphocytes in protection against human disease is unknown, some evidence supports their importance in protection against disease in animals.[105–108] Alternatively, after immunization, cytokines with antiviral activity that are produced by activated CD4-bearing T cells may be generated either earlier or in greater quantities than after primary infection. Studies have found that rotavirus-specific CD4-bearing T cells alone mediated protection against challenge in experimental animals.[109,110] Several cytokines have been found to block rotavirus replication in vitro.[111]

It has been reported in some instances that natural infection or immunization of children with one rotavirus serotype induced protection against challenge with a different serotype (heterotypic protection).[93,112] Heterotypic protection may be mediated by antibodies directed against cross-reactive epitopes on outer capsid proteins VP4 and VP7,[42] antigenically conserved inner capsid proteins that are actively transported through rotavirus-infected villous epithelial cells,[113] rotavirus-specific cytotoxic T lymphocytes that broadly cross-react with cells infected with different rotavirus serotypes,[114] or antiviral cytokines generated by activated CD4-bearing T cells.[109,110,115]

The relative importance of including all common human P and G types in a rotavirus vaccine remains undetermined. However, it is clear that after a primary, natural rotavirus infection infants develop virus-specific neutralizing antibodies in serum directed against the infecting G type at levels greater than those directed against other G types.[116–120] In a similar manner, it has been reported that children are more likely to be protected against disease after reinfection with a G type to which they have already been exposed.[89,93] Finally, protection against rotavirus disease in adults challenged with a virulent human rotavirus strain (G1P1A[8]) correlated with antibodies directed against homotypic VP4 and VP7.[121] This may explain, in part, why heterotypic protection following administration of vaccines is inconsistent (see below).

DIAGNOSIS

Electron microscopy, which once served as the reference standard for diagnosis of rotavirus infections, has now been replaced by enzyme-linked immunosorbent assay (ELISA). ELISA, which uses either polyclonal or monoclonal antibody preparations directed against the antigenically conserved inner capsid protein VP6, is as sensitive as electron microscopy but easier and less expensive to use.[122,123] However, false-positive ELISA results have been reported.[124]

The detection of rotavirus by polyacrylamide gel electrophoresis, although not commercially available, offers several advantages over ELISA. Assays that detect rotaviral genome, such as polyacrylamide gel electrophoresis and polymerase chain reaction, are absolutely specific for rotavirus. In addition, detection of migration patterns of segmented double-stranded RNA (electropherotypes) is of value in determining specific strains associated with community outbreaks or nosocomial infections, as is reverse transcriptase–polymerase chain reaction with partial sequencing of the gene coding VP7.[125] Indeed, because of difficulty in developing highly specific antisera against certain rotavirus P-serotypes, many have been classified only by characterization of their nucleic acid. Such strains are designated as *genotypes* with arbitrary numbered serotypes assigned based upon each P type newly identified by its genotype.

Reverse transcriptase–polymerase chain reaction is more sensitive than ELISA or electrophoresis in detecting small concentrations of rotavirus, particularly from clinical stool specimens.[126,127] A technique for VP7 typing of rotavirus in stool samples using partial genome product sequencing has proved consistently useful for characterizing specific serotype subtypes and allowing worldwide assessment of rotavirus strain relationships.[125]

EPIDEMIOLOGY

Rotavirus is a ubiquitous infection in young children.[2] In settings without rotavirus vaccines, nearly all children are exposed to rotavirus and acquire antibodies by 2 to 3 years of age and most rotavirus diarrhea occurs during the first 3 years. In the first 3 months, infections might be asymptomatic or less severe than those in older infants; this is most likely a result of the protective effect of passively transferred maternal antibodies or breastfeeding or both. Infections in neonatal nurseries are often asymptomatic.[83] First infections after 3 months of age are generally associated with diarrhea that can range from mild to severe, whereas subsequent exposures lead to milder illness or asymptomatic infections.[68,84,85,87,128] The observation that nearly all children throughout the world, regardless of socioeconomic status, were infected early in life suggests that rotavirus is not primarily transmitted through fecally contaminated water or food, as distinct from bacterial or parasitic pathogens. Therefore, improvements in water, sanitation, and hygiene are not likely to substantially alter the incidence of disease. The priority placed on prevention of rotavirus by vaccination is predicated on the great global disease burden of rotavirus, the recognition that natural immunity is protective, and the expectation that alternative public health measures, including the provision of clean food and water, are unlikely to fully prevent disease.[1,129] Also, although infants in developing countries experience many more episodes of gastroenteritis every year than those in developed countries, the percentage

of rotavirus disease in hospitalized infants with dehydration secondary to diarrhea (approximately 40%) was similar in developing and developed countries before vaccine introduction.[130] Clearly, rotavirus causes most of the life-threatening gastroenteritis, regardless of the economic status of a nation, if vaccine is not in use.

Little is known about the exact mode of transmission of rotavirus. Person-to-person contact or exposure to aerosolized respiratory droplets are the most likely modes of transmission. In a mouse model, airborne spread of epidemic diarrhea by a murine strain has been reported.[131] Furthermore, rotavirus disease has a distinct winter seasonality in temperate climates that is similar to that seen for viruses spread by the respiratory route, such as influenza, respiratory syncytial virus, and measles. Before implementation of rotavirus vaccination in the United States, annual rotavirus epidemics began in the Southwest and spread to the North and East. The reasons for this phenomenon are not clear, although a recent mathematical study correlated the earlier onset of rotavirus activity in the West with higher birth rates in Western U.S. states, possibly resulting in more rapid accumulation of susceptible infants through births in each rotavirus season.[132] Following implementation of rotavirus vaccination in 2006, postvaccine seasons have demonstrated a delayed onset and distortion of the geographic patterns seen in prevaccine seasons. A south-to-north migration of rotavirus activity has been documented in Europe, and has been correlated with variations in birth rates, temperature, and rainfall.[133]

Humans are believed to be the only reservoir of human rotaviruses and consistent transmission of animal rotavirus strains to humans seems unlikely. Nevertheless, although classical epidemiology has failed to identify transmission of virus from animals to humans, reassortant strains composed of genomic segments from both human and animal rotaviruses have been identified. The presence of wild-type animal–human reassortants indicates that some mixing of strains must occur in nature and that these reassortments might be important for virus evolution.[134–136]

Global surveillance has led to the characterization of at least 27 G types and 37 P types in humans, and because rotavirus has a segmented genome, gene reassortment could theoretically lead to almost 1000 different G and P combinations. Although more than 60 G-P combinations have been found in human beings, just five strains (P[8], G1; P[4], G2; P[8], G3; P[8], G4; and P[8], G9) cause 80% to 90% of childhood rotavirus disease globally (see Table 52.2). Certain histo–blood-group antigens expressed on enterocytes have recently been proposed as receptors or ligands for the P protein of rotaviruses and associations between a population's frequency of particular histo–blood-group antigens and disease (or lack of disease) from particular rotavirus P genotypes have been made.[137–141] The distribution of various rotavirus strains varies by region and over time within the same region[142]; these natural variations are important to keep in mind when monitoring ecologic changes in rotavirus strains following the implementation of rotavirus vaccination.

Certain clues about immunity and transmission of rotavirus can be gained from epidemic investigations and special studies. Whereas immunity is believed to protect adults from disease,[143–146] parents and caretakers of children with rotavirus diarrhea often get disease, perhaps because practices such as diaper changing expose them to a large infectious dose.[146–152] Outbreaks in childcare centers and hospitals can spread rapidly among nonimmune infants and young children, presumably because of person-to-person contact, airborne or droplet spread, or contact with contaminated toys.[150–152] In the elderly, outbreaks have been described that may be linked to waning immunity or institutional crowding or both. Of note, many of these outbreaks have been associated with G2 strains rather than other more common rotavirus serotypes.[153–155] In adults traveling from developed to developing countries (i.e., persons who would be expected to be immune to rotavirus), the virus has been identified as a cause of diarrhea, perhaps because the mode of transmission is different or the inoculum size is greater.[156] In addition, people immunocompromised by HIV infection,[157] hereditary immunodeficiency,[158,159] immunosuppression for organ transplantation,[160] or chemotherapy can develop rotavirus diarrhea with virus shedding that can persist for months.

Given the relatively high prevalence of HIV in some developing country settings, it is important to evaluate the safety of rotavirus vaccines in HIV-infected infants. It is anticipated that rotavirus vaccines will be well tolerated in HIV-infected infants, given that the vaccines are composed of highly attenuated viruses. In addition, wild-type rotavirus does not lead to more-severe disease in HIV-infected as compared with uninfected children. Studies conducted in the United States, Latin America, and several countries in Africa have shown that the presentation of rotavirus is similar in HIV-infected and uninfected infants.[161–166] The severity of symptoms, duration of hospitalization, and age at presentation does not appear to differ by HIV status. One study showed shedding of rotavirus up to 3 weeks after onset of symptoms in a higher proportion of HIV-infected than uninfected infants; however, the shedding was not associated with symptoms.[161] Furthermore, Rotarix (see below) was found to be safe in HIV-infected infants in South Africa.[167] Another factor that supports the safety of rotavirus vaccines in HIV-infected infants is the tolerability of oral polio vaccine (OPV) in this population. OPV is a live vaccine that replicates vigorously in the intestinal tract. There is no evidence of an increased risk of vaccine-associated paralytic poliomyelitis in HIV-infected infants.[168]

Several differences in the epidemiology of rotavirus diarrhea between developed and developing countries may affect future considerations concerning the use of vaccines. The distinct winter seasonality of rotavirus hospitalizations in temperate climates stands in contrast to the year-round disease seen in several tropical settings.[10] This means that a child born in a temperate climate immediately after the rotavirus season will have to wait many months before encountering the first possible natural infection during the following year's winter season. A child born in a tropical setting could be exposed any day of the year. Consequently, the average age at first infection is often younger in developing countries in tropical areas. In such tropical countries the first episode of rotavirus disease occurs most often during the first year of life, whereas frequently in developed countries the greatest prevalence of first rotavirus infections occurs in the second year of life. Therefore, an effective vaccine program in a developing country may require earlier and higher levels of coverage than a vaccine program in a developed country.[86a,168a] The Expanded Program on Immunization schedule, which is widely used throughout the developing world, calls for completion of the third vaccination visit by 14 weeks of age, which would provide such early coverage regardless of the rotavirus vaccine used. For unexplained reasons, fecal specimens from children from developed countries are typically infected with a single strain of virus of one of the common serotypes found globally,[155,169] while in developing countries, the rate of mixed infections with two or more strains can be as high as 30%. These mixed infections as well as single-virus infections in developing countries may include uncommon serotypes not found in other parts of the world.[171] Nevertheless G1 to G4 and G9 serotypes are still predominant (~80% of all strains); thus, a vaccine that provides protection against these strains would be expected to have broad coverage in the developing world.

TABLE 52.3 Burden of Rotavirus Disease in the United States and Worldwide Prevaccine

	United States		Worldwide	
Parameter	**Total**	**Risk Per Child**	**Total**	**Risk Per Child**
Births	3.9 million	—	130 million	—
Rotavirus gastroenteritis	2.7 million	1 in 1.4[a]	111 million	1 in 1.2
Physician, ED visits	600,000	1 in 6.5	2.5 million	1 in 52
Hospitalizations	55,000–70,000	1 in 70	2 million	1 in 65
Moderate to severe disease	—	—	16 million	1 in 8
Deaths	20–60	1 in 100,000	450,000–600,000	1 in 250
Medical costs	$400 million	—	—	—
Indirect and direct costs	$1 billion	—	—	—

[a]Meaning that 1 of every 1.4 children born in the United States will have at least one episode of symptomatic rotavirus infection.
ED, emergency department.

The burden of rotavirus diarrhea for children in developing countries became evident when the first diagnostic tests were used to study the etiology of diarrhea in hospitalized children (Table 52.3). In more than 100 studies conducted around the world among children younger than 5 years of age who were hospitalized for diarrhea, rotavirus was the most common cause of diarrhea (detected in 20–60% of cases). Prospective studies of children observed from birth to 2 years of age suggest that the incidence of rotavirus diarrhea varies directly with the intensity of surveillance. These studies indicate that 77% to 83% of children will have an episode of rotavirus diarrhea in their first 2 to 3 years of life and, depending somewhat on the locale,[172–174] seek medical attention or treatment.

In developed countries, rotavirus was originally considered to be a mild, inconsequential infection of children. This point of view was proved incorrect when early studies documented a clear winter peak of rotavirus-associated hospitalizations that accounted for as much as 80% of cases of winter diarrhea.[6,175,176] On the basis of these surveys, the Centers for Disease Control and Prevention (CDC) developed national estimates for rotavirus diarrhea. These estimates, prior to the use of rotavirus vaccines, indicated that by the end of the second or third year of life 60% to 80% of all children developed a rotaviral diarrheal illness (~2.7 million episodes per year), one in 6.5 sought medical attention, one in 70 (55,000–70,000 patients) were hospitalized, and one in 66,000 to one in 200,000 (20–60 children) died from rotavirus.[4,177] The estimated rate of hospitalizations for rotavirus gastroenteritis among U.S. children (~275 per 100,000 for children younger than 5 years of age) was lower than the estimated rates for the United Kingdom,[178] Australia,[179] Ireland,[180] Venezuela,[181] and Finland, which ranged from approximately 800 to 3000 per 100,000. The cost of medical care in the United States was estimated to be more than US$400 million; indirect costs were estimated to be more than US$1 billion.[7] However, because rotavirus can cause vomiting and fever in the absence of diarrhea,[182] estimates of disease burden from U.S. medical records that include a code for diarrhea alone may underestimate the actual incidence of disease.

PASSIVE IMMUNIZATION

Passive immunization with rotavirus-specific antibodies administered by the oral route ameliorates acute rotavirus infection in infants.[183–186] Sources of rotavirus-specific antibodies studied have included pooled commercial preparations of human serum immunoglobulin or colostrum from cows parenterally immunized with rotavirus. In both immunocompetent and immunocompromised children, oral inoculation with rotavirus-specific antibodies caused a decrease in the number of days with diarrhea as well as a decrease in the length of hospital stays. No commercial source of rotavirus antiserum for therapy is currently available.

ACTIVE IMMUNIZATION

Nonhuman Rotaviruses

Initially, researchers examined two bovine- and one simian-origin rotavirus as oral vaccines for humans. In each case, promising early clinical trials characterized by low pathogenicity and impressive protective efficacy were followed by more extensive trials yielding inconsistent results. They are described briefly below because many of their phenotypic characteristics are retained in reassortant vaccines. A single animal rotavirus strain of lamb origin is currently available in China.

Bovine Strain RIT4237

The first rotavirus vaccine to be evaluated in humans was the bovine strain Nebraska Calf Diarrhea Virus (P6[1]G6),[29] which was named RIT4237 for use in human trials.[187] This virus was highly attenuated by approximately 200 passages in bovine cell culture and most often was administered to infants at a dose of 10^8 median tissue culture-infective doses. Induction of immunity to the vaccine virus was associated with minimal replication and minimal shedding of vaccine virus in feces.[188] Between 50% and 70% of vaccine recipients had a humoral immune response measured by rotavirus-specific ELISA for serum IgG or IgA or by neutralization of the vaccine virus strain.[188–190] Adverse clinical events were not noted in infants as young as 2 weeks of age.

In two initial efficacy trials of RIT4237 in 6- to 12-month-old Finnish infants, 50% and 58% were protected against all rotavirus diarrhea and 88% and 82% were protected against clinically significant disease.[190,191] The natural challenge was serotype P1A[8]G1. In subsequent efficacy trials performed at other sites, little or no protection was observed.[192–194] As a result, RIT4237 was withdrawn from further development.

Several principles generally applicable to oral rotavirus vaccines in infants were established with RIT4237:

1. A live, attenuated oral vaccine could protect children against natural rotavirus challenge.

2. The highest rates of immune response were achieved in infants 5 to 12 months of age, a time when prevaccination serum antibody levels, which reflected passively transferred maternal antibodies, were minimal.[188,190,195]
3. The specificity of virus-neutralizing antibodies in serum was primarily restricted to the homologous (G6) serotype, but heterotypic immunity could protect children against human strains.[196,197]
4. Administration of infant formulas to buffer stomach acids given before the vaccine enhanced virus-specific immune responses.[198,199]
5. Coadministration of OPV with a bovine-origin rotavirus vaccine had the potential to inhibit rotavirus-specific immune responses.[200]
6. Protection against severe rotavirus disease was always more efficient than protection against all rotavirus disease.

Bovine Strain WC3

The parent rotavirus strain Wistar Calf 3 (WC3; P7[5]G6) was isolated from a calf with diarrhea in Pennsylvania in 1981 and passaged 12 times in African green monkey kidney cells.[201] Like RIT4237, WC3 vaccine was given at high titers (10^7 plaque-forming units [PFU] per child), was shed infrequently in feces, and was well tolerated.[201,202] Virus-specific neutralizing antibody responses in serum occurred in 71% to 100% of infants in trials in the United States and were primarily homotypic.[201]

In an initial double-blinded, placebo-controlled, efficacy trial in Philadelphia, WC3 vaccine caused a 76% reduction in rotavirus morbidity and 100% protection against moderate to severe rotavirus diarrhea when the natural challenge strain was P1A[8]G1.[112] Levels of virus-neutralizing antibodies did not correlate with protection from rotavirus illness. In a subsequent efficacy trial of WC3 conducted in Cincinnati, 100% of infant vaccine recipients developed WC3-specific neutralizing antibodies but none were protected against G1 rotavirus disease.[203] However, following immunization with WC3 vaccine, seropositive infants with evidence of prior infection exhibited booster responses of broad specificity (G1–G4).[204] In subsequent efficacy trials, WC3 gave little protection in the Central African Republic[205] and only 50% protection in Shanghai. Because protection against rotavirus disease was inconsistent, WC3 was eliminated as a potential vaccine candidate.

Simian Strain RRV

The third animal-origin rotavirus tested in infants was the rhesus rotavirus vaccine (RRV) (P5[3]G3), which was isolated from a young monkey with diarrhea[206] and tested as a vaccine candidate in the 16th cell-culture passage.[206] Although RRV is nominally type G3,[207] the G protein shares only moderate homology with human G3 strains.[208]

RRV was safe and immunogenic in children 2 to 12 years of age,[206] but in infants 5 to 20 months of age, a clustering of febrile responses was noted 3 to 4 days after inoculation.[209] In Finnish infants 6 to 8 months of age, postvaccine fevers (64%) and watery stools (20%) were associated with administration of RRV in quantities of 10^5 PFU.[210] In the same study, infants administered RIT4237 vaccine exhibited a lower rate of serum antibody responses but had no adverse reactions.[210]

The increased incidence of fevers was frequently absent in RRV vaccine recipients in developing countries[211] and in younger infants (4 months of age or younger) inoculated with a lower dose of vaccine. Immunogenicity was less at lower doses (i.e., 10^4 PFU) and in children who were breastfed or did not receive a buffer before vaccination.[211–213] RRV at a dose of 10^4 PFU was not reactogenic in newborn Venezuelan infants.[214] Coadministration of RRV and OPV did not appear to lead to significant inhibition of serum antibody responses to either vaccine.[214,215]

Results of clinical efficacy trials of RRV varied. Infants were given a single dose of 10^4 PFU between 2 and 12 months of age. Trials in Finland and Sweden identified modest protection (38% and 48%, respectively) against all cases of rotavirus diarrhea, but greater efficacy against severe rotavirus diarrhea (67% and 80%, respectively).[216,217] However, in three trials in the United States, much lower levels of protection were observed. In all these trials, the natural challenge viruses were predominantly G1.[194,218,219]

The greatest efficacy of RRV was observed in Venezuela. The vaccine protected 65% of children from all rotavirus disease and 100% of children from severe disease[220]; consistent with that observation, the natural challenge rotavirus strains were predominately serotype G3. Vaccine-associated reduction in cases identified as G1, G2, or G4 (67%) was similar to the efficacy against G3 (70%).[221] Because protection against rotavirus disease was inconsistent, RRV was not pursued as a vaccine candidate.

Lamb Strain Lanzhou Lamb Rotavirus Vaccine

The Lanzhou Lamb Rotavirus Vaccine (LLR), developed at the Lanzhou Institute of Biological Products, is licensed in China.[222] This live oral vaccine, which was derived from an ovine rotavirus isolated in 1985 and characterized as P[10]G12, was passaged 42 times in calf kidney cells. The vaccine is a liquid formulation with buffer containing $10^{5.5}$ infectious particles per dose in a volume of 3 mL and is given as a single dose between 2 and 24 months of age. In an efficacy trial, two cases of rotavirus diarrhea occurred among 1506 vaccine recipients and eight cases occurred among 1583 placebo recipients (73% efficacy). The rates of mild reactions were similar in the vaccine and placebo groups. The vaccine was licensed in China in 2000 and currently is being sold in several provinces. Postlicensure evaluations have found one dose, or one or more doses, of LLR to be 35.0% to 73.3% effective against rotavirus diarrhea requiring hospitalization.[223,224,224a]

Animal X Human Reassortant Rotaviruses

Because protection induced against rotavirus disease by animal rotavirus strains was inconsistent, vaccine researchers focused efforts on recombinant (reassortant) rotaviruses. Such reassortant strains were prepared by using simian (RRV) or bovine (WC3 or UK) rotaviruses. In vitro–derived reassortants were selected bearing a human VP7 protein (to evoke human G type-specific neutralizing antibodies) and the remaining genes from an animal rotavirus (to attenuate rotavirus virulence for infants).[62,225] One multivalent bovine (WC3) human reassortant vaccine (RotaTeq) also contains a reassortant strain containing human VP4 (to evoke human P serotype-specific neutralizing antibodies). In addition, naturally occurring bovine–human reassortants ("Indian strains") currently are being tested as vaccines.

The first generation of reassortants, including those in the first licensed vaccine (RotaShield), were designed to contain the gene coding for VP7 of a human virus and 10 genes of the animal virus. To prepare a reassortant, susceptible cell cultures, usually derived from African green monkeys, were coinfected with a human virus and an animal virus (Fig. 52.3). Reassortment of viral gene segments occurred by chance and a virus containing the desired human virus VP7 was selected, often by suppressing the VP7 of animal origin with a monotypic

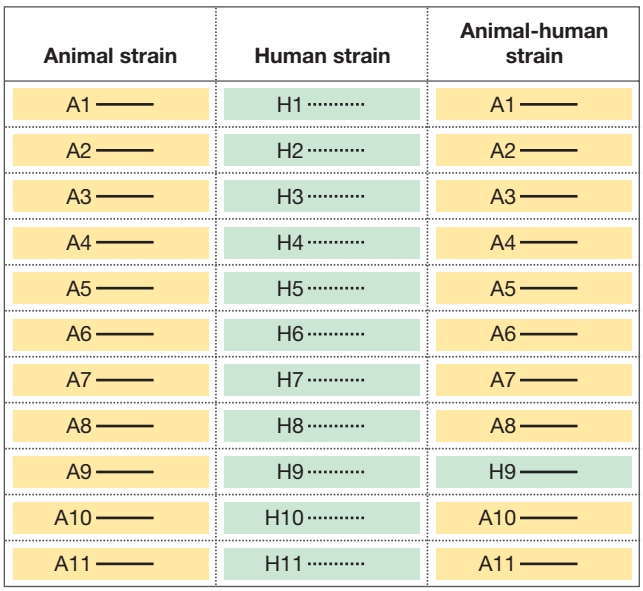


Figure 52.3. The rotavirus genome consists of 11 individual segments of double-stranded RNA (dsRNA). Segments can be distinguished by their pattern of migration following electrophoresis in polyacrylamide gels. Progeny virions resulting from coinfection of monkey kidney cells with an animal and a human rotavirus strain often contain gene segments from each parent. The animal-human reassortant rotavirus strain shown has 10 dsRNA segments from the animal strain and 1 segment (gene segment 9) from the human strain.

antiserum against that protein. Viruses with the human VP7 gene accompanied by 10 genes from the animal virus were selected by use of gels identifying all the genomic segments of the reassortants. In some cases, additional confirmation of the origin of each reassortant gene was obtained by sequencing the viral genome.

Simian (Strain RRV)–Human Reassortant Rotaviruses (RotaShield)

The first reassortant rotavirus vaccine candidates consisted of simian–human rotavirus reassortants that contained a single human gene (i.e., that coding for VP7) and the remaining 10 genes from RRV. These reassortant viruses were prepared at the Laboratory of Infectious Diseases at the National Institutes of Health.[226]

RotaShield was licensed for universal use for infants in the United States in August 1998. However, because the vaccine was later found to be a rare cause of intussusception, the recommendation to use RotaShield was withdrawn in October 1999.

Composition. The vaccine (RotaShield), produced by Wyeth-Lederle, contained RRV (P5[3]G3) and three simian–human reassortants of G types 1, 2, and 4, each in a virus containing all the other genes of RRV. The human virus genes were from strain D1 (RRV-G1), DS1 (RRV-G2), and ST-3 (RRV-G4). This quadrivalent combination of RRV with three simian–human reassortant rotavirus strains was called RotaShield.

Dosage and Route. Field trials of the quadrivalent RRV-human reassortant vaccine were initiated at an inoculum of 10^4 PFU of each of the four viruses (i.e., a total dose of 4×10^4 PFU) and completed at a dose of 1×10^5 PFU per strain (4×10^5 PFU total), the dose of the licensed vaccine.[226] In each of these trials, vaccine was administered orally in three doses, immunizations were separated by at least 3 weeks, and dosing was completed by the time a child was 6 to 7 months old. Protective outcomes were determined either by recording episodes of rotavirus diarrhea reported through active surveillance by field workers during the rotavirus season or passive catchment surveillance of children attending a clinic or hospital for diarrhea confirmed to be caused by rotavirus. In the United States, two separate but comparable multicenter trials using either the low (10^4 PFU per strain) or the high (10^5 PFU per strain) doses did not demonstrate a significant difference in efficacy.[103,227] However, in Latin America, two trials conducted in Peru[228] and Brazil[229] with the lower dose of vaccine each demonstrated a lower efficacy against severe disease than a trial with the same low dose in the United States and a trial with the higher dose conducted in Venezuela.[230]

Immunogenicity. RotaShield was developed with the hope that children would develop serotype-specific immunity to the four most common G types of rotavirus in global circulation at that time (G1, G2, G3, and G4). Whereas more than 90% of children developed an immune response, as measured by the appearance of virus-specific serum IgA or virus-neutralizing antibodies directed against RRV, few children (<50%) developed serotype-specific neutralizing antibodies to any of the four human G serotypes.[227] Nonetheless, studies of efficacy of RotaShield outperformed those using a single RRV-human reassortant (containing human G1) in seasons when strains other than G1 were circulating. This finding indicated that the strategy of using RotaShield to afford broad protection against several serotypes was correct even if there was not an adequate immunologic marker to predict protection against challenge or a complete understanding of the mechanism of this protection.[103,227,231] In a rare clinical trial in which the predominant natural challenge was not serotype G1 (but was G3), the quadrivalent RRV reassortant vaccine, which contained a strain (RRV) that was of G3 specificity, was clearly more protective than was a monotype G1 RRV reassortant, lending further evidence to the utility of multivalent vaccines.[232]

Efficacy. Seven efficacy trials of RRV-human vaccine were conducted, three using the vaccine in the lower dose and four using the vaccine in the higher dose. In all these trials, infants were enrolled for immunization between 6 weeks and 2 months of age and three doses were given by mouth. Efficacy was greater against severe disease than against mild disease and, in trials lasting 2 years, protection endured for both years.[232]

In general, the efficacy of the RRV-human reassortant vaccine was lower in developing countries. The protective efficacy of the low-dose vaccine was poorest in Peru,[228] intermediate in Brazil,[229] and best in the United States.[103] In a similar manner, the higher-dose preparations performed least well among Native Americans,[194] better among Venezuelans[230] and Americans,[232] and best among Finns.[233]

In trials using high-dose vaccine, efficacy ranged from 48% to 68% against any rotavirus disease and from 64% to 91% against severe disease. Of particular note, the efficacy reported in Venezuela (48% for mild disease and 88% for severe disease) was not significantly different from that reported in the multicenter trial in the United States using this higher dose (57% and 82%), suggesting that RotaShield might be well suited for use in developing countries. Furthermore, the observation that the higher dose worked well in Venezuela, when a lower dose did not work in Peru or Brazil, suggested that in developing countries vaccine dose might be critical.

Only two trials were large enough to examine protection against hospitalization. In the trial in Finland, all 22 children

hospitalized for rotavirus diarrhea were in the placebo group,[233] and, in Venezuela, efficacy against hospitalization was 70%.[230] A postlicensure effectiveness evaluation at three U.S. hospitals showed 100% protection against hospitalization for rotavirus disease.[234]

Adverse Reactions. A significant increase in mild fevers was observed 3 to 5 days after immunization with the RRV-human reassortant vaccine. Low-grade fevers (temperature >38°C and <39°C) were most common (up to 15%) after the first dose and a small group of children (1–2%) had higher temperatures (>39°C).[103,227,229,232,233] In the Finnish trial, fever was associated with diarrhea and abdominal cramping in approximately 3% of children.[235] No increase in the incidence of vomiting was associated with vaccination in the first 6 months of life. However, in earlier studies with RRV only, older children in Sweden did have an increased incidence of loose stools and fever, suggesting that replication of the virus in older children could lead to mild symptoms of diarrhea.[217] In Bangladesh, the RRV-human reassortant vaccine caused fever in 15% of vaccinees, compared with 2% in placebo recipients. In addition, vaccine strain virus was isolated from some of the latter, indicating contact spread from vaccinees.[236]

Prior to licensure, intussusception was reported in five of approximately 11,000 children who had received RRV-human reassortant rotaviruses compared with one of approximately 4500 children who had received placebo.[231] Intussusception was not found after the first dose in any child, but was observed within 7 days after receiving the second or third dose of vaccine in three of the five affected children. Differences in rates of intussusception between vaccine and placebo recipients were not found to be statistically significant and the incidence of intussusception in vaccine recipients was not greater than estimated background rates. However, both the CDC and the American Academy of Pediatrics warned in their recommendations for use of RRV-human reassortant vaccine that intussusception might be an adverse event associated with vaccination.[231,237] In addition, the possible relationship between the vaccine and intussusception was noted in the product insert.

In July 1999, after the RRV-human reassortant vaccine had been given to approximately 1 million children, 15 cases of intussusception following administration were reported to the Vaccine Adverse Event Reporting System (VAERS).[238,239] Although approximately 2000 cases of intussusception occur each year in the United States, these 15 cases were greater than what would have been predicted given the general underreporting to VAERS. Following these reports, RotaShield was temporarily suspended from use[238] pending results of a case-controlled analysis by the CDC.

In October 1999, the CDC completed its analysis and found that the relative risk 3 to 7 days after receipt of the first or second dose of RRV-human reassortant vaccine was 37 ($P < .001$) and 3.8 ($P < .05$), respectively.[233] Using case series and case-control analysis, the population-attributable risk was initially estimated to be approximately one case of intussusception per 10,000 children immunized.[240,241] The actual attributable risks were one in 4670 to one in 9474 for the case series and case-control studies, respectively. Following results of the CDC analysis, use of the vaccine was discontinued and the manufacturer ceased its production. Subsequent ecologic studies using hospital discharge diagnoses estimated that the attributable risk of intussusception following administration of RRV-human reassortant vaccine might have been much smaller than initial estimates.[242,243]

The cause of intussusception following RotaShield remains unknown. However, because the benefits of the vaccine likely outweigh its risks in the developing world, the vaccine was tested in Ghana, given as two doses administered in the first 2 months of life.[244] The two-dose efficacy against rotavirus gastroenteritis (any severity) to age 1 year was 64%.

Bovine (Strain WC3)–Human Reassortant Rotaviruses (RotaTeq)

RotaTeq (Merck and Co.) was licensed by the U.S. Food and Drug Administration (FDA) and recommended by the CDC and the American Academy of Pediatrics for universal use in U.S. infants in February 2006.[245] RotaTeq is also licensed in the European Union and many other countries.

Composition. RotaTeq is a live, oral vaccine that contains five reassortant rotaviruses developed from human and bovine parent rotavirus strains.[245,246] Four of the bovine (WC3)–human reassortant rotaviruses express human virus VP7 from serotypes G1, G2, G3, or G4 and VP4 (P7[5]) from bovine strain WC3. The fifth reassortant virus contains VP4 (P1A[8]) from a human rotavirus strain and VP7 (G6) from WC3. The human strains that provided surface proteins G1, G2, G3, G4, and P1A for the bovine–human reassortant vaccine were WI79, SC2, WI78, Bricout B, and WI79, respectively. The reassortants were propagated in Vero cells.

Each 2-mL vial of vaccine contains the following minimum concentration of each of the five reassortants: WC3-G1: 2.2×10^6 infectious units; WC3-G2: 2.8×10^6 infectious units; WC3-G3: 2.2×10^6 infectious units; WC3-G4: 2.0×10^6 infectious units; and WC3-P1A: 2.3×10^6 infectious units. Trace amounts of fetal bovine serum might be present. The vaccine does not contain thimerosal or other preservatives.

Buffer and Stability. WC3-human reassortant vaccine is suspended in a solution of buffer (sodium citrate and phosphate) and stabilizer (sucrose) that can be stored refrigerated at 2°C to 8°C for 24 months. The vaccine formulation contains sucrose, sodium citrate, sodium phosphate monobasic monohydrate, sodium hydroxide, polysorbate 80, and tissue culture media. This diluent was adapted following a bivalent (G1 and G2) WC3-reassortant experiment in which no loss of protective potency was observed in the reassortants suspended in the buffer-stabilizer formulation after 125 weeks.[247] The shelf life is 24 months at refrigeration temperature—6 months is reserved for packaging and shipping and 18 months for shelf life in the clinic. The vaccine does not require reconstitution into a liquid form; it requires only that the cap be removed from the squeezable plastic container and the contents then squirted into the infant's mouth.

Immunogenicity

Relative Capacity of VP4 and VP7 to Evoke Neutralizing Antibodies. Infants given one or two oral doses of the univalent G1 or G2 reassortant vaccine tended to have a more efficient neutralizing antibody response in serum to the bovine WC3 (the VP4 antigen in the reassortant) than to G1 or G2 (the human VP7 antigen in the reassortant).[248–252] This led to an early opinion that, unlike the response to parenterally inoculated rotavirus, the neutralizing antibody response to VP4 predominated following oral administration. This phenomenon also had been observed repeatedly in clinical trials with RRV-human reassortant rotaviruses.

However, when a WC3 reassortant was constructed with a human P1A (VP4) and administered orally to infants, the serum neutralizing antibody responses after two doses were predominately to the WC3 VP7 surface G6 component: 76% serum neutralizing antibody response to WC3 (VP7) and only 28% response to the P1A (VP4) surface component.[250,253] When two doses of a mixture of equal concentrations of the

G1 (VP7) and P1A (VP4) single-gene reassortants of WC3 were administered to infants, an excellent neutralizing antibody response in serum was obtained to both the P1AG1 parent (78%) and to the WC3 P7G6 parent (100%).[253] Conversely, less than 35% of infants developed neutralizing antibodies to either WC3 or the human P1AG1 parent after oral inoculation with two doses of one double reassortant (a single virus containing both P1A and G1 on a WC3 background).[250,253] These findings suggest that an animal rotavirus/human rotavirus reassortant (with regard to VP4 and VP7) may have the potential to induce certain antihuman rotavirus immune responses more efficiently than an entirely human rotavirus-origin vaccine; that is, one surface antigen of bovine virus origin in some manner enhances the immunogenicity of the reassortants in response to both surface antigens VP4 and VP7.

Importance of Dose. It was later determined in two separate clinical trials that, compared with two doses of the univalent G1 reassortant, three doses resulted in higher rates of neutralizing antibody responses in serum to both the WC3 (93% after two doses and 95% after three doses) and the P1AG1 (70% after two doses and 78% after three doses) parent viruses.[251] Most infants developed neutralizing antibodies to WC3 after the first dose, a few developed them after the second dose, and almost none developed them after the third dose. In contrast, a minority of infants responded to P1AG1 rotavirus after the first dose, but many more responded to the second and third doses. Infants with neutralizing antibodies to P1AG1 virus prior to the first dose were less likely to develop an antibody response of that specificity after the first dose, but, after three doses, they exhibited response rates similar to those of originally seronegative infants.[254] Therefore, it appears that one or two serial doses given to an infant who lost maternally acquired antibody gives a serum antibody response after the completion of the series that is equal to that of those who were seronegative prior to the first dose.

Efficacy. In a pilot efficacy trial of the WC3-G1 reassortant involving 77 infants given two doses, complete protection against rotavirus disease was observed. Cases of disease in the placebo cohort were caused by either serotype G1 or G3.[252] A subsequent efficacy trial employed three doses of the G1 reassortant administered to 312 infants in either New York or Philadelphia. The vaccine provided 64% protection against all episodes of rotavirus disease and 84% protection against moderate to severe rotavirus disease.[249] The predominant wild-type challenge rotavirus was serotype G1. No adverse effects followed administration of any dose of this vaccine.

Because of the success of the WC3-G1 reassortant vaccine in situations of primarily G1 challenge, additional WC3 reassortants of the other traditionally most common serotypes (i.e., G2, G3, and G4) were constructed, based on epidemiological evidence that protective immunity is at least partly G-serotype-specific. Each was individually evaluated in infants and found to be safe and immunogenic.

Prior to the availability of the G4 reassortant, the principle of using a multivalent WC3 reassortant vaccine was evaluated in an efficacy trial of a WC3–human reassortant vaccine containing human G1, G2, G3, and P1A.[255–258] Quadrivalent WC3–human reassortant vaccine containing G1, G2, G3, and P1A, given in three doses and containing 10^7 PFU of each reassortant, was safe, immunogenic, and protective in a trial involving 439 infants in 10 different U.S. sites. Rates of vaccine virus shedding in feces were less than 10%. There was no significant excess of fever, diarrhea, or vomiting in vaccine recipients. The vaccine provided 74.6% protection against all rotavirus disease and 100% protection against severe rotavirus disease.

In the clinical trial of quadrivalent WC3–human reassortant vaccine containing G1, G2, G3, and P1A, a rotavirus-specific serum IgA antibody response occurred in 88% of vaccine recipients and a fecal rotavirus-specific IgA antibody response occurred in 65% of vaccine recipients.[257,258] The proportion of children who developed virus neutralizing antibodies to serotype G1 was 57%. Similar rates of neutralizing antibody responses specific to types G3, G6, and P1A were observed; the neutralizing antibody response to G2 was slightly lower. The predominant wild-type rotavirus challenge strains as found in placebo cases of rotavirus disease were G1 (26 of 39 episodes) and G3 (10 of 39 episodes), while G2 and G4 were associated with two cases and one case, respectively. The breakthrough cases of rotavirus disease seen in quadrivalent rotavirus vaccine-immunized infants were predominately G1 (10 of 11 cases); the other breakthrough case was G2.

Because rotaviruses are highly acid labile, all the above-mentioned clinical trials involved prefeeding of vaccine recipients with breast milk, infant formula, or Maalox. These trials also involved a frozen product thawed immediately before use. Because prebuffering and use of a frozen product are impractical, a vaccine-diluent formulation was developed that contained both buffer and stabilizing components that permitted direct administration without reconstitution and storage of viable vaccine refrigerated in liquid form.[259,260] In a clinical trial of bivalent (types G1 and G2) reassortant vaccine, vaccine suspended in the buffer-stabilizer in varying volumes and concentrations, given without a previous oral buffer, was compared with traditionally prepared vaccine given after a separate administration of buffer.[247] Three doses were given and no adverse events were observed. Although the trial was not designed to demonstrate efficacy, immune responses in infants given vaccine in the new formulation without prebuffering were excellent and equal to those of the prebuffered vaccine cohort. For example, following administration of the vaccine in the buffer-stabilizer formulation, serum neutralizing antibody responses to G1 were 67% to 73% and rotavirus-specific IgA antibody responses were 84% to 91% in serum and 77% to 88% in feces.

Following the clinical success of a quadrivalent WC3–human reassortant vaccine containing G1, G2, G3, and P1A, a pentavalent reassortant rotavirus vaccine (RotaTeq) was developed by adding WC3-G4, to protect against the G4 serotype which, like G1, G2, and G3, was established to be of worldwide prevalence. In a multisite trial involving 1946 infants, the safety, immunogenicity, and efficacy of five different vaccine compositions (approximately 300 infants per group) were evaluated in comparison with placebo (Table 52.4). The test vaccines included three pentavalent vaccine combinations (G1, G2, G3, G4, and P1A) in concentrations of either 5×10^6, 1.6×10^6, or 5.5×10^5 PFU per reassortant. Also included were groups given the G1, G2, G3, and G4 reassortants alone and the P1A reassortant alone at concentrations of 5×10^6 PFU per reassortant.[260] In this trial, infants were prefed with infant formula for buffering before vaccine. The incidence of adverse events was comparable among vaccine and placebo recipients for all vaccines evaluated. All compositions were immunogenic and protective; however, the magnitude of the antibody responses varied with virus concentration. The immunogenicity and efficacy of the high and middle concentrations of the pentavalent vaccine and the quadrivalent vaccine (G1, G2, G3, and G4) were about equal and consistent with the efficacy of the previously evaluated quadrivalent vaccine (G1, G2, G3, and P1A). The low-concentration pentavalent rotavirus vaccine and the monovalent P1A vaccine were less effective. However, the approximate 50% efficacy of the P1A reassortant against rotavirus infection of any degree of severity suggested that P1A might provide protection against

TABLE 52.4 Effect of Dose and Composition of RotaTeq, Quadrivalent (G1, G2, G3, and G4) and Univalent (P1A) Bovine (WC3)–Human Reassortant Rotavirus Vaccine on Efficacy and Immunogenicity in Infants

Dose (PFU)	Strains	N	G1 SNA[a]	Serum IgA[b]	Efficacy (95% CI)
2.5×10^{7}[c]	G1–G4, P1A	375	86.2	98.7	68.0 (31.1–86.4)
8.0×10^{6}	G1–G4, P1A	328	73.3	97.3	74.3 (37.9–91.0)
2.5×10^{6}	G1–G4, P1A	324	61.7	93.4	57.6 (11.8–80.9)
2.0×10^{7}	G1–G4	270	84.4	98.2	74.0 (40.3–90.3)
5.0×10^{6}	P1A	327	3.6	68.7	43.4 (–1.7–69.2)
Placebo	—	322	1.1	13.5	—

[a]Percent of infants with G1-specific neutralizing antibody response determined by plaque-reduction neutralization assay.
[b]Percent of infants with serum rotavirus-specific IgA response (i.e., 3-fold rise in titer from baseline) determined by ELISA.
[c]Approximate combined dose of vaccine for all components.
CI, confidence interval; IgA, immunoglobulin A; PFU, plaque-forming units; SNA, serum neutralizing antibodies.

prevalent rotaviruses (e.g., P1AG9) expressing the P1A antigen but none of the G specificities in the vaccine (see Table 52.4). This trial conclusively demonstrated that the G antigens of these vaccine strains are the dominant molecular components in inducing serum antibody and protection. As a result of these studies, 1.6×10^{6} PFU per reassortant or 8.0×10^{6} PFU for all five reassortants (G1, G2, G3, G4, and P1A) was selected for further development.[261] These quantities of vaccine viruses also served as the basis for selection of the lowest dose acceptable at expiration.

The efficacy of RotaTeq was confirmed in a placebo-controlled trial involving 1312 infants randomized to receive three doses of either approximately 1.1×10^{7} infectious units of RotaTeq in a buffer stabilizer solution or placebo.[247] The incidence of adverse events was comparable among vaccine and placebo recipients with the exception of a statistically significant 4.6% excess of fevers greater than 38.1°C among vaccinees during the week after the first dose. A high proportion (96%) of infants had a significant increase in serum antirotavirus IgA and 57% had a significant increase in serum neutralizing antibody response to serotype G1. Efficacy against all rotavirus disease was 72.5% (95% confidence interval [CI], 50.6 to 85.6); efficacy against severe rotavirus disease was 100% (95% CI, 13.0 to 100).[247]

Although all rotavirus reassortants based upon a bovine WC3 rotavirus had been generally well tolerated in all clinical trials, new concerns for RotaTeq safety arose when the RRV reassortant-based vaccine RotaShield was found to be a rare cause of intussusception. It became necessary to perform a large (70,000 infant) Rotavirus Efficacy and Safety Trial (REST) to determine whether RotaTeq was also a rare cause of intussusception.[262]

The large sample size provided an opportunity to evaluate the efficacy of the vaccine in preventing healthcare encounters for rotavirus gastroenteritis of all serotypes including hospitalizations, emergency visits, and physicians' office visits. In addition, efficacy against all cases of rotavirus gastroenteritis regardless of whether they resulted in a healthcare encounter were evaluated in a subset of 5673 vaccinated subjects in this nested placebo-controlled study.

The efficacy of RotaTeq against rotavirus gastroenteritis caused by major rotavirus serotypes was also evaluated in a nested placebo-controlled study within the large-scale clinical trial. RotaTeq protected against the serotypes included in the vaccine (i.e., G1, G2, G3, and G4) (Table 52.5).[262] RotaTeq also protected against natural challenge with P1AG9 rotaviruses, demonstrating the contribution of the WC3–human reassortant containing human P1A. Compared with placebo, efficacy against any severity of rotavirus disease caused by serotypes G1, G2, G3, and G4 was 74.0% (95% CI, 66.8–79.9%); efficacy against severe rotavirus disease was 98% (95% CI, 88.3–100%).[45] RotaTeq reduced the rate of rotavirus-associated hospitalizations by 95.8% (95% CI, 90.5–98.2%), emergency visits by 93.7% (95% CI, 88.8–96.5%), and office visits by 86.0% (95% CI, 73.9–92.5%). Efficacy against all-cause gastroenteritis hospitalizations was 58.9% (95% CI, 51.7–65.0%), demonstrating the importance of rotavirus as a cause of severe gastroenteritis in children. The efficacy of RotaTeq in the second rotavirus season postvaccination was 63% (95% CI, 44–75%) against rotavirus gastroenteritis of any severity, and 88% (95% CI, 49–99%) against severe rotavirus gastroenteritis. RotaTeq has also shown sustained protective efficacy against rotavirus hospitalizations and emergency department visits, regardless of rotavirus serotype, for up to 3.1 years after vaccination, with rate reductions (95% CI) in hospitalizations and emergency department visits during the first, second, and third years of life of 94.0% (90.0–96.5%), 94.7% (90.7–97.2%), and 85.9% (51.6–97.2%), respectively.[263]

TABLE 52.5 Reduction in Hospitalizations and Emergency Department Visits (by G Type) in the Phase III Efficacy Study of RotaTeq

Serotype	Number of Cases of Rotavirus Disease		
	Vaccine (N = 34,035)	Placebo (N = 34,003)	Rate Reduction (95% CI)
G1	16	328	95.1 (91.6–97.1)
G2	1	8	87.6 (–0.0 to 98.5)
G3	1	15	93.4 (49.4–99.1)
G4	2	18	89.1 (52.0–97.5)
G9	0	13	100.0 (67.4–100.0)

CI, confidence interval.

Data on the efficacy of a partial series of RotaTeq are primarily based on protection conferred between vaccine doses (i.e., between dose 1 and 2 and between dose 2 and 3) as most vaccine recipients in the REST study received the full vaccine series. In the trial, RotaTeq reduced the rates of combined hospitalizations and emergency department visits for G1 to G4 rotavirus gastroenteritis or rotavirus gastroenteritis regardless of serotype between doses 1 and 2 by 100% (95% CI, 72–100%) or 82% (95% CI, 39–97%), respectively. Between doses 2 and 3, RotaTeq reduced the rates of combined hospitalizations and emergency department visits for G1 to G4

TABLE 52.6 Efficacy of RotaTeq and Rotarix Against Severe Rotavirus Gastroenteritis in the First Year of Life in Africa and Asia

Country	Incidence Per 100 Person-Years		Vaccine Efficacy (95% CI)
	Vaccine	Placebo	
ROTARIX			
South Africa	0.8	3.3	76.9 (56.0–88.4)
Malawi	4.0	7.9	49.4 (19.2–68.3)
ROTATEQ			
Ghana	2.7	7.7	65.0 (35.5–81.9)
Kenya	0.7	4.0	83.4 (25.5–98.2)
Mali	0.7	0.7	1.0 (–431.7–81.6)
Bangladesh	4.9	9.1	45.7 (–1.2–71.8)
Vietnam	0.8	2.8	72.3 (–45.2–97.2)

CI, confidence interval.

rotavirus gastroenteritis or rotavirus gastroenteritis regardless of serotype by 91% (95% CI, 63–99%) or 84% (95% CI, 54–96%).[264]

In the Phase III prelicensure study of RotaTeq, protection against severe rotavirus-induced gastroenteritis was 100%, 95.4%, and 100% in children who were never breastfed, sometimes breastfed, or exclusively breastfed, respectively.[265]

Phase III trials of RotaTeq were completed in developing countries of Asia and Africa (Table 52.6).[266,267] In three African clinical trial sites in Kenya, Ghana, and Mali, a three-dose regimen of RotaTeq administered at 6, 10, and 14 weeks of age was overall 64% (95% CI, 40–79%) effective against severe rotavirus gastroenteritis during the first year of follow-up. During the first year of follow-up in the two Asian trial sites in Vietnam and Bangladesh, three doses of RotaTeq were overall 51% (95% CI, 13–73%) effective against severe rotavirus gastroenteritis.

Effectiveness and Impact. Recently summarized,[268–270] effectiveness studies in the United States using the case-control approach were conducted primarily by performing active surveillance for children presenting to the emergency department or admitted to the hospital for acute gastroenteritis; the largest network for these evaluations comprised seven pediatric hospitals. Children whose stools tested positive for rotavirus by enzyme immunoassay comprise the cases; control groups consisted of children with gastroenteritis who tested negative for rotavirus (test-negative controls), children with acute respiratory illness, or children selected from city or state immunization registries. Statistically significant vaccine effectiveness estimates for three doses of RotaTeq against emergency department visits or hospitalization (considered moderate to severe illness) for rotavirus ranged from 83% to 92%, with similar estimates in infants aged 8 to 12 months of age and those older; there was with no evidence of waning protection up to 4 years of age.[271–279] Similarly high vaccine effectiveness estimates were found against moderate to severe rotavirus disease from all specific genotypes thus reported: G1P[8], G2P[4], G3P[8], G9, and G12P[8]. The statistically significant point estimates for effectiveness after one dose ranged from 69% to 74% and after two doses from 78% to 88%, demonstrating good protection for the time between doses.

Based on examination of medical claims databases, other RotaTeq effectiveness estimates in the United States have ranged from 89% to 100%.[280–282] In reports from other high income locations in Australia, Finland, France, Spain, Portugal (≥1 dose) and Taiwan, the statistically significant point estimates for vaccine effectiveness of three doses of RotaTeq against hospitalization for rotavirus disease has ranged from 92% to 97%.[283–288]

In Nicaragua, one of the poorest countries in Latin America and an early adopter of RotaTeq in 2006 (through a manufacturer-sponsored donation program), lower vaccine effectiveness was observed than in industrialized settings. In one evaluation, a full series of RotaTeq was 52% to 63% effective in preventing severe (Vesikari score ≥11) rotavirus gastroenteritis. However, RotaTeq was more effective (73–86%) in preventing very severe (Vesikari score ≥15) rotavirus gastroenteritis.[289] Another evaluation in Nicaragua found three-dose vaccine effectiveness against severe disease of 64% and 87%, depending on the control group.[290]

Consistent with the high effectiveness results, the use of RotaTeq in the United States (used exclusively before Rotarix was licensed) resulted in marked reductions in rotavirus hospitalizations in young children younger than 5 years of age: reductions ranged from approximately 62% to 83%.[281,291–299] This reduction in rotavirus disease translated to reductions in all-cause hospitalizations for gastroenteritis among children younger than 5 years of age, ranging from approximately 25% to 50%. Studies in Australia in areas that used RotaTeq also reported reductions of 50% to 83% in rotavirus hospitalizations and of 20% to 48% in total gastroenteritis hospitalizations among young children.[286,300,301]

An additional and somewhat unexpected benefit following the use of RotaTeq was identified by investigators with the U.S. Vaccine Safety Datalink (VSD). Given earlier clinical reports of the association of rotavirus disease and seizures, they performed a retrospective cohort analysis and found that children who received the rotavirus vaccine series had an 18% to 20% reduction in risk of seizures requiring hospitalization or emergency department care, compared with children who did not receive a rotavirus vaccine.[302] A protective effect against hospital or emergency department care for febrile seizures was found in Australia.[302a]

Transmission of Vaccine Virus. Reassortants of G1, G2, G3, and G4 specificity have been administered individually to infants and demonstrated to be as safe as WC3 and, like WC3, to be shed in feces in low incidence (<10%) and at low concentration.[248–251] Therefore, WC3-containing vaccine is much-less-well adapted to growth at the infant intestinal mucosal surface than were RRV-containing vaccines. As a result, horizontal transmission of WC3-based vaccine from immunized infants would seem unlikely. (The initial Phase I study of WC3 rotavirus in infants revealed virus shedding in seven of 22 infants inoculated with $10^{7.5}$ PFU; no shedding rate of similar magnitude has been noted in any subsequent clinical trial of WC3 or WC3 reassortants.) A report in 2009 described detection of a RotaTeq vaccine-derived strain in a child with gastroenteritis whose sibling had been recently vaccinated with RotaTeq, and further investigation indicated that this strain was generated by further reassortment between two of the reassortant rotavirus strains present in RotaTeq.[303] Additional patients with acute-gastroenteritis and vaccine-derived double-reassortant rotavirus strains have been identified through active surveillance, although it has often been difficult to determine the extent that the rotavirus detected contributed to the patients' symptoms.[304–306]

Adverse Reactions

Intussusception. Because RotaShield was found to be associated with intussusception postlicensure, a large trial was necessary to determine whether intussusception was causally

or coincidentally associated with administration of RotaTeq.[262] In the large Phase III REST study, one case of intussusception occurred in the vaccine group and one in the placebo group within 14 days of inoculation; six cases in the vaccine group and five in the placebo group occurred within 42 days of inoculation; and 13 cases in the vaccine group and 15 in the placebo group occurred within 1 year of inoculation. No clustering of cases in the RotaTeq group occurred at any time after vaccination.[303] There were no other cases of intussusception reported in the other Phase III studies. Therefore, RotaTeq did not appear to be associated with intussusception.

Postmarketing evaluations designed to evaluate whether RotaTeq was associated with intussusception have been conducted in the United States and Australia (Table 52.7).[307–313] In the United States, through VSD, analyses of data from March 2006 through March 2013 found no increased risk of intussusception following any of the three doses compared to that expected based on historical rates, and no significant clustering of cases following RotaTeq was detected.[309,314] In the U.S. Post-Licensure Rapid Immunization Safety Monitoring (PRISM) program, an increased risk of intussusception following dose 1 was detected in both the self-controlled risk interval and cohort designs.[310] Although with inherent limitations of a passive reporting system, the clustering of intussusception cases in U.S. VAERS following dose 1 has suggested an increased risk.[311] In Australia, an increased risk was detected following dose 1 and dose 2 in the self-controlled case series (SCCS) design, and dose 1 only using case-control methodology.[312]

Other Adverse Events. Among RotaTeq and placebo recipients in the Phase III trials of RotaTeq, the incidence of serious adverse events (2.4% vs 2.6%, respectively), including deaths (~0.1% [$n = 25$] vs <0.1% [$n = 27$], respectively), was similar.[246] No deaths were attributed to vaccination by blinded investigators. Sudden infant death syndrome accounted for 17 of the 52 deaths; cases were equally distributed among RotaTeq and placebo recipients [$n = 8$ and $n = 9$, respectively].

TABLE 52.7 Attributable Risk Estimates of Intussusception Associated With RotaTeq or Rotarix Among Infants, by Study

	Country	Study Period	Attributable Risk Based on This Study Design[a]	Attributable Risk Based on These Doses and Intervals[a]	Attributable Risk (Per 100,000 Vaccinated Infants)[b]		Reference
					Point Estimate	95% CI	
RotaTeq							
	U.S: six health care organizations in 5 states (VSD)	May 2006–March 2013	Vaccinated cohort vs expected rates		No statistically significant risk identified		309
	U.S: national network of 3 health plans (PRISM)	January 2004–September 2011	Self-controlled risk interval[c] Vaccinated cohort vs unexposed person-time[d]	Dose 1: days 1–7 Dose 1: days 1–21 Dose 1: days 1–21	1.1 1.5 1.2	0.3 to 2.7 0.2 to 3.2 0.2 to 3.2	310
	U.S.: national passive surveillance of adverse events (VAERS)	February 2006–April 2012	Self-controlled risk interval	Dose 1: days 3–6	0.7	0.2 to 1.7	311
	Australia: hospitals in 6 jurisdictions	July 2007–June 2010	Self-controlled case series	Dose 1: days 1–21 Dose 2: days 1–7	7.0 7.0	1.5 to 33.1 1.5 to 33.1	312
Rotarix							
	Mexico: 16 hospitals in 10 states	August 2008–August 2010	Self-controlled case series	Dose 1: days 1–7	2.0	NR	308
	Mexico: 221 hospitals	January 2008–October 2010	Self-controlled case series	Dose 1: days 0–6	3.7	1.2 to 7.3	313
	Brazil: 53 hospitals in 7 states	August 2008–August 2010	Self-controlled case series	Dose 2: days 1–7	1.5	NR	308
	U.S.: six healthcare organizations in 5 states (VSD)	April 2008–March 2013	Vaccinated cohort vs expected rates	Dose 1: days 1–7 Dose 2: days 1–7	5.3 5.3	NR NR	309
	U.S.: national network of 3 health plans (PRISM)	January 2004–September 2011	Vaccinated cohort vs unexposed person-time[d]	Dose 2: days 1–21	7.3[e]	0.8 to 22.5	310
	Australia: hospitals in 6 jurisdictions	July 2007–June 2010	Self-controlled case series	Dose 1: days 1–21 Dose 2: days 1–7	4.3 4.3	0.8 to 23.1 0.8 to 23.1	312
	Singapore: 1 hospital	October 2005–September 2012	Self-controlled case series	Dose 1: days 1–7	1.5	NR	314
	UK: National Health Service Hospitals	July 2013–October 2014	Self-controlled case series	Dose 1: days 1–21 Dose 2: days 1–21	3.4 3.4	NR NR	382 382[a]

[a]For the attributable risk presented in this table.
[b]Overall attributable risk based on doses and intervals in previous column.
[c]Primary study design.
[d]Secondary study design.
[e]Results from the secondary study design. Results from the primary study design (self-controlled risk interval) for Rotarix were not statistically significant.
CI, confidence interval; NR, not reported; PRISM, Post-Licensure Rapid Immunization Safety Monitoring; VAERS, Vaccine Adverse Events Reporting System; VSD, Vaccine Safety Datalink.
Modified from Rha B, Tate JE, Weintraub E, et al. Intussusception following rotavirus vaccination: an updated review of the available evidence. Expert Rev Vaccines. *2014;13(11):1339–1348.*

Approximately 11,700 children within the Phase III trials were studied in detail to assess other potential adverse experiences, such as fever, diarrhea, and vomiting.[246] Within 42 days of vaccination, vaccinees had a small but statistically significant greater rate of several symptoms than placebo recipients, including vomiting (15% vs 14%, respectively), diarrhea (24% vs 21%, respectively), nasopharyngitis (7% vs 6%, respectively), otitis media (15% vs 13%, respectively), and bronchospasm (1.1% vs 0.7%, respectively). Among RotaTeq and placebo recipients, the incidence of reported episodes of fever (43% vs 43%, respectively) and hematochezia (0.5% vs 0.3%, respectively) was similar. Although Kawasaki disease was reported in several children following RotaTeq in the Phase III trial, this association was not found postlicensure.[315]

In the 7-day period after vaccination, vaccinees had a statistically significantly greater rate of diarrhea after dose 1 (10% vs 9%, respectively), after dose 2 (9% vs 6%, respectively), and after any dose (18% vs 15%, respectively). Similarly, vaccinees had a statistically significantly greater rate of vomiting after dose 1 (7% vs 5%, respectively) and after any dose (12% and 10%, respectively). The incidence of fever and irritability during the 7-day period after any vaccine dose was similar among RotaTeq and placebo recipients.

Bovine (Strain UK)–Human Reassortant Rotaviruses

Bovine–human reassortant rotaviruses also have been generated incorporating the genes for VP7 of either G1, G2, G3, or G4 human serotypes and 10 genes from the bovine UK strain rotavirus, which (like strain WC3) is serotype P7[5]G6.[316–318] These reassortants are serotypically similar to the G1, G2, G3, and G4 reassortants of bovine rotavirus WC3. Phase I studies were performed on each of the four UK reassortants individually in small numbers of infants (11 to 20 per G serotype) at doses up to $10^{5.8}$ PFU per strain. The vaccine was well tolerated and was shed in feces at rates ranging from 10 to 64% per group. Serum neutralizing antibody responses to the human parent rotaviruses (representing G types 1, 2, 3, and 4) ranged from 0% to 30% per serotype, but the neutralizing antibody responses to the corresponding UK parent virus were higher (30–82%). Only 50% of a group of 14 infants given a G1 reassortant developed evidence of any immune response (serum neutralizing antibodies or serum IgA or IgG detected by ELISA) after the first dose of vaccine. However, following a second dose, all exhibited evidence of an immune response.[317]

Subsequently a quadrivalent vaccine containing G1, G2, G3, and G4 reassortants of UK was administered in three doses to 20 infants.[317] No adverse events occurred following vaccination. Nineteen (95%) of the infants developed neutralizing antibodies to UK bovine rotavirus; neutralizing antibody response rates to human rotaviruses of serotypes G1 through G4 ranged from 28% to 37%.[318,319]

The quadrivalent vaccine was tested for efficacy in a placebo-controlled trial involving 256 infants given two doses of 10^6 PFU (total) in Finland.[319] The first and second doses were given at mean ages of 95 days and 150 days, respectively. Efficacy in the first year postvaccination was 59% against all rotavirus gastroenteritis and 90% against severe gastroenteritis. The vaccine showed no protective efficacy in the second rotavirus season postimmunization. It has not been submitted for licensure in the United States. Despite limited information about the multivalent UK reassortant vaccine, the National Institutes of Health has now licensed it to seven non-U.S. research groups, including investigators located primarily in less-developed countries (including China, Brazil, and India). Both a quadrivalent (G1–G4) and pentavalent (G1–G4, G9) bovine–human reassortant vaccine have been shown to be safe and immunogenic in healthy Indian infants; efficacy testing is ongoing in India.[320,321] A recent randomized Phase III clinical trial of a pentavalent bovine-human reassortant vaccine (ROTASIIL) conducted in Niger found that the vaccine was not associated with any adverse events and had an efficacy of 66.7% (95% CI, 49.9–77.9%) against severe rotavirus gastroenteritis.[321a] Of note, the vaccine is reportedly thermostable for 24 months at 37°C and six months at 40°C, which may provide advantages for vaccine delivery in remote areas where cold chain capacity is limited.

Natural Human–Bovine Reassortant Rotaviruses (Rotavac)

Naturally occurring bovine–human reassortant rotaviruses were isolated in two sites in India—New Delhi (the 116E strain)[322] and Bangalore (the I321 strain).[323] Newborns infected with the 116E or I321 strains were found to be protected against severe disease on reinfection.[128,324] The 116E strain is a human G9 isolate with a single VP4 gene segment from a bovine strain (P[10]G9) while the I321 strain (P[11]G10) has a bovine backbone with two segments coding nonstructural proteins NSP1 and NSP3 from human strains. Both of these strains were developed for clinical trials. In Phase II trials, infants inoculated with strain 116E developed a more robust rotavirus-specific IgA response than those inoculated with I321.[325–327] Consequently, only the 116E candidate underwent further efficacy testing. In a multicenter Phase III trial that enrolled infants from three cities in India–New Delhi, Pune, and Vellore—the 116E vaccine (Rotavac) was administered in a three-dose schedule with doses given at 6, 10, and 14 weeks of age. The vaccine showed 56% efficacy (95% CI, 37–70%) against severe rotavirus gastroenteritis during the first year of life, with sustained efficacy in the second year of life (49%; 95% CI, 17–68%).[328,329] Rotavac provided protection against a wide variety of vaccine mismatch strains, including G1P[8], G2P[4], and G12P[6], which were the most common circulating strains during the trial. Rotavac was not associated with any serious adverse events, including intussusception, in the Phase III trial. Six (<1%) cases of intussusception were reported in the vaccine group and two (<1%) were reported in the placebo group (with a 2:1 allocation of vaccine and placebo recipients). All events took place after administration of dose 3. The minimum interval between dosing and intussusception was 112 days in the vaccine group and 36 days in the placebo group. However, since a relatively small number of infants (~4500) were vaccinated in the trial, a low risk of intussusception cannot be excluded. Rotavac was licensed for use in India in 2014 and was recommended for inclusion in the Universal Immunization Program of India.

Attenuated Human Rotaviruses

Newborn Rotavirus Strain M37

Rotavirus strains isolated from children in the first month of life have been studied as potential vaccines because infants in neonatal nurseries in several locales worldwide have been found to be infected with rotavirus with high prevalence but with little gastroenteritis; followed by resistance to subsequent episodes of severe disease.[83,330] Many of these newborn strains share a similar P gene.[41,330] These strains have been largely ineffective as vaccine candidates.[2,31,331–333] Only the Australian strain RV3 is currently in clinical testing.[88,334,335]

Attenuated Human Strain P1[8]AG1 (Rotarix)

The attenuated human rotavirus strain P1A[8]G1 (Rotarix) is now licensed in nearly 100 countries, including the European Union. The vaccine was licensed in the United States in April 2008, and recommended for use by ACIP in June 2008.

Origin. Symptomatic or asymptomatic infection of young children with a single circulating wild-type strain (serotype P1A[8]G1) in Cincinnati provided 100% protection against subsequent rotavirus disease caused primarily by the P1A[8]G1 serotype over a 2-year period.[84] A vaccine of this strain obtained from the stool of one of the study subjects (i.e., strain 89-12) was developed after 26 passages of the virus in primary African green monkey kidney cells and seven passages in serially passaged African green monkey kidney cells.[347] Initial safety and immunogenicity studies with this vaccine candidate indicated it was relatively safe and immunogenic in children less than 4 months of age.[336] Nearly every child developed an immune response to the vaccine virus after two doses.

Limited comparisons of the effects of dose on the immunogenicity of the 89-12 candidate vaccine have been conducted.[336] However, 19 of 20 infants inoculated with two doses of 10^5 fluorescent-focus units (FFU) of 89-12 developed rotavirus-specific antibodies and 16 did so after the first dose.

The strain 89-12 vaccine candidate was evaluated in an efficacy trial in healthy 10- to 16-week-old infants at four centers in the United States. Two doses of 10^5 FFU or placebo with a 4-week interval between doses were administered to 108 or 107 subjects, respectively.[337] Low-grade fever after the first dose was the only side effect more common in vaccine than placebo recipients (21 [19%] vs 5 [4.6%] subjects, respectively; $P < .001$). An immune response to rotavirus was detected in 94% of vaccinees and in only 4% of the placebo recipients. Rotavirus-specific serum IgA responses were detected in 91.6% and anti–89-12 serum neutralizing antibodies in 69.2% of vaccines.

During the first rotavirus season, rotavirus disease was detected in 18 placebo recipients and two vaccine recipients (efficacy of 89%). Vaccine episodes were scored for clinical severity using a 20-point system: in this system, cases scoring 8 or higher were considered "severe" and those scoring higher than 14 as "very severe." Ten placebo recipients but no vaccine recipients presented for medical care necessitated by rotavirus disease. In the second year, efficacy decreased to 59%, but only one case of severe rotavirus gastroenteritis occurred in vaccine recipients, while 10 severe cases occurred in placebo recipients. Vaccine efficacy for 2 years was 76% against any rotavirus gastroenteritis, 84% against severe disease, and 100% against very severe disease. As G1 rotaviruses predominated during both years, the efficacy against rotaviruses of other G or P types was not determinable.

To produce a homogeneous vaccine, strain 89-12 was purified by a series of three end point dilutions in Vero cells and passaged seven more times in these cells after it was sublicensed by GlaxoSmithKline in 1997. The final product, now at passage 43, was called RIX4414 and is being sold under the trademark name Rotarix.

Immunogenicity. Investigators orally inoculated 6- to 12-week-old infants in Finland with RIX4414 and found no increase in any elicited symptoms including fever.[338] Thus, it appeared that clonal selection and increased passage in tissue culture produced a more attenuated vaccine relative to its parental 89-12 strain. A dose range effect was seen for the immune response in Finnish infants with seroconversion detected in 73% to 96% after the second dose administered 4 weeks after the first dose (Table 52.8).[338]

Evaluation of RIX4414 began in Asia shortly thereafter, marking the initiation of what would become a global effort to evaluate this vaccine.[339,340] Unlike most vaccines, evaluation of RIX4414 was performed in developed and developing nations simultaneously (see Table 52.8). In a dose-ranging trial conducted in Singapore in 2464 infants, no increase in fever, diarrhea, vomiting, or irritability was detected—not even at the highest dose of $10^{6.1}$ FFU; seroconversion rates determined by rotavirus-specific serum IgA responses were 76% to 91% depending on the dose.[340] Interference with the other routine childhood vaccines administered concomitantly was not observed. In a multicenter study conducted in the United States, the vaccine appeared to be safe in infants 5 to 15 weeks of age, and did not interfere with the immune responses to concomitantly administered vaccines.[341] Concomitant use of Rotarix with OPV was reported to suppress the uptake of the rotavirus vaccine. This effect was eliminated following the second dose.[342] Vaccine-specific immune responses assessed by serum IgA response after two doses occurred in 81.5% to 88.0% of vaccinees, depending on the size of the dose.

TABLE 52.8 Clinical Studies of Rotarix in Infants

Site	N	Seroconversion[a]	Efficacy Against Severe Disease
Finland	92	73–96%	ND
Finland	405	80%	90%
Singapore	2464	76–91%	82%
United States	529	82–88%	ND
Latin America (Brazil, Mexico Venezuela)	2155	61–65%	66–86%
Latin America (11 countries) and Finland	63,225	ND	85%

[a]Percent of infants that developed an immune response after two doses as determined by rotavirus-specific IgA by ELISA.
IgA, immunoglobulin A; ELISA, enzyme-linked immunosorbent assay; ND, not determined.

Efficacy. The first efficacy study of RIX4414 was conducted in Finland during two rotavirus epidemic seasons following oral administration of two relatively low doses of the vaccine ($10^{4.7}$ FFU). In this trial of 405 infants, the vaccine was well tolerated with a seroconversion rate of 80% after the second dose (see Table 52.8).[343] Efficacy against any episode of rotavirus gastroenteritis was 73%, whereas efficacy against severe rotavirus disease was 90% in the first season and 85% after two seasons. Because 35 of the 38 cases of rotavirus gastroenteritis were associated with the same G1 serotype as the vaccine, this trial did not yield evidence about the potential of this vaccine to protect against heterotypic rotaviruses. In an initial placebo-controlled efficacy trial with RIX4414 in Latin America, seroconversion after two doses of either $10^{4.7}$, $10^{5.2}$, or $10^{5.8}$ FFU of vaccine virus based on serum rotavirus IgA responses, was a disappointing 61% to 65% and the dose administered had no significant effect on these responses.[344] Despite these low detectable serum antibody response rates, protection against severe rotavirus disease was 86% in vaccinees given the highest dose. Equally important was the observation that vaccine efficacy against non-G1 rotaviruses, most commonly P1AG9, was similar to that against G1 serotypes, confirming the hypothesis that a vaccine containing P1A could protect against P1A-containing viruses of a different G serotype from that of the vaccine.

A randomized, placebo-controlled safety, immunogenicity, and efficacy trial was next performed with the RIX4414 vaccine (now called by its commercial name Rotarix) in approximately 63,000 infants, primarily in 11 countries in Latin America (see Table 52.8).[345] The primary purposes of the study were to

determine if the rates of intussusception were equivalent in vaccinees and placebo recipients and, in a subset of 10,159 vaccinees and 10,010 placebo recipients, the efficacy of the vaccine against severe rotavirus disease and hospitalizations. The efficacy of Rotarix against hospitalizations resulting from rotavirus was 85% and reached 100% against the most-severe cases of rotavirus gastroenteritis. Overall efficacy against severe rotavirus disease was 91.8%. Efficacy against any severity of disease caused by non-G1 strains (G3, G4, and G9) that belonged to the same P1A[8] serotype and genotype as the vaccine was 87.3%. Although protection was only 41.0% against G2[P4] strains in this trial, a metaanalysis of the results of this trial combined with two previous trials of Rotarix[343,346] allowed the calculation of a protection rate against G2[P4] strain of 67%. Thus, although this vaccine appeared to be less protective against a rotavirus strain that differed serotypically in both its VP4 and VP7 neutralization proteins from that of the vaccine, it still provided protection. It is also important to note that protection against all cases of severe gastroenteritis or diarrhea-associated hospitalizations of any etiology in vaccinees in this trial was 40% and 42%, respectively, thus indicating that an effective rotavirus vaccine could dramatically reduce the numbers of cases of severe gastroenteritis in young children. In the other pivotal Phase III efficacy study, conducted among approximately 3800 infants from six European countries, the efficacy of two doses of Rotarix against rotavirus disease of any severity to age 1 year was 85%, with 100% efficacy against hospitalization for rotavirus diseases through the first rotavirus season.[347] The vaccine gave protection rates against severe gastroenteritis caused by G1, G2, G3, G4, and G9 strains of 96%, 86%, 94%, 95%, and 85%, respectively, through the second season. An efficacy trial was also conducted among approximately 10,700 infants in the high-income Asian locations of Hong Kong, Singapore, and Taiwan.[348] Two doses of Rotarix was 96% protective against severe rotavirus gastroenteritis (Vesikari score ≥11) to age 2 years. In the European, Latin American, and Asian trials, the efficacy of Rotarix was sustained in the second year of life.[349]

In the first Phase III trials of Rotarix in developing countries, Rotarix pooled efficacy for the 2- and 3-dose regimens ranged from 49% (95% CI, 19 to 68) in Malawi to 77% (95% CI, 56 to 88) in South Africa (see Table 51.6).[350] The number of severe rotavirus cases prevented per 100 infants vaccinated was substantial—seven cases per 100 vaccinated in Malawi and four cases per 100 vaccinated in South Africa—illustrating the large potential public health impact of even a moderately efficacious vaccine.

Effectiveness and Impact. The effectiveness of Rotarix has been evaluated in many countries.[268–270] Middle-income Latin American countries were among the first to introduce and evaluate the vaccine. Measured through case-control studies (described earlier), the overall effectiveness of two doses of Rotarix against hospitalization for rotavirus in Bolivia, Brazil, and El Salvador ranged from 40% to 77%, whereas an assessment from Columbia did not demonstrate effectiveness in all age groups combined, but did in infants 6 to 11 months of age (85%).[351–355] In a recent metaanalysis, the pooled estimate for effectiveness from these studies was 63% and 72%, with generally higher effectiveness in children 6 to 11 months of age (75% and 82%) than in those 12 months of age and older (56% and 66%).[356] The pooled estimate for one Rotarix dose was 50% and 55%, indicating moderately good protection in these countries at least during the period between doses.

The overall vaccine effectiveness of two doses of Rotarix against rotavirus hospitalization in the high-income countries of Belgium, Spain, Portugal, Taiwan, and the United States has ranged from 92% to 98% in case-control studies, with high effectiveness sustained in the second year of life in the two countries that reported results by separate age groups (i.e., Belgium and the United States).[274,276,284,285,357] In Central Australia with a large indigenous population, effectiveness estimate was 84% against hospitalization during a G9P[8] season, but was not statistically significant during a G2P[4] season—a genotype that is classified in a genogroup different from the vaccine strain.[358,359] However, in other middle- or in high-income settings where Rotarix effectiveness against hospitalization from genotype G2P[4] has been assessed, such as Belgium, Brazil, and the United States, the overall effectiveness against hospitalization has been 85% or greater, similar to that against P[8] strains; the Brazil study found the high effectiveness against G2P[4] challenge in infancy was not sustained in children 12 months of age or older.[276,353,357] In a metaanalysis, no differences were noted in Rotarix effectiveness against partly heterotypic or fully heterotypic strains, compared with homotypic strains, within country income levels.[360]

In the first evaluation of the effectiveness of Rotarix in the African region, two doses were found to be 57% effective against rotavirus hospitalization among South African children younger than 2 years of age, with similar results in the first and second year of life, and in HIV-exposed-uninfected and HIV-unexposed-uninfected children.[361] Importantly, in the low-income country of Malawi, two Rotarix doses were 64% effective against rotavirus hospitalization in young children.[362]

Several evaluations demonstrated marked reductions in hospitalizations for rotavirus among young children following the introduction of Rotarix into the immunization program, with reduction estimates ranging from 59% to 81% in Brazil and El Salvador, 68% to 88% in Australia, and 40% to 45% in S. Africa, including during early postintroduction periods when not all children younger than 5 years of age were age-eligible for vaccine.[363–367] In Blantyre, Malawi, the incidence of rotavirus hospitalizations among infants was reduced by 43% during the second season.[362] In the United States, in a retrospective cohort analysis based on a national insurance claims databases, a 96% rate reduction in the rotavirus-coded hospitalizations was observed in children who received Rotarix compared with children who were age-eligible but did not receive any rotavirus vaccine.[282]

Notably, Mexico was the first country to demonstrate a reduction in mortality from diarrheal disease following rotavirus vaccine introduction. Following the introduction of Rotarix, a sustained 46% decrease in overall diarrheal deaths has been detected among children younger than 5 years of age, with approximately 90% coverage in children younger than 2 years of age,[368,369] and similar diarrhea mortality rate reductions in high as well as low-income sectors.[370] Two studies in Brazil estimate diarrheal death rate reductions of 22%, and 33% to 39%, respectively, after Rotarix introduction.[371,372] In Panama, a reduction of 47% in diarrheal deaths was estimated, based on relatively small numbers.[373] Other studies from Latin America, some from single locations and others using regional or national data, estimated early annual reductions in overall diarrhea hospitalizations, generally among children younger than 5 years of age, with point estimates ranging from 17% to 55%.[367,371,372,374,375] In South Africa, diarrhea hospitalizations among children younger than 5 years of age were reduced by an estimated 33% in the first 2 years after Rotarix introduction.[364]

Transmission of Vaccine Virus. In Phase II or Phase III studies of Rotarix administered at $10^{6.5}$ to $10^{6.8}$ $CCID_{50}$ (median end point of the cell culture's infectious dose) per dose in various countries, after dose 1, rotavirus antigen shedding was detected by ELISA in 50% to 80% of infants at approximately day 7, 19.2% to 64.1% at approximately day 15, 0% to 24.3%

at approximately day 30, and 0% to 2.6% at approximately day 60.[376] Shedding was lower after dose 2, and was detected in 4.2% to 18.4% of infants at approximately day 7, 0% to 16.2% at approximately day 15, 0% to 1.2% at approximately day 30, and 0 at approximately day 45. Shedding of live rotavirus was assessed in two studies in which Rotarix was administered at $10^{6.5}$ $CCID_{50}$ per dose. In both studies, stool samples that were antigen-positive by ELISA at approximately day 7 after dose 1 were tested subsequently for live virus by focus forming unit assay. Live virus was detected in six (46.2%) of 13 and 15 (45.5%) of 33 rotavirus antigen-positive stools, for an estimated 26% of vaccinated infants shedding live virus at approximately day 7 after dose 1. The potential for transmission of vaccine virus to contacts was evaluated in a study of twin pairs conducted in the Dominican Republic. In this study, one twin from each pair received Rotarix and the other received placebo and fecal specimens were obtained thrice weekly for 6 consecutive weeks after vaccination. In 80 infants who received placebo, rotavirus vaccine strain was detected in at least one fecal specimen in 15 (19%) infants.[377] None of the transmission cases was associated with gastrointestinal symptoms.

Adverse Events. No excess of adverse effects in vaccinees compared with control infants was reported in any of the above-mentioned clinical trials.[378,379] However, the virus was shed in concentrations detectable by ELISA in 60% to 80% of infants[380,381] after dose 1 and often after dose 2, suggesting the possibility of horizontal transmission to nonvaccinees.

Intussusception. Because of the association of RotaShield with intussusception, a large Phase III safety trial was conducted with approximately 63,000 subjects randomized to receive either two doses of the Rotarix vaccine or placebo, as mentioned above. The primary end point of this large trial was the occurrence of intussusception within 31 days after each dose of vaccine.[345] A secondary objective was to assess the occurrence of serious adverse events, including intussusception, during the entire study period (i.e., a median of 100 days after the second dose). No increased risk for intussusception was observed after administration of Rotarix when compared with placebo. A total of 13 cases of intussusception were identified during the 31-day windows; six in Rotarix recipients and seven in placebo recipients. No clustering in the initial 1 to 2 weeks was identified after either dose. During the entire duration of the study, 16 cases of intussusception occurred in the placebo group compared with nine in the vaccine group.

Postmarketing studies to assess if Rotarix is associated with intussusception have been conducted in Mexico, Brazil, Australia, the United States, Singapore and the United Kingdom (see Table 52.7).[307–313,382,382a] An increased risk of intussusception was detected in a first study in Mexico, following dose 1 and dose 2 (SCCS and case-control methodology)[308]; a second study in Mexico, following dose 1 only (SCCS)[313]; in Brazil, following dose 2 but not dose 1 (SCCS and case-control)[308]; and in Australia, following dose 1 and dose 2 (SCCS) and dose 1 only (case-control).[312] In the United States, the VSD evaluation found an increased risk of intussusception following dose 2 compared with that expected based on historical rates; the statistical significance of the dose 1 relative risk could not be calculated.[309] The relative risk of intussusception within 7 days after Rotarix was greater than that following RotaTeq vaccination.[309] In the PRISM evaluation, the study of Rotarix was underpowered for the primary analysis (self-controlled risk interval design) but the secondary analysis (cohort design) suggested an increased risk following dose 2.[310] In Singapore, an increased risk of intussusception was detected following dose 1 (SCCS), and in the United Kingdom, following dose 1 and dose 2 (SCCS).[382,382a]

Inactivated Human Rotaviruses

Because administration of oral rotavirus vaccines in the developing world may induce a lesser response than in higher income countries—and because reduced immunogenicity and effectiveness in the developing world might be caused by antibodies in breast milk or interference by parasites or toxin-producing bacteria in the gut—parenteral rotavirus vaccines are being developed. A variety of approaches have been pursued, including inactivated whole rotavirus vaccine,[383] triple- and double-layered virus-like particle vaccines,[384,385] recombinant subunit VP6 protein vaccines,[386–388] and a truncated recombinant subunit VP8 protein vaccine candidate with P[8], P[4], or P[6] specificity expressed in *Escherichia coli*.[389] Preliminary studies in experimental animals show promise for some of these approaches[390]; further clinical development is ongoing.

Community Protection

Shortly after vaccine introduction in the U.S., declines in hospitalizations for rotavirus gastroenteritis were also observed among children under age 5 years that were not age-eligible for vaccination[268–270,281,291–294,297,299,391,392] suggesting indirect benefits from reduced transmission of rotavirus in the community. Similar observations were reported from Australia, countries in Europe and Latin America and in Israel, following national introduction of rotavirus vaccine.[286,301,363,367,393–398b] Interestingly, indirect protection from infant vaccination appears to extend to (unvaccinated) older children (United States, Australia, United Kingdom) and adults (United States, United Kingdom), revealing a previously unrecognized burden of moderate to severe rotavirus disease in these age groups.[398b,399–402] These indirect benefits were not anticipated prior to vaccine implementation and provide further encouragement for vaccine use, as do the early data on unanticipated benefits of rotavirus vaccination in protection against childhood seizures.[302,302a,402a]

Recommendations for Use of RotaTeq and Rotarix in the United States

Routine Administration. Recommendations for the use of rotavirus vaccines have been issued by several committees.[403–405] The ACIP recommends routine oral immunization of U.S. infants with either three doses of RotaTeq administered at 2, 4, and 6 months of age or two doses of Rotarix administered at 2 and 4 months of age.[404,405] The minimum age for the first dose is 6 weeks and the maximum age is 14 weeks and 6 days of age. Subsequent doses should be given with an interval of at least 4 weeks, and all doses of vaccine should be administered by age 8 months and 0 days. The first dose of vaccine was not recommended for infants more than 14 weeks and 6 days because of lack of safety data from the clinical trials in starting the series in infants older than this age and to avoid the peak age range at which idiopathic intussusception occurs. It was also recommended that no dose of vaccine be administered to infants more than 8 months and 0 days of age because of insufficient data on safety. It is unlikely that these age restrictions will be loosened any time soon.

Rotavirus vaccines can be administered together with diphtheria, tetanus, and acellular pertussis, *Haemophilus influenzae* type b, inactivated polio, hepatitis B, and pneumococcal conjugate vaccines. Rotavirus vaccine can be administered to infants with mild illness. Infants with moderate to severe illness should be vaccinated as soon as they recover from the acute phase of the illness.

The effect of simultaneous administration of both RotaTeq and Rotarix with OPV on the immune response to each of the

vaccines has been evaluated.[406–408] Neither RotaTeq nor Rotarix interferes with the immune response to any of the three antigens in OPV. OPV appears to interfere with the immune response of the first dose of rotavirus vaccine, but this interference is largely overcome after administration of the complete vaccine series. Bivalent and monovalent OPV also appear to interfere with the immune response to rotavirus vaccine.[408a,408b]

Breastfeeding. The efficacy of the vaccine is similar among breastfed and nonbreastfed infants. No restrictions are placed on the infant's feeding before or after receipt of rotavirus vaccine.

Premature Infants. Premature infants may be vaccinated if they are at least 6 weeks of age, have been discharged or are about to be discharged from the hospital nursery, and are clinically stable. The ACIP considers the benefits of rotavirus vaccination of premature infants to outweigh the theoretical risks.

Contraindications. Rotavirus vaccine should not be administered to persons who have severe hypersensitivity to any component of the vaccine or who have experienced a serious allergic reaction to a previous dose. Latex rubber is contained in the Rotarix oral applicator; consequently, infants with a severe (anaphylactic) allergy to latex should not receive Rotarix. The RotaTeq dosing tube is latex-free. Neither RotaTeq nor Rotarix should be administered to infants who have severe combined immunodeficiency disease; vaccine-acquired rotavirus infection and disease has been reported in infants with severe combined immunodeficiency disease following rotavirus vaccine administration.[409,410] RotaTeq and Rotarix are also contraindicated in infants that have had intussusception.[411]

Precautions. Practitioners should consider the potential risks and benefits when deciding whether to administer RotaTeq or Rotarix to the special populations listed below.

Altered Immunocompetence Other Than Severe Combined Immunodeficiency Disease. Children and adults who are immunocompromised because of congenital immunodeficiency, hematopoietic transplantation, or solid-organ transplantation sometimes experience severe, prolonged, and even fatal rotavirus gastroenteritis.[183–185] However, no safety or efficacy data are available for the administration of rotavirus vaccine to infants who are potentially immunocompromised.

Preexisting Chronic Gastrointestinal Disease. The safety and efficacy of RotaTeq or Rotarix have not been established for infants with chronic gastrointestinal diseases such as congenital malabsorption syndromes, Hirschsprung disease, short-gut syndrome, or persistent vomiting of unknown cause. However, the benefits of vaccination outweigh the theoretical risks in infants with preexisting chronic gastrointestinal conditions who are not undergoing immunosuppressive therapy.

Special Situations

Exposure of Immunocompromised Persons to Vaccinated Infants. Infants living in households with persons who have or are suspected of having an immunodeficiency disorder or impaired immune status may be vaccinated. Protection of the immunocompromised household member afforded by immunization of young children in the household likely outweighs the small risk of transmitting vaccine virus to the immunocompromised household member and any subsequent risk of vaccine virus-associated disease. To minimize potential virus transmission, all members of the household should employ measures such as good hand washing after contact with the feces of the vaccinated infant.

Exposure of Pregnant Women to Vaccinated Infants. Infants living in households with pregnant women should be vaccinated according to the same schedule as infants in households without pregnant women. Most women of childbearing age have preexisting immunity to rotavirus; therefore, the risk of infection and disease from potential exposure to the attenuated vaccine virus strains is low. In addition, immunization of young children would avoid potential exposure of pregnant women to wild-type virus if the unvaccinated infant suffers from rotavirus gastroenteritis.

Regurgitation of Vaccine. The practitioner should not readminister a dose of rotavirus vaccine to an infant who regurgitates, spits out, or vomits during or after administration of vaccine. The infant can receive the remaining recommended doses of rotavirus vaccine at appropriate intervals.

Hospitalization After Vaccination. If a recently vaccinated child is hospitalized for any reason, no precautions other than routine universal precautions need be taken to prevent the spread of vaccine virus in the hospital setting.

DETECTION OF PORCINE CIRCOVIRUSES IN ROTAVIRUS VACCINES

In 2010, Eric Delwart and colleagues at the University of California, San Francisco, using deep sequencing and microarray technologies, found nucleic acids from porcine circovirus (PCV) type 1 in Rotarix.[412] Subsequent studies performed by the U.S. Food and Drug Administration, Merck, and GlaxoSmithKline found evidence for live PCV type 1 in Rotarix and genetic fragments but not live PCV type 2 in RotaTeq.[412a,413,414] Neither PCV-1 nor PCV-2 has been found to cause an immune response or disease in humans.[415] Consistent with that observation, children inoculated with Rotarix or RotaTeq in Phase III trials were not found to have PCV-1 or PCV-2 antibodies in serum. The source of PCV in both vaccine preparations was probably the porcine trypsin used to grow rotaviruses in vitro. Although the presence of PCV does not pose a safety risk, both companies have committed to removing PCV from their vaccines.

PUBLIC HEALTH CONSIDERATIONS

After the withdrawal of RotaShield vaccine from the market, there was deep disappointment that, despite the licensing of an effective rotavirus vaccine, public health advocates were back at square one. A vaccine had been developed that had a rare flaw in its safety profile: intussusception in infants. The situation was made worse by the fact that the mechanism for the causation of intussusception was unknown and the incidence of intussusception attributed to the vaccine was extremely low (approximately one in 10,000). This meant that establishing apparent relative safety with a new and alternative vaccine could only be claimed by blindly vaccinating large numbers of infants and observing their health for an extended period, without the benefit of any biological markers known in the infant hosts to determine whether the vaccine trial in progress was safe (the numbers of infants tested by two companies that took up the challenge [GlaxoSmithKline and Merck] was more than 60,000 for each company). That two companies pursued this product at extraordinary cost and with absolutely no guarantee of success is just as remarkable as the fact that each created, produced, and licensed a new rotavirus vaccine, based on different biological approaches.

Many countries that have introduced rotavirus vaccines in their national childhood immunization programs have shown remarkable declines in severe rotavirus gastroenteritis post vaccine introduction.[268–270,415a] In the United States, for example, an estimated 177,000 hospitalizations, 242,000 emergency department visits, and more than 1,000,000 outpatient clinic visits for rotavirus gastroenteritis were averted during the 4-year period July 2007 through June 2011.[282] Data from a national network of sentinel clinical laboratories show the marked and sustained declines in U.S. rotavirus activity

after vaccine implementation and depict the biennial pattern of rotavirus activity that has emerged following vaccine introduction, with small peaks of rotavirus activity now alternating with years with very low levels (Fig. 52.4).[416]

Against these remarkable health benefits from vaccination, postlicensure evaluations have identified a safety issue, with a risk of intussusception following RotaTeq and Rotarix estimated between approximately one and six excess cases of intussusception per 100,000 vaccinated infants. Acknowledging the documented benefits as well as the small increased risk of intussusception from these vaccines (Table 52.9),[307,308,312,417] advisory bodies and health organizations continue to support the routine use of these vaccines. Evaluations based on ecological data do not demonstrate a consistent increase in the overall rate of intussusception in infants (younger than age 1 year) in early postintroduction data from the United States and Australia; however, ecological data have important inherent limitations for assessing possible changes in rate of such an uncommon condition.[417a,418,418a,418b] Given that three biologically different rotavirus vaccines are associated with intussusception, one could postulate that wild-type natural rotavirus infections might also be a rare cause of intussusception. If this is true, then one could argue that rotavirus vaccines, which prevent natural rotavirus infections, might also prevent intussusception caused by natural infection. U.S. investigators used VSD data to evaluate whether infants who received rotavirus vaccine have a lower intussusception risk in later infancy compared with infants who did not receive rotavirus vaccine; a statistically significant difference in rates in later infancy was not found.[419]

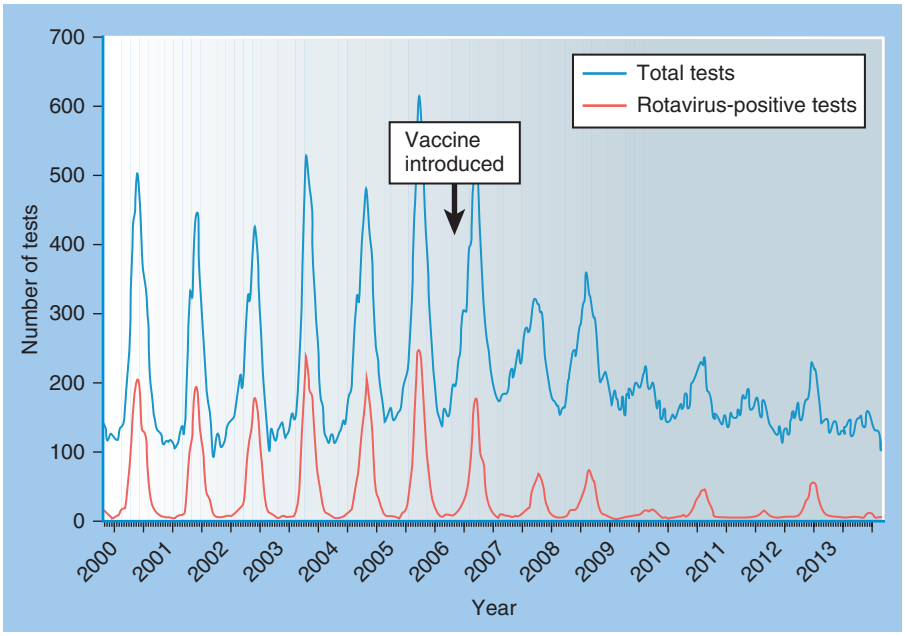


Figure 52.4. Number of total rotavirus enzyme immunoassay (EIA) tests performed and number of positive rotavirus EIA tests from 23 continuously reporting laboratories in the U.S. National Respiratory and Enteric Viruses Surveillance System (NREVSS), by week of year, July 2000–August 2014, 3-week moving average. *(Modified from Aliabadi N, Tate JE, Haynes AK, et al. Sustained decrease in laboratory detection of rotavirus after implementation of routine vaccination—United States, 2000–2014.* MMWR Morb Mortal Wkly Rep. *2015;64[13]: 337–342.)*

TABLE 52.9 Risk-to-Benefit Estimates of Rotavirus Disease vs. Intussusception Outcomes by Country[a]

Country		Rotavirus Outcome Averted	Intussusception Outcome Caused	Rotavirus Outcome Averted: Intussusception Outcome Caused	Reference
Mexico	Hospitalizations	11,551	41	282:1	308
	Deaths	663	2	331:1	
Brazil	Hospitalizations	69,572	55	1265:1	308
	Deaths	640	3	213:1	
Australia	Hospitalizations	6528	14	466:1	312
	Deaths	NR	NR	NR	
United States	Hospitalizations	53,444	35–166	322–1530:1[b] 251–1191:1[c]	417
	Deaths	14	0.1–0.5	28–134:1	

[a]Estimates based on one vaccinated birth cohort followed to age 5 years.
[b]Rotavirus hospitalizations averted per 1 excess hospitalized intussusception case.
[c]Rotavirus hospitalizations averted per 1 excess intussusception case.
NR, not reported.
Modified from Rha B, Tate JE, Weintraub E, et al. Intussusception following rotavirus vaccination: an updated review of the available evidence. Expert Rev Vaccines. *2014;13(11):1339–1348.*

In 2010, results of Phase III trials of both RotaTeq and Rotarix conducted in developing countries of Africa and Asia were reported (see Table 52.6). The efficacy of both vaccines in these trials was moderate compared with that reported in earlier trials in affluent settings.[262,267,350,415a,419a,419b] However, because of the greater baseline burden of severe rotavirus in developing countries, the public health benefits of vaccination in terms of absolute number of cases of severe rotavirus disease prevented were substantial. While the exact reasons for the somewhat diminished performance of rotavirus vaccines in developing countries are unclear, other live oral vaccines such as those against polio, cholera, and typhoid have also not worked equally well in populations in developed and developing country settings. Many factors in infants in the developing world—levels of maternal antibody, micronutrient malnutrition, the presence of interfering microorganisms, and companion diseases such as HIV and malaria—can alter the ability of the infant to process a live oral vaccine, lower the effective dose, and interfere with virus replication and impede a good immune response.[420,420a] Alternate vaccine schedules have also been explored.[423a,423b,423c,423d] Antibodies and other factors present in breast milk have also been postulated as factors that could impair rotavirus vaccine performance.[421–423,423e] However, several newer studies have not shown any beneficial effect of temporarily withholding breastfeeding for 30 to 60 minutes before and after vaccine administration on the immune response to rotavirus vaccine.[424–426]

RECOMMENDATIONS FOR ROTAVIRUS VACCINE USE IN THE EXPANDED PROGRAMME ON IMMUNIZATION

WHO recommends that rotavirus vaccines be included in all national immunization programs and be considered a priority in countries with high rotavirus fatality rates.[427,428] Two vaccines are currently widely available. Rotarix is recommended to be given in a 2-dose schedule at the time of DTP1 and DTP2; RotaTeq is recommended to be given in a 3-dose schedule at the time of DTP1, DPT2, and DPT3. For both vaccines, a minimum interval of 4 weeks is recommended between doses.

WHO recommendations state that although early immunization is still favored, with the first dose of rotavirus vaccine administered as soon as possible after 6 weeks of age, immunization programs can allow children to receive rotavirus vaccine together with DTP regardless of the time of vaccination (to age 24 months). These updated recommendations were made following review of data on the potential impact of the manufacturers' conventional age restrictions on the first and last doses of the series[427]; in countries where rotavirus mortality is the highest, delays in immunization are common and hence strict limits on maximum ages for administration of rotavirus vaccine can substantially reduce the number of children who can receive rotavirus vaccine. A modeling study had assessed the potential benefits of mortality reduction from rotavirus versus the risk of fatal intussusception in low- and low/middle-income countries when the first dose of the vaccine is strictly administered by 15 weeks of age and the last dose is administered by 32 weeks of age, compared with a unrestricted age strategy with rotavirus vaccine administered at the time of DTP administration (up to age 3 years).[429] This analysis demonstrated that removing the age restrictions in these countries (which would allow an additional 21% to 25% of children to receive rotavirus vaccine) would avert an additional 47,200 rotavirus deaths and cause an additional 294 intussusception deaths, for an incremental benefit-to-risk ratio of 154 deaths averted for every death caused by vaccine. Postintroduction intussusception assessments are underway in Africa.

Future development and introduction of rotavirus vaccines will require substantial input from the international donor community, including the Vaccine Alliance, Gavi, and the Bill & Melinda Gates Foundation. Many local vaccine makers in China, India, Indonesia, and other settings have begun their own programs for rotavirus vaccine development and introduction, and these efforts could provide an additional supply of vaccine at an affordable cost to ensure that they reach the poorest children in the world who need them the most. In addition to other candidate oral rotavirus vaccines, approaches such as inactivated rotavirus vaccines are being pursued, especially since this could help overcome some of the challenges to diminished performance of oral rotavirus vaccines in developing countries and also potentially avoid a risk of intussusception.[383] Such new vaccines are unlikely to be proposed for international use for many years.

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53 Rubella Vaccines

Susan E. Reef and Stanley A. Plotkin

RUBELLA

> *Just a dead comma of flesh where the ear should hang, a poxed hole mottled in bone, and she holds you, as you shiver curled against the muffled songs we sing as we pass by in our doctor's clothes and then leave you alone again, to redefine the white surgery waiting-room with glazed dum-dum eyes, not even warming the living gel of your pulped brain.*
>
> **Michael O'Reilly**[1]

First described in the late 18th century as a mild exanthematous disease of children and young adults, rubella became prominent in 1941 as a cause of congenital defects in the fetus after maternal infection during pregnancy. The development and use of a live attenuated vaccine has controlled rubella in developed countries during the last 40 years, even enabling elimination in Nordic countries and the Western Hemisphere, and more recently, use of the vaccine has been extended to large regions of the world. Nevertheless, congenital rubella continues to be an important problem in countries not using vaccine.

The first researchers to distinguish the disease from other exanthems were German physicians; hence, the common English language eponym *German measles*.[2] In 1841, a British physician reported an outbreak in a boys' school in India and coined the term *rubella*, a Latin diminutive meaning "little red."[3] For the next 100 years, rubella received scant attention, but in 1941, Norman McAlister Gregg,[4] an Australian ophthalmologist, linked congenital cataracts to maternal rubella. Gregg had noticed an unusual number of infants with cataracts coming to his practice, and he was curious enough to investigate. It is said that a crucial clue was a conversation he overheard in his waiting room between two mothers who were discussing the rubella they both had sustained in pregnancy during the Australian outbreak of 1940.[5] After several years of inattention and skepticism, Gregg's original observation was followed by reports of Australian,[6] Swedish,[7] American,[8] and British[9] epidemiologists and teratologists confirming the role of rubella in congenital cataracts and also noting the simultaneous association of heart disease and deafness in the infants. Thus, the characteristic congenital rubella triad was established.

During the next 20 years, efforts were made to isolate the causative agent and obtain statistics on the risk of fetal abnormality after maternal rubella. Various estimates of the risk were made, ranging from high to low. The disparity in estimates stemmed from the absence of a definitive diagnostic test and consequent misdiagnosis of rubella in the mother. In late 1962, a breakthrough came in the form of the first isolations of rubella virus by Weller and Neva[10] in Boston and by Parkman, Beuscher, and Artenstein[11] in Washington, DC. Weller and Neva detected the presence of rubella virus by cytopathic effect in human amnion cells, whereas Parkman, Beuscher, and Artenstein developed a technique dependent on interference with the growth of enteroviruses in African green monkey kidney (AGMK) cell culture.

Meanwhile, a pandemic of rubella started in Europe in the 1962 to 1963 season, with spread to the United States and pandemic disease in 1964 to 1965. As a result, from 1964 to 1966, thousands of pregnancies were affected by rubella, leaving behind a wake of medically induced abortions and abnormal infants.[12,13] The pandemic led to the recognition of an expanded congenital rubella syndrome (CRS), which added hepatitis, splenomegaly, thrombocytopenia, encephalitis, mental retardation, and numerous other anomalies to the already described deafness, cataracts, and heart disease.[2,3,14] The pandemic also made it obvious that a vaccine was needed, and many groups set to work.

Between 1965 and 1967, several attenuated rubella strains were developed and reached clinical trials.[15–17] In 1969 and 1970, rubella vaccine entered into commercial use in Europe and North America. Since the late 1970s, vaccination has had a major impact on the epidemiology of rubella and CRS.

BACKGROUND

Clinical Description

Acquired Rubella

Rubella is spread by large particle aerosols; primary implantation and replication occurs in the nasopharynx. The incubation period of rubella is 14 to 23 days, with most patients developing a rash 14 to 17 days after exposure.[18] During the first week after exposure, there are no symptoms, but in the second week, lymphadenopathy may be noted, particularly occipital and postauricular, and virus cultures reveal rubella virus in the nasopharynx. Later in the second week, virus appears in the blood. About this time, there may be a prodromal illness consisting of low-grade fever (<39.0°C), malaise, and mild conjunctivitis. If not already present, the aforementioned lymphadenopathy is likely to develop.

At the end of the incubation period, a maculopapular erythematous rash appears on the face and neck in about two thirds of cases, but the clinical to subclinical ratio is variable. The rash may be difficult to detect, particularly on pigmented skin, and is more prominent after hot showers or baths. During a course of 1 to 3 days, the rash spreads downward and begins to fade. Pharyngeal virus excretion is present in high titer up to 4 days after rash onset, and virus excretion in the pharynx and urine may continue for another 1 to 2 weeks, but viremia ends with the appearance of antibodies, which is more or less simultaneous with the onset of the rash.[19] Fig. 53.1 illustrates the evolution of acquired rubella.

Although acquired rubella is thought of as a benign disease, arthralgia and arthritis commonly are observed in adults, particularly women, associated with virus replication or latency in the synovia.[20,21] Chronic arthritis has been reported after rubella infection,[22] although the evidence is weak.[23,24] Other less-common complications are thrombocytopenia[23] and encephalitis, which may be fatal.[25,26] Encephalitis has been reported to occur in approximately one in 6000 cases and is of the postinfectious type, although the limited available pathologic data show little evidence of demyelination.[27] However, the incidence of encephalitis was one in 1600 cases of rubella in a Japanese outbreak,[28] and recently, in the South Pacific islands of Tonga and Samoa, the incidence was estimated as between one in 500 and one in 1000 cases (Ruben A: Rubella-associated encephalitis in Tonga, 2002; unpublished

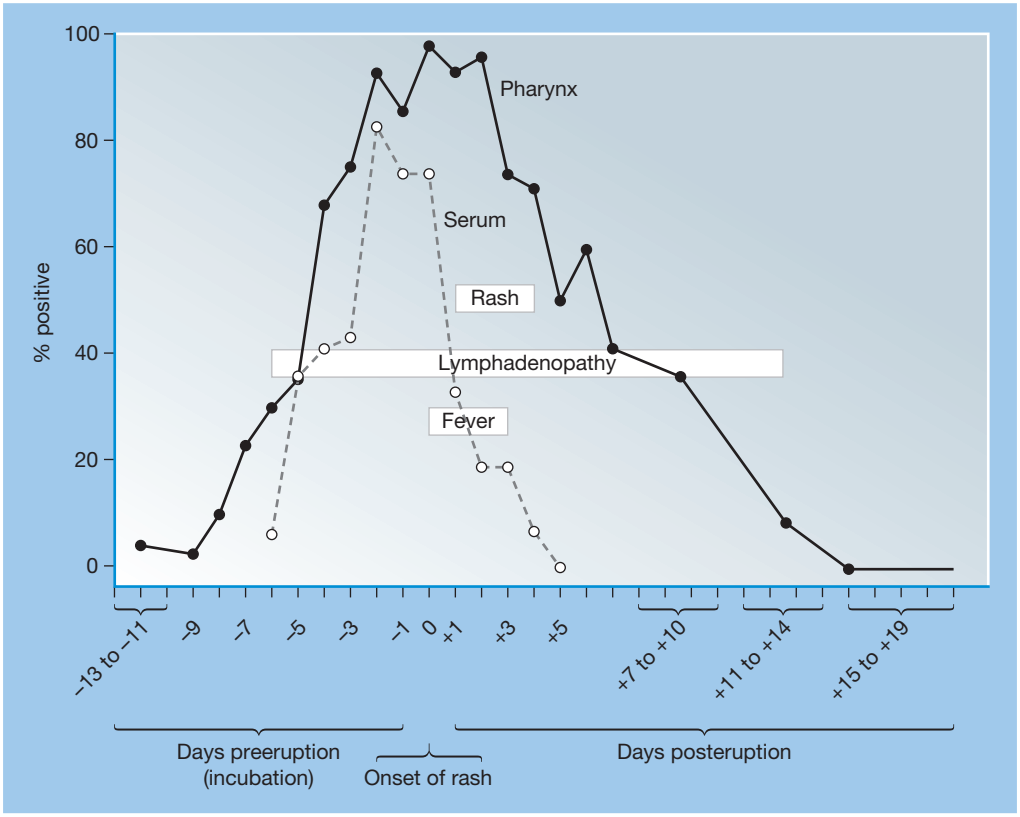


Figure 53.1. Sequence of events in acquired rubella infections, showing the relationship between onset of rash and other clinical symptoms and recovery of rubella virus from diagnostic specimens. Serum is noted by the dotted line; pharynx is noted by the solid line.

report, 2006). In 2011–12 in Tunisia, 280 cases of rubella were confirmed, including 39 cases of encephalitis that occurred mainly in adolescents and adults.[29] Another report described nine cases of encephalitis, most of which were in children.[30] A study done in China between 2010 and 2012 using a multiplex reverse transcription–polymerase chain reaction (PCR) showed that 3.3% of viral encephalitis was caused by rubella.[31] In addition, there is a rare late syndrome of progressive rubella panencephalitis that is inexorable and may also be seen in congenital infection.[32,33] By analogy to subacute sclerosing panencephalitis caused by measles, progressive rubella panencephalitis is thought to be related to persistence of viral antigens. Guillain-Barré syndrome after rubella has also been reported, although the causal relationship is uncertain.[34] Antibodies to rubella and measles structural proteins are elevated in autoimmune chronic active hepatitis, but there is no evidence for the presence of rubella virus in this disorder.[35]

Rubella virus may play a central role in the etiology of Fuchs heterochromatic anterior uveitis.[36,37] This condition is chronic and may lead to cataracts or glaucoma. Rubella virus RNA and high-titer antibodies have been demonstrated in intraocular fluid of patients with this condition.[38–40] Recently, intraocular rubella virus was detected in a 28-year-old survivor of congenital rubella syndrome, indicating the ability of the virus to persist and cause Fuchs uveitis.[40a] The prevalence of the syndrome has decreased in the United States since the introduction of vaccination.[41]

Congenital Rubella Syndrome

Rubella is the archetypical fetal infectious pathogen (see "Pathogenesis as It Relates to Prevention" later). Because all organs of the fetus are affected, it is not surprising that CRS comprises a lengthy list of abnormalities, including anatomic changes resulting from interference with organogenesis and inflammatory effects involving organs such as the liver and spleen (Table 53.1).[42] Histopathological study of several congenital rubella syndrome cases showed widespread presence of rubella antigen in fibroblasts lining the heart and blood vessels, alveolar macrophages, progenitor cells of the outer

TABLE 53.1 Prominent Clinical Findings in Congenital Rubella Syndrome

Cataracts
Retinitis
Microphthalmia
Glaucoma
Cochlear deafness
Central auditory imperception
Patent ductus arteriosus
Peripheral pulmonic artery stenosis
Encephalitis
Microcephaly
Mental disability
Autism
Intrauterine growth retardation
Metaphyseal rarefactions
Hepatosplenomegaly
Thrombocytopenic purpura
Interstitial pneumonitis
Late:
Diabetes
Hypothyroidism

Modified from Cooper LZ, Preblud SR, Alford CA. Rubella. In: Remington JS, Klein JO, eds. Infectious Diseases of the Fetus and Newborn Infant, *4th ed. Philadelphia, PA: WB Saunders; 1995:288.*

granular layer of the brain, as well as capillary endothelium in the placenta.[42a] Rubella virus replicates in the nasopharynx of affected infants and presents a risk to others. In a Japanese study, 17% of infected infants were still excreting rubella virus 1 year after birth.[42b]

The time of infection during gestation is important in relation to the fetal outcome; infection in the first 3 months tends to result in serious ocular or cardiac disease, whereas infection late in the first half of pregnancy is more likely to result in isolated hearing impairment. However, the relationship between gestational age and abnormality can be overemphasized because fetal infection, once established, spreads to all organs, and damage may be progressive.

Table 53.2 demonstrates that organ specificity is only generally related to the stage of gestational infection with rubella virus. The most common congenital defects are sensorineural deafness, cataracts, pigmentary retinopathy, and patent ductus arteriosus; however, malformations of the pulmonary artery or its branches can be demonstrated in 78% of CRS patients in whom cardiac studies are done, and patent ductus arteriosus is found in 62%. In addition, myriad other abnormalities occur, including glaucoma, endocrinopathies such as diabetes, hyperimmunoglobulinemia M,[43] microcephaly, and mental disability. Ocular defects are particularly varied, including abnormalities of the cornea, the lens, the retina, and uveitis.[44–47] An analysis of prospective studies for distribution of clinical manifestations of CRS is summarized in Table 53.3.[48]

A comprehensive review of the retrospective and prospective studies estimated that hearing, cardiac, ocular, and intellectual manifestations were found, respectively, in 70%, 31%, 16%, and 6% of CRS infants. However, if mixed-cohort and infant-case series are included, the percentages of cardiac, ocular, and intellectual manifestations increase to 46%, 35%, and 40%, respectively.[49] Various other estimates have been made of the incidence of fetal abnormalities after maternal infection.[8,50–56] Many prospective studies of the incidence of fetal rubella have accepted clinical diagnosis of rubella in the mother for case inclusion, but if only virologically confirmed maternal rubella is considered, the rate of transmission to the fetus during the first 10 weeks of pregnancy is as high as 90%.[51,57–59] Table 53.4 tabulates data from the two most reliable studies, one from United States data[52] and the other from United Kingdom.[51] The first 12 weeks of pregnancy are clearly the most dangerous time for rubella infection in the mother. The incidence of fetal disease declines during the next 4 weeks, and in the 16th to the 20th weeks, only deafness has been reported as a complication. Preconceptional rubella rarely results in fetal infection, but rashes that occur within 12 days

TABLE 53.3 Frequencies of Selected Defects in Infants With Congenital Rubella Syndrome: Comparison of Data From Prospective Studies and Data Presented in a Standard Pediatric Infectious Disease Textbook

Clinical Manifestation	No. of Studies	Study Subjects (%)[a]	Previously Reported Studies (%)[b]
Hearing impairment	10	68/113 (60)	80–90
Heart defect	9	45/100 (45)	–
Patent ductus arteriosus	3	9/45 (20)	30
Peripheral pulmonic stenosis	3	6/49 (12)	25[c]
Microcephaly	3	13/49 (27)	Rare
Cataracts	3	16/65 (25)	35
Low birth weight (<2500 g)	2	5/22 (23)	50–85
Hepatosplenomegaly	6	13/67 (19)	10–20
Purpura	5	11/65 (17)	5–10
Mental retardation	2	2/15 (13)	10–20
Meningoencephalitis	3	5/49 (10)	10–20
Radiolucent bone	3	3/43 (7)	10–20
Retinopathy	3	2/44 (5)	35[c]

[a]Number of infants with congenital rubella syndrome/number of study subjects (percentage in parentheses).
[b]Frequencies presented in textbook data.
[c]Includes pulmonary arterial hypoplasia, supravalvular stenosis, valvular stenosis, and peripheral branch stenosis.
From Reef SE, Plotkin S, Cordero JF, et al. Preparing for elimination of congenital rubella syndrome (CRS): summary of a workshop on CRS elimination in the United States. Clin Infect Dis. *2000;31:85–95.*

TABLE 53.2 Age of Gestation at Time of Rubella in Relation to Abnormalities Observed

	Month of Gestation Measured From Last Menstrual Period				
	0[a]	1	2	3	4
Birth weight <2500 g	0/1[b]	9/21	9/21	10/18	0/2
<38 wk gestation	0/1	5/21	2/21	4/18	0/2
Growth retardation	0/1	7/21	5/20	7/17	0/2
Ocular defects	0/1	14/21	9/21	9/18	0/2
Cardiac defects	0/1	17/21	13/21	6/18	0/2
Deafness	0/1	8/18	10/18	11/17	2/2
Mental retardation	1/1	7/20	7/20	8/16	0/2
Microcephaly	1/1	3/18	2/19	4/17	0/2

[a]Before conception.
[b]Ratio of number of patients with condition to total number for whom information is available.
From Plotkin SA, Cochran W, Lindquist J, et al. Congenital rubella syndrome in late infancy. JAMA. *1967;200:435–441. Copyright 1967, American Medical Association.*

TABLE 53.4 Fetal Abnormality Induced by Confirmed Rubella at Various Stages of Pregnancy

Stage of Pregnancy (Week)	United Kingdom Study (% Defective)[a]	United States Study (% Defective)[b]
≤4		70
5–8		40
≤10	90	
11–12	33	
9–12		25
13–14	11	
15–16	24	
13–16		40
≥17	0	8[a]

[a]Data from Miller E, Cradock-Watson JE, Pollock TM. Consequences of confirmed maternal rubella at successive stages of pregnancy. *Lancet*. 1982;2:781–784.
[b]Data from South MA, Sever JL. Teratogen update: the congenital rubella syndrome. *Teratology*. 1985;31:297–307.

of the last menstrual period carry proven risk.[60,61] In an Irish epidemic, 71% of fetuses infected early in pregnancy were affected.[62] Although some Japanese workers[63] have claimed that rubella virus is less teratogenic in their country, these claims have not been supported.[28]

Damage caused by congenital rubella infection is not always evident at birth: glaucoma may become apparent and late cataracts are possible. Retinal detachments and esophageal problems are common later in life.[64] Of interest, autism is a feature of some late-onset neurologic disease in CRS patients. In addition, a variety of syndromes thought to be autoimmune are seen, including diabetes mellitus and thyroiditis.[64,65]

Diabetes develops in 12% to 20% of American, European, and Australian CRS survivors.[66,67] However, a 40-year follow-up of Japanese CRS patients showed that only 1.1% were diabetics.[68] Although autoimmunity is involved in ordinary diabetes mellitus, a thorough study of CRS patients showed no increase in autoantibodies to pancreatic proteins.[69]

Virology

The agent of rubella is a cubical, medium-sized (50 to 85 nm), lipid-enveloped virus with an RNA genome belonging to the Togaviridae family and the genus *Rubivirus*.[69a] Although the other togaviruses are arthropod-borne, there is no evidence for such transmission of rubella. Frey[70] has reviewed the virology of rubella. Apart from the complex lipid envelope derived from the host cell, rubella virus is composed of three proteins: two embedded in the envelope in the form of spikes (E1 and E2) and one (C) composing the capsid. E1 is a glycoprotein with neutralizing and hemagglutinating epitopes, while the function of the E2 glycoprotein is unclear.[71] The three proteins, which have molecular masses of 60,000 kDa (E1), 42,000 to 47,000 kDa (E2), and 30,000 kDa (C), are derived from a polypeptide of 110 kDa that is translated from a 245-kDa messenger RNA.[72–74]

The positive-sense RNA genome of rubella virus contains approximately 9800 nucleotides and is infectious.[75,76] The replication strategy of rubella virus is similar to that of the alphaviruses in that both full-length and subgenomic RNAs are produced, and it is from the subgenomic RNA that viral structural proteins are translated. Three other proteins are produced by the virus in infected cells but are not incorporated into the virion.[77] The structural capsid protein interferes with cellular mitochondrial function, inhibiting transport of proteins into the mitochondria and causing morphologic alterations of those organelles.[78] A receptor for the virus has not been definitely identified, but myelin oligodendrocyte glycoprotein may be involved in spread to the placenta and fetal brain.[69a]

There is only one serotype of rubella virus, and analyses of sequence variation among occidental isolates show high conservation of amino acid structure (0% to 3.3% differences), with less conservation of isolates from Asia (up to 7%).[79,80] However, there are two clades and at least three genotypes.[81,82] For rubella isolates collected before 2000, isolates from North America, Europe, and Japan are closely related to each other and form clade I, whereas clade II comprises some strains from China, Korea, and India.[81] However, in China, between 2001 and 2007, there was a shift of genotypes toward predominance of genotypes 1E and 1F.[83] Similarly, in France, genotype 1E has become predominant.[84] Isolation of virus from cataracts in Indian CRS cases revealed genotype 2B.[85] Of rubella isolates collected between 2005 and 2010, three genotypes (1E, 1G, and 2B) had wide geographic distribution, whereas others occurred sporadically or were geographically restricted.[82] More recent studies continue to identify the circulation of multiple genotypes, including in Africa, with 1B, 1E, 1G, and 2B being prominent.[85a,85b]

The genetic differences between clades do not appear to translate into antigenic differences, despite amino acid changes of 3% to 6% in viral proteins.[86] Moreover, isolates from CRS cases are not genetically distinct from isolates from acquired rubella.[87] No significant antigenic drift has occurred in recent years, and isolates from vaccinees showed little variation from vaccine strains. However, recent data show many intercontinental transfers of clades I and II.[88] Because clade II was clearly of Asian origin, and clade I isolates from Asia are highly variable, an Asian origin of rubella virus has been suggested.[89] Genetic analysis allowed the confirmation of the epidemiologic data that implicated spread of endemic rubella strains from Greece to the United Kingdom in 1999.[90] Table 53.5 shows the distribution of rubella virus clades.[91]

Rubella virus grows in many different primary, semicontinuous, and continuous cells of mammalian origin. In human amnion cells, it produces a subtle cytopathic effect. More pronounced cytopathic effects, sufficient to allow plaque formation, are produced in continuous cell lines, such as rabbit kidney (RK13) and baby hamster kidney (BHK-21).[19] Even in those cell lines, fresh isolates frequently are not cytopathogenic and require adaptation by serial passage. High passage generates defective interfering RNA and particles.[92]

Virus isolation is generally performed in primary AGMK cell culture in which virus growth is detected by "challenging" the cultures with a cytopathogenic agent such as echovirus 11. If rubella virus has infected the cultures, replication of echovirus 11 is blocked, probably by interferon induction; the presence of rubella virus is inferred by this interference. Confirmation is performed by another technique, such as neutralization or fluorescence with specific antirubella serum.[93]

Pathogenesis as It Relates to Prevention

The pathogenesis of acquired rubella infection provides two points at which immune intervention could have an effect. The first point is in the nasopharynx, where the virus first replicates and from which it spreads to local lymph nodes. Secretory immunoglobulin (Ig) A antibody in the nasopharynx, induced by prior disease or vaccination, can block mucosal replication. The second point begins about a week into the incubation period, at which time the viremia can be blocked by the presence of antibody, either passively or actively acquired.

During viremia in a pregnant woman, the virus often infects the placenta. Placental replication appears to precede fetal infections, leading to entrance of virus into the fetal circulation, from which it infects fetal organs.[94] In vitro experiments show that human embryonic cells of many different lineages are susceptible to viral replication and develop chronic noncytopathic infection.[95] The same phenomenon occurs in vivo, except that only a few cells are infected at any one time.[96] If the infected cells are stimulated to divide, either artificially in vitro or in the course of embryologic development in vivo, there is an inhibition of mitosis[95,97] that may be mediated in part by a soluble protein inhibitor[98] or by the induction of apoptosis.[99–102] Although low-passage strains produce more pronounced apoptosis, the effect is only moderate in human fibroblasts in culture.[103] In a few organs, including the lens, cochlea, and brain, the damage caused by the virus is more cytopathic.[104] Viral infection of the ciliary body may contribute to cataractogenesis[105] and wild virus destruction of endothelial cells may lead to vasculitis and ischemia.[106] Cytologic studies show that the cell skeleton and mitochondria both are damaged by rubella virus replication.[107] Atreya and coworkers[108] proposed that rubella virus replicase interacts with retinoblastoma tumor suppressor protein and the enzyme citron-K kinase, leading to cell-cycle arrest in S phase

TABLE 53.5 Global Distribution of Reported Rubella Virus Genotypes, 2005–2010

Genotype, Country	Year(s)
1A	
Cambodia	2009
Japan	2008
Kazakhstan	2006
1B	
South Africa	2007, 2008
1C	
Chile	2005
Peru	2005
1E	
Belarus	2005, 2006
China	2005, 2006, 2007, 2008,[a] 2009, 2010[a]
China HK SAR	2008, 2009, 2010
France	2005[a]
Kazakhstan	2006
Laos	2009
Malaysia	2005[b]
Mongolia	2010
Poland	2007, 2008[b]
Russia	2005, 2006, 2007, 2008, 2010
South Africa	2008
Sri Lanka	2008
Sudan	2005
Thailand	2005, 2009
Tunisia	2008
Ukraine	2007
United Kingdom	2008
United States	2008[c]
Vietnam	2007[b]
Yemen	2008
1G	
Algeria	2007
Belarus	2005
Ghana	2005, 2008
Côte d'Ivoire	2005,[b] 2008
Kenya	2005, 2010[b]
Libya	2005[a]
Netherlands	2005,[b] 2006, 2008
Russia	2005
Sudan	2005
Uganda	2007[b]
Ukraine	2009
United Kingdom	2007
1H	
Belarus	2005, 2006
Kazakhstan	2008[a]
Kyrgyzstan	2009
Russia	2005, 2006, 2007, 2008, 2009, 2010
1J	
Brazil	2005[c]
Philippines	2010[a]
Spain	2005[c]
United Kingdom	2006[c]
United States	2010[c]
2B	
Argentina	2008
Bangladesh	2009
Bosnia and Herzegovina	2009, 2010
Brazil	2006, 2007, 2008, 2009
Chile	2007
China	2008
China HK SAR	2008, 2009
Dubai	2009[b]
Egypt	2007[b]
France	2009
India	2005, 2007, 2008,[b] 2010[b]
Italy	2008[b]
Japan	2007[c]
Kazakhstan	2008[b,c]
Mexico	2008[b]
Nepal	2008, 2009, 2010
Russia	2009[c]
South Africa	2007, 2008
Spain	2009
Sri Lanka	2008
Sudan	2006
Ukraine	2010
United Kingdom	2006, 2007, 2008, 2010
United States	2007,[c] 2009,[c] 2010[c]
Vietnam	2006,[b] 2009
Yemen	2008
2C	
Russia	2005

[a]Found endemically and as an export.
[b]Exported virus.
[c]Probable import, but links are unknown.
Note: Genotypes 1d, 1f, 1i, and 2a were inactive during this period. Country (or countries) and year (or years) of report are indicated for each genotype.
HK SAR, Hong Kong Special Administrative Region.

and activation of apoptosis. Adamo and coworkers[109] emphasized the importance of the innate immune response to rubella infection, leading to interferon induction that causes apoptosis.

In summary, the action of rubella virus on organogenesis is mediated by varying combinations of intracellular pathology, inhibition of cellular replication, and apoptosis.[107,110]

Diagnosis

Methods for the diagnosis of acquired and congenital rubella have been reviewed[111] and are summarized in Table 53.6. Clinical diagnosis of acquired infection is so inaccurate as to be useless without laboratory support. When suspected measles cases are subjected to laboratory tests, rubella infection is often identified.[110a] Isolation of virus can be accomplished from the blood and nasopharynx during the prodromal period and from the nasopharynx for as long as 2 weeks after eruption, although the likelihood of virus recovery is sharply reduced by 4 days after the rash. The Vero cell line, primary AGMK cells, or the RK13 cell line are generally used for virus isolation; but because of the slow growth of the virus in tissue culture, virus isolation is often bypassed in favor of serologic diagnosis.

The PCR has been adapted to the detection of rubella RNA by reverse transcription and amplification.[112] The method appears to be 100% sensitive and approximately 90% specific[113–115] and is particularly useful for prenatal detection of rubella infection of the fetus (see below).[116] Reverse transcription–PCR was shown to permit diagnosis of rubella during the rash before appearance of IgM antibodies.[117] The Taq Man real-time PCR assay has been applied to rubella virus detection.[118]

Initially, serologic diagnosis became feasible because rubella virus hemagglutinates red blood cells, particularly of avian origin, permitting measurement of antibodies by hemagglutination-inhibition (HI) testing.[119] Because HI and neutralization tests are labor intensive, other serologic examinations that are amenable to mass testing have come into use, such as latex agglutination, indirect hemagglutination, enzyme-linked immunosorbent assay (ELISA), latex agglutination, single radial hemolysis, and fluorescence inhibition.[59,120] Any of these methods may be used to measure antibodies to rubella. Detection of antibodies in saliva or urine could simplify testing in developing countries, but commercial kits are not available.[121–125] Nevertheless, identification of IgM antibody in oral or crevicular fluid permitted efficient diagnosis of rubella in the United Kingdom over a 10-year period.[126]

Serologic diagnosis depends on the demonstration of a fourfold rise in IgG titer between acute and convalescent specimens or a demonstration of IgM antibody in the acute specimen.[120] For IgM testing, ELISA, in a capture format to avoid false positivity because of IgG antibody, is the predominant assay in use; results may be positive for up to 6 weeks after the acute infection.[127]

Susceptibility to rubella infection has been defined as an HI antibody titer of less than 1:8, a hemolysis-in-gel result of less than 10 IU, or an optical density by ELISA at less than the limit set by the manufacturer. Waning antibody may be restimulated on exposure, such that protection results, and epidemics have not occurred in well-vaccinated countries even when significant numbers of vaccinees no longer have antibodies at the 10 IU level. Titers at the borderline level are difficult to evaluate, but the consensus is that, although in the majority of cases they reflect immunity, the safest response is to vaccinate the individual with equivocal levels of antibody.[128,128a,128b]

Assays for antibody avidity and for responses to specific proteins or peptides have come into use for diagnosis of recent infection.[129,130] Low-avidity antibodies indicate recent infection, with maturation to high avidity by about 2 months postexanthem. A careful study of antibody responses to primary rubella infection found an initial low-avidity IgM response, followed by low-avidity IgG_3 and IgA responses, and then, finally, IgG_1 responses maturing from low to high avidity.[131] Liebert and coworkers[132] studied various serologic modalities and found that low levels of antibody to the major neutralizing epitope on the E1 protein, poor response to the E2 protein, and low IgG avidity for rubella virus were highly predictive of recent infection in both mother and infant.[116,133] Antibodies to E2 develop months after infection or vaccination.[134] Rules for diagnosis of rubella in pregnancy have been well summarized.[134a]

In contrast to the situation with acquired infection, the recognition of the combination of cataracts, heart disease, and deafness provides a clinical means of diagnosis of CRS that is reasonably accurate.[135] However, laboratory confirmation is always desirable, both because isolated abnormalities may occur and for public health reasons.

Congenital rubella infection in the infant is associated with the detection of virus, viral genome, IgM antibodies by capture techniques, low-avidity IgG antibodies, lack of antibodies to the E1 protein,[136] or antibodies persisting beyond the predicted decay of passively transmitted maternal antibodies. Virus often can be isolated from tissues obtained at biopsy or autopsy or during surgical procedures such as cataract extraction,[137] but more often nasopharyngeal swabs, urine specimens, or cerebrospinal fluid serve as the sources. Almost always, one or more of these sources are positive for virus at birth, gradually becoming negative during the first year of life.[138] In severe cases, virus excretion may persist for several years.[139] A negative result from PCR performed on fetal samples

TABLE 53.6 Laboratory Diagnosis of Acquired and Congenital Rubella

		When Positive	
Test	**Specimen**	**Acquired Rubella**	**Congenital Rubella**
Virus isolation	Throat, urine, blood	First week of illness	At birth, declining thereafter
RT-PCR	Amniotic fluid, placenta	NA	Throughout pregnancy
IgM antibody	Serum	Up to 2 mo postillness	At birth and first year of life
IgG antibody rise	Sera	Fourfold increase between acute and convalescent	NA
Low IgG antibody avidity	Serum	Up to 2 mo postillness	At birth and years later
IgG antibody persistence	Serum	NA	Beyond 6 mo of age, until exposure to infection or vaccination

IgG, immunoglobulin G; IgM, immunoglobulin M; NA, not applicable; RT-PCR, reverse transcription–polymerase chain reaction.

or amniotic fluid during pregnancy is good evidence against intrauterine rubella.[61]

IgM antibodies are present in the infant for as long as a year after birth; however, the IgM titers decline over 6 months, whereas low-avidity IgG antibodies may persist for longer periods. Persistence of IgG antibodies beyond 6 months of age can be detected in 95% of infants with CRS.[140] Salivary specimens are also useful in CRS for detection of antibodies.[141] An infant with seropositive results for rubella after 6 months of age and who has not received rubella vaccine is likely to have been congenitally infected. However, CRS patients may lose their antibodies over time: 60 years after fetal infection, 40% were seronegative.[142] Moreover, sensitization to proliferation of lymphocytes after stimulation with rubella antigens is often negative in CRS infants, even when they are seropositive,[143] and this test may be used for diagnosis in children younger than 3 years of age. Congenital rubella can also be diagnosed by PCR tests of lens aspirates in cases of congenital cataracts.[144]

Diagnosis of CRS has been focused on the first year of life when IgM antibody and viral shedding are likely to be present. However, a recent study of school-age children with CRS identified several serologic markers of fetal infection, including higher rubella-specific IgG, higher relative concentrations of antibodies to the core C and E2 proteins of the virus, but lower relative concentration of antibodies to the E1 protein when compared with normal children. Using those markers, CRS could be identified retrospectively with 65% sensitivity and 100% specificity.[145] Reinfection with rubella virus has been documented to rarely cause CRS, in which case IgG3 antibody is usually absent, in contrast to CRS after primary infection.[145a]

EPIDEMIOLOGY

Acquired Infection

Until 2009, rubella was a worldwide infection, as may be inferred from serologic surveys conducted in many different countries.[122,146] With the successful implementation of the rubella vaccination strategies in the Region of the Americas, endemic rubella and CRS in the Region of the Americas was eliminated in 2009 as verified in 2015 by the International Expert Committee on the Documentation of Elimination of Measles and Rubella.[147,148] Before 2009, the Global Measles and Rubella Laboratory Network found rubella to be the cause of 15% of rash disease for which they received specimens.[91] The factors that give rubella its epidemiologic characteristics are respiratory spread that is greater in crowded societies, different age at infection, and periodic disappearance from geographic areas, only to reappear in epidemic form when susceptibles accumulate. The recent prevalence of rubella antibodies has recently been reviewed.[148a]

As is true of other diseases, some individual rubella patients excrete large amounts of virus in respiratory secretions and are highly infectious "spreaders."[149] The basic reproductive rate, Ro, before vaccination was estimated to be 6 to 7 in developed and most developing countries but up to 12 in crowded developing countries.[150,151] In Europe, there was wide variation in the contagiousness of rubella infection between countries, with reproductive rates ranging from 3 to 8.[152] In Mexico before vaccination, Ro varied among districts from 3.4 to 9.6 and mean age of infection from 3 to 10 years.[153] In the World Health Organization (WHO) African Region there is wide variation in the contagiousness of infection between countries, ranging from 3.3 to 7.9[154] Many rubella infections in childhood are asymptomatic or without rash and therefore unrecognized. Daycare centers also promote early infection with rubella. In island countries and in countries that are less crowded, the average age at rubella infection is older, and many children reach puberty still in a seronegative state.[155] Under these circumstances, introduction of the virus into places where young persons congregate results in epidemic spread.[149] Thus schools, colleges, and military camps are all places where rubella is likely to become epidemic. Other sites where outbreaks have also been reported include African refugee camps.[156]

Outbreaks at the stock exchange on Wall Street illustrate the potential effect of bringing seronegative individuals together in close quarters. In fact, rubella is highly efficient at infecting susceptible persons in certain epidemiologic situations.[157–159]

In the United States, the epidemiology of rubella before vaccination was both endemic and epidemic.[12] Rubella tended to occur each spring, primarily in schoolchildren 6 to 10 years of age, but also in older individuals. Superimposed on this occurrence was a cycle of major epidemics at 7-year intervals. Susceptibility in young adults varied from 10% to 20%, with the lower figure being found after an epidemic. Similarly, in Israel, approximately 9% of women were infected during a 1972 rubella outbreak[160]; however, even after the epidemic, a significant pool of susceptible individuals remained. A seroprevalence study of healthcare workers in Japan found 9.6% without rubella antibodies.[161] Although vaccination has sharply reduced rubella in Japan, immunity is incomplete and epidemics still occur.[161a]

The epidemiology of rubella in developing countries can be deduced from the seroprevalence of rubella antibodies. The large variation seen in seroprevalence suggests that rubella occurs in sporadic epidemics except where population density is high. In the metropolis of Saõ Paulo, Brazil, nearly everyone is seropositive by 20 years of age,[162] whereas in rural Mexico, seropositivity varies from 29% to 76%.[163] Surveillance of rash disease in South America shows that rubella infection is ubiquitous. Cutts and colleagues[164] reviewed rubella susceptibility data from 45 developing countries and found remarkable differences among them, not correlated with geography. For example, Malaysia, Peru, and Nigeria were among the countries where more than 25% of women were found to be seronegative. Studies done in Lagos and Ibadan, Nigeria, in Thailand, and in Izmir, Turkey, confirm the low seroprevalence in women of childbearing age.[165–168] Studies of rubella outbreaks in Kenya and Zimbabwe showed that rubella is endemic in those countries, and a serological survey in Cambodia showed only 73% seropositivity in women.[169–171] Thus, even in countries where little has been done in the way of vaccination and where low socioeconomic levels would predict high seropositivity, 5% to 25% susceptibility may be seen in women of childbearing age.[172–181] Vietnam has had a recent rubella epidemic with a high incidence of CRS,[181a] and studies in various African countries have revealed ubiquitous rubella infection.[181b,181c,181d,181e] The median age of infection in Africa is 7 years.[181f]

Most information on the epidemiology of CRS is available from the United Kingdom and the United States. Table 53.7 presents the tally of fetal damage caused by rubella subsequent to the 1963 to 1964 outbreak.[13] At least 30,000 fetuses were damaged by intrauterine rubella, for an incidence rate of 100 per 10,000 pregnancies. In Philadelphia, the rate was also as high as 1% of pregnancies.[182] After that outbreak, CRS rates fell to four to eight per 10,000 pregnancies until 1970, when the first vaccines were licensed. Since then, CRS declined further to an incidence of less than 0.01 per 10,000 pregnancies, with no indigenous cases,[183,184] and endemic rubella virus is no longer believed to be circulating. Table 53.8 gives the current surveillance criteria for CRS in the United States.[48] In the prevaccine era, the epidemiology of CRS in the United Kingdom was similar to that in the United States, with rates of approximately

TABLE 53.7 Estimated Morbidity Associated With the 1963–1964 Rubella Epidemic

Clinical Events	Cases
Rubella cases	12,500,000
Arthritis-arthralgia	159,375
Encephalitis	2084
Deaths	
Excess neonatal deaths	2100
Other deaths	60
Total deaths	2160
Excess fetal wastage	6250
Congenital rubella syndrome	
Deaf children	8055
Deaf-blind children	3580
Mentally disabled children	1790
Other congenital rubella syndrome	6575
Total congenital rubella syndrome	20,000
Therapeutic abortions	5000

From National Communicable Disease Center. Rubella Surveillance. *Bethesda, MD: U.S. Department of Health, Education, and Welfare; 1969.*

4.6 per 10,000 births.[185] In general, in Europe at present, CRS incidence is low, and surveillance is improving.[186,187]

Nevertheless, observations in Italy show that CRS was still a problem as of 2013, and a large outbreak of rubella occurred in Poland that same year.[188,189] As with other vaccine-preventable diseases, CRS in the United States has been associated with religious minorities that refuse vaccination. An epidemic of rubella among the Pennsylvania Amish resulted in a rate of more than 20 per 1000 live births.[190] An orthodox Protestant community in The Netherlands was the site of a large outbreak that spread to coreligionists in Canada, leading to 14 cases of CRS and two fetal deaths. Of the reported cases, 97% occurred in unvaccinated persons, but those vaccinated exhibited 99% protection.[191]

In recent years, the worldwide epidemiology of CRS is beginning to be known. Little published information is available for the countries in Africa, and most of Asia, although such data suggest that CRS is common even in the developing world.[192,193] In 2011–12, 279 CRS cases were identified in three Vietnamese hospitals.[194] CRS cases have been identified in Nigeria and in India it is estimated that CRS accounts for 10% to 15% of pediatric cataracts.[195,196] A thorough retrospective study done in the Maldives gave a CRS incidence of one to four per 1000 births during epidemics and 0.1 to 0.2 per 1000 births during endemic periods.[197] Observations made in Myanmar implied a CRS incidence of 0.1 per 1000 births.[198] Based on clinical virology studies, CRS seems to be common in Brazil.[199] In India, 26% of 90 infants investigated for congenital malformations had serologic evidence of CRS.[200] Several other Indian studies[201–205] showed that 6% to 25% of children with nontraumatic cataracts and 15% of infants

TABLE 53.8 Clinical Description and Criteria for Congenital Rubella Syndrome Case Definition for the United States

Definition	Case Classification
Clinical case definition: an illness, usually manifesting in infancy, resulting from rubella infection in utero and characterized by signs or symptoms from the following categories:	**Suspected:** a case with some compatible clinical findings but not meeting the criteria for a probable case **Probable:** a case that is not laboratory confirmed and that has any two complications listed in first paragraph of the clinical case definition or one complication from the first paragraph and one from the second paragraph, and lacks evidence of any other etiology **Confirmed:** a clinically consistent case that is laboratory confirmed **Infection only:** a case that demonstrates laboratory evidence of infection, but without any clinical symptoms or signs
• Cataracts and congenital glaucoma, congenital heart disease (most commonly patent ductus arteriosus or peripheral pulmonary artery stenosis), hearing impairment, pigmentary retinopathy	
• Purpura, hepatosplenomegaly, jaundice, microcephaly, developmental delay, meningoencephalitis, radiolucent bone disease	
Clinical description: presence of any defect(s) or laboratory data consistent with congenital rubella infection. Infants with CRS usually present with more than one sign or symptom consistent with congenital rubella infection. However, infants may present with a single defect. Hearing impairment is the most common single defect	
Laboratory criteria for diagnosis:	
• Isolation of rubella virus	
• Demonstration of rubella-specific IgM antibody	
• Infant rubella antibody level that persists at a higher level and for a longer period than expected from passive transfer of maternal antibody (i.e., rubella titer that does not drop at the expected rate of a twofold dilution per month)	
• Detection of rubella virus by RT-PCR	

CRS, congenital rubella syndrome; IgG, immunoglobulin G; IgM, immunoglobulin M; RT-PCR, reverse transcriptase–polymerase chain reaction.
Material from Reef SE, Plotkin S, Cordero JF, et al. Preparing for elimination of congenital rubella syndrome (CRS): summary of a workshop on CRS elimination in the United States. Clin Infect Dis. *2000;31:89.*

suspected of having congenital infection had rubella-specific antibodies. A history of maternal rash and fever was obtained in 40% of deaf Bangladeshi children.[202,206] Retrospective analysis of a recent epidemic in Kumasi, Ghana gave an incidence of 0.8 per 1000 live births.[207] An investigation in Nepal estimated a rate of CRS of 192 per 100,000 live births.[208] In Shenzhen, China, 22% of female migrant factory workers were seronegative.[209] After a rubella outbreak in Vietnam, several hundred congenital rubella infections and disease were confirmed in 2011–12.[194] Although not well described, rubella virus circulates widely in Africa, and serologic data suggest that 22,500 cases of CRS are estimated to occur yearly.[210]

An incidence of 0.1 per 1000 live births[198] was identified in Moroccco.[211] A review of worldwide data concerning CRS revealed rates in developing countries varying between 0.6 and 2.2 per 1000 live births, similar to rates seen in developed countries before universal vaccination.[151,211–214]

In the absence of systematic laboratory testing of newborns, detection of cataracts and of deafness with subsequent laboratory confirmation of rubella infection is the best method for estimating the incidence of CRS. As congenital cataracts occur in approximately 25% of CRS cases,[215] a rough estimate of incidence can be obtained by multiplying the number of patients with cataracts by four and dividing by the number of births.

A virulence factor has been postulated for rubella strains, and it has been argued from laboratory and clinical data that rubella virus is not teratogenic in Japan. Kono and colleagues[63] reported that Japanese strains had no effect on pregnant rabbits, whereas American strains were transmitted across the placenta from dams to fetal rabbits. In addition, they reported that on the main island of Honshu, no CRS was reported even though rubella outbreaks were evident.[216] However, by careful analysis of rubella on the southern Japanese island of Kyushu, Ueda and coworkers[217] showed that the rates of both rubella and CRS were high. They concluded that the apparently low rate of CRS on Honshu is the result of a high seropositivity rate in adult women and a low clinical reporting rate of CRS. More recently, in 2012–13 a large outbreak of rubella occurred in Japan involving thousands of cases and many CRS babies.[218]

CRS shows a predisposition to affect infants of young mothers, presumably because these women are more likely to enter pregnancy in a seronegative state. Women in contact with populations in which rubella outbreaks occur frequently, such as military recruits and school-age children, are more likely to be exposed. Military dependents and schoolteachers are thus at increased risk. Pregnant women with older children are also at greater risk.

Although rubella outbreaks may be explosive, they frequently do not exhaust all susceptible persons in large populations, and thus a history of having been exposed to a rubella outbreak does not necessarily indicate immunity to rubella. Even more important is the fact that 10% to 85% of infections in various outbreaks have been inapparent or at least have been without evidence of rash.[158] Infection without rash in pregnancy can still lead to fetal disease, although the risk may be lower than that after symptomatic infection with rash.[219]

Significance as a Public Health Problem

The seriousness of congenital rubella as a public health problem can be gauged by the results of the last major American epidemic in 1964 to 1965 (see Table 53.7). An estimated 12.5 million rubella cases occurred, including approximately 2000 cases of encephalitis. More than 30,000 pregnancies were affected by this epidemic. Of these pregnancies, approximately 5000 pregnant women chose to have surgical abortions, surely an underestimate, and approximately 6250 lost fetuses to spontaneous abortions. Another 2100 infants were stillborn or died soon after birth.[13] CRS occurred in 20,000 infants who survived pregnancy. Of these, 11,600 were deaf, 3580 blind, and approximately 1800 mentally disabled. The human misery imposed by such severe damage can well be imagined. The economic burden was estimated to be US$221,660 per child with CRS, and the total cost of the epidemic may have been US$1.5 billion in 1965 dollars.[13]

A more recent economic analysis of the impact of CRS calculated the disability-adjusted life-years and cost of care. For a CRS child in a low-income country, 29 disability-adjusted life-years and US$11,266 in cost of care would be incurred, whereas for a high-income country the figures are 19 disability-adjusted life-years and US$934,000 in cost of care.[220] Before the use of vaccine, rubella epidemics involved approximately 5% of the population, although only approximately 10% of these cases were reported to public health authorities.[221] In the years between epidemics, rates were about one-tenth of the epidemic peaks, but CRS continued at a low endemic rate even in those years.

Analysis of the age distribution of acquired rubella in the prevaccine era showed 60% of cases in children younger than 10 years of age and 23% in those older than 15 years of age.[222] As discussed subsequently, application of vaccine in children did not reduce the incidence in adolescents and adults by much, and CRS continued to occur for some years after the introduction of vaccination.

Lest it be thought that rubella infection in pregnancy has lost its danger, a more recent outbreak among unvaccinated Amish persons living in Pennsylvania serves as a corrective.[190] Young Amish women were 20% seronegative at the beginning of the outbreak, and infections in pregnancy were common. More than 8% of Amish infants gave laboratory evidence of infection, and more than 2% had CRS. Of infants born after first-trimester infection, 90% had confirmed or possible CRS.

In the United Kingdom, CRS estimates before the use of vaccine were only about 200 to 300 cases per year, but that figure was from a passive ascertainment system.[185] In Australia, about one baby with CRS per 2000 births was recorded before vaccine.[223] During an outbreak of rubella in Israel, there were 1441 confirmed infections in pregnant women, most of whom had abortions.[224] Projection of the experience to the United States would be equivalent to 75,000 pregnancies complicated by rubella. In France, 16% of congenital cataract cases were attributable to CRS.[225] A follow-up study of an epidemic in Poland showed that 15% of pregnant women had been infected. The rate of CRS after first, second, and third trimester infection was 78%, 33%, and 0%, respectively.[226] The picture of rubella in the developing world is complex.[151,227] Although a frequent seroepidemiologic finding has been a high rate of infection early in life,[159] there are many exceptions, including island populations,[228] several West African countries, the city of Calcutta, and Morocco.[229] Thus, the pattern is that of a disease that comes in epidemic waves, but that may be absent from an area for some time.

The epidemiology of CRS is known in detail for only a few countries of the world. Clusters of CRS have been reported in developing countries,[212,230,231] and there is little doubt that, wherever seronegative pregnant women are exposed to a rubella outbreak, CRS cases will follow. A study conducted in Chennai, India, confirmed the presence of rubella virus in 18% of congenital cataract cases, with serologic evidence of infection in 25%[232] (see "Epidemiology" earlier).

The most complete analysis of CRS globally was performed by Vynnycky and Adams (unpublished data, 2011). They

TABLE 53.9 Estimated Incidence Rate of CRS Per 100,000 Live Births and Number of Cases of CRS by WHO Region and Globally in 1996 and 2010[a]

	1996		2010	
WHO Region	**Number**	**Range**	**Number**	**Range**
Africa	29,692	6535–70,996	40,680	8923–97,483
Americas	9683	2577–19,081	3	0–394
East Mediterranean	9251	3007–22,297	5720	73–19,537
European	9749	5986–13,367	12	1–983
Southeast Asia	47,621	3651–138,674	47,527	3317–139,760
Western Pacific	11,707	6617–15,676	9127	4831–11,066
Global	117,703	28,372–280,090	103,068	17,146–269,223

[a]Adams E, Vynnycky E. Unpublished data, 2012.
CRS, congenital rubella syndrome; WHO, World Health Organization.

modeled the incidence of the disease in all WHO member states for the years 1996 and 2010 and derived the estimate of 103,068 annual cases of CRS globally, although the confidence limits were wide, ranging from 17,146 to 269,223 cases. Table 53.9 shows their estimates.

PASSIVE IMMUNIZATION

Immune Serum Globulin

Because most adults have had rubella, immune serum globulin (ISG) contains rubella antibody.[233] By the HI test, rubella antibody titers of approximately 1:16 are found in immunoglobulins.[234] Before the development of vaccine, ISG frequently was offered to pregnant women who had been exposed to rubella in the hope that it would prevent fetal infection. The results were equivocal, but if large doses (20 to 30 mL) of material were given, frank symptoms and viremia could be prevented.[235–239] Experimental studies confirmed the efficacy of passive antibody in preventing both viremia and clinical rubella,[240,241] but there were numerous failures of γ-globulin to prevent congenital fetal abnormality in actual practice.[237,241] In one study of CRS patients, 6% of mothers gave histories of receipt of γ-globulin after exposure.[140] Thus, whatever the real efficacy of ISG, it is unlikely to be complete.

The sole current indication for ISG is the exposure to rubella of a pregnant seronegative woman who will not accept abortion if infection is proved. If 1 week or less has elapsed since exposure and a serum specimen is taken first to confirm susceptibility, ISG in large amounts (20 mL) can be given intramuscularly. Convalescent specimens should be obtained 3 and 4 weeks later to search for IgM antibody and a rise in IgG titer. The absence of a rash in the exposed woman does not mean that viremia and fetal infection have been prevented. The newer intravenous γ-globulins do not have high concentrations of rubella antibodies, presumably because the antibodies are now the result of immunization rather than disease.[234]

Hyperimmune Rubella Globulin

To overcome the deficiencies of ISG, a hyperimmunoglobulin was prepared by Cutter Laboratories (Elkhart, IN) from the sera of normal individuals who had high rubella antibody titers. The titer of rubella virus-specific antibodies in this preparation was 1:8000 by HI. A controlled clinical trial of the preparation was performed with volunteers who were inoculated first with live unattenuated rubella virus and then given hyperimmune rubella globulin 24 to 96 hours later. With a challenge given intranasally, viremia was detected in two of five control subjects and in one of 10 subjects given γ-globulin. Pharyngeal excretion was similar in the two groups.[242] Although a previous experimental study with high-titered globulin had given better results,[240] the production of hyperimmunoglobulin was stopped, and it is not now commercially available.

ACTIVE IMMUNIZATION

Nonliving Vaccines

Advances in molecular biology allow consideration of a subunit-inactivated vaccine against rubella. The genome of the virus has been sequenced, in particular the genetic code for the E1 protein,[243,244] which carries multiple neutralizing epitopes located between amino acids 214 and 285 of the 481 amino acids contained in the polypeptide.[245–247] T-cell epitopes also were defined on the E1 protein, although no single epitope was recognized by a majority of individuals.[248–252] The E1 protein has been produced in quantity in baculovirus vectors and Chinese hamster ovary cells by truncating the C terminus to allow secretion.[253–257] The immunogenicity of bioengineered E1 has been moderate,[253] but strong adjuvants have produced good responses in animals.[258] Synthetic peptides also have generated neutralizing antibodies,[259] and virus-like particles containing the three main viral proteins have been produced from transfected cell lines, with retention of immunogenicity.[260,261] Other possible strategies for rubella vaccines include nucleic acid vaccine. Pougatcheva and coworkers[262] raised neutralizing antibodies in mice by injecting complementary DNA coding for the envelope glycoproteins. Although it is doubtful that an inactivated vaccine could provide protection from infancy throughout the childbearing period, it might be useful for immunizing adult women.

Live Virus Vaccine

Vaccine Strains: Origin and Development

Several vaccine strains were developed soon after the isolation of rubella virus in tissue culture.[263] Three vaccines were licensed in the United States in 1969 to 1970, as follows: HPV-77 (human papillomavirus 77; originally passaged 77 times in monkey kidney cells and then adapted to duck embryo),[264] HPV-77 (dog kidney),[15] and Cendehill (rabbit kidney).[16] In the same period, the RA27/3 human diploid fibroblast vaccine was licensed in Europe.[17] Between 1969 and 1979, the duck embryo derivative of HPV-77 was widely used in the United

States, in particular as part of the first measles-mumps-rubella (MMR) vaccine. The result was a sharp decline in rubella incidence commensurate with vaccine coverage in children of 60% to 70% by 1978.[265] HPV-77, in its original form and as a duck embryo–passaged virus, was immunogenic in more than 95% of subjects and, during outbreaks, gave protection to 65% to 94% of vaccinees.[15,266] Artificial challenge studies showed good protection against infection.[267] However, comparative studies of HPV-77 (duck embryo) versus RA27/3 conducted during the same period revealed lower antibody levels,[268,269] less persistent seropositivity,[270] less resistance to reinfection,[271,272] less herd immunity,[273] and more joint symptoms[274] in those vaccinated with the former strain, ultimately leading to replacement by RA27/3 in a new MMR vaccine (MMR-II). HPV-77 (dog kidney) and Cendehill had been previously withdrawn from American licensure when RA27/3 was licensed in the United States in 1979, and HPV-77 (duck embryo) was then withdrawn, leaving RA27/3 as the only American rubella vaccine. The RA27/3 strain is also the most widely used throughout the world, except in Japan and China (Table 53.10).[275] This strain was adopted because of its consistent immunogenicity, induction of resistance to reinfection, and low rate of side effects.[276] Accordingly, most of the information presented hereafter concerns the RA27/3 strain, with the addition of data on the other strains that can be extrapolated to the currently used vaccine.

RA27/3 was isolated from a fetus infected with rubella in early 1965.[263,277] Culture fluid from a tissue explant was passaged directly into WI-38 cells, and eight serial passages were made in WI-38 cultures incubated at 37°C. Additional passages then were done in cultures incubated at 30°C. After seven passages at 30°C, studies using human volunteers showed that the strain was attenuated. To reduce the pathogenicity even further, 10 additional passages were made.[278] The RA27/3 strain is produced as a vaccine strain between the 25th and 33rd passages in human diploid cells (WI-38 or MRC-5).[276] The relatively rapid attenuation by passage may be attributable to the use of cold adaptation, whereas the retention of high immunogenicity may be attributable to the low number of passages required to attenuate.[279] The nucleotide sequence of the envelope genes of RA27/3 has been sequenced, revealing 31 amino acid changes in the vaccine compared with the sequence of a wild strain.[280] In comparison, only five changes were noted in the HPV-77-DE strain.[281]

Strain-specific nucleotide sequences have been demonstrated in the RA27/3 strain, permitting specific identification.[282] Although some antigenic variations in rubella virus have been discerned with rabbit antibodies against *Escherichia coli*–expressed proteins,[283] studies employing a panel of monoclonal antibodies showed no significant differences (see "Virology" earlier).[284]

In Japan, five Japanese strains were attenuated by passage in the following substrates: Matsuba—AGMK, swine kidney, rabbit kidney; Matsuura—AGMK, chick embryo amniotic cavity, quail embryo fibroblasts; Takahashi—AGMK, rabbit testicle, rabbit kidney; TCRB19—AGMK, bovine kidney, rabbit kidney; and TO-336—AGMK, guinea pig kidney, rabbit kidney.[285] As of 2011, only three of these rubella vaccine strains (Takahashi, Matsuura, and TO-336) are still being administered.[286] One Japanese vaccine strain, TO-336, has been sequenced before and after attenuation.[287] Nucleotide mutations were discovered at 21 sites, 13 in the genes for nonstructural proteins, five in the genes for structural proteins, and three in noncoding regions. Although these mutations gave rise to 10 amino acid changes, none could be associated with attenuation. Moreover, the mutations were different from those of two other attenuated strains (RA27/3 and Cendehill). Two papers on Japanese strains attributed temperature sensitivity to mutations in the p150 nonstructural gene.[288,289] In China a strain called BRD-II is in use, but no details of attenuation are published in English.[290] The RA27/3 viruses being used by Merck (Whitehouse Station, NJ) and by GlaxoSmithKline (Research Triangle Park, NC) as part of their MMR combination have been shown to be identical by nucleotide sequencing.[291]

TABLE 53.10 Current Manufacturers of Rubella Vaccines

Manufacturer	Virus Strain	Cell Substrate
Merck (United States)	RA27/3	HDCS
GlaxoSmithKline–RIT (Belgium)	RA27/3	HDCS
Sanofi Pasteur (France)	RA27/3	HDCS
Institute of Immunology (Yugoslavia)	RA27/3	HDCS
Serum Institute of India	RA27/3	HDCS
Kitasato Institute (Japan)	Takahashi	Rabbit kidney
Biken (Japan)	Matsuura	Quail embryo fibroblast
Takeda Chemical Industries (Japan)	TO-336	Rabbit kidney

HDCS, human diploid cell strain.
Modified from Perkins FT. Licensed vaccines. Rev Infect Dis. *1985;7:S73–S76.*

Dosage and Route of Administration

The vaccine dose of RA27/3 is required to be at least 1000 plaque-forming units (PFU) of virus delivered subcutaneously. However, titration studies in humans showed that, in keeping with its being a live vaccine, even small subcutaneous doses (<3 PFU) of RA27/3) are immunogenic.[292] A peculiar attribute of RA27/3, not so far demonstrated with any other rubella vaccine strain, is its immunogenicity when administered intranasally.[293–299] Some studies suggested that intranasal administration might confer an advantage on the vaccinees in terms of quality of the immune response.[300] However, the subcutaneous administration of vaccine gave similar humoral antibody with only slightly less secretory antibody.[277,300] Moreover, the intranasal dose of RA27/3 required for consistent immunization is high: 10,000 PFU.[295] Lower doses result in frequent failures,[301] particularly in children, possibly because of the mechanics of administration. Nevertheless, under careful conditions, some workers have been able to achieve 95% seroconversion rates, only slightly inferior to those rates achieved with subcutaneous injection of RA27/3.[298]

Mexican workers have attempted vaccination against measles, mumps, and rubella by a small-particle aerosol, and in preliminary studies have achieved seroconversion rates indistinguishable from those obtained with subcutaneous inoculation.[302,303] A trial was also done in young adults comparing a MMR vaccine given subcutaneously or by aerosol. Seroconversion of the small percentage of seronegative subjects and geometric mean titers in the two groups were quite similar after vaccination by either route.[304]

Aerosol administration may have public health advantages and is being explored further by the WHO.

Combination With Measles and Mumps

In most countries, rubella vaccination is accomplished with a triple vaccine that also contains measles and mumps vaccine viruses (MMR). The American triple formulation (MMR II; Merck Sharp & Dohme, Whitehouse Station, NJ) contains the

Moraten attenuated measles virus (1000 median tissue culture infective doses [$TCID_{50}$]), the Jeryl Lynn mumps virus (5000 $TCID_{50}$), and the RA27/3 rubella virus (1000 $TCID_{50}$). Three formulations are available in Europe and elsewhere. Pluserix (GlaxoSmithKline) contains the Schwarz measles virus (1000 $TCID_{50}$), the Jeryl Lynn–like mumps strain (20,000 $TCID_{50}$), and RA27/3 rubella virus (1000 $TCID_{50}$). Trimovax (Sanofi Pasteur, Swiftwater, PA) contains the Schwarz measles virus (1000 $TCID_{50}$), the Urabe mumps virus (20,000 $TCID_{50}$), and the RA27/3 rubella virus (1000 $TCID_{50}$). Morupar (Chiron, Emeryville, CA) contains the same three viruses, but the mumps virus concentration is given as at least 5000 $TCID_{50}$. For those who prefer not to vaccinate against mumps, a measles and rubella combination is produced by Sanofi Pasteur and the Serum Institute of India (Mumbai), which manufactures three different rubella-containing formulations: rubella-only vaccine (Wistar RA27/3; 1000 $TCID_{50}$), measles-rubella (MR) (with the addition of Edmonston Zagreb measles virus; 1000 $TCID_{50}$), and MMR (Tresivac, with the addition of L-Zagreb mumps strain; 5000 $TCID_{50}$).

Production and Constituents of Vaccine

RA27/3 is manufactured on a human diploid cell substrate, either WI-38 or MRC-5 fetal lung fibroblasts. Cell cultures inoculated with the seed virus are incubated at 30°C. After 4 to 7 days of initial incubation, there is sufficient virus in the supernatant medium to harvest. Fresh medium is added, and subsequent harvests can be made every 2 to 3 days for several weeks. Stabilizer is added to the harvest fluids, which are frozen for later safety testing and pooling before eventual lyophilization.[305,306] The final RA27/3 vaccine is essentially free of animal serum but does contain 0.4% human albumin, 25 to 50 µg/mL neomycin, and, in one case (Chiron), 50 µg/mL of kanamycin. However, human albumin is being replaced with recombinants-produced albumin. The lyophilization medium varies according to the manufacturer; however, it generally contains sucrose or sorbitol, glutamic acid and other amino acids, and buffering salts. When lyophilized, the vaccine is hypertonic. Reconstitution is accomplished with preservative-free sterile distilled water (0.5 to 1.0 mL) according to the manufacturer's directions, which restores the vaccine to a normal or slightly hypertonic state.

RA27/3 is produced in the United States, the United Kingdom, France, Belgium, Italy, Switzerland, Yugoslavia, and India by the manufacturers listed in Table 53.10. Despite minor differences in dose, antibiotic content, and other details among manufacturers, differences in vaccine efficacy or in the nature or severity of side effects have not been reported.

Stability of Vaccine

Rubella vaccine is highly stable in the frozen state at approximately −70°C or approximately 20°C. At 4°C, the viability of the virus and the potency of the vaccine also are maintained for at least 5 years. At room temperature, there is significant loss after 3 months; at 37°C, a 3-week period is sufficient to damage vaccine potency.[307] The vaccine should be stored at 2°C to 8°C and protected from light. The virus is labile after reconstitution and should be used within 8 hours.

Results of Vaccination

Immune Responses

Vaccination induces antibodies of both IgM and IgG classes and cellular immune responses. Secretory IgA responses are also induced.

Most studies of immunogenicity have been done by measuring HI responses, although the neutralizing responses may be more important biologically. By the HI technique, 95% to 100% of RA27/3 vaccinees experience seroconversion by 21 to 28 days after vaccination, with geometric mean antibody titers ranging from 1:30 to 1:300, depending on the method of titration.[17,278,308,309] Some of the apparent failures, at least in young adults, may be explained by preexisting low levels of antibody that neutralize the vaccine virus but are detectable only by sensitive tests.[310]

Several direct comparative immunogenicity studies have been done with different strains. A trial comparing the administration of the RA27/3 and Cendehill strains to Scottish schoolgirls resulted in a 98% seroconversion for the former and 90% for the latter.[311] In Sweden, Bottiger and Heller[312] found a 98% response after inoculation with RA27/3 and 96% after inoculation with Cendehill. In another Swedish trial, Grillner[313] tested neutralizing titers after vaccination, and 95% of RA27/3 vaccinees were seropositive compared with 56% of Cendehill vaccinees. Menser and colleagues[314] tested RA27/3 and Cendehill in Australian schoolgirls and adults; they found no differences in seroconversion but better boosting of seropositive individuals by RA27/3. Weibel and associates[315] conducted large comparative studies of RA27/3 and HPV-77DE that showed higher immunogenicity of the former strain. The Japanese TO-336 and the Chinese BRD-II strains compared favorably with RA27/3 in parallel studies.[316,317] A vaccination study conducted in India using MMRV showed 99% to 100% seroconversion to rubella after one dose.[318] RA27/3 elicits complement-fixing and precipitating antibody titers in nearly all vaccinees.[277] In a precipitin test system, RA27/3 was the only vaccine strain that evoked antibodies to the iota internal antigen of rubella virus.[319] Vaccination induces antibodies predominantly binding the E1 protein as detected by immunoblot, but those antibodies mature in avidity less rapidly than after natural infection and do not reach the same level.[320]

The induction of neutralizing antibody is particularly significant and is seen regularly and promptly after the administration of RA27/3. Fig. 53.2 and Table 53.11 present comparative data obtained in New Haven, Connecticut,[222] and in Sweden.[312] Immunoblot analysis confirmed the persistence of antibodies for at least 3 years to the E1 protein that bears neutralizing epitopes. Antibodies to the C protein also persisted, but antibodies to E2 were often absent.[321]

A crucial property of RA27/3 is its ability to induce secretory IgA antibody in the nasopharynx, which, as discussed subsequently, may prevent reinfection with wild virus. This property makes vaccination with RA27/3 similar to natural infection, which also induces local immunity. Although secretory IgA responses are higher after intranasal vaccination, they also are induced by subcutaneous vaccination with RA27/3

TABLE 53.11 Neutralizing Antibody Response in 114 Rubella-Vaccinated Women Who Demonstrated Seroconversion With Hemagglutination-Inhibiting Antibodies

Vaccine	Before Vaccination[a]	After Vaccination	
		8 Weeks	2 Years
Cendehill	1/45	24/43 (56%)	27/33 (82%)
HPV-77 duck	2/29	23/29 (79%)	16/17 (94%)
RA27/3	1/40	37/39 (95%)	20/20 (100%)

[a]Number positive/number tested.

From Grillner L. Neutralizing antibodies after rubella vaccination of newly delivered women: comparison between three vaccines. Scand J Infect Dis. *1975;7:169–172.*

because of replication of the virus in the nasopharynx.[322–324] IgA antibodies were detected 5 years after vaccination with RA27/3, when they had disappeared subsequent to other vaccine strains.[325]

Not surprisingly, vaccination, like disease, is followed by the early production of IgM-class antibodies. These antibodies reach a peak at 1 month after vaccination[287] and last approximately l month more but may persist longer at low levels.[325–327]

Cellular immune responses to vaccination have been studied, although they have no known significance to protection. A proliferation of lymphoblasts in response to rubella antigen appears 2 weeks after vaccination, without suppression of tuberculin hypersensitivity.[328] Relatively short-lived cellular responses also were seen in other studies.[329–332] Human leukocyte antigen (HLA)-restricted T-cell cytotoxicity was shown to increase after immunization.[333] Morag and coworkers[334] showed that cytotoxic T-lymphocyte activity was high in tonsillar lymphocytes after intranasal vaccination (with RA27/3) but was low after subcutaneous vaccination (with HPV-77DE). Unfortunately, subcutaneous administration of RA27/3 was not evaluated. An association of rubella antibody response with class II but not class I HLA antigens has been demonstrated.[335,336] Elevation of tumor necrosis factor-α (TNF-α), interleukin (IL)-4 (IL-4), and IL-10 after vaccination has been reported.[337] A study of second-dose MMR revealed effects on the distribution of lymphocyte classes, but no functional changes.[338]

A group from the Mayo Clinic has studied single nucleotide polymorphisms (SNPs) in relation to both antibody and cellular responses to rubella revaccination and have found numerous SNPs associated with higher or lower responses, although none that eliminated responses entirely. The SNPs were located in HLA genes and cytokine receptor genes.[339–343] These extensive studies have recently been summarized.[69a] Males may have somewhat higher immune responses than females.[344] Viremia has been documented between 7 and 11 days after RA27/3 inoculation.[345] However, the viremia is low and inconstant. Pharyngeal excretion of virus is more frequent, occurring from approximately 7 to 21 days after vaccination, in low titer of usually less than 10 PFU per swab. Excretion peaks on approximately the 11th day after vaccination; if properly tested, essentially all vaccinees will be shown to excrete virus from the nasopharynx.[346,347]

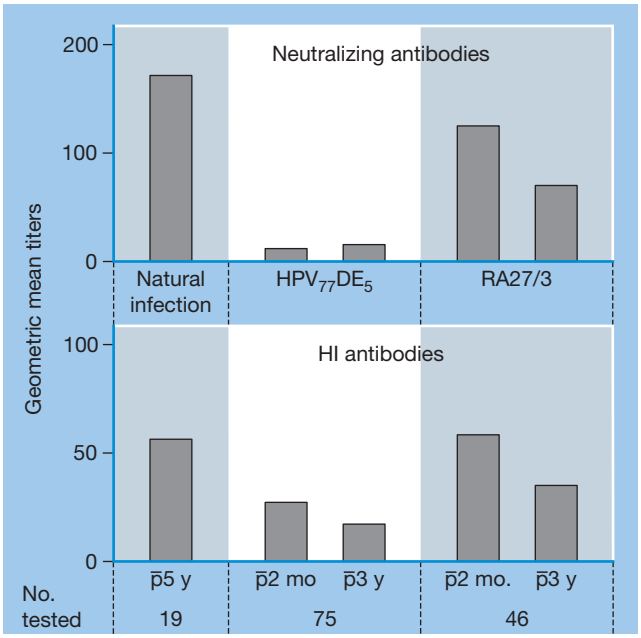


Figure 53.2. Comparison of neutralizing antibodies and hemagglutination-inhibiting (HI) antibodies in three groups of persons: naturally infected children, children given human papillomavirus 77 duck embryo ($HPV_{77}DE_5$) vaccine, and children given RA27/3 vaccine. *(From Horstmann DM. Viral vaccines and their ways.* Rev Infect Dis. *1979;1:502–516.)*

Contact Spread. In view of the excretion of rubella virus by vaccinees, considerable effort has been made to detect the spread of vaccine virus to susceptible contacts. Initially, contact studies were focused on children in institutions and on families, and no evidence for spread of vaccine virus was found.[16,17,275] For example, none of 393 seronegative family members was infected by contact with RA27/3 vaccinees.[17] Veronelli[348] studied 347 familial contacts of HPV-77DE vaccinees and also found no evidence of spread.

Experience in studies of contact spread produced largely negative results, with the rare asymptomatic seroconversion that could not be explained fully.[349,350] In the early 1970s, many studies were conducted in contacts of HPV-77DE or Cendehill strain vaccinees, including pregnant women, marital partners, school teachers, and the general population, with little or no evidence of spread.[351–357] The general lack of evidence for spread by vaccine virus may reflect the maintenance of attenuated markers by excreted virus, as demonstrated for RA27/3.[358]

Rubella in the Measles-Mumps-Rubella Combination. Responses to rubella as part of MMR combinations are equal to those seen after rubella vaccination as a single antigen. Table 53.12 is taken from a study by Weibel and colleagues[315] that compared MMR formulated with RA27/3 to RA27/3 alone. The excellent responses in both groups of vaccinees

TABLE 53.12 Antibody Responses in Initially Seronegative Children Who Received Combined Measles (Moraten)-Mumps (Jeryl Lynn)-Rubella (RA27/3) or Monovalent RA27/3 Rubella Vaccine

	Antibody Responses Versus								
	Measles (HI) Conversion			(Mumps Neutralizing) Conversion			Rubella (HI) Conversion		
Vaccine	N/Total	%	Geometric Mean	N/Total	%	Geometric Mean	N/Total	%	Geometric Mean
Combined	64/68	94	57	65/68	96	8	68/68	100	136
Monovalent	–	–	–	–	–	–	67/67	100	159

HI, hemagglutination inhibition; N, number.
From Weibel RE, Carlson AJ, Villarejos VM, et al. Clinical and laboratory studies of combined live measles, mumps, and rubella vaccines using the RA27/3 rubella virus. Proc Soc Exp Biol Med. *1980;165:323–326.*

have been confirmed by other investigators,[359,360] who also found RA27/3 to be superior to the earlier HPV-77DE component.[359] Seroconversion to rubella vaccination with any of the other triple combinations (see "Immune Responses" earlier) is usually 97% to 98%.[361,362] Bivalent measles-rubella and mumps-rubella vaccines also produce rubella antibody levels that are equivalent to those of the monovalent vaccine.[359,363] Comparative studies of the Merck and GlaxoSmithKline triple combinations showed excellent seroconversion to rubella after both vaccines.[364,365]

In summary, rubella vaccines induce immune responses that are similar in quality but lesser in quantity than those after natural disease.[366,367] The live virus produces viremia and pharyngeal excretion, but both are of low magnitude and are noncommunicable. IgG and IgM antibody responses follow vaccination. Natural infection elicits nasal secretory antibody that may be useful in the prevention of reinfection, and RA27/3 vaccine also has the same property.

Effect of Maternal Antibody. On average, maternal rubella antibody levels are detected only for approximately 2 months after birth in the infant, and thus rubella vaccine does not suffer the same problem as measles vaccination in early life, where maternal antibodies interfere with infant vaccination.[368] Studies have documented that the seroconversion rate in infants at 9 months of age is similar to that of children older than 12 months of age.[318,369,370]

Immunocompromised Subjects. Children who have recovered from chemotherapy for hematologic malignancies and solid tumors usually lose their rubella antibodies that were generated by prior vaccination. One year after chemotherapy a booster dose will restore their immunity.[371] Rubella revaccination of children with juvenile rheumatoid arthritis using MMR induced good responses despite the children being treated with methotrexate and antibody to TNF-α receptor.[372]

A study of children who had received bone marrow transplants also showed frequent loss of antibodies (33%), but revaccination 2 years later induced an anamnestic response with high-avidity antibodies.[373] Vaccination of children with the DiGeorge congenital immune deficiency was not followed by severe adverse reaction, and 93% of subjects seroconverted at titers equal to controls.[374] Patients with HIV infection are discussed later under "Contraindications."

Protective Effects

The protective efficacy of rubella vaccination has been assessed (a) by observation of vaccinees and control subjects during natural epidemics and (b) by intranasal challenge of vaccinated volunteers with unattenuated or attenuated viruses. No double-blind efficacy study has been done, but there is much evidence for effectiveness.

During an institutional outbreak of rubella, Davis and colleagues[267] were able to evaluate the effect of natural rubella exposure on persons who had been vaccinated with HPV-77DE. Clinical rubella occurred in 22 of 33 unvaccinated seronegative individuals but in none of 22 vaccinees and 66 naturally immune individuals. Asymptomatic reinfection without viremia was noted in five vaccinees (23.0%) and in one naturally immune subject (1.5%). Grayston and associates[375] compared the effect of three HPV-77 vaccines with a placebo during an epidemic on Taiwan. Rubella incidence began to drop in the vaccinated group within 2 to 3 weeks, and vaccine efficacy was estimated at 94%. In a separate study undertaken during the same outbreak, RA27/3 vaccine gave a 97% protection rate. A group of children in daycare was observed by Chang and colleagues[376] after some had been vaccinated with Cendehill. The vaccine afforded complete protection against disease, but 50% of the vaccinees were reinfected.

A dramatic example of protection by vaccine was reported from Japan.[377] An outbreak started in a school for apprentices at the Toyota automobile factory. RA27/3 vaccine was given randomly to a third of the boys. As shown in Fig. 53.3, the epidemic continued for 7 weeks, but rubella ceased to occur in vaccinees 2 weeks after they were vaccinated. Observation of an outbreak that occurred in Chinese school children allowed calculation of 99.5% efficacy of prior RA27/3 vaccination.[378]

An outbreak of rubella in a primary school in France during 1997 permitted a calculation of effectiveness because only approximately 75% of children had been vaccinated. Effectiveness of RA27/3 against clinical rubella was 95% (85% to 99% confidence interval) and almost 100% for laboratory-confirmed rubella.[379] An effectiveness study done with Italian military recruits gave a result of 94.5% for rubella vaccination.[380]

In Olmstead County, Minnesota, adolescent girls were vaccinated with Cendehill; unvaccinated boys were used as control subjects, which permitted a calculation of efficacy at 94% during a 1972 outbreak.[381] The introduction of rubella vaccination of recruits at Lackland Air Force Base resulted in a 95% reduction in rubella by 1979.[382] A 1980–81 outbreak in Sanford, Maine, allowed an assessment of vaccine efficacy 8 years after vaccination; it was 90%.[383]

In Finland, five cases of rubella encephalitis were seen at Helsinki Children's Hospital during a 15-year period, starting in the late 1960s, whereas none has been seen since the advent of MMR vaccination in 1982.[384] The Chinese BRD-II strain was shown to have high efficacy in an outbreak in Guangzho.[290] It is possible that rapid administration of rubella vaccine to contacts of a case would provide prophylaxis, but this possibility has not been tested in any controlled fashion.

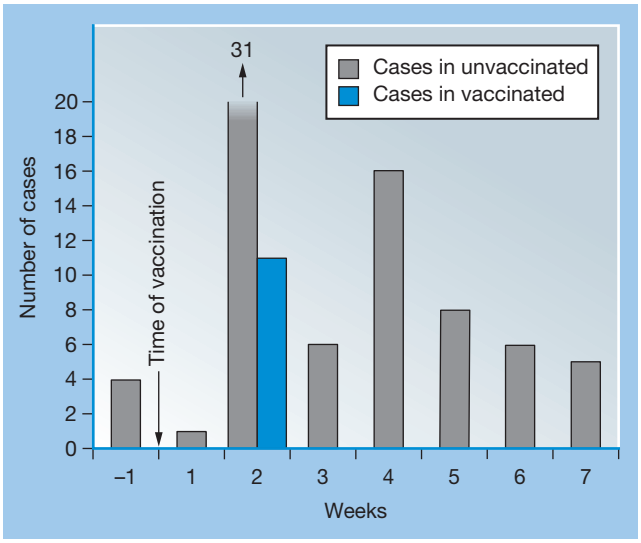


Figure 53.3. Demonstration of protection against rubella during an epidemic in a boys' school at the Toyota car factory near Nagoya, Japan. Boys were selected at random to receive RA27/3 vaccine at the time indicated, and about one-third received the vaccine. *(From Furukawa T, Miyata T, Kondo K, et al. Rubella vaccination during an epidemic.* JAMA. *1970;213:987–990. Copyright 1970, American Medical Association.)*

Correlates of Immunity

The immune response that correlates best with protection is neutralizing antibodies.[385] However, on the one hand, even vaccinees with low levels of serum antibodies are often protected,[386] while on the other hand, reinfection may take place in some individuals with measurable antibodies. Because measurement of neutralizing antibodies is not routinely available, 15 IU of antibodies measured by other methods has been proposed as a correlate of protection.[387] However, infants with CRS have been born to mothers who have antibody levels of 15 IU or greater. Review of epidemiologic data in the United States demonstrated protection at 10 IU, so the cutoff for protection was set at 10 IU in the United States.[128] Although there is some variation among countries in terms of the concentration of IgG antibodies considered to be protective, the presence of rubella IgG antibodies greater than or equal to 10 IU/mL is commonly considered to provide evidence of protection (WHO immunologic basis).[134]

Although cellular immunity seems to have little correlation with protection from replication, arrest of viral excretion by CRS patients correlates with the appearance of specific lymphoproliferation responses (Best J. Personal communication to SA Plotkin, 2001).

Reinfection and Herd Immunity

Early in rubella vaccine studies, it became evident that, under conditions of exposure to wild virus, reinfection of persons vaccinated with HPV-77 and Cendehill could occur with a frequency of 50% or greater.[271,272,388–390] Herd immunity was shown to be ineffective when rubella broke out in a company of military recruits.[391] Although most recruits had antibodies as a result of vaccination or prior infection at the start of the epidemic, 100% of the remaining susceptible individuals were infected. Moreover, 80% of the recruits vaccinated with HPV-77DE were reinfected. The same conclusion was reached in another study of recruits, in which rubella singled out the susceptible individuals despite high levels of immunity in the group.[392]

Bermuda experienced a rubella outbreak in 1971. A vaccination campaign with Cendehill resulted in prompt termination of the epidemic, and vaccine efficacy was estimated to be 94%.[393] However, a rubella epidemic in Casper, Wyoming, in the same year, showed that although the vaccine (HPV-77DE) prevented rubella in immunized elementary school children, spread to unimmunized adolescents and adults resulted in an attack rate that was 50% of that expected without vaccination.[394,395] Fogel and colleagues[242] used intranasal RA27/3 as a challenge to RA27/3, Cendehill, and HPV-77DE vaccinees. Their results showed a higher rate of reinfection in the last two groups compared with that in RA27/3 vaccinees (Table 53.13).

TABLE 53.13 Reinfection[a] of Vaccinees After Virus Challenge

Vaccinees	Previous Vaccine Given	Vaccinees Showing Reinfection	
		Total No.	Percentage
Cendehill	27	18	66.7
HPV-77	30	14	45.7
RA27/3	28	2	7.1

[a]As revealed by a positive booster in at least one of four serologic tests.
From Fogel A, Gerichter CB, Barena B, et al. Response to experimental challenges in persons immunized with different rubella vaccines. J Pediatr. *1978;92:26–29.*

Among RA27/3 vaccinees, approximately 5% had low titers 6 to 16 years after vaccination.[396] A 9.8% incidence of asymptomatic reinfection was reported 5 years after vaccination.[397]

Harcourt and coworkers[388] concluded that reinfection is dependent on a number of immunologic factors, including, but not restricted to, the presence of nasopharyngeal IgA rubella antibody. When the RA27/3 virus itself was given intranasally as a challenge virus, O'Shea and coworkers[386] found that reinfection, as determined by titer rises, was infrequent in those who had significant titers (>15 IU) of rubella antibody from previous infection or vaccination, but was frequent in vaccinees who had low titers (<15 IU). However, reinfection was not associated with lack of neutralizing antibodies, and viremia as part of the reinfection was rare (Table 53.14).

In some studies, reinfection led only to an IgG booster response, without IgM,[398] whereas in others, the appearance of IgM antibody suggested significant viral replication.[399,400] The distinction between primary infection and reinfection can be more accurately assessed by avidity antibody testing than by IgM tests. In primary infection, low-avidity antibody is found, whereas in reinfection, antibody is of high avidity.[387,399,401]

Clinical rubella has been documented during reinfection of vaccinees[402,403] and naturally immune individuals.[404,405] Moreover, maternal reinfection during pregnancy has resulted in congenital rubella.[62,168,406–422] In a prospective study, Morgan-Capner and associates[423] found definite evidence of passage of virus from reinfected mothers to their fetuses in two of three cases in which exposure had occurred in the first trimester and fetal tissue was available. However, follow-up of seven live-born infants did not reveal CRS. O'Shea and colleagues[424] measured rubella immunity in reinfected women and reinfected seropositive volunteers undergoing a challenge with rubella virus. The presence or absence of serum neutralizing antibodies or cellular immunity did not correlate with the likelihood of reinfection. However, Matter and coworkers[387] suggested that reinfection may be more likely at low levels of antibody as measured by hemolysis in gel (10 to 15 IU), and Mitchell and colleagues[425] reported that reinfection was correlated with lack of prior antibodies to a peptide of the E1 protein.

The best conclusion at this time is that reinfection with fetal transmission of wild rubella virus is a fact in the presence of both natural and vaccine-induced immunity, but that the

TABLE 53.14 Viremia and Virus Excretion in Volunteers After Subcutaneous or Intranasal Challenge With RA27/3 Rubella Virus Vaccine

Antibody Status Before Vaccination	No. of Vaccinees	Viremia	Virus Excretion
Seronegative	21	15	12 (6–14)[a]
Seropositive (>15 IU)	10	0	1 (7)
Low titer (≤15 IU)			
Natural infection	12	0	0
Previous vaccination			
RA27/3	7	0	0
Other strains	12	1 (12)	3 (6–9)

[a]Numbers in parentheses indicate days after vaccination on which findings were positive.
Modified from O'Shea S, Best J, Banatvala JE. Viremia, virus excretion, and antibody responses after challenge to volunteers with low levels of antibody to rubella virus. J Infect Dis. *1983;148:639–647.*

risk of fetal transmission is probably less than 5% in the first trimester of pregnancy; however, the risk of CRS is even rarer.

Persistence of Immunity and Revaccination

Numerous data are available on persistence of antibody after vaccination. Liebhaber and colleagues[426] found antibodies in all 18 vaccinees 2 years after vaccination with RA27/3. In response to a challenge with unattenuated rubella virus, 16 of the 18 vaccinees showed resistance to reinfection. Amazonian Indians given RA27/3 by Black and coworkers[427] had persistent antibodies in the absence of any possible reexposure to the virus, although there was a twofold drop during 2.5 years. A 98% to 99% persistence of antibody was noted in schoolchildren 4 years after vaccination.[428] Zealley and Edmond[429] reported on a long-term study of Edinburgh schoolgirls, 97% of whom retained antibodies 6 to 7 years after vaccination with RA27/3. The longest observation periods after vaccination with RA27/3 thus far have been 12 to 17 years,[279] 15 years,[430] and 10 to 21 years.[431] In the latter study, 97% of subjects retained their positive antibody status. In one long-term study, 75 (96%) of 78 vaccinees retained measurable antibody (Fig. 53.4).[279] In a follow-up of Baltimore schoolchildren, there was a decline of less than fourfold in mean titer during a 15-year postvaccination period, and 97% remained seropositive.[432]

Miller and colleagues[433] studied 475 children 4 years after vaccination with MMR and found 97% to be positive for rubella antibodies. A Canadian study of a similar number of children tested 5 to 6 years after vaccination with MMR gave identical results.[434] Bottiger[435] observed 220 Swedish children given MMR at 18 months of age. At 12 years of age, 97% had rubella antibodies, although not at the same levels seen immediately after vaccination. A second dose given at 12 years of age raised the titer levels to those seen after primary vaccination. Christenson and Bottiger[436] also observed a cohort of almost 500 girls vaccinated at age 12 years. After 8 years, 96% were still seropositive, and 94% were still so at 16 years after vaccination, although the mean titers had dropped from 1:110 to 1:18 (Table 53.15). On a population level, between 1988 and 1994, 96% of 6- to 11-year-old children and 94% of adolescents in the United States were seropositive to rubella.[437]

Different results were reported by Johnson and coworkers,[438] who studied 95 children given MMR at 15 months of age. Children studied at age 4 to 6 years were 100% positive for rubella-neutralizing antibodies, but only 63% of children ages 11 to 13 years were positive. They suggested that immunity had waned. After a second dose of MMR, 100% of children became seropositive, with almost a threefold rise in the geometric mean titer of rubella antibodies. In Newfoundland, Ratnam and associates[439] found that 16.5% of children 8 to 17 years of age were seronegative after prior vaccination at 1 year of age. Elsewhere, in Canada, Mitchell and coworkers[440] found ELISA antibodies, "subprotective" neutralizing antibodies, and lymphocyte proliferation responses in 92%, 77%, and 74%, respectively, of adolescents 14 to 16 years after their first dose. Le Baron and coworkers[441] showed that 9% of vaccinated children were seronegative when given a second dose before admission to school, that almost all responded to the second dose, but that 12 years later 10% had become seronegative.

A Dutch seroprevalence study showed that 9 years after the introduction of universal rubella vaccination of infants more than 95% of vaccinees were still seropositive. Moreover, only 5% of reported rubella cases occurred in vaccinees.[442] Another Dutch study of 17- to 23-year-old subjects vaccinated as children showed antibodies in 71% of those who had one dose and 99% in those who had two.[443]

In Finland, titrations were done in children followed over 15 years. Five to 7 years after a single dose, 98% to 100% of the children were still seropositive. They were given a booster dose, after which seroprevalence continued to be 100%. However, at 15 years after a first rubella vaccination and 9 years after a second dose, 30% of children had low titers. Children given a second dose at 11 to 13 years still had elevated titers.[444] Twenty years postvaccination, 100% of children were seropositive.[445] Follow-up of Swedish adult women vaccinated as children showed 96% with rubella antibodies,[446] whereas follow-up of Saudi children in Saudi Arabia and residents in Luxembourg showed rather uniform persistence of antibodies after vaccination.[447,448] Also, in Saõ Paolo, Brazil, and in Korea, 15% to 20% loss of antibodies was seen 3 to 8 years postvaccination,[179,449] A follow-up study conducted in Luxembourg 15 to 20 years after first vaccination in infancy showed 94% seropositivity in those who had received one dose of rubella vaccine and 100% in those who had received two doses.[450]

Reports of persistence of antibody to Cendehill and HPV-77DE strain vaccines, no longer in use, were summarized in the fourth edition of this book, to which the reader is referred.

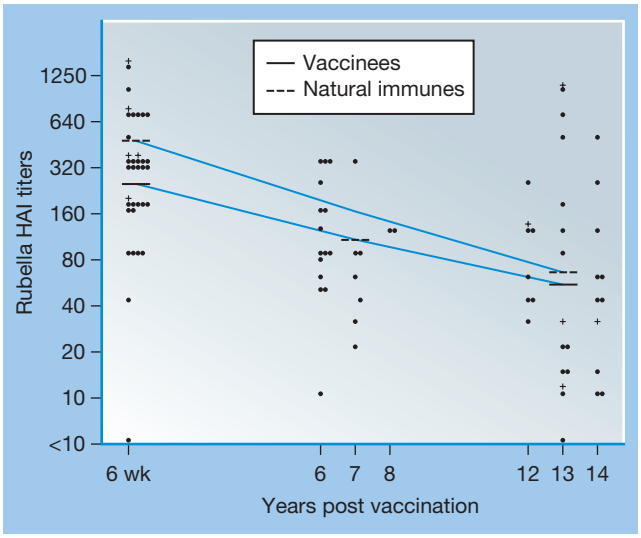


Figure 53.4. Persistence of hemagglutination-inhibiting antibody (HAI) to rubella after natural infection or the administration of RA27/3 vaccine. *(From Plotkin SA, Buser F. History of RA27/3 rubella vaccine.* Rev Infect Dis. *1985;7[Suppl]:S77–S78.)*

TABLE 53.15 Persistence of Rubella Antibodies After Vaccination of Seronegative Schoolgirls

Time After Vaccination	Number	% HI titers ≥1:8	GMT (HI)
8 wk	486	100	110
2 y	346	99	80
4 y	136	99	53
8 y	486	96	34
16 y	190	94	18

HI, hemagglutination inhibition; GMT, geometric mean (antibody) titer.
Data from Christenson B, Bottinger M. Long-term follow-up study of rubella antibodies in naturally immune and vaccinated young adults. Vaccine. *1994;12:41–45.*

However, several observations are of continued interest. Best[451] summarized published studies of antibody persistence 9 to 21 years after vaccination and found a seronegativity rate of 1% for RA27/3 vaccinees, 2.7% for Cendehill vaccinees, and 7.3% for HPV-77DE vaccinees. An epidemic of rubella occurred during 1981 to 1982 among students at the University of California at Los Angeles. Strassburg and colleagues[452] performed a case-control study and found that prior immunization with unspecified rubella vaccines conferred 97% protection, even though some students had been immunized 10 years previously. Natural immunity to rubella lasts at least 26 years.[453]

A follow-up of women vaccinated prepubertally in Taiwan showed that after 20 years approximately 7% were seronegative and 9% weakly seropositive.[454] One study reported good persistence of antibody 10 years after inoculation with the Japanese TO-336 virus.[455] Persistent antibody and protection also followed the use of the Matsuba strain.[456] In a small group of vaccinees followed 23 years after receipt of the Matsuba strain, 15% were negative by HI, but reimmunization nevertheless induced secondary responses.[457] A follow-up study of subjects given the Matsuba strain also showed good persistence of antibodies and T-cell activation.[458]

Table 53.16 attempts to summarize most of the studies done on persistence after vaccination. It appears that antibodies wane after each of the two doses of rubella vaccine, although most vaccinees retain antibodies. Judging from the absence of outbreaks in well-vaccinated countries, such as the United States, persistence of protection may be the result of anamnestic responses that are protective. A recent small study showed good persistence of memory B cells in rubella-vaccinated adults.[459]

Reimmunization has been advocated by some of those who have studied the persistence of antibodies to HPV-77DE and Cendehill,[460,461] and subcutaneous RA27/3 could be given as a booster vaccine for those previously immunized.[462,463] However, ostensibly seronegative individuals usually show secondary immunologic responses after boosters, indicating that they had been immune.[464,465] A study of two doses of rubella vaccine (as part of MMR) showed persistent immunity in 95% of children 2 to 4 years after the first dose, with a boost in geometric mean titers from 41 IU to 72 IU after a second dose.[466] The authors commented that within 3 years of the booster, antibodies fell to prior levels. In any case, the question has now been rendered partially moot by the adoption in many countries of a routine second dose of measles vaccine, usually as a triple vaccine containing rubella. Revaccination is a suggested strategy to augment rubella immunity before the age of reproduction. Individuals who fail to seroconvert after rubella vaccination should be given a single repeated dose. Failure to respond to the second dose may be evidence for tolerance to rubella virus antigens,[136] which is seen in survivors of CRS.[467]

In summary, the need for a second dose of rubella vaccine is unproven. The rare cases of rubella occurring in the United States are in unvaccinated persons, without signs of spread to the general population and suggest that a second dose of rubella vaccine may not be needed. Although titers decrease with time, high rates of seropositivity are maintained. Indeed, considering the long incubation period of rubella (14 to 21 days) an anamnestic response in vaccinees after exposure may offer sufficient protection, making the presence of detectable antibodies at the time of exposure unnecessary. However, the routine indication for a second dose of a measles-containing vaccine, the general use of combination vaccines containing rubella for that purpose, the innocuousness of a second dose of rubella vaccine, the success of two-dose national strategies in eliminating rubella, and the need to maintain protection during the years of childbearing all argue for a two-dose schedule. Although a second dose of rubella vaccine would be preferable just before adolescence, measles control and convenience are better with a second dose at school entry.[468]

Combined and Associated Vaccines

As mentioned previously, when rubella vaccine is given to preschool-age children it is almost always given in combination with measles and mumps vaccines as MMR vaccine, or, more recently, in measles-mumps-rubella-varicella (MMRV) vaccines. Excellent results have been reported for both RA27/3 and Cendehill as components of triple vaccines.[315,469] There is no contraindication to giving MMR even in the presence of immunity to one or two of its components, and American

TABLE 53.16 Long-Term Persistence of Rubella Antibodies After Vaccination With the RA27/3 Strain

References	Country Where Study Carried Out	Years After Vaccination (No. of Doses)	No. Seropositive/ No. Tested (%)	Serologic Method Used
Christenson and Bottiger, 1994[805]	Sweden	16 (1)	184/190 (96.8%)	HI
Enders and Nickerl-Pacher, 1988[60]	Germany	14 (1)	115/115 (100%)	HI
Hillary and Griffith, 1984[806]	Ireland	15 (1)	20/21 (93.3%)	HI
Horstmann et al, 1985[807]	United States	11–12 (1)	35/35 (100%) 33/35 (95%)	NT HI
O'Shea et al, 1988[431]	United Kingdom	10–21 (1)	47/48 (97.9%)	SRH, EIA, latex aggl.
Zeally and Edmond[808]	United Kingdom	12 (1)	93/94 (99%)	SRH
Plotkin and Buser, 1985[279]	United States	12-14 (1)	29/29 (100%)	HI
Johnson et al, 1996[438]	United States	10-12 (1)	36/57 (63%)	NT
Kremer et al, 2000[809]	Luxembourg	7(1)	1224 (92%)	EIA
Vandermeulen et al, 2007[443]	Netherlands	8 (2)	119/119 (100%)	EIA
Davidikin et al, 2008[810]	Finland	20 (2)	275/275 (100%)	EIA
LeBaron et al, 2009[441]	United States	7–12 (2)	521/613 (85%)	NT
Kakoulidou et al, 2010[811]	Sweden	22 (2)	1707/1870 (91%)	EIA

aggl., agglutination; EIA, enzyme immunoassay; HI, hemagglutination inhibition; NT, neutralizing antibody; SRH, single radial hemolysis.

authorities prefer to use MMR exclusively in vaccination programs. Rubella or MMR vaccine can be given simultaneously (but separately) with diphtheria and tetanus toxoids and pertussis vaccine, *Haemophilus influenzae* type b vaccine, inactivated poliovirus vaccine, hepatitis B vaccine, oral poliovirus vaccine,[470] and varicella vaccine.[471]

Adverse Reactions

Common Side Effects

Vaccinees sometimes develop mild rubella, including rash, lymphadenopathy, fever, sore throat, and headache. The incidence of each side effect varies directly with age, being almost absent in infants but present in up to 50% of women, particularly in those older than age 30 years. Fortunately, the minor side effects are seldom severe enough to cause days to be lost from school or work.[312,314,472,473]

A double-blind study of vaccination with MMR in children gave a 1% incidence of arthropathy and little evidence of other reactions.[474] This study was conducted in twins, one of whom received vaccine and the other placebo. The results are given in Table 53.17, which shows that the differences in reactions between vaccinees and placebos were small. The twin study by the same Finnish workers was repeated in 6-year-old children receiving second doses of MMR. The only symptom appearing to be associated with vaccination was arthralgia.[475] Another controlled study revealed no significant differences in reactions of children receiving either MMR or measles vaccines.[476] Female children are more likely than males to have fever and rash after vaccination.[477] Reactions attributable to rubella after second doses of MMR or MMRV vaccines are much more uncommon.[478,479]

TABLE 53.17 Symptoms and Signs Caused by Measles-Mumps-Rubella Vaccination and Day of Peak Occurrence in Finnish Twin Study

Symptoms and Signs	Maximum Difference in Rate[a] (%)	95% CI	Peak Frequency (Days After Vaccination)
Local erythema (>2 cm)	0.8	0.1–1.4	2
Other local reaction	0.4	0–1.4	2
Mild fever (≤38.5°C rectal)	2.7	0–6.1	10
Moderate fever (38.6°C–39.5°C)	2.9	1.6–4.3	9
High fever (≥39.5°C)	1.4	0.7–2.1	10
Irritability	4.1	2.1–6.1	10
Drowsiness	2.5	1.4–3.6	11
Willingness to stay in bed	1.4	0.5–2.3	11
Generalized rash	1.6	0–3.0	11
Conjunctivitis	2.1	0.9–3.2	10
Arthropathy	0.8	0.2–1.3	7–9
Peripheral tremor	0.4	0–0.9	9
Cough and/or coryza	–1.5[b]	–4.6–1.6	9
Nausea and/or vomiting	–0.8[b]	–1.6–0	7–8
Diarrhea	0.7	0–1.7	11

[a]Between measles-mumps-rubella group and placebo group.
[b]More in placebo-injected children.
CI, confidence interval.
From Peltola H, Heinonen OP. Frequency of true adverse reactions to measles-mumps-rubella vaccine: a double-blind placebo-controlled trial in twins. Lancet. *1986;1:939–942.*

Serious Reactions

In 1991, the Institute of Medicine (IOM) of the National Academy of Sciences published a committee report on four possible adverse effects of rubella vaccine: acute arthritis, chronic arthritis, neuropathies, and thrombocytopenia.[480] The committee concluded that RA27/3 causes acute arthritis. With regard to chronic arthritis, the committee stated, "The evidence is consistent with a causal relation between the currently used rubella vaccine strain (RA27/3) and chronic arthritis in adult women, although the evidence is limited in scope and confined to reports from one institution." However, the committee found insufficient evidence to indicate a causal relationship between use of the vaccine and incidence of radiculoneuritis, other neuropathies, and thrombocytopenia. In 2011, the IOM published a committee report on the evidence of adverse health events associated with several vaccines including MMR.[481] As discussed in the following sections, some of these conclusions remain controversial.

Arthritis and Arthralgia

A review of adverse events in Canada gave a reaction rate for rubella vaccine of 28.7 per 100,000 doses distributed, of which 0.3 per 100,000 was arthritis or arthralgia.[481] Acute arthropathy is part of the disease caused by rubella virus, at least in adults, and is also the most important side effect of vaccination.[482] Although the HPV-77 strains are no longer in use, much information on joint reactions was obtained by studying reactions to vaccination with this strain. The HPV-77 dog kidney vaccine was particularly likely to cause reactions in joints, even in children.[21,484] At times, the reactions included neuropathies, such as the "catcher's crouch" syndrome.[25,484–488] The HPV-77DE vaccine produced reactions that were age related; for example, in one study, 0% of girls younger than age 13 years, 2% of those 13 to 16 years old, 6% of those 17 to 19 years old, 25% of women 20 to 24 years old, and 50% of women older than 25 years of age developed reactions in the joints.[489,490] Symptoms could be prolonged.[491] In two different studies, Cendehill vaccine was reported to cause these reactions in 23% and 16%, respectively, of women.[492,493] RA27/3 produced infrequent reactions in children,[494] but approximately 25% of adults had temporary joint symptoms.[472]

Polk and associates[495] performed a comparative trial of rubella vaccines in adult women. Table 53.18 presents some of their comparative data. In addition, they reviewed other reports and concluded that the incidence of transient arthralgia or arthritis was 35% to 63% with the use of HPV-77 (dog kidney), 27% to 33% with HPV-77DE, 8% to 10% with Cendehill, and 13% to 15% with RA27/3. Although many different joints can be involved in the reaction to rubella vaccines, knees and fingers are the most common, whereas the hips are seldom involved. Conflicting evidence has been reported regarding the influence of the stage of the menstrual cycle on joint symptoms.[316,489,491]

The mechanism of joint inflammation appears to be direct infection of the synovial tissue by the virus, if one extrapolates from recoveries of the virus from joints of patients with arthritis after occurrence of natural rubella[496] and after inoculation with HPV-77 (dog) vaccine.[497] Laboratory studies suggesting that rubella RNA and rubella peptides might induce

TABLE 53.18 Side Effects Among Seronegative Adult Female Vaccinees Receiving Measles-Mumps-Rubella or Measles-Mumps-Rubella II and Among Seropositive Control Subjects

	MMR (HPV-77:DE-5)	MMR II (RA27/3)	Control Subjects
N			
Age (y)	59	53	60
X ± SD	29.29 ± 9.09	30.00 ± 9.81	30.70 ± 10.36
Median	26	27	27
Range	19–58	19–58	20–58
Any joint manifestation	17 (28.8%)	14 (26.4%)	2 (3.3%)
Arthritis only	9 (15.3%)	6 (11.3%)	0 (0)
Paresthesias	2	0	0
Fever	5	1	4
Rash	4	4	2
Days of work missed because of joint pains[a]	8 (4)	3 (1)	0

[a]Number of vaccinees who missed work in parentheses.
MMR, measles-mumps-rubella; SD, standard deviation.
From Polk BF, Modlin JF, White JA, et al. A controlled comparison of joint reactions among women receiving one of two rubella vaccines. Am J Epidemiol. *1982;115:19–25.*

TABLE 53.19 Frequencies of Acute and Chronic Reactions to Rubella Vaccine or Placebo in Adult Women

	Groups		
	Placebo (N = 275)	Vaccine (N = 268)	Odds Ratio (95% CI)
ACUTE REACTIONS			
Sore throat	32	34	1.09 (0.75–1.59)
Lymphadenopathy	10	19	2.21 (1.31–3.76)
Rash	11	25	2.57 (1.58–4.21)
Myalgia	16	21	1.36 (0.88–2.10)
Paresthesias	7	7	1.09 (0.57–2.09)
Arthralgia	16	21	1.42 (0.92–2.19)
Arthritis	4	9	2.36 (1.13–4.92)
Arthralgia or arthritis	20	30	1.73 (1.17–2.57)
CHRONIC REACTIONS			
Myalgia	9	15	1.68 (0.99–2.84)
Paresthesias	4	5	1.12 (0.50–2.50)
Arthralgia or arthritis	15	22	1.58 (1.01–2.45)

CI, confidence interval.
Modified from Tingle A, Mitchell L, Grace M, et al. Randomised double-blind placebo-controlled study on adverse effects of rubella immunisation in seronegative women. Lancet. *1996;349:1277–1281.*

autoimmunity also have been published.[498,499] Women who possess class II HLA DR2 or DR5 have elevated risk of acute joint reactions after rubella vaccine.[500] Human joint tissue infected in vitro by Miki and Chantler[501] supported high-titer replication of wild viruses and of HPV-77DE but 100- to 1000-fold less replication of RA27/3. The Cendehill strain grew hardly at all in joint tissue, and genetic mapping showed that the mutations giving restriction were mostly located in the 5′ noncoding region.[502] Although RA27/3 has not been recovered from joints, Tingle and associates[503] reported isolating vaccine virus from the peripheral blood mononuclear cells of two women who had prolonged arthritis after administration of the vaccine. Joint reactions were not associated with elevated IgG or prolonged IgM responses.[503] Bosma and coworkers[23] tested synovial fluids or biopsies from 79 patients with chronic inflammatory arthritis and detected rubella RNA in only two specimens, one from a patient who had rheumatoid arthritis and the other from an immunodeficient patient with arthritis caused by mycoplasma. They concluded that rubella was not etiologic for chronic arthritis, but viral antigens might rarely persist within the joints.

Tingle and coworkers[504] reported a prospective study conducted in British Columbia of arthritis after RA27/3 vaccination or natural disease. The incidence of acute arthritis was 52% in the disease group and 14% in the vaccinees. Recurrent arthropathy developed in 30% of women who had the disease and 5% of women who had been vaccinated. Symptoms were not associated with circulating immune complexes.[505]

In 1991, the IOM committee suggested the need for prospective, double-blind, controlled trials of rubella vaccine in adult women, associated with attempts to isolate rubella virus, to resolve the controversy.[506] Six studies touching on this issue have since been reported. Phillips and coworkers,[507] and subsequently Frenkel and coworkers[508] and Nielsen and coworkers,[509] were unable to confirm the presence of viremia in vaccinees complaining of chronic arthritis. Rubella genomic RNA was not demonstrated by Zhang and coworkers[510] in blood and synovial fluid from cases of rheumatoid arthritis. Slater and coworkers[511] observed two cohorts of Israeli women, one of which had received rubella vaccine. Five to 10 years later, the incidence of arthritis in vaccinees and control subjects was 3.9% and 3.2%, respectively, an insignificant difference. At the Kaiser Permanente Northern California Health Maintenance Organization, Ray and coworkers[512] linked rubella vaccination records with cases of chronic arthritis and found no association between the two.

Finally, a British Columbia group performed a placebo-controlled prospective study of approximately 500 women, half of whom were vaccinated and observed for 1 year.[513] Table 53.19 summarizes their results. Not surprisingly, acute arthralgia and arthritis were more common in vaccinees, although the excess attributable to vaccine was only 5%. In evaluating women with persistent arthropathy, they defined "Persistent arthropathy…as occurrence of arthralgia or arthritis at any time during the 12 months after vaccination in women who experienced acute arthropathy and for whom joint complaints could not be attributed to other causes." The frequency of chronic arthropathy was 15% in the placebo group and 22% in the vaccine arm. However, whereas 58 (72%) of 81 women in the vaccine group with acute arthropathy later developed chronic arthropathy, 41 (75%) of the 55 women in the placebo arm with acute arthropathy also developed chronic arthropathy. Thus, chronic arthropathy was associated with prior acute arthropathy but not with rubella vaccine. In addition, there was no description of the severity or frequency or duration of the symptoms of the women in either group. Moreover, by the authors' case definition, a woman who had an episode of acute arthralgia for 3 days and then one episode of arthralgia of any duration more than a month later, would meet the chronic or persistent arthropathy case definition. Thus, it is unclear what relationship these findings have to the chronic rubella arthropathy previously reported.

More recently, the British Columbia group retested the seronegative women in the original study with several additional tests for antibodies and found that 21.7% were positive.[514] The group then revised its hypothesis, arguing that low, nonfunctional responses to prior rubella infection predisposed women to joint complaints when vaccinated. However, the original placebo group apparently was not retested, and the statistical significance of the new conclusions remains doubtful. Moreover, the large vaccination campaigns conducted in millions of Latin Americans, including women of childbearing age, has not been accompanied by additional reports of chronic arthropathy (WHO rubella position paper).[515]

Thus, taking the evidence as a whole, one is left with the conclusion that although chronic arthropathy caused by RA27/3 is plausible biologically, it is unconfirmed by virology or epidemiology, and is rare, if it occurs at all. However, despite extensive use of RA27/3 vaccine in millions of subjects including many adults in Latin America, articles confirming the British Columbia reports have not been published. In their 2011 report, the IOM concluded that the evidence is inadequate to accept or reject the causal relationship between MMR vaccine and chronic arthralgia or arthritis in women.[481] Nevertheless, on the basis of the IOM report, the National Vaccine Injury Compensation Program has been accepting claims of chronic arthropathy resulting from rubella vaccination, and compensation has been awarded to 23 of 56 completed cases.[516]

Chiba and colleagues[517] reported a depression of rubella-specific cellular immune responses in children with arthritis. Circulating immune complexes containing rubella antigen were found in 11 of 33 vaccinees who developed arthralgia after vaccination but in only three of 19 who did not.[518]

Neurologic Adverse Events

Although few neurologic events after vaccination with RA27/3 have been reported, it is useful to review this type of information for all vaccine strains.[519] Polyneuropathy is part of natural rubella and is among the most frequent of the unusual complications of rubella vaccination. Schaffner and coworkers[520] reviewed the problem in 1974 and found 299 reports of polyneuropathy after vaccination. The cases they reviewed followed immunization with HPV-77DE, HPV-77DK, and, less frequently, Cendehill vaccines. The symptoms began about 40 days after vaccination, appearing in the form of two syndromes, one consisting of paresthesias and pain in the arms and the other involving pain in the knee and preference for a crouching position. The second syndrome tended to recur. Carpal tunnel syndrome and Horner syndrome also were seen. Children with polyneuropathy showed impairment of motor and sensory nerve conduction. Cusi and coworkers[521] studied a case of a young seronegative woman with mild peripheral neuropathy that began 4 weeks postvaccination. Antibodies to myelin basic protein were detected in the patient, and the authors induced antirubella binding antibodies in mice by immunization with a peptide derived from myelin that is homologous with a peptide in the rubella C protein. Attempts to demonstrate rubella virus or genome in the patient's white blood cells were negative.

Other possible adverse neurological events following vaccination have been reported. Two cases of optic neuritis after vaccination for rubella have been reported, the vaccine being HPV-77DE in one instance[522] and an unstated vaccine in the other.[523] Two cases of anterior uveitis after MMR vaccination were reported from Saudi Arabia.[524] Transverse myelitis has been reported on two occasions[525] and diffuse myelitis on three occasions after inoculation with RA27/3, Cendehill, and an unstated vaccine.[141,526] Six cases of transverse myelitis have been reported after vaccination with MMR or MR containing RA27/3, although other associated vaccines may have been the cause.[527–529] Facial paresthesias were reported after vaccination with RA27/3.[530] A case of disseminated encephalomyelitis was reported in Japan after vaccination with the Takahashi strain.[531] Two cases of Guillain-Barré syndrome have been noted after the use of HPV-77DE, but an etiologic association could not be confirmed.[525,532]

Guillain-Barré syndrome after rubella vaccination given in combination with other vaccines has been reported,[533] but an epidemiologic study in the United Kingdom after an MR vaccination campaign revealed rates of Guillain-Barré less than that expected by the background rate (1 per 100,000 children).[534] In addition, a large retrospective study of Guillain-Barré syndrome was conducted in Finland. No clustering of cases occurred after MMR vaccination, nor did subsequent MMR vaccination cause relapses in patients with the syndrome.[535] Another Finnish study showed no relationship of MMR vaccination to encephalitis, aseptic meningitis, or autism.[536] Chantler and associates[537] showed that rubella viruses grow well in human astrocytes but poorly in oligodendrocytes, suggesting that demyelinating disease is not likely to be associated with rubella virus replication.

Other Adverse Events

A study of transfusions from vaccinated persons gave no evidence of transmission of vaccine virus.[538]

A case of "bone changes" after rubella vaccination was reported,[539] but the negative virus isolation, absence of IgM antibodies, and lack of detail make the association dubious. One case of mild orchitis after the administration of rubella vaccine was reported.[540]

Thrombocytopenia has followed vaccination for rubella on several occasions[541–543] and has been reported frequently after the administration of triple vaccine.[544,545] A decrease in platelets may be seen in some asymptomatic vaccinees after vaccination, and symptomatic thrombocytopenia has been reported after natural rubella. A rate of one in 3000 is accepted for thrombocytopenia after wild-type rubella virus infection,[546] whereas rates 10 times lower have been reported after rubella vaccination.[544] It appears that either measles or rubella vaccines may be responsible for thrombocytopenia after MMR.[547] Platelet-binding antibodies to rubella were demonstrated in some cases of thrombocytopenia after vaccination with a Japanese strain.[548] In addition, exacerbation of chronic thrombocytopenia has been reported after MMR,[549] as has recurrent thrombocytopenia after repeated administration.[550] A British study concluded that the risk of thrombocytopenia within 6 weeks of MMR vaccination was one in 22,300 doses, but only two-thirds of cases were attributable to the vaccine, for a risk of one in 33,000 doses.[551] Another British study concluded that the rate of thrombocytopenia after MMR was one in 50,000 doses.[552] An American Vaccine Safety Datalink study of more than a million doses of MMR calculated a one per 40,000 rate of immune thrombocytopenia,[553] and a systematic review of reported cases calculated a similar rate.[554] Both the review and case reports[555] found no problem with vaccination of children with chronic thrombocytopenia.

Revaccination is not recommended by the Advisory Committee on Immunization Practices for children who suffered thrombocytopenic purpura after MMR,[556] but the balance of risk and benefit should be weighed, particularly because the British study found no recurrences after MMR in children with prior thrombocytopenia.[557]

A Swedish study examined autoantibodies to pancreatic islet cells and to thyroid cells before and after MMR

vaccination and found no increase, making it unlikely that vaccination contributes to diabetes or thyroiditis.[558]

Recently, a French group reported that rubella vaccine virus was a trigger for cutaneous granulomas in T-cell–deficient children. RA27/3 genome was recovered from those patients, whose biopsies revealed the presence of rubella virus by immunofluorescence.[559] This observation has been confirmed at the U.S. Centers for Disease Control and Prevention (CDC). The rubella vaccine was found to persist in keratinocytes and particularly in M2 macrophages.[559a] A second paper from France confirmed that this complication of vaccination is not rare.[559b] However, as with all live vaccines, immunodeficiency is a contraindication to vaccination.

Rubella vaccines have been noted to depress nonspecific cellular immunity transiently,[560] including the tuberculin reaction, cell-mediated immunity to *Candida*, phytohemagglutinin responses, and delayed-type hypersensitivity to recall antigens; they also increase suppressor T cells.[561–563] A decrease in lymphocytes was noted at 7 days postvaccination, followed at 30 days by increased production of TNF-α, IL-10, and IL-4 and decreased production of interferon-γ.[337] A follow-up study of RA27/3 vaccinees has shown no increase in cancer incidence.[564] A summary of findings from the U.S. Vaccine Adverse Events Reporting System (VAERS) for the 2003–13 period relative to adults who received MMR turned up no unusual safety signals. Pyrexia, rash, pain, and arthralgia were the most common complaints.[565]

Indications for Rubella Vaccine

Table 53.20 lists the targets of rubella vaccination.

Infants

Rubella vaccine is given to preschool-age children in the United States and many other developed countries in an effort to immunize them for the future and to protect their mothers through reduction of rubella virus circulation. In view of the lack of evidence of contagiousness of vaccinees, infants whose mothers are pregnant may be vaccinated.

Rubella vaccine is usually given at 12 to 15 months of age as part of the MMR or MMRV vaccine. Children who miss the vaccine at that time can receive it at any later age and, in most states, must receive it at the time of school entry. The age at first vaccination does not seem to be as critical for rubella as for measles vaccine. Although passive maternal antibodies are well transmitted to both term and preterm infants, they were lower in those born before 28 weeks of gestation. In general, rubella antibodies disappeared rapidly, being present in only 13% of infants at 6 to 12 months, 5% of infants from 9 to 12 months, and 2% from 12 to 15 months of age.[566–568] From 9 months of age on, vaccination is almost 100% successful,[361,569–574] and a study done in Brazil showed reliable seroconversion after rubella vaccine from about 6 months of age.[162] However, there is little need to prevent rubella in those younger than age 1 year.[575] Three separate studies showed that the presence of acute respiratory infection at the time of vaccination of infants 12 to 18 months of age was shown to have no influence on seroconversion.[576–578]

TABLE 53.20 Target Groups for Rubella Vaccination

Infants ≥12 mo
Older unvaccinated children and adolescents
College students
Childcare personnel
Healthcare workers
Military personnel
Adult women before pregnancy
Adult seronegative women postpartum
Adult men in contact with pregnant women
All the above as part of a two-dose elimination strategy

Adolescents

Vaccination is recommended for all adolescents, male and female, who have not been previously vaccinated. In addition, revaccination with rubella vaccine as part of MMR is recommended in many countries, either at entry to primary school or at 11 to 13 years of age. The justification for this practice lies mainly in the prevention of measles outbreaks caused by accumulation of susceptibles resulting from primary vaccine failure, but it has been argued that revaccination also provides protection to adolescents who did not initially seroconvert to rubella and also boosts waning antibody in those who did. Although the evidence for the need to revaccinate against rubella is slim, two-dose regimens are now standard in the United States and other countries (see "Persistence of Immunity and Revaccination" earlier and "Epidemiologic Results of Vaccination" later).

In the United Kingdom, public health policy formerly emphasized vaccination of 11- to 14-year-old girls, with the idea that a cohort of immune women could be created during a period of 10 to 20 years. The success of this policy is considered subsequently (see "Epidemiologic Results of Vaccination"): in brief, its advantage was narrowing the target group to potential mothers, and its disadvantages were a high refusal rate and the lack of effect on the circulation of virus. However, Schiff and colleagues[579] succeeded in vaccinating 97% of the high school girls in a rural town in Wisconsin, with excellent results. Immunization of adolescent girls has been done with and without the addition of contraception, depending on the known sexual activity in the particular population.[580,581]

Adults

The most direct vaccination program, in the sense of having the most immediate impact on CRS, is vaccination of women. The principal problem with this approach is the possibility of unsuspected pregnancy, although, as described subsequently (see "Pregnancy" later), the observed risk to the fetus has been zero.

Identification of seronegative women cannot be done accurately on the basis of history because clinical diagnosis of prior rubella is unreliable.[582] Vaccination of women with negative histories of prior vaccination is advocated without confirmatory serologic testing.[575] However, a frequent practice has been to test women for immune status and then to vaccinate them while oral contraceptives or other forms of pregnancy precaution are taken.[583–586] Vaccination can be practiced as part of routine gynecologic care, premarital screening, and occupational healthcare or at other medical opportunities, as long as pregnancy can be avoided for 1 month. A frequently expressed concern is whether to vaccinate women with low titers, such as those with ELISA values just below the cutoff value, with hemolysis-in-gel values of 10 to 15 IU or with HI titers of 1:8. Current evidence suggests that those women usually are immune but may be at higher risk of reinfection,[587] and thus, one dose of vaccine should be offered (see "Reinfection and Herd Immunity" earlier). There is no evidence that vaccination of seropositive women is attended by increased risk of untoward reactions.

The use of rubella vaccine during the puerperium (postpartum vaccination) has been widely advocated.[492,588–594] For example, because 56% of CRS children in the United Kingdom were born to multiparae,[595] this practice should prevent a significant portion of CRS. Indeed, Edmond and Zealley[596] observed that CRS was prevented in Edinburgh by vaccination of girls and postpartum vaccination of women, with most of the failures occurring in women who had been screened but not vaccinated. However, pregnancy can occur even in the immediate postpartum period,[595,597] and contraceptive measures, such as the use of depot progestogen, have been advocated.[598] Administration of blood or blood products such as Rh_0(D) immunoglobulin before vaccination requires postvaccination tests for seroconversion because of the rubella antibodies in those products.

Although the postpartum genital tract is not susceptible to rubella vaccine virus,[599] vaccinated parturient women do excrete vaccine virus in their breast milk.[600] The vaccine virus then may be transmitted to their newborns, who remain asymptomatic and do not develop tolerance to subsequent vaccination.[601–603] Thus, breastfeeding is not a contraindication to vaccination.

Adult men not in high-risk groups are not generally targeted for vaccination, although they are also a reservoir of susceptibles and may serve as the source of infection of pregnant women.[604] An outbreak in Croatia showed that infection can propagate among men even if they do not live in a collectivity situation, despite routine vaccination of infants and women.[605]

In Latin America, rubella vaccination of both men and women has resulted in rapid control and elimination of the virus.

Military Recruits and College Students

Vaccination is routinely practiced in the armed forces of the United States, with the consequence being the virtual eradication of rubella.[382] Rubella also was eradicated in the armed forces of Singapore several years after commencement of routine vaccination.[606] The consequences of failure to vaccinate were illustrated by an epidemic in British troops in Bosnia.[607] The French army, which until recently has not practiced routine rubella vaccination of its recruits, has had perennial epidemics.[608] In view of the congregation of large numbers of susceptible young adults in colleges and universities, routine vaccination with MMR at matriculation also is advocated.[575] In principle, these vaccinations will be extra doses, but they are considered important to prevent outbreaks of measles and perhaps rubella on college campuses.

Hospital Employees

Outbreaks of rubella in hospitals,[609–612] with the resultant exposure of pregnant women, have led to the recommendation of compulsory rubella vaccination for both male and female hospital employees.[613] In one hospital outbreak,[614] those departments that had a compulsory vaccination policy escaped unscathed, whereas those departments that had a voluntary vaccination policy suffered numerous cases, including infections of pregnant women. A comprehensive evaluation reconfirmed the recommendation for vaccination of hospital personnel.[615]

International Travelers

Because the United States has eliminated endemic rubella and CRS, travelers should be immunized against rubella before they travel overseas if they have not been previously immunized; many imported cases result from U.S. residents returning from countries where rubella vaccination is not widespread. In particular, unimmunized women of childbearing age who travel overseas should be vaccinated to protect against the greater likelihood of exposure to the disease in countries that do not use rubella vaccine.

Contraindications

General Contraindications

Rubella vaccine can be given to patients with minor respiratory illnesses, whether febrile or afebrile (see "Indications for Rubella Vaccine" earlier). Those with congenital immune deficiencies should never receive a live virus vaccine, although family members should be vaccinated to protect the patients against rubella, measles, and mumps.[575] It also would be prudent to wait until systemic immunosuppressant drug therapy has been terminated for 3 months before vaccinating patients receiving such regimens. A 16-year-old boy with acute lymphoblastic leukemia who received RA27/3 rubella vaccine developed chronic infection of leukocytes and high antibody titers. Symptoms of arthritis required temporary interruption of immunosuppression, but fortunately, the patient recovered spontaneously.[616] Short-term (less than 2 weeks) corticosteroid therapy is not a contraindication to the administration of rubella vaccine. Patients whose immunity had been ablated during bone marrow transplantation 2 years previously were given rubella vaccine in MMR by Ljungman and coworkers.[617] They observed 75% seroconversion to the rubella component with no safety problems. King and colleagues[618] performed a similar study and found 91% seroconversion.

Children with chronic renal disease who are on hemodialysis can be immunized successfully before transplantation.[619]

Persons who have had anaphylactic reactions to neomycin should not receive rubella vaccines containing that antibiotic.[620] Whereas monovalent RA27/3 vaccine is grown in human diploid cells, MMR does contain other viruses grown in avian tissue, reactions in egg-sensitive persons may occur, but are very rare.

Concurrent Immunoglobulin G

Vaccination within 2 weeks before receipt of IgG or 3 months after receipt of IgG is inadvisable.[234,575] However, anti-Rh_0(D) globulin did not interfere with vaccination of postpartum women.[594] Women who are vaccinated after receiving anti-Rh_0(D) globulin should be tested 6 weeks later for rubella antibodies. Because most rubella vaccine is administered with measles vaccine (MR, MMR) in many countries, the precautions pertaining to measles-containing vaccine should be followed. Thus, the administration of immunoglobulins or other antibody-containing blood products may neutralize the effect of the measles component for 3 to 11 months, depending on the dose of measles antibody.

Human Immunodeficiency Virus Infection

There are not many data concerning the results of rubella immunization in HIV-seropositive children, but one study reported 80% seroconversion.[621] A similar result was obtained in another study, with a rise to 100% after revaccination.[622] A recent study of children with perinatal HIV infection given MMR showed a 65% seroconversion rate.[623] Although relatively asymptomatic HIV-positive children respond well to rubella vaccine, those in late stages may have a poor response.[624] Vaccination of HIV-seropositive women has been reported to be safe.[625,626]

HIV-infected children who are asymptomatic should receive rubella vaccine as part of MMR. Virologic study of 10 HIV-infected patients who received MMR failed to detect rubella virus.[627] MMR also should be considered for children with AIDS who are not severely immunosuppressed. However, in a small study, children with symptomatic infection had a significantly poorer response than asymptomatic children, who responded normally.[628] Older children and adults who were rubella seropositive before becoming infected with HIV maintain high-avidity rubella antibodies and do not need revaccination.[629]

Of interest is the report that rubella vaccination may give transiently positive ELISA tests for HIV antibodies.[630]

Juvenile Idiopathic Arthritis

A study of 314 patients with this condition revealed no exacerbations of disease after rubella vaccination.[631] Even treatment with methotrexate and etanercept was not associated with vaccine failure or reactions.[372] A relapse was reported in a single patient after vaccination with a Japanese rubella vaccine.[372,632]

Acute Lymphoblastic Leukemia

Children with acute lymphoblastic leukemia lose rubella antibodies after therapy but respond well to revaccination.[633]

Pregnancy

Although there is now abundant evidence for the safety of RA27/3 for the fetus, pregnancy remains a contraindication to rubella vaccination, and women are advised to take precautions against pregnancy for 1 month (28 days) after vaccination. On the basis of a case of wild virus transmission to a fetus conceived 7 weeks after vaccination,[629] a prior recommendation had been for a 3-month delay, but evidence for damage to the fetus by rubella vaccine strains is nonexistent.[634] Several summaries of the accumulated American, British, and German data have been published (Table 53.21).[635–637] Transplacental passage of vaccine virus is a rare event, having been documented four times in 708 pregnancies complicated by RA27/3 vaccination. More important, there was no CRS case demonstrated in more than 2750 pregnancies during which rubella vaccines were given. Hoffman and coworkers[638] reported the case of a woman vaccinated 3 weeks after conception who gave birth to an infant who excreted RA27/3 virus until 8 months of age. Nevertheless, the infant was free of abnormalities.[638]

Data from Germany and Latin America are particularly supportive of a lack of teratogenic effect of rubella vaccine given during pregnancy. In Germany, cord blood was positive for rubella IgM in only six of 126 (4.6%) infants born to rubella seronegative mothers, and none of those infants, plus 131 other infants from whom cord blood had not been obtained, had abnormalities compatible with CRS (Enders G. Personal communication to SA Plotkin, 2006). In the course of efforts to eliminate rubella from Latin America (see "Latin America and the Caribbean" later), women who were unknowingly pregnant at the time of vaccination were surveyed in Costa Rica, Brazil, Ecuador, El Salvador, Paraguay, Argentina, and Mexico.[639–641] Although cord blood was positive for rubella IgM antibodies in 3.6% of cases, no case of CRS was confirmed in either country, nor was there evidence for increased fetal loss.[642,643] A smaller study in Israel reached the same conclusion.[363] Iran performed a mass measles-rubella vaccination in December 2003. Infants born to 535 women who were inadvertently vaccinated during pregnancy were studied, including women known to be seronegative before vaccination and women who had not been tested. Only five newborns had IgM antibodies at birth, and none had evidence of abnormality on follow-up.[401]

The results of the studies conducted in six Latin American countries provide the largest collection of new evidence to support existing documentation of a negligible or absent risk for CRS after administration of rubella vaccine shortly before or during pregnancy. Of the 1980 susceptible pregnant women followed, 70 (3.6%) of the infants had congenital rubella infection, but none had congenital defects associated with CRS.[640] In the Brazilian study, one rubella IgM antibody–positive infant had clinical features of CRS but had wild-type

TABLE 53.21 Summary of Data on Vaccination of Unknowingly Pregnant Rubella-Susceptible Women

	Live Births to Women Receiving Rubella Immunization		
Country	Within 3 Months Before Conception or During Pregnancy	Evidence of Infection	Abnormalities Compatible With CRS
United States	324[a]	6/222 (2.7%)	0/324
Germany (West BRD)	280[a]	3/69 (4.3%)	0/279
Sweden	5[a]	NK	0/5
United Kingdom	71[a]	4/52 (7.7%)	0/71
Brazil	1647[b]	67/1647 (4.1%)	0/1647
Ecuador	43[b]	2/43 (5%)	0/43
El Salvador	59[b]	1/59 (1.6%)	0/59
Paraguay	119[b]	0/119	0/119
Iran	117[c]		0/117
Costa Rica	93[b]	0/93 (0%)	0/93
Mexico	175[d]	0/174	0/174
Total	2933[e]	83/2478 (3.3 %)	0/2931

[a]Data from Centers for Disease Control and Prevention (CDC). Rubella prevention: recommendation of the Immunization Practices Advisory Committee. *MMWR Recomm Rep*. 1990;39(RR-15):1–18; Centers for Disease Control and Prevention (CDC). Rubella vaccination during pregnancy: United States, 1971–1988. *MMWR Morb Mortal Wkly Rep*. 1989;38:289–293; Enders G. Rubella antibody titers in vaccinated and nonvaccinated women and results of vaccination during pregnancy. *Rev Infect Dis*. 1985;7(Suppl):S103–S107.

[b]From Castillo-Solórzano C, Reef SE, Morice A, et al. Rubella vaccination of unknowingly pregnant women during mass campaigns for rubella and congenital rubella syndrome elimination, the Americas 2001–2008. *J Infect Dis*. 2011;204(Suppl 2):S713–S717.

[c]From Hamakar R, Jalilvand S, Abdolbaghi MH, et al. Inadvertent rubella vaccination of pregnant women: evaluation of possible transplacental infection with rubella vaccine. *Vaccine*. 2006;24:3558–3563.

[d]From Reyna J, Herbas I, Gómez M, et al. Perinatal outcome of inadvertent immunization with the measles-rubella vaccine in pregnant Mexican women during the campaign for the eradication of congenital rubella in 2008. *World J Vaccines*. 2011;1:1–4.

[e]Confirmed susceptibles; actual number much higher.

BRD, Bundesrepublik Deutschland; CRS, congenital rubella syndrome; NK, not known.

rubella virus identified by molecular genotyping of viral culture.[641] Badilla and coworkers[644] followed more than 3000 women who were vaccinated during a rubella campaign in Costa Rica, of whom 1191 were followed closely. Comparison of subgroups that were seronegative, seropositive, or of unknown serologic status before vaccination showed no differences in fetal outcome, no association with defects compatible with CRS, and no IgM seropositivity in newborns. In Iran, 106 pregnant women vaccinated with MR were followed, and again no infant had clinical or serologic evidence of infection.[645] Infants born to 60 Iranian women who received vaccine around the time of conception also showed no CRS.[646] Finally, a prospective study of 175 susceptible Mexican women studied show that all the 174 infants studied were IgM negative and had no CRS-compatible defects.[639]

On the basis of data in Table 53.21, the theoretical maximum risk for CRS after the administration of vaccine is 0.2%, considerably lower than the risk with wild rubella virus, or indeed the risk of non–CRS-induced congenital defects in pregnancy. If only the American women known to be seronegative and who received RA27/3 are considered in the calculation, the theoretical risk to the fetus still does not rise to more than 2.1%. The newer data recorded above substantially reduce the theoretical risk. Because the observed risk has been zero, rubella vaccination during pregnancy is no longer considered an indication for abortion.[647,648]

These data are reassuring because pregnant women continue to receive rubella-containing vaccines. In a review of the VAERS database between 2003 and 2013, there were 131 reports of pregnant women vaccinated with MMR alone or in combination with other vaccines. Of the pregnancies that went to delivery, no infant was born with CRS-associated manifestations.[565] Based on the information on pregnancies between 2002 and 2009 in the U.S. Vaccine Safety Link Database, pregnant women had received MMR or rubella monovalent vaccine at rates of 1.19 and 0.16 vaccinations per 1000 pregnancies, respectively.[649] In fact, there is little evidence for danger of vaccination in pregnancy for other live and killed vaccines.[650]

Concomitant Yellow Fever Vaccine. A report from Brazil recorded lower seroconversion to rubella, mumps, and yellow fever when the vaccines were given concomitantly then separately, although the rubella response may have been measured too early after simultaneous administration.[651]

Children With Congenital Rubella Syndrome

Children with CRS probably will not respond to parenteral rubella vaccine, even when they are seronegative.[652] The reason for resistance of CRS children is unknown, and probably involves immune factors other than antibodies, but immune tolerance is not responsible. In fact, 81.5% of CRS children were seropositive 5 years after birth, although the loss of antibody in 18.5% is faster than in normal children postinfection.[652] Even though they could be immunized with RA27/3 intranasally,[653] there is no official recommendation to vaccinate these children.

PUBLIC HEALTH CONSIDERATIONS

Epidemiologic Results of Vaccination

The goal of rubella vaccination programs is the prevention of the intrauterine infection that causes CRS, and, incidentally, the occasional complication of the disease in adults. Since the licensure of the rubella vaccine in 1969, the global epidemiology of rubella and CRS has changed dramatically. In 2000, the WHO convened a meeting to review the worldwide status of CRS and its prevention.[654,655] This meeting was prompted by the availability of more data on the CRS disease burden in developing countries, an increase in the number of countries with national rubella immunization programs, and advances in laboratory diagnosis since the last international meeting on CRS and rubella in 1984. In 1996, only 83 countries and territories used rubella vaccine in their national immunization programs. Since the 2000 meeting, additional countries have introduced rubella-containing vaccine, two WHO regions (Regions of the Americas and Europe) have established rubella elimination goals by 2010 and 2015, respectively, and one WHO region (Western Pacific) has established an elimination goal for rubella and CRS. As of September 2016, 149 (77%) this number had increased to 140 (72%) of the 194 reporting countries and territories. The proportion of countries using rubella vaccine varies markedly by WHO region (Fig. 53.5).[656] Much of the recent increase occurred in Asia and Africa. Even

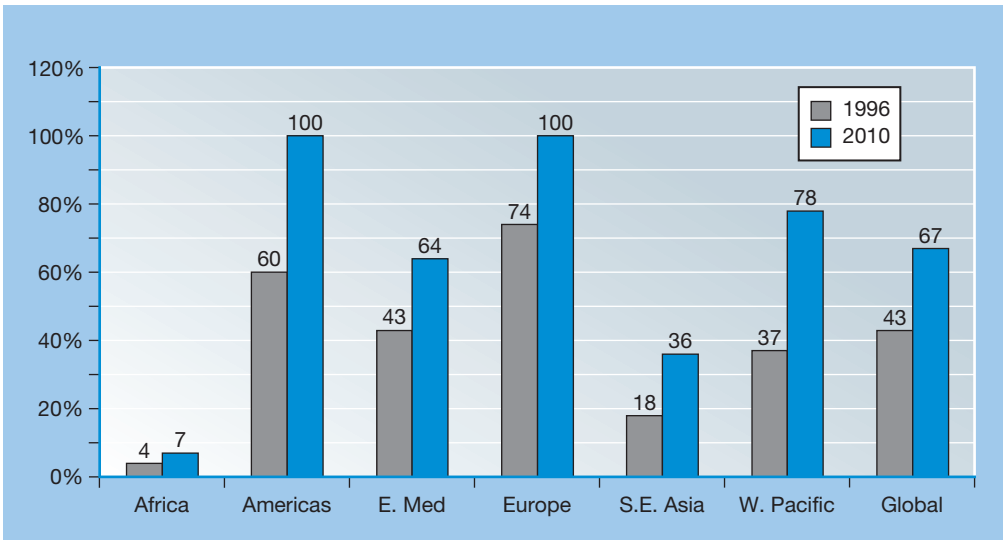


Figure 53.5. Percentage of member states by World Health Organization (WHO) region, with routine rubella vaccination, 2009. *(From Reef SE, Strebel P, Dabbagh A, et al. Progress toward control of rubella and prevention of congenital rubella syndrome: worldwide, 2009.* J Infect Dis. *2011;203:S24–S27.)*

though only one dose is required, most countries use a two-dose schedule because rubella vaccine is generally combined with measles vaccine, which is recommended as a two-dose schedule.[657]

United States

Rubella vaccine has had spectacular success in the United States, in terms of the number of persons vaccinated and the declining numbers of rubella cases reported. Since the licensing of the vaccine in 1969, no major epidemic of rubella has occurred, despite the previously observed 6- to 9-year cycle.

These impressive results were obtained initially by the vaccination of children, with a dependence on herd immunity to protect pregnant women. However, assessment in 1977–78 revealed that although the program was having a major impact on rubella in children, rubella rates in those older than 15 years were not substantially different from prevaccination rates.[234] Specific experiences in institutional or citywide outbreaks showed that herd immunity was limited, giving a protection of perhaps 50%.[394,658,659] Moreover, children were not the sole source of infection for their mothers.[660] Serologic surveys showed a persistence of 12% to 24% seronegativity in adolescents and adults. As a consequence, programs to expand vaccination of these groups were increased. Subsequently, the incidence of rubella dropped to less than one per 100,000 population, and the incidence of CRS dropped to less than 0.1 per 100,000 births (Fig. 53.6).[183]

In 1988, a nadir was reached in reported postnatal rubella, and only one case of CRS was registered in 1989. Large outbreaks of rubella occurred in unimmunized immigrants to California and among persons in religious communities that do not accept vaccination, which were followed by the expected cluster of CRS cases.[190,661] A serologic survey conducted between 1988 and 1994 showed 92% seropositivity in 6- to 11-year-old children, but only 85% in persons 20 to 29 years of age.[662]

In the mid-1990s, rubella occurred mainly among foreign-born Hispanic adults who were unvaccinated or whose vaccination status was unknown, with very limited spread and circulation among the U.S. resident population.[663] From 1997 to 1999, 20 (83%) of 24 infants with CRS were born to Hispanic mothers, of whom 91% were foreign-born.[180] Mothers' countries of birth include mainly Mexico, Central American countries, and countries of the Spanish-speaking Caribbean. Genomic sequencing confirmed that the strains circulating earlier were sporadic, while imported strains featured in remaining small outbreaks.[215] Finally, elimination of endemic rubella was achieved in 2001 and has been maintained, with rubella and CRS becoming an imported disease, as in a recent case of a mother immigrating from Liberia.[664,665]

Cost-benefit analyses showed that for every dollar spent on childhood rubella vaccination in the United States, US$7.70 was saved.[666]

Accumulating evidence that there was no more endemic circulation of rubella virus allowed the CDC and an expert committee to declare in 2004 that rubella had been eliminated from the United States.[401] Since the meeting in 2004, elimination of rubella and CRS has been maintained in the United States. As part of the verification of elimination of measles, rubella, and CRS in the Region of the Americas, the CDC convened an external expert panel in December 2011, which reviewed the evidence. The panel concluded that rubella and CRS had been eliminated from the United States.[667] Recent serologic data show that 95% of Americans have rubella antibodies.[668]

Meanwhile, the growing resurgence of measles resulted in a recommendation for a routine second dose of measles vaccine, usually in the form of MMR.[669,670] Thus, revaccination with rubella became standard, practiced at either entrance to primary school (4 to 6 years of age) or entrance to secondary school (11 to 12 years of age). Although there are theoretical arguments in favor of revaccinating older preadolescent children,[468] the United States has moved to uniform revaccination with MMR at 4 to 6 years of age. An economic analysis revealed a ratio of benefit to cost of 14.2 for direct costs and 26.0 for societal costs.[671]

Canada

The provinces of Canada adopted a policy either of mass vaccination of infants or of selective vaccination of preschool-age girls.[242,639] Total rubella incidence dropped in the provinces that adopted mass vaccination of infants but was not much changed in those adopting vaccination of preschool-age girls. However, reported CRS decreased throughout Canada. As of 1983, all provinces give vaccine to infants and also to 12-year-old girls who had not been immunized previously.[672] During 1996 to 1997, a nationwide two-dose MMR policy was implemented. As part of measles elimination strategy, several of the

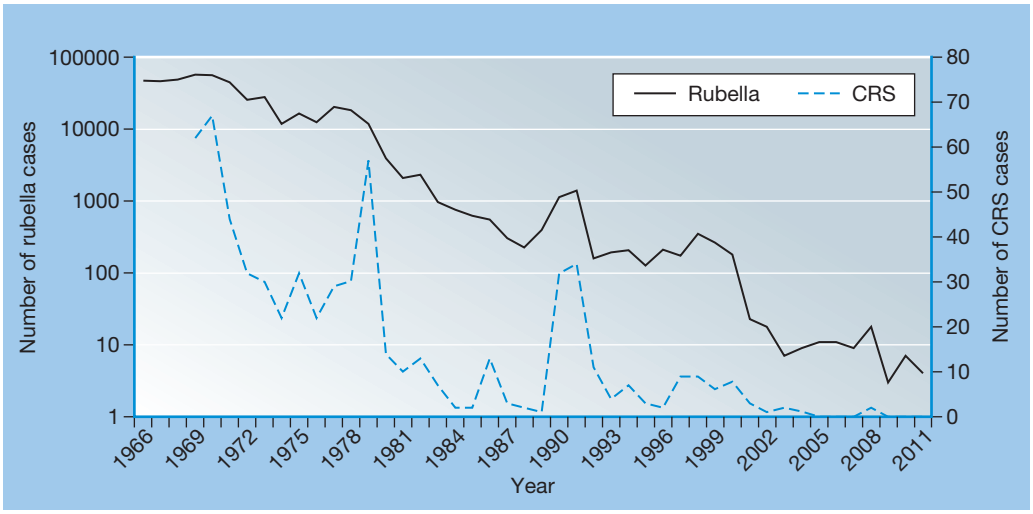


Figure 53.6. Reported rubella and congenital rubella syndrome (CRS) cases, United States, 1966–2009.

provinces conducted MR catch-up campaigns. With the success of the measles-rubella vaccination program, rubella incidence has been at an all-time low since 1999, except during 2005 when there was an outbreak among a religious community in Ontario. The review of epidemiologic and laboratory evidence indicates that endemic rubella transmission has been interrupted.[673]

Europe

The WHO European Region is composed of 53 member states, including Western and Eastern Europe, Russia, and the Newly Independent States. WHO-Europe had set a goal of eliminating rubella and thus reducing CRS incidence in the European Region to less than one per 100,000 births by 2010,[674] and by 2004, 90% of countries in the region were using rubella vaccine.[675] By 2009, all 53 member states had included rubella vaccination in routine childhood vaccination as part of MR/MMR.[676,678] Most countries using MMR recommend two doses. The Scandinavian countries have succeeded in eliminating rubella (see "Scandinavia" later). Not surprisingly, rubella incidence is relatively low in Western Europe, more frequent in Central and Eastern Europe, and very common in the Newly Independent States.[678,679] However, in 2010, vaccine coverage with MMR was low in Southern Europe and epidemics of measles and rubella occurred. Thus, the elimination goal had not been met. The goal for rubella elimination target date was changed to 2015.[680] The number of rubella cases in the European Region decreased from 621,039 in 41 countries in 2000 to 640 cases in 37 countries in 2014.[147] In November 2015, at the regional verification meeting, 23(43%) of the member states were verified to have interrupted endemic rubella transmission.[681]

United Kingdom. The policy of vaccinating schoolgirls was adopted by the British in 1970.[265,682] During subsequent years, the number of reported rubella cases decreased only slightly, although the reported cases of CRS decreased approximately 75%.[683] Approximately 15% of schoolgirls did not accept the vaccine,[684,685] which explained why 88% of women reporting for assessment for rash disease had not been immunized.[686] Although serologic studies showed that vaccination had reduced seronegativity of young women,[686–689] many susceptible persons still remained.[689,690]

After original hesitation, opinion in Britain changed to favor the inclusion of young children in rubella vaccination practice, as is now done in the United States.[682,691–694] Since October 1988, rubella vaccine as part of MMR has been recommended to all infants,[695] and in 1994, a large-scale vaccination campaign was conducted with MR combined vaccine. Congenital rubella and terminations of pregnancy for rubella decreased markedly in England and Wales,[696] with only one CRS case reported in 1995.[697,69] In 1996, however, the United Kingdom experienced a resurgence of rubella, primarily in adult and adolescent males who were unvaccinated.[698] As might be predicted, cases of CRS followed the outbreak. In response, during 1994 to 1995, a catch-up campaign was conducted in both boys and girls of 5 to 16 years of age, with considerable reduction in susceptible youths and in rubella infections.

Pregnant women immigrating into the United Kingdom from Asia and Africa are at increased risk of being seronegative.[699,700] Additional outbreaks in universities have resulted from imported rubella in Greek students.[701] In 2011, 7% of British women were seronegative; the figure for women born after 1986 was 18%,[702] possibly the result of public concern relating to allegations of a relationship between MMR vaccination and autism that reduced rubella coverage, but CRS remains rare.[703]

Scandinavia. In 1982, Sweden adopted a two-stage vaccination scheme involving the use of MMR at two ages: 18 months and 12 years.[704,705]

An acceptance rate of 88% was achieved among Swedish schoolgirls and an even higher rate among other children. Some problems with seroconversion for mumps and measles among the 12-year-old girls were reported, but rubella vaccine gave good results in both age groups.[706] Christenson and Bottiger[707] studied the rubella serology of 1343 susceptible 12-year-olds in the Swedish program and found that 100% seroconverted after vaccination. Before 1974, a yearly average of 14 CRS cases was recorded in Sweden; there were two cases per year between 1975 and 1985, and there have been no cases since 1985.[708]

Nevertheless, some resistance to vaccination has caused vaccination to be done later in life than infancy[709] and in 2012 an outbreak of rubella resulting in 50 cases occurred largely in an anthroposophic community.[710]

In Denmark, no case of rubella has been reported since 1996.[711] A survey of congenital cataracts identified 26 cases resulting from congenital rubella before rubella vaccination was introduced in 1987, but only one indigenous case subsequently.[712] A review of deafblind persons born in Denmark before 2008 revealed that those cases caused by congenital rubella was 32% in adults but only 3% in children.[713]

The Finns vaccinate with MMR at 14 to 18 months and at 6 years of age. The peak incidence has shifted to adults, whereas children appear to be protected.[714] The success of the MMR two-dose vaccination policy in Finland has been spectacular. Peltola and colleagues[715] summarized the results of the program that succeeded in eliminating indigenous cases of rubella, measles, and mumps. This program was supported by improved surveillance of disease and by ancillary studies demonstrating lack of significant reactions to vaccine and persistence of antibodies. Ukkonen[716] provided data on the disappearance of rubella and the state of rubella immunity in the Finnish population. As shown in Fig. 53.7, the two-dose policy has erased seronegativity in adolescents and caused rubella to disappear. Since 1986, no case of CRS has been reported. The last indigenous rubella case occurred in 1996. In 1997 and 1998, rubella was eliminated from Finland.[625,717] Only one case of rubella was reported between 2003 and 2007.[718]

France. Between 1982 and 1994, during which MMR was recommended for all 1-year-old French children, rubella infections in pregnancy dropped from 45 to nine cases per 100,000 births.[719] Vaccine coverage is now greater than 90% in children younger than 10 years, but considerably lower in adolescents.[720] Moreover, areas of low vaccine coverage persist, resulting in large numbers of measles cases and probably unreported rubella, as well. Although French authorities have instituted a second dose of MMR at 6 years of age, and much congenital rubella has been prevented, susceptibility remains high in adolescents and adults.[312,721,722]

The Netherlands. The Netherlands replaced selective vaccination of girls with mass vaccination of infants in 1987. Although serologic immunity to rubella is high in the Dutch population,[723] circulation of rubella virus persists and rare cases of CRS continue to occur, perhaps because some religious communities have low coverage and export infection (Van Doornum GJ. Personal communication, 2005).[724,725] A population-based serosurveillance study performed in The Netherlands in 2006–07 found that orthodox Protestants reached a seroprevalence above 85% only at 5 years of age, whereas in the nonorthodox community that level was reached at 2 years of age.[726] In 2013 an outbreak of rubella comprising

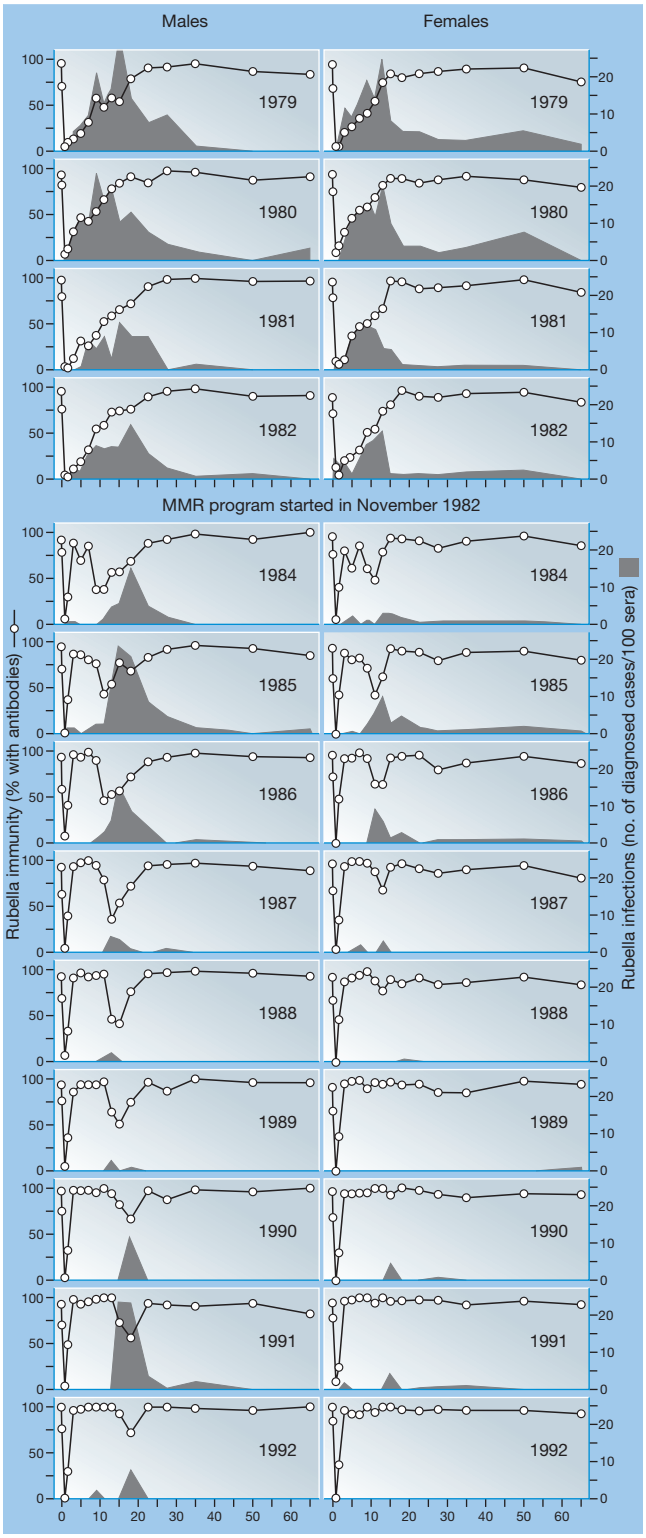


Figure 53.7. Gradual increase in rubella immunity and disappearance of rubella infections as a consequence of measles, mumps, and rubella (MMR) vaccination. *Top*, Before the MMR program (1979–1982). *Bottom*, During the MMR program (1984–1992). *(From Ukkonen P. Rubella immunity and morbidity: impact of different vaccination programs in Finland 1979–1992.* Scand J Infect Dis. *1996;28:31–35.)*

54 cases was linked to an orthodox Protestant primary school.[726]

Italy. Although adolescent girls have been targeted for rubella vaccination since 1972,[727] routine childhood vaccination began in 1990 with MMR vaccine being administered at 12 to 24 months. In 1999, Italy introduced a two-dose MMR schedule. However, measles-containing vaccine coverage was less than 80% until 2002. In 2002, MMR vaccine was provided free of charge. Recent efforts designed to eliminate CRS by 2007 have decreased the incidence of rubella in women of childbearing age from 14.1 to 2.8 cases per 100,000.[728] CRS continues to occur in Italy,[729,730] as substantial percentages of women are seronegative.[731]

Between 2005 and 2013 a total of 75 congenital rubella infections were reported, amounting to a national mean incidence of 1.5 per 100,000 live births. Two peaks occurred in 2008 and 2012, giving incidences of 5.0 and 3.6 per 100,000, respectively. Rubella and CRS continue to be a public health problem in Italy.[188,188a]

Spain. Serologic data show that 97% of children in Spain have rubella antibodies because of high vaccine coverage.[732] In the Basque region, there have been no rubella cases since 1997 and no CRS since 1984.[733] However, in 2003, there was an epidemic of rubella in Madrid, centering on women who had immigrated from Latin America.[734–736] Nevertheless, in Catalonia, MMR vaccination has achieved elimination of indigenous rubella, and only 6% of pregnant women are seronegative.[737,738]

Greece. Vaccination against rubella was introduced slowly into Greece through the private market in 1975. In 1989, MMR was introduced into the routine childhood program, with a second dose of MMR added in 1991. An outbreak in 1993 was attributed to the reductive effect of insufficient rubella vaccination in children on the circulation of rubella virus and thus greater susceptibility of adult women. Since then, high rates of coverage have been achieved in infants, but many adolescents are still susceptible.[739]

Other European Countries. Ninety-six percent of children and adolescents in Luxembourg are rubella seropositive.[447]

Coverage rates in Switzerland are uncertain, and Matter and colleagues[740] reported the persistence of rubella and CRS cases.

A large rubella outbreak in 2002–03 caused Romania to adopt MR vaccination of adolescent girls and subsequently MMR vaccination of infants in 2004.[741] Again, in 2011–12, Romania experienced an outbreak involving 24,727 cases, of which 98% were unvaccinated and more than 75% were in 14 to 19 year olds. The majority of cases occurred in those who had not been recommended to receive vaccine. In 2012, 119 pregnant women were infected by rubella, resulting in 55 cases of laboratory-confirmed CRS, nine infant deaths, and one stillborn CRS. Poland also experienced epidemics until 2008 but has now undertaken rubella elimination.[676] However, a report from 2013 recounted an epidemic of more than 21,000 cases centered in 15- to 29-year-old boys, who had not been vaccinated because Poland had previously vaccinated only adolescent girls. Since 2004, a universal two-dose combined MMR vaccination program has been implemented.[726] In Austria, vaccination of children has reduced rubella incidence in children, but unvaccinated adolescents are still being infected.[742]

In Albania, a mass vaccination campaign was conducted in 2000, covering children 1 to 14 years of age. In 2001, women of childbearing age were targeted. Since then rubella and CRS have been rare.[743]

Israel

Israel at first adopted the British policy of vaccination of schoolgirls. Subsequent observations showed that although the youngest girls were being protected, epidemics could still infect large numbers of pregnant women. Accordingly, family health clinics, which most Israeli women attend, began to screen for rubella antibodies and to vaccinate seronegative women, and universal vaccination of children with MMR was started in 1989.[190] Economic analysis supported that step.[744] The problem of eliminating rubella in Israel has been exacerbated by the early emphasis on vaccination of women only, by immigration of unvaccinated populations, and by the existence of religious communities that do not accept vaccination. An epidemic of rubella in the Israeli Army led to an analysis that showed only 69% in seropositivity in male soldiers, probably because of insufficient catch-up vaccination.[745] The earlier vaccine policy is also reflected in a low rate of seropositivity in the civilian 13- to 17-year-old age group.[746] Nevertheless, since the occurrence in 1992 of five CRS cases, only one further CRS case has been reported, and that was in the baby of an immigrant woman (Slater P. Personal communication, 2006).[747,748]

Russia and the Newly Independent States

In 2000, a survey in the Perm region of Russia showed a 17% susceptibility rate in Russian women and a CRS rate of 3.5 per 1000 live births. An estimated 15% of all birth defects were attributed to rubella.[749] Since then, more rubella vaccination has been done in Russia, but coverage is far from complete, and epidemiologic information is scarce.

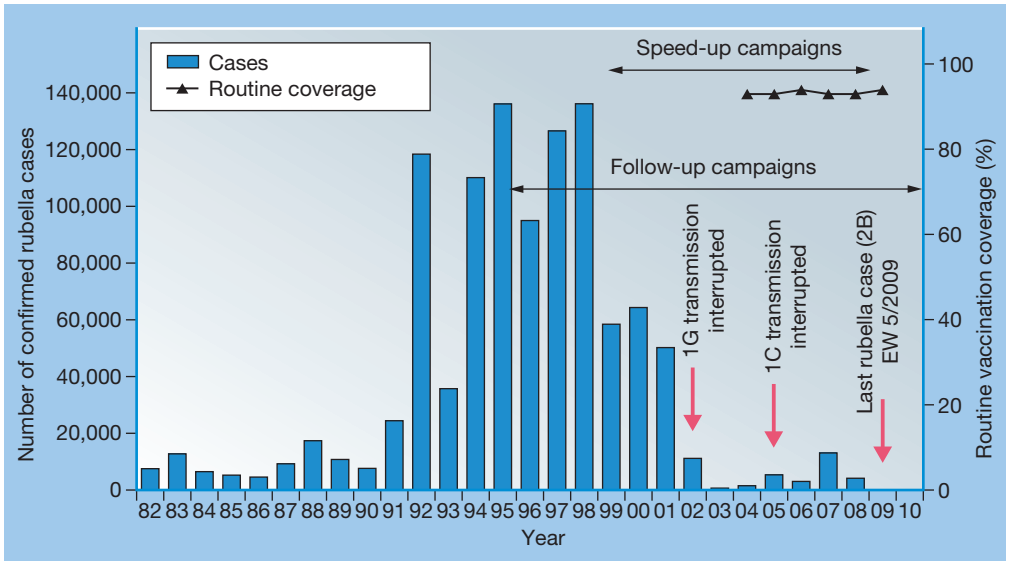
All the newly independent countries have introduced rubella-containing vaccine, with Tajikistan being the last country to do so in 2009. Kyrgyzstan served as the model. Early studies showed that rubella was endemic and epidemic in that country and that 13% of women 15 to 39 years of age were susceptible.[173,177] Subsequent vaccination campaigns were highly successful, and similar campaigns have been conducted in Belarus, and many of the other Newly Independent States.

Latin America and the Caribbean

The Technical Advisory Group (TAG) of the Pan-American Health Organization (PAHO) recommended routine rubella immunization throughout the Americas in 1997 after concluding that 20,000 infants with CRS are born each year in that region even in the absence of an epidemic.[750] In 1999, the PAHO TAG recommended an accelerated rubella control and CRS prevention initiative. Countries wishing to eliminate rubella and CRS rapidly were advised to conduct a one-time mass campaign that targeted adolescent and adult females and males with MR vaccine. Countries wishing to prevent and control CRS were advised to conduct a one-time campaign in females only, 5 to 39 years of age.

In 2003, the Directing Council for the Member States for the Americas established a goal for the elimination of rubella and CRS by 2010. Rubella and CRS elimination was defined as the interruption of endemic rubella virus transmission in all the countries of the Americas for a period greater than or equal to 12 months, without the occurrence of CRS cases associated with endemic transmission, in the presence of high-quality surveillance. In 2003, the TAG recommended that mass campaigns be conducted in both males and females. The target age group (5 to 39 years of age) was selected on the basis of year of vaccine introduction, previous campaigns conducted, epidemiology, and fertility rates in the countries. In 2007–09, the three countries (Chile, Argentina, and Brazil) that initially conducted female-only mass campaigns documented outbreaks in adult males.[751,752] All three countries subsequently conducted additional mass campaigns, two in males only (Argentina, Chile) and one in both males and females (Brazil).

With the successful implementation of the PAHO strategy for rubella vaccine, endemic transmission of rubella virus in the WHO Region of the Americas was interrupted in 2009 (Fig. 53.8).[148] Since 2010, countries in the Region of the Americas



Source: Country reports to PAHO/WHO.
*Data until EW 52/2010

Figure 53.8. Reported routine coverage (2004–2009), time frame of campaigns conducted, and reported rubella cases (1982–2010), Region of the Americas. PAHO, Pan American Health Organization; WHO, World Health Organization.

have started the documentation of elimination of measles, rubella, and CRS using standardized guidelines.[752] In addition to the United States, Costa Rica and Canada have published their evidence for the elimination of rubella and CRS.[753] In 2015, it was declared that rubella had been eliminated from the Region of the Americas.

Costa Rica has been a pioneer in the elimination of rubella, having introduced the vaccine as early as 1972. However, because of low coverage of infant vaccination for more than one decade, the epidemiology changed with a shift in the peak age of rubella toward adults. Serologic studies showed that coverage was incomplete, particularly in young adults. In 1999, a large rubella outbreak occurred, with 84% of cases in persons more than 15 years old. A mass vaccination campaign in adults with MR finally eliminated rubella in 2001.[754–756]

Brazil has also had an extensive experience with vaccination. A model immunization program for rubella was conducted in Saõ Paulo, Brazil in 1993 by Massad and coworkers.[757,758] All children 1 to 10 years old were included in a mass campaign with MMR, accompanied by routine MMR vaccination of infants at 15 months of age. Immunity to rubella in the population jumped from 40% to 97%, and, more importantly, the number of CRS cases dropped from 29 confirmed and suspected in 1992 to none in 1994. However, immigration of new children into Saõ Paulo and the absence of a vaccination program for older persons led to a subsequent increase in the overall incidence of rubella. Although the highest incidence in 1997 was still among children 1 to 9 years of age, by 1999 to 2000, the incidence in the older age groups (15 to 29 years) had risen from seven to 13 per 100,000 and exceeded that of the younger age groups. Serologic studies conducted 8 years later showed that previously vaccinated adolescents were 94% seropositive, and 100% if they had received two doses, but in general, the population older than 20 years was 30% seronegative.[449,754,758,759]

Accordingly, a two-phase vaccination plan for Brazil as a whole was developed to accelerate CRS prevention. In 2001–02, in a two-phase campaign, Brazil targeted women 12 to 29 years of age. During the first phase in 2001, a total population of 16,437,881 females in 13 states received MMR vaccine.[760,761] The incidence of rubella dropped to a low level; however, in 2006, a rubella outbreak occurred that spread to other major cities in 2007 and was concentrated among adolescent and adult males who had not been included in the previous vaccination strategy. In 2007, 8753 rubella cases were reported.[762] As a consequence, 47 CRS cases were reported between 2007 and 2008. To reach the 2010 elimination goal, in 2008 Brazil conducted a mass campaign targeting mainly adult males and females, but also included some adolescents. The estimated target population was greater than 70,000,000.[763]

Chile began MMR vaccination of infants in 1990 and booster vaccination of 6-year-old children in 1993. However, rubella outbreaks were observed in 1997 and 1998 involving adolescents and adults. The Chileans launched a mass vaccination program for women 10 to 29 years of age in 1999. Pregnant women were excluded from the mass vaccination, but were vaccinated postpartum.[754,764] Similar to Brazil, Chile experienced rubella outbreaks mainly among adolescent and adult males. During these epidemics, pregnant women were infected and infants with CRS were born. To achieve the rubella elimination goal, Chile conducted a mass campaign in adult males. After the completion of these mass campaigns, the last endemic rubella case in Argentina was in February 2009.[751,752,765]

Australia

Australia also adopted the policy of vaccinating schoolgirls. By 1983, 12 years after the program had started, 96% of pregnant women were seropositive, and in 2000, a serologic survey showed 97% seropositivity.[766] MMR vaccination of children began in 1988–89, followed by vaccination of adolescents of both sexes in 1994–96. A second dose of MMR at age 4 to 5 years has been recommended.[767] Vaccine coverage is 93%, and 97% of children with documented rubella immunization are seropositive.[768] Cases of reported CRS have fallen from approximately 120 per year (prevaccine) to two cases in 2003.[142] Vaccine-induced immunity is high,[769] and since then, there has been only 1 imported case (Burgess M. Personal communication, 2006). In the province of Victoria, rubella incidence fell by 90% between 1995 and 2000,[770] and overall, the incidence in 2001–02 had fallen to 1.3 per 100,000 population.[771]

Between 2008 and 2012 only two CRS cases were recorded, and rubella incidence has fallen so low that rubella may have been eliminated from Australia.[772]

Taiwan

Taiwan has made extensive efforts to vaccinate both girls and women of childbearing age. A serologic survey showed that only 4% of women covered by both programs were seronegative, but older women were still 23% seronegative, showing the need for more vaccination of adults.[773] A recent evaluation pointed out the need to immunize immigrants and to improve vaccination of women before pregnancy.[774]

Japan

An epidemiologic analysis showed that Japan had suffered rubella epidemics every 5 years and reported 1600 CRS cases between 1965 and 1985. Vaccination has been focused on young girls, because of the absence of an MMR vaccine in Japan. Because vaccination is voluntary, 27% of schoolgirls were found to be seronegative in 2003.[775] Nevertheless, a survey by Katow[87] in 2004 found that CRS cases in the past were typical of those found in other countries but that a decrease in CRS cases to one to three per year had occurred because of the application of vaccination in infancy with monovalent products. However, because rubella has not been eliminated from Japan and there has been a recent epidemic, the government launched campaigns with MR vaccine in young adolescents.

Developing Countries

The facts concerning rubella and CRS described earlier under "Epidemiology" suggest that many developing countries have a significant incidence of CRS and could benefit from rubella vaccination. Robertson and colleagues[776] have reviewed the use of rubella vaccine in developing countries.

Most developing countries with middle income, notably Malaysia, Thailand, Hong Kong, and the Middle Eastern states, are vaccinating against rubella. Singapore has made good progress and has not had recent CRS cases.[777] Malaysia has achieved high rubella vaccine coverage, including women of childbearing age.[778] However, in Korea, only vaccination of female infants has been practiced.[779] Large countries such as China and India are vaccinating few of their children against rubella; one estimate of CRS incidence in South India is 32 to 97 cases per 100,000 live births.[728] A recent review concluded that the burden of CRS in India is high but undefined.[780] Modeling analysis concluded that CRS will increase in China, but that routine vaccination of infants would eliminate rubella by 2051.[781] Iran undertook a mass vaccination program of the 5- to 25-year-old population in 2003 because of high levels of seronegativity, resulting in 98% seroconversion.[782] In Sri Lanka, multiple age cohorts of children and women

have been vaccinated.[728] Egypt has conducted a national MR vaccination campaign, achieving 95% coverage,[783] and Taiwan now has high seropositivity in pregnant women, except for immigrants.[784]

Africa has been moving slowly toward inclusion of rubella vaccine in measles campaigns. Nevertheless, only 46% of the world's birth cohort was vaccinated against rubella in 2014. In South Africa, the rubella component of an MMR vaccine elicited seroconversion in 94% of black African infants, and in Oman and Iran, vaccination resulted in 98% seropositivity.[95,571,785]

Rubella outbreaks have recently been reported in two Pacific island countries: Tonga and Samoa. In 2002, the Kingdom of Tonga experienced an outbreak of more than 1000 rubella cases, including 42 with encephalitis. In response to the outbreak, a mass rubella vaccination campaign was conducted, targeting all children from 1 to 14 years of age and women from 15 to 44 years of age. In 2003, Samoa had a similar outbreak, with more than 1000 cases, mainly in school-age children and, to a lesser extent, in younger children. Encephalitis also occurred in Samoa. Thus, encephalitis was much more common in the Pacific island outbreaks than previously reported elsewhere.[29]

Hinman and colleagues[786] reviewed 17 studies of cost-benefit and cost-effectiveness in both developed and developing countries. They found that the economic argument for rubella vaccination was as strong as for hepatitis B and *H. influenzae* type b vaccination, certainly in developed countries, Latin America, and the Caribbean, and, probably, in Asia and Africa, although more data are needed. Considerable progress is being made toward the worldwide prevention of CRS.[677]

In 2000, Babigumura et al.[787] reviewed the economics of rubella vaccination. Twenty-seven studies, 11 cost-benefit analyses, four cost-effectiveness analyses, and one cost-utility analysis were identified. Of these, 20 were conducted in high-income countries, five in middle-income countries, two in lower-middle-income countries, and none in low-income countries. CRS was estimated to cost (in 2012 U.S. dollars) between US$4300 and US$57,000 per case annually in middle-income countries and up to US$140,000 over a lifetime in high-income countries. Rubella vaccination programs, including the vaccination of health workers, children, and women, had favorable cost-effectiveness, cost-utility, and cost-benefit ratios in high- and middle-income countries.[787]

Strategies for Rubella Vaccination

Although many possible strategies for the prevention of CRS have been used, there are two basic approaches: CRS reduction and rubella/CRS elimination. CRS reduction focuses exclusively on immunizing adolescent girls or women of childbearing age, or both groups. This approach will provide direct protection to women of childbearing age; however, the impact of this strategy is limited by the coverage achieved and the range of age groups targeted. The second approach focuses on interruption of rubella virus circulation, thereby eliminating rubella and subsequently CRS. In principal, assuming high coverage, routine vaccination of infants alone would eliminate CRS in 25 to 30 years. Countries can shorten the time frame to elimination by vaccinating wider age ranges. Rubella and CRS will be eliminated within approximately 10 to 20 years when vaccination at high coverage is provided to young children and adolescents (e.g., children 1 to 14 years of age), and within 10 years when vaccination at high coverage is provided to young children, adolescents, and adults.

In 2011, the WHO rubella vaccine recommendations were updated.[515] The WHO recommends that countries that have not introduced rubella vaccination take the opportunity offered by accelerated measles control and elimination to introduce rubella vaccine. The measles vaccine strategy platform provides the opportunity to use combined vaccine and an integrated measles-rubella surveillance system. The preferred strategy for introduction of rubella vaccination is to begin with MR/MMR vaccine in a campaign targeting a wide range of ages together with immediate introduction of MR/MMR vaccine into the routine program.

Countries planning to introduce rubella vaccine into their routine programs should review the epidemiology of rubella/CRS. Depending on the burden of CRS and available resources, a country should define its goal and the time frame for achieving it. Considerable progress has been made on a global level.

By 2014, 140 countries were using rubella vaccine and elimination of rubella is now a goal. Rubella vaccination is being accomplished using combinations containing measles vaccine in 98% of those countries. Vaccine is being administered at 8 to 11 months of age in 11% of countries, at 12 to 18 months of age in 85% of countries, and at older than 18 months of age in 4% of countries.[147]

In the United States, a decision was made to eliminate rubella and CRS.[788] The strategies for elimination were essentially childhood vaccinations supplemented by vaccination of women and other groups as outlined in Table 53.20. As described above, this policy has been successful, and rubella has been eliminated. Nevertheless, some lacunae remain, as shown, for example, by a study of poultry-plant workers born in Mexico and American women coming for obstetrical care, of whom, respectively, 20% and 9% were rubella susceptible.[663,789]

In the United Kingdom, rubella control has emphasized surveillance of rash disease and serologic status of women. Voluntary vaccination of schoolgirls reached approximately 80%, and routine immunization of infants with MMR was continued, despite some defections of parents because of the false accusations of a connection with autism. These strategies have made CRS a rarity, but rubella virus continues to circulate, largely through importation from Asia.[790]

Of importance, Knox,[791] Anderson and May,[792] and Gay and coworkers[793] have shown by mathematical modeling that institution of infant vaccination with inadequate vaccine coverage will decrease rubella virus circulation sufficiently to augment the susceptibility of adult women and thus allow a paradoxic increase in CRS. Another calculation found that a private-sector coverage of less than 60% might increase the incidence of CRS in developing countries with a high force of infection.[794] Indeed, as described above, it appears that the phenomenon may have occurred in Greece, where low-level introduction of MMR in infants through vaccination in the private sector only was followed by increased seronegativity of Athenian women and a large cluster of CRS in 1993.[795] The same phenomenon may also have operated in Costa Rica. In both countries the shift to the older age group was gradual over a 10+-year period during the low-level routine programs. Certainly, as demonstrated in Brazil, vaccination of children only does not entirely eliminate the risk for women of having babies with CRS.[796] Fig. 53.9, based on the mathematical modeling of Nigel Gay, gives the relation between rubella vaccine coverage and the predicted incidence of CRS, and shows that 80% infant coverage is needed to prevent the paradoxic effect (Gay N. Modeling the Impact of Strategy Options. Unpublished document, 2002). From country experiences, incorporating rubella vaccine into the measles strategy has rapidly controlled rubella and reduced CRS. Mathematical modeling of this strategy has also highlighted the impact of this strategy. Using this strategy, countries can introduce rubella vaccine into their programs if they can achieve 80% or greater coverage either through the routine program or regular supplementary

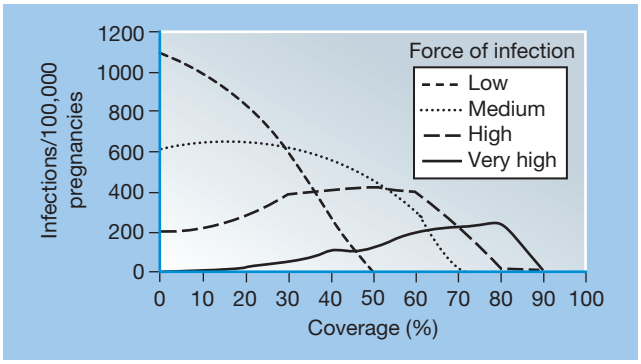


Figure 53.9. Predicted long-term effect of coverage by rubella vaccination of infants on the incidence of congenital rubella syndrome (CRS) according to the force of infection in a given population. *(Courtesy Nigel Gay, Consultant, Berkshire, UK.)*

immunization activities or both. If the force of infection is lower, the paradoxic effect can be avoided with lower coverage, as illustrated in Fig. 53.9.

Thus, to prevent the introduction of rubella vaccination in a country from temporarily increasing CRS, two options are possible: (a) rapid vaccination of all prepubertal children to quickly decrease rubella virus circulation or (b) vaccination of postpubertal females through routine vaccination to render them nonsusceptible. If a country conducts a campaign in postpubertal women, adult males must be included. However, the difficulty of adult vaccination should not act as a barrier to moving forward with elimination of rubella.[655] Measles elimination should be done with combined vaccines (MR or MMR) so as to take advantage of mass campaigns in infants that also deal with rubella. Of course, there must be a commitment to continue universal routine use of the same vaccines in infancy. In fact, many countries have been able to conduct campaigns both in children and in adults as demonstrated in the region of the Americas.[797]

The elements necessary for rubella and CRS elimination include virologic and epidemiologic surveillance for rubella and CRS,[798] high coverage of infants with MMR or MR vaccine, a second dose later in life, and a program for vaccination of adults, either at the time of routine visits for other reasons or in mass campaigns. The latter may include only young women or women up to 39 years of age, depending on resources, but for most complete control, men should also be vaccinated.

THE FUTURE

New manufacturers in developing countries such as the Serum Institute of India have made rubella vaccine more available in the form of MR or MMR. Aerosol administration may also help immunization programs.[304]

Although work continues on recombinant rubella E1 protein produced in Chinese hamster ovary cells transfected with the gene, and the protein is immunogenic in mice,[257] the live attenuated vaccine is likely to be the weapon for rubella control in the future.

Rubella virus and the congenital syndrome it causes are legitimate targets for eradication by vaccination for several reasons[799]: the virus is carried only by humans; the sole reservoir is chronically infected babies with CRS, who do eventually stop excreting; the vaccine is highly effective; and combination vaccination with measles or measles and mumps reduces the cost and avoids any additional expenses of administration.[800,801] There is a steady rise in the number of countries using rubella vaccine, with two WHO regions with rubella elimination goals. Using the measles elimination or control strategies, rubella vaccine can be rapidly introduced into the remaining countries.

In 2011, the Global Alliance for Vaccines and Immunizations (GAVI) board approved the decision to open the funding window for rubella vaccine. Of the remaining 63 countries that have not introduced rubella vaccine, 51 (81%) are GAVI eligible. GAVI will be supporting MR vaccine for catch-up campaigns targeting children 9 months to 14 years 11 months of age. Between 2013 and 2018, it is anticipated that all GAVI-eligible countries will introduce rubella vaccine. With GAVI support, the goal of rubella eradication may be within reach.[802] The implementation of universal immunization throughout the world would mean the end of CRS within 100 years of its description.[803,804]

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54 Smallpox and Vaccinia

Richard B. Kennedy, J. Michael Lane, Donald A. Henderson,† and Gregory A. Poland

The World Health Assembly certified the eradication of smallpox on May 8, 1980,[1] an event that many hoped would consign smallpox to history. However, the former deputy director of the Soviet Union's BioPreparat activities has written that manufacturing facilities had been constructed that were capable of producing smallpox virus in ton quantities.[2] These claims also specified that the State Research Center of Virology and Biotechnology (the VECTOR Institute), in Novosibirsk, Russia, pursued the possible use of recombinant products of smallpox virus as biological weapons. The veracity of these claims is unclear. In addition, since the breakup of the former Soviet Union, there has been no documentation about the whereabouts of scientists previously involved with biowarfare involving smallpox. There have been no public statements by nation states or terrorist groups about plans to develop or use smallpox as a bioweapon, but there is no way to prove the lack of such motivation. Therefore, the United States and several other nations have rebuilt smallpox vaccine supplies and improved diagnostic laboratory capabilities.

Such actions are prudent because the release of an aerosol of smallpox virus and its subsequent spread could result in an international catastrophe. Accordingly, the United States and a number of other countries have developed and stored supplies of new vaccines and planned for an appropriate emergency response should smallpox recur. These countries are working with the World Health Organization (WHO) to devise means for coping with smallpox outbreaks wherever they might occur, recognizing that an outbreak anywhere threatens all countries. These efforts have been strengthened by the development of second- and third-generation vaccines,[3,4] improved antiviral agents,[5] more sophisticated laboratory and diagnostic techniques,[6–8] and a greater understanding of the biology and immunology of orthopoxviruses in general.[9]

HISTORY OF DISEASE

Smallpox is an exanthematous viral disease that was once prevalent throughout the world, existing as an endemic infection wherever population concentrations were sufficient to sustain transmission. Outbreaks of variola major, the only known variety until the end of the 19th century, resulted in case-fatality rates of 30% or more among unvaccinated persons. Most people who survived had distinctive residual facial pockmarks, blindness, or other residua. A less-severe variety, variola minor (known also as alastrim), produced a milder illness with case-fatality rates of 1% or less. Variola minor was first described in South Africa late in the 19th century by de Korte[10] and in the United States in 1897 by Chapin.[11] Variola minor eventually became the prevalent variety throughout the United States, parts of South America, Europe, and some areas of eastern and southern Africa.[12]

Fortunately, there is no animal reservoir of smallpox and no long-term human carrier state; thus, the virus must spread continuously from human to human to survive. Historians and virologists speculate that smallpox emerged sometime after the first agricultural settlements, about 10,000 BC.[12,13] The first certain evidence of smallpox in the ancient world comes from mummified remains of the 18th Egyptian dynasty (1580–1350 BC), evident in the characteristic skin pustules still visible in the mummified remains of Ramses V (1157 BC).[14] Possible epidemics of disease are described in the Bible and in Greek and Roman literature, but descriptions of clinical signs are sparse. More specific written descriptions of the disease did not appear until the 4th century AD in China, the 7th century in Egypt, and the 10th century in southwestern Asia and the Middle East.[12,15–17] Smallpox seems to have progressively spread from the populated endemic areas of Asia and Africa into less-populous areas of these continents and into Europe, becoming established as an endemic infection when populations increased sufficiently in size.[16] Smallpox began to be imported into the Western Hemisphere in the early 16th century by explorers. Catastrophic epidemics followed that seem to have decimated the Amerindian peoples and likely contributed to the collapse of the Aztec and Incan empires.[13] It is likely that smallpox in central and southern Africa probably became endemic about this time or soon thereafter. The impact of smallpox on history and human affairs has been profound.[17] Deities to smallpox became a part of the cultures of India, China, and parts of Africa. In Europe, by the end of the 18th century, an estimated 400,000 persons died annually of smallpox, and survivors accounted for one-third of all cases of blindness. During the 18th century, five reigning European monarchs died of smallpox, and the Austrian Hapsburg line of succession shifted four times in four generations.

The name *variola* was first used during the sixth century by Bishop Marius of Avenches (Switzerland).[18] The term is derived from the Latin *varius* (spotted) or *varus* (pimple). In the Anglo-Saxon world, by the 10th century, the word *poc* or *pocca*, a bag or pouch, described an exanthematous disease, possibly smallpox, and English accounts began to use the word *pockes.* When syphilis appeared in Europe in the late 15th century, writers began to use the prefix "small" to distinguish variola, the "smallpox," from syphilis, the "great pox."[19]

In Europe in the late 18th century, the notion that people who contracted cowpox were thereafter protected from smallpox was fairly common, especially in dairy communities in England and Germany.[20] There is evidence that physicians in these communities were aware, decades before Jenner's work, of the protective effect that prior cowpox infection provided patients, but these physicians did not follow through on the observations or disseminate the findings. A prominent farmer in Dorset county, Benjamin Jesty, inoculated his wife and two sons with material from cowpox lesions in 1774.[21] These persons remained free of smallpox despite local epidemics. In fact, Jesty's sons were variolated 15 years later without any signs of infection. Jesty, however, made no attempt to publicize these observations.[22] Two decades later, in 1796, Edward Jenner (Fig. 54.1) conclusively demonstrated that material could be taken from a human pustular lesion caused by cowpox virus and inoculated into the skin of another person, producing a similar localized and limited infection.[23] He showed that an inoculated person was protected from infection with smallpox after recovery. He called the material vaccine, from the Latin *vacca*, meaning cow, and the process vaccination. Pasteur, in recognition of Jenner's discovery, later

†Deceased.

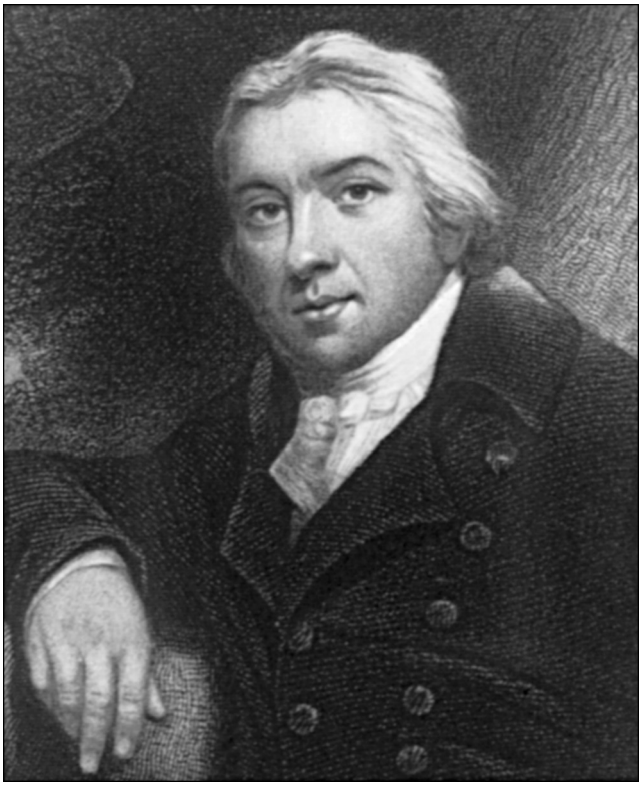


Figure 54.1. Edward Jenner (1749–1823) demonstrated that a person inoculated and infected with cowpox was protected against smallpox. The procedure, which he called vaccination, represented the first use of a vaccine in the prevention of disease. *(Courtesy the Institute of the History of Medicine. The Johns Hopkins University, Baltimore, MD.)*



Figure 54.2. Document signed on December 9, 1979, by members of the World Health Organization and Global Commission, certifying that smallpox had been eradicated. *(From Fenner F, Henderson DA, Arita I, et al.* Smallpox and its Eradication. *Geneva, Switzerland: World Health Organization; 1988, frontispiece, with permission.)*

broadened the term to denote preventive inoculation with other agents.[24] Jenner may not have been the first to observe that cowpox protected against smallpox, but he was the one to follow through on the observations, conduct experiments, and publish his findings.

Toward the end of the 19th century in the United States, vaccinia virus began to be propagated on the flanks of calves, making vaccine more readily and widely available. By 1897, the numbers of smallpox cases had decreased sharply as a result of vaccination and outbreak control.[25] That summer, however, an outbreak of variola minor occurred in Pensacola, Florida. Within 4 years, this variety of smallpox had spread across the country.[26] Routine childhood vaccination continued in the United States until 1972 as a protection in case smallpox was imported and was enforced in most states by compelling vaccination for school entry.

Through the 1800s, other industrialized countries had experiences with vaccination similar to that in the United States. After the initial surge of enthusiasm for vaccination in the early 1800s, vaccination was less uniformly and extensively practiced in most countries until near the close of the century, when the vaccinia virus began to be propagated on calves. By 1900, a number of countries in northern Europe had become smallpox free. By 1914, the incidence in most countries had decreased to comparatively low levels. Even so, during the period from 1910 to 1914, Russia reported 200,000 deaths, and nearly 25,000 deaths were recorded in other European countries.[27,28] World War I led to a resurgence of smallpox in Russia, and it spread from there to many other countries. During the 1920s, vaccination programs again led to the interruption of smallpox transmission in many European countries. By the mid-1930s, smallpox occurred only after importations except in Spain and Portugal, where endemic smallpox persisted until 1948 and 1953, respectively.

In the mid-1900s a concerted effort on part of the WHO and national governments worldwide led to the Smallpox Eradication Program (1966–80) culminating in the eradication of this disease in 1980.

Why Smallpox Is Important

Smallpox was widespread and had a relatively high mortality rate, making it one of the most deadly of all infectious diseases. Its impact on human civilization cannot be overstated. It also represents the only, hopefully the first, human disease to be eradicated (Fig. 54.2). This was made possible by the availability of a heat-stable vaccine, a vaccination technique that was simple and effective, intensive surveillance and containment procedures, and a united, global effort.[29] Although it has been 40 years since the last naturally occurring case of smallpox, the disease and vaccine remain relevant due to outbreaks of zoonotic orthopox viruses and concerns about the use of variola major in bioterrorism.

BACKGROUND

Clinical Description

Smallpox is caused by either of two closely related viruses, variola major or variola minor, that can be distinguished by polymerase chain reaction (PCR) analysis. Clinically they are similar, but variola minor cases are associated with fewer systemic symptoms, less-extensive rash, little persistent scarring, and far fewer deaths. The disease has an incubation period of approximately 12 days, with a range of 7 to 17 days. A 2- to

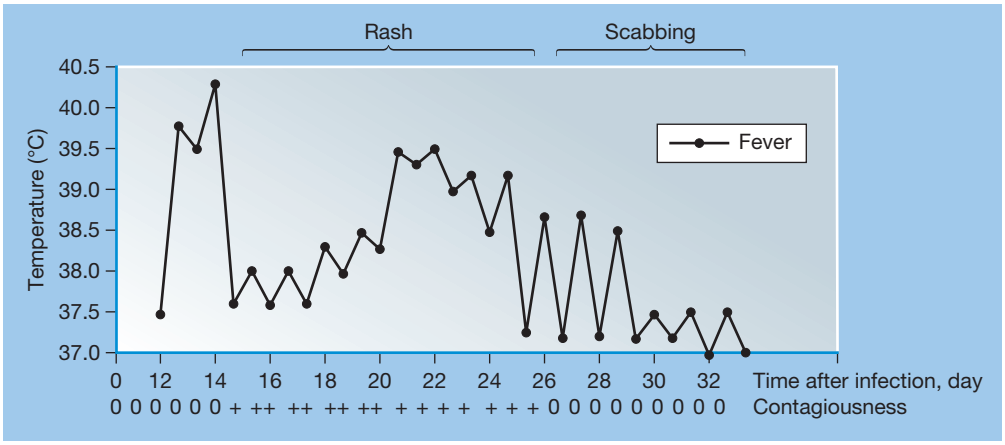


Figure 54.3. Typical temperature chart of a patient with smallpox infection showing the approximate time of appearance and evolution of the rash and the period in which the patient is contagious after acquisition of infection. *(Modified from Henderson DA, Inglesby TV, Bartlett JG, et al. Smallpox as a biological weapon: medical and public health, management.* JAMA. *1999;281:2427–2137.)*

3-day prodrome of high fever (Fig. 54.3), malaise, and prostration with headache and backache is followed by the development of a maculopapular rash (Fig. 54.4). The rash appears first on the mucosa of the mouth and pharynx, then the face and the forearms, and then spreads centrifugally to the legs and trunk. There is a slow evolution over approximately 6 or more days as the rash becomes vesicular and then pustular. The pustules are characteristically round, tense, and deeply embedded in the dermis. Crusts begin to form about the eighth or ninth day. When they separate, they leave pigment-free skin and frequently pitted scars. The eruption is characteristically more extensive on the cooler parts of the body, perhaps because the virus grows better at 35°C than 37°C. Lesions are most numerous on the face, arms, and legs (Fig. 54.4) and are often found on the palms and soles. Death usually occurs late in the first week or during the second week of the illness, and although the cause of death is not definitively known, evidence suggests that it is not the result of bacterial sepsis[30]; rather it is probably from the effects of an overwhelming viremia, the formation of immune complexes, and the associated immunopathology or "cytokine storm."[30,31]

The WHO classification scheme for smallpox[32] describes five types: (a) ordinary, (b) flat (also known as "malignant"), (c) hemorrhagic, (d) vaccine modified, and (e) variola sine eruptione. Rao[33] used this classification scheme to analyze the frequency and case-fatality rates of vaccinated and unvaccinated cases in Madras, India. Among persons not previously vaccinated, the vast majority of variola major cases (approximately 89%) were of the ordinary type and had a fatality rate of approximately 30%. Flat smallpox accounted for 6.7% of cases and had an associated mortality of 96%. The hemorrhagic type accounted for 2.4% of cases and was more common in pregnant women; 96% died, with nearly 100% fetal wastage. Extensive bleeding into the skin and the gastrointestinal tract preceded death; in retrospect, hemorrhagic cases almost certainly represented disseminated intravascular coagulation. Cases "modified" by prior vaccination had many fewer lesions and, in the series by Rao,[33] accounted for 2.1% of the cases and resulted in no deaths. The rash in such cases was usually more scant and atypical, and the evolution of lesions more rapid. Variola sine eruptione (without rash) was rare and occurred in highly immune persons who had laboratory evidence of infection but were not infectious to others.

Complications

Complications of smallpox include bacterial infections of the skin and other organs, pneumonia, generalized sepsis, destructive arthritis in young children and arthropathies, corneal ulceration resulting in blindness (most frequent in patients with trachoma), keratitis, osteomyelitis, and encephalitis. The latter, present in approximately one in 500 cases,[33] is indistinguishable from the acute postinfectious perivascular demyelination observed as a complication of infection with vaccinia, measles, and varicella.[34] Cases of smallpox among pregnant women usually result in spontaneous abortion or, occasionally, a stillborn infant with evidence of lesions on the skin.[12]

The most common sequelae among survivors are facial pockmarks, blindness, and limb deformities.[35] The scars (Fig. 54.5), present in 65% to 80% of survivors, are usually depigmented and most evident on the face and result from the destruction of sebaceous glands followed by shrinking of granulation tissue and fibrosis.[12]

Virology

Variola virus belongs to the genus *Orthopoxvirus*, family Poxviridae, which includes vaccinia, monkeypox, cowpox, camelpox, and ectromelia (mousepox).[36] All species exhibit extensive serologic cross-reactivity in vitro and in experimental animals. The poxvirus genome, the largest of all virions that infect humans, is an ovoid brick-shaped structure with a diameter of approximately 200 nm, consisting of a single molecule of a double-stranded DNA (Fig. 54.6). It differs from most other DNA viruses in that it multiplies in the cytoplasm rather than in the nucleus of susceptible cells.

The orthopoxviruses grow and produce a cytopathic effect in cultured cells derived from many species,[37,38] although they generally grow best in cells from humans and other primates. The four that infect humans (variola, vaccinia, cowpox, and monkeypox viruses) cannot be differentiated readily from one another in most cell cultures. For diagnostic purposes, therefore, they are customarily grown on the chorioallantoic membrane of 10- to 12-day-old chick embryos, on which they produce pocks characteristic of their species.[39] Newer PCR techniques have been developed that permit their differentiation (see "Diagnosis" later).

Although inactivated fairly rapidly by direct sunlight, especially under hot or humid conditions, Variola virus exhibits



Figure 54.4. Images of smallpox infection. A–C, Progression of the rash from days 3 to 5 to 7. **D–F,** Lesions in the same stage of development regardless of anatomical location. **G–H,** Prominent lesions on the hands and feet. **I,** Close-up of umbilicated lesions. **J,** Confluent lesions. *(Courtesy Public Library of Health and the Centers for Disease Control and Prevention.)*

considerable durability in lesion scabs (hundreds of days, and in one case up to 13 years)[40,41] or embedded in cotton (months to years).[42] A variety of historical artifacts (Egyptian mummies from 1200–1100 BC, 17th to 19th century corpses exhumed from permafrost, corpses exhumed from crypts or coffins from the 1500s to 1800s, a museum exhibit containing a scab from a child infected in 1876) have also been tested for the presence of variola virus.[43–46] Although in some cases viral particles have been identified by electron microscopy and viral DNA isolated by PCR, none of these relics has yielded infectious virus.[47]

Pathogenesis as It Relates to Prevention

Smallpox generally follows the progression in the host described by Fenner and colleagues[12] for ectromelia. Smallpox infection occurs by implantation of variola virus on the respiratory mucosa. The virus is usually transmitted as virions in droplets expressed from nasal and oropharyngeal secretions. Higher concentrations of virus are believed to be expelled by patients with the hemorrhagic and malignant forms of the disease. When cough is present, the virus can be expelled as a fine-particle aerosol,[48] but cough is not a typical symptom of ordinary smallpox. After migration to and multiplication in regional lymph nodes, asymptomatic viremia develops about the third or fourth day, followed by multiplication of virus in the spleen, bone marrow, and lymph nodes. A secondary viremia occurs some 8 to 10 days later, accompanied by high temperature, toxemia, and prostration. The virus, contained in leukocytes, then localizes in small blood vessels of the dermis and beneath the oral and pharyngeal mucosa, where it subsequently infects adjacent cells. In the skin, this process causes the characteristic maculopapular lesions that

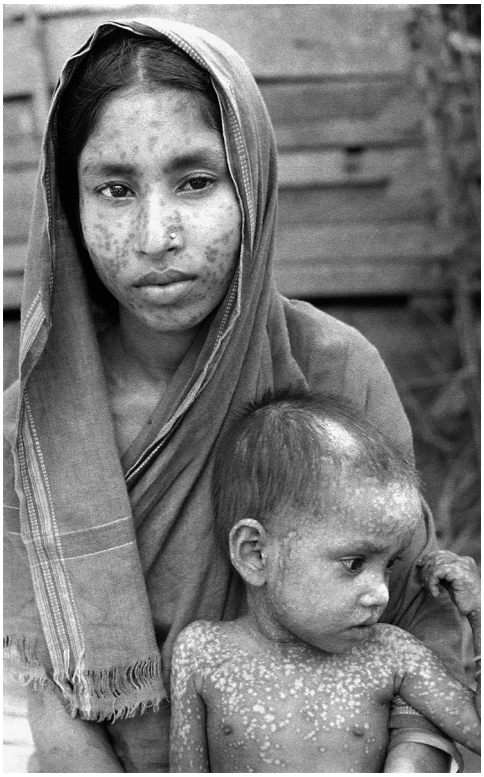


Figure 54.5. Mother and child exhibiting characteristic residual scarring and depigmentation after smallpox. *(Courtesy Public Library of Health and the Centers for Disease Control and Prevention.)*

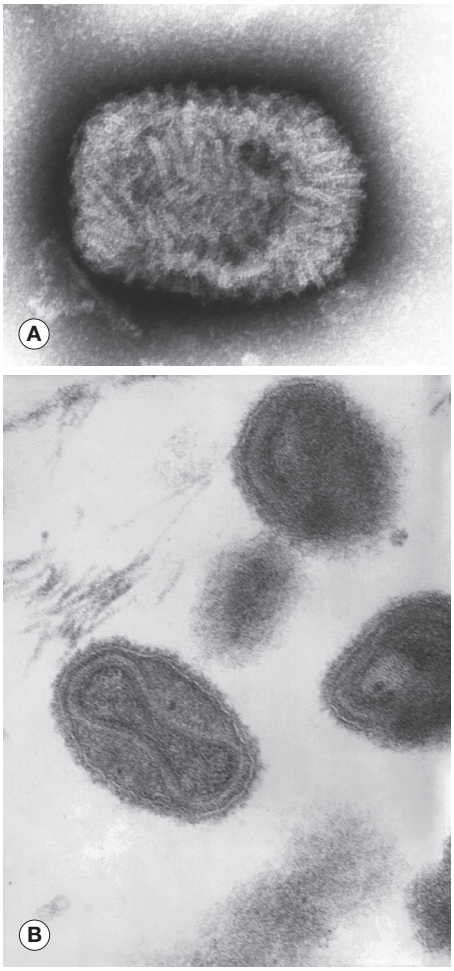


Figure 54.6. Variola virion. A, Transmission electron micrograph of a variola virion. **B,** Transmission electron micrograph showing a cross-sectional view of the variola virion including the dumbbell-shaped core. *(Courtesy Centers for Disease Control and Prevention.)*

then evolve into vesicular and pustular lesions. Lesions are more extensive on the face and extremities, perhaps because the virus grows most readily at temperatures slightly below 37°C. Lesions in the mouth and pharynx ulcerate quickly because of the absence of a stratum corneum, releasing large amounts of virus into the saliva about the time the cutaneous rash first becomes visible. Virus titers in saliva are highest during the first week of rash, corresponding with the period during which patients are most contagious.

Rudimentary immunologic studies of smallpox were done during the eradication era, but little is known about the immune response to variola major in modern terms. Hemagglutinin-inhibiting (HI) and neutralizing antibodies can be detected beginning about the sixth day of illness or, on average, about 18 days after infection, and complement-fixing (CF) antibodies, approximately 2 days later.[49,50] Neutralizing antibodies are long lasting. The HI antibodies decline to low levels within 5 years, and the CF antibodies rarely persist for longer than 6 months. Little is known about the development of cell-mediated immunity following smallpox, but it likely has a significant part in immunity. Modern studies of the immune response to vaccinia and other orthopoxviruses show a complex array of humoral and cellular immune responses, many with manifestations detectable in the first 3 or 4 days after infection.[51–55]

Except for the lesions in the skin and mucous membranes, other organs are seldom significantly involved in variola transmission. Secondary bacterial infection is not common. Death, when it occurs, probably results from the toxemia associated with circulating immune complexes and soluble variola antigens.[56]

Modes of Transmission

Transmission of variola virus, with few exceptions, results from droplets expressed from the oral, nasal, or pharyngeal mucosa of an overtly ill patient that are inhaled by susceptible persons in close contact with the patient. Such transmission is possible from the time of the end of the prodrome and onset of rash and was most frequent during the first week of the exanthem. Spread is generally through large droplets and, thus, was not widely dispersed.[12,57] Virus also was present in scabs that had separated from the skin lesions of convalescing patients,[58] but epidemiologic evidence shows that infected scabs had a negligible role in transmission of infection, presumably because the virus was tightly bound in its fibrin matrix.

Airborne infection over distances of more than a few meters was uncommon, although two outbreaks within hospitals demonstrated this to be possible as they probably resulted from an aerosol dispersal.[48,59] In the outbreak that occurred in a hospital in Meschede, Germany, in 1970, a patient initially presumed to have typhoid fever (and cared for under contact isolation precautions) gave rise to 19 other cases of smallpox in patients who had not been in close contact with the index patient.[48] Dissemination in this outbreak was favored by low relative humidity, prevailing air currents within the hospital

TABLE 54.1 Comparison of Common Conditions and Distinguishing Features of Smallpox

Conditions That Might Be Confused With Smallpox	Clinical Clues
Varicella (primary infection with varicella-zoster virus)	Most common in children <10 y; children usually do not have a viral prodrome
Disseminated herpes zoster	Immunocompromised or elderly persons; rash looks like varicella, usually begins in dermatomal distribution
Impetigo *(Streptococcus pyogenes, Staphylococcus aureus)*	Honey-colored crusted plaques with bullae are classic but may begin as vesicles; regional not disseminated rash; patients generally not ill
Drug eruptions	Exposure to medications; rash often generalized
Contact dermatitis	Itching; contact with possible allergens; rash often localized in pattern suggesting external contact
Erythema multiforme minor	Target, bull's eye, or iris lesions; often follows recurrent herpes simplex virus infections; may involve hands and feet (including palms and soles)
Erythema multiforme (including Stevens-Johnson syndrome)	Major form involves mucous membranes and conjunctivae; may be target lesions or vesicles
Enteroviral infection, especially hand, foot, and mouth disease	Summer and fall; fever and mild pharyngitis 1–2 days before rash onset; lesions initially maculopapular but evolve into whitish gray, tender, flat, often oval vesicles; peripheral distribution (hands, feet, mouth, or disseminated)
Disseminated herpes simplex	Lesions indistinguishable from varicella; immunocompromised host
Scabies; insect bites (including fleas)	Itching is a major symptom; patient not febrile and otherwise well
Molluscum contagiosum	May disseminate in immunosuppressed persons

Data courtesy Centers for Disease Control and Prevention. Evaluating Patients for Smallpox poster. Available at: https://emergency.cdc.gov/agent/smallpox/diagnosis/evalposter.asp.

building, and the presence of cough in the patient. Although cough is not a typical finding of ordinary smallpox, in this case, it would have facilitated viral aerosolization.

There have been instances in which a patient spread the disease to large numbers of contacts, but these were infrequent. In an outbreak in Yugoslavia in 1972, a patient with hemorrhagic smallpox was misdiagnosed as having a severe allergic reaction to penicillin. He was hospitalized because of the development of hemorrhagic disease. In all, he infected 38 healthcare workers and patients before dying.[60] In another patient in the same Yugoslavian outbreak, a woman who had vaccine-modified smallpox spread disease to 16 others. She was a neighborhood matriarch who was visited by many friends and neighbors in her community during her illness and had prolonged face-to-face conversations with many.[12]

Infection of persons such as mortuary workers who handled dead bodies and laundry workers who handled linen from patients has also been repeatedly documented.[60] However, various older accounts that purport to document transmission over great distances on other fomites, such as carpets, letters, and cotton rags, are suspect because the virus does not survive for long periods at customary ambient temperatures or with exposure to ultraviolet sunlight.[61]

Another method of transmission, the ancient practice of variolation (inoculation into the skin of material from pustules or scabs from patients), continued in a number of remote areas until August 1976 and was responsible for many cases in Afghanistan and Ethiopia. In persons inoculated by these methods, extensive rash sometimes developed, and they transmitted infection to susceptible contacts by respiratory droplet infection.[12]

Diagnosis

In endemic areas or when smallpox was known to be circulating, most cases could be readily clinically diagnosed by a history of direct contact with a patient with smallpox, the presence of a prodromal febrile illness, the appearance of a typical deep-seated rash, the centrifugal distribution of lesions, and the fact that all lesions were at the same stage of development on any given area of the body (unlike varicella infection). The infrequent hemorrhagic cases were often initially misdiagnosed as meningococcemia, acute leukemia, or drug toxicity. The diagnosis often was assisted by examination of patients who were the source of their infection or to whom they had transmitted disease. Varicella was by far the most frequent disease confused with the ordinary cases of smallpox (Table 54.1).

Patients with smallpox who had previously been vaccinated and patients with variola minor sometimes had a sparse and atypical rash with minimal systemic symptoms that resembled varicella. In contrast with smallpox, in varicella, the prodromal illness is typically mild or absent, lesions are superficial, appear first on the face or trunk, evolve rapidly and appear to be in different stages of development, and only rarely involve the palms and soles. Severe cases of varicella in adults with extensive rash were also sometimes mistaken for smallpox.[62] For this reason, the Centers for Disease Control and Prevention (CDC) has produced and distributed a chart with an algorithm to assist in the differential diagnosis of acute febrile illness associated with rash.[63] Patients are classified as having a low, moderate, or high likelihood of smallpox based on the presence or absence of a febrile prodrome, the appearance of skin lesions, and other clinical criteria, such as whether the patient appears "toxic." Only patients reporting a febrile prodrome and who have classic smallpox lesions in the same stage of development are classified in the high likelihood category.

A suspected case of smallpox must be reported immediately to the appropriate local, state, or territorial health department and, through them, to the CDC and the WHO. Electron microscopic examination of vesicular or pustular fluid or scabs will reveal the characteristic, brick-shaped orthopox virions.

The presence of poxvirus DNA can be rapidly established by PCR testing. Differentiation among variola, vaccinia, and monkeypox is usually apparent from the patient's history of possible exposure and the growth characteristics of the virus on the chorioallantoic membrane of chick embryos. PCR techniques for rapid identification and differentiation of orthopoxviruses have been developed,[64,65] and continue to be updated, as new poxvirus strains are sequenced, to provide rapid and reliable disease confirmation for public health responses to outbreaks.[66] These methods are available at the CDC and designated member laboratories of the national Laboratory Response Network. Specimens that are required include vesicular or pustular material (scraped or swabbed from the base of a lesion), punch biopsies, scabs, or venous blood. Detailed specimen collection guidelines are available from the CDC.[67] According to the emergency smallpox response plan available on the CDC website,[67] only personnel immunized against smallpox within the past 3 years or personnel wearing appropriate respiratory and barrier protective equipment should obtain specimens from patients suspected of having smallpox.

Treatment and Prevention With Antivirals

There has been considerable progress in development of antiviral drugs effective against orthopoxviruses in recent years.[5] Cidofovir and its more bioavailable analog, CMX001, are nucleoside analogs that are active against viral DNA polymerase. They are effective against vaccinia and variola in vitro.[68] Cidofovir is licensed for treatment of cytomegalovirus retinitis, but the administration of cidofovir is complicated by the fact that it is available only for intravenous administration, requires prehydration with saline and probenecid to minimize nephrotoxicity, and requires close monitoring of renal function.[69] CMX001 seems considerably less toxic, but is currently available for human use only as an investigational new drug. Brincidofovir, a lipid conjugate of cidofovir, has been coadministered with both ACAM2000 and ACAM3000 in a mouse model and found to reduce the severity of vaccination-association lesions with only a slight attenuation in immune response.[70]

ST-246 (tecovirimat) is a small-molecule compound that targets a gene that produces p37, a major envelope protein required for the production of extracellular virus.[71,72] Early evidence suggests it is safe for humans, with minimal manifestations of toxicity even at very high doses. It has proved efficacious against several orthopoxviruses in a variety of animal models as a prophylactic agent and in substantially reducing the pathology of established infections.[72–76] There are animal data that suggest ST-246 can curtail the replication and spread of vaccinia without diminishing the immune response, which would make it ideal for simultaneous treatment and vaccination of close contacts of patients with smallpox (Dennis Hruby, PhD, Oregon State University, Corvallis, personal communication, 2011).[77,78] Currently, ST-246 is available only as an investigational new drug.

Three human patients with immunologic abnormalities and severe life-threatening vaccinia infections have been treated with CMX001 and ST-246. The patients recovered, but were treated with such a wide array of therapies that it is difficult to isolate the specific role of the antivirals used.[79,80] Ribavirin, another antiviral that has some in vitro activity against vaccinia and variola,[5] was used in a patient with progressive vaccinia along with high doses of vaccinia immunoglobulin (VIG).[81] The role of ribavirin in this patient's recovery is uncertain. Animal studies using ribavirin to treat cowpox or vaccinia infections have not shown great promise.[82,83]

EPIDEMIOLOGY

Incidence and Prevalence Data

Smallpox is among the most lethal of all diseases. Unlike malaria and other infections that claim millions of lives annually, smallpox occurred globally when it was endemic (i.e., it affected the world's entire population). In the century before its eradication, it is estimated to have claimed 300 million lives and maimed countless more.[12] As late as the beginning of the smallpox program in 1967, it is estimated that there were 10 to 15 million cases and 2 million deaths in that year alone.[12] Routine childhood vaccination ceased in 1972 in the United States,[84,85] and by 1983, most of the rest of the world had followed suit. As a result, vaccine production facilities closed, and although many countries retained their smallpox vaccine stockpiles, those have been gradually depleted. Now, decades after the eradication of smallpox, a large proportion of the world's population has never been vaccinated or was vaccinated so long ago that immunity has likely waned; thus, the global population is extremely vulnerable to the disease should it reoccur.

The seasonal pattern of smallpox is similar to that of varicella, measles, and other epidemic respiratory diseases. Its incidence was highest during winter and spring. This seasonal peak was consonant with the observation that the duration of survival of an orthopoxvirus in the aerosolized form is inversely proportional to temperature and humidity.[86] Seasonal variation undoubtedly was amplified in many countries by social events, such as the congregation of large numbers of people at festivals and marriage parties during the dry season, and the seasonal movement of nomads.

The age distribution of smallpox cases depended on the immunity of the population, whether the immunity was acquired by vaccination or by infection. Because the disease is less infectious than other childhood diseases such as measles, cases regularly occurred among adults. As recently as 1974 to 1975 in India, where vaccination was widely practiced and smallpox was endemic, adults constituted 21% of a carefully documented series of 23,546 patients; approximately 2% (or 412) of the patients were older than 50 years. In western Africa between 1967 and 1969, most cases occurred in rural villages, and the age distribution of cases approximated the age profile of the population.[87] Men and women were equally affected in all endemic countries.

The severe Asian form of variola major customarily had overall case-fatality rates that ranged from 40% to 50% for persons younger than 1 year of age and approximately 30% among unvaccinated persons. Variola major in Africa and Indonesia was a somewhat milder disease, with age-standardized case-fatality rates of approximately 10% to 15%. Variola minor, which after 1967 was present only in Brazil and southern and eastern Africa, resulted in case-fatality rates of 1% or less (Table 54.2).[12]

Most estimates for the R_0 (the average number of secondary cases infected by each primary case) of smallpox are between 3 and 6,[12,88–90] but have ranged as high as 10.[91] Newer reports indicate that vaccine coverage of at least 66% to 70% is necessary to reach herd immunity levels that will prevent outbreaks,[90,92] and the Smallpox Eradication Program's initial goal was to obtain 80% vaccination coverage.[93,94] However, outbreaks have occurred in populations with high (>90%) vaccine coverage[12,95] and the first WHO Expert Committee on Smallpox recommended 100% vaccine coverage.[96]

Within the household, smallpox was as infectious as varicella but less infectious than measles.[97–99] With few exceptions, however, smallpox spread less widely and rapidly than these diseases. This finding can be accounted for by the fact that transmission of variola virus does not occur until 2 or 3 days

TABLE 54.2 Mortality of Smallpox Infections by Geographic Location

Strain	Country	Case-Fatality Rate (%)[a]
Variola major	Bangladesh	18.5
	India (Tamil Nadu)	26.0
	Burma	17.0
	Afghanistan	16.0
	India (Punjab)	15.0
	Indonesia (Jakarta)	13.0
	Indonesia (West Java)	8.0
	West Africa	13.0
	Togo	8.0
	Pakistan (Sind Province)	9.4
	Pakistan (Karachi)	23.9
	Tanzania	9.2
	Uganda	5.2
Variola Minor	Brazil	0.8
	Ethiopia	1.5
	Sudan	1.2
	Somalia	0.4
	Botswana	0.2

[a]Case-fatality rates for all age groups. These numbers include vaccinated and unvaccinated cases because precise proportions between the two groups are unobtainable. However, vaccinated persons generally have less-severe disease and survive. Fatality rates among unvaccinated persons would be substantially higher.
Data from Fenner F, Henderson DA, Arita I, et al. Smallpox and Its Eradication. *Geneva, Switzerland: World Health Organization; 1998.*

after the onset of the prodromal fever, as attested to by numerous epidemiologic observations. By then, most patients already were confined to bed because of the high fever and malaise, and, thus, secondary cases usually were restricted to the few who came in contact with patients in the household or hospital. For this reason, smallpox outbreaks tended to be clustered in a segment of a town or village and in localized areas of a province or district.[100–103]

High-Risk Groups

With routine immunization being stopped in nearly every country by 1983, nearly all children and the majority of adults have little or no protection against smallpox. If smallpox were to be released today, vaccination would be indicated for all people considered to be at immediate risk of exposure. The people at highest risk would be healthcare providers who might have contact with patients before the disease has been diagnosed and laboratory workers.

Reservoirs of Infection

Variola virus is a uniquely human pathogen and since its eradication in 1980, there are no reservoirs of infection. Today, there is concern that smallpox virus could be released accidentally from a laboratory or deliberately as a biological weapon. Although the risk of these events is not quantifiable, they are generally acknowledged as possible. There are also concerns regarding outbreaks of other zoonotic orthopoxviruses such as monkeypox, and, indeed, a monkeypox outbreak occurred in the midwestern United States in 2003 as a result of exotic small pet mammal trade.[104–106] Fortunately, it was quickly recognized and dealt with appropriately.

SIGNIFICANCE AS A PUBLIC HEALTH PROBLEM

Smallpox as a Biological Weapon

There are anecdotal reports that Spanish explorers gave Amerindians contaminated clothing, leading to devastating epidemics among the Aztecs and Incas, among others.[107] During the French and Indian Wars (1754–67) the British General Lord Jeffery Amherst suggested to his staff that they distribute smallpox-infected blankets to Amerindians.[107] It is not certain that they were successful, however, as smallpox was already raging among some tribes in the Hudson and St. Lawrence River valleys at the time.[108] During the Revolutionary War, smallpox became a serious problem for the Continental Army, forcing George Washington to institute the mandatory variolation for all American soldiers.

The media have raised concern about the risk of a terrorist biological attack using smallpox. Such risks are unknown but probably quite low. Much of the concern stems from a book written by the former deputy director of the Soviet Union's BioPreparat[2] that states that during the height of the Cold War there existed manufacturing facilities capable of producing tons of smallpox virus and that the State Research Center of Virology and Biotechnology (the VECTOR Institute), Novosibirsk, Russia, pursued the possible use of recombinant products of smallpox virus as biological weapons. There have been no public statements by nation states or terrorist groups about development of smallpox as a bioweapon. The United States did not find evidence that smallpox virus existed in Iraq, despite the claims of the aforementioned book's author that Saddam Hussein had the means and plans to use smallpox as a weapon of mass destruction. No published evidence suggests that smallpox is being illegally grown or produced anywhere else. Furthermore, efforts to recover virus from bodies buried in the tundra or from mummified corpses have been unsuccessful.[44–46,109]

In terms of weaponization, variola virus may be lyophilized and is relatively stable as an aerosol.[86] The infectious dose, as suggested from the epidemiology of a hospital outbreak in which aerosol spread was documented,[48] may be only a few virions and may be effectively disseminated through aerosolization. The resulting disease has high mortality and morbidity rates. The current world population is immunologically naïve or has waning levels of protection. There is no effective treatment for the disease. Even a small outbreak would cause global panic and disruption.

Since the terrorist events of September 11, 2001, and the anthrax attacks of fall 2001, the U.S. federal government has procured a large stock of vaccine to meet the needs of the nation in case of an outbreak of smallpox.[110] As of 2015, the U.S. Strategic National Stockpile contained three smallpox vaccines: (1) sufficient doses of ACAM2000 to vaccinate the entire U.S. population, (2) 85 million remaining doses of Aventis Pasteur Smallpox Vaccine (APSV, or "wetvax," a liquid calf-lymph product), and (3) more than 20 million doses of Imvamune (a modified vaccinia virus Ankara [MVA]-based vaccine) to be used in populations for whom live vaccinia vaccines are contraindicated.[110a] Studies indicate that both APSV and Imvamune can be diluted up to fivefold and retain immunogenicity.[110b] In 2002, the CDC released a guidance plan to public health agencies detailing the necessary steps to be taken to contain an outbreak of smallpox, including vigorous surveillance for cases, rigorous isolation of cases, and identification and vaccination of close contacts.[111] Nevertheless, most experts believe that a deliberate release of smallpox remains a low-likelihood but high-consequence event.

PASSIVE IMMUNIZATION (VACCINIA IMMUNOGLOBULIN)

In 1960, Kempe[112] introduced the use of VIG for treatment of severe infections with vaccinia. There have been no formal controlled trials of VIG for treatment of smallpox or vaccinia.[113] In 1961, Kempe and colleagues[114] claimed a reduction of the incidence of smallpox in close contacts of index patients

to approximately one quarter that of a comparison group, although there was no randomization and the trial would not stand up to scrutiny by modern standards. Among 326 contacts receiving VIG, there were five cases of smallpox; among 379 control subjects, there were 21 cases of smallpox. Vaccination or revaccination of all contacts was also performed at various intervals after exposure. Marennikova[115] also conducted a small and poorly controlled trial. These trials suggested there might be some reduction in the incidence and severity of smallpox, but they were too small and inadequately controlled to draw firm conclusions.[12] With the development of more effective antivirals, VIG seems to have no place in the prophylaxis or treatment of smallpox.

Despite the absence of controlled trials of efficacy, VIG has continued to be used to treat adverse events following vaccinia. Historical controls and the opinion of expert clinicians suggest that VIG is effective, however, in the treatment of eczema vaccinatum and some forms of progressive vaccinia, but not in accidental implantation or benign maculopapular rashes. Progressive vaccinia was universally fatal before the introduction of VIG.[112] After VIG was introduced, the case-fatality rate for progressive vaccinia dropped to approximately 25% to 50%, although data may be confounded in that other interventions were often simultaneously used, such as antiviral agents, transfusions of blood from recently vaccinated donors, and surgical debridement.[116] Severe eczema vaccinatum in the pre-VIG era had a case-fatality rate of 8% to 30%.[112,117] After the institution of VIG, the case-fatality rate fell to 1% or less.[118] Note that VIG is contraindicated in patients whose ocular vaccinia is complicated by keratitis; in rabbits, such therapy causes corneal scarring presumably from precipitation of antigen–antibody complexes.[119]

In 2005, the U.S. Food and Drug Administration (FDA) licensed an intravenous form of VIG manufactured by Cangene Corporation.[110] The recommended dose is 6000 U/kg of body weight; severely ill patients may require repeated doses.[120] In addition, the CDC holds investigational new drug protocols for the use of cidofovir and ST-246 in the treatment of adverse effects after smallpox vaccination that fails to respond to treatment with VIG,[121] although there are no animal studies to support their use or benefit in treating adverse vaccine reactions.

ACTIVE IMMUNIZATION

History of Vaccine Development

Although early smallpox vaccines were cowpox virus, a virus from a disease called horsepox was sometimes used. Eventually, however, vaccinia virus became the poxvirus of choice in most major vaccines. Although its exact origins are unclear,[122] vaccinia is not a mutant of variola virus; rather, it may have arisen as a hybrid of cowpox and another orthopoxvirus or through thousands of serial passages under artificial conditions of culture. It is also possible that the species represents a laboratory survivor of a now naturally extinct species of orthopoxvirus.[123] Characterization of strains is further complicated by the fact that a seed lot system for vaccine production was not used widely until the 1960s. Thus, even the strains with common names and ancestors have different passage histories, having been passed sequentially through a variety of vaccinifers, such as cows, sheep, and water buffalo, with periodic passages through rabbits, horses, and even humans. Indicative of the scientific ignorance of vaccine technology until recent decades is a statement of the Ministry of Health of Great Britain, which, in 1928, advised that seed lymph could be obtained from (a) "smallpox direct"; (b) cowpox; (c) horsepox, sheep pox, or goatpox; and (d) vaccinia in the human body.[123]

At various times, attempts were made to create vaccine products based on inactivated virus.[124] Inactivated vaccines elicited high titers of neutralizing antibodies but were ineffective at preventing disease in animal models and human populations.[125–127] Inactivated vaccines consist almost entirely of a single type of poxvirus virion: mature virus (MV) particles, which lack an additional membrane, and associated proteins found on the other major type of poxvirus virion, extracellular virus (EV) particles. Consequently, these vaccines are unable to induce responses to EV proteins, a defect that likely accounts for their lack of protective efficacy.[128–130]

Attenuated strains of vaccinia were created in an effort to diminish the incidence of serious adverse events.[12] Several of these strains based on the New York City Board of Health (NYCBOH) strain (Rivers, CVI-78) produced less-severe reactions but also had reduced immunogenicity, calling into question the level of protection provided against smallpox.[131–136] Another attenuated vaccine, the MVA strain, was produced by a German research group[137] through more than 500 passages in chick embryo fibroblast cells.[138,139] During passage, multiple genetic deletions occurred (approximately 15% of its genome), and it became much more narrowly restricted in its host range. Laboratory studies demonstrated unimpaired MVA gene expression in human cells and a block in virion morphogenesis.[140] The ability to achieve high expression of recombinant genes despite abortive replication is a significant characteristic of this mutant virus.[140] MVA was only developed during the latter part of the eradication effort and, consequently, was never used in a smallpox-infected area. Nevertheless, the potentially improved safety profile of MVA and other attenuated vaccinia strains has led to renewed development of attenuated smallpox vaccine candidates and their clinical testing (see "Future Vaccines" later).

Development of the Smallpox Vaccine

A method for protection against naturally acquired smallpox infection seems to have been discovered in India sometime before 1000 AD, although there is evidence for its use in China as well.[141–144] There it became the practice to deliberately inoculate, into the skin or by nasal insufflation, scabs or pustular material from lesions of patients. This practice (now called variolation) resulted in an illness usually less severe than that acquired naturally by inhalation of droplets. From India, variolation spread to China, western Asia, Africa, and finally, in the early 18th century, to Europe and North America.[145] Case-fatality rates associated with variolation were considerably lower than fatality rates with naturally acquired infection, but infected persons were still capable of transmitting smallpox to others.[146] After cowpox (an orthopoxvirus closely related to variola virus) began to be used as a protective vaccine, the practice of variolation diminished, but continued to be practiced in Ethiopia, western Africa, Afghanistan, China, and Pakistan as recently as the 1960s and 1970s.[12]

Variolation was gradually replaced with vaccination. In fact, within 5 years of publication, Jenner's seminal paper had been translated into six other languages[147] and vaccination was widely used in many countries of Europe. Within a decade, this process began to be used in countries throughout the world. Historically, the chronicles of the de Balmis expedition of 1803 to 1806 vividly describe the transport of the vaccine by sea to Spanish colonies in the Americas and Asia by serial arm-to-arm vaccination of orphaned children.[148,149]

As the 19th century progressed, the initial wave of enthusiasm for vaccination subsided. Occasionally, syphilis, hepatitis, and other infections were transmitted in the process of arm-to-arm vaccination.[150,151] In some areas, significant opposition occurred among religious and governmental leaders and antivaccinationist societies that opposed the principle of

infecting humans with an animal disease, especially under civil mandate.[152–156] Despite Jenner's claims of lifelong immunity, smallpox can and did occur in patients who had previously been successfully vaccinated, and the need for revaccination was demonstrated early in the 19th century.[157] Other issues included difficulty obtaining fresh vaccination material and difficulty transporting vaccine over long distances. These problems were eventually solved by growing vaccinia on the flanks of calves or other animals, providing an adequate and safer supply of vaccine material. This approach was initially used in Italy as early as 1805[158] but did not become widespread until the mid-1800s.[159,160] In the 18th century an English physician, John Haygarth, carefully studied local outbreaks and disease transmission, then published simple rules for the prevention of smallpox. These rules (avoid entering homes of infected individuals, quarantine yourself when sick, clean everything that comes into contact with the patient, do not allow patient to approach susceptible people) proved to be highly effective when followed.[161]

Vaccine Use and Disease Occurrence in the United States

In the United States, variolation was promoted by a prominent minister, Cotton Mather, in collaboration with a Boston physician, Zabdiel Boylston.[17,152] General George Washington instituted variolation among the Continental Army in 1777 in an effort to minimize the drastic effect smallpox outbreaks were having on soldiers' combat readiness.[16] Thomas Jefferson and his family underwent variolation in 1782.[162] Benjamin Franklin lost a 4-year-old son to smallpox in 1736 and later became an outspoken proponent of variolation, even setting up an organization to provide free variolation to poor people.[163,164]

Benjamin Waterhouse performed the first vaccinations in Boston, Massachusetts, in July 1800 using material provided by Jenner[165] and is widely credited with introducing vaccination to the United States.[12,16,165,166] President Thomas Jefferson actively promoted vaccination,[167] although its routine use did not occur until the 1900s owing to difficulties sustaining the virus through arm-to-arm inoculation. Epidemics of variola major continued to occur at intervals, depending on population density and frequency of importations.

Although outbreaks of variola major continued to occur until about 1927, most 20th-century cases of smallpox in the United States were caused by variola minor. The origin of this strain is unknown. However, a similarly mild strain of smallpox, called Kaffir pox, had begun to circulate widely in South Africa in the early 1890s. What, if any, connection there was between these two foci of variola minor infections is unknown. Because the disease was mild and the case-fatality rate was less than 1%, interest in vaccination waned and smallpox incidence increased sharply. To control the disease, public health authorities sought to compel vaccination as a requirement for school entry, an action upheld by the U.S. Supreme Court.[168] However, antivaccinationist sentiment and antipathy toward compulsory measures prevailed in many states, most of which did not support the legislation or actually prohibited compulsory vaccination. Reported cases of smallpox declined from 108,487 in 1921 to 30,151 in 1931. Between 1932 and 1939, 5,000 to 15,000 cases were reported annually, with 21 to 52 deaths. During the following decade, reported U.S. cases steadily diminished, with the last occurring in 1949. This progress occurred in the absence of a nationally coordinated smallpox control effort. Leake attributed improved smallpox control and its eventual elimination to the wider availability of refrigeration and, consequently, better preservation of the vaccine.[169] Routine childhood vaccination continued in the United States until 1972 as a protection in case smallpox was imported and was enforced in most states by compelling vaccination for school entry. In the early 1960s, many importations of smallpox occurred in Europe; almost half of all subsequent cases occurred as a result of contact in hospitals. Accordingly, beginning in the 1960s, the CDC urged the routine vaccination of hospital staff, but few hospitals complied. After the global eradication of smallpox, distribution of vaccine was restricted to the military and to the few laboratories working with orthopoxviruses.

Vaccine Use and Disease Occurrence in the Industrialized World

Of the other major industrialized countries, Canada interrupted transmission of smallpox in the early 1940s, and Japan about 1950. Vaccination continued in all industrialized countries, as it did in the United States, until the mid to late 1970s, as a protective preventive measure in case smallpox was reintroduced. Australia and New Zealand were notable exceptions. These countries, protected by distance and strict quarantine measures, never vaccinated widely but also never became endemic for smallpox.

In 1958, a WHO study group first recommended that a seed lot system be used in vaccine manufacture.[170] Beginning in 1967, an increasing number of vaccine producers began to use one of two strains. Most common was the Lister strain from the Lister Institute in England, which was propagated as seed virus by the National Public Health Institute of The Netherlands for distribution by the WHO. The second strain was the NYCBOH strain, propagated by Wyeth Laboratories, Radnor, PA, using the trade name Dryvax. Three of the largest countries, Russia, China, and India, used other strains: EM-63 in the Soviet Union, the Temple of Heaven strain in China, and the Patwadanger strain in India.

Vaccine Use and Disease Occurrence in the Developing World

With an assured source of vaccinia, the numbers of vaccinations in Europe increased and smallpox was largely controlled in Europe and North America by the end of World War II. Unfortunately, the disease remained endemic throughout tropical and semitropical countries, in part because the vaccine was heat-labile and difficult to keep viable in tropical areas. It had long been noted that dried vaccines were more stable than liquid formulations. Two of the more common methods involved (a) placing vaccine lymph with sulfuric acid under vacuum conditions and allowing it to dry at room temperature and (b) mixing vaccine lymph with stabilizing and antibacterial agents such as peptone and phenol, followed by centrifugal freeze-drying techniques originally pioneered for human plasma.[12] In the 1940s, techniques were developed for large-scale and commercially feasible processes for the manufacture of stable, freeze-dried smallpox vaccine,[171] leading to increased success with vaccination and eradication programs in Indonesia and parts of Africa.

CONSTITUENTS OF CURRENT VACCINES

In the United States, two smallpox vaccines are available for use in an emergency. ACAM2000, developed by Acambis and manufactured by Sanofi Pasteur, is a second-generation lyophilized preparation of purified virus (grown in Vero cell line) in HEPES (hydroxyethylpiperazine ethanesulfonic acid)-buffered saline, pH 7.4, containing 1% to 4% human serum albumin USP, 5% mannitol, and trace amounts of antibiotics

(100 U/mL polymyxin B and 100 µg/mL neomycin) (John Becher, RPh, CDC, personal communication, 2006). It must be reconstituted before use by the addition of a diluent.

A second preparation, nicknamed "wetvax," manufactured by a predecessor company of Sanofi Pasteur in the 1950s, is a liquid preparation, unlike the lyophilized ACAM2000 vaccine. There are no plans to license wetvax owing to lack of availability of complete documentation of manufacture and storage over the years; it would be used only under an investigational protocol and only in an emergency at a 1:5 dilution.

The United States originally intended to stockpile at least 20 million doses of IMVAMUNE, an MVA-based vaccine developed by Bavarian Nordic.[172] This vaccine has gained fast-track status at the FDA for licensure and is available for government use under national emergency rules (see "Future Vaccines" later).[173] The vaccine is currently available as a frozen liquid suspension containing no less than 5×10^7 $TCID_{50}$ (tissue culture infectious dose) with the following excipients: trometanol, sodium chloride, and water. A freeze-dried version with potentially longer shelf life is under development.

Bavarian Nordic is also producing a vaccine in tissue cell culture based on the Lister strain. In The Netherlands, the National Institute of Public Health and the Environment is producing a vaccine in calves based on the Lister strain. In Japan, Kaketsuken has opened a large manufacturing facility in Kumamoto to produce LC16m8 vaccine.[174] There are also indications that the Russians are producing smallpox vaccine, but no official report has been issued.

PROCESS OF VACCINE MANUFACTURE

Classical Vaccine Manufacture

Most vaccine was grown on the skin of a calf, sheep, or water buffalo and harvested after the animal was humanely killed (Fig. 54.7). The vaccine usually was purified by the addition of fluorocarbon and differential centrifugation, and its bacterial content was reduced by the addition of phenol. Peptone was added as a stabilizing agent, and the vaccine was freeze dried. Because of its source, calfskin vaccine inevitably contained some bacteria but, when properly prepared, the number of bacteria was 10 organisms per milliliter or fewer. Microbiologic examination was required to confirm the absence of staphylococci, streptococci, bovine tuberculosis, *Clostridium perfringens,* and *Clostridium tetani*. For reconstitution of the vaccine for multiple puncture vaccination, a solution of 50% (volume/volume) glycerin in McIlvaine solution was used. The vaccine in its freeze-dried form was required to retain a potency of not less than 10^8 pock-forming units per milliliter after incubation at 37°C for 1 month.



Figure 54.7. Inoculation: lymph from calf. *(Courtesy National Library of Medicine, created by C.J. Staniland, 1883.)*

During the 1960s and 1970s, laboratories in Brazil, New Zealand, Sweden, and the United States (Texas State Health Department) harvested vaccinia membranes of chick embryos, a simple process that permits production of a bacteria-free vaccine. However, vaccine from this source proved difficult to produce in a satisfactory thermostable, freeze-dried form, and, as far as is known, only Sweden produced vaccine in eggs that were free of avian leukosis virus.[12,175]

Vaccinia virus grown in tissue culture initially proved difficult to produce as a thermostable, freeze-dried product but, in the 1970s, Hekker and colleagues[176] eventually achieved good results using Lister-strain vaccinia virus grown primarily in rabbit kidney cells. In field trials, the vaccine was comparable to vaccine grown on calf skin,[177] but because of the impending conclusion of the smallpox eradication program, no further use was made of it.

Current Vaccine Manufacture

Because of the eradication of smallpox and the cessation of routine immunization, smallpox vaccine production eventually ceased, and national stockpiles were used for laboratory workers and military personnel. However, increasing concern about the possible use of smallpox as a biological weapon caused the United States, in 2001, to contract with a United Kingdom–based company, Acambis, for a joint effort with Baxter Laboratory in Austria to develop and produce a freeze-dried, plaque-purified, bacteria-free, tissue-culture vaccine using the NYCBOH strain.[178] The United States has also contracted with Bavarian Nordic to produce 20 million doses of an MVA-based smallpox vaccine for use in immunocompromised persons.[179] In all, 200 to 300 million doses of the Acambis vaccine have been delivered to the U.S. national stockpile. Several vaccine manufacturers have begun development and production of next-generation smallpox vaccines (see "Future Vaccines" later). In 2004, the U.S. government passed the Project Bioshield Act (Public Law 108–276) to acquire countermeasures against chemical, biological, radiological, and nuclear terrorism agents. As of 2014, Project Bioshield has spent $1,100,000,000 on smallpox countermeasures. This includes: $625 million for 24 million doses of IMVAMUNE and $433 million for 1.7 million doses of the antiviral Arestvyer (ST-246).[180,181]

Producers and Trade Names

Although some doses of first-generation vaccines may still exist, the currently available vaccines are all second- and third-generation vaccines. In the United States, the following formulation is FDA approved: ACAM2000 (Sanofi Pasteur Biologics Co.). The MVA-based vaccine, IMVAMUNE (Bavarian Nordic; also known as IMVANEX) has been approved in the European Union and Canada. In the United States, IMVAMUNE recently completed Phase II and Phase III clinical trials.

Dosage and Route

Jenner realized that the skin had to be abraded to produce a successful vaccination. Over the years, a number of methods were developed to break open the skin and introduce the virus. Early vaccine preparations undoubtedly had variable titers, and fairly vigorous techniques sometimes were used to enhance the chance of successful vaccination. A variety of knives, needles, and scalpels were used to scarify the skin.

Some techniques, most notably the rotary lancets used in India, Pakistan, and Bangladesh, were painful and caused significant lesions even in the absence of viral replication. In the United States, during the 1920s, Leake[169] vigorously promoted a multiple pressure technique in which virus was pressed into the skin by multiple firm pressures with the tip of a needle held flat against the skin surface.

In the early days of the worldwide eradication campaign, specially adapted jet-injector guns were used to accelerate mass vaccination efforts, particularly in West Africa and Brazil. The guns proved to be cumbersome and expensive to maintain and not well adapted for use in sparsely populated areas. They were abandoned in favor of the bifurcated needle, which was invented in 1965 by Dr. Benjamin Rubin of Wyeth Laboratories.

Presently, first- and second-generation vaccines and the attenuated but replication-competent LC16m8 vaccine are inoculated intradermally with a bifurcated needle. If alcohol, acetone, or other skin preparation is used, it must be allowed to evaporate fully, or it may inactivate the live virus. The vaccine should have a titer of not less than 10^8 pock-forming units per milliliter by assay on the chorioallantoic membranes of 12-day-old chick embryos. Approximately 0.0025 mL of vaccine adheres by capillarity action between the tines of the bifurcated needle when it is dipped into the vaccine. The needle is positioned vertically to the skin surface, usually the lateral surface of the upper arm (Fig. 54.8), with the heel of the hand resting on the arm. Three (for primary vaccination) or 15 (for revaccination) rapid strokes are made sufficiently forcefully so that a trace of blood appears at the vaccination site within 20 to 30 seconds. Good technique produces more than 95% "takes" in vaccinia-naïve persons. The process of scarification with the bifurcated needle has been shown to elicit a strong, local, non-specific, innate immune response that boosts vaccinia-specific immunity and contributes to protection.[182]

Following terrorist attacks in the early 21st century, the limited availability of smallpox vaccine doses spurred a number of vaccine dilution studies.[183–190] Most of these efforts indicated that live virus vaccine doses of first-generation vaccines could be diluted 1:10 with little effect on take rate, seroconversion, immunoglobulin (Ig) G titer, or T-cell response.

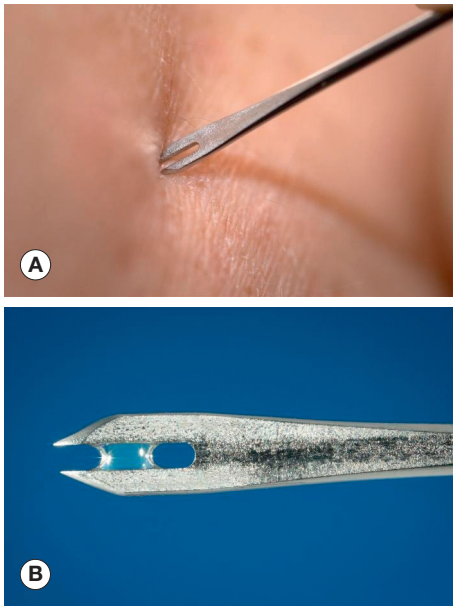


Figure 54.8. Bifurcated needle used for smallpox vaccination. A, Use of the needle to administer the vaccine. **B,** Small volume (≈2.5 µL) of reconstituted vaccine held between the tines of the needle. *(Courtesy Centers for Disease Control and Prevention.)*

Availability in Combinations

Smallpox vaccine is not available as a combination vaccine; however, it can be administered at the same time as a number of other antigens, usually at a different site, with levels of safety and efficacy comparable to those observed when the vaccines are given separately. Simultaneous administration of oral poliovirus, measles, and diphtheria-tetanus-pertussis (DTP) vaccines became a routine practice in many countries beginning in the 1970s.[191,192] Smallpox and bacille Calmette-Guérin (BCG) vaccines began to be administered to newborns in Hong Kong in the 1960s.[191] This became a common practice in many African countries in the late 1960s. Yellow fever and smallpox vaccines were mixed and administered successfully in many French-speaking areas of West Africa,[193] and measles and smallpox vaccines were simultaneously administered throughout western Africa from 1967 to 1972.[194] Mixing of smallpox, yellow fever, and measles vaccines for inoculation by jet injection resulted in a diminished immune response to yellow fever vaccine,[195] but responses were satisfactory when different sites of inoculation were used. Ruben and colleagues[196] extended the studies to the simultaneous administration by jet injection, but at different sites, of smallpox, yellow fever, measles, and DTP vaccines. They found that systemic reactions were no more frequent or severe than those that occur after measles or smallpox vaccination alone, although there was a diminished immune response to measles vaccine. However, this last observation was not confirmed in subsequent studies. From these and other observations, Foege and Foster[197] concluded in 1974 that it was safe and efficacious to administer simultaneously all vaccines (oral poliovirus, DTP, measles, and BCG) then used in the WHO Expanded Programme on Immunization, as well as smallpox and yellow fever vaccines. The Advisory Committee on Immunization Practices (ACIP) recommends that varicella and smallpox vaccines be administered at least 4 weeks apart[198] because if post-vaccination skin lesions occur following the concomitant administration of these two vaccines, it may not be possible to determine which vaccine was responsible and to provide appropriate care.

Vaccine Stability

Freeze-dried smallpox vaccine has proved to be the most stable of the currently available vaccines. The vaccine can be preserved indefinitely at −20°C, and most batches can be preserved for many months to years at 2°C to 8°C. International standards require that the vaccine in its freeze-dried form maintain full potency when it is incubated at 37°C for 1 month. Studies of vaccine produced at the Lister Institute demonstrated that it retained full potency for 64 weeks when incubated at temperatures of up to 45°C and for 104 weeks at 37°C.[199] Not all vaccines are this stable: during the eradication campaign, assay of vaccines produced in India and the former Union of Soviet Socialist Republics (USSR) and retrieved from the field revealed batches of vaccine that met potency standards after 6 to 9 months of exposure at high ambient summer temperatures.[12]

After reconstitution, the vaccine is much more sensitive to temperature and exposure to direct ultraviolet light. During the eradication program, unused reconstituted vaccine was routinely discarded at the end of each day, but, in fact, it will remain potent for a much longer time. Studies on the stability

of undiluted and diluted (1 : 5 and 1 : 10) reconstituted Dryvax showed that vaccine retained satisfactory potency at room temperature (<24 °C) for 1 month with a minimal decrease in viral titer[200] and that vaccine retained satisfactory potency for 2 weeks when subjected to alternate 12-hour cycles of 4 °C and 25 °C temperatures, much like temperature conditions to which the vaccine might be subjected in an immunization campaign.[201] However, the vaccine must be kept out of any extended period of direct ultraviolet light exposure.

IMMUNOGENICITY OF VACCINE

Successful primary vaccination results in virus proliferation in the basal cells of the epidermis, producing a typical lesion known as a Jennerian vesicle (Fig. 54.9). A papule with surrounding erythema develops in 3 to 5 days, rapidly becoming a vesicle, and by day 7, a pustule. It reaches its maximum size after 8 to 10 days. A scab forms that traditionally was expected to separate after 14 to 21 days, leaving a typical vaccination scar. Recent experience showed that the scab sometimes stays attached somewhat longer if the site is bandaged much of the time.[202] A low-grade fever may accompany the development of the pustule, and swelling and tenderness of the draining lymph nodes is often observed. When certain European vaccine strains were used, viremia occasionally occurred between the 3rd and 10th days,[203] and the virus was sometimes isolated from tonsillar swabs.[204] Recent efforts to grow virus from the blood or the oropharynx of NYCBOH strain vaccine recipients postvaccination were unsuccessful.[205,206]

A person's response to revaccination depends on the person's prior degree of immunity. In highly immune persons, erythema typically develops within 24 to 48 hours as a classical delayed hypersensitivity reaction. This reaction can be elicited with both live and inactivated vaccine in previously vaccinated patients.[207] Persons with some residual humoral or cell-mediated immunity, but not enough to inhibit viral replication, develop erythema and sometimes a pustule at the site that evolves more rapidly than a primary vaccination reaction. If erythema or induration is present at 6 to 8 days, it is considered evidence of virus proliferation and is termed a "major reaction." It should be noted that persons with substantial immunity may experience no more than a delayed hypersensitivity reaction. It is impossible at day 5 or earlier after vaccination to distinguish clinically between a delayed hypersensitivity reaction resulting from the use of impotent vaccine or poor vaccination technique and a similar reaction resulting from a high level of immunity. Therefore, the WHO Expert Committee on Smallpox recommended that such a response be termed an "equivocal reaction."[32] Persons with equivocal reactions should be revaccinated with vaccine of known high potency using a vigorous technique.

Vaccinia-induced antibody responses are slightly more rapid than those following smallpox. They can be detected as early as the 10th day after primary vaccination and even more quickly with anamnestic response following revaccination.[208] Evidence of cell-mediated immunity also occurs about the fifth or sixth day.[12,209–211] This accelerated response is associated with complete or partial protection from smallpox infection in persons vaccinated within 3 or 4 days after exposure.[212–215] A considerable body of animal work shows that exogenous antibodies may be effective in treating vaccinia, monkeypox, and ectromelia.[216–219]

Contemporary studies in human vaccine recipients and animal models have found that cellular and humoral immune responses fulfill important roles in protection (Fig. 54.10).[52,130,220] Cytotoxic T-cell activity has a predominant role in the control of primary infection (i.e., clearance of the virus after infection), while antibody and memory B-cell responses are critical to protection during secondary infection.[221–223] Poxvirus immunology research is also focused on determining the genetic basis for variations in smallpox vaccine response or susceptibility to adverse events,[224–229] gene expression studies to determine host–pathogen interactions and the key drivers of immune responses,[230–236] and exploring animal models of infection, disease, immunity, and therapeutic interventions.[230,237–245]

Humoral Responses

After primary vaccination, neutralizing and HI antibodies develop about the 10th day and are present in almost all persons by the end of 2 weeks; CF antibodies develop in fewer than half of vaccinees.[208] The HI and CF antibodies are usually not detectable beyond 6 months. Antibody response after revaccination is more rapid, usually within 7 days, and antibody titers are generally higher. However, some persons who exhibit a substantial rise in neutralizing antibody titer after revaccination do not have a rise in HI or CF antibody levels.

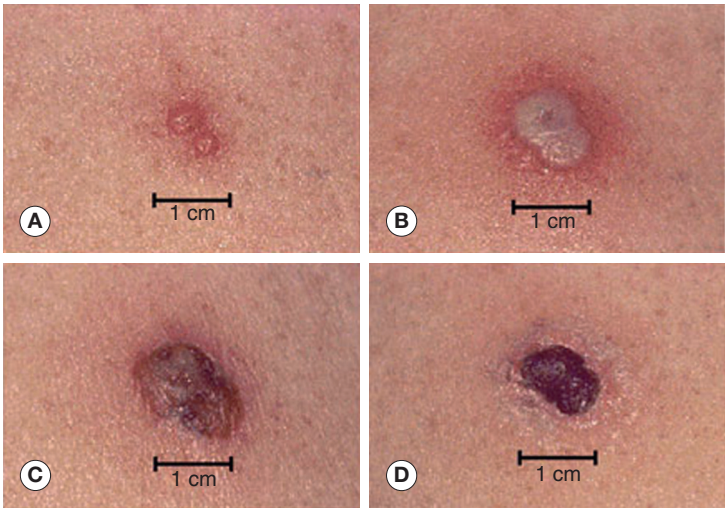


Figure 54.9. Evolution of a normal primary vaccination site reaction. A, Day 4. **B,** Day 7. **C,** Day 14. **D,** Day 21. *(Courtesy the Public Library of Health and Centers for Disease Control and Prevention.)*

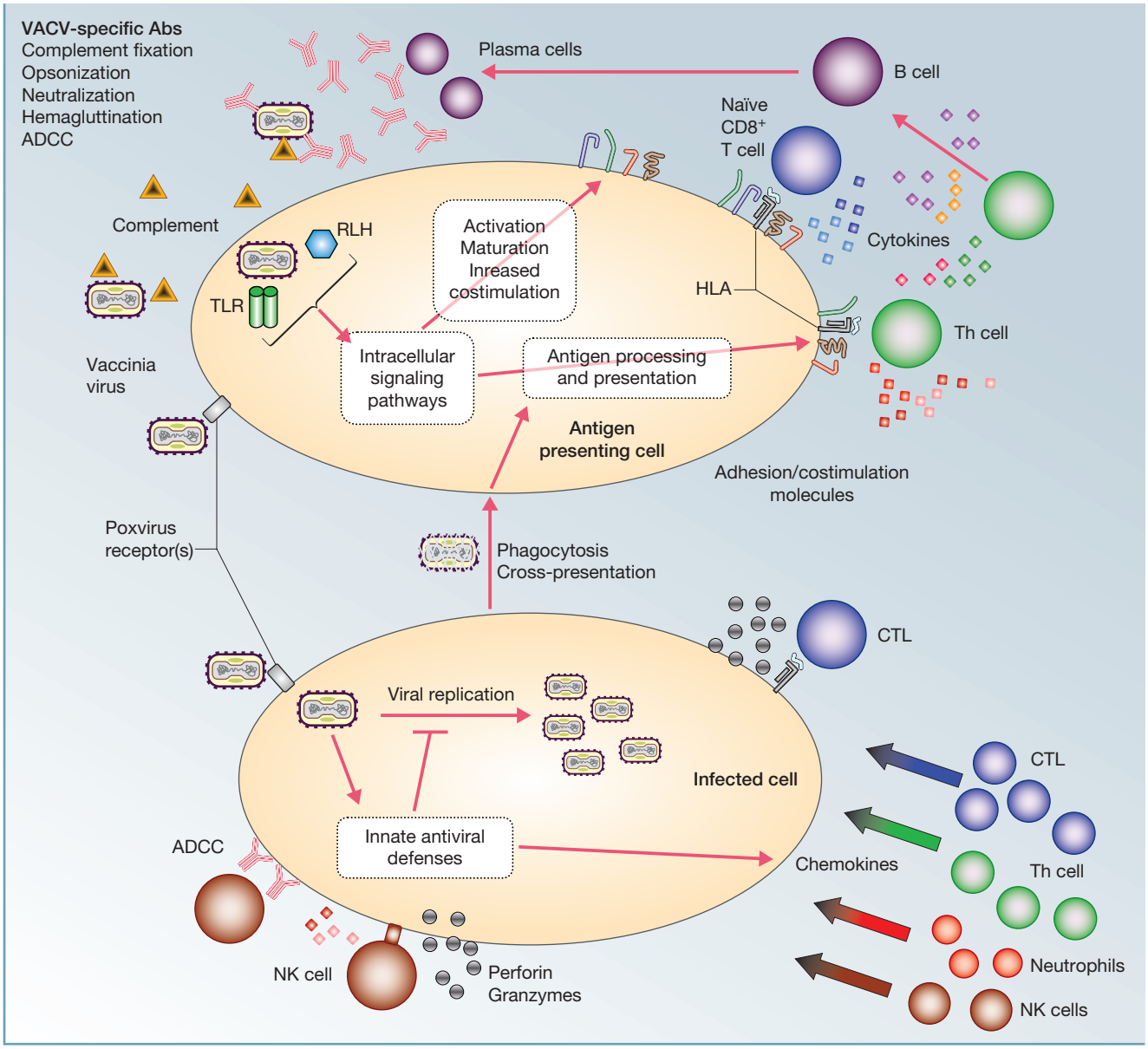


Figure 54.10. Immune response pathways activated by smallpox vaccines. Abs, antibodies; ADCC, antibody-dependent cell-mediated cytotoxicity; CTL, cytotoxic T lymphocyte; HLA, human leukocyte antigen; NK, natural killer; Th, T-helper; VACV, vaccinia virus. *(From Kennedy R, Ovsyannikova I, Jacobson R, et al. The immunology of smallpox vaccines.* Curr Opin Immunol. *2009;21:314–320.)*

Poxviruses have two major forms: MV, the intracellular mature virion, and EV, the extracellular virion containing one additional membrane and several unique proteins.[128] Humoral responses induced by the smallpox vaccine target MV and EV proteins. Antibodies to either form can be protective, but it is thought that optimal protection and disease resistance require antibodies to both forms of the virus.[246] As EV is difficult to neutralize, protection against this form of the virus may be mediated by other, isotype-dependent antibody functions such as opsonization, or antibody-dependent, cell-mediated cytotoxicity.[247] One study shows that an early, neutralizing IgM response appears by day 4 postvaccination.[51] This early response is dependent on T-cell help, is capable of neutralizing the EV and MV virions, and may be important in controlling poxvirus infections.[248–250]

Neutralizing antibodies develop in almost all primary vaccinees by day 4 and can persist for 30 years or more.[51,129,251–255] Titers of neutralizing antibodies measured in primary vaccinees at 1 year were only 23.7% of the titers measured on day 28 after vaccination.[256] Neutralizing antibody titers to EV continue to decrease over time; in fact, 25 years postvaccination, 20% of vaccinees have little to no EV neutralizing activity.[246] In contrast, titers to MV decrease slightly within 5 years of vaccination and then remain stable for up to 75 years postvaccination.[246,254] Revaccination increases total serum antibody titer in most subjects but has little effect on neutralizing antibody titer.[253] Revaccination every 10 years is the current recommendation,[257] but longitudinal studies indicate that protection may last far longer than 10 years.[253,258,259] In the absence of endemic smallpox,

the question of how long after vaccination one remains protected may never be answered.

Cellular Responses

Although Jenner first noticed cellular immunity in the form of the delayed-type hypersensitivity reaction and pock formation at the site of vaccination, T-cell immunity was largely ignored during the eradication efforts. This was because of several factors, including the ease of measuring antibody titer and vaccine take and the lack of proper scientific tools to dissect cellular immune responses. In the last decade, T-cell immunity to smallpox vaccination has received far more attention. Following vaccination, sequential waves of cytokines can be detected in recipient's serum including: granulocyte colony-stimulating factor at days 6 and 7; tumor necrosis factor α, interferon γ, interferon-inducible protein 10, and interleukin 6 on days 8 and 9; soluble tumor necrosis factor receptor 2 and CXCL9 on days 10 and 11; and granulocyte-macrophage colony-stimulating factor on days 12 and 13.[260] Cell-mediated immunity to vaccinia may develop as early as 2 days after primary immunization,[207] peaks approximately 1 month after vaccination,[252,261] and correlates with vesicle formation.[256] In animal models, T-cell help is crucial for optimal formation of antibody responses.[211] Immunization induces extremely robust $CD4^+$ and $CD8^+$ T-cell responses, with up to 3% of circulating T cells being vaccinia-specific.[252] This T-cell population then gradually declines over decades, with $CD4^+$ T lymphocytes exhibiting greater longevity than their $CD8^+$ counterparts.[262–267] Newer work also indicates that the quantity of pathogen-specific T cells matters less than the functional capabilities of those T cells.[268] Although some studies found that antibodies are sufficient for protection against subsequent poxvirus infection,[222,269,270] other studies purport to show efficacy of cellular immunity alone in combating orthopoxvirus infections,[211,271–273] but an insufficient database makes it too preliminary to make strong statements about the comparative efficacy of the two main modes of immunity.

Viral Targets of Immune Responses

Advances in antigen prediction algorithms, pathogen proteome arrays, mass spectrometry, computational modeling, and other techniques allow researchers to comprehensively examine humoral and cellular immune responses to complex pathogens such as poxviruses. The ability to determine which of the many viral proteins are targeted by immune responses and which of these myriad responses provide clinical protection is critical to the development of next-generation, subunit-based smallpox vaccines (see "Future Vaccines later").

Unlike humoral responses to some viruses, smallpox vaccine–induced antibodies target a broad spectrum of viral proteins, with no clear immunodominant pattern. Antibody responses can be found to early and late proteins and predominantly target structural (membrane and core) proteins but can recognize secreted proteins as well (Fig. 54.11).[54,274–278] Convalescent serum samples from smallpox victims and from vaccine recipients show considerable overlap, further evidence of the immune cross-reactivity between vaccinia and variola.[278]

Despite the large genome size and complexity of poxviruses, T-cell epitope identification efforts by a number of laboratories have resulted in the characterization of hundreds of human leukocyte antigen (HLA) class I and class II epitopes.[53,271,272,279–306] The National Institute of Allergy and Infectious Diseases supports a public online epitope database and associated tools that can be found at http://www.iedb.org.[307] As with humoral responses, T cells recognize a wide variety of viral proteins, with $CD8^+$ T cells targeting proteins expressed early in infection[303] and $CD4^+$ T cells recognizing predominantly late antigens and structural proteins.[300] In mice, T-cell responses exhibit clear patterns of immunodominance that can be altered by the route of immunization.[308,309]

Consistent with the T-helper–dependent nature of poxvirus humoral responses, there is a strong correlation between CD4 T-cell epitopes and antibody targets.[302,303] Although it has been reported that there is a deterministic linkage between T-helper and antibody antigens (antigen-specific T cells were found to provide help to B cells specific to the same viral protein),[304] not all studies support this linkage.[310]

Correlates of Protection

During a primary infection, B cells, $CD4^+$ T cells, and $CD8^+$ T cells all play complementary roles in viral clearance and recovery. Upon secondary exposure following vaccination antibody responses are likely to neutralize viral particles before infection, whereas T cells control viral replication after infection by eliminating the infected cells before they can produce additional viral particles. Animal studies in vaccinated mice and nonhuman primates (challenged with mousepox and monkeypox, respectively) clearly show protection in the absence of $CD8^+$ T cells, but not in the absence of B cells and antibody responses.[211,302,303,311] However, vaccine-induced protection wanes at about the same rate as cellular immunity wanes and multiple studies show that $CD8^+$ T-cell peptide-based vaccines can induce protective immunity,[271–273] raising the question of what role T-cell responses fulfill in protection against infection.

The level of humoral antibody necessary to provide protection against smallpox or the development of a major reaction following revaccination is unknown.[312] The best estimate that we have comes from small studies performed during the eradication effort indicating that a neutralizing antibody titer of more than 1 : 20 or more than 1 : 32 conferred some protection against disease.[313,314] Studies of immune responses in vaccine recipients demonstrate a wide range of humoral and cell-mediated immunity.[189,252,256,265,315]

There are even fewer data concerning protection and T-cell immunity. Animal studies involving peptide immunization with a single T-cell epitope range from no protection to complete resistance to disease, with no discernible mechanism behind the differences in protection.[273]

These data and epidemiologic observations suggest the following about the probable protection provided against acquiring smallpox and fatal outcomes:

1. Patients who survive smallpox are fully protected for life from experiencing the disease a second time.[12,16,17,33,316]
2. Persons who responded to primary vaccination and who later experienced a subclinical smallpox infection (variola sine eruptione) are likewise protected for life.[12,16]
3. Persons who responded to primary vaccination and one or more successful revaccinations, even if more than 30 years ago, probably sustain substantial protection against a fatal outcome and some protection against acquiring the disease.[12] Repeated immunizations temporarily boost humoral and cellular immunity.[251,265] Even though total vaccinia-specific antibody levels remain elevated in recipients of multiple vaccinations, neither neutralizing antibody titers nor cellular immune measures appear significantly higher.[252,253] It must be noted, however, that a "successful" revaccination requires that the vaccination has been properly performed

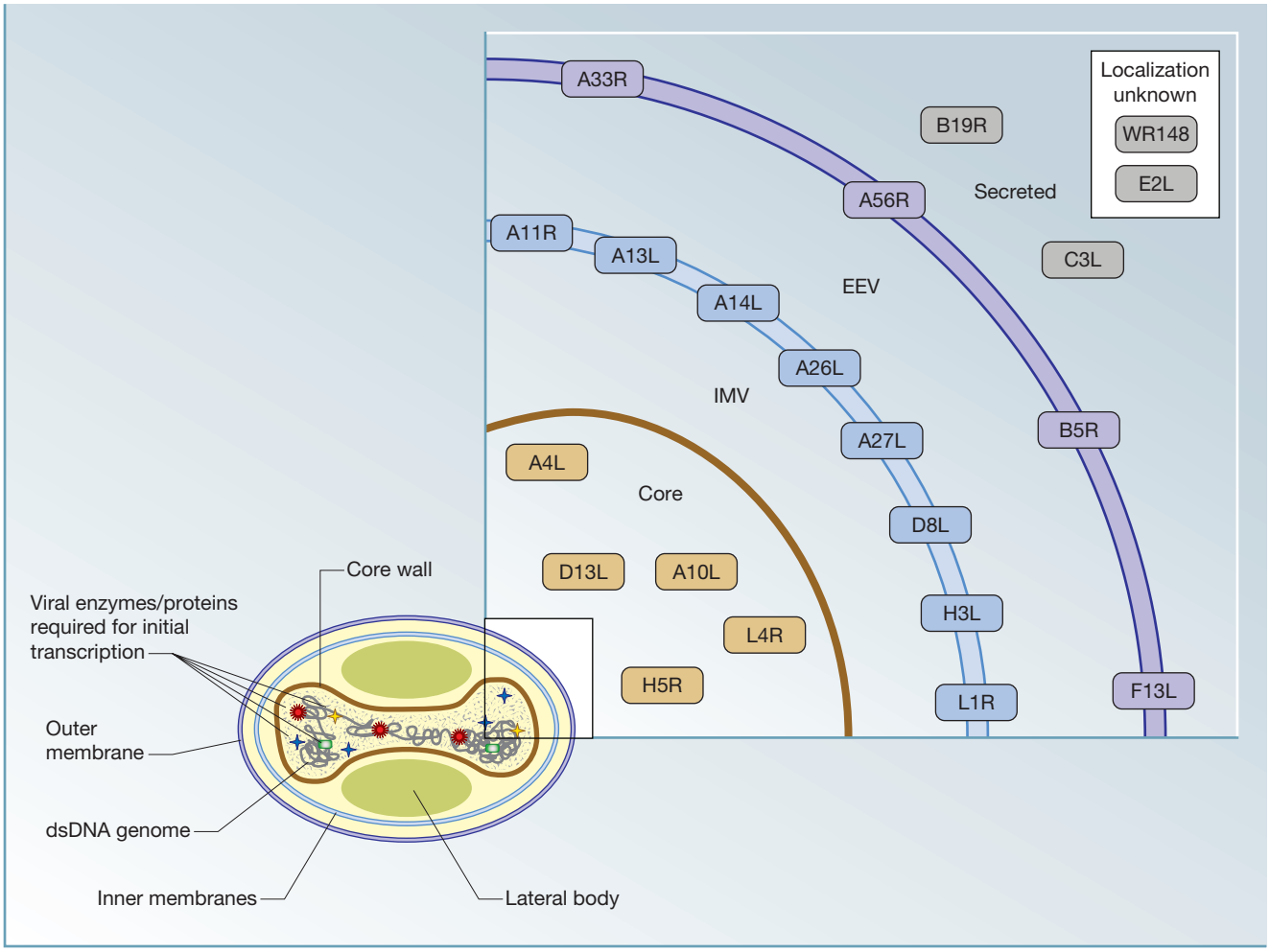


Figure 54.11. Schematic of poxvirus virion showing viral core and double membrane. *Inset* at upper right denotes the location of viral proteins targeted by humoral immune responses. dsDNA, double-stranded DNA.

so that the person has experienced a "major" reaction (see "Immunogenicity of Vaccine"). Because of the past practice in the United States of vaccinating very carefully so as not to draw blood, many revaccinations failed to result in virus proliferation and vaccinees did not have major reactions. It is possible that many of the revaccinations administered in the United States during that period were not successful. In the contemporary military and civilian vaccination programs, high take rates among revaccinees have been documented.[317–319]

4. Persons who have a primary vaccination only will have reasonably complete protection against acquiring smallpox for perhaps as long as 10 years, but protection against symptomatic infection steadily wanes and is probably minimal to nil after 30 years.[12,16] Protection against a fatal outcome of smallpox, however, may persist for a longer time and may, indeed, for some, extend beyond 30 years.[253,258,259,320,321]

In Special Groups (Infants, Immunocompromised)

Vaccination is contraindicated in premature infants and in immunocompromised individuals because of the high risk for adverse reactions (see "Safety [Adverse Events)]" later).

EFFICACY AND EFFECTIVENESS

For the Individual Vaccine

First-generation smallpox vaccine efficacy was typically measured by the development of the characteristic blister (or vaccine take) at the site of immunization. The vast majority (>97%) of individuals developed this take following vaccination. Because it was easily visible and required no special training to document, vaccine take was used as an indicator of successful vaccination, a policy that was successfully used during the eradication campaign. However, this blister is an indicator of local viral replication and not the development of humoral immunity. Preexisting humoral immunity can attenuate or even block the development of this skin lesion upon immunization.[322] Individual immune responses vary widely, and this variability is partly a result of genetic differences between individuals.[224–227,323–326]

For the Community

During the early eradication efforts, mass vaccination campaigns endeavored to reach 80% or more of the population. It was believed that herd immunity levels of 80% to 85% are necessary to stop smallpox transmission.[327] Later, it was found that surveillance and targeted vaccination of primary

contacts for each case was equally effective at containing outbreaks.

EXCRETION OF VACCINE VIRUS, IF ANY

The CDC ACIP recommends that recently vaccinated non–healthcare workers cover the site with a loose gauze bandage and adhere to strict hand hygiene after contact with the vaccinated site during the 2 to 3 weeks in which virus is shed.[198,328] Coverage can delay scab formation, drying, and separation,[202] but reduces the chance of vaccinial transmission, prevents clothing from being soiled, and prevents accidental abrasion of the lesion. More occlusive dressings, even if semipermeable, may cause maceration of the skin and the development of satellite lesions. Studies of site care options to prevent the spread of vaccinia and to avoid excessive local reaction (such as skin maceration) have been published.[329–331] The application of povidone iodine ointment on day 7 after vaccination has been shown to reduce viral shedding without altering the immune response.[332] Bathing is acceptable, but the site should be kept dry otherwise. A separate towel should be used to dry the vaccinated site until a scab forms.

For recently vaccinated healthcare workers, the ACIP recommends avoidance of contact with high-risk patients, if possible, until the scab separates. If contact is unavoidable, meticulous hand hygiene and a dressing comprising gauze (or a similar absorbent material) covered with a semiocclusive dressing or a product that combines an absorbent base with an overlying semipermeable layer are recommended during direct patient care until the scab separates.[198] The ACIP does not believe that healthcare workers need to be placed on administrative leave after being vaccinated unless extensive skin lesions develop, which would preclude adherence with the aforementioned infection control precautions. The contemporary experience of the U.S. military vaccination program supports this conclusion.[257,333–335]

Contact Vaccinia

Contact vaccinia may occur from the accidental mechanical transfer of vaccinia virus from a recently vaccinated person to close contacts. Primary vaccination lesions shed vaccinia from about the third day until a scab forms.[336] Several population-wide studies of contact vaccinia have been performed; they have been reviewed by Neff and colleagues.[337] Two of the studies dealt with the incidence of contact transmission during the course of emergency vaccination campaigns in the United States and Great Britain. During the 1947 New York City outbreak of smallpox, 5 to 6 million persons were vaccinated using the NYCBOH strain. In all, 28 persons developed eczema vaccinatum as a result of close contact with a vaccine recipient, and two of them died.[338] During the 1962 smallpox outbreak in Great Britain, at least 3,250,000 persons were vaccinated using the Lister strain vaccine. There were 185 cases of eczema vaccinatum, of which 89 resulted from contact with a vaccine recipient; seven with contact vaccinia died.[117] Note that compared with contemporary programs, screening to identify persons at risk and the covering and care of the inoculation site are likely to have been much less sophisticated.

National surveys to assess the frequency of complications were conducted in 1963 and 1968 by, respectively, Neff and colleagues[118] and Lane and colleagues.[339] Both used several methods to detect possible cases, but most reports included data for persons who had received VIG for the treatment of complications. The two surveys yielded similar results: the rates for the respective years were 8.7 and 10.7 cases of contact eczema vaccinatum per 1 million primary vaccinees. The incidence of accidental infection resulting from contact was somewhat lower than that for eczema vaccinatum. In all, three deaths were recorded among 114 persons with eczema vaccinatum acquired by contact. There were no cases of progressive vaccinia or postvaccinial encephalitis resulting from transfer of vaccinia virus to contacts (Table 54.3). In statewide surveys

TABLE 54.3 Complications of Smallpox Vaccination in the United States, 1968

Vaccination Status and Age (y)	Estimated No. of Vaccinations	No. of Reported Cases (Deaths)					
		Postvaccinial Encephalitis	Progressive Vaccinia	Eczema Vaccinatum	Generalized Vaccinia	Accidental Infection	Other
PRIMARY VACCINATIONS							
<1	614,000	4 (3)	—	5	43	7	10
1–4	2,733,000	6	1	31	47	91	40
5–9	1,553,000	5 (1)	1 (1)	11	20	32	8
10–19	406,000	—	1 (1)	3	5	3	1
≥20	288,000	1	2	7	13	4	5
Unknown		—	—	1	3	5	2
Total	5,594,000	16 (4)	5 (2)	58	131	142	66
REVACCINATIONS							
<1	—	—	—	—	—	—	—
1–4	478,000	—	—	1	—	1	1
5–9	1,643,000	—	1 (1)	4	1	3	2
10–19	2,657,000	—	1	3	—	—	—
≥20	3,796,000	—	4 (1)	—	9	3	6
Total	8,574,000	—	6 (2)	8	10	7	9
Unvaccinated contacts		—	—	60 (1)	2	44	8
Total	14,168,000	16 (4)	11 (4)	126 (1)	143	193	83

From Lane JM, Ruben FL, Neff JM, et al. Complications of smallpox vaccination, 1968. N Engl J Med. *1969;281:1201–1208.*

in 1963 and 1968,[337,340] physicians were individually contacted and asked to report cases. The rates for contact eczema vaccinatum were approximately double those derived from the national surveys. It was thought that the higher rates reflected more complete reporting, especially of less severe complications.

In their review, Neff and colleagues[330] noted that contact vaccinia required very close contact, seldom occurred outside the home, and rarely occurred as a result of hospital contact. Persons transmitting vaccinia were predominantly children. During the 1960s, it occurred comparatively infrequently, at the rate of only two to six per 100,000 primary vaccinations.[330]

Data from the 1960s are informative but provide only limited insight as to what might be anticipated as a result of large-scale vaccination today, especially of adults. During the 1960s, most persons were vaccinated at about 1 year of age or before school entry. Not surprisingly, most cases of contact vaccinia resulted from transfer of vaccinia from young children to others in the household. Most adults who received the vaccine had previously been vaccinated, with a correspondingly smaller vaccine take and diminished viral shedding. Today, in contrast, a proportionately larger number of persons are at higher risk of complications, and yet the complication rates observed in intense surveillance systems detected adverse events that were largely at or below historic rates.[317,341,342] There were a few exceptions to this rule, as a 2002 study found that the autoinoculation rate for ACAM2000 was 20.6 per 100,000 vaccinations and the contact transmission rate was 4.4 per 100,000 vaccinations.[343] Furthermore, these rates were similar between ACAM2000 and Dryvax. The prevalence of atopic dermatitis in the current population is considerably higher,[344–346] as is the prevalence of serious immunodeficiency in the population because of HIV,[347] organ transplantation,[348] cancer chemotherapy,[349] and the availability of corticosteroid and immunomodulatory therapy for a range of prevalent inflammatory and musculoskeletal diseases.[350] That few cases of contact transfer occurred after 2 million vaccinations of U.S. military personnel who used simple bandages, not semipermeable dressings, suggests the primacy of human factors and postvaccination discipline on infection control and the relative difficulty of transferring this virus in nonintimate settings.

DURATION OF IMMUNITY AND PROTECTION

Reliable data about the protection provided by vaccination are sparse. Furthermore, the degree of protection among a population is difficult to quantify with the passage of decades. Before 1967, revaccination every 3 to 10 years was considered essential to ensure protection. This practice was based on early data largely from the United Kingdom, such as those provided by Hanna and Baxby[213] and on newer data from India.[33] These studies compared the frequency of smallpox cases among persons with and without vaccination scars. However, the vaccine in use in the populations studied had a far lower titer than that used after 1967, and most of the vaccine was heavily contaminated with bacteria. In India, the vaccination instrument used was the rotary lancet, which frequently produced localized sepsis and an apparent scar even when only the diluent was applied. The estimates of protection after successful vaccination in these studies are probably understated.

From studies conducted since 1967, it is probable that vaccinial immunity is more durable than most investigators believed. The evidence is indirect, however, because there are no proven serologic correlates of protection. Unfortunately, resistance to intradermal inoculation with vaccinia virus has been mistakenly equated by some with resistance to variola virus acquired by droplet inhalation. With the available higher titer vaccines, studies show that major reactions can be induced in persons successfully vaccinated as recently as 3 to 6 months before and, indeed, in more than 10% of persons who experienced smallpox only 1 year previously.[351] Because natural infection confers permanent immunity, the ability of vaccinia virus to proliferate in the basal cells of the dermis is not known to correlate well with the level of protection provided against natural infection.

In most countries, 90% or more of smallpox cases occurred among persons without vaccination scars. This finding led to surveys in endemic countries that disclosed vaccine efficacy ratios of 80% or more among persons vaccinated 20 years previously. Heiner and colleagues[99] showed that this protection could not be attributed solely to the vaccine. They discovered that previously vaccinated persons often developed noncontagious inapparent infections (termed variola sine eruptione) with substantial increases in antibody levels. Immunity in the endemic countries was, thus, a composite of past experiences with vaccinia and variola infections.

Data from countries where smallpox was introduced after an absence of many years provide insufficient information to permit a calculation of duration of vaccine protection, but they suggest that vaccinia provides long-term protection against a fatal outcome.[352] Among 680 cases of variola major occurring after importations of smallpox into Europe, the case-fatality rate was 52% among persons who had never been vaccinated, 1.4% among persons vaccinated up to 10 years before exposure, 7% among persons vaccinated 10 to 20 years before exposure, and 11.1% among persons vaccinated more than 20 years before.[352] Other studies show a similar trend of decreasing protection resulting from waning immunity over time.[33,213,353]

As mentioned, studies on smallpox vaccine–induced immunity have found detectable antibody titer and memory B-cell and memory T-cell populations for decades after vaccination. These measures of immune function wane at differing rates,[251–253,255,258,264,354] with antibody responses exhibiting greater longevity. A similar situation occurs in persons who recover from smallpox infection.[264] According to these studies, only 30% to 50% of vaccine recipients maintain neutralizing antibody titers more than 1:32 for 20 years after vaccination. Without established correlates of protection (for either humoral or cellular immunity), it is not known precisely how much protection remains at a given time after vaccination. Complete protection against infection and clinical illness is likely short-lived, lasting for perhaps 5 to 10 years after vaccination, followed by 10 to 20 years of diminishing partial protection (exposure to smallpox then resulting in mild illness such as variola sine eruptione), with protection against fatal disease lasting much longer, possibly for life.[258,259,320,355]

POSTEXPOSURE PROPHYLAXIS AND THERAPEUTIC VACCINATION

Antibody response after primary vaccination usually occurs 4 to 8 days earlier than the response after naturally acquired smallpox infection[356]; thus, primary vaccination, even after exposure, can modify or abort the wild virus disease. The available data indicate the likelihood of substantial protection against disease even when vaccination takes place as long as 3 to 4 days after infection and at least partial protection against a fatal outcome when administered as late as 4 to 5 days after infection occurs.[161,212–215]

SAFETY (ADVERSE EVENTS)

Common Adverse Events

The most frequent symptoms after receipt of the smallpox vaccine are similar to those experienced with other vaccines: pain at the vaccination site, muscle aches, and fatigue, with the addition of pruritus at the vaccination site. Slight temperature elevations are also common, with overt fever occurring in a minority of subjects. Historically, for every 1 million primary vaccine recipients, there would be approximately five to 10 persons with adverse reactions serious enough to require hospitalization and one or two deaths. In the absence of smallpox (or other poxvirus) exposure, these risks are unnecessary. However, they pale in comparison with those encountered during a smallpox epidemic. In fact, a smallpox outbreak with 1 million cases among an unvaccinated population would result in hundreds of thousands of deaths and long-term sequelae (blindness, limb deformities, facial scarring, and depigmentation) among the majority of the survivors.

The frequency of the minor but common adverse events in four contemporary trials of Dryvax and Lister vaccines are shown in Table 54.4. These studies are smaller than the large-scale data available from the 1970s but are more applicable to the current U.S. population. Rates of adverse events are generally similar, and disparities in adverse event rates between these studies likely reflect differences in study design and reporting. For example, the National Institutes of Health Vaccine and Treatment Evaluation Units and the CDC study defined fever as a temperature of 37.7°C or higher, but reported different rates of fever (2.3% and 21.5%, respectively) in different time frames (0 to 6 days and 0 to 20 days postvaccination, respectively). An important note from the data in Table 54.4 is the fact that "repeat" vaccinees had far fewer adverse events than did primary vaccinees. Fever (temperature >38.3°C) occurred in 3.3% to 20% of subjects. In all, 14% of subjects had minor rashes at other than the vaccination site, usually maculopapular or vesicular, affecting the chest and back, with spontaneous resolution in all. A total of 36% of subjects were sufficiently affected by the vaccine to have trouble sleeping or miss at least 1 day of work, school, or recreational activities.[190] In military cohorts, 2.4% reported diminished work activities, 0.4% missed work, and 0.1% were hospitalized.[317,357] Of some concern are generalized vaccinia, erythema multiforme, vaccinia keratitis, and accidental implantation of vaccinia in distal sites. More serious and occasionally life-threatening are eczema vaccinatum, progressive vaccinia, congenital vaccinia, and postvaccinial encephalitis. Estimates of the frequency for these reactions are shown in Table 54.3 (U.S. historical data from the 1960s) and in Table 54.5 (data from the recent U.S. Department of Defense

TABLE 54.4 Common Minor Adverse Events Seen After Immunization in Recent Smallpox Vaccine Trials[a]

Cohort	Nih Vteu (2001)[b]	CDC (2002)[c]		Taiwan (2003)[d]		Dod (2004)[e]
No. of subjects	106	1006		219		628,414
Vaccine tested	Dryvax	Dryvax		Lister[f]		Dryvax
Vaccine recipients[g]	Primary	Primary	Repeat	Primary	Repeat	All
Adverse Event (%)						
Swelling at site	–	63.1	50.0	–	–	62
Pain at site	54.7	52.3	37.5	–	–	–
Lymphadenopathy	30.5	73.8	41.1	65	20	20.0
Fever	2.3	21.5	7.1	20	5	6.6
Headache	44.2	43.1	23.2	50	20	23
Backache	–	20.0	7.1	–	–	–
Muscle pain	39.8	50.8	17.9	40	15	27
Joint pain	–	23.1	12.5	–	–	–
Fatigue	52.8	56.9	35.7	55	25	–
Itching	–	92.3	83.9	–	–	11
Abdominal pain or nausea	15.8	13.8	1.8	–	–	–

[a]The data provide examples of the frequency of common adverse effects of smallpox vaccination. For some of the trials the numbers are very low. Furthermore, much of the data are for Dryvax, which is no longer available. The frequency of side effects from the ACAM2000 replacement may differ. Dashes mean that the specific adverse event was not monitored in the study and no data are available for that adverse event.

[b]Data from Frey SE, Couch RB, Tacket CO, et al. National Institute of Allergy and Infectious Diseases Smallpox Vaccine Study Group. Clinical responses to undiluted and diluted smallpox vaccine. *N Engl J Med*. 2002;346:1265–1274. Data from Table 3, days 0–6.

[c]Data from Baggs J, Chen RT, Damon IK, et al. Safety profile of smallpox vaccine: insights from the laboratory worker smallpox vaccination program. *Clin Infect Dis*. 2005;40:1133–1140. Data from Table 2, subjects ≤30 y old.

[d]Data from Hsieh SM, Chen SY, Sheu GC, et al. Clinical and immunological responses to undiluted and diluted smallpox vaccine with vaccinia virus of Lister strain. *Vaccine*. 2006;24:510–515. Data from Table 4, primary vaccinees receiving undiluted vaccine and repeat vaccinees ages 24–40 receiving undiluted vaccine.

[e]Data from Neff J, Modlin J, Birkhead GS, et al. Advisory Committee on Immunization Practices; Armed Forces Epidemiological Board. Monitoring the safety of a smallpox vaccination program in the United States: report of the joint Smallpox Vaccine Safety Working Group of the advisory committee on immunization practices and the Armed Forces Epidemiological Board. *Clin Infect Dis*. 2008;46(Suppl 3):S258–S270. Data from Table 5, all DoD vaccinees. Lister variant strain unknown.

[f]*Primary* is the first vaccination of vaccinia-naïve subjects; *repeat* is the subsequent vaccination of previously immunized subjects (total number of vaccinations received varies by individual); all includes primary and repeat vaccinees.

CDC, Centers for Disease Control and Prevention; DoD, Department of Defense; NIH VTEU, National Institutes of Health Vaccine and Treatment Evaluation Units.

TABLE 54.5 Complications Per 1 Million Smallpox Vaccinations in the United States[a]

	Dryvax (Sep 2002–Feb 2008)	ACAM2000 (Mar 2008–Jan 2011)	Total[b]
Total vaccinated	1,554,407	502,981	2,057,388
CUTANEOUS AEFI			
EV	3	0	3
PV	0	1	1
Contact transmission	67	19	86
NEUROLOGIC AEFI			
Encephalitis	5	0	5
CARDIOVASCULAR AEFI			
Myopericarditis	171	70	241
VIG treatments	1 for burn, 3 for EV, 1 for PV	3	8
Deaths	1	0	1

[a]Data are for the Department of Defense Smallpox Vaccination Program, Smallpox Vaccine Adverse Events, December 2002 to January 2011. Population details: 2,253,771 screened, 89% primary vaccinees, 88% male, and 70% white. Adverse event numbers include cases defined as confirmed, probable, possible, and suspected (based on surveillance definitions).
[b]Total includes active duty service members, beneficiaries, and nonbeneficiaries.
AEFI, adverse events following immunization; EV, eczema vaccinatum; PV, progressive vaccinia; VIG, vaccinia immunoglobulin.

Smallpox Vaccination Program, 2002–11). Note that during the 1960s, far more vaccinations were given; however, it was a routine vaccination with no subject screening, whereas the data from the 2000s reflect somewhat smaller cohorts that underwent prevaccination screening and include data on Dryvax and ACAM2000. Furthermore, note that Table 54.5 shows a similar frequency of adverse events with ACAM2000 and Dryvax; all recent smallpox vaccine recipients are healthy adults, and we do not have any contemporary data regarding adverse reaction rates among children or the general U.S. population.

Rare Adverse Events (By Organ System)

Skin Reactions

Vaccinees may have a variety of skin rashes after vaccination. Most are benign, but two can be life threatening: eczema vaccinatum and progressive vaccinia (see Tables 54.3 and 54.5).

Eczema vaccinatum (Fig. 54.12A) follows vaccination (or accidental implantation from vaccinated contacts) of persons with active or quiescent atopic dermatitis (including eczema). Concurrently with or shortly after the development of the local vaccinial lesion, or after an incubation period of about 5 days in an unvaccinated eczematous contact, a vaccinial eruption occurs at sites that are eczematous or that had previously been so. The areas become intensely inflamed, and the eruption can spread to involve normal skin. Constitutional symptoms can be severe, with high fever and generalized lymphadenopathy. Subjects may lose fluid and electrolytes through exudation. Deaths are uncommon if the condition is properly treated with VIG and supportive care. The deaths that occur are usually in young children with severe eczema who have acquired vaccinia through contact.

Progressive vaccinia (sometimes called vaccinia necrosum, vaccinia gangrenosa, or disseminated vaccinia) (see Fig. 54.12B) is the most severe complication of vaccination and solely affects immunodeficient persons. These include, among others, persons with agammaglobulinemia, defective cell-mediated immunity, and immune deficiency associated with tumors of the reticuloendothelial system or from the use of immunosuppressive drugs. In the 1960s, this population was composed mostly of persons with agammaglobulinemia or chronic lymphocytic leukemia, and rates were extremely low (≈1 per 1 million vaccinees). Today, patients with serious immunosuppression from HIV, organ transplantation, cancer chemotherapy, or the use of a variety of immune-modifying drugs would constitute most of this at-risk population. In patients with these conditions, the vaccinia lesion fails to heal and usually does not show the cardinal signs of inflammation. Secondary lesions sometimes appear elsewhere on the body and then gradually spread. VIG and methisazone (*N*-methylisatin β-thiosemicarbazone) are reported to have been partially effective in treatment during the 1960s.[358] Data are sparse, however, and methisazone is no longer available for use. Cidofovir, an antiviral agent licensed in 1996 for the treatment of cytomegalovirus retinitis in people with AIDS, shows in vitro activity against orthopoxviruses and may prevent disease in animal models when given shortly before or at the time of vaccinia infection,[359] but it has not been shown to have therapeutic value in treating an established infection of any orthopoxvirus. Likewise, ST-246, another antiviral agent, has shown promise of therapeutic value in the treatment of orthopoxviruses and may be made available for emergency use.

Generalized vaccinia (see Fig. 54.12C) occurs in immunocompetent persons from the systemic spread of vaccinia from the vaccination site. It is a benign reaction in which one to multiple lesions develop within 6 to 9 days after vaccination at locations other than the vaccination site. The evolution of these lesions follows the same temporal course as that of the vaccination lesion itself. Although occasionally patients may experience high temperature and malaise, an uneventful recovery without the need for specific therapy is the norm. Contemporary surveillance suggests that clinicians may be inclined to casually describe a variety of benign maculopapular rashes after smallpox vaccination as generalized vaccinia, but the incidence rate using a strict case definition is far lower than historical reports of about 25 per 1 million vaccinees.[317,341]

Accidental implantation (or autoinoculation) of vaccinia virus occurs when virus from the primary site is transferred to other parts of the body (or to other persons). Accidental implantation is the most common adverse event following vaccination and occurs most frequently in young children who cannot refrain from scratching the pruritic vaccination site and scratching other areas of the body. The most common sites for implantation are the vulva, perineum, and eyelids (see Fig. 54.12D). Lesions resulting from accidental implantation usually heal without therapy at the same time as the primary site lesion. Occasionally, implantation on the eyelids leads to vaccinia keratitis. In the only large series available, 6% of patients with ocular implantation had corneal involvement.[360] Although VIG frequently was given to patients with ocular vaccinia in the hope that it would reduce inflammation, VIG is contraindicated in patients with corneal lesions because antibody–antigen precipitates could cause corneal scars and blindness.[119]

Erythema multiforme (see Fig. 54.12E) is the term used to identify several types of skin rashes that require symptomatic therapy. Rarely, bullous lesions of Stevens-Johnson syndrome develop, requiring aggressive supportive, and even steroid,

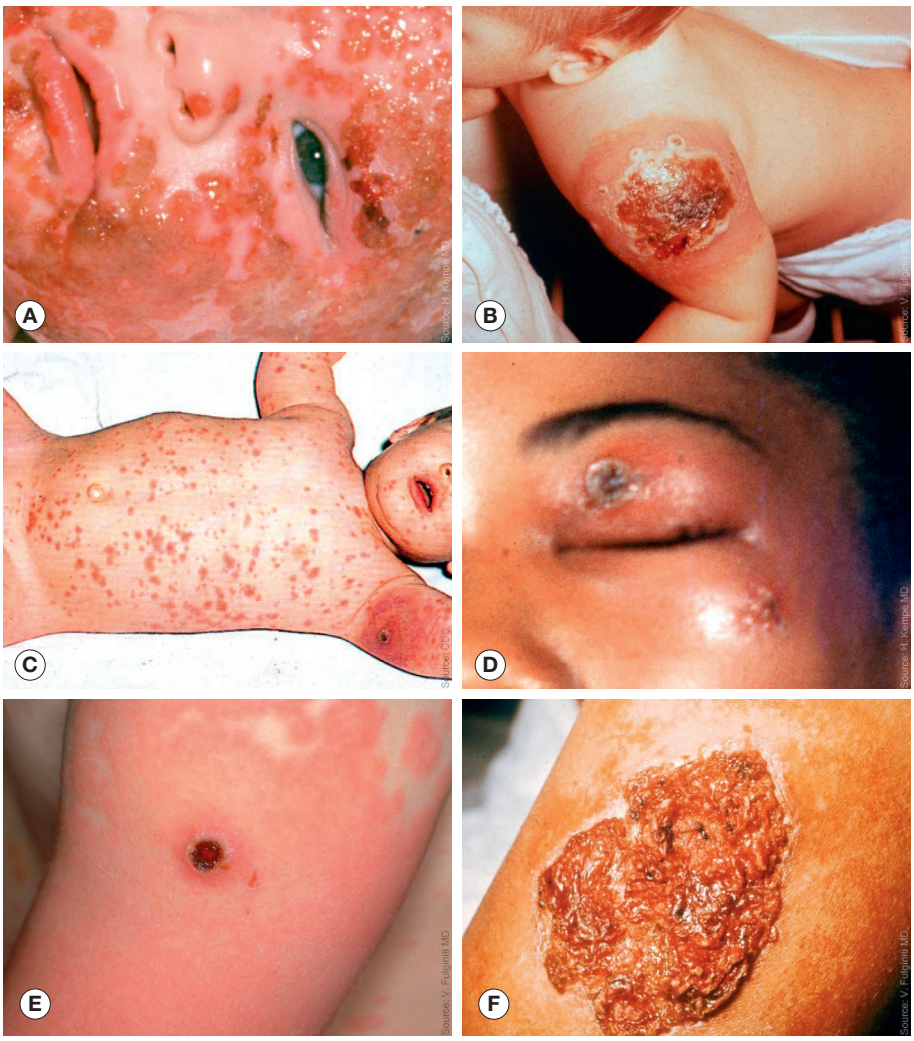


Figure 54.12. Complications of smallpox vaccination. A, Eczema vaccinatum. **B,** Progressive vaccinia. **C,** Generalized vaccinia. **D,** Accidental implantation of vaccinia virus on the eyelids. **E,** Erythema multiforme. **F,** Bacteria superinfection of the vaccinated site. *(A and D, Courtesy Dr. H. Kempe; B, E, and F, courtesy Dr. V. Fulginiti; C, courtesy Centers for Disease Control and Prevention.)*

therapy. Pyogenic infections (see Fig. 54.12F) sometimes occur and may be difficult to distinguish from normal but vigorous primary takes. With improved methods, such infections have ceased to occur. True bacterial infections may require antimicrobial therapy for resolution.

Postvaccinial Encephalopathy and Encephalitis

Among persons without known contraindications to vaccination, postvaccinial encephalopathy and encephalitis are the most serious adverse events, although these were quite rare in the civilian and Department of Defense contemporary programs.[361] The incidence of these complications was substantially higher in Europe, where especially reactogenic strains were in common use in most countries before the 1960s.[362] DeVries[363] distinguished two pathologic forms: encephalopathy primarily in children younger than 2 years of age, and encephalitis or encephalomyelitis in individuals older than 2 years.

Postvaccinial encephalopathy is characterized by general hyperemia of the brain, lymphocytic infiltration of the meninges, widespread degenerative changes in ganglion cells, and perivascular hemorrhage. Symptoms are severe and begin abruptly within 6 to 10 days after vaccination,[364] with fever and seizures, usually followed by hemiplegia and aphasia. Death, when it occurs, follows within a few days. Recovery is seldom complete; the patient is left with mental impairment and some degree of paralysis.

Postvaccinial encephalitis is characterized by perivenous demyelination and microglial proliferation and primarily afflicts persons older than 2 years. It is similar to the form of postviral encephalitis observed after some vaccinations against rabies or after measles infection. Illness usually begins between 8 and 15 days after vaccination and is accompanied by fever, vomiting, headache, malaise, and anorexia followed by disorientation and drowsiness and sometimes seizures and coma. Death occurs in 10% to 35% of cases, usually within a week. Survivors may have residual paralysis or mental impairment. Paralysis, when it is present, tends to be of the upper motor neuron type. Among patients who recover fully, symptoms and signs resolve within 2 weeks.[365–371]

Many reports give the frequency of cases of postvaccinial encephalopathy and encephalitis in Europe and the United States. The rates are difficult to compare because of differing criteria for diagnosis and variability in the completeness of reporting (Table 54.6). The rest of the chapter does not

TABLE 54.6 Combined Incidence Rates of Neurologic Adverse Events

Country and Investigator	Date	No. of Cases[a]	No. of Vaccinations	Rate Per Million
GERMANY AND AUSTRIA				
Austria (Berger and Puntigam[365])	1948–53	32	79,761	401
Bavaria (Herrlich[369])	1945–53	68	1,148,800	59
Dusseldorf (Stuart[370] and Femmer[368])	1948	14	95,836	146
Hamburg (Seeleman[372])	1939–58	46	394,103	117
NETHERLANDS				
Netherlands (van den Berg[371])	1924–28	133	704,150	189
Netherlands (Stuart[370])	1940–43	78	602,069	130
UNITED KINGDOM				
England and Wales (Conybeare[366])	1951–60	66	3,820,369	17
UNITED STATES				
10 states (Lane et al.[340])	1968	16	5,594,000	3
National (Neff et al.[118])	1968	8	650,000	12

[a]Number of cases includes postvaccinial encephalopathy and encephalomyelitis (see text for description of each).
Data from Fenner F, Henderson DA, Arita I, et al. Smallpox and Its Eradication. Geneva, Switzerland: World Health Organization; 1988.

distinguish between the two events. The incidence rates reported from The Netherlands, Germany, and Austria were much higher than those in the United Kingdom, which, in turn, seemed to be somewhat higher than those found in the United States.[118,339,340] Whatever the criteria and methods, differences between the rates seemed to be real. This fact caused a number of countries, during the 1960s, to begin using the Lister strain, then in use in the United Kingdom. A dramatic reduction in the incidence of postvaccinial encephalitis subsequently occurred.[367,373] Only five cases of postvaccinial neurologic adverse events were identified among the 2,057,388 U.S. military personnel vaccinated between 2002 and 2011. Analysis of other neurologic events such as vertigo, meningitis, Bell palsy, and seizures in military and civilian recipients of the smallpox vaccine between 2002 and 2004 found incidence rates of less than two per 100,000 vaccinations for each event. None of the neurologic syndromes were reported at rates above baseline estimates in nonvaccinated populations.[361]

No single laboratory test correlates with strain virulence, but Marennikova and colleagues,[374] using the results of a series of studies in mice and rats, provided a broad classification of a number of strains as follows: (a) least pathogenic: NYCBOH and EM-63 (a derivative of this strain); (b) moderately pathogenic: Lister, Berne, and Patwadanger (from India); and (c) highly pathogenic: Denmark, Tashkent (an older Russian strain), and Ikeda (an older Japanese strain).

Myopericarditis

Occasional cases of myopericarditis (dyspnea, palpitations, and/or chest pain of probably cardiac origin in the absence of any other likely cause of symptoms) after smallpox vaccination were reported through the 1970s when routine vaccination ceased, but whether they were associated causally or temporally was often unclear as myopericarditis has been recognized to occur in association with a number of viral infections.[375] The terrorist attacks in New York and Maryland in September 2001, spurred renewed interest in smallpox vaccination and a reevaluation of adverse event risks in a contemporary population (with higher numbers of individuals with contraindications).

Multiple investigations led by the Department of Health and Human Services identified a causal relationship between myopericarditis and smallpox vaccination in both military and civilian cohorts.[376–380] By May 2006, following the reinstitution of smallpox vaccination of U.S. military personnel, 113 cases of myopericarditis had been reported among approximately 710,000 persons who had received primary vaccinations, whereas four cases occurred among 300,000 revaccinees. These events occurred disproportionately among young adult male white primary vaccinees, clustered in the second week after vaccination, and were associated largely with full recovery of cardiac function as assessed by electrocardiography, echocardiography, and exercise stress tests.[379,381,382] Affected persons experienced fever, fatigue, and myalgia, accompanied by chest pain, beginning between 7 and 12 days postvaccination. Electrocardiograms showed ST-segment changes and an increase in circulating cardiac enzymes (troponin), consistent with myopericarditis. Except for one person in whom severe heart failure developed, patients were generally able to be discharged back to active duty within 7 to 10 days. In more than 2 million vaccine recipients, 241 cases of myopericarditis or pericarditis were noted. This rate is similar to the rate (1.3 per 1000) observed in the recent Department of Health and Human Services civilian program that vaccinated some 40,000 persons.[341] Newer studies benefited from a larger data set that included more vaccinees, and were able to examine the new ACAM2000 vaccine. A study published in 2015 found that 10.6% of new ACAM2000 recipients experienced new-onset cardiac symptoms, compared to only 2.6% of the control group receiving trivalent inactivated influenza vaccine.[383] Using active surveillance, the incidence rate for myopericarditis was estimated to be 463 per 100,000 for smallpox vaccine recipients, 0 per 100,000 for trivalent inactivated influenza vaccine recipients, and 2.2 per 100,000 for unvaccinated, healthy controls. Subclinical myopericarditis rates (defined as elevated cardiac troponin T levels) were estimated to be 2868 per 100,000 for smallpox vaccine recipients and 0 per 100,000 for trivalent inactivated influenza vaccine recipients. These estimates are far higher than the studies performed in the early 2000s and likely reflect the active, prospective nature of the study compared to the earlier, passive surveillance efforts.

Another study, published in 2014, reviewed 1149 reports submitted to the Vaccine Adverse Event Reporting System after ACAM2000 vaccination over the 5-year period between 2008 and 2013.[384] This study found that 54.4% of the serious adverse events and 31% of the nonserious events reported were for cardiac issues. The authors identified 16 cases of myopericarditis and 15 acute ischemic cardiac events.

At least two studies examining cardiac events in subjects receiving MVA-based vaccines did not find any evidence of vaccine-linked myopericarditis.[385,386] A study involving 125 recipients of the LC16m8 vaccine did not identify any cardiac events related to this vaccine.[387] Because of the limited number of subjects in these studies, one cannot rule out a potential risk for myopericarditis. The bottom line is that the first- and second-generation smallpox vaccines do carry a risk of cardiac adverse reactions, which typically occur within 30 days of immunization. Most cases are mild and self-limited. Because individuals with cardiac risk factors were excluded from the clinical trials, safety information on ACAM2000 in persons with heart disease is not available. Persons with known heart disease or cardiac risk factors who are at high risk of infection or with known exposure should be vaccinated because the risk of smallpox is greater than the risk of cardiac reactions.[388]

Other Types of Heart Disease

Among healthcare workers who were vaccinated in the United States, several ischemic incidents (e.g., angina, myocardial infarction) occurred after vaccination.[341,389] These events were detected by a sensitive system designed to monitor adverse events of all types. Many of the afflicted persons had known heart disease or significant risk factors. However, the incidence of ischemic cardiac events identified in civilian vaccinees, including the incidence of sudden death, does not seem to be greater than that expected in a comparable nonvaccinated population.[341] In analyses of several military cohorts, including more than 62,000 vaccinees age 35 years or older, the risk of ischemic cardiac events was comparable among smallpox-vaccinated and unvaccinated groups (Dr. Eric Shry, U.S. Army Medical Department, personal communication, 2006).

While awaiting definitive analyses of the risk of cardiac events postvaccination, the CDC, on the advice of the ACIP, recommended that people with known heart disease or with three or more risk factors for heart disease (such as hypertension, hyperlipidemia, diabetes mellitus, tobacco use, or a first-degree relative with premature cardiac disease) defer being vaccinated.[198] It is now apparent that these contraindications were not warranted based on actual experience.[390]

Unusual Adverse Events

Congenital vaccinia is rare, having been documented on approximately 50 occasions.[391,392] Vaccination during pregnancy does not seem to result in an increase in the incidence of abortions or stillbirths.[393–395] No study has implicated vaccinia virus as a teratogen.[396] The development of a malignant skin tumor, such as a melanoma, in the vaccination scar many years later is a rare occurrence and likely represents coincidence.[397] Vaccinial osteomyelitis occasionally has been recorded and sometimes confirmed by recovery of vaccinia virus.[398]

INDICATIONS FOR VACCINE—WHO AND WHY

In endemic countries, which consisted of most of the world until after World War I, vaccination was recommended for everyone, with revaccination every 3 to 10 years. The only exceptions were infants, for whom primary vaccination customarily was delayed until they were 3 to 12 months old, mainly because frequent vaccination failures occurred at an earlier age. As higher-titer vaccines became available in the 1920s, French, then German, physicians showed that a high proportion of successful vaccinations could be achieved at birth and, in some hospitals, this became routine practice.[399] At least one city in the United States—Detroit, Michigan—mandated neonatal vaccination in the mid-1920s.[400]

As time passed and smallpox incidence declined, smallpox-free countries began to delay primary vaccination until children were older. This resulted in part from the demonstration that maternal antibody inhibited virus proliferation[401] and in part from the belief that older children could better handle the fever and systemic symptoms of vaccinia inoculation. Vaccination at a later age was also less likely to be mistakenly associated with other events, such as sudden infant death syndrome, which might be temporally, but not causally, related. Some European countries recommended that vaccination be delayed until the second year of life to avoid the possible erroneous attribution to vaccinia of cases of encephalopathy resulting from other causes.[367] The United States started vaccinating infants at 12 months of age when studies suggested a higher frequency of postvaccinial encephalitis among infants vaccinated at 9 to 12 months than among children vaccinated between 1 and 4 years of age.[339] No studies were subsequently performed to show that complications became less frequent after these changes in policy.

Vaccination practices in developing countries tended to parallel those in Europe and North America. By 1967, most countries, even those with endemic smallpox, delayed vaccination until the child was 3 to 9 months old. Notable exceptions included Hong Kong,[402] where neonatal vaccination had been traditional at least since World War II, and Madras, India,[33] where neonatal vaccination had been introduced in the late 1950s. During the late 1960s, vaccines that met international standards of potency consistently produced high levels of vaccination takes in newborns. Newborn vaccination became widely recommended, although not all countries followed the practice. There are no adequate studies that compare the efficacy and durability of immunity provided at birth with that provided at older ages, nor are there data that permit a comparison of the relative frequency of vaccination complications at infancy and older ages.

Primary vaccination was provided for adults if required, although there were concerns that adults might have a higher incidence of postvaccinial encephalitis and other serious complications. Earlier European data suggested this to be the case,[362] although studies conducted in the United States, where primary vaccination of adults was rare, did not confirm it.[118,339] A review of U.S. military medical records between 1946 and 1962, conducted by the CDC, found no cases of central nervous system complications among an estimated 2 million basic trainees given primary vaccinations. Data from U.S. military personnel who were vaccinated between 2002 and 2011 indicate that there were five cases in more than 2 million vaccine recipients. The differences between European and U.S. studies probably reflect differences in the pathogenicity of different strains and/or different diagnostic criteria.

Routine vaccination ceased in all countries in the early 1980s, although a number of countries continued to provide vaccination to military forces in case variola virus was to be used as a biological warfare agent. U.S. military vaccination was restricted to trainees in 1984 and discontinued in 1990, until reintroduced in late 2002 for forces being deployed for duty in certain high-risk geographic areas.[317] Otherwise, vaccination, until recently, has been recommended only for persons working in laboratories and animal handlers working in facilities where orthopoxviruses are used.[198]

Recommendations for wider use of the vaccine are under review in some countries because of the threat of use of smallpox as a biological weapon. By the end of 2002, the United States had acquired sufficient vaccine to permit, if necessary, vaccination of the entire country. Because of the substantial risks associated with vaccination, this was not considered desirable unless the risk of release of smallpox virus was substantial. As additional vaccine has become available, it has been possible to consider several options for its use. In a preevent setting (zoonotic orthopox outbreak, bioterrorism act involving variola or another orthopoxvirus), normal contraindications should be followed and the vaccine is only currently recommended for laboratory workers handling orthopoxviruses and for military personnel deployed to southeast Asia. In a postevent setting, everyone except severely immunocompromised individuals should be vaccinated with a replication-competent vaccine while individuals with immune deficiencies should receive an attenuated vaccine (IMVAMUNE) unless effective antivirals are immediately available.[388] The ACIP recommends that vaccination be undertaken to protect persons deemed to be at highest risk should an outbreak occur.[198] These would include smallpox public health response teams at federal and state levels who would be expected to investigate cases and smallpox healthcare teams composed of healthcare workers in acute care hospitals who would be expected to provide 24-hour care for acutely ill patients with smallpox in hospitals. In this plan, every acute care hospital would have the opportunity to establish smallpox healthcare teams, comprising emergency department, intensive care unit, and general medical unit staff; selected medical house staff from a variety of disciplines; medical subspecialists; infection control professionals; respiratory therapists; radiology technicians; security personnel; and housekeeping staff.

As a result, a national smallpox vaccination program was established.[333] It was initially hampered by concerns about possible risks of vaccination and workmen's compensation issues should vaccine-induced medical complications arise. Eventually, almost 40,000 civilian U.S. workers were vaccinated,[341] and some states continue to provide vaccine to select personnel, but this policy is no longer actively pursued. By vaccinating these groups, experience in vaccination was gained by those who would be responsible for vaccinating larger numbers should an outbreak occur and for caring for persons with complications of vaccination.[403] Similarly, this program allowed for the development of surveillance and review procedures to track adverse events related to smallpox vaccination.[404,405] At the same time, several million U.S. military personnel considered at elevated risk have been vaccinated since late 2002.[317,333]

The current risk of natural exposure to smallpox is zero and the risk of exposure from intentional release of variola as a biological weapon is considered to be low; therefore, current ACIP recommendations are to limit preexposure vaccination to selected groups with work-related contact with poxviruses. In case of a smallpox release, ACIP recommends vaccination of the following groups: (a) persons exposed at the site of initial release; (b) persons who come into face-to-face contact with, proximity of, or household contact of persons exposed at the site of initial release; (c) personnel involved in the medical care, public health evaluation, or transport of patients with known or suspected smallpox; (d) persons involved in the collecting or processing of tissues, fluids, or other samples from patients with known or suspected smallpox; (e) any persons with an increased risk of contact with potentially infectious materials from a patient with smallpox (medical waste disposal, laundry services responsible for linen disinfection or disposal, janitorial services responsible for room cleaning and disinfection); and (f) other groups deemed necessary for appropriate response activities (military personnel, law enforcement, and emergency response). Insofar as possible, vaccination of persons in group 6 should be limited to those with no contraindications to vaccination.[198] Contraindications for administration of the smallpox vaccines (including immune deficiencies, cancer, use of immunosuppression drugs, skin disorders, and heart conditions) are discussed later in this chapter. In case of known exposure to smallpox, there are *no* contraindications, although individuals with a significant immunodeficiency might be better treated with VIG and antiviral medications rather than a vaccine to which they will likely not respond.

Meanwhile, some countries in Europe, Asia, and Latin America are contracting for supplies of vaccine, and a few vaccine production facilities are being prepared. The WHO is exploring mechanisms and resources to deal with an outbreak of smallpox wherever it might occur and is establishing a modest stockpile of second-generation vaccines. Constant vigilance and the availability of vaccine will be requirements far into the distant future.

CONTRAINDICATIONS AND PRECAUTIONS

The WHO recognized no contraindications to vaccination in areas that were endemic for smallpox for two reasons. First, the risk associated with smallpox infection was significantly greater than the risk of complications. Second, most vaccinations were performed by persons without medical training who could not be expected to recognize conditions such as eczema or to identify patients with immune deficiency syndromes. The WHO recommended that only patients who were extremely sick not be vaccinated because their subsequent death might be attributed mistakenly to vaccination.

In nonendemic areas, several conditions were and currently are accepted as contraindications. The major conditions include immune deficiency, atopic dermatitis and other skin conditions with open lesions or abraded dermis, pregnancy, and certain hypersensitivities.[257,389]

Immune Deficiency Disorders

Primary or acquired immune deficiency disorders include agammaglobulinemia, hypogammaglobulinemia, a variety of neoplasms, organ transplantation, certain autoimmune diseases, and AIDS. Persons with such disorders may be at risk for development of the sometimes fatal progressive vaccinia and should not be vaccinated. The ACIP does not require mandatory HIV testing before smallpox vaccination but recommends that HIV testing be readily available to persons who want to be tested or who have risk factors for HIV infection.[198] It is not known whether persons infected with HIV with higher CD4 cell counts could be safely immunized, although this is probable. Ten HIV-infected military personnel with median CD4 lymphocyte counts of 483 cells/mm^3 who were inadvertently vaccinated developed Jennerian lesions that healed without sequelae.[406] First- and second-generation vaccines are also contraindicated for persons receiving immunosuppressive drugs for the treatment of cancer or for organ transplant maintenance or receiving systemic steroid therapy (≥2 mg/kg per day or ≥20 mg per day for longer than 14 days).[198] The recent increase in the number and use of immune-modifying drugs also raises concern about vaccinia vaccination in such cases. No data are available on the safety of this vaccine in such cases, although substantial trials of MVA vaccine have been completed in atopic adults and patients with HIV with CD4 counts of more than 250 cells/mm^3, with no cases of eczema vaccinatum or progressive vaccinia.[179,407,408] In terms of immune response, at least one study found that vaccination

with MVA elicits similar antibody titers in HIV-infected subjects and uninfected controls.[409] Animal models show that vaccination with MVA also elicits robust, long-lasting $CD8^+$ T-cell memory.[410–413] These findings are supported by similar results in human vaccine studies.[268,414]

Eczema, Atopic Dermatitis, and Other Skin Disorders

Persons with a history of or active eczema or atopic dermatitis, irrespective of disease severity, are at special risk for the development of eczema vaccinatum after they or their close contacts are vaccinated.[198] Family members with eczema are at risk from contact spread of vaccinia virus, so the healthy vaccinee or the eczematous family member should live apart, if possible, until the lesion has fully scabbed over. Otherwise, strict infection control precautions, as described earlier (see "Excretion of Virus"), should be observed. Other skin conditions that may be of concern, if extensive, include acne, burns, impetigo, varicella zoster, herpes, psoriasis, open or surgical incision wounds, Darier disease, and contact dermatitis. It is best for vaccination to be deferred until the condition is resolved. If vaccine is administered, patients should be counseled in ways to prevent the transfer of vaccinia from the primary site to the affected area.[198] In a mouse model of eczema vaccinatum, MVA immunization did not cause satellite lesions and protected greater than 83% of animals against a lethal intranasal challenge.[415] These promising results indicate that third-generation attenuated vaccines may provide protective immunity for those individuals with contraindications for the conventional live virus smallpox vaccines.

Disorders of the Central Nervous System

Some countries considered disorders of the central nervous system in potential vaccinees to be contraindications to vaccination. It was hoped that this might decrease the risk of postvaccinial encephalitis, but there is no evidence that this diminishes the risk.

Pregnancy

Pregnant women are not vaccinated on the general principle that live viral immunizations should be avoided during pregnancy and because of the very low risk of fetal vaccinia. The ACIP does not recommend routine pregnancy testing of women of childbearing age before smallpox vaccination (other than verbal screening), but recommends the exclusion of women who are pregnant or intend to become pregnant within a 4-week period following vaccination.[198] Because fetal vaccinia seems to be an extraordinarily rare complication, inadvertent vaccination of a pregnant woman is not a reason to terminate pregnancy. Pregnant women who have direct contact with a patient with smallpox should be vaccinated.

Other Contraindications

Vaccine is contraindicated in persons with a history of acute hypersensitivity to vaccine components such as polymyxin B sulfate, streptomycin sulfate, chlortetracycline hydrochloride, and neomycin sulfate.[416] Because persons with certain inflammatory eye disorders (that predispose to itching or rubbing the eye) might be more likely to autoinoculate their eyes following vaccination, it may be best to avoid vaccinating them.

Some authorities recommended withholding vaccination from patients with various serious acute or chronic illnesses, hypothesizing that the response to vaccination might be abnormal. There is no evidence for this occurrence except that people with leprosy sometimes developed erythema nodosum leprosum or neuritis after primary vaccination.[417,418] In endemic areas, however, people with leprosy were vaccinated because the risk of smallpox substantially outweighed the risk of adverse events.

As mentioned, exemptions based on cardiac risk factors were adopted by the CDC in spring 2003, after several ischemic events occurred among recent vaccinees. Further assessment has not identified any elevated risk among vaccinees in the contemporary program or in retrospective evaluations of a mass vaccination program in New York City in 1947.[419,420]

PUBLIC HEALTH CONSIDERATIONS

Epidemiological Effects of Vaccination

Disease Control Strategies

As a consequence of the eradication of smallpox, disease control strategies are no longer needed. These efforts have been replaced with sensitive diagnostic assays and regular training for first responders to quickly and accurately identify, diagnose, and contain potential outbreaks.

Cost-to-Benefit Information

In the years preceding the eradication program, all countries were expending substantial funds to control smallpox and care for its victims or to vaccinate and operate quarantine programs to prevent the importation of disease. In India alone, the estimated cost of smallpox in 1967 was US$722 million (US$10 million used to control smallpox through vaccination, US$12 million to care for sick patients, and US$700 million in lost economic productivity). Even in the absence of endemic disease, the costs were tremendous. In the United States, the estimated cost associated with smallpox protection in 1968 was US$150.2 million (US$93.5 million to vaccinate and treat complications, US$42.2 million in lost productivity, and US$14.5 million for international traffic surveillance and related to delays in clearance of vessels).[12] Other countries, such as the United Kingdom and the Federal Republic of Germany, maintained special hospitals to be opened to receive patients when imported cases of smallpox occurred.

For the entire global eradication program, the overall cost was estimated at approximately US$300 million, of which US$100 million represented international assistance. The savings, as a result of cessation of vaccination and quarantine measures, has been estimated to be more than $1 billion annually.[12] The United States is estimated to realize in savings the total of all its contributions to the eradication program every 26 days.[421]

Smallpox Eradication

The first commitment to smallpox eradication was made in 1950 by the Pan American Sanitary Organization, which decided that year on a hemisphere-wide effort.[422] Freeze-dried vaccine was used in mass vaccination campaigns that, during the succeeding decade, eliminated smallpox from all countries except Argentina, Brazil, Colombia, and Ecuador.

The World Health Assembly considered smallpox eradication programs in 1950, 1952, 1954, and 1955. The first resolution for smallpox eradication, introduced by Dr. Brock Chisholm (WHO Director General), was rejected. In 1958, Dr. Victor Zhdanov, Vice Minister of Health of the Union of Soviet Socialist Republics (USSR), proposed to the World Health Assembly that a global smallpox eradication program be undertaken.[423] This second resolution was approved the

following year at the 12th World Health Assembly in May 1959.[424] During the succeeding 7 years, a number of countries embarked on mass vaccination campaigns, and several, including China, eliminated the disease. Overall, however, progress was disappointing, especially in Africa and the Indian subcontinent. Few countries voluntarily contributed resources, and the WHO, then preoccupied with a costly and disappointing global malaria eradication program, provided few of its own resources and little support.

Frustrated by lack of progress in the program, although skeptical about the feasibility of the concept of eradication itself, the World Health Assembly in 1966 decided to provide to the WHO a special allocation of $2.4 million annually for an intensified global smallpox eradication effort.[425] It expressed hope that the task might be accomplished within a 10-year period, that is, by December 1976.[426]

During the first year of this program, an estimated 10 to 15 million smallpox cases occurred in the 31 countries in which the disease remained endemic.[1] The eradication campaign was based on a twofold strategy: (a) vaccination campaigns in each country, using vaccine of ensured potency and stability, that would reach at least 80% of the population (a coverage level that would be assessed by independent teams); and (b) special programs to detect and isolate cases, and contain outbreaks,[427,428] called "surveillance and containment." Progress was not to be measured in terms of the number of vaccinations performed, as had been the practice, but in terms of a declining incidence of smallpox. This principle required the development of an effective case notification system and focused attention on measures to reduce incidence.

The strategy of surveillance and containment proved highly effective in controlling the spread of smallpox throughout the global eradication program, whether in villages or major cities.[152] It is the strategy that is given priority for the control of an outbreak should one occur in the United States, whether or not large-scale vaccination is also undertaken. The reason for this concept is straightforward—to detect cases as quickly as possible, to isolate them, and to vaccinate all persons who have been in contact with any cases since the time they became ill and the persons to whom the contacts might transmit infection if they become ill. The method originally was called "ring vaccination" by Dixon,[16] a term that some have erroneously interpreted to mean a geographic ring when, in fact, it means erecting a barrier of immune persons around the case and his or her contacts (following onset of symptoms) as quickly as possible.

The surveillance-containment strategy worked as well as it did because of a number of unique clinical and epidemiologic features of smallpox. First is the fact that smallpox virus, to sustain itself, must pass from one person to another, each of whom experiences a clinically obvious infection with rash. There are no persons with subclinical infection who can spread the disease unnoticed, and there is no animal reservoir. Thus, it is a simple matter to identify where smallpox is at any time and to know how and by whom it has been spread. An outbreak may be envisaged as a continuing chain of cases with control measures being directed at breaking the chain. Second is the fact that the person is able to transmit infection only from the time of onset of rash until scabs form. Although a patient normally experiences a 10- to 12-day asymptomatic incubation period after the patient is infected, the patient cannot transmit virus during this period. Thus, although an infected patient may contact many persons during the incubation period, the patient will not infect them. Next, the infected patient experiences a 2- to 3-day prodromal period before rash develops. With variola major, the Asian form of smallpox and the one of most concern, patients during the prodromal phase experience a very high temperature and serious systemic symptoms and generally take to bed. Even though they are very ill, they still cannot transmit infection to others until oropharyngeal and skin lesions begin to develop and the virus begins to be shed into the oropharynx. Not surprisingly, most patients are successful in transmitting smallpox only to household members and to persons with whom they have contact in the hospital.

The strategy of containment, therefore, is to identify contacts of a patient since the patient first became ill and to vaccinate the persons to whom an infected patient is most likely to transmit the disease. This strategy works because vaccination after exposure provides protection to the contact even when given 4 to 5 days after infection has been transmitted. Usually, the numbers of such contacts are comparatively few and include members of the infected person's household but not persons such as fellow passengers on trains or subways or fellow workers in an office complex. Hospital contacts are likewise of major importance. They include not only hospital staff, but also other patients and visitors. Most person-to-person contact results from droplets expressed by the patient from the mouth during speaking or coughing. The patients and visitors most likely to become infected are those who come within 6 feet of the patient. Such persons must be identified as soon as possible and vaccinated.

Patients who are severely ill with malignant or hemorrhagic smallpox and who may be coughing pose a special risk. Patients in such condition constitute only a small percentage of patients in whom smallpox develops, but they can generate fine-particle aerosols that can drift over very long distances within a building. Should such cases be admitted to a hospital without special infection control precautions, large numbers throughout the hospital might need to be vaccinated.

Provision of adequate supplies of fully potent vaccine was a critical initial problem.[429,430] Early surveys revealed that 90% of the vaccine produced in or provided to endemic countries failed to meet accepted international standards. Laboratories in Canada and The Netherlands agreed to test samples of all vaccine to be used in the program. Manufacturers collaborated in developing a detailed production manual, and consultants and equipment were provided to vaccine production laboratories in endemic countries. Donations of vaccine, primarily from the Soviet Union and the United States, partially met initial needs. By 1973, more than 80% of all vaccine for the program was produced in the developing countries.

During 1967, the first year of the program, 44 countries, 31 of which had endemic disease, reported 217,218 cases of smallpox. The endemic countries were Brazil, most countries of Africa south of the Sahara, and five countries in Asia: Afghanistan, India, Indonesia, Nepal, and Pakistan (which then included Bangladesh). Later surveys revealed that only approximately 1% of all cases were being reported. Thus, it is estimated that probably 10 to 15 million cases occurred that initial year in countries with a total population of about 1.2 billion.

Vaccination technique also improved, and, thus, results improved.[431] The WHO encouraged abandonment of traditional methods of vaccination by scarification or rotary lancet. Jet injectors were introduced in 1967 for programs in Brazil and western and central Africa, but they proved cumbersome, expensive to maintain, and not well adapted for use in sparsely populated areas. One year later, the bifurcated needle, developed by Wyeth Laboratories, proved extremely effective in multiple-puncture vaccinations, inexpensive, and easy to use.[432] By 1969, bifurcated needles were in use in all countries. Vaccination with the bifurcated needle required only one-fourth as much vaccine, and even illiterate village volunteers required less than an hour's training in proper use, and workers could vaccinate, on average, 500 persons per day.

Vaccination programs were developed or strengthened in all endemic and neighboring countries, the last of them beginning in 1971. Although the strategy also called for the improvement of national reporting systems and containment of outbreaks by special teams, such activities were slow to begin. It quickly became apparent, however, that these activities, referred to as the surveillance-containment strategy, could interrupt smallpox transmission more easily and quickly than anyone had imagined, even where vaccinial immunity was low.[100,212,428]

With increasingly greater emphasis on surveillance-containment activities, endemic smallpox steadily receded. It was eliminated from 20 countries of western and central Africa by 1970,[87] from Brazil in 1971, from Indonesia in 1972, and from the entire continent of Asia in 1975. Ethiopia halted transmission in 1976 and Somalia in 1977. The last naturally occurring case of smallpox developed less than 1 year after the originally projected 10-year target date, on October 26, 1977, in Merka, Somalia.[433] Two further cases occurred in 1978 as a result of a laboratory infection in Birmingham, England[434] and were the last known cases. Subsequently, many rumors of other cases have been investigated but found not to be smallpox.

To confirm eradication, WHO-organized international commissions visited each of the endemic countries and areas 2 years or more after the last recorded smallpox cases. In May 1980, the World Health Assembly, acting on the recommendation of a WHO global commission, announced that worldwide eradication had been achieved (see Fig. 54.2) and recommended that routine smallpox vaccination be discontinued.[1] The WHO established an international stockpile of vaccine should it ever be required and encouraged laboratories to destroy their stocks of variola virus or transfer them to one of two designated reference laboratories (WHO Collaborating Centres on Smallpox and Other Poxvirus Infections), at the CDC, Atlanta, Georgia, or the Research Institute of Viral Preparations, Moscow, Russian Federation.[435] All countries reported voluntary compliance with the WHO recommendations.[436] The last country to destroy its stocks was South Africa, in 1983.[12] By 1984, only the CDC and the Research Institute of Viral Preparations were known to have retained variola virus isolates. In 1994, the Russian isolates were moved to the State Research Center of Virology and Biotechnology (the VECTOR Institute), Novosibirsk, Russia.

For a more complete and detailed discussion of the history of smallpox and the eradication effort readers are encouraged to review this chapter in the sixth edition of *Vaccines* and are directed to several additional sources: *Smallpox and Its Eradication*,[12] which provides a detailed account of the eradication campaign, *The Greatest Killer: Smallpox in History*, by Hopkins (originally issued in 1983 under the title *Princes and Peasants*),[17] recent books by epidemiologists DA Henderson and William Foege who were intimately involved in the eradication campaign,[437,438] and detailed accounts of national eradication programs,[20,84,421,439–443] including a 2011 special supplement in the journal *Vaccine* to commemorate the 30th anniversary of the eradication of smallpox.[220]

FUTURE VACCINES

Smallpox vaccine use has seen an evolution over time because of disease eradication, changes in public perception of risk, concerns about bioterrorism, increasing outbreaks of zoonotic poxviruses, and increases in understanding of poxvirus immunity. Table 54.7 depicts the main categories of smallpox vaccines, each of which is described in subsequent paragraphs. In the absence of endemic smallpox, all new vaccines are routinely compared against products such as Dryvax or Lister that are known to induce protective immunity. Approval for clinical trials and eventual production are dependent on noninferiority to the previously licensed products and on test results from multiple animal models according to guidelines from the FDA's "animal rule."[444]

First-Generation Vaccines

The relative risks and benefits of vaccination have changed with the eradication of smallpox. The most successful vaccines contained live vaccinia virus and were crude products. Vaccinia virus was applied to the shaved and abraded skin on the flank of a calf or sheep; the animal was killed after 7 to 8 days, and the crude vaccinia pulp was scraped off; the pulp was purified; and the product was freeze-dried. Inevitably, the vaccine contained some bacteria, but the number was minimized through use of carefully cleansed animal facilities, and the final product was always tested for possible pathogens. Most smallpox vaccines that were produced from the late 1800 s through 1980 are called first-generation vaccines.

After smallpox was eradicated, vaccination ceased and vaccine production facilities were dismantled because they were of no use for producing other vaccines. Such limited quantities of vaccine as were required were drawn from stockpiles stored at −20°C. With the recognition that smallpox might be used as a biological weapon, the United States decided that a large supply of vaccine should be produced to avert a potential catastrophe should smallpox virus be released. It was decided that a contemporary vaccine should be produced using tissue cell culture production. The product, called ACAM2000, is referred to as a second-generation smallpox vaccine.

Second-Generation Vaccines

As the perceived threat of smallpox as a biological weapon grew, there were efforts to replace dwindling old vaccine stocks to protect largely naïve populations. They are tissue culture–based products containing the same viral strains used in earlier smallpox vaccines. The most prominent of these new formulations is ACAM2000. This vaccine was derived from the original NYCBOH strain[445] and subsequently underwent safety testing in small animal models.[178,446] Vaccines based on tissue culture production methods are better characterized, free of adventitious agents, and possibly somewhat safer than lymph-based preparations. These products contain live vaccinia virus, so it is not surprising that immunogenicity testing and clinical trials have shown take rates, resulting antibody titers, and induction of cellular immunity similar to those of first-generation vaccines.[179,316,447] However, these vaccines also induce a similar spectrum of immediate (fever, headache, lymphadenopathy, malaise) and cutaneous reactions,[448] and can result in the same more serious adverse events discussed earlier. The majority of the present U.S. national strategic stockpile of smallpox vaccine consists of ACAM2000.[449]

Several additional second-generation vaccines have shown promise in clinical trials. Dynport has developed a cell-culture smallpox vaccine grown in MRC-5 human diploid fibroblasts. In a clinical trial directly comparing cell-culture smallpox vaccine with Dryvax, both vaccines induced similar levels of neutralizing antibody titer and similar quantities of interferon-γ–producing T cells and had similar rates of local adverse events.[450] Another cell-culture–derived product, CJ-50300, developed in South Korea,[210] was highly immunogenic, with the vaccine take rate and the rate of induction of high-titer antibody responses at 99%. Cellular immunity developed in 89% of vaccinees. Although this study did not directly compare CJ-50300 and a first-generation vaccine,

TABLE 54.7 Historical and Modern Smallpox Vaccines[a]

Name	Strain/Antigen Source	Production Method	Description
First-Generation (Classical, Live Virus Vaccines Used During Eradication Efforts)			
Dryvax	NYCBOH	Freeze-dried, lymph	Live vaccine used in United States
SPSV	NYCBOH	Frozen liquid, lymph	Live vaccine used in United States
Lister	Lister/Elstree	Chick chorioallantoic membranes	Live vaccine used worldwide
Lancy-Vaxina	Lister	Freeze-dried, lymph	Live vaccine used worldwide
EM-63	NYCBOH	Freeze-dried, lymph	Live vaccine used in Russia
Temple of Heaven	Tian Tian	Freeze-dried, lymph	Live vaccine used in China, greater percentage of adverse events than NYCBOH or Lister vaccines
Second-Generation (Live Virus, Cell Line–Produced Vaccines Based on First-Generation Vaccine Strains)			
ACAM1000	NYCBOH	MRC-5 cells	Interim Acambis product no longer in production
ACAM2000	NYCBOH	Vero cells	Acambis product with similar immunogenicity as Dryvax
Elstree BN	Lister/Elstree	Chick embryo fibroblasts	Bavarian Nordic product based on Lister strain
CCSV	NYCBOH	MRC-5 cells	Dynport cell-cultured smallpox vaccine
CJ-50300	NYCBOH	MRC-5 cells	Korean vaccine with similar reactogenicity and immunogenicity as Lancy-Vaxina
Third-Generation (Live Attenuated Virus Vaccines)			
MVA	Ankara	CEF	Lost 15% of genome during passage; limited use during eradication efforts
LC16m8	Lister	Cell line	B5R deletion mutant, used in Japan, improved safety over live vaccines
NYVAC	Copenhagen	Cell line	Missing 18 immunomodulatory genes
dVVL	NYCBOH	Cell line with UDG activity	Missing UDG; nonreplicating
ACAM3000	Ankara	Cell line	MVA-based formulation developed by Acambis
IMVAMUNE	Ankara	Cell line	Bavarian Nordic MVA-based vaccine
TBC-MVA	Ankara	Cell line	MVA-based vaccine in development at Therion
Fourth-Generation (Subunit Vaccines, Including Protein, Peptide, DNA Based)			
Protein-vaccine	A33, B5, L1, A27 proteins	Recombinant protein	Quadrivalent, alum-adjuvanted protein vaccine
4pox-VRP	A33, B5, A27, L1 genes	Alphavirus replicon vectors	Viral-like particle vaccine encoding for four poxvirus membrane proteins
VennVax	Multiepitope	DNA vector	T-cell peptide vaccine based on 25 HLA class II epitopes
Others	Various	Expression vector or cell line	Vaccine candidates are in various stages of research and development

[a]This table describes the different types of smallpox vaccines, the source of antigen or parental viral strain, the production method, and a brief description. Several of these vaccines represent temporary products no longer being pursued (e.g., ACAM1000), and others are at very early stages, requiring several years to a decade of additional research and development.

CEF, chicken embryo fibroblast; HLA, human leukocyte antigen: MVA, modified vaccinia virus Ankara; NYCBOH, New York City Board of Health; UDG, uracil-DNA-glycosylase.

measures of humoral and cellular immunity in vaccine recipients were similar to those elicited in subjects receiving Dryvax.[451]

Third-Generation Vaccines

Attenuated vaccinia strains form the basis for the third-generation vaccines. These include replication-competent (LC16m8) and replication-defective (MVA) vaccinia viruses. Attenuated vaccines would be particularly suited for use in persons who are immunocompromised and thus, at special risk of developing progressive vaccinia, and in persons with severe eczema or exfoliative skin conditions. A modern understanding of the genetics of vaccinia and the ability to manipulate the genome have led to the development of additional vaccine strains to complement the products based on the earlier strains.

LC16m8 was produced by Hashizume in the 1970s by repeated passage of Lister-strain vaccinia at low temperature in rabbit kidney cells.[452–454] The attenuated vaccine virus replicates in the skin but is replication-deficient at human body core temperatures and is thus expected to have less potential for inducing adverse events in humans. This strain produced HI and neutralizing antibodies in humans and, in a field trial of 50,000 children, produced markedly less-severe cutaneous reactions and less fever than other strains.[455] Studies in immunocompromised animals show that LC16m8 is much safer than unattenuated vaccinia strains.[456] A clinical trial

involving the administration of LC16m8 to several thousand participants was recently conducted in Japan and showed similar immunogenicity to Lister strains, no serious adverse events, and lower frequencies of minor adverse events (fever, swelling, itching, and headache) than seen with first-generation vaccines.[457] Mice, rabbits, and monkeys vaccinated with LC16m8 have been protected against challenge with, respectively, ectromelia, rabbitpox, and monkeypox.[458–460] This strain has been licensed for use by Japanese government authorities, who have purchased some 56 million doses for Japan's national stockpile.

Progress is also being made in developing nonreplicating third-generation vaccines such as MVA.[138,461] A modern understanding of the genetics of vaccinia and the ability to manipulate the genome have led to the development of additional vaccine strains to complement the products based on the earlier strains. There are concerns that these attenuated vaccines induce lower levels of humoral and cellular immunity that may not last as long as the responses elicited by replication-competent vaccines.[462,463] On the other hand, nonhuman primate studies have shown protection against lethal challenge with monkeypox.[462,464] These vaccines would likely be used in a multidose regimen: the first dose of attenuated vaccine used to induce partial immunity and reduce the frequency and severity of side effects upon receipt of a subsequent dose of live virus vaccine (such as ACAM2000). Because 6 weeks would be required for full immunity to develop, such vaccines would be of limited use in coping with epidemic smallpox.

MVA is a live, nonreplicating form of vaccinia virus developed in Germany in the 1950s and 1960s.[12,465] The virus originates from a Turkish vaccine strain (Ankara) that had been used for routine vaccination for many years and was propagated at the Ankara Vaccination Station. The MVA form was derived by more than 500 serial passages of the Ankara strain in primary chick embryo fibroblasts resulting in the loss of approximately 15% of its genome and the ability to replicate in most mammalian cell types.[466,467] It was originally intended to be administered as a prevaccination intradermal injection to be followed by scarification with the replicating Lister vaccinia virus 1 to 2 weeks later. Published German reports from the 1960s indicate that some 120,000 persons had been given MVA vaccine without known complications.[12] However, the titer of the MVA vaccine used in the German studies was some 100 times less than is now intended, and the MVA vaccinia strain, since the 1960s,[138] has undergone multiple additional passages. Newer studies (see next paragraph) also indicate that the vaccine is safe, but it is probable that not more than 20,000 have been vaccinated in the course of these studies, far too few to examine the rates of serious adverse events.

Several companies have MVA-based products in development, including IMVAMUNE,[463] TBC-MVA,[414] and ACAM3000.[468] IMVAMUNE has been found to be safe, well tolerated, and immunogenic in Phase I clinical trials[138] and has completed Phase II clinical testing[469] during which it was determined that two doses are necessary to elicit immune responses similar to those of Dryvax or ACAM2000.[470] Animal studies supported the need for a prime-boost vaccination schedule.[471] Further work is being performed to ascertain the usefulness of IMVAMUNE as a priming vaccine preparatory to immunization with the second-generation ACAM2000 vaccine in the hope that priming would reduce local and systemic reactivity to the live virus vaccine.[472] Phase I/Ib trials of TBC-MVA have shown that vaccination with MVA reduces the severity of local symptoms and lesions on subsequent Dryvax immunization.[414] A recent clinical trial testing increasing doses and different immunization routes with ACAM3000 found that all doses and routes were well tolerated.[473] No serious adverse events occurred, while self-limiting, local and systemic reactions (headache, local swelling and discomfort, fever, malaise, nausea) occurred in half of the subjects. A separate study found that doses above 2×10^8 plaque-forming units [PFU] resulted in a significant increase in malaise, nausea, and "flu-like" symptoms.[474] Neutralizing antibody response and cellular immunity (interferon-γ enzyme-linked immunosorbent spot [ELISPOT]) were detectable at least 180 days after vaccination. A companion trial examining the effect of ACAM3000 priming on subsequent Dryvax inoculation found similar results as the TBC-MVA study discussed previously, providing further support for the prime-boost vaccination strategy.[414] If an outbreak of smallpox does occur, (a) there will be relatively few contraindications for the live virus vaccines (e.g., severe immune deficiency), and (b) rapid containment is critical and IMVAMUNE does not provide protection until approximately 2 weeks after the second dose. These conditions, as well as cost considerations, make it difficult to determine the utility of widespread use of the stockpiled MVA-based vaccines in a biological attack or outbreak scenario.

Additional attenuated vaccinia strains are also under investigation as potential vaccines. NYVAC, was developed in the United States in the 1980s. It stemmed from the observation that 50 or more of the nearly 200 genes of vaccinia virus are dispensable for replication in tissue culture cells and that deletion of some of these genes reduced virulence in animal models.[475–477] In all, 18 genes were deleted from the Copenhagen strain of vaccinia virus to produce this strain.[478] Several studies indicate that NYVAC has potential for a human vaccine, perhaps to be used as would the nonreplicating MVA strain.[479,480] dVV-L is a Lister derivative missing the uracil-DNA-glycosylase gene.[481] This strain can grow only in cell lines complementing the deleted enzyme. As with other attenuated strains, it too requires high doses and multiple vaccinations to induce the same levels of immunity seen with first- and second-generation vaccines.[482,483] Selective combination of these vaccines with appropriate adjuvants may serve to boost immunogenicity to the levels seen with second- and third-generation vaccines.[484]

A significant problem in developing attenuated strains is that a definitive assessment of their efficacy is now impossible. Because of the absence of human smallpox cases, a determination of efficacy under the conditions of a natural challenge is not possible; in addition, there is no suitable animal model of variola virus infection, and surrogate serologic measures of protection are unknown. The evaluation of efficacy thus will have to rely on comparison of serologic responses with those induced by the NYCBOH (ACAM2000) or Lister strains and challenge of vaccinated monkeys with aerosols of monkeypox virus.

Fourth-Generation Vaccines

Increased understanding of poxvirus immunology, the identification of T- and B-cell epitopes, and advances in reverse immunology approaches have made feasible the development of fourth-generation subunit smallpox vaccines. Numerous vaccine candidates are in various stages of research and development. Among the most promising candidates are combinations of MV and EV proteins (most notably, A27, A33, B5, and L1) that, when injected as DNA or protein formulations, elicit strong, protective immune responses to a number of poxviruses, including vaccinia, ectromelia, and monkeypox.[485–489] Other subunit vaccine examples include a multiepitope, peptide-based DNA vaccine,[490] a B5R protein–based vaccine produced in plants,[491] and an inactivated, mucosal vaccine.[492] Peptide vaccines based on HLA supertype motifs capable of inducing immune responses in large portions of the

population are also being considered.[272,273,289,290,292,493] These subunit vaccines have several notable advantages: well-defined composition, lack of viral/bacterial contaminants, easy adaptability to multivalent applications, rapid and cost-effective production, and increased safety profiles.

Despite the eradication of smallpox, poxviruses remain a public health threat as an agent of bioterrorism and because of an increasing number of zoonotic outbreaks such as those involving monkeypox in Africa and the United States,[104,494–496] vaccinia viruses in South America,[497–499] buffalopox in India and Pakistan,[500] and cowpox in Germany.[501,502] Furthermore, previously uncharacterized poxviruses are being seen for the first time as they cause human infections.[503–506] Thus, these new vaccine candidates (and antiviral compounds) may be used in biodefense and to protect against and contain natural disease outbreaks.[507] The current consensus view is that of a need for a vaccine with fewer complications, that is more antigenic (preferably requiring a single dose), is cost-effective, has a long shelf life, and can be readily stockpiled. As we move forward with research and product development in this area, it will be important to align global and local public health objectives with various national security goals and realistic financial considerations given the immense costs involved.

DESTRUCTION OF THE VIRUS

After 1980, when the World Health Assembly declared that eradication had been achieved, many countries questioned whether it would be prudent to destroy the known remaining laboratory stocks of variola virus to provide added assurance that the virus might not accidentally or deliberately be released into an unprotected world. The first official recommendation to destroy the known stockpiles of variola virus was made in 1994 by the WHO Expert Committee on Smallpox.[508] This recommendation was made after almost 10 years of scientific review and expert consultations. This recommendation was then discussed at multiple World Health Assemblies as arguments were advanced supporting[509] and objecting to[510] destruction of the virus stocks. Arguments against destruction stemmed from concerns that it would prohibit significant advances in understanding the pathogenesis of variola virus and that destruction of the two known repositories would not guarantee that forgotten or hidden stocks of virus were not preserved elsewhere.[511] This point was brought into focus in July 2014, as researchers at the National Institutes of Health discovered several vials containing viable smallpox virus in a freezer on their Maryland campus.[512] Arguments for destruction of the virus stemmed from concerns that an unintentional release of virus in an increasingly susceptible global population would lead to a serious outbreak, that all should be done to ensure that smallpox never again would afflict humankind, and that the available cloned DNA fragments and sequence information of several strains of variola were sufficient to allow scientific inquiry into the virus and an understanding of its properties.

Since that original recommendation, the destruction of the viral stocks has been postponed multiple times to complete specific research projects requiring variola virus.[513–516] Dissatisfaction by a number of countries with the progress of these research programs resulted in further debate at the World Health Assemblies of 2006 and 2007. The WHO Director General then arranged for the publication of a comprehensive report documenting published and unpublished research involving variola virus during 1999 to 2010.[9,517,518] Subsequently, a separate advisory group of independent experts from outside the variola virus field reviewed this material and reported to the 2011 World Health Assembly.[516,519,520] This Assembly reaffirmed the need to destroy the remaining stocks of smallpox on completion of crucial variola virus research. Progress in this regard was reviewed in 2014 at the 67th World Health Assembly but the decision was delayed yet again.[521] We agree that the elimination of the last remaining stocks of variola virus will reduce (but not necessarily eliminate) the risk of a smallpox virus release; nevertheless we believe that this outweighs the potential benefits of further research requiring live variola virus and are in favor of destroying the official virus stocks.[522,523]

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55 *Staphylococcus aureus* Vaccines

Robert S. Daum

More than a century ago, the Scottish surgeon Alexander Ogston first noticed an association between skin abscesses and an organism that formed structures resembling clusters of grapes when seen by light microscopy.[1] The organism he saw, *Staphylococcus aureus*, is now known to be a commensal that is also responsible for many serious community and nosocomially acquired infections and several more recently recognized toxin-mediated diseases. It is also the most frequently isolated bacterial pathogen from patients with hospital-acquired infections.[2] Among staphylococci, the defining characteristics of this species are the production of the extracellular enzyme coagulase and protein A. Staphylococci lacking coagulase are grouped under the designation coagulase-negative staphylococci (CoNS). Once thought to be nonpathogenic constituents of human bacterial flora, the approximately 16 species of CoNS isolated from humans are now also known to cause nosocomial infections and urinary tract infections in children, catheter-related infections, prosthetic joint infections, endocarditis, and septic episodes in premature infants and other immunocompromised hosts.[3] Although CoNS can be important pathogens in certain settings, vaccine development has been focused primarily on *S. aureus*, the subject of this chapter. CoNS isolates also colonize human skin universally,[4] and multiple strains may be isolated from the same person.

ECOLOGY

Staphylococci usually have a symbiotic relationship with their human hosts. The traditional view is that asymptomatic *S. aureus* colonization occurs intermittently in between 10% and 40% of children and adults, with children having somewhat higher colonization rates than adults.[5–9] Nasal carriage is widely believed to be a risk factor for invasive disease.[10,11] In addition, the skin, hair, nails, axillae, perineum, rectum, and vagina (in approximately 10% of menstruating women) may be colonized.[12–14] Three patterns of colonization by *S. aureus* have been reported: approximately 20% of the population is persistently colonized, 60% is intermittently colonized, and approximately 20% is never colonized.[15] Hospital personnel, persons with chronic skin conditions, individuals with implanted medical devices, people who indulge in intravenous substance abuse,[16] patients with type 1 diabetes, patients undergoing hemodialysis, and patients with AIDS have higher rates of asymptomatic *S. aureus* colonization than do persons in the general community.[5]

As a result of using new methodology for detection, higher rates of asymptomatic colonization have been reported, even among healthy people. A rate of 65% to 75% has been documented,[13] and some believe that colonization may even be universal, depending on the method used for sampling. Most of these strains associated with asymptomatic colonization are bacteria from genetic backgrounds that have not been isolated from infectious material. Thus, *S. aureus* is a commensal that is occasionally responsible for active infection.[17]

Conversely, most methicillin-resistant *S. aureus* (MRSA) strains that asymptomatically colonize are from genetically pathogenic backgrounds; the explanation is unclear. Moreover, the nose is not the commonest site of asymptomatic colonization of any *S. aureus* strain and colonization of a single individual by multiple *S. aureus* strains is common.[18–28]

An epidemic of *S. aureus* infections in the United States, with onset in the mid- to late-1990s intensified interest regarding this important pathogen. The emergence of several *S. aureus* genetic backgrounds, mainly USA300 MRSA, circulating in the community as methicillin-resistant clones, so-called community-acquired MRSA (CA-MRSA), exponentially increased the burden of disease in many areas.[29] *S. aureus* is now the most frequent pathogen isolated from children.[30] The rate of asymptomatic colonization with methicillin-resistant strains has increased and was reported to be as high as 22% in children in Texas.[31] Virulence determinants in these CA-MRSA strains have been recognized that are apparently new to the species, such as the ACME element with its attendant gene[32]; increased in frequency, such as the Panton-Valentine leukocidin; or increased in expression, such as alpha toxin.[33] This increase in the clinical burden of *S. aureus* disease associated with this epidemic has initiated discussions about the possible need for a universal immunization strategy.

CLINICAL SPECTRUM

When not acting as a commensal, *S. aureus* is a major cause of morbidity and mortality. It is the most virulent member of the genus *Staphylococcus.* Unlike other members of the genus, *S. aureus* is well endowed with a variety of virulence factors (Fig. 55.1).

Superficial and invasive *S. aureus* infections occur in previously healthy persons. Infections of the skin range from impetigo to abscess formation, cellulitis, or lymphadenitis, particularly of the cervical lymph nodes in children or adjacent to an infectious focus. *S. aureus* also may cause several important ocular infections, including conjunctivitis, preseptal cellulitis, and endophthalmitis. *S. aureus* is an important cause of endocarditis and is the leading cause in many regions of the world.[34] The infected heart valves may have been previously normal, especially when the mitral or aortic valves are involved and the clinical manifestations may be particularly severe. Pericarditis may be an isolated syndrome or may accompany endocarditis.

Hematogenous seeding of a bone or joint may result in osteomyelitis, septic arthritis, or even bursitis. *S. aureus* is a cause of several respiratory tract infections, including an occasional otitis media and pneumonia. The latter may be a severe, necrotizing process with high mortality. Central nervous system infections are infrequent and usually involve an assisted portal of entry for the organism, such as extension from an infected sinus, a dermal sinus, or a meningomyelocele. Central nervous system infectious syndromes also include subdural and epidural empyemas and even meningitis.[35] A spinal epidural abscess adjacent to the dura also may be caused by *S. aureus*. *S. aureus* may rarely infect the urinary tract or be recovered from the urine of a patient with high-grade bacteremia or a renal abscess.

S. aureus frequently complicates surgical procedures in which the integument was breached and indwelling foreign bodies are left in place.[36] The insertion of plastic, metal, or Gore-Tex devices provides an opportunity for *S. aureus* to

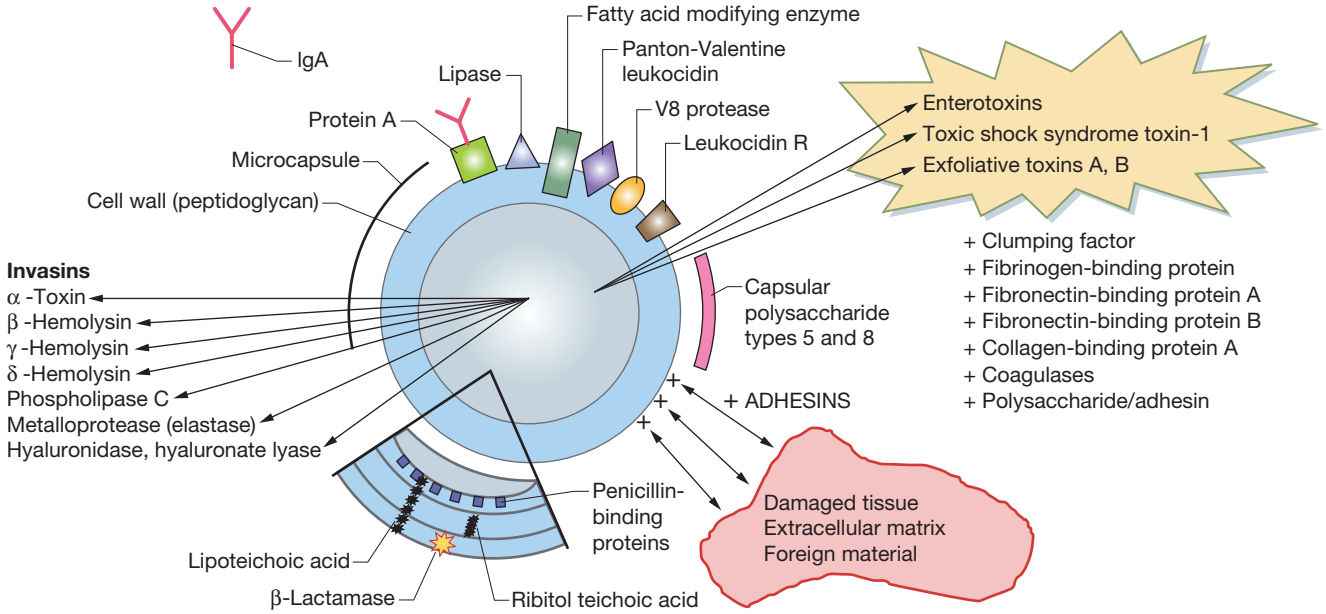


Figure 55.1. Virulence factors of *Staphylococcus aureus*. IgA, immunoglobulin A. *(Modified from Lowy FD. Medical progress:* Staphylococcus aureus *infections.* N Engl J Med. *1998;339:520–532.)*

adhere, causing persistent infection. Success in eradicating the organisms from the inserted device often poses major challenges, even when the isolate is susceptible to the antibiotic used. Thus, certain patients, such as those needing hemodialysis, those with indwelling venous catheterization, indwelling intravascular Gore-Tex patches, artificial prostheses, or cerebrospinal fluid flow diversionary devices, are at high risk for *S. aureus* infections.

A variety of veterinary infections are also caused by *S. aureus*. For example, mastitis in cows is an economically important disease. Horses, chickens, dogs, and cats are among the animals known to be colonized or infected with *S. aureus*, with documented transmission to humans from horses.[37] Mounting evidence suggests that pigs may represent a new important reservoir for CA-MRSA strains. ST398 is the most commonly reported sequence type among livestock in Europe and the United States. Transmission to pig-farm workers has occurred, although invasive disease has rarely been documented.[38–41]

Although veterinary vaccines have been developed for *S. aureus*, particularly in cows,[42,43] they are not discussed in this chapter.

S. aureus is also the cause of a number of toxinoses with clinical manifestations that reflect the effects of one or more elaborated and released exotoxins. Examples include toxic shock syndrome (TSS), scalded skin syndrome, and food poisoning.

Attention has been called to septic illnesses caused by *S. aureus* with multiorgan failure that likely reflect the activity of one or more *S. aureus* toxins. Examples include so-called severe sepsis,[44] an illness with a sometimes fulminant course, purpura fulminans,[45] and the Waterhouse-Friderichsen syndrome.[46]

PROBLEM OF ANTIMICROBIAL RESISTANCE IN *STAPHYLOCOCCUS AUREUS:* EPIDEMIC COMMUNITY-ACQUIRED METHICILLIN-RESISTANT DISEASE

Before the introduction of antimicrobials in the early 1940s, the mortality rate of invasive *S. aureus* infections was approximately 90%; this rate decreased markedly following the introduction of penicillin G into clinical practice.[47] Almost immediately, however, a few *S. aureus* isolates were noted to be resistant to penicillin.[48–50] By the end of the 1940s, most strains isolated from hospitalized patients were penicillin-resistant[51]; currently, resistance rates exceed 90%, rendering penicillin virtually ineffective in treating *S. aureus* infections. The mechanism of resistance is the elaboration of a β-lactamase, usually encoded by a transposon borne on a plasmid; there is also cross-resistance with other β-lactams that are susceptible to β-lactamase hydrolysis.

In the early 1960s, methicillin, representative of a new class of semisynthetic β-lactam antibiotics that are relatively resistant to hydrolysis by staphylococcal β-lactamases, was marketed. However, resistance to it was immediately recognized.[52,53] Such resistance is still referred to as MRSA, although methicillin is uncommonly used in clinical practice. MRSA isolates survive β-lactam exposure by elaborating a peptidoglycan-synthesizing enzyme called penicillin-binding protein (PBP) 2a that, in concert with native PBP2, allows peptidoglycan synthesis to occur despite the presence of a β-lactam compound. This resistance mechanism conveys cross-resistance to all β-lactam antibiotics, including cephalosporins except for ceftaroline.[54] Since their recognition, the prevalence of MRSA isolates has been slowly but steadily increasing,[55–57] but has now probably plateaued and may be declining. Institutional prevalence rates exceeding 45% are not uncommon.[58] One development has been the recognition of epidemic CA-MRSA infections in children and adults in many regions of the United States, elsewhere in North America, Western Europe, Asia, Australia, and South America who lack traditional MRSA risk factors such as frequent contact with healthcare facilities.[59] The MRSA isolates responsible for these community-acquired infections are less resistant to multiple antibiotics than their hospital counterparts; the genetic backgrounds of these isolates differ from their hospital counterparts, and the isolates usually contain novel, smaller chromosomal elements called staphylococcal cassette chromosome mec (SCC*mec*) IV or V[60,61] that may be more transferable to other staphylococcal organisms than the larger SCC*mec* elements found in hospital MRSA isolates. CA-MRSA isolates have been associated with

dramatic upsurges in the prevalence of *S. aureus* infections in general and rates of MRSA infections in particular.[62,63]

Vancomycin, a glycopeptide antibiotic, until recently had been the only agent to which MRSA isolates had remained uniformly susceptible. In the late 1990s, this susceptibility changed with the recognition of glycopeptide intermediate-resistant *S. aureus* (minimal inhibitory concentration of vancomycin, >2 and <16 μg/mL) isolates in Japan,[64] the United States,[65–68] and several other countries.[69–74] Often, patients with glycopeptide intermediate-resistant *S. aureus* isolates have been receiving dialysis and, therefore, have prolonged low-level serum vancomycin levels, an in vivo environment similar to that used to select for vancomycin-intermediate resistant mutants in the laboratory,[75,76] and have failed vancomycin treatment. More recently, higher level vancomycin-resistant *S. aureus* isolates have also been isolated from patients in the United States[77] with minimal inhibitory concentrations of vancomycin of more than 16 μg/mL. These isolates bear genes from vancomycin-resistant enterococcal strains that produce a structurally altered peptidoglycan precursor that does not bind vancomycin.[77] The occurrence of these isolates represents an important event that will greatly impact healthcare throughout the world.[78–80] The ever-evolving resistance mechanisms in *S. aureus* suggest that any antimicrobial therapy for this agent will be met with a resistance mechanism by the staphylococcus species. The situation with antibiotic resistance in *S. aureus* is certainly one issue that has spurred vaccine development.

Resistance to other antimicrobials active against *S. aureus*, such as linezolid,[81,82] tedizolid, daptomycin,[83] dalbavancin,[84] oritavancin,[85] and mupirocin,[86] has also been identified and is a source of clinical concern. This continuing saga of antimicrobial resistance in *S. aureus* and the slowing of the development of new antimicrobials is reminiscent of the clinical alarms sounded by resistance of *Haemophilus influenzae* type b to ampicillin and chloramphenicol, and resistance of *Streptococcus pneumoniae* to penicillin,[87] which suggested that infections caused by these important pathogens had become increasingly difficult to treat. In both instances, the deployment of an effective vaccine decreased these concerns. Effective prevention outpaced new concerns about therapy.

MICROBIOLOGY AND PATHOGENESIS

Staphylococci are hardy aerobic or facultatively anaerobic gram-positive bacteria that can persist in distressed environments such as acidic conditions, high sodium concentrations, and wide temperature variations; they can survive on fomites, in dust, and on clothing from several days to more than a week. Once *S. aureus* infection is established, local and systemic effects result from direct invasion, hematogenous dissemination, and/or toxin release.

Initially the organisms were thought to be nonencapsulated, but it is now clear that many clinical *S. aureus* isolates, and some CoNS isolates, have a polysaccharide capsule, sometimes called a microcapsule because of its thinness and adherence to the bacterial cell surface.[88] The prevalence of two capsular polysaccharide types (5 and 8)[89] in most collections of clinically important human and veterinary isolates suggests an important role for these polysaccharides in pathogenesis, although, to date, its nature is uncertain. These capsular antigens were targeted in a staphylococcal vaccine to be described subsequently.

The cell wall of *S. aureus* is composed of capsular polysaccharide, peptidoglycan, lipoteichoic acid (LTA), ribitol wall teichoic acid, and numerous surface proteins. Protein A, also an important surface protein, binds the Fc region of the immunoglobulin (Ig) G molecule[90–93] and decreases the efficiency of antibody binding to *S. aureus* isolates, reducing opsonization and phagocytosis.[94–97]

The virulence of *S. aureus* is the result of a combination of many elaborated virulence proteins that include extracellular products, such as α-,[98] β-,[99] γ-,[100] and δ-[101] hemolysins (or toxins); leukocidins[102]; proteases[103]; lipase[104]; deoxyribonuclease, a fatty acid-modifying enzyme[105]; and hyaluronidase.[106] Coagulase is an extracellular protein that binds host prothrombin to form staphylothrombin; this, in turn, activates thrombin and results in the formation of fibrin from fibrinogen.[107] A small amount of coagulase remains cell-bound. Clumping factors are distinct cell surface proteins that bind fibrinogen, thereby producing the typical clusters of staphylococci when mixed with plasma.[108] The pathogenic roles of coagulase and the clumping factors[109] have been uncertain; they may protect against host defenses by causing localized clotting, and clumping factors may aid in adherence to traumatized skin, endothelial structures, and foreign surfaces. Recognition of this role for *S. aureus* clumping factors has prompted investigation into their use as potential vaccine antigens, as reviewed subsequently.

α-Hemolysin, the best studied of the staphylococcal exotoxins, hemolyzes erythrocytes, leads to skin necrosis, and triggers the release of cytokines and eicosanoids that can produce shock. It is lethal when injected into animals,[110] and mutants lacking α-toxin are less virulent.[111] However, pathogenic *S. aureus* isolates that do not produce α-hemolysin have been identified. β-Toxin is a sphingomyelinase that damages membranes rich in this lipid, but its role in disease pathogenesis is uncertain. Although most human isolates do not express β-toxin, its elaboration by a majority of isolates associated with mastitis in cows suggests a potentially important pathogenic role, at least in that species.[112] δ-Toxin and leukocidins are synergohymenotropic toxins that damage membranes of certain cells as a result of in-concert action by several elaborated proteins. Interestingly, the transcription of the individual protein components is separate.[100,113] The high percentage of γ-leukocidin-producing isolates from necrotizing skin infections suggests the importance of this toxin in dermonecrosis. Another leukocidin, the Panton-Valentine leukocidin, has been found in cases of severe community-acquired pneumonia in children; the majority of those cases were fatal, and all were marked by hemorrhagic necrosis of the lung.[114] Controversy persists as to the role of this toxin in pathogenesis.

Other toxins, produced by specific *S. aureus* isolates, mediate specific clinical syndromes. For example, epidermolytic (exfoliative) toxins A and B cause sloughing of skin that occurs in staphylococcal scalded skin syndrome.[115] The mechanism of epidermal splitting is unknown, although the toxins have esterase and, possibly, protease activity.[116] The clinical characteristics of TSS can be attributed to the actions of two different types of toxins: TSS toxin-1 (TSST-1) and certain enterotoxins. TSST-1 can be elaborated by *S. aureus* and is responsible for most TSS cases and nearly all of cases associated with menses; antibody to TSST-1 is protective,[117] but patients with TSS often do not make antibody during convalescence from TSS. The family of *S. aureus* enterotoxins may cause vomiting and diarrhea when ingested and is responsible for staphylococcal food poisoning; these enterotoxins also may cause TSS when their entry is via a nongastrointestinal route. Enterotoxins B and C account for about half the TSS cases not associated with menses. The enterotoxins and TSST-1 can have superantigen activity, that is, they can stimulate T cells nonspecifically.[118] This nonspecific antigen T-cell stimulation results in release of immunomodulators, particularly cytokines, an event that produces the clinical picture of TSS. Superantigens bind to class II major histocompatibility complex receptors but recognize

only the V_β element of the receptor, resulting in nonspecific polyclonal T-cell activation.[119]

Locally, *S. aureus* organisms may invade or necrose tissue and evoke a potent inflammatory response that largely affects the interaction of bacteria and polymorphonuclear leukocytes. Abscess formation is common, with a necrotic center consisting of pus and a fibrin wall that makes penetration by antibiotics difficult. The infection may spread locally by the formation of sinus tracts and secondary abscesses. Hematogenous dissemination and infection of distant bones, joints, cardiac valves, or other tissues may result.

CoNS strains readily adhere to foreign bodies and cause infections that are difficult to eradicate without removal of the prosthetic material. Like *S. aureus*, CoNS have a cell wall composed of peptidoglycan and teichoic acid but with a glycerol backbone. The teichoic acid and surface proteins have a role in adhering to plastic and other foreign material.[120] In addition, secretion of material commonly called "slime" is thought to be important in the pathogenesis of infection because it coats foreign bodies, thereby insulating the organism; this, in turn, makes penetration by antibiotics and phagocytosis by host cells difficult. It has been surmised that *Staphylococcus epidermidis* isolates may be more likely than isolates of other CoNS species to produce slime.[121]

DO WE NEED A STAPHYLOCOCCAL VACCINE?

Available epidemiologic data suggest that *S. aureus* infections now constitute a public health imperative. MRSA infections have been the subject of recent population-based epidemiologic studies. Klevens and colleagues[122] estimated that there were 94,360 invasive MRSA infections in the United States in 2005 and 80,461 in 2011.[123] Liu and colleagues[124] found that one in 316 people in San Francisco sought medical care for a MRSA skin and soft tissue infection in 2004–05. At Fort Benning, Georgia, it was recently estimated that the attack rate for medically attended MRSA infections peaked at 42 per 1000 soldiers per year and exceeded 35 per 1000 soldiers per year in the two subsequent years that the study encompassed.[125] In 2008–10, the rate remained high at 27 per 1000 soldiers annually.[126] These data suggest an urgent need for improved strategies for control and prevention of *S. aureus* infections.

Although all would agree that *S. aureus* is an important pathogen with protean clinical manifestations, substantial controversy exists about whether *S. aureus* infections can be prevented by vaccination and, if so, who should be targeted to receive this preventive strategy. Various vaccine approaches have been used with varying levels of promise and success. Interpretation of the data should be done with an understanding of whether the chosen vaccine antigen was relevant, what immune response was measured, what clinical end point was sought, if the work was performed with the use of an animal model, and whether the pathophysiology in the animal model was relevant to the pathogenesis of human disease. With these thoughts in mind, the available data regarding vaccine development in *S. aureus* are reviewed.

VACCINE DEVELOPMENT

Live Whole-Cell Vaccines

Several investigators have used whole-cell vaccines with varying success at preventing certain *S. aureus* syndromes. A live attenuated vaccine strain has been derived from a *S. aureus* isolate from bovine mastitis mutated with nitrosoguanidine. The mutant was selected for its ability to grow well at low temperatures, such as those found in breast tissue, and to replicate poorly at 37°C. The vaccine was injected into the mammary gland of pregnant or lactating mice and produced IgA and IgG antibody responses in milk and serum as measured by an enzyme-linked immunosorbent assay using killed *S. aureus* as the coating antigen. This vaccine also primed CD4 and CD8 lymphocyte populations capable of responding to staphylococcal antigens in vitro and in vivo challenge.[127] Immunized mice had a 2-$\log_{10}$ decrease in the quantity of *S. aureus* recovered from milk after challenge. A similar decrease was not observed when the vaccine was given parenterally.[128] Thus, this whole-cell vaccine, injected locally, provided some protection against an infection with pathogenesis that involves access by the microorganism to the breast from the exterior. It is unlikely that a live bacterial vaccine, even if efficacious, would receive serious consideration today.

Killed Whole-Cell Vaccines

There was no vaccine efficacy when a formalin-killed whole-cell vaccine prepared clinical *S. aureus* isolate[129,130] was given parenterally to rabbits before *S. aureus* challenge in a model of experimental endocarditis. Following immunization, antibodies to *S. aureus* protein A and bacterial agglutination titers were detectable in serum at high levels. However, after intravenous challenge, the incidence of endocarditis, the concentration of *S. aureus* in infective vegetations, incidence of renal abscess, and the concentration of bacteria in infected kidneys[130] were not significantly different in the immunized animals and the unimmunized controls. Thus, systemic immunity against an invasive bacteremic disease syndrome was not achieved by systemic immunization with this killed whole-cell vaccine.

A similar vaccine, called Staphypan, consisting of a killed suspension of *S. aureus* cells combined with a "toxoided" α-hemolysin (α-toxin) was developed. After a complex immunization schedule consisting of 10 injections, the effectiveness of Staphypan was assessed in patients undergoing continuous ambulatory peritoneal dialysis. Among vaccine recipients, Staphypan was immunogenic stimulating a robust serum antibody response to α-hemolysin and to *S. aureus* cells in the dialysate of vaccine recipients. However, vaccine recipients had rates of peritonitis, catheter-associated infection, and *S. aureus* asymptomatic carriage that did not differ significantly from the unimmunized control group.[131] Although this patient population may have posed challenges for the demonstration of vaccine efficacy, and there were issues regarding choice of outcome parameters, the failure of this vaccine reinforces the difficulties faced in the choice of vaccine components. Put simply, if the whole-cell vaccine had poor efficacy, how does one proceed to selection of components for a component vaccine? Also, given this experience, it is unlikely that a killed whole cell vaccine would be evaluated for possible licensure.

Component Protein Vaccine Antigens

The choice of a relevant *S. aureus* protein in a component vaccine has obvious advantages over a whole-cell vaccine, particularly in an era of enhanced concern about vaccine safety. Investigators have attempted to identify one or more protective antigens among the many toxins or "virulence factors" elaborated by *S. aureus* as targets for vaccine development (Table 55.1). Substantial effort spanning many years has suggested that a fully efficacious single protective antigen is not likely to be the ideal vaccine candidate. Thus, efficacy of a candidate vaccine antigen often is measured by a decrease in disease severity without preventing infection per se. For example, immunization with either purified α-toxoid or β-toxoid in experimental model of mastitis in lactating rabbits,[132] demonstrated that both vaccines were immunogenic. In this model, mastitis, typified by abscesses at or near

TABLE 55.1 Virulence Factors as Potential Targets for Vaccine Development

Factor	Gene
VIRULENCE FACTORS INVOLVED IN ATTACHMENT	
Clumping factors	*clfA*, *clfB*
Fibrinogen-binding protein	*fbpA*
Fibronectin-binding protein A	*fnbA*
Fibronectin-binding protein B	*fnbB*
Collagen-binding protein	*can*
Coagulase	*Coa*
Polysaccharide/adhesin	*ica* locus
VIRULENCE FACTORS INVOLVED IN EVASION OF HOST DEFENSES	
Enterotoxins A, B, C1–C3, D, E, H, etc.	*sea-set* or *entA-T*
Toxic shock syndrome toxin-1	*tst*
Exfoliative toxins A, B	*eta*, *etb*
Protein A	*Spa*
Lipase	*Geh*
V8 protease	*sasP*
Fatty acid-modifying enzyme (FAME)	
Panton-Valentine leukocidin	*lukF-PV*, *lukS-PV*
Leukocidin R	*luk-F-R*, *lukS-R*
Capsular polysaccharide type 5	*cap5* locus
Capsular polysaccharide type 8	*cap8* locus
Staphylokinase	*Sak*
VIRULENCE FACTORS INVOLVED IN INVASION/TISSUE PENETRATION	
α-Toxin	*Hla*
β-Hemolysin	*Hlb*
γ-Hemolysin	*hlgA*, *hlgB*, *hlgC*
δ-Hemolysin	*Hld*
Phospholipase C	*Plc*
Metalloprotease (elastase)	*sepA*
Hyaluronidase, hyaluronate lyase	hysA

Modified from Projan SJ, Novick RP. The molecular basis of pathogenicity. In: Crossley KB, Archer GL, eds. The Staphylococci in Human Disease. *New York, NY: Churchill Livingstone; 1997:57.*

one or more teats, occasionally was complicated by a rapidly fatal, edematous, hemorrhagic mastitis with systemic manifestations termed "blue breast." Three clinically relevant *S. aureus* isolates were used for challenge of immunized animals. However, for two of the three isolates, α-toxoid prevented blue breast but not mastitis. The β-toxoid vaccine had no apparent efficacy against either clinical outcome.[132]

Protein A, a 40- to 60-kDa *S. aureus* cell wall protein encoded by the *spa* gene, is produced by nearly all *S. aureus* isolates. It binds to the Fc region of human IgG_1 and IgG_2 to mouse IgG_{2a} and IgG_{2b} with high affinity. It also binds with moderate affinity to human IgM, IgA, and IgE, and to mouse IgG_3 and IgG_1, but not to human IgG_3 or IgD or to mouse IgM, IgA, or IgE. The binding of protein A to the Fcγ region of the immunoglobulin molecule through interaction with the heavy chain results in improper orientation (when compared to normal antibody binding) and, thus, disrupts the role of the antibody in opsonization and phagocytosis. Protein A can therefore be considered a protective mechanism for the species shielding it from antibody "attack." Protein A has also been termed a B-cell superantigen[133] because it also binds to V_H3 B-cell receptors and limits their antibody production. Furthermore, marginal zone B cells and B-1 cells undergo, preferentially, induced cell death with supraclonal depletion and immune tolerance on exposure to protein A.

Thus, protein A likely impedes development of a vaccine that depends on the traditional approach of finding the protective antigen(s), demonstrating that it is immunogenic and generating opsonophagocytic antibody. It may also decrease antibody production by virtue of B-cell binding with resulting diminution in antibody production and B-cell apoptosis. Kim and colleagues[134] engineered a mutant protein A molecule that had mutations targeted to the immunoglobulin binding site and the V_H3 B-cell binding site and this mutant protein A SpA_{KKAA} did not bind the Fc fragment of immunoglobulins or trigger B-cell apoptosis. Immunization of mice with SpA_{KKAA} elicited antibodies that neutralized immunoglobulin binding activities of protein A, augmented the immunogenicity of unrelated antigens, and provided some protection in a mouse model of bacteremic *S. aureus* infection.[134]

Another approach for staphylococcal vaccine development has been targeting the bacteria–host cell adhesion. In the 1980s, the results of several studies indicated that *S. aureus* adhered to certain components of the host extracellular matrix.[135–138] Substantial effort has been invested in identifying these externally expressed bacterial proteins that mediate bacterial binding to host extracellular matrix. The proteins are collectively referred to as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), and they mediate *S. aureus* binding to host proteins including fibronectin, fibrinogen/fibrin, elastin, vitronectin, collagen, laminin, decorin, and heparan sulfate–containing proteoglycans.[139]

A recombinant collagen adhesin fragment was used to immunize mice, the animals were challenged with intravenous *S. aureus*, and the mortality in the immunized animals was decreased from 87% to 13%.[140] Immunization with another MSCRAMM, fibronectin-binding protein, effected a 2-log (99%) decrease in bacterial density in heart valve vegetations in experimental endocarditis.[141] Similarly, a fusion protein vaccine consisting of the fibrinogen-binding domain of fibronectin-binding protein A effected a 99.9% decrease in *S. aureus* density in the mammary glands of experimentally challenged mice after immunization.[142]

Another MSCRAMM, clumping factor A (ClfA), a surface protein that binds fibrinogen and fibrin, has been evaluated as a vaccine candidate.[143,144] ClfA differs from coagulase, which also binds fibrinogen, in that it promotes clumping of bacterial cells (the defining staphylococcal morphology of clusters) and bacterial adherence to blood clots and to plasma-conditioned biomaterials and catheter-damaged heart valves. In a mouse model of *S. aureus* arthritis, mice immunized with purified recombinant ClfA and subsequently challenged with *S. aureus* developed less-severe arthritis. In addition, passive administration of anti-ClfA antibodies was protective against experimental septic arthritis and death.[145] A DNA vaccine directed against ClfA with genetic adjuvants elicited IgG_2 anti-ClfA antibody and decreased *S. aureus* adherence to mammary gland epithelial cells.[146] Thus, ClfA is an important MSCRAMM that has induced partial or complete protection against invasive *S. aureus* infections in experimental animals. In human studies, a polyclonal anti-ClfA antibody administered to premature infants delayed the onset of naturally occurring *S. aureus* infections. However, this observation could not be confirmed in a prospective, randomized trial (S. Hetherington, personal communication, May 2006).

Perhaps a multiple MSCRAMM-containing vaccine would hold more promise. DNA vaccine directed against both ClfA and fibronectin-binding protein provided modest protection against experimental mastitis in dairy cows.[147]

Efforts are underway to identify other protective antigens. Merck has identified the iron-scavenging, cell wall–anchored protein isdB as a potential vaccine candidate.[148] Patients with *S. aureus* infections were shown to have a brisk antibody response to this protein during convalescence. The protein also was immunogenic in mice and rhesus monkeys.[148] A Phase I dose-range study[149] of the Merck adjuvanted isdB vaccine in 124 healthy adults demonstrated that a single 30- or 90-μg dose was more immunogenic than a 5-μg dose and that the immune response appeared by 10 to 14 days after vaccination and persisted through day 84.[149]

A Phase IIb/III double-blind, randomized, placebo-controlled, event-driven trial was conducted with a novel vaccine candidate called V710 that contained the unadjuvanted isdB. This trial, conducted between December 2007 and August 2011, enrolled 8031 patients 18 years of age or older who were scheduled to undergo a median sternotomy for heart surgery within 14 to 60 days after vaccination. It was an international trial conducted at 165 sites in 26 countries. However, the Data and Safety Monitoring Board recommended termination of the study in 2011 after concerns about safety and low efficacy. Although V710 vaccination induced a significant but modest and transient increase in functional antibodies, it was not significantly more efficacious than placebo in preventing *S. aureus* bacteremia or mediastinitis through postoperative day 90. In addition, the mortality rate and the occurrence of multiorgan failure was higher among the vaccinees, a finding that has not been explained.

Interfering with Nasal Colonization: A Valid Vaccine End Point?

Because the nares are believed to be the principal site of *S. aureus* colonization and because colonization appears to be a risk factor for invasive disease, several investigators have focused on novel immunization strategies aimed at decreasing nasal colonization as a method of decreasing invasive disease. ClfB is an *S. aureus* MSCRAMM that binds to fibrinogen and type 1 cytokeratin molecule K10 on the surface of desquamated human epithelial cells.[150,151] In mice, *S. aureus* mutants deficient in ClfB had decreased nasal colonization compared with controls,[152] suggesting that ClfB might be an attractive vaccine component to decrease nasal colonization. However, it is not clear whether ClfB decreases asymptomatic colonization at other body sites or if it selectively decreases nasal colonization of the more pathogenic *S. aureus* strains.

Carbohydrate Surface Molecules as Vaccine Candidates

Two lines of investigation have suggested that polysaccharides elaborated by *S. aureus* may be important protective antigens. The first concerns an in vivo–expressed[153] polysaccharide formerly called poly-*N*-succinyl-β-1-6-glucosamine, or PNSG, now termed poly-*N*-acetylglucosamine (PNAG). This intercellular adhesin was first recognized among strains of CoNS.[153] However, *S. aureus* isolates obtained from the lungs and sputum of patients with cystic fibrosis reacted with antirabbit anti-PNAG antibody as well. Additional study revealed that eight examined isolates all had the *ica*ADBC locus whose gene products synthesized PNAG among CoNS isolates. It was also appreciated that *S. aureus* strains elaborated PNAG in vivo, whereas in vitro, expression was minimal or undetectable. Rabbits immunized with purified PNAG produced high-titer anti-PNAG IgG antibody and passive administration of rabbit anti-PNAG antibody to mice significantly decreased or eliminated organisms after experimentally induced *S. aureus* kidney infection.[153] Active PNAG immunization of mice produced similar results.[154]

PNAG is structurally distinct from types 5 and 8 *S. aureus* capsular polysaccharides, studied in earlier vaccine studies. Interestingly, the elaboration of the type 5 or 8 capsules was found to be inversely related to the expression of PNAG, suggesting that staphylococci may choose to preferentially express PNAG in vivo. Moreover, cultivation of the *S. aureus* isolates in aerobic trypticase soy broth resulted in high capsular polysaccharide expression with an associated decreased PNAG expression. Although most clinical strains of *S. aureus* did not express PNAG in vitro,[153,154] subsequent data revealed that PNAG expression could be induced in vitro by growing the bacteria in a brain–heart infusion broth supplemented by glucose.[154]

Deletion of the *ica* locus resulted in decreased virulence of several *S. aureus* isolates in a murine model of *S. aureus* infection.[155] Human monoclonal antibodies directed against deacetylated or backbone PNAG epitopes (dPNAG) were shown to be opsonophagocytic and were modestly protective in a mouse model (high inoculum, intraperitoneal challenge, lethality end point).[156]

Thus, dPNAG, but not PNAG, is an immunogen that may be a protective antigen against *S. aureus* invasive disease. Both a monoclonal antibody prepared against dPNAG and a dPNAG vaccine will be evaluated in a planned clinical trial in 2016. Evaluation will be by human challenge experiments among bacterial infections that will not include *S. aureus*.

Recognition of the crucial role of the polysaccharide capsule in the virulence of *S. pneumoniae* and *H. influenzae* type b led to the development and deployment of highly successful preventive vaccine strategies currently in use in the United States and other countries. These vaccines rely on the notion that antibody to the capsular polysaccharide is opsonic and, thus, counters the antiphagocytic properties imparted by the capsular polysaccharide. The possibility that the *S. aureus* capsular polysaccharide is a similarly protective antigen and that a vaccine directed against capsule can greatly decrease the incidence of invasive staphylococcal disease has created enthusiasm for this approach, but the available data raise doubts about this concept.

Many *S. aureus* isolates are said to produce capsular polysaccharide. Four polysaccharide types—1, 2, 5, and 8—have been chemically characterized. Isolates elaborating the type 1 and type 2 polysaccharides are heavily encapsulated with mucoid colony morphology, but they rarely cause clinical disease. However, most clinical isolates do not elaborate a mucoid capsule like *H. influenzae* type b and *S. pneumoniae*, which shed capsular polysaccharide into the surrounding environment. In contrast, *S. aureus* capsular polysaccharide is shed only minimally if at all. Two types, 5 and 8, account for approximately 85% of encapsulated isolates from clinical sources. Because it was shown that antibody to the type 5 and type 8 capsular polysaccharides provided type-specific opsonophagocytosis[157] and conferred protection in animals,[158,159] although conflicting data exist,[160] a vaccine was prepared consisting of the type 5 and type 8 capsular polysaccharides, each bound to detoxified recombinant *Pseudomonas aeruginosa* exotoxin A expressed in *Escherichia coli*. This vaccine was evaluated in two clinical trials.[161]

The initial trial enrolled adults undergoing hemodialysis, a population at high risk for invasive *S. aureus* infections, and was conducted in 1998–99 among 73 hemodialysis centers. A total of 1804 subjects were randomized to receive either an *S. aureus* capsular polysaccharide–protein conjugate vaccine (100

μg of the types 5 and 8 polysaccharides) or placebo. Of the participants, 22% were asymptomatic *S. aureus* carriers. The primary end point for the study was the occurrence of *S. aureus* bacteremia at any time in the first year after immunization beginning 2 or more weeks after immunization.[161]

Although the vaccine was well tolerated, there was no significant efficacy observed from 2 to 20 weeks after immunization; 28 episodes of *S. aureus* bacteremia occurred (10 episodes in vaccine recipients and 18 in placebo recipients). However, at the 30- and 40-week postimmunization time points modest efficacy was noted (63%; 95% confidence interval, 14% to 86%, and 57%; 95% confidence interval, 10% to 81%, respectively). Furthermore, efficacy dissipated when observed at the 50-, 54-, and 91-week postimmunization time points, and was not significantly different in the vaccine recipients and controls. The investigators interpreted these data to indicate that the vaccine had provided some protection for about 40 weeks after immunization.

First, analysis of anticapsular antibody responses following immunization in the vaccine recipients was 80 μg/mL, a value vastly exceeding concentrations believed to be protective against other encapsulated bacteria, such as *H. influenzae* type b and *S. pneumoniae*. Second, there were no significant differences between the peak antibody levels achieved in the vaccinees who became bacteremic and those who did not. Third, the antibody concentrations peaked 6 weeks after immunization and declined thereafter, although significant protection was not observed until sometime later. Finally, approximately 20% of the isolates obtained from study subjects had neither the type 5 nor type 8 capsule, suggesting that other *S. aureus* antigens will have to be targeted in addition to types 5 and 8 for a successful vaccination program.

A second randomized, placebo-controlled clinical trial with this same bivalent capsular polysaccharide–protein conjugate vaccine was conducted in adult patients receiving dialysis at 200 sites in the United States. Efficacy was evaluated 3 to 35 weeks after immunization. A booster dose was administered, and participants were followed for 6 more months. This trial produced a clearer result in that there was no decrease in types 5 and 8 *S. aureus* bacteremia among vaccinees when compared with controls.[161]

Why did the types 5 and 8 conjugated capsular polysaccharide vaccine prove unsuccessful? There are several possible explanations. First, and most simply, the capsule may not be the protective antigen. Unlike the case with *H. influenzae* type b, *S. pneumonia* and *N meningitidis* in which it was clear that circulating anticapsular antibody was protective, no such demonstration has been made for *S. aureus*. Second, a relatively high frequency of isolates lacking the type 5 and type 8 capsule was found in the clinical trial.[162] It should be noted that USA300, the genetic background most responsible for the CA-MRSA outbreak currently affecting the United States, also does not elaborate either type 5 or type 8 capsule.[163,164] Third, it is possible, although less likely, that people undergoing dialysis were too immunocompromised to generate a protective antibody titer against *S. aureus* bacteremia.

OTHER POTENTIAL VACCINE TARGETS

The notion that LTA may be a protective antigen to prevent *S. aureus* sepsis has been evaluated. Initial efforts have focused on an anti-LTA monoclonal antibody, pagibaximab, that underwent clinical trials in neonates.[165] However, protection against *S. aureus* sepsis did not occur.

Antibody against PBP2a surprisingly protected against infection of the kidney after intravenous challenge with a MRSA strain. No protection occurred after challenge with a methicillin-susceptible isolate.[166] Immunization with a chimera-like heteropolymer consisting of an antibody conjugate targeting *S. aureus* and the type 1 complement receptor found on erythrocytes protected mice from death after intravenous *S. aureus* challenge by both of two challenge isolates.[167]

The role of T cells, a new chapter in *S. aureus* vaccine development, was recently opened by the recognition that lymphocyte-mediated pathways have a role in immunity to *S. aureus* infections. Spellberg and colleagues[168] and Lin and colleagues[169,170] found that interferon-γ–deficient mice were very susceptible to *S. aureus* infection after intravenous inoculation. Others found that mice deficient in interleukin (IL)-17A and IL-17F developed spontaneous *S. aureus* skin infections.[171,172] Hence, it may be possible to induce memory T cells that are capable of increasing phagocyte recruitment to sites of infection and facilitate clearance of *S. aureus* from tissues. In support of this concept, a novel recombinant N-terminus vaccine molecule containing the candidal adhesion, rAls3p, is being evaluated (Table 55.2).[168–170] This vaccine induced high antibody concentrations, but they were not protective when used to passively protect animals against *S. aureus* intravenous challenge[168] and the antibody titers did not correlate with protection against lethal *S. aureus* infection.[170] Furthermore, the vaccine was equally effective in B-cell–deficient and wild-type mice but had no efficacy in T-cell–deficient mice.[168] Adoptive transfer of immune $B220^+$ B cells did not transfer protection, but transfer of $CD4^+$ T cells did. The vaccine was ineffective in interferon-γ– and IL-17A–deficient mice and in $gp91^{phox-/-}$ mice that are unable to produce superoxide.[170] Cross-adoptive transfer experiments confirmed that functional phagocytes were operative in vaccine-mediated protection at the downstream effector stage. Finally, vaccination increased the recruitment and activation of phagocytes at sites of tissue infection in mice, and cytokines produced by vaccine-primed lymphocytes improved the ability of phagocytes to kill *S. aureus*. Hence, the rAls3p-N vaccine demonstrates that it is feasible to induce a protective immune response in mice against *S. aureus* in the absence of induction of protective antibodies, and by inducing a protective T-helper (Th) 1/Th17 response.[173–175]

These observations do not preclude development of a humoral-based vaccine against *S. aureus*. Rather, they suggest that cell-mediated vaccines merit additional study and raise the possibility of combining antigens that stimulate both humoral and cellular responses against *S. aureus*. Indeed, this

TABLE 55.2 *Staphylococcus aureus* Vaccines Receiving Clinical Evaluation as of December 2015

Manufacturer	Vaccine	Type of Study	Composition of Vaccine
Novartis	4 Components	Safety and immunogenicity–COMPLETE	Not publicly disclosed; components are all proteins
Pfizer	4 Components	Safety and immunogenicity–COMPLETE. Efficacy after spinal surgery trial ongoing	Conjugated capsular polysaccharides, types 5 and 8; clumping factor A; manganese transporter C
Novadigm	Recombinant protein	Safety and immunogenicity–COMPLETE	rAls3p-N
GlaxoSmithKline	4 Components	Safety and immunogenicity–COMPLETE	Capsular polysaccharides conjugated to tetanus toxin, α-toxoid, clumping factor A

strategy may be the most likely to result in a protective vaccine against *S. aureus*.

EPILOGUE

Table 55.2 summarizes clinical trials in progress or recently completed. However, many questions remain. Is the *S. aureus* disease burden sufficient in the general population to justify development of a universal vaccine? Recent data suggest that the answer is yes. Administration of an *S. aureus* vaccine to young children during the time when they are receiving other vaccines (first year of life) would be most convenient, and, perhaps, appropriate, as the attack rate is so high at that time. Can one vaccine formulation protect against the three major clinical scenarios, skin and soft tissue infection, pneumonia, and invasive disease? This is a difficult question for which the answer is not fully known. Can an antibody eliciting vaccine protect against any *S. aureus* clinical syndrome? Antigens that have elicited opsonophagocytic antibody have not elicited protection against *S. aureus* disease. The reason has remained a mystery. If it is possible to elicit protective antibody(ies), what is (are) the protective antigen(s)? Is protein A a barrier to this approach? If so, how can this conundrum be dealt with? Can any active immunization strategy protect immunocompromised persons at high risk for *S. aureus* disease? If not, can a passive vaccination approach be delivered with sufficient regularity to be helpful to persons with such conditions with reasonable ease and at a reasonable cost? What is the role of the polysaccharide capsule? Its presence suggests that it is important, but defining its place in the pathophysiologic cascade has not been easy. Its absence from USA300, the epidemic genetic background of the last decade in the United States, does not lend confidence to believing that this antigen is crucial for protection against currently circulating invasive strains. The recent identification of important T-cell pathways, particularly the Th17/IL-17 pathway in *S. aureus* immunity, suggests that they might have a role in eliciting protection. An additional problem is understanding immunity to *S. aureus*, a commensal organism that causes disease in a small subset. Is there an immunologic gap in persons who acquire disease that can be traversed with a vaccine? Despite extensive investigation, we do not know the answers to many of these questions, and *S. aureus* remains a complex enigmatic pathogen. Understanding the answers will be necessary to the development of a rational vaccine formulation.[176]

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56 *Streptococcus* Group A Vaccines

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Streptococcus pyogenes (group A β-hemolytic *Streptococcus* [GAS]) has challenged scientists and clinicians throughout modern medical history. Descriptions of epidemics of fulminant illnesses in previously healthy individuals date back to the 1600s.[1,2] Until the early 1900s, scarlet fever and acute rheumatic fever (ARF) were a major cause of child death in the United States and Europe. Thereafter, the incidence and severity of GAS-related diseases declined. In the United States, the case-fatality rate from scarlet fever fell from nearly 30% to less than 2%, well before the advent of antibiotics, for reasons that are not fully understood.[3] Institution of antibiotic therapy of GAS pharyngitis produced further improvements. In the 1980s, amid discussion about the vanishing threat of GAS pharyngitis,[4] outbreaks of severe invasive disease and ARF and a newly described streptococcal toxic shock syndrome (STSS) emerged around the world.[5] These invasive infections carried a high mortality, even in the face of appropriate antibiotic therapy.

In addition to its unpredictable epidemiology, GAS has presented a diagnostic dilemma requiring a series of incisive observations to link its manifold manifestations with a single etiology. Circa 1880, Pasteur identified the microbe in specimens from puerperal fever victims.[6] In 1898, Cheadle linked the major features of ARF that comprise the five major criteria that we know today (i.e., carditis, polyarteritis, chorea, erythema marginatum, and subcutaneous nodules).[3,7] In 1903, Brown established that complete (β) hemolysis on blood agar could distinguish streptococci that were pathogenic for humans.[8] Although β-hemolytic streptococci were among suspected etiologic agents of ARF, it was the landmark studies of Coburn in 1930 that firmly established the direct link between GAS pharyngitis and the "rheumatic state."[9] Lancefield pioneered the serologic classification of β-hemolytic streptococci according to the group-specific polysaccharide. She determined that most human pathogens belonged to group A, and further categorized group A strains according to the trypsin-sensitive surface-exposed M protein[10,11] and a trypsin-resistant T antigen.[12] The ability to classify streptococci serologically provided the foundation for studies to characterize the clinical features, epidemiology, and protective immunity, and to devise strategies for vaccine development.

BACKGROUND

Clinical and Epidemiologic Features

Pharyngitis and impetigo are the most common uncomplicated manifestations of GAS infection. Serious infections result when the organism spreads to contiguous sites or disseminates to normally sterile spaces or deep tissues, which may be accompanied by necrotizing fasciitis or STSS or both. Major nonsuppurative complications of GAS infections are ARF and poststreptococcal glomerulonephritis (PSGN), both considered immune-mediated.[13,14] In industrialized countries, the burden of uncomplicated infections is high; an estimated 2% to 6% of U.S. schoolchildren visit a physician for GAS pharyngitis annually.[15] Despite their benign nature, these illnesses incur substantial costs related to missed school and work and use of healthcare resources. The incidence and mortality from invasive infection has remained stable in the United States for more than a decade, causing approximately 15,360 cases (4.8 per 100,000 population) and 1640 deaths (case fatality: 10.7%) in 2014.[16] Localized, episodic increases of invasive infection are seen, as has been the case in Utah.[17] Exceptionally high rates occur in northern Australia among nonindigenous (8.8 per 100,000 population) and indigenous populations (69.7 per 100,000 population).

Cases of ARF continue to occur in some U.S. communities,[17] but the overall incidence is less than 0.5 per 100,000 population.[4,18] In contrast, ARF persists in causing substantial morbidity and mortality in many developing countries and in indigenous populations within developed countries. The incidence of ARF reaches 650 per 100,000 population in some remote Aboriginal communities.[19] The prevalence of rheumatic heart disease reported from South Asia and sub-Saharan Africa ranges from 130 to 640 per 100,000 children 5 to 14 years old.[20]

PSGN has received less attention than ARF because of its generally benign outcome, even in those who develop renal insufficiency during the acute episode.[21] However, in areas where nephritogenic strains are prevalent, PSGN can follow 10% to 15% of episodes of GAS pharyngitis or pyoderma.[22]

Epidemic waves that characterize the epidemiology of severe GAS infection have been attributed both to the host and the organism. Circulating strains produce herd immunity, resulting in decreased transmission and disease severity. GAS acquires virulence factors via transmissible agents, such as bacteriophages,[23] or via genetic mutations.[24] The upsurge of severe GAS disease in the 1980s occurred during a time when the incidence of uncomplicated disease remained constant, suggesting that highly virulent organisms created by genetic alterations in critical regulatory and virulence genes emerged in an immunologically susceptible population.[5,25] One virulence factor that has been strongly associated with these hypervirulent invasive strains (regardless of M type) is the extracellular enzyme nicotinamide adenine dinucleotidase, which has chemotactic, cytotoxic, and vasoactive properties.[26] Accordingly, genome sequencing of population-based M1 strains demonstrated the acquisition of the chromosomal region containing the gene for nicotinamide adenine dinucleotidase just before the pandemic surged.[27]

Bacteriology, Virulence Factors, and Pathogenic Mechanisms

An assortment of secreted and surface-expressed molecules mediate the four pathogenic processes that characterize acute infection: adhesion, immune evasion, inflammation, and tissue invasion (Fig. 56.1). The virulence factors that are being explored as possible vaccine antigens are highlighted.

Group A β-hemolytic streptococci adhere to epithelial cells in the pharynx, cervix, or skin by binding to plasma and matrix proteins,[28] forming microbial aggregates and producing a biofilm that protects against host defenses and nutrient deprivation.[29] Among the best described adhesins are M protein,[30] hyaluronic acid capsule,[31] and fibronectin binding proteins such as SfbI[32] and FBP54.[33] R28 is found in GAS

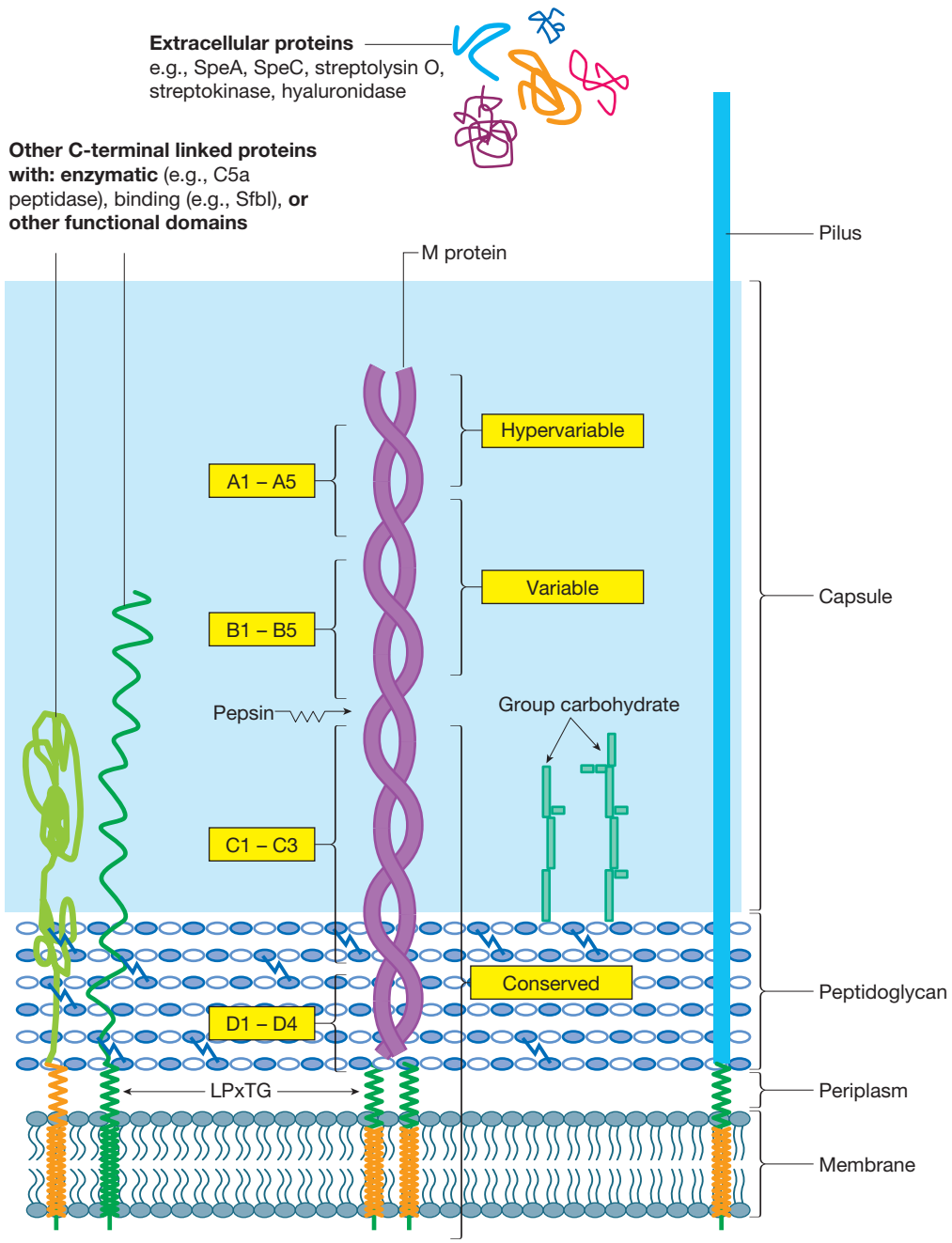


Figure 56.1. Major surface structures of group A β-hemolytic *Streptococcus*. The main categories of proteins that have been exploited in vaccine development are shown. Most proteins are anchored at the C terminus via a highly conserved LPxTP consensus motif followed by a hydrophobic region that spans the cell membrane and terminates with a short charged tail within the cytoplasm. After translocation to the surface, the LPxTP motif is cleaved and the mature molecule is anchored into peptidoglycan. The majority of proteins are multifunctional, usually with domains that bind molecules found in extracellular matrix, such as fibronectin (SfbI), but also domains that exhibit enzymatic activity, such as C5a peptidase, or have other functions. In the case of proteins with multiple repeats, such as M protein, each repeat domain (designated as A, B, C, and D) has a different function. The pepsin cleavage site of M protein is marked with a crooked arrow. Extracellular proteins that exhibit toxin and/or superantigen properties include streptococcal pyrogenic exotoxin (Spe) A and SpeC, which have been targeted for vaccine development, as well as anti–streptolysin *O*, streptokinase, and hyaluronidase.

strains associated with puerperal fever and is thought to promote binding to cervical epithelium.[34]

GAS pilus, identified in 2005, contributes to adhesion and biofilm formation.[29,35] Interestingly, these filamentous structures protruding from the cell surface bear the T antigens described by Lancefield and thus present a potential avenue for vaccine development. All GAS isolates tested thus far carry and express the *bp/tee* pilus genetic locus.[29]

After colonization, GAS persists in the host by deploying a multi-pronged approach to disarm the innate immune response. C5a peptidase (also called SCPA, or surface-bound C5a peptidase) inactivates a chemokine of the complement

system,[36,37] while SpyCEP (also called ScpC or Spy 0416), a cell envelope serine protease, cleaves the neutrophil attractant interleukin-8,[38] both contributing to inhibition of neutrophil recruitment. Extracellular DNAases degrade chromatin webs produced by neutrophils to entrap GAS bacteria.[39] The secreted serine esterase (SsE, or Spy 1718) inhibits neutrophil recruitment and contributes to skin invasion and systemic dissemination.[40] The hyaluronic capsule promotes resistance to host defense peptides and to phagocyte killing within the extracellular neutrophil traps,[41,42] and streptococcal inhibitor of complement, found only in M1 and M57, inhibits complement-mediated lysis.[43] M protein, encoded by *emm*, impedes neutrophil phagocytosis in the absence of type-specific antibody by binding plasma proteins and inhibiting opsonization via the alternate complement pathway.[44–48] Antigenic heterogeneity of the amino terminus of M protein further enables GAS to escape recognition by specific antibody.

GAS elaborate several extracellular products, including streptolysin *O*, deoxyribonuclease B, streptokinase, and hyaluronidase, that induce tissue liquefaction and facilitate invasion.[49] Streptolysin *O* kills eukaryotic cells by forming membrane pores.[50]

M protein and the streptococcal pyrogenic exotoxins (SPEs) have potent proinflammatory properties.[51,52] The SPEs form a family of superantigens that induce profound immunologic changes[53,54] as well as fever, tissue damage, and clinical manifestations of endotoxic shock.[55–57] SPE A and SPE C, formerly known as erythrogenic toxins because of their association with the rash of scarlet fever, are bacteriophage-encoded and contain distinct T-cell receptor and class II major histocompatibility complex binding sites.[58] Severe scarlet fever and STSS has been associated with SPE A– and SPE B–producing strains.

The pathogenesis of the nonsuppurative postinfectious sequelae of GAS is not well understood. ARF follows approximately 3% of episodes of untreated GAS pharyngitis under epidemic conditions[59] but is considerably less frequent in endemic situations.[60] Observations of Aboriginal populations experiencing high rates of ARF have led some investigators to suggest that skin infections lead to ARF in these communities.[61] Strains causing pharyngitis and ARF were long thought to bear different M types and other antigenic and virulence properties than those that cause impetigo/pyoderma, although some overlap exists.[62,63] Proof that skin strains are responsible for a substantial burden of ARF would alter the necessary composition of M protein–based vaccines.

Appreciation of the structure of M protein helps explain its function as the major virulence factor of GAS and also the major protective antigen (see Fig. 56.1).[64] These α-helical coiled–coil fibrillar rods consist of two polypeptide chains anchored in the cell wall peptidoglycan by a C-terminus LP×TG motif.[65] Each polypeptide contains up to four segments of repeating amino acids (labeled A to D). The C repeats contain epitopes that are exposed on the surface of the cell wall and are largely conserved among different M types.[66] During infection, opsonic antibodies that confer protection are directed predominantly at the N-terminus,[67] a highly variable region that constitutes the basis for Lancefield's serotyping classification of GAS into approximately 100 M serotypes. Currently, group A β-hemolytic streptococci are classified based on sequence analysis of the portion of the *emm* gene that encodes M serospecificity. The more than 220 distinct *emm* types have been further classified into 48 distinct *emm* clusters based on phylogenetic, structural, and binding properties of M protein.[68,69] Epitopes found within the B repeats and adjacent segments of the A and C repeats cross-react with human antigens and have been proposed as a mechanism in the pathogenesis of ARF. Moreover, a segment within the B-repeat region bears homology with known superantigens.[70]

The concept of molecular mimicry has been invoked to explain the pathogenesis of ARF and PSGN. M protein bears antigenic similarities with human fibrillar proteins, such as cardiac muscle myosin and molecules of the extracellular matrix of joints and kidneys.[71–74] A mechanism for carditis is postulated whereby GAS infection stimulates cross-reactive antibodies that bind to the valvular endothelium resulting in inflammation[74] and infiltration by $CD4^+$ T cells that recognize GAS M protein and cardiac antigens.[75,76] Antibodies evoked by the immunodominant epitope of group A carbohydrate, *N*-acetylglucosamine, cross-react with GM_1 gangliosides on the surface of neuronal and valvular endothelial cells and have been proposed in the pathogenesis of Sydenham chorea[77] and carditis,[74] respectively. Seroreactivity with components of the cell membrane, SPE B, and other GAS antigens has been suggested in the etiology of PSGN.[49,78] It is important to note that antibodies that cross-react with human tissues and somatic components of GAS are found in many healthy children, so their presence alone does not explain the nonsuppurative sequelae of GAS.[79,80] Further elucidation of the pathogenesis of nonsuppurative sequelae will facilitate rational development of a safe GAS vaccine.

Treatment and Prevention With Antimicrobials

In the 1950s, Denny and colleagues demonstrated that a course of penicillin prevents more than 90% of ARF episodes (primary prevention) if initiated within 9 days of the onset of GAS phayryngitis.[81] Furthermore, the observation that nearly 30% of patients with rheumatic carditis experience additional valvular injury when reinfected with GAS[82] prompted recommendations to administer prophylactic penicillin during the years when the risk of exposure to GAS is greatest (secondary prevention). In contrast, although antibiotics speed recovery of skin lesions, they do not appear to prevent glomerulonephritis following either pharyngitis or impetigo. Severe invasive infections are treated with a combination of penicillin and an antibiotic that inhibits protein synthesis such as clindamycin plus surgical debridement of necrotic tissue.[83] Intravenous immunoglobulin therapy has been advocated as ancillary therapy for STSS based on its ability to neutralize superantigen toxins, but its efficacy remains inconclusive.[84]

There are well-recognized limitations to the treatment of GAS disease. Nearly one-third of episodes of ARF are preceded by a GAS infection that does not receive medical attention.[85] In many parts of the world, resources are not available for effective primary and secondary prevention. Even in the face of adequate therapy, the outcome of invasive disease may be poor.[86,87] Development of a safe, effective, and affordable GAS vaccine has the potential to overcome many of these barriers.

ACTIVE IMMUNIZATION

Feasibility of Developing a Safe and Efficacious Group A β-Hemolytic *Streptococcus* Vaccine

The feasibility of developing a GAS vaccine depends on adequate resolution of several safety and immunogenicity concerns. The principal safety concern is the theoretical risk that a GAS vaccine, like natural infection, might trigger an immunopathological event such as ARF. Thus an important advance for the development of M-protein–based vaccines was the discovery in the 1980s that type-specific aminoterminal regions of the M protein elicited the strongest bactericidal immune responses and could be separated from the potentially harmful cross-reactive epitopes.[88–92]

Evidence suggests that there is immunity to GAS, so it is reasonable to expect that a vaccine could be constructed to do the same. The declining incidence of GAS pharyngitis with age implies the acquisition of immunity following natural exposure.[93] Lancefield established the role of type-specific immunity in preventing experimental infections in animals[94] and later showed that humans develop long-lasting type-specific immunity following natural infection.[95] Beachey and colleagues protected mice against M24 infection using serum from human volunteers immunized with a type-specific peptide fragment of type 24 M protein.[96] The most direct evidence comes from clinical trials performed by Fox and colleagues (described below), which demonstrated that vaccinated volunteers were significantly protected against challenge with virulent GAS bearing the homologous M protein.[97–99]

Correlates of Protection

Knowledge of the precise immune responses that mediate clinical protection could be exploited in vaccine development, but these remain incompletely elucidated. The findings of Lancefield and Fox bespeak type-specific immunity recognizing the N-terminal region of M protein,[97–99] presumed to be mediated by bactericidal antibodies. Nonetheless, a direct correlation between bactericidal antibodies and clinical immunity has not been observed consistently.[97] Type-specific mucosal immunity may confer protection against colonization.[98,100,101] Natural infection in children and adults induces strong antibody and cellular responses that may contribute to protective immunity. T-helper type 1 $CD4^+$ cells produce cytokines (e.g., interferon-γ) that stimulate complement-fixing, bactericidal immunoglobulin G_3 antibodies as well as cells of the innate immune system (macrophages) that are able to limit bacterial dissemination.[102] On the other hand, some investigators argue that immune responses evoked by repeated exposure to GAS antigens that are shared among different M types would be required to account for the observed age-related decreases in GAS pharyngitis, given the large number of M types in circulation.[103] Antibodies to the conserved regions of M protein and to a variety of surface expressed or secreted antigens have been shown to elicit protection in animals.[101,104,105]

Even if a safe and effective vaccine were developed, the feasibility of introduction and uptake will be driven by factors such as cost-to-benefit analysis, cost-effectiveness analysis, public perception, and market forces in a given country. The Institute of Medicine performed a cost-to-benefit analysis of GAS vaccination in the United States in which it was estimated that the annual healthcare costs for GAS in the United States reach US$493 million.[106] If a vaccine were provided to 95% of infants and conferred 75% efficacy, the committee predicted a net cost savings of US$314 million. Although similar cost-to-benefit analyses are not yet available for developing countries, the World Health Organization estimated that rheumatic heart disease accounted for 5.9 million disability-adjusted life-years during 2002.[107]

Historical Perspective: Clinical Group A β-Hemolytic *Streptococcus* Vaccine Trials

The history of modern GAS vaccine development dates back to the 1940s, when approximately 4000 young adults were injected with whole-cell vaccines inactivated by heat or ultraviolet light.[108] These vaccines produced reactions, some severe, but did not prevent GAS disease. In the 1960s, clinical trials involving children and adults were initiated using parenteral vaccines consisting of partially purified M protein or cell wall preparations containing high M protein content.[97,99,109–114] The crude preparations of whole cell or cell wall were unacceptably reactogenic and inconsistently immunogenic, presumably because the toxicity limited the total amount of protein that could be administered. However, when injected with mineral oil adjuvant, partially purified M protein induced bactericidal antibodies in some subjects.[109] These findings suggested that a vaccine based on M protein may be an effective strategy if a safe and reliable delivery system could be identified.

The 1960s saw an unfortunate turn of events. Investigators conducting a clinical trial involving a partially purified M3 protein reported an apparent increase in the attack rate of ARF among vaccinated children compared with historical controls.[115,116] The vaccine regimen consisted of increasing doses (up to 1 mg) generally administered subcutaneously at weekly intervals for 18 to 33 weeks. Recipients were siblings of ARF patients. The ARF episodes appeared 37 days to 23 months after the previous vaccination and occurred among subjects who were experiencing high rates of intercurrent GAS pharyngitis. Whether these cases were triggered by vaccination remains uncertain. Nevertheless, a federal regulation prohibiting licensure of GAS organisms or their derivatives for human administration was issued in 1979 and endured until 2006.[117]

Meanwhile, the sentinel studies conducted by Fox and colleagues clearly demonstrated that a vaccine could induce protective immunity. These investigators immunized seronegative volunteers with three monthly injections of purified M1, M3, or M12 protein[97–99] given either subcutaneously with alum or via aerosol to the nose and pharynx. One to 2 months after immunization, a cohort of vaccinated and unvaccinated subjects was challenged by painting virulent GAS bearing the homologous M type onto their tonsils and pharynx. Volunteers were evaluated clinically and microbiologically for 6 days and then treated with penicillin. Both parenteral and mucosal vaccines prevented GAS pharyngitis, although the level of protection achieved statistical significance only for M1 vaccine. Furthermore, mucosal vaccination significantly prevented acquisition of throat infection.

Around this same time Beachey and colleagues discovered techniques for creating highly purified M protein fragments as vaccine antigens[96] and demonstrated that M24 peptide adsorbed to alum was well-tolerated and elicited bactericidal antibodies in volunteers. None of the sera contained antibodies that cross-reacted with myocardial tissue. This trial provided further proof that the use of well-defined M protein epitopes could be safe and elicit type-specific immunity.

Current Prospects for a Group A β-Hemolytic *Streptococcus* Vaccine

M-Type–Specific Vaccines

The advent of molecular biology techniques enabled a new generation of GAS vaccines with rational, well-defined components and allowed clinical trials to resume after a hiatus of nearly 30 years (Table 56.1). Dale and colleagues constructed a recombinant fusion protein containing N-terminus fragments from six GAS M types of clinical and epidemiologic importance (1, 3, 5, 6, 19, and 24) and excluding tissue cross-reactive epitopes.[118] A Phase I, proof-of-principle trial was conducted to evaluate three dosage levels (50 μg, 100 μg, and 200 μg), with 10 subjects per group.[119] This intramuscular vaccine appeared safe, well-tolerated, and did not induce antibodies that cross-reacted with human tissues. Three spaced doses of 200 μg (at 0, 4, and 16 weeks) elicited a fourfold or greater rise in antibody by enzyme-linked immunosorbent assay (ELISA) in 57 (95%) of 60 possible assays (i.e., six antigens times 10 subjects). Postvaccination rises in functional (bactericidal or opsonophagocytic) antibody were common.

TABLE 56.1 Group A Streptococcal Candidate Vaccine Antigens That Evoke Immune Responses in Humans After Natural Infection and Demonstrate Efficacy in Animal Models

Antigen	Function in GAS Virulence
M PROTEIN-BASED VACCINES	
Type-specific M peptides[a,118–120,123,128]	Adhesion; inhibits opsonization by the alternate complement pathway
Conserved M protein epitopes Mucosal synthetic peptide vaccine[126] *Streptococcus gordonii* vector[127,135,184] J8-DT B-cell epitope[a,136–138] with SpyCEP[139,185] StreptInCor[140]	Inhibits opsonization SpyCEP inhibits neutrophil chemotaxis
NON-M PROTEIN ANTIGENS	
Fibronectin-binding proteins	
SfbI,[143–146,186–188] FBP54[147]	Adhesion to pharyngeal epithelium
R28[34]	Adhesion to cervical epithelium
Spy 1536 (and 8 other common antigens identified via antigenomics)[105]	Binding to extracellular matrix proteins
C5a peptidase (SCPA)[105,150,151]	Adhesion; inactivates a chemokine of the complement system
Serine protease (SpyCEP or ScpC)[153,154,168]	Cleaves interleukin-8
Serine carboxylic esterase (Sse)[156]	Tissue invasion
Streptococcal pyrogenic exotoxins (SPE)[157–160]	Superantigens, tissue damage, shock
Group A carbohydrate[79]; synthetic oligosaccharide conjugates[161]	Impedes phagocytosis
Pilus (T serotype antigens)[35]	Adhesion and biofilm formation
Spy 1536 (and 8 other common antigens identified via antigenomics)[105]	Binding to extracellular matrix proteins
Multi High-Throughput Approach identified 6 antigens: Spy0167, Spy2010, Spy0146 (SpyCEP), Spy0269 (mediates cell division, among the 9 strains identified by antigenome analysis and reported to be protective[105]), Spy0019, and Spy1361[168]	Spy0167: streptolysin *O* precursor (kills eukaryotic cells by forming membrane pores) Spy2010 (C5a peptidase precursor) Spy0416 (SpyCEP) (above) Spy0269 (mediates cell division) Spy0019 (unknown) Spy1361 (internalin A precursor)

[a]Only these candidates have been tested in clinical trials.
GAS, group A β-hemolytic *Streptococcus*.

TABLE 56.2 Response of 30 Volunteers to Immunization With a 200-µg Dose of 26-Valent GAS Vaccine at 0, 4, and 16 Weeks[123]

M Epitope or Antigen	Percent of Subjects With Fourfold Rise in Antibody by ELISA After Immunization
3, 12, 24, 75, 76, 89, 92, Spa	90–100%
1, 1.2, 11, 19, 28, 29, 59, 94, 101, 114	80–89%
2, 22, 77	70–79%
5, 14, 43	60–69%
6, 18, 33	<50%

ELISA, enzyme-linked immunosorbent assay; GAS, group A β-hemolytic *Streptococcus*.

In an effort to develop a broadly protective vaccine, Dale and colleagues next constructed fusion proteins containing aminoterminal peptides of 26 M types[120] that account for 79% to 90% of cases of pharyngitis and invasive disease from various populations in the United States, Canada, Mexico, and Israel (Table 56.2)[120–126]; serotype coverage in low-resource countries is estimated to be 32% to 68%.[127] Thirty subjects received a 200-µg dose at 0, 4, and 16 weeks. Local and systemic reactions were common but tended to be mild. No evidence of ARF was seen. Seventeen of the 26 M antigens (65%) elicited a fourfold or greater antibody response by ELISA in at least 80% of subjects, many of whom exhibited bactericidal activity (see Table 56.2). The proportion of subjects responding to M types responsible for the most severe infections in the United States in recent history (1, 3, 5, 6, and 18)[18] varied (see Table 56.2). A 30-valent vaccine has also been constructed; it targets 98% of pharyngitis cases in the United States and Canada, 90% of invasive disease in the United States, and 78% of invasive disease in Europe, but has far less homology with the broad spectrum of *emm* types seen in many developing countries.[128] However, evidence that the vaccine evokes cross-opsonic bactericidal antibodies against a significant percentage of nonvaccine M serotypes tested to date may further enhance the vaccine's spectrum of efficacy.[129] Clinical trials with the 30-valent vaccine have begun in Canada. To assess whether M peptides might be immunoprotective when delivered mucosally, a prototype vaccine comprising food-grade *Lactococcus lactis* expressing an aminoterminal peptide of M9 GAS was administered intranasally to mice. The vaccine appeared immunogenic and prevented colonization with a homologous wild-type intranasal challenge strain.[130]

Vaccines Using Conserved M Antigens

Several investigators have constructed vaccines from the conserved region of M protein with the hope of identifying antigens with broad protection. Antibodies to epitopes in this region have been found among persons from highly endemic communities[131] and exhibit opsonic potential.[132] In animal models, mucosal vaccines comprising the C-terminal region of the M6 strain induced serum and mucosal antibodies that did not cross-react with human heart tissue and conferred protection against challenge with both homologous and heterologous GAS M types.[133] The oral commensal bacteria *Streptococcus gordonii* was evaluated in animals as a mucosal vector for surface expression of the conserved fragment of M protein.[134] A *S. gordonii* vector strain bearing no GAS antigens was shown to be well-tolerated and able to achieve short-term colonization when delivered orally and nasally to healthy adults.[135] These studies were conducted to guide the design of future Phase I trials to evaluate a *S. gordonii* live vector vaccine expressing the conserved region of GAS M protein. However, the GAS antigen-bearing strain has not been constructed.

Good and colleagues identified the 12-amino-acid synthetic J8 peptide as a vaccine candidate; it contains a minimal conserved murine protective B-cell epitope in the C-repeat region within a non–M protein helix-forming sequence (to maintain conformational integrity), and lacks cross-reactive T-cell epitopes on valvular endothelium. Parenteral immunization with J8 conjugated to diphtheria toxoid (DT) in concert with adjuvant (alum or SBAS2) produced opsonic antibodies and memory B cells that protected mice against systemic challenge.[136,137] Intranasal administration of J8-DT plus cholera toxin B subunit protected mice against mucosal challenge.[138] The J8-DT vaccine was evaluated in a Phase I clinical trial in humans and a publication is in preparation (M.F. Good, personal communication, 2015). This group further combined J8-DT with a recombinant fragment of the antineutrophilic protease SpyCEP.[139] Whereas J8-DT alone was protective against multiple GAS strains, it was minimally protective against challenge with epidemic CovR/S mutants (which overproduce SpyCEP and thus avoid neutrophilic killing) in a mouse pyoderma/bacteremia model; however, the addition of SpyCEP to J8-DT resulted in significant enhancement of efficacy against these hypervirulent strains. The bivalent vaccine produced a synergism by allowing both neutrophil killing and opsonization.[140] These investigators are also exploring a strategy of presenting peptide vaccine antigens in a synthetic lipopeptide delivery system that is self-adjuvanting.[141,142]

By searching 79 overlapping synthetic peptides from the C-terminus of M5 protein for reactivity to a large library of human sera and peripheral blood mononuclear cells, researchers have identified a 55-amino-acid peptide designated StreptInCor that contains both T- and B-cell immunodominant epitopes. Subcutaneous delivery of StreptInCor with alum protects mice against intraperitoneal challenge.[140]

Non–M-Protein Vaccine Candidates

Several virulence antigens not borne by the M protein that are surface expressed or secreted among M types have undergone preclinical testing but have not yet reached clinical trials. Those discussed here in general are immunodominant (i.e., composed of antigens that elicit immune responses following natural infection), shared by multiple M types, evoke both homologous and heterologous protective immunity in animal models, and have no known epitopes that cross-react with human tissues. It is likely that a broadly protective GAS vaccine based on this approach would require a combination of these antigens.

One strategy for preventing GAS infection is to block GAS attachment to epithelial surfaces with the use of adhesins such as fibronectin-binding proteins. The most investigated of these is SfbI,[143,144] an antigen that possesses intrinsic adjuvanticity.[145] Intranasal immunization with either purified recombinant SfbI or DT conjugated polypeptides encompassing linear T- and B-cell epitopes of the SfbI protein, along with cholera toxin B subunit or the (TLR)2/6 agonist MALP-2 as mucosal adjuvants, protect mice against lethal challenge with homologous and heterologous GAS strains.[146] Recombinant FBP54 also induces heterologous protection in mice when delivered subcutaneously with Freund's complete adjuvant, and homologous protection when administered by the mucosal route with cholera toxin adjuvant.[147] The putative cervical epithelial cell adhesion R28 was protective in passive immunization murine experiments[34] and thus has potential as an immunogen for prevention of puerperal fever.

C5a peptidase, long considered an attractive vaccine candidate, exemplifies a vaccine approach intended to disarm the ability of GAS to evade immune surveillance.[148,149] In mice, intranasal immunization with recombinant enzymatically inactive protein without adjuvant prevented colonization following intranasal challenge in a serotype-independent fashion[150] and enhanced clearance of GAS from the nasopharynx of infected mice,[141] while a subcutaneous formulation adjuvanted with alum and monophosphoryl lipid A promoted clearance from both nasopharynx and lung.[151,152] The antiphagocytic protease SpyCEP also appears very promising as a vaccine antigen[153,154]; mice immunized with recombinant CEP protein plus Freunds' incomplete adjuvant had reduced bacterial dissemination after intramuscular and intranasal challenge.[155]

The secreted esterase SsE has two antigenic variants, together encompassing at least 10 M serotypes. The M types comprising each SsE variant share greater than 98% amino acid homology. Accordingly, subcutaneous vaccination with recombinant SsE plus alum protected mice against lethal subcutaneous infection with virulent M1 and M3 strains and inhibited GAS invasion of mouse skin tissue.[156]

Vaccine-mediated prevention of STSS and severe invasive disease has focused on SPEs as antigens. Vaccination with SPE A protects rabbits not only from STSS, but also from necrotizing fasciitis-myositis.[157] SPE A and SPE C toxoids and recombinant antigens lacking regions thought to mediate T-cell receptor or major histocompatibility complex class II interaction, given subcutaneously with adjuvant (either Freund's incomplete adjuvant or unspecified), lost their superantigenic properties yet protected against lethal challenge.[158–160]

The GAS cell wall polysaccharide is surface-exposed, conserved, immunoreactive, abundantly expressed in most GAS M serotypes, and forms the basis of the group A antigen used in rapid tests to diagnose GAS pharyngitis.[79] Several parenteral formulations of alum-adjuvanted synthetic oligosaccharide vaccines conjugated to CRM_{197}, when compared to purified native polysaccharide glycoconjugates, elicited comparable opsonophagocytic antibody and similar protection against lethal injection of wild-type GAS in mice.[161] Nonetheless, potential safety issues related to possible cross-reactivity of these antigens with valvular endothelial and neuronal tissues will have to be addressed.[74,77] A potential alternative approach is to use purified polyrhamnose backbone lacking the immunodominant (and potentially autoimmune) *N*-acetylglucosamine side chain.[162]

More recent searches for novel vaccine candidates have utilized proteomic techniques, reverse vaccinology, and genome-wide selection of surface-expressed antigens.[105,163,164] The discovery of the GAS pilus is one example. Recombinant pilus proteins administered intraperitoneally with Freund's

adjuvant conferred protection against lethal mucosal GAS challenge in mice.[35] Because there are only 21 known Lancefield T serotypes (based on pilus antigens), broader strain coverage with fewer vaccine antigens might be possible compared with M-protein–based vaccines. A subset would be needed for a vaccine to protect against greater than 90% of GAS strains from the United States, Europe,[165] and New Zealand,[166] but genetic diversity elsewhere remains unknown.[167]

Nine promising candidates for a GAS vaccine were identified by antigenomics. Potential antigens were selected from genome libraries that are surface expressed, conserved, reactive with convalescent human serum, confer virulence, and induce protection in at least one of several murine models. These modes varied by challenge route (intravenous or intranasal), challenge strain, and vaccination regimen (subcutaneous injection with either Freund's or alum adjuvant or intranasal inoculation with either IC31 or MALP-2 adjuvant).[105] Bioinformatic analysis excluded homology with human proteins. Sequence conservation was assessed with published GAS strain sequences and an international collection of clinical isolates. Spy 1536, which binds extracellular matrix proteins, emerged as a promising candidate, as did SpyCEP.[153,154]

A combination of three high-throughput technologies (mass spectrometry–based proteomics, protein array, and flow cytometry) identified six vaccine candidates that were well-expressed in a high number of strains, surface-associated or secreted, immunogenic during natural infection, and efficacious in murine models when administered with monophosphoryl lipid A–synthetic trehalose dicorynomycolate adjuvant. These include Spy0167 (streptolysin *O* precursor), Spy2010 (C5a peptidase precursor), Spy0146 (SpyCEP), Spy0269 (mediates cell division, among the nine immunoprotective strains identified by antigenome analysis),[105] Spy0019, and Spy1361.[168] The progress in clinical development of these promising antigens is eagerly awaited.

Indirect Effects

Viral infections, in particular varicella and influenza, may predispose to invasive GAS infection. The ability of vaccines that prevent viral infections to diminish the occurrence of severe GAS disease should not be underestimated.[169–171]

Future Challenges

Although promising, M type-specific vaccines must address the antigenic variability posed by the numerous distinct *emm* types. Less-developed and transitional countries show a much higher diversity and a different hierarchy of circulating *emm* types compared to the Americas (excepting Hawaii,[172] Europe, and Japan), limiting coverage rates for the proposed 30-valent vaccine. Moreover, *emm*-type distributions can change over time.[173–177] Careful analyses will be required to determine the overall level of efficacy and serotype coverage that would make a type-specific GAS vaccine desirable for public health use.

Challenges face each stage of the clinical development of a GAS vaccine, many resulting from the need to exclude the theoretical risk of a vaccine triggering ARF.[178,179] Preclinical assessments are handicapped by the lack of a reliable animal model for ARF but should at least provide assurance that the candidate does not contain B- and T-cell epitopes that cross-react with host tissues and does not possess superantigenic properties. Recent clinical trials provide a template for intensive safety evaluations but more expedient tools would be useful for larger Phase II and Phase III trials. Whether Phase III trials must be designed to exclude a potential rare occurrence of ARF remains to be determined.

The choice of primary clinical end points for Phase III trials has engendered debate; considerations such as all pharyngitis, medically attended pharyngitis, impetigo (in Aboriginal populations), invasive infections, primary ARF, and recurrent ARF each have merits. Other considerations for Phase III study design include potential confounding effects of herd immunity and high asymptomatic colonization rates. Implementation of vaccine programs will face issues such as emergence of newly characterized *emm* types[180,181] and the possibility that nonvaccine serotypes may replace those in the vaccine via so-called immune escape.[182,183] Finally, it will be critical to determine a level of vaccine efficacy that would sufficiently assure practitioners that they can safely abandon the practice of culturing throats, empirically treating pharyngitis with antibiotics, or recommending secondary antibiotic prophylaxis in individuals with ARF.

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57 *Streptococcus* Group B Vaccines

C. Mary Healy and Carol J. Baker

In 1938, Fry[1] reported three cases of fatal puerperal sepsis caused by group B β-hemolytic streptococci. Sporadic reports appeared until the 1970s, when group B β-hemolytic *Streptococcus* (GBS) sepsis and meningitis in young infants increased dramatically,[2–4] followed by recognition of invasive GBS infections in pregnant women and in nonpregnant adults; the latter infections occasionally were severe and fatal.[5,6] Explanations for the emergence of GBS as a prominent human pathogen have been traced to bovine origin through a series of elegant genomic studies.[7] Despite implementation of intrapartum antibiotic prophylaxis (IAP) to prevent peripartum maternal morbidity and early-onset neonatal disease,[8] GBS infections continue to occur, albeit at a reduced incidence.[9,10] Among infants 7 to 89 days old, incidence, case-fatality rates, and short-term and long-term neurodevelopmental sequelae have remained stable for decades.[10–13] Finally, invasive GBS disease increasingly impacts adults with underlying medical conditions and elderly people.[14–16]

BACKGROUND

Clinical Description and Complications

Invasive GBS disease in infants is characterized by age at onset. Early-onset GBS (EOGBS) occurs before age 7 days (≈90% have signs of infection within 12 hours of birth), frequently manifesting as sepsis without a focus (80% to 95%), pneumonia (10% to 15%), or meningitis (5% to 10%). Clinical findings are nonspecific. Severity of illness ranges from fatal multiorgan failure to healthy-appearing infants who are evaluated because of maternal GBS colonization and inadequate duration of IAP before delivery. Focal findings, except pneumonia or meningitis, are rare. In contrast, late-onset GBS (LOGBS) occurs in infants 7 to 89 days of age, and more often manifests with fever, poor feeding, and lethargy and less commonly with overwhelming sepsis. Meningitis is more frequent with LOGBS (21% to 35%), and approximately 30% to 40% of cases have permanent neurological sequelae (e.g., vision or hearing loss, cerebral palsy, developmental delay).[11–13] Other focal manifestations include septic arthritis, osteomyelitis, cellulitis, and adenitis.[17–19] Late–LOGBS occurs in infants older than 89 days (typically very preterm infants whose corrected gestational age is younger than 3 months) and accounts for approximately 20% of LOGBS cases.[19,20] Recurrent GBS is reported in an estimated 0.5% to 3% of GBS cases.[19,21] Invasive GBS is rare after age 6 months unless an underlying immunodeficiency is present.[22]

Lower vaginal and/or rectal colonization with GBS in pregnant women is a prelude to maternal invasive disease and EOGBS in the infant. The risk of invasion of amniotic fluid or the neonatal bloodstream is related directly to the density of maternal colonization and virulence of the organism.[23–25] Maternal GBS infection manifests during pregnancy as asymptomatic bacteriuria, urinary tract infection, or pyelonephritis and during the peripartum period as bacteremia, chorioamnionitis, or postpartum endometritis; these infections can result in spontaneous abortion, preterm delivery, and stillbirth.[26] Two-thirds of GBS disease cases in adults are not associated with pregnancy; rates in nonpregnant adults have increased twofold to fourfold during the last 25 years.[16] Skin and soft-tissue infections are the most common manifestations of GBS infection in this population, but urinary tract infection, pneumonia, bacteremia, bone and joint infection, endocarditis, meningitis, peritonitis, and catheter-associated infections are described. The incidence of GBS is directly related to increasing age and coexistent morbid conditions.[14–16,25,27]

Bacteriology and Diagnosis

Group B β-hemolytic streptococci are aerobic gram-positive diplococci that are divided into 10 serotypes (Ia, Ib, and II though IX) based on their capsular polysaccharides (CPSs); types VI, VII, and VIII, however, rarely cause human infection. Many strains have surface proteins (e.g., α- and β-C, R1–R4, rib, alpl-3, Sip, pilus islands 1, 2a, and 2b) that allow further characterization for epidemiologic and virulence studies. They form gray-white, mucoid, 3- to 4-mm diameter colonies on sheep blood agar medium, and produce a narrow zone of β hemolysis. Infection is diagnosed by isolation of GBS from a normally sterile site.[19]

Pathogenesis

Virulence factors characterized as important for attachment and invasion in human infection are CPS, β-hemolysin, C proteins, and pilus-like proteins. Experimental models of infection have demonstrated that antibodies to these surface structures are protective.[28–31] Alpha- and β-C proteins and pili allow epithelial cell adherence, and β-hemolysin and other factors lyse epithelial and endothelial cells, leading to direct tissue damage to and spread through host tissues. Immune clearance of GBS is resisted by CPS and other virulence factor–mediated effects at the cellular and molecular levels, leading to inhibition of neutrophil recruitment, inhibition of opsonophagocytosis and impairment of phagocytosis, and oxidative burst killing. Finally, cell wall lipoteichoic acid, cell wall peptidoglycan, and β-hemolysin/cytolysin trigger cytokine activation and release.[19] High-virulence clonal complexes, such as ST-17 of type III GBS, are also described; the latter has a tropism for meninges and is typically found only among invasive cells.[32,33]

Treatment

Penicillin is the drug of choice for treatment of systemic infection.[34,35] GBS also is susceptible to other β-lactam antibiotics and to vancomycin. GBS isolates with point mutations in penicillin-binding proteins and reduced β-lactam susceptibility have been reported from the United States and Japan; the clinical significance of these is unclear.[36–39] Resistance to erythromycin and clindamycin is reported in approximately 32% and 20% of isolates, respectively, although higher rates of clindamycin resistance in serotype IV (up to 80%) and V recently have been described.[40] While typically resistant to aminoglycosides, synergistic killing of GBS occurs in vitro and in vivo when gentamicin is combined with penicillin or ampicillin.[19] Treatment duration depends on the severity of illness and site involved, but a minimum of 10 and 14 days is

recommended for bacteremia and meningitis, respectively, and longer for more complicated infections.[14,35] Despite advances in intensive care and prompt initiation of antimicrobials, GBS infant disease overall mortality (5%) and morbidity exceeds that of *Haemophilus influenzae* type b before the use of routine infant immunization to prevent this infection in infants.[41,42]

Prevention

Current preventive strategies target neonatal EOGBS disease. IAP was adopted in the United States in 1996 following randomized trials demonstrating that intravenous ampicillin or penicillin G given intrapartum to GBS carriers prevented EOGBS. Two methods, based on culture screening of pregnant women or presence of one or more risk factors predisposing to neonatal EOGBS disease were recommended.[43–45] In 2002, the Centers for Disease Control and Prevention revised the recommendations because universal culture-based IAP had superior efficacy (relative risk of EOGBS, 0.46 [95% confidence interval, 0.36–0.6]) compared with the risk-factor strategy.[46] These recommendations were updated in 2010 (Table 57.1) to incorporate additional diagnostic methods to detect GBS colonization and revised algorithms for screening and prophylaxis of women and management of newborns.[47] IAP remains an interim strategy while vaccine development proceeds.[8]

EPIDEMIOLOGY

Incidence and Prevalence

Group B β-hemolytic streptococci colonize mucous membranes, leading to intermittent or chronic carriage. The most common sites of colonization in adults are the rectum, lower vagina, and urethra (men). Isolation rates increase 10% to 15% in pregnant women if rectal cultures are assessed in addition to lower vaginal specimens, although two-thirds of colonized women are positive at both sites. Differences between ascertainment techniques make direct comparisons of reported prevalence impossible, but group B β-hemolytic streptococci carriage rates are approximately 10% in prepubertal children, 30% in women of childbearing age regardless of trimester of gestation, and 22% and 25%, respectively, in men and healthy elderly.[19,48]

The incidence of EOGBS in the United States decreased dramatically following the introduction of IAP, from 1.7 per 1000 live births in 1993 to 0.24 to 0.26 per 1000 live births between 2009 and 2013.[49] The incidence of LOGBS has remained unchanged at 0.25 to 0.32 per 1000 live births between 2009 and 2013. Late-late onset disease numbers are increasing in association with increased survival of preterm infants.[10,49] IAP has reduced invasive infection in pregnant women to 0.12 per 1000 deliveries.[16] A recent systemic review of the literature, largely prior to 2000, showed that GBS-related stillbirth rates varied from 0.04 to 0.9 per 1000 births, accounting for 0 to 12% of all stillbirths.[26] Invasive GBS infections are increasing in nonpregnant adults (4.1 to 7.2 per 100,000 population), especially in elderly people (25.4 per 100,000).[14,15] GBS infections in all age groups are overrepresented in black populations.[11,16,49] Europe, where national surveillance and IAP are not uniform, reported neonatal EOGBS rates of 0.3 to 0.4 per 1000 live births.[50–56] There was a fivefold reduction in Australasian rates for EOGBS (from 1.43 to 0.25 cases per 1000 births) as a result of IAP during the 1990s,[57] but recent reports show that EOGBS in Australia has increased to 0.38 per 1000 births, possibly a consequence of uneven compliance with prevention through IAP.[58] Data from low-income countries are lacking, precluding a reliable estimation of the GBS-associated global burden on rates of pregnancy loss, as well as EOGBS and LOGBS.[26,59]

In the United States and Europe, invasive disease in pregnant women and infants is caused predominately by five CPS types: III, Ia, V, Ib, and II, in decreasing order of prevalence (Table 57.2).[16,51,54,55,60–62] More recently, there has been a 10-fold increase in the proportion of serotype IV in carriage studies of nonpregnant American women; serotype IV also has emerged as a cause of EOGBS and LOGBS in Minnesota, suggesting that this serotype may be emerging.[40,63] In adults and elderly people, serotype data from the United States and Canada demonstrate that the same five CPS types cause more than 95% of disease, although type V has replaced type III as most common, and type IV is causing an increasing proportion.[14,16,25,62,64]

TABLE 57.1 Recommendations for IAP[a] to Prevent Perinatal GBS Disease

IAP Recommended	IAP Not Recommended

• Previous infant with invasive GBS disease
• GBS bacteriuria in the current pregnancy
• Positive GBS screening culture in current pregnancy (unless planned cesarean delivery performed without labor or rupture of membranes)
• GBS culture status not known and any of the following:
  ◦ Delivery at <37 wk gestation
  ◦ Rupture of membranes ≥18 h
  ◦ Intrapartum fever ≥38°C
  ◦ Intrapartum NAAT[b] positive for GBS
	• Previous pregnancy with GBS colonization
	• Previous pregnancy with GBS bacteriuria
	• Planned cesarean delivery in the absence of labor or rupture of membranes, regardless of GBS colonization
	• Negative vaginal and rectal GBS screening cultures at 35–37 wk gestation

[a]Vaginal and rectal GBS cultures are recommended at 35–37 wk gestation for all pregnant women (unless there is a history of GBS bacteriuria in the current pregnancy or a previous infant with GBS disease). For IAP, intravenous penicillin G is preferred and ampicillin is acceptable at onset of labor or rupture of membranes and every 4 h until the infant is delivered. For penicillin-allergic women at low risk for anaphylaxis, intravenous cefazolin every 8 h is preferred.

[b]If intrapartum nucleic acid amplification test (NAAT) is negative but any other intrapartum risk factor is present, IAP is indicated.

GBS, group B *Streptococcus*; IAP, intrapartum antibiotic prophylaxis.

Modified from Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, 2010. MMWR Recomm Rep. *2010;59(RR-10):1–36.*

Groups With Additional Risk of Group B β-Hemolytic *Streptococcus* Infection

In the United States, the incidence of EOGBS and LOGBS infection in African American infants is two to three times higher than in white infants.[11,16,47,49] Infants of Hispanic ethnicity also have somewhat higher incidence rates.[65] This may be a result of reduced access to prenatal care, higher rates of preterm birth, or higher colonization rates attributable to vaginal hygiene practices. Among adults, blacks have twice the rate of invasive GBS infection as whites, and disease rates rise in elderly people.[15,16,66] Nursing-home residents have a greater risk than do community-dwelling persons (72.3 vs 17.5 per 100,000 population).[67] After controlling for age, diabetes mellitus, cirrhosis, stroke, breast cancer, decubitus ulcer, and neurogenic bladder, all increase risk for invasive GBS infection.[15,16,68]

TABLE 57.2 Serotype Distribution of GBS Isolates Causing Invasive Infection and GBS CPS Conjugate Vaccine Responses in Healthy Adults

Serotype	Estimated Percentage of Isolates From Infants[a]	Estimated Percentage of Isolates From Nonpregnant Adults[b]	Vaccine[c]	Approximate CPS Dose (μg/mL)[c]	Percentage of Adults With ≥4-Fold Increases in CPS-Specific IgG 8 wk Postvaccination[c]	GMC, μg/mL, of CPS-Specific IgG (95% CI) 8 wk Postvaccination[c]
Ia	27	24	Ia-TT	15	80	18.3 (6.0–55.4)
Ib	7	12	Ib-TT	16	80	11.1 (3.3–37.0)
II	8	12	II-TT	4	87	11.2 (6.1–20.8)
III	39	17	III-TT	13	93	16.2 (7.4–35.8)
V	16	28	V-TT	10	73	5.7 (1.5–21.5)

[a]Data from references 16 and 60–62 (the remaining isolates are nontypeable by serologic methods).
[b]Data from references 14–16, 25, 60, and 62 (the remaining isolates are nontypeable by serologic methods).
[c]Data from references 60, 78, 79, and 81–83.
CI, confidence interval; CPS, capsular polysaccharide; GBS, group B β-hemolytic *Streptococcus*; GMC, geometric mean concentration; TT, tetanus toxoid.

Disease Burden

Case-fatality rates of 3% and 20% are reported for term and preterm infants with EOGBS, respectively. Case-fatality rates for LOGBS are lower (2% and 6%), but LOGBS more often is accompanied by meningitis, which results in permanent neurologic sequelae in up to 30% of survivors.[11–13,16] In adults, invasive GBS infection is associated with a 15% case-fatality rate, but this rate approaches 25% or higher in elderly people and people with underlying medical conditions.[15] In most countries, including the United States, CPS types Ia, Ib, II, III, and V account for approximately 85% to 95% of invasive disease isolates.[60]

ACTIVE IMMUNIZATION

History of Vaccine Development

Lancefield's observations demonstrated protection against lethal GBS infection in a mouse model by antibodies to the CPS of GBS. In the 1970s, Baker and Kasper[69] reported that mothers of infants with GBS type III disease had significantly lower serum III CPS-specific antibodies at delivery than mothers of healthy infants, a finding confirmed and extended for serotypes Ia and Ib.[70,71] Human serum containing sufficient concentrations of Ia, Ib, II, III, and V CPS-specific immunoglobulin (Ig) G promotes efficient opsonization and phagocytosis of homologous strain in vitro and provides protection from lethal experimental infection in mice. Once the CPSs of Ia, II, and III GBS were purified and immunochemically defined, an effective polysaccharide vaccine seemed imminent. It was hoped that active immunization of pregnant women would elicit protective levels of antibodies that would prevent maternal disease and, through placental transport, protect their infants from EOGBS and LOGBS. However, studies of unconjugated GBS polysaccharide vaccines in healthy adults were disappointing. The first purified CPS (type III) was tested in 1978 and was followed by types Ia and II. Although well tolerated, CPS Ia, II, and III elicited variable immune responses: 40%, 88%, and 60%, respectively.[72] Nearly 90% of subjects had low preimmunization CPS-specific antibodies, reflecting immunological naïveté, and this predicted no or less robust immune responses to CPS vaccines. In the few subjects with higher initial CPS-specific IgG, brisk, reliable booster responses occurred.

The single maternal immunization study performed using type III CPS vaccine demonstrated that it was well tolerated and had comparable immunogenicity to that in nonpregnant adults (63% immune response).[73] Among infants of maternal vaccine responders, 90% had presumed protective levels of CPS-specific IgG in their serum samples at 2 months of age that promoted opsonophagocytosis and killing of type III GBS in vitro. Furthermore, these functionally active specific antibodies persisted in the majority of infants through age 3 months. Although disappointing, these early studies demonstrated that vaccine prevention of GBS disease was feasible but that improved immunogens were needed.

The success of polysaccharide–protein conjugate vaccine technology heralded more hope to control GBS through maternal immunization.[74,75] In addition, GBS glycoconjugates could generate T-cell–dependent responses and potentially long-term protection. Theoretically, a GBS CPS-protein glycoconjugate also would induce higher maternal CPS-specific IgG concentrations, favor an IgG_1 subclass response, thereby improving placental transport, and increase likelihood of protection of mother and infant.

Constituents of Candidate Conjugate Vaccines

Most candidate GBS conjugate vaccines for clinical trials were developed at the Channing Laboratory, Harvard Medical School (Boston, Massachusetts). The first glycoconjugate was type III CPS covalently linked to monomeric tetanus toxoid (TT) using reductive amination coupling chemistry.[76,77] Monovalent types Ia, Ib, II, and V CPS and one bivalent type II/III CPS were produced using the same method with TT as the carrier protein for all but one type V CPS conjugate lot in which diphtheria mutant cross-reactive material 197 (CRM_{197}) was used.[78–81] The preclinical studies defined optimal size, percentage of oxidation, and degree of crosslinking to retain epitope specificity for each CPS-TT conjugate and to optimize immunogenicity. They also established the relationship between binding antibody to CPS by quantitative enzyme-linked immunosorbent assay (ELISA) and protection in the maternal-neonatal mouse model. Each vaccine then was evaluated in healthy adults (Table 57.3).[60,78,79,81–83] In a Phase I study of type III CPS-TT conjugate, adding aluminum hydroxide adjuvant to type III CPS-TT conjugate did not enhance immunogenicity.[82]

Dosage and Route of Administration

Phase I and Phase II trials used decreasing doses of type-specific CPS to define the optimal immune responses (see Table 57.3). Candidate vaccines were administered as a single intramuscular injection in a volume of 0.5 mL, using

TABLE 57.3 Summary of Adverse Events and Immunogenicity of GBS Conjugate Vaccine in Healthy Adults[a]

GBS Vaccine (CPS Carrier, μg/mL)	No. of Subjects[b]	Local Reactions (%)[c]			Systemic Symptoms (N)[d]	GMC, μg/mL, of CPS IgG (95% CI) at 8 wk[e]	% With ≥4-Fold Increase	GMC, μg/mL, of CPS IgG (95% CI) at 1 y
		Mild	Moderate	Severe				
Ia-TT								
60	30	20	60	0	0	26.2 (13.0–52.9)	93	12.4 (5.9–25.9)
15	15	46.7	13.3	0	0	18.3 (6.0–55.4)	80	9.1 (2.9–28.6)
3.75	15	13.3	6.7	0	0	1.9 (0.7–5.4)	80	1.5 (0.6–4.3)
Ib-TT								
63	30	33.1	40.1	3.3	0	12.9 (5.2–32)	78	5.8 (1.6–20.7)
15.75	15	20	40	0	0	11.1 (3.3–37)	80	7.0 (2.1–23.4)
3.94	15	33.3	20	0	0	2.9 (1.2–7.1)	47	2.2 (0.9–5.5)
II-TT								
57	30	26.7	50.0	0	0	34.3 (18.7–62.4)	97	ND
14.3	13	14.3	33.3	0	1	29.5 (13.9–62.4)	100	ND
3.6	15	20	33.3	0	0	11.2 (6.1–20.8)	87	ND
III-TT								
50	15	73.3	0	0	0	41.8 (16.5–106)	97	ND
12.5	15	33.3	13.4	0	0	3.6 (1.1–12.3)	87	ND
V-TT								
38.5	15	53.3	26.7	0	0	11.1 (3.8–31.9)	100	3.2 (1.5–7)
9.6	15	66.7	0	0	0	5.5 (1.5–20.5)	93	1.7 (0.6–5.4)
2.4	15	33.3	0	0	0	5.1 (1.3–20)	80	1.5 (0.4–5.2)
II/III-TT								
3.6/12.5	25	52	12	0	2	13.1 (5.6–30.6)/ 6.9 (3.2–14.9)	80/92	ND

[a]Data from references 60, 78, 79, and 81–83.
[b]Subjects were women age 18–40 y for Ia-TT, Ib-TT, and III-TT trials; women age 18–45 y for II-TT trial; either sex, age 18–45 y for II/III-TT trial; and either sex, age 65–85 y for V-TT (38.5) trial.
[c]Mild, tenderness, sore to move, or <5-cm redness or swelling; moderate, ≥5- to 10-cm redness or swelling; severe, ≥10-cm redness or swelling.
[d]Fever ≥38°C, chills, myalgia, headache, or fatigue.
[e]GMCs shown for III-TT are at 4 wk.
CI, confidence interval; CPS, capsular polysaccharide; GBS, group B β-hemolytic *Streptococcus*; GMC, geometric mean concentration; ND, not done; TT, tetanus toxoid.

a sodium chloride diluent containing 0.01% thimerosal or 0.9% saline.

IMMUNOGENICITY

Immune Response

Clinical trials measured CPS-specific antibodies before and 2, 4, 8, 26, and 52 weeks after immunization. Antibodies were measured by ELISA with type-specific CPS covalently linked to human serum albumin as the coating antigen.[84] Although another investigator group questioned the epitope specificity of the type III CPS-specific IgG ELISA, both investigator groups agreed that sialic acid is critical for conformational epitope specificity, that opsonophagocytosis of type III GBS in vitro is a functional correlate of protective activity, that "protective" antibodies are directed at this sialic acid–dependent epitope, and that a sufficient quantity of type III CPS-specific IgG in human serum is protective against infant GBS disease.[85,86] No controversy arose over the specificity of the types Ia, Ib, II, and V CPS-specific IgG ELISAs, but assay sensitivity varied substantially.

The first clinical evaluation of a GBS conjugate was in 100 women, 18 to 40 years old, who were randomized to receive III-TT at one of three doses, unconjugated type III CPS, or placebo.[77] This trial and those evaluating types Ia, Ib, and II CPS-TT conjugates demonstrated significantly higher geometric mean concentrations (GMCs) of CPS-specific IgG in serum samples from conjugate vaccine recipients compared with recipients of unconjugated CPS or placebo.[78,81] Immune responses were dose-dependent for each conjugate except for type V CPS-TT. Dose–response studies defined total amount of polysaccharide needed to optimize immune response (quantitative ELISA and opsonophagocytosis in vitro) while minimizing local and systemic symptoms. Fourfold or greater increases in CPS-specific IgG were demonstrated in a majority of recipients (see Table 57.2).[60] In addition, vaccine-induced IgG in adults promoted opsonization of homologous GBS strains by human neutrophils in the presence of endogenous human complement and correlated with the concentration of vaccine-induced CPS-specific IgG. When type III CPS-TT conjugate was administered to women at 30 to 32 weeks' gestation, the GMC III CPS-specific IgG rose from 0.18 μg/mL before to 9.98 μg/mL at delivery; 77% of maternal levels were

transported to infant cord sera, and maternally acquired CPS-specific IgG and endogenous infant complement in sera at age 2 months promoted opsonophagocytosis and killing of type III GBS. Furthermore, the type III CPS-specific IgG_1 subclass predominated in infant cord serum samples.[87] These data proved that conjugation results in T-cell–dependent responses.

Duration of Immunity and Protection

GBS CPS-TT conjugate vaccines induce increases of fourfold or more in CPS-specific IgG in 80% or more of volunteers; maximum levels peak 4 to 8 weeks later and then decline gradually to approximately 50% of peak values 1 year after immunization (see Table 57.3). Limited data 2 and 5 years later demonstrate GMCs substantially higher than baseline and above the presumed protective levels for types Ia, Ib, II, and III in most conjugate vaccine recipients. In the absence of Phase III efficacy trials, two observations suggest that GBS glycoconjugate vaccine-induced CPS-specific IgG is protective. First, postvaccination serum concentrations as low as 0.5 μg/mL (Ia, Ib, and III) and 1.0 μg/mL (II and V) promote significant opsonophagocytosis of homologous strains in vitro (>1 $\log_{10}$ reduction) and although this activity declines over time, substantial functional activity is retained 18 to 24 months post immunization (Ia, III, and V).[77,78,80,81,88] Second, in case-control studies, similar concentrations in maternal serum samples at delivery are 90% protective against types Ia and III and 70% protective against type V EOGBS neonatal disease.[89] This strongly suggests that the immune response to GBS glycoconjugate vaccines is sufficient to induce durable protection, similar to other conjugate vaccines.[56]

SAFETY

Adverse Events

Candidate GBS CPS-TT glycoconjugate vaccines have been evaluated in more than 600 healthy adults. Each vaccine was well tolerated, and no vaccine-related serious adverse events occurred. The most common dose-dependent adverse effects reported were mild or moderate tenderness or swelling at the injection site in the highest-dose recipients. Systemic reactions consisting of low-grade fever, chills, headache, or myalgia were reported in only five volunteers (0.8%), and in almost all, these symptoms resolved in 24 to 36 hours. When a type III glycoconjugate vaccine was tested in 20 pregnant women, mild local reactions occurred in 5% of recipients.[87] No vaccine-related adverse event was noted in any infant.

PUBLIC HEALTH CONSIDERATIONS

Invasive GBS disease causes morbidity and mortality in pregnant women, neonates, young infants, and nonpregnant adults with underlying medical conditions, including elderly people. GBS infection also contributes to adverse pregnancy outcome including stillbirth.[26] The cost of treating these conditions is considerable. Only maternal peripartum disease and EOGBS can be prevented through IAP. Ongoing cases of EOGBS and LOGBS, cost, and concern for emergence of resistant GBS isolates suggest that IAP is an interim measure.

The health benefits of GBS vaccination were predicted in an analysis of potential chemoprophylaxis and/or GBS vaccination (adolescent, maternal, or postpartum) strategies, assuming an immunogenic multivalent GBS vaccine.[90] In this model, adolescent and maternal immunization strategies were superior to IAP. Maternal immunization strategies were more effective and projected to prevent 4% of preterm births, 61% to 67% EOGBS disease, and 70% to 72% of LOGBS disease. Maternal immunization is the only strategy that will reduce not only maternal and EOGBS infection, but also LOGBS.

Once safe and immunogenic GBS vaccines are developed commercially, appropriate regimens for their administration will be necessary. The regimen likely to have the most profound and immediate effect on human disease rates would be to vaccinate women in the early third trimester of pregnancy with a single dose of vaccine, thus preventing maternal and infant (EOGBS and LOGBS) disease.[87,90] Recent cost-effectiveness analysis of this strategy assuming a trivalent GBS conjugate (Ia, Ib, III) vaccine with 85% coverage in addition to IAP, predicted that an additional 899 cases of invasive infant GBS disease and 35 deaths would be prevented in the United States annually at a cost of US$91.31 per quality-adjusted life-year gained.[91] Surveys of pregnant and recently delivered women indicate that 79% to 83% would be accepting of such a strategy if it was accompanied by a provider recommendation, and as influenza and pertussis booster vaccines are already recommended in pregnancy, the platform for implementation exists.[92–95] However, such a regimen would not prevent GBS-associated maternal morbidity or preterm births earlier in pregnancy. An alternative strategy in the longer term may be to administer a priming GBS vaccine dose to young adolescent girls at 11 to 12 years of age with other recommended adolescent vaccines (i.e., tetanus, diphtheria, acellular pertussis [Tdap], meningococcal conjugate [MCV4], and human papillomavirus [HPV] vaccines) followed by a booster dose preconception as part of "preconceptional health" recommendations[96–99] or in the early third trimester of pregnancy. Further studies would need to define the optimal frequency of vaccination throughout the childbearing years and whether vaccination is required with each pregnancy. There are few data on immune response in nonpregnant adults with underlying medical conditions and elderly people, who account for ever more of the disease burden.[14,15,100] Given the increasing number of cases in this population caused by poorly encapsulated strains and the distinct difference in capsular types compared with those causing invasive perinatal disease, it is possible that vaccine constructs other than glycoconjugates will be needed for prevention (Baker C.J., personal communication, 2016).

FUTURE VACCINES

Efforts to develop GBS conjugate vaccines continue. The European Union's 7th Framework Program for Research and Technological Development funded the DEVANI project with the objective of developing GBS immunization strategies from 2008 to 2011.[101] A candidate GBS trivalent (Ia, Ib, III-CRM_{197}) glycoconjugate vaccine has been tested in Phase I and Phase II studies in pregnant and nonpregnant women, both HIV positive and negative, in Europe, Canada, United States, and Africa.[102] Although published data are limited, this candidate vaccine was safe, well-tolerated and appeared immunogenic when tested in 51 mother-infant pairs in Belgium and Canada. In 2015, after acquisition of Novartis Vaccines by GlaxoSmith-Kline, further development of this trivalent glycoconjugate vaccine was discontinued to concentrate on developing a glycoconjugate covering additional GBS CPS types. Also in 2015, Pfizer Inc. announced that it was exploring development of a multivalent GBS vaccine. It is likely that both companies will target infant GBS disease prevention using multivalent glycoconjugates that focus on maternal immunization, and that it will take many more years for new clinical trials to document immunogenicity, safety, and efficacy of these candidate vaccines.

Alternative strategies to develop GBS vaccines focus on GBS surface-expressed proteins as candidates themselves or

TABLE 57.4 GBS Vaccine Development

Target Antigen(s)	Approach	Candidate Vaccine	Correlate of Protection	Status
CPS	Conventional vaccinology	Multivalent glycoconjugate	Case-control studies of CPS IgG (μg/mL) in maternal sera; OPK; mouse protection[a]	Awaiting trials during pregnancy[b]
SURFACE PROTEINS				
C5a peptidase	Conventional vaccinology	C5a peptidase in PLGA microsphere	OPK; protection of mice from lethal injection, but not colonization	Unknown
α or β C protein	Conventional vaccinology	As protein carrier with CPS	Mouse protection	Unknown
Sip	Conventional vaccinology	Recombinant Sip	Mouse protection	Unknown
LrrG			Mouse protection	Unknown
Rib-α				Phase I trial
BibA	Reverse vaccinology		OPK; mouse protection	Unknown
Pilus 2a chimeric protein			OPK; mouse protection	Unknown

[a]Immunized dams → protection of pups from lethal GBS inoculum.
[b]Phase I and Phase II trials with monovalent CPS (Ia, Ib, II, III, V)-tetanus toxoid published.
BibA, GBS immunogenic bacterial adhesion (to human epithelial cells); CPS, capsular polysaccharide; GBS, group B β-hemolytic *Streptococcus*; LrrG, leucine-rich repeat protein; OPK, opsonophagocytic killing; PLGA, poly(lactide-co-glycolide); Rib-α, Rib protein combined with α-C protein; Sip, surface immunogenic protein.

as carrier proteins for multiple CPSs (Table 57.4). Examples are α- and β-C proteins (found in 50% and 10% of clinical isolates, respectively), C5a peptidase, rib protein, GBS immunogenic bacterial adhesion (BibA), and surface immunogenic protein (Sip). C5a peptidase is a cell-surface-localized serine protease that inactivates human C5a, a neutrophil chemoattractant produced during complement activation. The protein is a conserved, cell-surface–localized protein found in all GBS strains. Immunization of mice with purified SCPB/ScpB, derived from C5a peptidase, resulted in enhanced clearance of GBS from lungs of mice inoculated intranasally. Although this study used only a single GBS strain (the uncommon serotype VI), the results indicate the C5a peptidase construct might be a vaccine candidate, even if the antibodies produced did not inhibit the enzymatic activity of the surface-localized protein.

Another potential vaccine candidate is a well-conserved, cell wall–anchored protein called GBS immunogenic bacterial adhesion or BibA.[102] The *bibA* gene appears to be present in all GBS strains, and BibA binds human C4 binding protein and promotes the adhesion of GBS to human epithelial cells. Pups of BibA variant immunized pregnant mice survive lethal challenge by multiple GBS capsular types and antibodies to this moiety promote opsonophagocytosis in vitro.

Another possible candidate is a single-component protein vaccine based on a fusion of N-terminals of α-C and Rib, GBS-NN, which theoretically covers 95% of GBS CPS types. GBS-NN is in a Phase I trial in Northern Ireland.[103] The initial evaluation will investigate safety and dose escalation in 70 healthy, nonpregnant women and then enroll another 240 healthy, nonpregnant women to further evaluate safety and initial dose and schedule. It is possible that preliminary results will be available in mid 2017.

The genome sequences of eight GBS isolates have been analyzed and cloned.[104,105] This led to use of reverse vaccinology to identify 312 GBS surface proteins, and four of these in combination were highly protective against all circulating GBS serotypes in a mouse model of infection.[105] Protection correlated with antigen accessibility on the bacterial surface and with the induction of opsonophagocytic antibodies. GBS pili and other surface proteins also could be exciting carrier proteins with the potential of providing additional protection against virulent GBS strains.[28–31,106–108] Using structural vaccinology methods, the backbone subunit of pilus 2a, common to many CPS types, is another potential protein carrier. This backbone (BP-2a) is present in six immunogenically different but structurally similar variants. The three-dimensional structure of one of the variants has been demonstrated to elicit protective antibodies that specifically recognize one of the four domains that comprise the protein. A synthetic chimeric protein, constituted by the protective domain of each one of six variants, protects mice against lethal challenge with all type 2a pilus-carrying GBS strains.[109,110] Achievements in GBS vaccine development, albeit painfully slow, promise the advent of effective vaccines to at last decrease perinatal and adult GBS disease burden. Studies from Soweto, South Africa, demonstrating that GBS disease is related to high colonization and maternal antibody deficiency, strongly suggest that such GBS vaccines would significantly impact the global burden of GBS-associated morbidity and mortality.[111,112]

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58 Tetanus Toxoid

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Tetanus is unique among vaccine-preventable diseases in that it is not communicable. *Clostridium tetani*, the causative agent of tetanus, is widespread in the environment; many animals, in addition to humans, can harbor and excrete the organism and its spores. When spores of *C. tetani* are introduced into the anaerobic conditions found in devitalized tissue or punctures, they germinate to vegetative bacilli that elaborate toxin. The clinical presentation results from the actions of this toxin on the central nervous system (CNS). Many animal species besides humans are susceptible to the disease.

The clinical characteristics of tetanus were recognized as distinct early in human history because of the constancy and severity of the symptoms in animals and humans. A description of tetanus was found as early as 1550 BC in the medical papyri of ancient Egypt, and detailed descriptions were included in the writings of Hippocrates and other ancient Greeks.[1,2] The etiology of tetanus was unknown until 1884, when Carle and Rattone[3] demonstrated that injecting the contents of a pustule from a fatal human case into the sciatic nerve of a rabbit resulted in the typical symptoms of tetanus. The disease could subsequently be passed to other rabbits from infected nervous tissue. Inoculation of soil samples into animals also resulted in tetanus. Gram-positive bacilli were often noted in the exudate at the inoculation site but generally not in nervous tissue, leading Nicolaier[4] to hypothesize that a poison produced at the site of inoculation caused the nervous system symptoms. In 1886, spore-forming bacilli were observed in the exudate obtained from a human patient.[5] In 1889, the spores of *C. tetani*, in contrast to the vegetative organisms, were shown to survive heating and to germinate under anaerobic conditions; injection of pure cultures reproducibly caused the disease in animals.[6] After identification and purification of the toxin in 1890, repeated inoculation of animals with minute quantities of toxin led to the production of antibodies in survivors that neutralized the effects of the toxin.[7] Preparations of antibodies derived from animal sera, particularly from horses, became the first means to prevent and treat tetanus; efficacy was demonstrated in World War I by a sharp decline in tetanus cases among wounded soldiers once tetanus antiserum was introduced.[8,9] In 1924, these efforts culminated in the preparation of an anatoxin—a chemically inactivated toxin, now termed a toxoid—that induced active immunity against the disease before exposure.[10]

The impetus to prevent tetanus through immunization was the striking and highly fatal disease in both high and lower income nations, predominantly associated with injuries to otherwise healthy persons, and particularly during military conflicts. In the lower-income countries, tetanus in neonates continues to represent a substantial proportion of the health burden from tetanus. Prevention of tetanus is now almost universally achievable by use of highly immunogenic and safe toxoid-containing vaccines. Tetanus also can be prevented or modified by proper wound care, sterile surgical and obstetric practices, and use of exogenous antibody.

CLINICAL DESCRIPTION

Although the incubation period for tetanus varies from 1 day to several months after a wound, the majority of cases occur within 3 days to 3 weeks after inoculation of spores. In the United States during 1972 to 2001, the median interval between the injury and onset of tetanus was 7 days (range: 0 to 178 days) for 1191 nonneonatal cases with reported information. The time between injury and the onset of symptoms was 30 days or less for 97% of the cases, and 2 days or less for 10% of the cases.[11]

A direct relationship exists between the site of inoculation and the incubation period: the longest intervals occur after injuries farthest from the CNS; injuries of the head and trunk are generally associated with the shortest incubation periods.[12,13] The incubation period is inversely related to severity of illness[8,14–19] and has historically been considered one of the best prognostic indicators.[20,21] Incubation periods of 10 days or more tend to result in milder disease, whereas incubation periods within 7 days of injury tend to result in more severe disease.

Three clinical syndromes are associated with tetanus infection: (1) localized, (2) generalized, and (3) cephalic.[12] Localized tetanus, which is uncommon, consists of spasm of muscles in a confined area surrounding the site of the injury.[22,23] Painful contractions may persist for several weeks to months before gradually subsiding. Localized disease is thought to occur when transport of toxin produced at the site of the injury is restricted to the local nerves. The symptoms can be produced experimentally by simultaneously injecting toxin into a muscle and antitoxin into blood to prevent hematogenous dissemination.[13] Although localized tetanus per se is generally mild, with fatality rates of less than 1%, progression to generalized tetanus and its complications can occur.[22]

More than 80% of cases of tetanus are generalized. The most common initial sign is spasm of the muscles of mastication—trismus, or lockjaw—occurring in up to 90% of patients on presentation.[12,24,25] Spasm of the facial muscles produces a characteristic facial expression—*risus sardonicus*—consisting of raised eyebrows, tight closure of the eyelids, wrinkling of the forehead, and extension of the corners of the mouth laterally. Other early presentations of tetanus include pain and muscle spasm of the neck, shoulders, back, and abdomen. Dysphagia can be the initial symptom, particularly in the elderly.[26,27] Initially, spasms are triggered by external stimuli such as touch or sudden noises, but they occur spontaneously as the disease progresses. Sustained spasm of back muscles gives rise to the arched posture of opisthotonos. Generalized tonic seizure-like tetanospasms consist of sudden excruciating contraction of all muscle groups, resulting in opisthotonos, adduction at the shoulders, flexion of the elbows and wrists, and extension of the legs. Frank convulsions can occur in severely affected patients. Spasm of the glottis can result in immediate death. Patients exhibit generalized hyperreflexia; temperature elevations of 2°C to 4°C are often associated with severe spasms. Cognitive functions are not overtly affected.

Generalized tetanus is frequently accompanied by respiratory failure as a result of chest wall rigidity and spasm,

†Deceased.

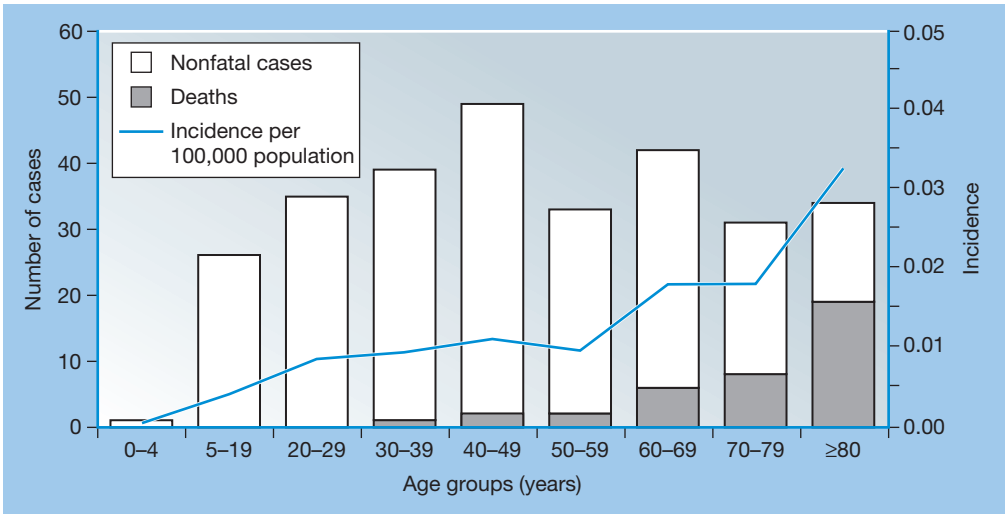


Figure 58.1. Number of reported cases of tetanus, survival status of patients, and average annual incidence rates by age group in the United States, 2000–09. More recent data show no change in trends. *(From Centers for Disease Control and Prevention, unpublished data.)*

diaphragmatic dysfunction, airway obstruction from laryngeal or glottis spasm, aspiration pneumonia, and fatigue.[1,12] Before the availability of effective medications to control spasms and mechanical ventilation, respiratory failure was the most common cause of death in patients with tetanus, and it continues to be so in lower income countries.[1,28,29] Tetanus also can be accompanied by severe autonomic nervous system abnormalities, usually developing several days to a week after symptom onset, which include labile or sustained systemic arterial hypertension, hypotension, flushing, diaphoresis, tachycardia, bradycardia, and arrhythmias.[1,30–35] Development of autonomic dysfunction carries a worse prognosis and has become the most common cause of death in countries where intensive care facilities and respiratory support are available.[1,36,37] Other complications associated with tetanus include vertebral and long bone fractures, dysphagia, traumatic glossitis, urinary retention, catheter-associated infections, pulmonary embolism, decubitus ulcers, and contractures. Long-term consequences—including prolonged muscle fatigue, hyperostoses and osteoarthritis, and difficulties with speech, memory, and mental capacity—have been documented but are uncommon.[38–41] Neonatal tetanus is a form of generalized tetanus occurring in the first month of life, most often as a result of an infected umbilical stump.[12,42,43] Symptoms begin 3 to 14 days after birth in approximately 90% of cases, but can occur from 1 to 28 days of age.[41,42,44–46] The predictable incubation period led several cultures to apply time-related names to the condition, such as "three-six disease" in China, "disease of the 7th day" in Latin America, and "8-day disease" in Scotland, the Pacific Islands, and other locations.[42,47–49] Illness begins with poor sucking and excessive crying in an infant with normal ability to suck in the first 2 days of life.[12,42,50,51] This is followed by variable degrees of trismus, difficulty swallowing, rigidity, and spasms. In survivors of neonatal tetanus, neurologic damage, including spastic paresis/paralysis and severe psychomotor retardation, as well as more subtle intellectual and behavioral abnormalities, has been reported in some studies when long-term sequelae have been assessed.[52–55]

The clinical course of generalized tetanus is highly variable. The disease frequently remains intense for 1 to 4 weeks and then gradually subsides. Case-fatality rates for reported cases of generalized tetanus have varied from 25% to 70% overall and approach 100% at the extremes of age.[18,19,41] With modern intensive care, mortality can be reduced to 10% to 20%[21,29,47,56–63]; however, high mortality persists where intensive care is limited.[64–68,68a] In the United States from 1972 to 2009, reported case-fatality rates declined from approximately 50% to 10% overall, but varied depending on age (Fig. 58.1) and immunization status (Centers for Disease Control and Prevention [CDC], unpublished data).[11,68–71] Higher case-fatality rates have been reported when tetanus results from intramuscular injection of quinine, or after deliveries and abortions.[41,42,72–74]

Cephalic tetanus is a rare manifestation of the disease generally associated with lesions of the head or face, especially in the distribution of the facial nerve and the orbits,[12,50] and with chronic otitis media.[75] The incubation period is usually 1 to 2 days after an acute wound. In contrast to generalized tetanus, which is associated with spasm, cephalic tetanus is manifested by atonic cranial nerve palsies involving nerves III, IV, VII, IX, X, and XII, singly or in combination. Nonetheless, trismus can be present. The disease may progress to generalized tetanus and has a similar prognosis if it does.[12,76]

BACTERIOLOGY

C. tetani is a gram-positive, spore-forming, motile, anaerobic bacillus.[77–80] Typically measuring 0.3 to 0.5 µm in width and 2 to 2.5 µm in length, the vegetative form often develops long filament-like cells in culture. Motility is produced by peritrichous flagellae coating the cell surface. With sporulation, *C. tetani* loses its flagellae and takes on the more characteristic drumstick-like appearance reflecting spore formation in the terminal position. *C. tetani* is a strict anaerobe that grows optimally at 33°C to 37°C; however, depending on the strain, growth can occur at 14°C to 43°C. It can be cultured in a variety of anaerobic growth media such as thioglycolate, casein hydrolysate, and cooked meat. Growth is enhanced in media supplemented with reducing substances at a neutral to alkaline pH. On blood agar, the organism produces characteristic compact colonies extending in a meshwork of fine filaments. Growth is usually accompanied by the production of gas and is associated with a fetid odor.

Sporulation depends on a variety of factors that include pH, temperature, and medium composition. Spores can be promoted at 37°C and in the presence of oleic acid, phosphates, 1% to 2% sodium chloride, protein, and magnesium.[77,78] In

contrast, acidification, high (≥41°C) or low (≤25°C) temperatures, glucose, assorted saturated fatty acids, antibiotics, and potassium inhibit spore formation. The germination of spores requires anaerobic conditions and is enhanced by the presence of lactic acid and chemicals toxic to cells.[80]

If not exposed to sunlight, *C. tetani* spores can persist in soil for months to years.[12,77,78] Spores are resistant to boiling and a variety of disinfectants.[12,81] Inactivation of spores requires 15 to 24 hours in a solution of phenol (5%), formalin (3%), chloramine (1%), or hydrogen peroxide (6%). Use of aqueous iodine or 2% glutaraldehyde at pH 7.5 to 8.5 kills spores within 3 hours; autoclaving at a temperature of 120°C and a pressure of 15 pounds per square inch destroys them within 15 to 20 minutes.[1,28,81]

The most common source of environmental exposure to *C. tetani* bacilli and spores is soil, where the organism is widely but variably distributed. It is difficult to compare studies of the distribution of the organism in nature because of differences in methodology.[82] Most studies suggest that viable spores are more common in soils with an alkaline pH and in nutrient-rich soils in warm, moist climates that could more easily support multiplication of the bacillus.[74,83] In the contiguous United States, however, a limited study in 1975 found spores in 30% of the samples without any apparent geographic or chemical influence on the distribution.[84]

Animals are also *C. tetani* reservoirs. Both herbivores and omnivores can harbor tetanus bacilli and spores in their intestines, disseminating the organism in their feces. Fecal carriage has been reported in 10% to 20% of horses and 25% to 30% of dogs and guinea pigs; fecal specimens from several other species, including sheep, cattle, and small mammals, also were found to contain *C. tetani*.[81,85] Attempts to quantify the frequency of human intestinal colonization have produced varied results ranging from 0% to 40%.[80,86–92] Rural residents tend to have higher rates of intestinal carriage than city dwellers. *C. tetani* spores have also been detected in street dust and the dust and air of surgical operating theaters.[92,93]

PATHOGENESIS

C. tetani produces two exotoxins: tetanolysin and tetanospasmin.[12,79,80] Tetanolysin is an oxygen-sensitive hemolysin related to streptolysin and the θ-toxin of *Clostridium perfringens*. It may play a part in establishing infection at the site of inoculation, but it has no other known role in pathogenesis of the disease.[94] Tetanospasmin, referred to as tetanus toxin, is the neurotoxin that causes the manifestations of tetanus. The toxin is a protein produced intracellularly during the logarithmic phase of bacterial growth and released on autolysis. The highly conserved genes for tetanus toxin and its transcriptional regulator are present on a plasmid.[95–97] Observed differences in *C. tetani* reference-strain toxigenicity are related to heterogeneity in the strains' constituent clones; lower toxigenicity results when there are mixtures of organisms with and without the plasmid.[98]

Tetanus toxin has an approximate molecular weight of 150,000 kDa and is synthesized as a single polypeptide prototoxin chain. When released by cell death, the prototoxin is cleaved by bacterial or tissue proteases into its active form, consisting of a 50,000-kDa light chain, the toxic domain, and a 100,000-kDa heavy chain with two domains related to membrane binding and translocation. The light and heavy chains are held together by a single disulfide bond; a second disulphide bond connects two heavy chain sites.[99–101] The three distinct domains of the toxin are related to the separate steps of neuron receptor binding, internalization, intraaxonal transport, membrane translocation, and target enzymatic activity. The carboxyterminal end of the heavy chain (HC, or fragment C) binds to receptor sites and is required for internalization by endocytosis[102]; the aminoterminal end (HN) of the heavy chain mediates intraaxonal transport and, in central interneurons, the translocation of the toxin molecule into the neuronal cytosol.[101,103,104] The light chain is an endopeptidase that is responsible for tetanospasm's specific toxic properties (discussed later).[101,105]

Tetanus toxin is one of the most potent known poisons on a weight basis because of its absolute neurospecificity and enzymatic function at the site of action.[105] As little as 1 ng/kg will kill a mouse; 0.3 ng/kg will kill a guinea pig.[106] The estimated minimum human lethal dose is less than 2.5 ng/kg.[106] Various species have different levels of responsiveness to the toxin: cats, dogs, birds, and poikilotherms are relatively resistant to its effects; mice, guinea pigs, monkeys, sheep, goats, and particularly horses are sensitive. Humeau and coworkers described evidence that these differences are the result of specific differences in toxin binding and neurotransmitter blocking activity.[107]

Infection usually begins with the inoculation of spores into wounds, accompanied by tissue injury, necrosis, and the anaerobic conditions necessary for spore germination and bacterial replication. Ionic calcium appears to increase local necrosis and the likelihood of *C. tetani* infection; its presence in soil contaminating wounds may enhance spore germination and bacterial replication.[108]

Transport of toxin from the infected site into the CNS is complex (Fig. 58.2). After extracellular release, toxin diffuses to neuromuscular junctions of α-motor neurons in adjacent muscle tissue or to the lymphatic system, which transports the toxin to the bloodstream, leading to systemic dissemination and widespread uptake.[12,109] The toxin gains entry into α-motor neurons via a double-receptor sequential binding process involving specific polysialgangliosides, and possibly glycoproteins, in lipid raft microdomains, followed by internalization by clathrin-mediated endocytosis.[104,110–117] Once internalized, toxin is transported by a specific retrograde axonal transport system to the motor neuron cell body at a rate of 3 to 13 mm/h.[105,115,118–121] The mechanism of its release into contiguous synaptic junctions is unclear.[105,111,115] Entry into adjacent inhibitory interneurons in the CNS occurs via synaptic vesicle recycling, whereby the toxin binds to synaptic vesicle membrane protein SV2 and is endocytosed in the synaptic vesicle lumen, and also by clathrin-mediated endocytosis and bulk endocytosis.[115,117,118,122–124]

Once inside an inhibitory neuron, the light chain is transferred to the neuronal cytosol by membrane translocation through a channel created in the vesicle membrane. Channel formation, and the conformational changes in heavy chain subdomains that are required for translocation, are still incompletely understood.[117,125] In the cytosol, the disulphide bond between the heavy and light chains is broken, freeing the light chain to begin its toxic enzymatic activity.[105,111,123,126] The light chain is a zinc endoprotease that cleaves peptide bonds in synaptobrevin, a synaptic vesicle protein necessary for vesicle fusion with the plasma membrane. Without intact synaptobrevin, synaptic vesicles accumulate at the nerve ending, unable to release their contents of the inhibitory neurotransmitters glycine or γ-aminobutyric acid (GABA).[105,127–129] With inhibition blocked, increased excitatory neuron baseline firing rates lead to muscle rigidity, followed by multiplication of unopposed excitatory impulses that cause characteristic tetanic spasms, and, less commonly at the cerebral level, convulsions.[1,12,101,130–132]

Tetanus toxin does not cross the blood–brain barrier[109]; neuronal transport is the sole means of entry into the CNS.[12,133–135] Transsynaptic transport of intact toxin allows transfer to inhibitory neurons in the spinal cord and wider

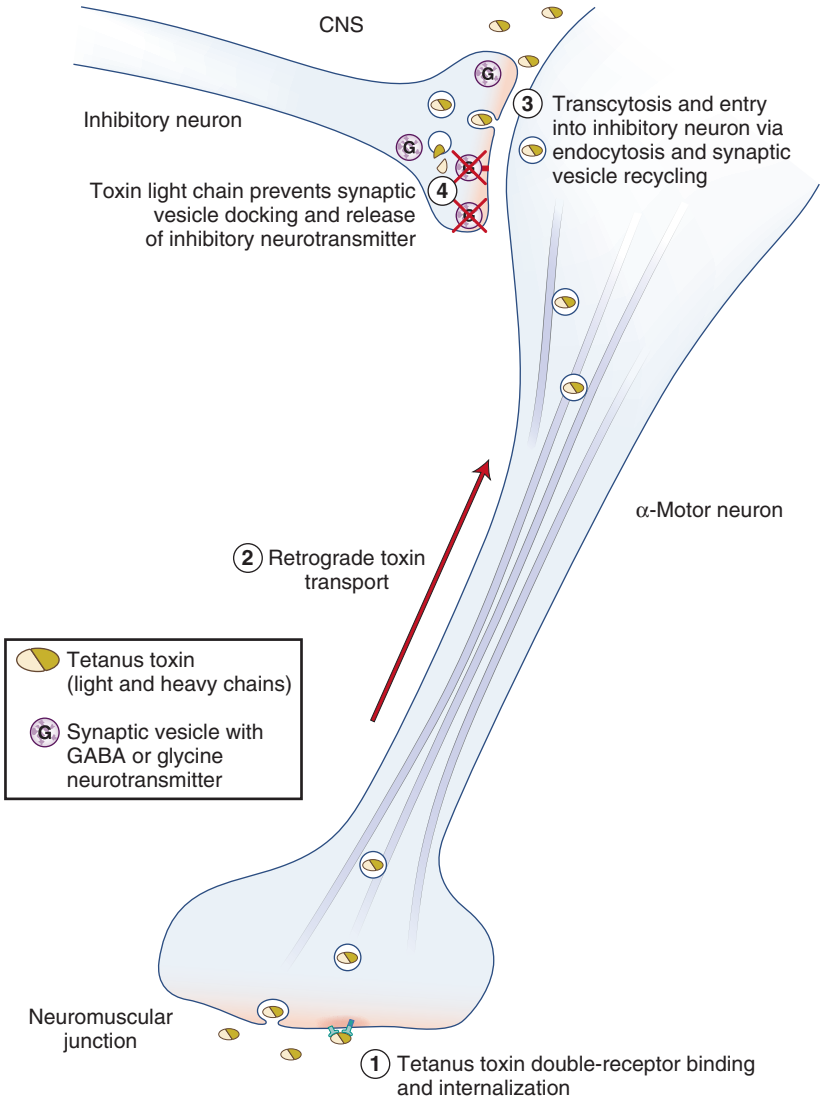


Figure 58.2. Route of tetanus toxin, from entry into an α-motor neuron to its site of action in an inhibitory neuron in the central nervous system (CNS). The steps are as follows: (1)Toxin binding with two specific receptors in the α-motor neuron membrane and internalization into the motor neuron. (2) Retrograde axonal transport of toxin to the motor neuron cell body. (3) Toxin release from the motor neuron, followed by entry into the inhibitory neuron via synaptic vesicle recycling, and also clathrin-mediated and bulk endocytosis. (4) Toxin translocation from the vesicles in which it entered the inhibitory neuron; the bond between heavy and light chains is then broken, freeing the light chain to cleave synaptobrevin, thereby preventing synaptic vesicle docking and release of inhibitory neurotransmitter. GABA, γ-aminobutyric acid.

dissemination in the CNS. Recovery may depend on new functional connections or toxin degradation.[136–138]

Tetanospasmin can act at the peripheral neuromuscular junctions, the spinal cord, the brain, and the autonomic nervous system.[12,30–33,104,131,132] With local tetanus, transport of toxin is from the neuromuscular junction of the affected muscle without hematogenous dissemination; the predominant effect is on spinal glycinergic inhibitory neurons.[1,139] In generalized tetanus, hematogenous dissemination of toxin allows more widespread uptake at neuromuscular junctions; the predominant effect is on the GABAergic supraspinal inhibitory neurons.[1] The clinical syndrome appears almost identical to strychnine poisoning, which acts by competitively binding to postsynaptic glycine receptors at the motor neurons.[140] Tetanospasmin also interferes with release of a variety of other neurotransmitters, including acetylcholine in peripheral somatic and autonomic nerves.[107,123] This accounts for autonomic dysfunction in cases of severe disease, flaccid cranial nerve palsy in cephalic tetanus, and peripheral muscle weakness, which often is masked by the more dramatic manifestations of the central inhibitory blockade. More detailed information on tetanus toxin can be found in recent reviews.[101,105,107,111,115,117,141]

DIAGNOSIS

The diagnosis of tetanus is established primarily on clinical grounds and secondarily supported by epidemiologic setting.[1,28,142] A history of a wound contaminated by soil or other material and the presence of a local skin infection are helpful in the diagnosis, although these criteria may not be present.[12] Bacteriologic investigations are frequently negative. Characteristic gram-positive bacilli, some with terminal or subterminal spores, occasionally may be seen in aspirates from the affected area, although anaerobic cultures of tissues or aspirates are usually not positive[57,143]; conversely, detection of the organism at a wound site does not confirm the clinical diagnosis.[1,144,145] Low or undetectable levels of circulating

antitoxin at the time of onset of symptoms are compatible with the diagnosis; however, there are a number of case reports in which moderately high levels of antitoxin were noted when the patient sought treatment.[146–153] Changes in antitoxin levels in convalescence are not reliably seen.[154] Given the mild nature of some presentations, electromyography has been suggested to aid in the diagnosis,[155] and elicitation of trismus by posterior pharyngeal stimulation ("spatula test") has been reported to help in clinical differentiation.[156]

The differential diagnosis depends on the clinical form of tetanus and the presenting symptoms. Cephalic tetanus may be confused with Bell palsy and trigeminal neuritis. However, cephalic tetanus often is accompanied by other cranial nerve symptoms, including dysphagia, and signs of trismus and nuchal rigidity.[12,50] Trismus has a variety of causes, including dental infection, tonsillitis, peritonsillar abscess, temporomandibular joint dysfunction, parotitis, and CNS disturbances other than tetanus. Patients with rabies can also have hyperreflexia; however, rabies is more likely to be associated with hallucinations, hydrophobia, mania, stupor, and a history of an animal bite, and it is unlikely to be accompanied by trismus. In addition, seizures with rabies are usually clonic, whereas tetanospasms are prolonged and tonic. Encephalitis is rarely associated with trismus and is much more likely to be accompanied by disturbances of consciousness than is tetanus. Because of nuchal rigidity, bacterial meningitis could be confused with tetanus. Stiff Person Syndrome, which is very rare, has been mistaken for tetanus (CDC, unpublished data).[1,157]

A variety of metabolic conditions and poisonings can resemble tetanus. Although muscle spasm may be seen with hypocalcemic tetany, it is not generally associated with trismus.[158] A determination of low serum calcium can confirm tetany. Strychnine poisoning mimics generalized tetanus.[140] However, such poisoning is characterized by (a) rare association with persistent trismus, (b) greater muscle relaxation between spasms, (c) normal body temperature, and (d) the presence of detectable strychnine in gastric contents or in urine. Phenothiazine toxicity may be associated with a variety of dystonias, including trismus. Detection of phenothiazines in the blood or amelioration by treatment with diphenhydramine confirms the diagnosis. Hysteria can mimic tetanus; however, hysterical patients usually relax during prolonged observation or when they are distracted, and they are more likely to display clonic rather than tonic spasms.[159]

Because of the distinctive presentation of neonatal tetanus, a postmortem history determining the nature and timing of symptoms, with verification that the child was normal at birth, permits accurate classification of tetanus as the cause of death with a high degree of probability.[160] The World Health Organization (WHO) defines neonatal tetanus as an illness occurring in a child who has the normal ability to suck and cry in the first 2 days of life, loses this ability between 3 and 28 days of life, and becomes rigid or has convulsions (see Chapter 76).[161]

TREATMENT

By the time a patient with tetanus presents for treatment, toxin has entered the nervous system, is circulating in the lymphatics and bloodstream, and is continuing to be produced by *C. tetani* at the site of infection. The purposes of tetanus therapy are: (a) to inactivate circulating toxin; (b) to prevent further toxin production by eliminating the organism; and (c) to give supportive care for the duration of the illness. General principles for use of human tetanus immunoglobulin (TIG) and pharmacotherapy are outlined here. Detailed information on treatment can be obtained from review articles and chapters.[1,28,61,130,162–167]

Patients with tetanus may initially have few symptoms and signs, but these will generally progress over subsequent days; in a substantial proportion of cases, the clinical status deteriorates quickly after hospital admission.[1,12] Although clinical scoring methods have been developed to predict prognosis and the need for aggressive management, these methods were derived empirically and have been used primarily in clinical research to stratify disease severity to balance treatment groups.[20,168] In addition, the scales were developed before major improvements were made in therapeutic interventions and mortality reduction. A tetanus severity score developed by Thwaites and associates was derived by logistic regression and found to be more specific and sensitive overall than other scales in their setting.[169] The primary indicators of rapid progression were age, time from first symptom to admission, entry site, and hemodynamic indicators on the first day.

TIG should be given at the time of diagnosis to neutralize circulating toxin before it reaches the nervous system.[1,13,163] The optimal dosage of therapeutic TIG has not been established. When TIG became available in the 1960s, a dosage range of 3000 to 6000 units was chosen based on calculations of the quantity of immunoglobulin necessary to achieve antibody levels higher than those found to be minimally protective against the effects of tetanus toxin.[170] Despite the absence of evidence to support this choice of dosage range, 3000 to 6000 units of TIG given intramuscularly in a single administration was adopted as the standard for TIG therapy.[12] A retrospective analysis of tetanus cases reported in the United States from 1965 to 1971 subsequently suggested that a TIG dosage of 500 units is as effective as higher dosages in reducing mortality.[171] Similarly, the results of an observational study involving 236 patients with severe tetanus admitted to two hospitals from 1981 to 2001 also indicated that the reduction in TIG dose from 3000 to 6000 units to 500 units midway through the study did not appear to adversely affect patient outcome.[21] In addition to apparently being as effective as higher dosages, 500 units constitutes a smaller volume of antitoxin, thereby reducing the number of injections required when given intramuscularly and avoiding repeated painful spasm-inducing stimuli to the patient. Thus, a total dosage of 500 units of TIG is now commonly recommended,[1,136,163–166,172,173] although some authorities continue to prefer 3000 to 6000 units of TIG.[28,61,174,175]

Equine antitoxin can be given intravenously but is associated with serious allergic side effects such as anaphylaxis and serum sickness.[176–178] After intramuscular TIG administration, peak serum levels of antitoxin are achieved by 48 to 72 hours, a theoretical disadvantage of TIG, which, according to the product label, cannot be given intravenously in the United States.[179] Regardless, no evidence to date suggests that intravenous equine antitoxin is more efficacious than intramuscularly administered human TIG, and therefore equine antitoxin is not recommended when TIG is available.[180] Intravenous immunoglobulin (IVIg) has been proposed as an alternative to TIG when TIG is unavailable or when intramuscular injections must be avoided.[173,179,181,182] Although the quantity of tetanus antitoxin in IVIg produced by different manufacturers varies, it appears that commercial IVIg preparations contain sufficient antitetanus antibody to achieve protective antibody levels when given in dosages of 200 to 400 mg/kg.[179,182,183] IVIg is not licensed for this indication in the United States.

Use of intrathecal antitoxin for therapy has been controversial.[184,185] Systemically administered antibody does not cross the blood–brain barrier; intrathecal administration theoretically offers the possibility of neutralizing extracellular toxin in the CNS as it traverses synaptic spaces. This was supported by animal studies and early clinical series.[186,187] However, the

results of studies evaluating the benefits of intrathecal therapy are conflicting. The majority of published controlled trials with intrathecal administration of equine antitoxin or TIG to treat either adults or neonates reported failure to significantly decrease mortality.[185,187–190] However, even though many of these trials were limited in size, they did show a trend toward decreased mortality. Significantly decreased mortality, lowered by one-third to one-half, was demonstrated in six studies.[191–196] A metaanalysis of English-language studies by Abrutyn and Berlin in 1991 on the topic concluded that there was no net proven benefit, safety was not established, and well-designed controlled clinical trials were needed.[185] Subsequently, two randomized controlled clinical trials evaluating intrathecal TIG in adult patients with tetanus demonstrated significant improvement in clinical course and shortened duration[197,198] (as did some earlier trials[186,191]); the larger study demonstrated a trend toward decreased mortality and a clear reduction in treatment costs.[198,199] A 2006 metaanalysis of controlled clinical trials added these studies and concluded that intrathecal therapy is superior to intramuscular antitoxin alone in decreasing mortality[200]; inexplicably, the analysis did not include all studies used in the 1991 analysis and had other irregularities. Three newer studies again supported improved outcome with intrathecal therapy.[201–203] Because overall tetanus mortality has been decreasing over time as a result of improved medical care, evaluating the impact of individual therapeutic interventions on tetanus mortality is increasingly difficult and progressively less important. Although additional randomized controlled trials of sufficient sample size could provide definitive proof of decreased mortality, most studies suggest that intrathecal TIG can improve tetanus morbidity and mortality when given early in the course of disease. The added benefit of intrathecal therapy in reducing mortality, even if limited, may be more relevant to settings where critical care resources are scarce. Because TIG is not licensed for this indication and lacks clear benefit, intrathecal TIG use cannot be recommended in the United States.

All patients suspected to have tetanus should start or complete a primary series of vaccination with tetanus toxoid, or receive a booster dose, at the time of diagnosis. The quantity of toxin sufficient to produce disease is so minute that an immune response may not be elicited.[154] Relapsing or recurrent cases have been reported.[75,150,204–210]

When patients with tetanus have identifiable sites of ongoing infection, continued production of tetanus toxin may be prevented by careful surgical drainage or debridement,[211] and appropriate antimicrobial therapy. In the absence of active infection, the utility of antibiotics in treating tetanus is unclear,[1,28] but a study from Ghana demonstrated that when antibiotics were unavailable, mortality from tetanus increased.[65] In the past, penicillin administered for 5 to 10 days as procaine penicillin, 1.2 million units daily, or aqueous crystalline penicillin G, 4 million units daily divided (every 6 hours), was the antibiotic of choice to kill the vegetative form of the organism.[12] However, because penicillin is a central GABA antagonist and may potentiate the effects of tetanus toxin,[1,163] metronidazole given as 500 mg every 6 hours either intravenously or orally has been preferred more recently.[1] One controlled trial from Indonesia found significantly improved prognosis with use of metronidazole compared with penicillin[212]; another could not show differences in mortality, but did find reduced requirements for muscle relaxants and sedatives in patients randomized to metronidazole therapy,[28] and a third randomized controlled trial showed no advantage at all.[213] Thus, preference for metronidazole over penicillin may be more theoretical than real. Other antibiotics that are effective against *C. tetani* include macrolides, tetracyclines, and clindamycin.[1]

Meticulous supportive care is critical to the treatment of patients with tetanus.[57,58,184] To minimize spasms, the patient should be kept in a quiet, dimly lit room equipped to avoid sudden environmental stimuli, including loud noises. Pharmacologic treatment of hypertonicity and spasms depends on the severity of disease. The objective is to control spasms and increased tone without impairing voluntary movement, consciousness, or spontaneous respiration, if possible. Because tetanospasmin blocks GABA release from inhibitory neurons in the CNS, the ideal therapeutic agent would reverse or counteract this blockade.[1,130,163,214] Benzodiazepines such as diazepam are $GABA_A$ agonists that indirectly counteract the effects of the toxin.[1,167,215,216] Intravenous diazepam is generally given in dosages of 0.5 to 15 mg/kg per day.[143,217–220] Some clinicians use standard dosages administered every 2 to 8 hours; others give dosages at the time of spasms, using 5 to 10 mg as often as three times per hour or more.[12,58] Higher dosages may be used if the lower dosages fail; some adults require and tolerate dosages of more than 600 mg in 24 hours. Lorazepam is also effective and may be preferred because of its longer duration of action.[1] Midazolam given as a continuous infusion also has been effective and has the advantage of being free of propylene glycol, a preservative present in parenteral diazepam and lorazepam preparations that can produce lactic acidosis.[1,162,163]

Other muscle relaxants that have been used include dantrolene and intrathecal baclofen.[1,61,162,163,166,221,222] Propofol by continuous infusion has been used as an adjunct sedative to benzodiazepine therapy.[1,61,163,223] Magnesium sulfate, a presynaptic neuromuscular blocker, has also been used by some to control or reduce muscle spasms in generalized tetanus as well as manifestations of autonomic dysfunction (see later).[43,61,166,167,224–229] Short-acting barbiturates (secobarbital sodium and pentobarbital) can be used when diazepines are not available, bearing in mind they are more likely to result in respiratory depression and coma than benzodiazepines.[12,130,165] Chlorpromazine has also been used with and without benzodiazepine and barbiturate therapy.[12,28]

If conservative therapy fails to control muscle spasms, or if the patient has severe spasms compromising respiration, neuromuscular blockade with assisted ventilation is indicated. Vecuronium is now the agent of choice because it causes minimal autonomic instability; an alternative is atracurium.[1,61,130,163] Older agents can be used in their absence.[12,130] When neuromuscular blocking agents are used, sedation is still required to suppress consciousness and memory of the period of paralysis.[1,163]

Although effective in controlling spasms, the agents just discussed may not decrease the sympathetic overactivity that can complicate the course of tetanus and increase the risk of fatal outcome. Autonomic dysfunction should be treated with labetalol or morphine.[33,130,163,230–232] β-Blockers should not be given in isolation, because their use can result in unopposed α-adrenergic activity leading to severe hypertension. Other agents used for managing autonomic dysfunction include continuous intravenous magnesium sulfate, clonidine, and fentanyl.[61,162,167,224,226,227,233,234]

Acute respiratory failure is a common early complication of generalized tetanus. Respiratory decompensation in tetanus can be precipitous; close observation of patients early in the course is essential. Assisted ventilation may be required. Indications for tracheotomy include: likely prolonged mechanical ventilation, endotracheal tube exacerbation of upper airway spasm and inability to cough or swallow.[12,28,57,166,235]

Moderate or severe tetanus often results in high metabolic demands and a protein-catabolic state. Parenteral alimentation is sometimes required, best initiated early in treatment.[1,61,130,166,236,237] Other supportive measures include

prophylaxis for thromboemboli, gastrointestinal hemorrhage, and pressure sores.

EPIDEMIOLOGY

Incidence

The true public health impact of tetanus was largely unappreciated until the second half of the 20th century, despite its high mortality and dreadful symptoms. Possible reasons for the lack of recognition include the rural predominance of the disease, the occurrence of cases singly rather than in clustered epidemics, and the lack of compulsory reporting.[41,238] A 1966 review by Bytchenko of data reported to the WHO, supplemented by scientific publications from the 1950s, suggested overall tetanus mortality rates of two to three deaths per 100,000 population in Africa, Central and South America, and Asia, with rates as high as 25 to 100 per 100,000 in some countries and subnational areas.[74] He estimated a total of at least 50,000 annual tetanus deaths worldwide, but cautioned that this was likely to be a substantial underestimate.

A more accurate understanding of global tetanus burden came 20 years later when public health interest in tetanus, especially neonatal tetanus (NT), was spurred by the easy availability of inexpensive and highly effective tetanus toxoid vaccine. Before the 1980s, the incidence of NT in lower-income nations, based on reported disease, was estimated to be approximately 0.1 per 1000 live births.[74] Using hospital-based reporting, an estimate of 600,000 NT deaths worldwide was made for 1981, corresponding to approximately five NT deaths per 1000 live births.[239] However, because most NT cases were not brought to health facilities for treatment, a realistic assessment became possible only when community-based, house-to-house surveys of neonatal deaths were conducted in more than 40 countries in Africa, Asia, South America, and the Middle East. These surveys documented that actual NT mortality was far greater, with more than 50 deaths per 1000 live births in some areas.[41,44,240,241] Comparisons of survey data to reported data demonstrated that national NT reporting efficiencies were generally less than 5% in lower-income countries.[41,242,243] Using the survey results, global NT mortality was estimated to be approximately 1 million deaths per year in the early 1980s, corresponding to approximately 10 deaths per 1000 live births (Fig. 58.3).[42,244] At the same time, 310,000 to 700,000 non-NT cases with 122,000 to 300,000 deaths were estimated to occur in low- and middle-income countries, more than half in children.[245] In contrast, in high-income countries, only an estimated 2000 tetanus cases and 1000 resultant deaths occurred annually during that period.

A decline in tetanus incidence in higher-income countries began in the early 1900s. By the mid-1930s, many European countries had tetanus mortality rates of less than one per 100,000 population.[74] Factors that contributed to the decline included urbanization, mechanization of agriculture, adoption of aseptic surgical and medical techniques, hygienic childbirth and wound care practices, use of prophylactic tetanus antitoxin, and, ultimately, use of antibiotics.[41,74,245] However, in lower-income countries, tetanus continued to constitute a major health burden until tetanus toxoid was introduced into national immunization programs.

After more than 50 years of use of tetanus toxoid–containing vaccine in higher-income countries, and more than 30 years

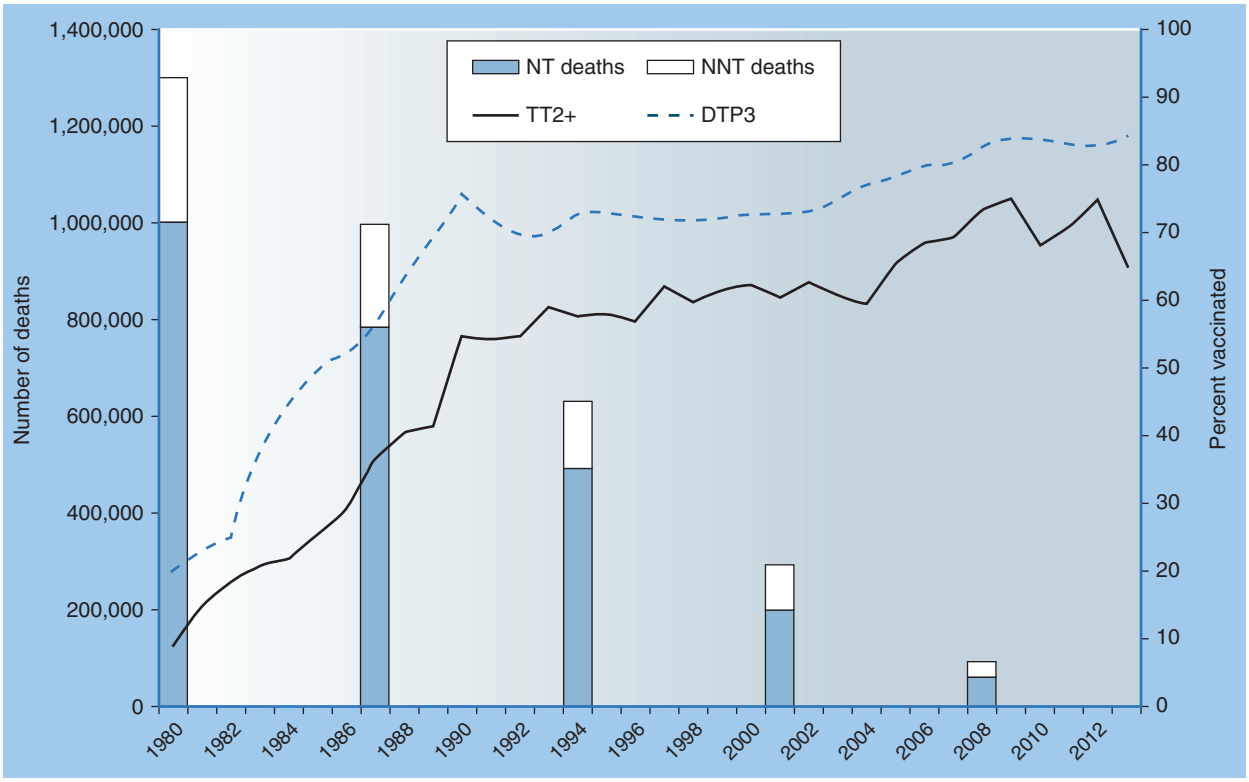


Figure 58.3. Estimated annual deaths from neonatal tetanus (NT) and nonneonatal tetanus (NNT), and coverage with combined diphtheria, tetanus toxoid, and pertussis-containing vaccine (DTP3) among children age 12 to 23 months and tetanus toxoid (TT)2+ (two or more TT doses) among pregnant women, 1980–2013. *(Vaccination coverage from the WHO/IVB [World Health Organization's Department of Immunization, Vaccines, and Biologicals] database, 2014. Estimated tetanus deaths from references 244, 245, 247, 249, 263, 795, and 804; World Health Organization, UNICEF, World Bank; State of the World's Vaccines and Immunization, Geneva, Switzerland, World Health Organization, 2002; and from M. Roper [unpublished data].)*

in lower-income countries, the number of tetanus cases and deaths has decreased dramatically (see Fig. 58.3).[246,247] The most recent estimates of total global tetanus (derived from different models and perhaps underestimated) are 66,129 deaths for 2012[248] and 58,900 deaths for 2013.[249] Fewer than 1000 of these tetanus deaths occurred in high-income countries. Total reported tetanus incidence rates for 2014 are less than 0.03 per 100,000 population in most European countries[250]; U.S. incidence from 2000 to 2012 was 0.01 per 100,000 population.[251,252]

Tetanus is also becoming rare in low- and middle-income countries with well-established immunization programs delivering both primary and booster tetanus toxoid doses.[247,253–255] For example, the 2006 incidence of tetanus in Thailand was 0.23 per 100,000 population, and in Brazil it is approximately 0.17 per 100,000 population; in both countries the rates are steadily declining.[256,257] Countries where primary immunization coverage has lagged, or where booster doses have not yet been introduced, still have incidence rates of one to two per 100,000 population (M. Roper, unpublished data). In such countries, tetanus still accounts for a significant proportion of hospital admissions and deaths.[62,258–262] Although global NT mortality has decreased by more than 90% since the 1980s,[263] 49,000 NT deaths were estimated to have occurred in 2013 (see "Public Health Perspective" later).[264]

Contemporary tetanus incidence estimates are still uncertain; reporting is little better now than in the 1950s. Many low- and middle-income countries report only NT cases, and NT reporting fractions are still low.[243,265–267] Even in developed countries, tetanus reporting efficiency is 60% or less[41,268–272]; in the United States, only 40% of tetanus deaths were reported in 1989,[268] and overall reporting was estimated to be less than 20%.[11] In the absence of reliable surveillance data, estimates of tetanus burden are generated by mathematical modeling.

Nonneonatal Tetanus

Prior to the availability of tetanus toxoid, the epidemiology of tetanus in a given area depended on the risk of infection with *C. tetani* and the availability of prophylactic tetanus antitoxin. The advent of widespread use of tetanus toxoid modified the distribution of disease, as has socioeconomic development in many parts of the world.

Sources of Infection

Acute wounds, particularly puncture wounds and lacerations, account for more than 50% of lesions associated with tetanus in most series (range: 47% to 82%). The severity of injury varies from superficial and trivial to extensive and deep; often, antecedent conditions are considered too minor to require medical attention. Burns, open fractures, abrasions, and excoriations also are commonly associated with tetanus.[11,17,74,273–277] Historically, tetanus was a dreaded consequence of war, with incidence rates on the order of two cases per 1000 injured troops[8,278]; similarly high rates were described in wounded civilians in Asia during World War II.[279] Large clusters of tetanus cases also have occurred in conjunction with natural disasters resulting in mass casualties,[166] including the 2004 tsunami in Aceh, Indonesia (106 cases, 20 deaths)[280,281] and the earthquakes in Pakistan (2005: 139 cases, 41 deaths)[282,283] and Indonesia (2006: 81 cases, ≥25 deaths).[281,284]

Tetanus also is associated with a wide variety of nontraumatic conditions, including chronic skin ulcers, abscesses, gangrene, dental infections, insect and animal bites, snake and stingray envenomations, and carcinomas.[11,41,71,73,277,285–287] In South Asia and Africa, otitis media is a common cause of tetanus in children, accounting for up to half of cases in some pediatric tetanus series.[73,201,210,277,288–292] Tungiasis (skin inflammation and secondary bacterial infection caused by *Tunga penetrans* flea infestation) is often associated with tetanus in Africa, South America, and the Caribbean, especially in children.[21,285,291,293,294]

Medical procedures, including surgeries and injections, were frequent causes of tetanus in high-income countries prior to the use of sterilized equipment, solutions, and dressings; they continue to account for a significant proportion of cases in lower-income countries.[74,261,273,295–299] Even vaccination, especially against smallpox, was associated with tetanus.[74,273,277] Intramuscular quinine injections are associated with especially high mortality, likely related to quinine-induced muscle necrosis that enhances bacterial growth and toxin production.[72,261,300] Tetanus associated with deliveries and abortions (maternal tetanus) has been a frequent cause of maternal mortality where unclean deliveries and abortions are common, accounting for up to 5% of maternal mortality.[43,74,301–303] Circumcision, both in the neonatal period and at older ages (male and female) has been associated with tetanus.[60,240,258,293,298,304–306] Tetanus also has been identified following voluntary male medical circumcision for HIV prevention.[307,307a,307b,307c] Traditional surgeries, such as uvulectomy, ear piercing, and ritual scarification, also are associated with tetanus.[41,60,74,298,308] In the United States, cases associated with body piercing and tattoos have been reported.[70,71,309,310] Injection drug use, long appreciated as a significant tetanus risk factor,[11,70,311–314] has accounted for 15% to 89% of tetanus cases in recent series from more affluent countries.[11,71,254,255,315,316] Substantial clusters have occurred in California and England in association with contaminated illegal drug supplies.[316–318] Failure to identify an antecedent wound or condition is not uncommon; up to 25% of tetanus cases in published series have no known associated cause (M. Roper, unpublished data).[74]

Seasonal and Geographic Distribution

Tetanus generally follows a seasonal pattern in temperate countries, with a midsummer peak that may reflect soil and spore conditions as well as agricultural and other outdoor activities associated with injuries during warmer months.[17,74,238,273,319,320] In tropical climates, tetanus tends to occur year round, although in some areas an increase in cases is seen during the wet season. Geographic distribution is uneven, with a higher incidence in lowland areas with a moist, warm climate and fertile soil, especially where land is used for cultivation or to pasture domestic animals,[9,80,321] although cases have been documented in desert environments.[322] High altitude has been associated with lower tetanus rates, although not consistently.[74,323] The highest rates of tetanus are found in rural communities, particularly those lying nearest the equator.[41,73,74]

Age and Sex Distribution

In the absence of vaccination, children were at highest risk of non-NT, accounting for 50% to 70% of all cases; age-specific incidence was highest in children younger than 10 years of age.[41,74,238,245,273,324] Despite the greater frequency of pediatric tetanus, mortality in children and adolescents was (and continues to be) substantially lower than in adults, ranging from 5% to 30% compared to 50% and higher in adults.[245,277,321,325] Second to pediatric disease, tetanus historically affected young adults, usually with a male predominance (2:1 or higher) except in societies where women are responsible for agricultural work and where septic deliveries and abortions are common.[74,326]

Vaccine Era

Tetanus has become rare wherever systematic use of tetanus toxoid has been implemented in large proportions of the population over many birth cohorts. In higher-income countries, cases predominate in the elderly who are most likely to be unvaccinated or undervaccinated.[11,41,58,59,245,269,272,325,327–331] Many countries have given tetanus toxoid to male military recruits since the 1940s, contributing to a female predominance among tetanus cases in older-age groups.[269,272,327,330,331] Other important risk groups are immigrants from countries with less-developed vaccination programs, persons with religious or philosophic objections to immunization, and injection drug users.[11,71,254,332,333] This distribution is remarkably consistent in higher-income countries, and is mirrored in population-based serosurveys that demonstrate highest susceptibility in the elderly, foreign born, and groups of vaccine objectors.[272,334–341]

In lower-income countries, coverage with three doses of DTP (diphtheria and tetanus toxoids and pertussis vaccine) in infancy and TT (tetanus toxoid) for pregnant and childbearing-age women has steadily improved (see Fig. 58.3); consequently, tetanus in young children is rare and is increasingly uncommon in women. Data from a longitudinal study of tetanus in a major medical center in Ho Chi Minh City, Vietnam, demonstrate this impact.[62] Vietnam has had high coverage with three doses of DTP (DTP3) since the late 1980s, exceeding 90% since the early 1990s; TT immunization for pregnant and childbearing-age women was widely implemented later in that decade. The age- and sex-specific pattern of tetanus admissions showed a small proportion of cases in children younger than 10 years of age (5.8%) and a 90% reduction in adult women over the study period (10% of cases in 1993; 1% in 2002), whereas tetanus in older children and adult men remained unchanged. The same pattern has been documented in other Asian and African countries: the most affected groups are persons in their 20s and 30s, with a male predominance as high as 12:1 (M. Roper, unpublished data).[258,342–345,307a] The few tetanus serosurveys available from lower income countries mirror this pattern: loss of protection is apparent in older childhood and, by adolescence and young adulthood, the prevalence of seroprotection is below 65% (often well below 50%), except among women who receive TT to prevent NT.[346–353,353a] Where booster doses in childhood and adolescence are used, the median age of patients with tetanus shifts to mid- and late adulthood and male predominance is modest.

Neonatal Tetanus

NT clusters in poor, rural, and disadvantaged communities where access to immunization is low, and where delivery conditions and postpartum cord care practices are unclean.[43,243] Studies in highly affected areas documented NT mortality rates as high as 50 to 110 per 1000 live births.[241,354–356] In community-based studies from the 1970s and 1980s, 23% to 72% of neonatal mortality was caused by NT; in 39% of the 46 surveyed countries, NT accounted for more than half of all neonatal deaths in at least one surveyed area.[41,44]

In contrast to the situation in the lower-income countries, NT rates in high-income countries prior to the use of TT were orders of magnitude lower. In Denmark, as hospital and clinic deliveries became increasingly common and aseptic practices became the norm, NT incidence fell from 0.79 per 1000 live births in the 1920s to less than 0.05 per 1000 live births by the mid-1960s.[357] Similarly, in the United States, NT mortality dropped from 0.64 per 1000 live births in 1900 to less than 0.02 per 1000 live births in the early 1950s.[48,320] Once tetanus vaccination became widespread in high-income countries in the second half of the 20th century, NT became exceedingly rare (see "Public Health Perspective" later).

Neonatal Tetanus Risk Factors

Roper and colleagues have reviewed documented NT risk factors in detail.[42] Increased NT risk is associated with home deliveries with unclean birthing surfaces, untrained birth attendants, lack of attendant hand washing, unclean tools used to cut the umbilical cord, and the use of traditional substances on the umbilical stump.[45,60,358–369] In Africa and South Asia, where animal dung was commonly used for postpartum cord care (to confer "strength" or "purity"), NT mortality was particularly high; when the practice was substituted with culturally acceptable cleaner alternatives, rates decreased dramatically.[370,371] Where domestic animals are prevalent, and especially if animals are kept close to or inside homes, NT is more common.[360,372,373] Use of dried cow dung as fuel may result in contamination of hands and substances placed on the umbilical stump.[374,375] Traditional surgeries or piercings are also associated with NT.[60,74,304,308,368]

Although immunization against tetanus per se is more effective in reducing NT mortality than deliveries performed by attendants trained in clean delivery practices in the absence of immunization, training of birth attendants reduces overall neonatal mortality resulting from tetanus and other infections.[356,362,370,376–379] Measures to prevent infection of the umbilical stump also have a powerful effect on reducing NT (and other neonatal infections).[370,371] Three case-control studies have indicated that the use of topical antibiotics at the time of delivery and in postnatal care decrease the risk of NT; antiseptics were also protective, while dry cord care in itself was not.[380–382] Recent systematic reviews of randomized, controlled trials evaluating the effect of chlorhexidine use on the umbilical stump in rural community settings in South Asia showed a 50% reduction in omphalitis and a 20% reduction in neonatal mortality.[383,384] The included trials were conducted in areas where maternal TT immunization coverage is high; thus impact on NT prevention per se could not be evaluated. Provision of clean delivery kits for home deliveries can have a positive impact on neonatal outcomes, even in the absence of trained birth attendants.[385,386] A systematic review of published studies on the effects of clean delivery and cord care practices on NT, neonatal sepsis and all-cause neonatal mortality underscored the difficulties in separating out and measuring the independent effects of specific hygienic obstetric and postnatal practices on the prevention of NT and other causes of neonatal death.[387] Based on the available evidence and a Delphi expert opinion exercise, it was concluded that clean delivery and postnatal practices are effective in preventing deaths from NT and neonatal sepsis and should continue to be promoted. Clean delivery practices can also reduce maternal tetanus and other forms of post-partum infections. China, a country that does not administer TT after childhood, eliminated maternal and NT* through near universal provision of clean delivery services.[388,389]

In many published community- and hospital-based studies, a male predominance in NT incidence has been observed (1.5:1 male-to-female ratio or higher).[41] However, this has not been a universal finding or, when found, is often minor and consistent with the known slightly higher frequency of male

*Elimination of maternal and neonatal tetanus is defined as an incidence of less than 1 NT case per 1000 live births in every district. See "Public Health Perspective" later.

births (~1.05 : 1).[42,360,369,379,390] Some authors attribute male NT predominance to culturally based differences in care seeking or recall bias resulting in better case/death detection in males.[42,44,364,391,392] Indeed, many of the higher ratios (up to 4 : 1) have been reported in hospital-based studies. Others propose a true male predominance in NT incidence and death attributable to sex-related differences in cord care practices and neonatal male circumcision,[240,304] or biologic differences, including later cord separation in males.[41,74,393]

Tetanus Epidemiology in the United States

Nonneonatal Tetanus

The epidemiology of tetanus in the United States illustrates patterns occurring in all high-income countries. Death certificate data from the early 1900s and national surveillance data starting in 1947 document a relatively constant decline in the annual rate of deaths from tetanus (Fig. 58.4). The decline accelerated somewhat in the mid-1920s with introduction of equine antitoxin for prophylaxis and treatment, as well as with improvements in living standards and medical care.[325] In 1947, when national reporting began, the incidence of reported cases was 0.39 per 100,000 population.[319] By 2000–09, overall incidence had declined to 0.01 per 100,000 population.[251] Declines in incidence occurred in all age groups (Fig. 58.5), with the greatest proportional reductions in infants and those older than age 60 years. There continue to be 25 to 37 tetanus cases per year reported through 2015, per the CDC (https://www.cdc.gov/mmwr/mmwr_nd/index.html). In the 1950s, more than one-third of the deaths from tetanus in the United States were among neonates and infants,[319,320] as compared with 2000–12, when there were only three infant cases (0.8% of 373 total cases; all NT), and more than 80% of tetanus deaths occurred among persons 60 years of age and older (CDC, unpublished data) (see Fig. 58.1). Tetanus in children and adolescents is now rare: in 2001–09, only 21 cases and no deaths occurred in those 1 to 19 years of age, corresponding to an incidence of 0.002 per 100,000 population. This trend has continued through 2012.[252] A review of neonatal and pediatric tetanus cases from 1992 to 2000 found that 85% of the children were unprotected because of parental religious or philosophic objection to vaccination[332]; homeschooling also has been identified as a risk for undervaccination and tetanus in children.[333]

Among all tetanus cases reported from 1972 to 2009, 52% were in males and the overall incidence of tetanus has been similar for males and females (0.022 per 100,000 and 0.019 per 100,000, respectively). However for all except the elderly (70 years of age or older), the incidence in males was higher than in females throughout the 37-year series, including 1.7 times higher in 2000–09 (0.13 vs 0.08 per 100,000). Among cases of tetanus in the elderly, women have predominated (64% of cases in 2000–09) with correspondingly higher incidence rates (0.42 per 100,000 vs 0.38 per 100,000 in elderly men) (CDC, unpublished data).[11,251] This pattern has persisted through 2014.

Serosurveys in the United States have demonstrated similar age- and sex-related patterns of immunity.[340,394–399] A national, population-based serosurvey of more than 18,000 people older than 5 years of age from 1988 to 1994 reaffirmed the heightened susceptibility in older ages.[340] Overall, 72% of the population 6 years of age and older had protective levels, falling from more than 90% in children 6 to 11 years of age to 31% in people 70 years of age or older.[340,398] At age 70 years, only 45% of men and 21% of women had protective levels of antibody to tetanus.[340] A similar pattern of lower levels of protective antibody with increasing age was identified in a 1990 to 2000 study of emergency department patients. Although protective levels were noted in 90% or more of

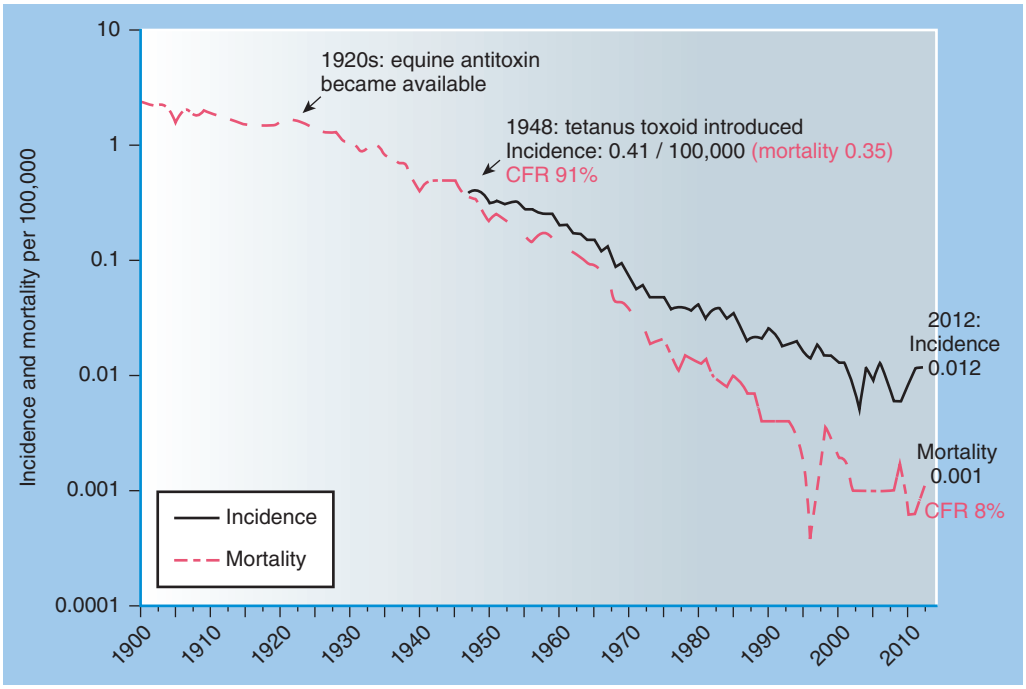


Figure 58.4. Mortality and incidence rates of tetanus reported in the United States, 1900–2012. Not all states reported deaths from tetanus until after 1932. Estimated rates shown are based on the population of the reporting states. National reporting of cases began in 1947 when widespread use of tetanus toxoid (TT) began. While tetanus mortality was declining prior to the availability of TT (see text), following its introduction, reduction in incidence, mortality, and case-fatality rates (CFRs) accelerated. *(From Centers for Disease Control and Prevention, unpublished data.)*

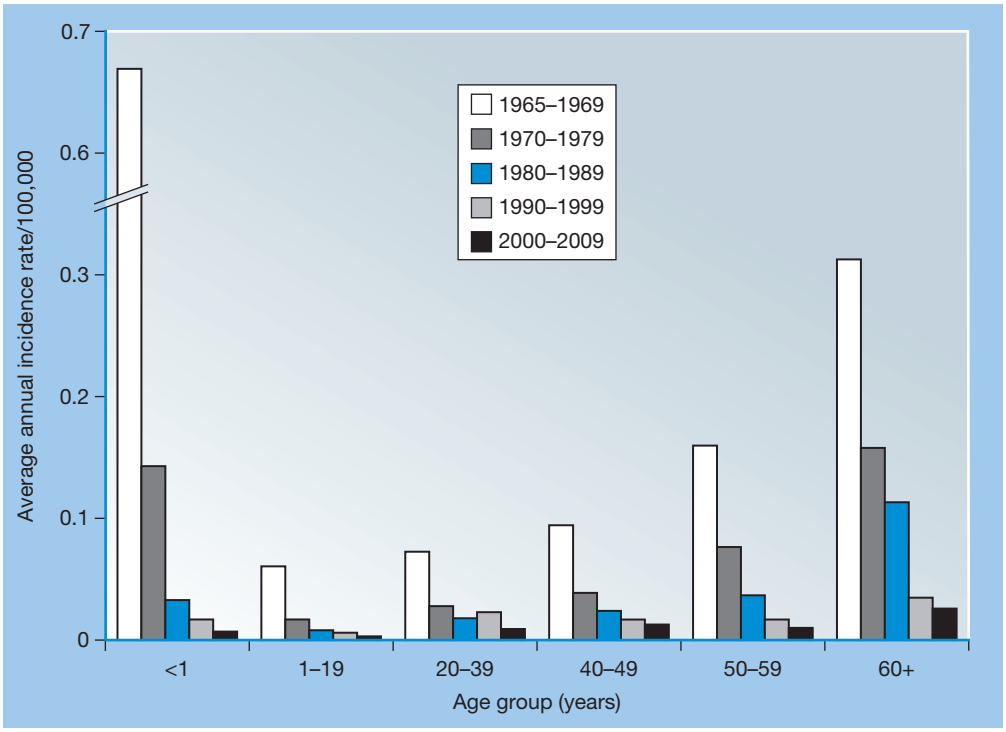


Figure 58.5. Age group–specific incidence rates of reported tetanus cases in the United States, 1965–2009. Data from 2010–12 show the same patterns seen in 2000–09. *(From Centers for Disease Control and Prevention, unpublished data.)*

patients up to 50 years of age, only 60% (both men and women) had protective antibody levels by age 70 years in that study population.[400]

Historically in the United States, tetanus was predominantly reported by states in the Southeast; incidence had a distinct peak during the summer.[17–19,319,320,325] In recent years, cases have been routinely reported from throughout the United States, and both geographic and climatic distinctions in incidence have been less apparent.[11,68–71]

In the 1950s, nonwhite people had an incidence of tetanus more than five times higher than that of whites.[319,320] By the 1990s, no substantial difference in incidence rates remained between whites (0.01 per 100,000 population) and nonwhites (0.01 per 100,000 population). However, the incidence in the Hispanic population (0.02 per 100,000 population) continues to be twice that of non-Hispanics (0.01 per 100,000 population).[11,252] Adult immigrants to the United States may be at substantially increased risk for tetanus compared with the rest of the population. A serosurvey involving 1988 patients seeking treatment at five major metropolitan emergency departments for wound care from 1998 to 2000 identified those born in Central and South America as being at significantly higher risk for nonprotective tetanus antitoxin levels.[400] Other studies in Washington, DC, Los Angeles, and New York City demonstrated increased tetanus risk in adult immigrants from Asia and Africa as well as the Americas.[397,401–404]

Clinical characteristics of non-NT cases are derived from available information on 2,012 cases reported from 1972 to 2009 (CDC, unpublished data).[11] Of 736 cases with clinical presentation reported, 88% were generalized, 12% were localized, and less than 1% were cephalic. Acute trauma of diverse severity was reported in 76% of the cases, including puncture wounds, lacerations, blunt trauma, crush injuries, burns, frostbite, surgery, gunshots, and animal bites or scratches. Seventeen percent were associated with chronic wounds and infections (e.g., skin ulcers, gangrene, abscesses, blisters, and cellulitis) or illegal injection drug use. Dental infections accounted for 0.5% of cases. Between 1987 and 2009 (when data on diabetes history were collected), patients with diabetes made up 13% of 882 cases reported and accounted for 29% of the tetanus deaths in that period. The estimated average annual incidence of tetanus among known diabetics[405] was 0.043 per 100,000 (0.061 per 100,000 among known diabetics older than 65 years of age). Patients with a history of injection drug use accounted for 10% of cases from 1990 to 2009; many had multiple subcutaneous abscesses from subcutaneous injections.[11,68–71] The steady decline in tetanus incidence among persons 20 to 39 years of age was interrupted in the 1990s by the resurgence in this cause of tetanus (see Fig. 58.5).[11,71]

Immunization histories were available for 986 (49%) of the tetanus cases reported from 1972 to 2009 (CDC, unpublished data): 163 (16%) had received at least a three-dose primary series of TT, and 62 (6%) reported that the last dose of TT was received less than 10 years before the illness.[71,251] Cases in previously immunized persons generally exhibit a milder clinical course and lower case-fatality rate than those in unimmunized persons.[406–408] Among the 163 patients who reported having had at least a three-dose primary series of TT, five deaths occurred (case-fatality rate: 3%); all of those five patients had received their last dose of TT 10 or more years before the onset of tetanus. All other deaths (526) occurred among 1849 patients with fewer than three doses of TT or with unknown vaccination history (case-fatality rate: 28%) (CDC, unpublished data).

Of the 514 patients from 1990 to 2009 with acute wounds and available medical history, 197 (38%) sought medical care for the injury; of these, 182 (92%) were eligible for TT prophylaxis with wound care, but only 79 (43%) received it (CDC, unpublished data). Prophylactic TIG was administered

to only eight (9%) of 91 patients for whom it was indicated. A review of U.S. cases reported in the 1980s and 1990s demonstrated that fewer than 60% of persons with injuries for which a tetanus booster was indicated received a dose during wound management.[409] Most patients in that series who sought medical care for the acute injury were also candidates for, but did not receive, TIG (see "Tetanus Prophylaxis in Wound Management" later). Similar findings were noted in a 2004 prospective study of wound patients treated in the emergency department of five academic medical centers: 58% of patients needing a TT dose were not vaccinated; only one of 504 patients for whom prophylactic TIG was indicated was actually given TIG.[400]

Neonatal Tetanus

In the United States, deaths from tetanus among infants declined from 0.64 per 1000 live births in 1900, to 0.07 per 1000 live births in 1930 and to 0.01 per 1000 live births by the 1960s.[48] Because tetanus among infants occurs almost exclusively in the neonatal period, tetanus in this age group has been used as a surrogate measure of neonatal disease in the United States. This decline reflected an increased prevalence of maternal immunity acquired through TT vaccination beginning in the late 1940s, as well as more hygienic birth practices, including hospital delivery. Monitoring of the incidence of tetanus among infants began in 1961; trends in incidence paralleled those in mortality. From 1967 to 1968, a precipitous decrease occurred in the incidence and mortality of reported tetanus among infants. This decrease corresponded to a sharp decline in cases reported from Texas following a rigorous vaccination campaign targeting high-risk mothers. After 1968, the incidence of tetanus among infants continued to decline but more gradually (see Fig. 58.5).

Of the 32 cases of NT reported in United States from 1972 to 2009, 28 (88%) were among infants born outside of a hospital. Nineteen of the cases (59%) occurred in Texas, which had fewer than 9% of U.S. births during that period. Only six mothers (19%) had a history of having received TT, and only one mother was reported to have received more than one dose of toxoid. Of the 28 infants with known outcome, 21 (76%) survived (CDC, unpublished data).

From 1985 to 2012, only six cases of NT have been reported in the United States: one each in 1989, 1995, 1998, 2001, 2011, and 2012 (CDC, unpublished data).[252,410–412] Three of the six mothers were foreign-born women who were undervaccinated or had unknown vaccine status; three were U.S.-born and declined tetanus vaccination on religious or philosophic grounds. In the well-documented 1998 case, the mother chose not to receive toxoid because of philosophic objection to vaccination. The infant was delivered in a hospital by cesarean section; at home, the umbilical stump was treated with a nonsterile, commercial clay powder thought to accelerate healing.[412] These cases highlight the importance of obtaining vaccination histories from all pregnant women, particularly those who are foreign-born, and of counseling women who decline vaccination about the risk of NT.

NATURAL IMMUNITY

There has been debate over whether humans can develop circulating antibody against tetanus in the absence of vaccination, an idea that is now considered discredited.[413] In the era before active immunization and standardization of the neutralization test, humans colonized with *C. tetani* were rarely and inconsistently found to have antibodies to toxin and other *C. tetani* antigens.[81,84,86,88] The majority of subsequent studies purporting to show "natural immunity" did not use an in vivo toxin neutralization assay, the definitive method of testing for biologically active antibody that correlates with clinical protection (see "Assessment of Immune Responses" later). Rather, using in vitro assays (e.g., enzyme-linked immunosorbent assay [ELISA] and passive hemagglutination), often with inappropriately low immunity thresholds, investigators reported that substantial proportions of allegedly unimmunized populations in Brazil, China, Ethiopia, India, Italy, Israel, Spain, the former Soviet Union republics, and the United States had detectable levels of antitoxin.[394–396,414–420] However, in most of these studies, detected antibody levels were 0.01 IU/mL or less, far too low to be distinguishable from nonspecific binding. "Naturally immunized" subjects failed to demonstrate the rapid rise in antitoxin characteristic of an anamnestic or "booster" response in the first week after administration of high-dosage TT (100 or 250 limit of flocculation [Lf]) in the one study assessing immunologic memory.[415] Also, the prevalence of seropositive people in these studies did not increase with increasing age as would be expected from a theoretically cumulative exposure.[413,414] Finally, vaccination histories were not confirmed and may have been inaccurate.[395,396,413,421]

PASSIVE IMMUNIZATION

Early researchers were able to prevent, modify, or treat tetanus in animal models using antitoxin prepared from large animals. The therapeutic dosages required were much larger than the prophylactic ones.[422] Passive immunization with equine tetanus antitoxin for treatment and for prophylaxis after wounds became common practice in World War I; data collected during the war provided evidence of prophylactic efficacy.[8,9,80,278] However, equine antitoxin is frequently associated with adverse reactions, including anaphylaxis and serum sickness.[176–178,423] As a means of decreasing the antigenicity of equine antitoxin for human use, proteolytic treatment was introduced to obtain cleaved Fab fragments with preserved neutralizing activity.[424] Further techniques to produce purified equine IgG antitoxin were developed, and a pasteurization step to reduce the risk of virus transmission has been tested.[425] Despite improvements in the quality of equine antitoxin, the passive immunity it confers is of limited duration. The half-life of refined equine antitoxin in humans is less than 2 weeks, and may be considerably less in previously sensitized persons.[425–427]

Human TIG was introduced in the early 1960s and was found to have a fairly constant circulating half-life of 28 days.[423,428] The frequency of local and systemic reactions associated with TIG is considerably lower than with equine antitoxin.[170,423] The greater safety and longer half-life of TIG has made it the preferred tetanus antitoxin for passive immunization. Even in the lower-income countries, TIG is beginning to replace equine antitoxin as it becomes more widely available, although the high cost of TIG compared with equine antitoxin precludes its use in many countries.[28,429] TIG is produced by cold-ethanol fractionation of the plasma of hyperimmunized adults. This preparation has been shown not to pose a risk of hepatitis transmission. It is distributed in 1-mL vials with 250 IU/mL. Recommendations for the use of TIG in patients with tetanus-prone wounds or conditions are given in "Tetanus Prophylaxis in Wound Management" later. TT is always given with TIG to induce long-lasting immunity in those with past exposure to toxoid, and to initiate active immunization in those without any prior exposure.[430–433]

Quantitation of the potency of antitoxin of animal or human origin has been standardized by comparison with an internationally standard antitoxin using fixed dosages of toxin. The international antitoxin unit defined in 1928 represented half the potency of the unit defined by the U.S. National

Institutes of Health (NIH). In 1950, the WHO reset the international standard unitage to agree with that of the NIH. Assay results before that time were generally reported in antitoxin units (or American units) per milliliter (AU/mL) using the NIH reference standard. Results using the current standard are reported as international units per milliliter (IU/mL). Before 1992, the only international reference antitoxin was of equine origin; in that year, the WHO Expert Committee on Biological Standardization established the first international standard for human anti-TIG.[434]

ACTIVE IMMUNIZATION: TOXOID

Early Approaches

The purification of tetanus toxin led to efforts to inactivate it chemically without eliminating immunogenicity. Initially, researchers used iodine trichloride. An early human challenge study and seroconversion studies supported the applied use of TT for prophylaxis in humans.[10] Later, formaldehyde emerged as the most convenient and efficient means of inactivation. In 1927, Ramon and Zoeller combined TT with diphtheria toxoid and demonstrated that there was no antigenic competition for immune responses; they also recognized NT as a disease to be prevented by immunization of pregnant women.[435] TT became commercially available in the United States in 1938, but was not widely used until the military began routine prewound prophylactic inoculation in 1941.

Toxoid Description

Currently, a high-yielding strain of *C. tetani* is cultured in liquid medium in large-capacity fermenters (up to 1000 L) to produce commercial toxoid. The medium, modified by Latham from that of Mueller and Miller, consists of a tryptic digest of casein, free of Berna and Witte peptones and other allergenic substances.[78,436] Media containing human blood group–specific substances, such as beef heart infusion broth, are specifically avoided. Data suggest that tetanus toxin production can be enhanced by bubbling nitrogen through the liquid media.[437] Extracellular toxin is harvested by filtration, purified, and detoxified for 4 to 6 weeks[438] or longer with 40% formaldehyde at 37°C. Some manufacturers detoxify the crude toxin before purification to enhance the safety of the production process.

In 1965, the WHO standardized the calibration of the potency of TT-containing vaccines and established the first international standard for TT, adsorbed, shortly after the establishment of the first international standard for TT, plain.[439] Comparison with a standard preparation using mouse bioassays allowed the establishment of international units of toxoid potency. The international standard assay was not adopted in the United States for licensure and lot standardization of TT content because of inconsistencies in results obtained from mouse assays.[440–442] The immune response in mice varies greatly depending on the mouse strain used.[441,442] In the United States, TT potency regulations require antitoxin induction of at least 2 IU/mL in the guinea-pig potency test which yields more consistent results than the mouse assay.[443,444] In late 2010, the fourth WHO international standard for TT, adsorbed, was approved.[445] The WHO now exclusively uses guinea-pig challenge assays for calibration.

In 1979, the WHO set the required potency standard for TT preparations at 30 IU per human dose. This was changed in 1982 to 40 IU per dose (60 IU in preparations of DTP).[446] Since 2003, WHO standards call for the potency of monovalent TT to be no less than 40 IU per 0.5 mL dose (determined in guinea pigs or in mice), and at least 40 IU (determined in guinea pigs) or 60 IU (determined in mice) per dose when TT is used in combination with diphtheria toxoid and whole-cell pertussis vaccine.[436,447–449] The Lf test is sometimes used as a surrogate measure of potency. Manufacturers of TT licensed in the United States indicate the quantity of toxoid in preparations by Lf content and the purity by Lf per milligram of protein nitrogen (pure toxoid has 3000 Lf/mg).[450] However, the Lf assay measures overall antigen content, which may not perfectly correlate with the level of antibody elicited. U.S. requirements for antigen content and potency for commercial TT products are discussed later (see "Dosage and Route" later).

WHO standards[436] apply as recommendations for commercial and governmental producers, and also serve as a requirement for supplies to United Nations agencies, including WHO and the United Nations Children's Fund (UNICEF).

Producers

In the 1970s to mid-1990s, local production of TT-containing products was common; in 1992, there were 63 producers of DTP/TT vaccines in 42 countries (WHO, unpublished data). The discovery of subpotent TT in use for NT prevention played a central historical role in the adoption of the 1992 World Health Assembly resolution that all vaccines used in national immunization programs should meet WHO requirements.[451–453] The effectiveness of maternal TT immunization in conferring protection against NT tetanus was well established by that time,[354–356,454–458] yet cases of NT were being reported in infants of vaccinated mothers.[149,151,359,413,459–462] A 1990 study in Bangladesh found the effectiveness of two doses of TT to be 45%; a small serosurvey supported those findings.[359] Subsequent testing at a WHO reference laboratory of TT samples from three consecutive lots produced in Bangladesh indicated no detectable potency. This prompted the WHO to review the quality-control procedures for commercial and state producers in 22 countries with a high NT burden. Only four had a functioning national control authority. Eighty lots of toxoid from 21 manufacturers were tested; 15 of the 80 failed to meet minimal potency standards.[451] Major efforts then were made to standardize the production of vaccines and to initiate and strengthen the biologics control authorities in the 22 countries. The volume of local products and producers decreased substantially as regulatory requirements to ensure vaccine quality were instituted. The regulatory requirements, and pre- and postqualification processes, developed by the WHO that are in place today ensure consistent effectiveness and safety for all vaccines procured by United Nations agencies.[453]

In the United States, TT is available in various combinations with diphtheria toxoid, acellular pertussis vaccine, and other antigens such as *Haemophilus influenzae* type b (Hib) conjugate vaccine, hepatitis B, and inactivated poliovirus vaccine. Similar preparations are available outside the United States. (See Chapter 15 for specific preparations and manufacturers.) Vaccine producers whose TT-containing products meet WHO requirements[436] and are prequalified, thus eligible for use by United Nations agencies, can be found on the WHO Immunization Standards website.[463]

Dosage and Route

As with other inactivated vaccines and toxoids, more than one dose of TT is required to confer protection and persisting immunity. When administering a primary series, there is no need to repeat doses if scheduled doses are delayed.

In North America, and universally when produced according to WHO recommendations,[436] TT preparations are given as 0.5-mL doses. Adsorbed toxoid is administered intramuscularly; fluid TT can be given subcutaneously. Either can be

given by jet injector. The toxoid content of commercial products is assessed by flocculation with standard antitoxin and measured in Lf units. This measure of toxoid protein content does not necessarily correspond to immunogenicity as measured by potency in guinea pigs. Adsorbed products available in the United States have a content of 2 to 10 Lf/dose. Potency, determined by measuring the amount of neutralizing antitoxin in serum pools from immunized guinea pigs must exceed 2 IU/mL.

Available Preparations

Whole-cell DTP, DTaP (diphtheria and TTs and acellular pertussis vaccine), and diphtheria and TTs (DT) are combinations used in infants and children younger than 7 years of age. Universal use of DTP/DTaP in infancy and childhood is recommended unless there are contraindications to pertussis vaccine, in which case DT is recommended.[173,464,465] In the United States, combination vaccines may be used when any component of the combination is indicated and if the other components are not contraindicated (see Chapter 15).

Products with reduced diphtheria toxoid content (2 to 2.5 Lf, compared with 6.7 Lf in pediatric preparations) are licensed for older children and adults. In the past, a combination of tetanus and diphtheria toxoid (Td) was used for persons 7 years of age and older in the United States. Since 2005, two Tdap products (reduced content of pertussis antigens and diphtheria toxoid) licensed for one-time use in adolescents and adults to boost immunity against pertussis as well as tetanus and diphtheria have been available.[466–469] (See Fig. 9.1 in Chapter 9 for details on recommendations and restrictions.[467]) Td or Tdap (tetanus, diphtheria, and pertussis) is preferred over single-antigen TT for tetanus prophylaxis in older children, adolescents, and adults under almost all circumstances because most persons in need of TT are likely to be susceptible to diphtheria.[466,468,469] In the United States, single-antigen TT is no longer available.

Adsorbed TT preparations are available with various precipitating salt adjuvants. In the United States, aluminum hydroxide, aluminum potassium sulfate, or aluminum phosphate is used as the adjuvant. These adjuvants induce an adequate immune response after fewer doses of toxoid than with the fluid preparation.[470] The primary immunization schedule for fluid toxoids requires four doses, whereas the adsorbed toxoid requires three. In NT prevention trials, three doses of fluid toxoid for pregnant women were necessary to achieve protective levels of antitoxin, as opposed to two doses of adsorbed toxoid.[471] In combined active–passive immunization, TIG does not substantially alter the response to adsorbed toxoid as it does with fluid toxoid.[428,433,472] Because fluid and adsorbed preparations are essentially equivalent with respect to the frequency of adverse events after immunization, adsorbed toxoid is preferred.[471,473] In the United States, Td preparations are available only as adsorbed products. Outside the United States, a calcium phosphate–adsorbed product is also available.[474] There is a theoretical but unproved possibility that calcium-adsorbed preparations may be associated with fewer reactions than the aluminum-adsorbed products.[475–477]

MenAfriVac (PsA-TT), a new Group A meningococcal conjugate vaccine with TT as a carrier protein, was designed to provide a tetanus booster dose in addition to immunization against meningococcal disease. Confirmation of its effectiveness is available.[478]

Constituents of Tetanus Vaccines

(See Chapters 5, 6, and 7 for more details on adjuvants and preservatives.)

According to WHO standards, the final bulk-purified lot of adsorbed TT should contain less than 0.2 mg/mL of free formaldehyde.[436] In the United States, regulatory requirements stipulate residual formaldehyde content of less than 0.1 mg per dose. In adsorbed products, amounts of precipitating calcium or aluminum salt adjuvant are also restricted. In the United States, a single human dose must contain less than 1.25 mg of aluminum. Preparations outside the United States may contain larger amounts of aluminum. Multiple-dose preparations of DTaP (and combinations), DT, Td, and TT products must contain an additive to prevent bacterial contamination; single-dose packaging does not require a preservative. Historically, mercury-containing thimerosal was the most common preservative, used in a final concentration of up to 0.1%, but more typically 0.01%. In response to concerns about mercury exposure from thimerosal raised in the 1990s, and to the 1999 U.S. Food and Drug Administration (FDA) call for eventual availability of thimerosal-free vaccines for infants and children, phenoxyethanol has been substituted as a preservative in preparations for children, usually to a final concentration of 0.01%. Currently, all DTaP, DT, and Tdap products used in the United States, and some preparations of Td, are either thimerosal-free or contain only trace residua from the manufacturing process (<0.00012%, corresponding to <0.3 μg of mercury per dose). Multiple-dose packaging includes 2-phenoxyethanol as a preservative, which is present in some single-dose preparations. Some Td products, TT fluid, and adsorbed multiple-dose preparations contain 0.0033% to 0.01% thimerosal as preservative, corresponding to 8.3 to 25 μg of mercury per dose. DTaP vaccines may also contain trace amounts of residual formaldehyde, residual glutaraldehyde, gelatin, and polysorbate 80.

Stability

Preparations should be stored at 2°C to 8°C and generally have a 2- or 3-year expiration date. The relative thermostability of tetanus vaccines make them less dependent on the cold chain than other immunobiologics. Ideally, higher ambient temperatures should be avoided, particularly for periods longer than 7 days, but exposure to ambient temperature for weeks does not substantially reduce the potency of the toxoid except at temperatures of 45°C or greater.[479] A recent study in rural Chad demonstrated that TT can safely be maintained in a "controlled temperature chain" at temperatures up to 40°C for 30 days or less without appreciable loss in potency.[352] However, TT exposed to 60°C is destroyed in 3 to 5 hours. Freezing can also reduce potency, particularly when the toxoid is adsorbed.[480,481] This effect is variable, but repeated freezing has been shown to reduce mean antitoxin response.[482] Adsorbed TT, whether monovalent or combined, can appear granulated after freezing has induced changes in the adsorbent, but granulation is not a reliable way to determine if the toxoid was previously frozen. The available data on temperature stability were reviewed in 1998,[483,484] with an update in 2006.[485]

RESULTS OF ACTIVE IMMUNIZATION

Assessment of Immune Responses

Active immunization confers immunity to tetanus by stimulating production of serum antitoxin. Primary immunization with TT also induces cellular immune responses (T-helper cell type 1, or type IV hypersensitivity) in 74% to 90% of recipients, which can be useful in assessing a person's capacity to mount an anamnestic response.[486] Intradermal skin testing

with TT is often used as a screen for anergy,[487–489] although false-positive reactions occur.[490]

In Vivo Assays

The gold standard for assessing a serologic immune response to TT is the in vivo toxin neutralization test, which measures biologically active antitoxin in serum.[420,471,491–493] (See "Serologic Correlates of Protection" later for a discussion of the relevance of antitoxin levels.) Neutralization tests are performed in mice injected with serial dilutions of test serum that have been preincubated with a lethal dose of tetanus toxin and standardized to a reference serum specimen. Toxin neutralization tests detect antitoxin titers as low as 0.001 IU/mL. This assay has been considered the most reliable and relevant because it assesses actual neutralization in a living host, although there is no universally accepted protocol.

In Vitro Assays

Because in vivo neutralization tests are time consuming and expensive, a variety of in vitro serologic tests have been developed. Among these are passive hemagglutination (PHA), ELISA, radioimmunoassays, immunofluorescent assays, latex agglutination, and a variety of methods using agar gel precipitation. The advantages and disadvantages of each technique have been reviewed.[413,494] In general, any of the in vitro techniques for tetanus antitoxin detection can be useful, provided that correlation with toxin neutralization has been confirmed. However, most in vitro assays have the important limitation that they do not discriminate between biologically active antibody and nonneutralizing antibody, leading to a lack of specificity that is most apparent at low antibody titers.[413] PHA and ELISA are currently the easiest to perform and the most commonly used in vitro techniques to determine tetanus antitoxin levels; the following discussion is limited to these two types of assays.

PHA techniques were the first widely used in vitro alternatives to neutralization assays.[420,494–500] Although results vary among PHA techniques, there is a good correlation with toxin neutralization at high and moderate titers of antibody. Consistency in testing among laboratories has been aided by the use of turkey erythrocytes in the assay.[494,498] However, PHA tests measure both immunoglobulin (Ig) G and IgM, perhaps preferentially IgM.[500] Only IgG has biologically relevant neutralizing activity.[491,501] Thus antibody detected at low titers by PHA, particularly those produced early in a primary immunization series, may not represent neutralizing antitoxin.[413]

The standard (indirect) ELISA measures antibody binding to tetanus toxin or toxoid that has been passively adsorbed to wells in microtiter plates (solid-phase antigen). Studies comparing the results of standard ELISA with neutralization assays in mice have demonstrated strong correlation between the two tests when ELISA titers are greater than 0.16 to 0.2 IU/mL; ELISA titers below that level significantly overestimate effective antibody concentrations.[413,502–508] Factors contributing to this lack of specificity include nonspecific antibody binding to contaminant proteins adhering to the surface of the test plate, detection of IgM in addition to IgG, and detection of antibody with little or no neutralization activity. Biologically inactive antibody can result from low avidity, asymmetric (monovalent) structure, or binding sites that recognize biologically unimportant epitopes, including those created on the toxin or toxoid molecules by the denaturing effects of solid-phase binding.[505,506,508–510] In reviewing serologic data for the licensure and regulation of TT-containing products in the United States, the FDA uses 0.1 IU/mL when determined by ELISA as the surrogate measure of the minimal level of protection.[511]

Several modifications to the standard ELISA have been used to overcome the problem of detecting biologically inactive antibody. In competitive-antigen ELISA, soluble toxin or toxoid antigen is mixed with the test serum solution, and the amount of antibody binding to soluble antigen is calculated. This assay produces results that correlate well with neutralization assays at titers as low as 0.004 IU/mL.[510,512] Another modification of ELISA that increases specificity at low antibody titers is toxin-binding inhibition. In this technique, a measured amount of toxin is preincubated with the serum specimen and subsequently exposed to antitoxin-coated plates to detect unbound toxin.[509,513,514] The most recent modification is the double-antigen ELISA, in which biotin-labeled toxin or toxoid is added to test serum that has been incubated in microtiter wells precoated with toxoid. In this assay, only antibody that is bound to both solid-phase antigen and the labeled soluble antigen is detected. The double-antigen method also shows good correlation with in vivo neutralization at antibody titers below 0.10 IU/mL.[515] Double-antigen ELISA has been recently applied in large serosurveys[272,341,516,517] and other studies.[351,518,519] Multiplex bead assays using tetanus toxoid have also been used in large serosurveys and have been shown to be accurate at detecting antibodies titers below 0.10 IU/mL.[353,519a]

A commercially available rapid test using fingerstick blood for tetanus antibody determination is discussed later in "Tetanus Prophylaxis in Wound Management." While efforts are under way to develop assays measuring tetanus antibody in oral fluid,[520] only serum assays reliably determine immunity to tetanus at present.

When reporting results of tetanus antitoxin tests, the assay method used should be stated, as well as the correlation with a neutralization assay, if known. Serosurveys to determine the prevalence of protective tetanus antitoxin in United States and Canadian populations have used standard ELISA to measure antitoxin levels. Protective immunity was defined as an antitoxin level greater than 0.15 IU/mL.[340,398,521] The use of this relatively high cutoff value underestimates the level of protection in the study populations because some people with lower antitoxin levels will be protected; however, use of a cutoff value substantially below 0.16 IU/mL would clearly overestimate the level of protection. The use of a more specific modified ELISA technique as discussed earlier may permit more accurate determinations of protective immunity against tetanus in future serosurveys.

Serologic Correlates of Protection

The minimal level of tetanus antitoxin needed to confer protection against tetanus is generally considered to be 0.01 IU/mL as measured by in vivo neutralization assay.[420,428,471,522] This cutoff level is based primarily on animal studies of the protective effects of active and passive immunization. A 1964 review by McComb of data on the reduction of tetanus in horses after homologous passive immunization found an absence of clinical tetanus in horses after injection of 1500 IU of antitoxin after acute injury (approximately 2.5 IU/kg), corresponding to a serum antitoxin level of 0.01 IU/mL.[428] These results were in agreement with earlier work showing that guinea pigs with serum antitoxin levels of 0.01 AU/mL or higher after active or passive immunization did not succumb to challenge with inocula of fixed lethal doses of *C. tetani* spores.[523] Of interest, although none of the guinea pigs with antitoxin levels of 0.01 AU/mL or higher died, 13% (with levels up to 0.5 AU/mL) developed mild, nonfatal tetanus; 7% with levels less than 0.01 AU/mL did not develop tetanus symptoms at all. As reviewed by Looney and coworkers, other animal studies of active immunization suggested incremental protection at 0.01 to 0.1 AU/mL.[524] The only experimental study in human

subjects was by Wolters and Dehmel, who immunized themselves with toxoid, achieving serum levels of 0.007 to 0.01 AU/mL, which allowed them to resist challenge with "two or three fatal doses" of tetanus spores. The actual challenge dosage is unknown.[525] Two observational studies of mothers immunized in pregnancy, and their newborns, suggested that an antitoxin level of 0.01 AU/mL was sufficient to protect infants from NT.[420,471] When standard in vitro assays are used, the minimal level of protection is taken to be 0.1 to 0.16 IU/mL,[413,510] while the 0.01 IU/mL cutoff can be used with modified ELISAs.

A number of reports describing cases of tetanus in neonates and adults with tetanus antitoxin levels of 0.01 IU/mL or higher challenge the assumption that an antitoxin level of 0.01 IU/mL (in vivo assay; 0.1 IU/mL for in vitro assay) is always protective.[146–148,150–153,526] Serum antitoxin levels detected at the time of presentation ranged from 0.15 to 25 IU/mL. The measurement of serum antitoxin levels in many of the reports was performed with in vitro methods that are known to overestimate the amount of neutralizing antibody present. In one study, the patient was shown to have a level of 0.2 IU/mL by ELISA but less than 0.01 IU/mL by neutralization assay.[150] Thus, in some of these cases, levels of biologically active antitetanus antibody may have been less than 0.01 IU/mL. However, a number of other patients had levels well above 0.01 IU/mL by neutralization assay.[146,148,151,152] Some of these patients had deep necrotic wounds. It is possible that, when large quantities of toxin are produced, a serum concentration of antitetanus antibody of 0.01 IU/mL or greater is insufficient to provide protection. Alternative explanations for these findings are that the wounds in these patients may have been sufficiently sequestered from circulating antitoxin, that toxin gained access to the nervous system before neutralization could occur, or that, in previously vaccinated persons, large dosages of tetanus toxin could lead to an anamnestic response before antitetanus antibody testing was performed. Nonetheless, these case reports suggest that protection at a 0.01 IU/mL level of antitoxin is not absolute.

Efficacy and Effectiveness

Tetanus Toxoid for Nonneonatal Tetanus

The early development of TT led to its use without the systematic safety and immunogenicity testing that is mandatory for licensure and WHO prequalification today. Some of the first data supporting the efficacy of active immunization with TT came from the self-experimentation by Wolters and Dehmel described above.[525] The first indisputable demonstration of the effectiveness of TT immunization came from its use in World War II. American troops were given a two- or three-dose primary immunization series plus postwound booster doses. Only 12 cases of tetanus occurred among 2.73 million wounded U.S. Army personnel on all fronts (0.44 per 100,000) compared with 70 of 520,000 wounded in World War I (13.4 per 100,000), and only four of the 12 had completed primary immunization.[527] Similarly, among British troops immunized with TT, tetanus incidence was four to nine per 100,000 wounded soldiers, with cases occurring predominantly in those who had evaded immunization, whereas in earlier conflicts, incidence was 22 to 147 per 100,000.[278]

Formal assessments of the efficacy of TT against non-NT conforming to the current standard of randomized controlled clinical trials have not been conducted. Instead, routine use of toxoid has been assumed to be highly effective on the basis of results of the studies on NT prevention presented in the next section, the impact that immunization programs have had on reducing both neonatal and non-NT, and the correlation of immunogenicity with protection.[356,420,471]

Tetanus Toxoid for Neonatal Tetanus

The success of TT in preventing tetanus during World War II inspired investigation into the possibility that immunizing pregnant women could prevent tetanus in their newborns. The first study to assess this possibility was a nonrandomized, controlled trial in New Guinea in 1959, that evaluated the effect of fluid TT administered during pregnancy on preventing NT. Vaccine efficacy for three doses was 94%; for two doses, 65%; and one dose of fluid toxoid had no effect.[355] A subsequent double-blind, randomized, controlled clinical trial in rural Colombia showed that two or three doses of TT administered to women of childbearing age protected their babies against NT for up to 4 years after immunization.[356] Control infants had an NT mortality rate of 78 per 1000 live births, whereas no NT cases occurred in the children of women who received at least two doses.

Subsequent field assessments of the efficacy of two or more TT doses using data collected during NT mortality surveys demonstrated effectiveness of 70% to 100%.[413,456,457,528–533] A recent systematic review and metaanalysis concluded that immunization of pregnant or childbearing-age women with two or more doses of TT reduces NT mortality by 94% (95% confidence interval, 80–98%).[534]

Factors Associated With Reduced Effectiveness

A variety of factors have been associated with apparent TT failure in addition to the use of subpotent preparations (see "Producers" earlier). These include inaccurate or unverified histories of prior vaccination, use of inappropriately short intervals between doses,[483] and poor toxoid handling, particularly repeated freezing.[12,480–483] Immunosuppression can be associated with diminished response to vaccination or accelerated loss of protection (see "Immunization in Immunodeficiency" later). If tetanus antibody levels already are high, little or no additional antibody production is induced.[524,535–537] Dietz and colleagues[483] reviewed other factors that may affect the response to TT, including malarial infection, which can decrease the response; malarial chemoprophylaxis, which can enhance it; and malnutrition, which appears not to substantially affect induction of immunity.

TT prevention of NT depends on a mother's ability to produce antibody in response to vaccination, and normal transplacental antibody transfer to the developing fetus.[42] Substantial reductions in transplacental transfer of antitetanus IgG occur in the presence of hypergammaglobulinemia, maternal HIV infection, chronic placental malaria, and prematurity.[538–547] Where HIV infection, malaria, and premature births are common, the number of newborns without adequate protection against tetanus may be unexpectedly high despite immunization programs that are apparently functioning well. A final factor that may be at work in some apparent failures of TT to prevent NT is an unusually high amount of toxin production in heavily infected umbilical stumps that overwhelms normally protective amounts of maternal antitoxin. Case investigations are critical to determine whether the predominant cause of NT in a given area is vaccine failure or failure to vaccinate. Lack of adequate vaccination is a far greater cause of NT than is vaccine failure.

INDICATIONS FOR USE

Because of the success of active immunization in the military, ubiquitous *C. tetani* spore distribution, high tetanus case-fatality rates, and frequent reactions with and incomplete efficacy of equine antitoxin,[548] routine inoculation in childhood was recommended in 1944 by the American Academy of

Pediatrics (AAP). In the mid-1940s, TT was combined with diphtheria toxoid and pertussis vaccine, which permitted administration of all three antigens with a single injection of DTP. In 1951, the AAP recommended routine use of DTP in infancy and widespread use by practitioners began. Since that time, advisory groups in the United States, including the Advisory Committee on Immunization Practices (ACIP), have recommended that all people receive three or more doses of age-appropriate TT-containing vaccine followed by routine booster doses every 10 years.[173,464,549]

Administration to Infants and Children

In higher-income countries, primary tetanus immunization schedules consist of three doses of TT in the first year of life, often followed by a fourth dose approximately 6 months to 1 year afterward.[145,173,550–553] The recommended schedule in the United States includes a primary series at 2, 4, 6, and 15 to 18 months of age with DTaP, followed by booster doses at 4 to 6 years of age (DTaP), and 11 to 12 years of age (Tdap), with decennial Td boosters thereafter (see also Chapter 9, or the CDC website, https://www.cdc.gov/vaccines/acip/recs/index.html).[549,554]

The Expanded Programme on Immunization (EPI) of the WHO, with its emphasis on protection in early infancy, recommends an accelerated schedule for primary immunization in lower-income countries with doses at 6, 10, and 14 weeks of age.[449,555,556] Other accelerated schedules (doses at 2, 3, and 4 months, or at 3, 4, and 5 months) are used in some European countries[552] and elsewhere, and are discussed in more detail later (see also Chapter 44, Table 44.5). Because combination vaccine use in infancy is the norm, the schedule for tetanus immunization is tied to the immunization requirements for diphtheria toxoid, pertussis vaccines, and other included vaccines, which in turn are driven by epidemiologic risk. The diversity of national schedules in use today is based on varying exposure or mortality risks and available health resources.[553]

TT is one of the most potent immunizing agents used routinely in children. Protective levels can be obtained with schedules starting in the newborn period.[443,557–562] Premature infants have immune responses at a given chronologic age comparable to those of term infants.[561,563]

In contrast to the immunologic response to diphtheria toxoid, which may be impeded in the presence of passively transferred maternal antitoxin, the immune response to TT has historically been considered to be minimally inhibited by maternal antitoxin.[559,560,564–566] However, many of the studies examining potential inhibition of response to TT were performed at a time before most mothers were likely to be highly immune.[567] The vast majority of women of childbearing age in the United States today have previously been immunized and have received preschool, adolescent, and possibly additional booster doses; increasing numbers of adult women in the lower income countries have also been previously immunized in infancy and childhood, or in adult life. Studies in the United States have demonstrated that term infants who have high geometric mean titers (GMTs) of circulating tetanus antitoxin before immunization at 2 months of age have an adequate response to the three-dose series.[568–572] Edwards and colleagues[570] evaluated 13 candidate acellular pertussis (DTaP) vaccines (with a U.S. schedule of doses at 2, 4, and 6 months of age) and compared the serologic responses with a whole-cell DTP vaccine licensed in the United States. Before the first dose, GMTs varied from 1.11 to 7.43 IU/mL as measured by modified PHA. After the third dose, GMTs ranged from 3.09 to 22.51 IU/mL, again demonstrating that high preimmunization antitoxin titers in children of mothers vaccinated in pregnancy do not inhibit the induction of active immunity. Although the response to TT may be lower in infants with higher preexisting maternal antitoxin who receive an accelerated primary series, this appears to be clinically irrelevant; at 6 months after completing an accelerated schedule, mean titers are still well above the minimal level of protection.[573–575]

During the first year of life, the older a child is at the time of receipt of the third dose of TT, the higher the level of antitoxin produced. Brown and coworkers studied infants beginning immunization at 3 to 7 months of age who received three doses of DTP inoculated at 1- or 2-month intervals.[576] In general, regardless of interval, GMTs were higher in infants completing the schedule at older ages (21.2 to 29.1 AU/mL; in vivo neutralization assay) versus younger ages (13.5 to 24.0 AU/mL). However, these differences are probably of no clinical significance: all infants, regardless of schedule, had antitoxin titers much higher than 0.01 AU/mL for at least 12 to 18 months after completing the initial three-dose series (the lowest was 0.125 Au/mL), and both groups had comparable GMTs. Response to the booster dose was similar under all schedules tested (GMTs of 1.2 to 2.9 AU/mL), confirming that immunization against tetanus can begin at an early age with good results. More recent studies support the results of Brown and coworkers. In the United Kingdom, the routine schedule for DTP was changed in 1992 from three doses at 3, 4.5 to 5, and 8.5 to 11 months, to 2, 3, and 4 months.[557,575] Children vaccinated with the accelerated schedule (completed at 4 months of age) had only about one-sixth the GMT of tetanus antitoxin at approximately 8 weeks after the third dose, compared with children who completed immunization at the older age (0.522 vs 3.43 IU/mL, as assessed by radioimmunoassay). By 1 year after the third dose, the differences had narrowed (0.197 IU/mL for the accelerated schedule vs 0.341 IU/mL). No child with either schedule had levels less than 0.01 IU/mL. While combination vaccines containing acellular pertussis vaccine instead of the whole-cell formulation tend to induce lower responses to TT, the difference is not clinically relevant in populations that receive recommended booster doses.

Single doses of standard- or high-potency TTs have induced protective levels in some studies.[476,577–580] In general, however, a substantial proportion of subjects did not attain minimal protective levels of antibody; those subjects who did attain a level greater than 0.01 IU/mL had levels that only slightly exceeded this minimum, and long-term follow-up was lacking. Because most antibody induced by the first dose of TT is nonneutralizing IgM, and the avidity of neutralizing IgG antibodies increases with subsequent doses,[413] a minimum of two doses of standard-potency TTs is considered necessary to reach minimal protective levels of antitoxin during the first year of life.[443,581–585] Data collected in trials of DTP vaccines in the United States indicate that three doses may be needed in infants before production of substantial antitoxin takes place and persists.[568–570]

Recommended intervals between the first two or three TT doses are generally 1 to 2 months, and there appears to be little reason to prefer one interval over another with regard to tetanus antitoxin production. Data from Brown and colleagues indicated that, whereas infants vaccinated at 2-month intervals made higher levels of antitoxin than infants vaccinated at 1-month intervals, all groups of infants had similar GMTs at the time of reinforcing doses 12 to 18 months later.[576]

After the three-dose primary series during infancy, antibody levels tend to wane relatively rapidly.[565,575,576,586–588] Although children who receive three doses of vaccine at 1- or 2-month intervals have rarely lost protective levels of antibody 1 year after the last dose, reinforcing doses at this time (a fourth dose) leads to high levels of antitoxin production and protection through school entry.[565,568,575,576,585,587–591] The

WHO Pertussis Vaccine Position Paper notes that providing the fourth DTP dose (first booster) during the second year of life is preferred for optimal protection.[592]

In children older than 1 year of age, giving two doses at intervals of 1 to 2 months appears to induce protective levels of antitoxin that persist at least 6 to 12 months.[586] A third dose given 6 to 12 months after the first two is associated with a marked booster response and persistent high levels of antitoxin. Therefore, most immunization schedules for children older than 1 year call for three doses: either two doses separated by 1 to 2 months and a third dose 6 to 12 months later, or three doses at least 1 month apart.[551,593] However, when TT is combined with pertussis vaccine, which requires a minimum of three closely timed doses (4 to 8 weeks apart), a schedule similar to that for infants is often used, with the three doses given 1 to 2 months apart.

For tetanus protection in all age groups, the WHO recommends that, ideally, all children who begin immunization in infancy with DTP should receive at least three tetanus toxoid–containing vaccine booster doses, preferably in the second year of life, between 4 and 7 years of age, and between 9 and 15 years of age.[449,555,592,592a]

Administration to Adults

The immune response to TT appears to decrease with increasing age. In comparative studies, children generally develop higher levels of antitoxin than adults.[597] Despite the decrease in immunogenicity, the majority of adult vaccinees achieve and maintain protective levels of antitoxin for many years.[473,594] Most adult primary immunization schedules in use in the world today call for two doses 1 to 2 months apart, followed by a third dose 6 to 12 months later.[449,464,595,596] Some recommend three doses with a minimum of 1 month apart.[551]

After two doses 4 weeks or more apart, almost all adults produce antitoxin levels higher than 0.01 IU/mL (or 0.10 IU/mL by in vitro assay).[597] GMTs vary according to type of vaccine and schedule but in general are below 1.0 IU/mL. In one large-scale field trial in New Guinea, titers persisted at or above minimal protective levels for 40 months in 78% of adult women given two doses of aluminum phosphate–adsorbed vaccine, and for 54 months in 33%, although it should be noted the $AlPO_4$ adjuvant concentration was twice the maximum amount used today (2.5 mg).[454] Other studies, particularly in older adults, have confirmed that maintenance of antitoxin at or above minimal protective levels is short lived without a third dose.[421,598]

A third dose 6 to 12 months after the first two doses is associated with production of high levels of antitoxin (>5 IU/mL) that have long-term duration.[597] In the elderly, the immune response may be somewhat impaired. Only 77% of elderly subjects in one study had protective levels of antitoxin 8 years after a three-dose primary series.[421] Other investigators have also indicated a weaker immune response in elderly persons.[599–604] Nevertheless, in most adults, routine boosters every 10 years are sufficient to maintain immunity.[473,591,594,600]

In the United States, to reduce pertussis risk, the ACIP recommends that a single dose of Tdap be substituted for one of the recommended Td doses in the three-dose adult primary series, or as a booster dose (see Chapters 9 and 44, or the CDC website, https://www.cdc.gov/vaccines/acip/recs/index.html).[549,554]

Administration for Prevention of Maternal and Neonatal Tetanus

For previously unvaccinated women, a minimum of two doses of TT administered at least 4 weeks apart, with the last dose given at least 2 weeks before the estimated date of delivery, provides protective levels of antibody for well above 80% of newborns. The second dose is preferably given 4 or more weeks before delivery to ensure high maternal antibody levels and maximal transplacental antibody transfer.[42,413,579]

Early clinical trials found that the effects of a single maternal TT dose for NT prevention were limited, if any were observed at all.[355,356,420,577] Thus, data do not support sufficient protection against NT when only one dose of standard potency is administered in pregnancy.[413,457] The contrary findings of partial effectiveness of a single dose for up to 4 years from a long-term follow-up study of women in Bangladesh must therefore be held in some doubt.[605] The women's immunization histories were not confirmed and may have excluded TT doses actually received, and the findings were inconsistent with an earlier report from the same study site.[456]

The estimated duration of protection against NT from two maternal TT doses is up to 3 years for most recipients; after a third dose, protection lasts up to 10 years.[42,413,454] Efficacy above 80% and long-term protection are desired, so the WHO schedule of five properly spaced doses for previously unvaccinated women is designed to elicit high antitoxin titers during pregnancy to prevent maternal and NT (MNT) (Table 58.1; see "Duration of Immunity" later).[42,413,449,606]

In low- and middle-income countries where maternal and NT burden has been greatest, national EPI programs have been delivering routine immunization with TT-containing vaccine to infants, and in some countries booster doses to children, since the mid- to late-1970s. Many women now entering their reproductive years have thus received prior TT doses. A TT schedule that considers the woman's prior history of vaccination in infancy, childhood, and adolescence has been designed to avoid giving an excessive number of TT doses—a maximum of six doses is administered from infancy through the reproductive years to prevent NT (see Table 58.1).[413,449]

In the United States, Td has long been recommended for use in pregnancy for women who are not vaccinated, did not complete a primary series against tetanus, or received the last dose of Td-containing vaccine more than 10 years previously.[607] The ACIP now recommends that Tdap be given in the third trimester of every pregnancy between 27 and 36 weeks, or immediately postpartum, to reduce the risk of pertussis in young infants (see Chapter 44).[608] If more than one dose of tetanus-containing vaccine is indicated to complete a primary series, a combination of Td and Tdap should be used, with the Tdap dose given in the third trimester. Women requiring a third dose to complete the primary series should receive follow-up to ensure the series is completed.

Duration of Immunity

After each subsequent TT injection, antitoxin levels peak within 2 weeks, fall rapidly during 2 months, and then fall more gradually in the years following (Fig. 58.6).[413] A constant log-linear decline in antitoxin level has been described.[473,590,591,609] Data on long-term protection reviewed by Simonsen generally support long-lasting persistence of protective antitoxin levels but with the need for booster doses after a primary series throughout life.[610] In studies conducted in high income countries where infant immunization starts at 2 months of age or later, an acceptable immune response is achieved after booster doses of TT even when intervals of longer than 20 years have elapsed since the last dose, although the briskness and height of response and duration of protective antitoxin levels are somewhat dependent on the length of time since the primary vaccination.[519,524,590,600,609,611–613] Work from Cameroon raises concern that when a primary series is given on the accelerated EPI schedule (doses at 6, 10, and 14 weeks), immunologic

TABLE 58.1 WHO Recommendations for Routine Immunization With Tetanus Toxoid Vaccine[a]

	Primary Series: Age/Interval Between Doses[b,c]			Booster Doses[b]		
Age at First Dose	**Dose 1**	**Dose 2**	**Dose 3**	**Dose 4**	**Dose 5**	**Dose 6**
Infancy	6 wk (min) or at 1st contact	4 (min)–8 wk	4 (min)–8 wk by age 6 mo	2nd year of life, ≥6 mo after 3rd dose (min)	4–7 y	9–15 y
Childhood (1–6 y)	At 1st contact	2 mo	2 mo	≥6 mo after 3rd dose (min)	4–7 y ≥1 y after 4th dose (min)	9–15 y
Older children (≥7 y), adolescents, and adults	At 1st contact	2 mo	6–12 mo	≥1 yr after 3rd dose (min)	9–15 y ≥1 yr after 4th dose (min)	
Women of childbearing age/pregnancy (MNT prevention) with no previous immunization[d]	At 1st contact or as early as possible in pregnancy	6–8 wk (min, 4)[e]	6–12 mo or in next pregnancy[e]	≥1 yr after 3rd dose (min) or in next pregnancy[e]	≥1 yr (min) after 4th dose, or in next pregnancy[e]	
Women of childbearing age/pregnancy who have received 3 childhood DTP doses				At 1st contact or as early as possible in pregnancy	6–8 wk (min, 4)[e]	≥1 y (min) after 5th dose, or in next pregnancy[e]

[a]For the U.S. recommendations, see Chapter 9 or http://www.cdc.gov/vaccines/schedules/index.html.
[b]Vaccine used should be appropriate for age: DTP/DT for children ≤6 y; Td/TT for doses given to individuals age ≥7 y. For children aged ≥4 y, Td may be used and is preferred. From 7 years of age only Td combinations should be used.
[c]Individuals whose vaccination series has been interrupted should have the series resumed without repeating previous doses.
[d]For details on use of Td/TT for women of childbearing age who received DTP/DT/Td/TT in childhood or adolescence, see the sources below.
[e]If administered during pregnancy, optimally given in the third trimester, at least 2 weeks before due date.
DT, diphtheria and tetanus toxoids; DTP, diphtheria and tetanus toxoids and pertussis vaccine; MNT, maternal and neonatal tetanus; Td, tetanus and diphtheria toxoids adult formulation; TT, tetanus toxoid.
From WHO Recommendations for Routine Immunization: Summary Tables: http://www.who.int/immunization/policy/immunization_tables/en/index.html and Tetanus vaccines: WHO position paper – February 2017. Wkly Epidemiol Rec 2017;92:53–76, available at http://apps.who.int/iris/bitstream/10665/254582/1/WER9206.pdf?ua=1.

memory may be lost if there are delays of 10 years or more before administering a first booster dose, necessitating a second booster dose to achieve desired antibody levels and duration of protection.[614] Further research is needed.

Studies in Denmark indicated that, after a three-dose primary series consisting of two doses separated by 1 month, followed by a third dose 9 to 12 months later, protective levels of antitoxin persisted in 96% of recipients for 13 to 14 years, and in 72% of recipients for more than 25 years.[589,609,610] For the Danish population, which had received toxoid of higher potency than that used elsewhere at the time, Simonsen and colleagues proposed a school-age booster after primary immunization and subsequent routine boosters at least every 20 years.[609]

A study in Sweden found that, after a series of three doses 4 to 6 weeks apart, antibodies at or above minimal protective levels were retained by 94% after 10 years.[615] An American study of children vaccinated at 2 to 15 years of age showed persistence in 91% of recipients for 7 to 13 years after a three-dose primary series 1 month apart without a reinforcing dose.[616] In U.S. children who received four or more doses of TT, none were found to have an antitoxin level below 0.08 IU/mL in the 90 months of follow-up.[591] A study from Finland found that, after 4 to 5 DTP doses in the first 4 years of life, a booster dose of Tdap at age 10 to 14 years protected more than 97% of individuals for up to 10 years.[616a] On the basis of this information and other studies with similar results, immunization advisory bodies in the United States have recommended that, after infant and childhood toxoid doses, boosters for routine, preexposure prophylaxis are needed no more frequently than every 10 years.[173,464]

The need for booster doses every 10 years has been questioned both because recent population-based serosurveys have supported duration of protective immunity for at least 20 years, if not substantially longer,[334,336,517,617,617a] and because few cases of tetanus, and even fewer associated deaths, are reported among people who received a primary series, regardless of whether they have received booster doses.[68–71,251,610,618–623] This low morbidity and mortality in vaccinated populations has occurred despite poor adherence to the recommendation for decennial boosters. In 2013, only 63% of adults in the United States reported having received a tetanus booster during the previous 10 years.[624] Proposed alternatives for the United States are that, after a primary childhood series and adolescent booster, no further routine assessment of tetanus immunization status should occur until the age of 30 or 50 years.[595,622,617a] TT prophylaxis in wound management would still be recommended. Gardner has argued that this alternative schedule is justified on the basis of excess adverse events attributed to TT use, and because this schedule could be more effectively monitored for compliance.[622] Some countries have revised their adult booster dose recommendations to reflect the longer expected duration of protection. For example, the United Kingdom recommends five doses of TT-containing vaccine delivered in infancy, childhood, and adolescence with no further boosters but rather TIG administration with wound care when indicated.[551,623] France has adopted an age-based approach, recommending adult booster doses at ages 25 years, 45 years, 65 years, and then every 10 years thereafter.[537,552]

Because medical care is not always sought for wounds that can result in tetanus, and tetanus boosters are not always administered for wounds and other conditions for which TT

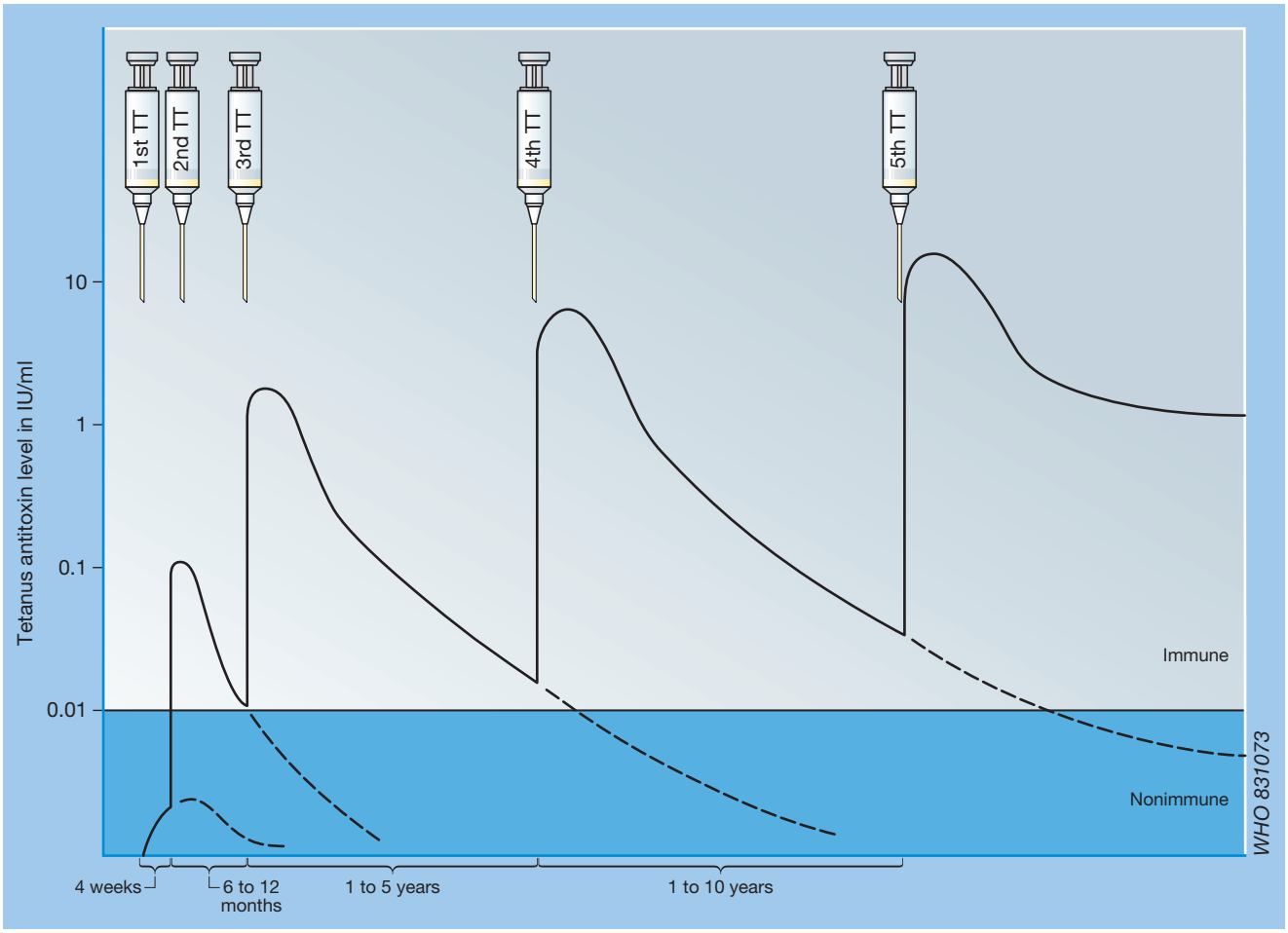


Figure 58.6. Antibody response to tetanus toxoid (TT). After five properly spaced doses, protection lasts at least 20 years and probably substantially longer for most recipients. (Threshold of protection of 0.01 IU/mL applies to values measured by in vivo neutralization or modified ELISA; for standard ELISA, the minimum level of protection is 0.10 to 0.16 IU/mL.) *(Modified from Borrow R, Balmer P, Roper MH. The immunologic basis for immunization. Module 3: Tetanus (update 2006). Geneva, Switzerland: WHO; 2007. Available at: http://apps.who.int/iris/bitstream/10665/43687/1/9789241595551_eng.pdf.)*

is indicated (see "Epidemiology" earlier), reliance on prophylaxis provided during wound care does not guarantee that those at high risk will be protected.[625] In addition, antibody response to a booster dose given at the time of wound care may not be sufficiently prompt to protect against an incubating infection.[413]

The most important rationale for the current recommendation of decennial adult tetanus boosters in North America has been to maintain serum antitoxin well above protective levels for nearly all members of the population who receive them. Although primary vaccination alone has an effect on disease severity, severe tetanus cases and deaths have been reported despite prior receipt of toxoid doses.[17,71,152,153,251,548,626] The consequences to population immunity of lack of compliance with the decennial booster policy are evident in population-based tetanus antitoxin serosurveys: the proportion of the population with antitoxin at or above minimal protective levels declines with increasing age, leaving substantial proportions of older age groups unprotected.[338,340,341] In addition, the elderly may not have as great or long-lasting a response to a single dose of toxoid as younger persons; a single booster dose at 50 years of age may not provide adequate protection,[394,421,601,603,604,627] although immunization histories in these studies were not generally verified, and some older "booster" recipients may have never received primary doses. The proportion of the unprotected in older age groups may be declining as increasing numbers of people who received childhood and adolescent immunization reach their later years.[400,517]

A cost-effectiveness analysis comparing the decennial booster strategy with that of a single booster in later life was performed by Balestra and Littenberg.[621] They estimated that decennial boosting prevented four times as many cases as a single booster at 65 years of age. They also estimated that decennial boosters were substantially less cost-effective—US$143,138 in 1993 dollars for every year of life saved with decennial booster, versus US$4527 with a single booster at age 65 years. However, the analysis did not take the costs of prophylactic tetanus boosters with wound care into account and may have overestimated the costs of complications after immunization, thereby overestimating the difference in cost between the two strategies.

The final consideration underlying the recommendation to give adults decennial boosters specifically with Td is the fact that diphtheria antitoxin levels decline more rapidly than tetanus antitoxin levels.[464,628] Given the greater health impact of decennial boosters, the ACIP has continued to recommend them.[136,173,596]

Immunization in Immunodeficiency

Because all TT-containing vaccines are inactivated, they are safe for use in immunocompromised persons, including those with congenital immunodeficiencies, HIV infection, hematologic or other malignancies, stem cell transplants or organ transplants, and chronic renal failure (CRF).[607,629,630] Most of these conditions are associated with reduced responsiveness to TT vaccination, shorter duration of protection, or both, although data are incomplete for some conditions.[630–649] Except for modified guidelines for stem cell transplant recipients and patients with CRF, general schedules for primary and booster vaccination with TT-containing vaccine are also recommended for persons with known or suspected immunodeficiency.[607,629,630] However, antitetanus antibody determinations to assess responses to vaccination and duration of protection may be indicated for immunocompromised persons to guide the use of supplemental TT doses.[643] See also Chapters 69, "Vaccination of Immunocompromised Hosts," and Chapter 70, "Vaccination of Human Immunodeficiency Virus–Infected Persons."

HIV and Tetanus Toxoid

Adults and infants infected with HIV generally respond to TT vaccination with increases in serum antitoxin levels, but antitoxin levels tend to be lower than in uninfected controls, particularly in those whose $CD4^+$ cell counts are less than 300×10^6/L.[518,543,630,632,633,635,650] Although most infants with perinatally acquired HIV infection respond normally to TT-containing vaccines, serologic responses to vaccination decline as HIV disease progresses.[634,635,651] The duration of tetanus antitoxin at or above minimal protective levels in HIV-infected patients is uncertain, although there is evidence that antibody levels are shorter lived than in those who are uninfected.[630,652,653] A 2010 review of immune responses in HIV-infected children on highly active antiretroviral therapy (HAART) concluded that immune reconstitution did not guarantee long-term protection. Children on HAART require monitoring and additional booster doses as indicated.[654] The same is quite likely true for adults on HAART: poor responsiveness to TT has been demonstrated in older subjects on HAART.[655] The AAP recommends that TIG be administered to HIV-infected patients with tetanus-prone wounds, regardless of history of tetanus immunization.[173] See also "Factors Associated with Reduced Effectiveness" earlier and Chapter 70.

Transplantation and Tetanus Toxoid

Vaccination recommendations for transplant patients differ for stem cell transplant recipients and solid-organ recipients. After stem cell transplantation, patients are initially severely immunocompromised and require reconstitution of immunity against vaccine-preventable diseases once their acquired immune system has matured.[656,657] Three doses of age-appropriate TT-containing vaccine are recommended; the doses should be given at 12, 14, and 24 months after transplantation.[638,656] There is evidence that vaccinating the stem cell donor 6 to 10 days before marrow harvest results in the transfer of antibody-synthesizing B cells and of antigen-specific T cells to the recipient, thereby conferring some protection during the first year after stem cell transplantation and augmenting the response to active immunization given during the second year.[638]

In contrast to the situation with stem cell transplantation, information about tetanus immunity and responsiveness to TT after solid-organ transplantation is sparse. Solid organ transplantation patients require lifelong immunosuppression to prevent organ rejection and to preserve allograft function, and are therefore at increased risk for infections. Children who undergo successful renal or hepatic transplantation appear to retain normal responses to TT despite immunosuppressive therapy.[658,659] Adult renal transplant patients also retain responsiveness to TT, although the level of antitoxin produced is significantly lower than in healthy controls.[639] Vaccination strategies vary among transplant centers, and sufficient information is not currently available to mandate a change in the formal recommendations for vaccination in solid-organ transplant recipients. Thus, the standard schedule for primary and booster TT vaccination continues to be recommended for both child and adult solid-organ transplant recipients.[638] See also Chapter 69.

Renal Failure and Tetanus Toxoid

CRF is associated with immune deficits caused by uremia-induced alterations in cell-mediated immunity and IgG_1 production.[639,658,660] Studies of adults with CRF, including those receiving chronic dialysis, demonstrate a variety of defects in responsiveness to TT. The proportion of patients with CRF with protective levels of tetanus antitoxin at baseline is lower than in healthy populations, as is the proportion of patients who seroconvert in response to TT.[639,640] Mean antitoxin levels produced by patients with CRF who respond to toxoid are lower than those produced by healthy controls, and the duration of protective immunity in patients with CRF is reduced.[639,641,642] More than one booster dose may be necessary to achieve protective antitoxin levels in adult patients with CRF, and boosters may be required more frequently than every 10 years to maintain protection. Monitoring antitoxin levels after vaccination and at periodic intervals thereafter is indicated in this population to direct individual vaccination strategies. In contrast to adults, children receiving hemodialysis for CRF have a normal initial response to vaccination with DTP, although the duration of protection has not been determined.[661] Most infants receiving peritoneal dialysis also have a normal response to routine DTP vaccination, maintaining antitoxin at or above minimal protective levels for 24 months.[662]

Adverse Events After Immunization

Whereas mild local reactions are relatively common after receipt of TT, more serious reactions, including neurologic and hypersensitivity reactions, are exceedingly rare.[663] The rates and severity of adverse events in recipients of TT are influenced by the number of prior doses and the level of preexisting antitoxin, perhaps the amount of toxoid in the dose, the type and quantity of adjuvant, the route of injection, the presence of other antigens in the preparation, and perhaps the presence of organomercurials used as preservative. Causality assessments of the role of TT in adverse reactions are complicated by the frequency with which it is given in combination with other antigens. Systematic reviews by the Institute of Medicine and the Agency for Healthcare Research and Quality concluded that evidence was adequate only to convincingly support a causal relationship between TT and anaphylaxis among the 16 potential serious adverse reactions examined.[663,664]

The most common adverse event after injection of TT is a local reaction. Local reactions are reported in 0% to 95% of recipients, depending on the definition. Most studies suggest that rates of local reactions increase with an increasing number of doses.[597,624,665,666] This occurred when annual boosters were given for wound management.[591] However, boosting with Tdap with every pregnancy is now recommended because of the risks of pertussis morbidity and mortality in the newborn

and the lack of apparent increased risk of local reactions when given to nonpregnant subjects as close as 4 to 5 years apart.[666a,666b] Studies on short intervals (i.e., within 21 days or ≤2 years) between administration of Td and Tdap or Tdap-inactivated polio vaccine in healthy, nonpregnant adolescents and adults found no serious adverse events.[666c,666d,666e] Among recipients of a booster dose of adsorbed toxoid, 50% to 85% experience pain or tenderness at the injection site, and 25% to 30% experience edema and erythema.[667–671] More severe local reactions characterized by marked swelling occur in fewer than 2% of vaccinees.[665,672]

Some investigators claim that increasing antigen content (Lf) increases local reactions, but there is no clear evidence of this.[665,666,673] However, higher levels of preexisting antitoxin are associated with higher rates of local reactions and with reactions of greater severity.[324,665–668,673–676] Persons who have a rapid immunologic response (within 4 days), suggestive of priming by past doses, have higher rates of local reactions than persons whose immunologic response is slower.[324,666,673,675,676] Massive local reactions, such as swelling from elbow to shoulder, have occurred most often in persons with a history of multiple booster doses of toxoid. Typically, these reactions began within 2 to 8 hours after toxoid administration in the deltoid. Serum antitoxin levels in these persons ranged from 2 to 160 IU/mL, manyfold higher on average than levels in persons without reactions, or in those with systemic adverse events.[673,677–680] The pathogenesis of massive local reactions is not fully understood. Preformed antibody apparently forms complexes with the deposited toxoid to induce an inflammatory response (i.e., Arthus reaction, or type III hypersensitivity).[677,680] Other undefined host factors are involved because the range of antitoxin levels in persons who exhibit this response is wide and includes levels seen in persons without such a response. In persons with a history of massive local reactions after TT, boosting with lower than standard doses of TT (e.g., 1 Lf) has been proposed by some researchers to lead to an adequate immune response without significant adverse events.[666,673,681] Antitoxin levels in such persons should be evaluated before booster doses are given to ascertain whether there is a need for additional doses. Most other severe local reactions can be prevented by avoiding unnecessary boosters of TT in wound management (see "Tetanus Prophylaxis in Wound Management" later).

There are conflicting reports as to whether aluminum adjuvants increase the incidence of local reactions compared with fluid toxoids.[470,667,668,676,682] Aluminum adjuvant in adsorbed products can theoretically invoke local inflammatory responses more frequently than fluid toxoid through adjuvant-induced complement activation and macrophage stimulation.[683] Histologic evidence suggests that aluminum hydroxide crystals may remain at the injection site after intramuscular injection of aluminum hydroxide–adsorbed vaccines in some persons, possibly creating local inflammation. However, the clinical implications of this finding, if any, are unclear.[684–686] Differences in study results could arise from manufacturing differences resulting in variable adsorption and adjuvant activity.[683] Alternatively, toxoid potency may have varied (with or without equivalent Lf content), a problem found in all comparisons of adverse events after TT administration. A 2004 systematic review found the quality of evidence from studies evaluating aluminum adjuvant–induced reactions to be low, but concluded that there was significantly more local erythema and induration in young children and more injection site pain in older children who received vaccines with aluminum adjuvant compared with fluid vaccine.[687] However, there was no evidence of increased serious adverse reactions, and the authors recommended that concerns about aluminum adjuvant be laid to rest.

The frequency of local reactions is increased when toxoid is administered subcutaneously rather than intramuscularly.[665] This is particularly true for adsorbed toxoid[688]; aluminum adjuvant can cause sterile abscesses when given subcutaneously.[683] Use of jet injectors, which deposit some toxoid in the subcutaneous tissue, results in twofold higher rates of edema at the administration site than intramuscular injection by needle.[689]

In the United States, TT is generally given in combination with diphtheria toxoid, with or without pertussis antigens.[145,173,607] (Adverse effects of diphtheria toxoid- and pertussis-containing vaccines are addressed in Chapters 19 and 44.) In some controlled trials, minor adverse events such as swelling or pain occurred more frequently after Td than after TT alone (Williams JJ, Ellingson HV. Field trial of commercially prepared diphtheria-tetanus toxoid: immunization reactions among recruits. Unpublished report to the Armed Forces Epidemiologic Board, 1954),[669] whereas other studies have not found a substantial difference in the rates of local reactions with Td compared with TT, or found that TT was associated with higher rates of local reactions than Td.[670,671,690] Increasing frequencies of large local reactions after use of DTaP vaccines have been observed; the pathogenesis of these reactions is not well understood but may be related in part to gelatin content.[691,692]

Reactogenicity data indicate that Tdap is well tolerated in older children, adolescents, and adults. In large, randomized, controlled trials evaluating a single booster dose of Tdap conducted in the United Kingdom and North America, the most commonly reported side effect was local injection site reaction in 5% to 78% of recipients. Other adverse events were mild and resolved without sequelae.[693] Comparative studies of Tdap preparations with Td indicate that although local reactions were more frequent in the groups receiving Tdap products, the increases were not of clinical importance (e.g., "any pain" occurred in 75% of recipients after Tdap, and in 72% after Td).[692] Tdap also is well tolerated when concurrently administered with other vaccines such as influenza and meningococcal vaccines.[694]

Up until 1999, the preservative used in U.S. preparations of TT was exclusively thimerosal. This mercury-containing compound has been linked to delayed-type hypersensitivity reactions in some people.[681,695] It is uncertain to what degree hypersensitivity to thimerosal influences local reactions when given intramuscularly. Reports have rarely linked reactions with an exaggerated delayed-type hypersensitivity response to TT itself.[677,696–698] No long-term effects of thimerosal use have been identified.[699] Nonetheless, concerns over exposure to mercury led the FDA to undertake a review of all medicinal products containing the metal, including vaccines. Similarly, the European Medicines Evaluation Agency's Committee for Proprietary Medicinal Products requested vaccine manufacturers to ultimately eliminate organomercurials as preservatives in their products.[700] In the United States, all DTaP and DT products administered to infants and children younger than 7 years of age are either thimerosal-free or contain trace amounts (see "Constituents of Tetanus Vaccines" earlier).[701,702] Other TT-containing products that are thimerosal free or contain only trace amounts are also available in the United States.

Systemic reactions after TT inoculation are less common than local reactions. Fever can accompany a local response, particularly when there is a marked local reaction or when antitoxin levels are high.[669,672,680] Uncommonly, persons with high antitoxin levels experience high fever and malaise without experiencing substantial local reactions, primarily after receiving booster doses of toxoid.[680] Clinically significant reactions are not substantially more frequent after receipt of Td than single-antigen TT[703,704]: booster doses of Td are associated with

fever in 0.5% to 7% of recipients, and temperatures above 39°C are rare (Williams JJ, Ellingson HV. Field trial of commercially prepared diphtheria-tetanus toxoid: immunization reactions among recruits. Unpublished report to the Armed Forces Epidemiologic Board, 1954).[669,672] Other systemic symptoms such as headache or malaise are reported less frequently than fever.[666,672] Lymphadenopathy also can occur after toxoid inoculation.[668,677] Comparative studies of Tdap and Td indicate that although local reactions, fever, and headache were significantly more frequent in the groups receiving Tdap products, the increases were not of clinical importance (e.g., fever after Tdap in 5% of recipients vs 3% after Td).[466,469,692,693]

Peripheral neuropathy, particularly brachial plexus neuropathy, has been reported to occur hours to weeks after TT administration.[705–712] These reports and laboratory findings have been reviewed.[712–714] Although anecdotal, the reports are consistent with neuropathy as a manifestation of immune complex disease similar to that occurring after equine tetanus antitoxin.[715] The Vaccine Safety Committee of the U.S. Institute of Medicine concluded in 1994 that there is evidence to support a causal association between TT and brachial plexus neuropathy.[714] The evidence was considered inadequate to accept or reject a causal relationship of TT with mononeuropathy. On the basis of two published case series from the Mayo Clinic of brachial plexus neuropathy that included cases after TT exposure,[710,711] the Institute of Medicine committee estimated that 0.5 to 1 case of brachial neuropathy per 100,000 recipients was attributable to TT within 1 month of immunization.[714] Given that this estimate was based solely on open case series that include six toxoid-exposed patients with unknown total immunization histories,[710,711] the calculated attributable risk may have been overestimated. Passive surveillance of events following immunization in the United States for 1991 to 2003 detected 49 verified reports of brachial neuritis occurring 0 to more than 60 days (median: 2 days) after administration of TT-containing vaccines (38 toxoid alone, 11 in combination). The ratio of reported cases to doses distributed was 0.69 cases per 10 million doses.[716] This strongly suggests that the true frequency of occurrence is much less than 0.5 to 1 case per 100,000 recipients.

There have been reports of at least 25 cases of Guillain-Barré syndrome (GBS) after TT.[705–708,714] One patient had relapsing GBS signs and symptoms after each of three injections of toxoid.[706] Although there may be a causal relationship between TT and GBS, the occurrence is rare and available evidence neither adequately supports nor excludes the association.[663,664] An estimate of the incidence of GBS after TT administration is 0.4 per million doses.[714] Population studies do not support a causal role for TTs in GBS. A study of 700,000 children who received DTP found a frequency of GBS after immunization that was similar to expected background rates.[717] After administration of TTs to an estimated 1.2 million children and adults, another study found a lower frequency of GBS after immunization than expected by chance alone.[718] The ACIP has recommended that GBS occurring less than 6 weeks after receipt of a TT-containing vaccine is a precaution for subsequent TT-containing vaccines.[550]

Other neurologic events, including seizures and acute encephalopathy, have been reported after TT or DT.[680,714,719,720] The data are insufficient to support a causal relationship with any of these other illnesses.[663,664,714] As is true with all immunizations, the occurrence of systemic symptoms soon after inoculation does not in itself indicate causation; the association may instead reflect an unrelated chance occurrence. A metaanalysis performed on case-control studies reported conflicting conclusions of the relationship of multiple sclerosis with tetanus immunization[721]; this analysis and additional reports suggest that TT administration may lower the risk of developing multiple sclerosis.[722,723]

TT occasionally induces an IgE response, particularly with aluminum-adsorbed toxoid.[475,724–726] Although true anaphylactic (type I hypersensitivity) reactions to purified TT are very rare,[714,727–729] the Institute of Medicine has concluded that evidence is sufficient to accept a causal relationship between TT and anaphylaxis.[664] Before 1942 in the United States, contaminating peptones from the culture media and silk fibers from the filtering process were present in toxoid preparations. Two cases of anaphylaxis were reported after administration of 61,000 doses (0.003%) of these vaccines.[730–733] In a large series using Air Force recruits in the early 1960s, serious allergic reactions attributable to toxoid occurred in 0.001%.[734] Passive surveillance in the United States from 1991 to 1995 for serious allergic reactions including laryngospasm, bronchospasm, or anaphylaxis yielded a rate of 1.6 serious allergic reactions reported per million doses of publicly distributed Td (CDC, unpublished data).

Skin testing has been suggested as an aid to assessing the risk of anaphylaxis with TT administration in persons with a history of hypersensitivity reactions after tetanus vaccine. However, skin tests are of limited benefit and confer some risk themselves.[681,735] In a study of Air Force recruits with a history of an anaphylactic reaction after a prior TT dose, an intradermal skin test was negative in 94 of 95. All recruits tolerated full-dose challenges of toxoid, suggesting lack of IgE-mediated hypersensitivity in the prior reaction. Positive skin test responses were found in study subjects who subsequently tolerated full-dose challenges.[681] However, in another study, anaphylaxis was reported in three of 200 persons undergoing skin testing who had a history of sensitivity; similar reports have been made by others.[729,735] Thus, undue weight should not be put on the results of the tests alone because of the lack of specificity,[736] and caution must be used if skin testing is performed.

When eliciting a history of allergic reactions to TT, attention should be paid to the details of the injection and the reaction. One potential problem with histories of reactions in older subjects is confusion between equine antitoxin and TT. An adverse reaction after a "tetanus shot" received before TT became widely available may suggest that equine antitoxin or another product was received. When the history is consistent with an immediate hypersensitivity reaction, testing for serum antitoxin level may be helpful to evaluate the actual need for a booster of toxoid. In the context of emergency wound care, such results are unlikely to be immediately available. In that case, skin testing before challenge could be attempted in a setting equipped to manage anaphylaxis (see Jacobs et al.[681] for the protocol).[681] If skin testing is not feasible, or is deemed imprudent, TIG can be used for immediate tetanus prophylaxis, and decisions about the approach to booster administration can be deferred until the results of serum antitoxin testing are available.

Simultaneous Administration With Other Vaccines

TT has been combined with diphtheria toxoid, pertussis vaccines (both whole cell and acellular), hepatitis B, inactivated poliovirus, and Hib conjugate vaccines without compromising the immune response or substantially enhancing adverse events associated with the tetanus component (see Chapter 15). Combination with whole-cell pertussis vaccines enhances the serologic response to diphtheria and TTs because of the adjuvant properties of whole-cell vaccines.

With the use of Hib–polyribosylribitol phosphate (PRP) polysaccharide conjugate vaccine covalently linked to TT (PRP-T), theoretical concerns were raised whether TT in the

conjugate vaccine could inhibit or enhance a response to TT. These concerns have not been shown to be clinically relevant. Initial antitoxin responses are slightly higher after simultaneous administration of DTP vaccines with PRP-T compared with DTP alone, but overall antitoxin levels are well above protective levels regardless of whether DTP is administered with PRP-T. Maternally acquired tetanus antitoxin does not interfere with the immune response to PRP-T.[737] PRP-T alone induces a tetanus antitoxin response that is substantial but lower than the response to TT;[738] thus, PRP-T vaccines cannot substitute for TT. Use of simultaneous PRP-T with DTP does not appear to increase the frequency of common adverse events and does not appear to differ in this respect from other Hib conjugate vaccines.[739] DTP combined with PRP-T vaccine in the same syringe is equivalent in immunogenicity and safety to DTP with PRP-T vaccine administered separately.[740,741]

Contraindications and Precautions

A careful history of possible adverse events is necessary because some patients may have confused equine antitoxin with toxoid. Conditions recognized by the ACIP and the Committee on Infectious Diseases of the AAP as contraindications to the use of TT include a history of severe hypersensitivity or a neurologic event after a prior dose.[173,464] The evaluation should distinguish between a neurologic illness, for which further doses are contraindicated, and other syndromes, such as syncope, for which they are not. When toxoid is contraindicated, TIG should be given if immune status is unknown and a wound occurs that is not clean and minor. TIG is not necessary for clean and minor wounds. Delayed-type hypersensitivity to thimerosal is not a contraindication to products containing this preservative because it would be manifested as a subcutaneous or cutaneous reaction without systemic effects.

Studies consistently fail to identify teratogenic or any other harmful effects when TT is given in pregnancy, with or without diphtheria toxoid and pertussis vaccine.[742–747] However, in settings where providers are confident that women will return for visits later in pregnancy, it may be prudent to delay immunization to the second trimester to minimize any concern of a perceived relationship with any observed birth defect.

TETANUS PROPHYLAXIS IN WOUND MANAGEMENT

All people seeking treatment for care of skin wounds or infections should be evaluated for tetanus prophylaxis. The lesion should be treated as medically indicated, including removal of foreign bodies, debridement of devitalized tissue, incision and drainage, and irrigation to prevent or eliminate an anaerobic environment and bacterial contamination.[211,748–751] The patient's history of TT immunization should be obtained, with attention to the number of prior doses as well as the interval since the last dose, bearing in mind that histories collected during emergency department and office visits may not be accurate, potentially overestimating protection among those claiming up-to-date status and underestimating protection in those whose histories suggest incomplete immunization.[400,752–758] The critical issue is to determine whether the person has completed a course of primary immunization; if that is in doubt, consideration must be given to administering TIG if warranted.

In the 1950s, a booster dose of toxoid was recommended for every wound if more than 1 year had elapsed since the last dose. The association of Arthus-type reactions with frequently repeated tetanus boosters, and a better understanding of the kinetics of antibody responses after booster administration, led to a reappraisal of this practice.[473,587,591,616,679] Table 58.2 summarizes the guidelines for the use of TT and TIG for tetanus prophylaxis in wound management based on the current recommendations of the ACIP.[173,464,549,751] Tdap should replace a single dose of Td in wound management for adults who have not previously received a dose of Tdap.[145,554,759,760]

TABLE 58.2 Summary Guide to Tetanus Prophylaxis in Routine Wound Management, United States

History of Adsorbed Tetanus Toxoid (Doses)	Clean, Minor Wounds		All Other Wounds[a]	
	Td[b]	TIG	Td[b]	TIG
Unknown or <3	Yes	No	Yes	Yes
≥3[c]	No[d]	No	No[e]	No[f]

[a]Including, but not limited to, wounds contaminated with dirt, feces, soil, saliva; puncture wounds; avulsions; and wounds resulting from missiles, crushing, burns, or frostbite (see text).
[b]For children age <7 y, Tdap (DT, if pertussis vaccine is contraindicated) is preferred to TT alone; whole-cell DTP may be used in other countries. For children age 7 to 9 y, Td is preferred to TT alone. Tdap is preferred to Td for persons age ≥10 y who have never received Tdap; Td is preferred to TT for persons aged ≥10 y who received Tdap previously or when Tdap is not available. (If TT and TIG are both used, Tetanus Toxoid Adsorbed rather than Tetanus Toxoid for Booster Use Only [fluid vaccine] should be used.)
[c]If only three doses of fluid toxoid have been received, then a fourth dose of toxoid, preferably an adsorbed formulation, should be given.
[d]Yes, if >10 years since last dose.
[e]Yes, if >5 years since last dose. (More frequent boosters are not needed and can exacerbate side effects.)
[f]Yes for persons with severe immunodeficiencies, including HIV infection.
DT, diphtheria and tetanus toxoids for pediatric use; DTP, diphtheria and tetanus toxoids and pertussis vaccine; Td, tetanus and diphtheria toxoids for adult use; Tdap, tetanus toxoid and reduced content diphtheria toxoid and acellular pertussis vaccine for adult use; TIG, tetanus immunoglobulin; TT, tetanus toxoid.
From Advisory Committee on Immunization Practices (ACIP): ACIP Vaccine Recommendations. Available at: http://www.cdc.gov/vaccines/acip/recs/index.html, and recommendations of the American Academy of Pediatrics in Tetanus (lockjaw). In: Kimberlin DW, Brady MT, Jackson MA, et al., eds. Red Book: 2015 Report of the Committee on Infectious Diseases. *30th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2015:775–776.*

Although any wound can give rise to tetanus, clean wounds are considered to have a low likelihood of tetanus spore contamination and of developing the anaerobic and acidic conditions that promote spore germination. People with a history of three or more doses of toxoid but without detectable antitoxin develop detectable antibodies within 4 to 7 days after the receipt of a subsequent dose.[524,611,613,761,762] Thus, for people with clean wounds, a TT booster is recommended if the patient has received fewer than three doses of adsorbed toxoid in the past, or if more than 10 years has passed since the previous toxoid dose. TIG administration is not necessary. Except for the elderly and some immigrants, most people in the United States will have antitoxin at or above minimal protective levels before exposure (for groups more likely to be unprotected, see "Epidemiology" and "Duration of Immunity" earlier).

Wounds contaminated with dirt, feces, or saliva; deep wounds and punctures; and wounds with devitalized tissue, such as burns, frostbite, gangrene, and crush injures, are considered more likely to develop *C. tetani* infection and toxin production. Abscesses, cellulitis, chronic ulcers, and other wounds in patients with diabetes mellitus or illicit injection-drug use should also be considered "tetanus prone." Because antitoxin levels may have fallen to barely protective or to nonprotective levels in some patients after 5 years, the current

recommendation for patients with wounds that are other than clean and minor is to receive a dose of toxoid if more than 5 years have elapsed since the last dose.[145,173,549,591,751] In addition, patients who have received fewer than three prior toxoid doses, those whose vaccination histories are uncertain, and those with immunodeficiencies, including HIV infection (see "Immunization in Immunodeficiency" earlier) should receive prophylactic TIG for such wounds. When administered at a separate site, TIG does not interfere with the immune response to adsorbed TT.[428,433,472]

The dosage currently recommended by the ACIP and the AAP for prophylactic TIG is 250 IU given intramuscularly.[173,464] This dosage will provide antitoxin levels above 0.01 IU/mL beginning 2 to 3 days after administration and lasting for at least 4 weeks in almost all persons.[178,433,763–767] A concern that higher TIG doses are necessary to guarantee protection has led some authorities to recommend the range of 250 to 500 IU for wound prophylaxis.[163,751,768] The uncertainty about the optimal dosage for prophylactic TIG stems from reports of tetanus in people with circulating levels of antitoxin greater than 0.01 IU/mL (see "Serologic Correlates of Protection" earlier), and the report of fatal tetanus despite prophylactic administration of 250 IU of TIG.[769] Dosages of 400 to 500 IU may induce protective levels earlier than a dosage of 250 IU,[770] and may produce serum antitoxin levels of up to 1.0 IU/mL.[178,423,771] Because patients who receive prophylactic TIG do so in the context of professional wound care, they are less likely to develop tetanus, or tetanus infection with high levels of toxin. Thus, a TIG dosage of 250 IU is appropriate in most circumstances; however, if wound care has been delayed or if a wound appears to present a high risk of tetanus, the higher dosage of 500 IU may be considered.

Before TIG was widely available, antibiotics were used for tetanus prophylaxis in wound management as a substitute for, or as an adjunct to, equine antitoxin.[772] Smith and earlier researchers demonstrated the efficacy of prophylactic antibiotics in animal models[773]; however, efficacy superior or equal to that of antitoxin in the prevention of tetanus in humans was never proven.[774,775] Antimicrobials are not currently recommended for tetanus prophylaxis per se.[748,774] Instead, wounds should be observed for signs of infection and treatment should be initiated promptly if any infection occurs.

Reviews of tetanus cases reported in the United States from 1972 to 2009 demonstrated that many people who subsequently developed tetanus did not seek wound care or received less than the recommended prophylactic care when medical treatment was sought for wounds.[11,68–71,251,409] Several studies suggested that, when patients with wounds do seek care in the United States, substantial proportions (up to 96%) receive fewer prophylactic measures than recommended, whereas 8% to 17% receive Td, with or without TIG, when not indicated.[68,251,776–778] Failure to provide recommended prophylaxis was most common for patients who had not received primary immunization and who had tetanus-prone wounds—that is, for those in greatest need of appropriate prophylaxis.[400,776]

Failure to comply with recommended prophylactic guidelines has been described outside the United States as well.[758,779–782] A rapid immunochromatographic test that identifies tetanus antitoxin levels above 0.1 IU/mL has been evaluated for performance and cost-effectiveness in several emergency departments and outpatient travel and geriatrics clinics.[754–758,783–789] While specificity was very high, sensitivity ranged from 55% to 92%, with a median of 86%. Most false-negative tests occurred with lower antibody levels (between 0.1 and 0.5 IU/mL by standard ELISA), levels for which a tetanus booster would be reasonable.[780,786,788,789a] A more recent validation of tetanus rapid tests against in vivo neutralization assay showed a specificity of 1%, suggesting further validation studies are needed. Although use of the test could reduce errors in both over- and undertreating patients, the price is sufficiently high (and the cost of a tetanus booster is sufficiently low) that its cost-effectiveness may be limited to subgroups of patients, such as those younger than 65 years of age with tetanus-prone wounds for whom substantial unnecessary TIG administration could be averted.[756–758,783,787] Given that the major problem in appropriate prophylaxis is physician unfamiliarity with guidelines and the tendency to ignore them, substantial efforts are needed to educate healthcare providers and improve overall use of prophylactic measures before cost savings from a point-of-care rapid tetanus antibody test can be realized.

PUBLIC HEALTH PERSPECTIVE

Elimination of environmental exposure to *C. tetani* is impossible. However, TT is one of the most effective and safest biologics available, so the health burden of tetanus is almost completely preventable through routine immunization. Although advances have been made in the care of tetanus patients, tetanus remains a severe and lengthy disease with high mortality, especially for patients who do not have access to modern intensive care. It also is a very expensive disease: the average direct cost of care for tetanus patients in the United States from 1988 to 2001 was estimated to be US$84,000 (range: US$1700 to US$925,000 in 2005 dollars); the estimated total annual cost exceeded US$12.6 million.[11] In California, the median cost of hospital charges from 2008 to 2014 was US$166,259 (range: US$22,229 to US$1,024,672).[778] In France, the 2010 cost (direct and indirect) of a tetanus case was estimated to be €209,000 (about US$277,000 in 2010 dollars).[787] Data from Ibadan, Nigeria indicate that the cost of treatment for tetanus there was one to three times the average yearly income of the patients.[790]

Nonneonatal Tetanus Disease Control

In high-income countries, and those low- and middle-income countries that provide tetanus booster doses, the lowest seroprevalence of tetanus antitoxin and the highest incidence rates of tetanus disease are in the oldest age groups and in immigrants from countries with less-developed immunization programs (see "Epidemiology" earlier).[334–336,338,340,341,400,403,404,517,623] To achieve further progress in preventing tetanus in these more affluent nations, efforts are needed to increase compliance with national recommendations for primary immunization and booster doses, with particular attention to the elderly and foreign born.[595,596]

The WHO recommendations for use of TT-containing vaccine reflect the changing global picture of tetanus, with decreasing tetanus in neonates and young children and persistent burden in adolescents and adults, especially males (see "Epidemiology" earlier).[449] The recommendations now encompass tetanus prevention in all ages and both sexes (see Table 58.1). Because non-NT is beginning to predominate globally, the public health gains and reductions in tetanus-specific costs could be substantial if countries are able to phase in the recommended booster doses.

Maternal and Neonatal Tetanus Elimination

History of Neonatal Tetanus Elimination, 1974–1999

Efforts to decrease the incidence of NT in the lower-income countries began in earnest when the EPI was launched by the WHO in 1974; TT for women was among the original six recommended antigens. The WHO recommended NT prevention through training of traditional birth attendants to provide

clean delivery services and administration of TT to pregnant women.[606] Uptake of TT for women was slow compared with DTP for infants: reported administration of a second or higher dose of TT in pregnancy (TT2+) was 9% in 1980 and 26% in 1985, while coverage with DTP3 rose from 20% to 50% over the same period (see Fig. 58.3).[42]

The recognition in the 1980s of the enormous neonatal mortality caused by tetanus, coupled with the availability of an inexpensive and effective vaccine, led to the 1989 World Health Assembly resolution to eliminate NT worldwide by 1995.[791,792] The annual number of NT deaths at that time was estimated to be 787,000, or about six deaths per 1000 live births (see "Epidemiology" earlier).[792–794] The core strategies proposed by the EPI Global Advisory Group included rapidly increasing both the TT coverage for all women of childbearing age and the proportion of deliveries respecting the "three cleans" (clean hands, clean delivery surface, and clean cutting and care of the umbilical cord), adopting a "high-risk approach" to designate high-priority geographic or socioeconomic areas for elimination activities, and encouraging the adoption of NT incidence rate as a health indicator.[792]

In 1993, a specific definition for elimination of NT as a public health problem was adopted: less than one case of NT for every 1000 live births per year in all districts (third administrative level) of a given country.[242] Because the disease tends to cluster in hard-to-reach areas whose high disease burden may be obscured at the national or provincial level, elimination efforts had to be brought to the most remote and inaccessible communities to meet the intent of the World Health Assembly resolution.

Countries with relatively well-established health services and adequate internal resources made rapid progress toward NT elimination in the 1990s, principally by increasing routine use of TT in pregnancy. By 1996, reported TT2+ coverage had increased globally to 57%; the estimated number of global NT deaths dropped 44% to approximately 438,000; and 27 of the 90 countries that did not meet NT elimination criteria in 1990 had achieved that goal.[795] By 1999, an additional five countries attained elimination status; NT remained a public health problem in 57 countries.[796,797]

High-Risk Approach

The "high-risk" approach, initially pioneered in the Americas in the early 1990s, was instrumental in progress toward the global elimination goal.[42,242,792,795,796,798–803] The high-risk approach focuses available resources on districts or subdistrict areas with reported NT incidence of more than one case per 1000 live births or, if surveillance is unreliable, on areas where relevant EPI and Maternal and Child Health indicator data suggest substantial disease risk.[42,792] Once high-risk areas are identified, all women of childbearing age (usually 15 to 45 years of age) are targeted for TT immunization through mass campaigns (supplemental immunization activities [SIAs], or TT SIAs). Three rounds of immunization are performed to provide protection for up to 10 years. The immunization of all childbearing-age women circumvents limitations in access to routine maternal health services[241,804] and addresses tetanus after septic miscarriages, abortions and other wounds.[301,805] Because NT often clusters in areas consistently missed by routine services, the high-risk approach can reduce NT burden dramatically even though national-level indicators do not change appreciably (Fig. 58.7).[803]

Program Monitoring and Evaluation

To monitor the progress in TT delivery, the first programmatic indicator adopted was TT2+, the annual proportion of pregnant women who had received a second or higher dose of TT during pregnancy. At the outset of the program, few women in countries with significant NT burden had received prior immunization with TT, so immune protection was almost exclusively obtained from doses received in pregnancy. However TT2+ now often underestimates the true level of protection against MNT because TT-containing vaccine received in childhood, prior pregnancies, SIAs, and at other health encounters, is not usually taken into account. Thus, reported TT2+ coverage now underestimates the true proportion of women with protective antibody levels.[242,351,806–808]

An alternative indicator, protection at birth (PAB), was developed to improve the accuracy of monitoring program effectiveness for countries with high DTP1 coverage.[792,808] With the PAB method, health workers determine if infants coming for a first DTP dose were protected against tetanus at birth based on their mothers' full immunization history with TT-containing vaccine. An additional benefit of the PAB method is that mothers who are eligible for a TT dose can be immunized on the spot, thereby protecting future pregnancies. Although PAB is more reliable than TT2+, its relative complexity has prevented its routine use in most countries.[808]

PAB measurement can also be performed during nationally representative surveys investigating immunization coverage and other health indicators. A mother's cumulative TT immunization history is obtained and used to judge her immunity status at the time of her last delivery.[808,809] Although survey PAB measurement provides a more accurate measure of protection than TT2+, in populations where a high proportion of women received infant DTP doses and/or TT doses later in childhood, protection still will be underestimated because those doses are usually not taken into account.

Serosurveys have provided an immunologic assessment of the true protection of mothers and infants, and the correlation between maternal histories and actual protection.[353,512,543,807,810,811] A 1989 study in Burundi demonstrated good correlation of serologic protection with PAB estimated from maternal recall.[807] Similar studies in the Central African Republic in 1996 and Cambodia in 2012 showed that maternal history underestimated true protection: PAB by maternal recall was 74% and 83%, respectively, whereas seroprevalence of protective antitoxin levels (≥0.01 IU/mL, double-antigen ELISA) was 89% and 97% among parous women.[351,353]

Reliable surveillance for NT is the best method for monitoring program success. After implementing the high-risk approach, countries are encouraged to strengthen their NT surveillance systems to monitor intervention results and focus future activities on populations in greatest need. NT case investigations are performed to determine whether the case occurred as an isolated failure of the mother to access health services, or as a more systematic failure of the health system to provide immunization and clean delivery services to the community in which the case occurred.[161,797]

Maternal and Neonatal Tetanus Elimination Initiative: 2000 to Present

By 1999, progress toward NT elimination had slowed. Reported TT2+ coverage had stagnated at around 60% (see Fig. 58.7),[812] and the proportion of pregnant women with access to clean deliveries had not changed greatly since 1989, remaining well below 50% in many low-income countries.[42,796,797,813] Many of the 59* countries that had not achieved elimination faced

*The total number of high-risk countries increased from 57 to 59 when Timor Leste and South Sudan gained independence in 2002 and 2011, respectively.

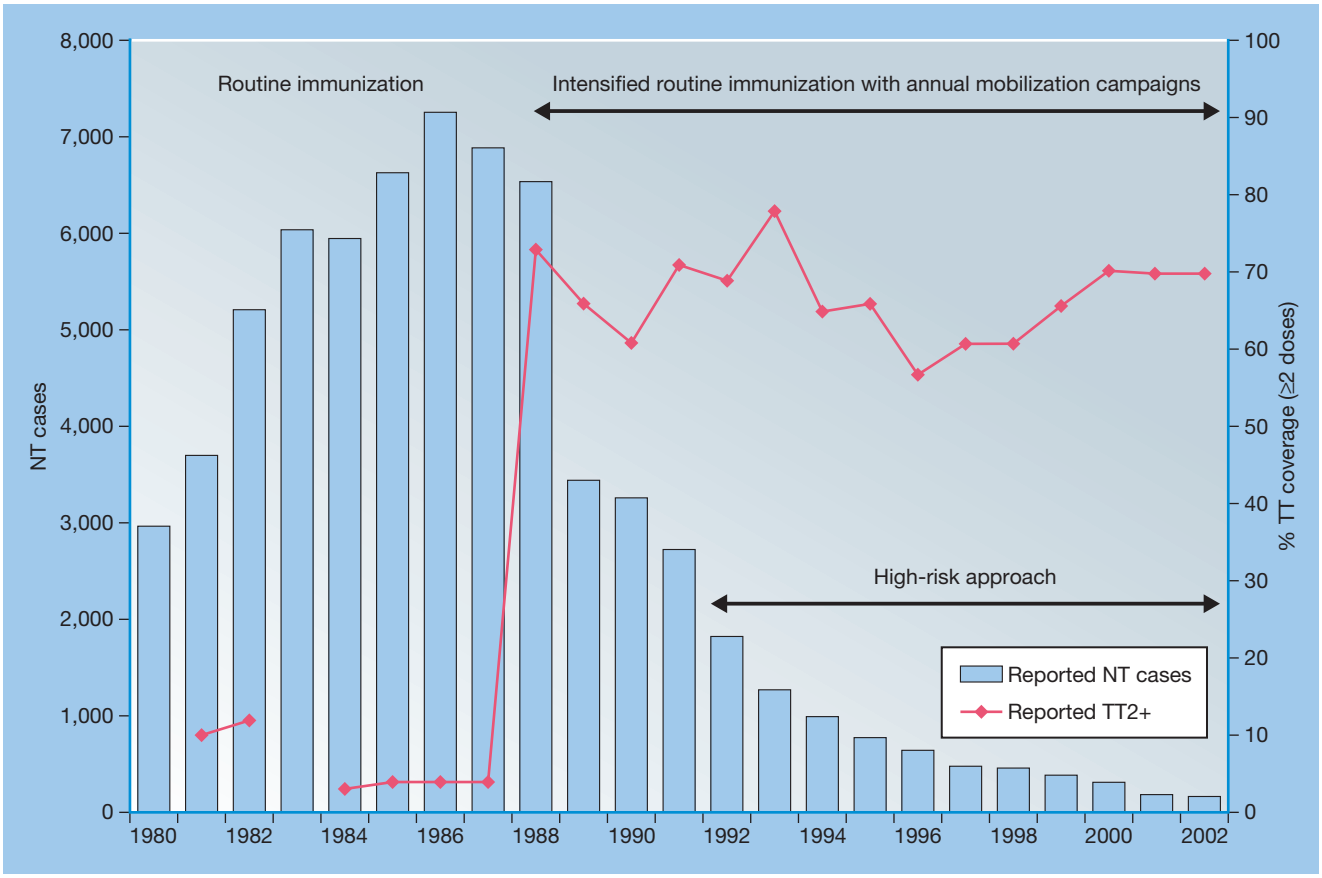


Figure 58.7. High-risk approach in Egypt. Tetanus toxoid (TT) supplemental immunization activities (SIAs) can produce a rapid reduction in neonatal tetanus (NT) cases and deaths when SIA coverage is high. Because SIAs target a limited number of communities with high NT burden, the reduction in NT incidence is often more dramatic than improvements seen in national-level TT or protection at birth coverage estimates. *(Modified from the World Health Organization's Department of Immunization, Vaccines, and Biologicals database and Expanded Programme on Immunization. Progress towards neonatal tetanus elimination, 1988–1994. The "high risk" approach: the WHO-recommended strategy to accelerate elimination of neonatal tetanus. Egypt. Wkly Epidemiol Rec.1996;71:33–36.)*

substantial health infrastructure weaknesses, ongoing civil unrest or conflict, and severely limited health budgets.[42,43,243]

In that context, UNICEF, the WHO, and the United Nations Population Fund forged a stronger partnership, with UNICEF taking the lead. Because maternal tetanus (tetanus during pregnancy or within 6 weeks of the end of pregnancy) shares the same risk factors and means of prevention as NT, the partnership amended the global goal to include elimination of maternal tetanus, with an operational target of 2005[243,796,797]; the program name was revised to the Maternal and Neonatal Tetanus (MNT) Elimination Initiative.[814] The traditional strategies of routine immunization of pregnant women, clean delivery and cord care practices, and effective NT surveillance continue to be promoted. Use of the high-risk approach was vigorously expanded using a more standardized method to identify high-risk areas requiring TT SIAs, and campaign achievements are closely monitored.[42,243,796,797,814]

Since 2000, 53 countries have used the high-risk approach, delivering at least two TT doses to more than 130 million women; 23 countries conducted TT SIAs in 2010 alone.[243,814] By the mid-2000s, reported TT2+ again began to rise, reaching 75% in 2009. A formal survey method was developed to accurately assess whether a country had met the definition of NT elimination.[815] If NT elimination has been achieved, elimination of maternal tetanus is assumed.[796] Since 1999, 41 of 59 countries at risk for MNT were validated as having eliminated MNT (Fig. 58.8). Among the remaining 18 countries, MNT elimination was confirmed subnationally for more than 90% of the populations of Ethiopia (except Somali Region), for the Philippines (16 of 17 regions) and for the Punjab province of Pakistan; many others also are nearing the validation process.[243,814]

A major impediment to program implementation in some countries has been dissemination of misinformation about the purpose of vaccination campaigns. Allegations that TT is a covert contraceptive or sterilizing agent disrupted immunization of adolescents and women in Cambodia, Cameroon, Kenya, Mexico, Nicaragua, Papua New Guinea, the Philippines, and Tanzania.[816–819] Such allegations, which circulate freely on the Internet, are based on suspicions and fears that the true aim of tetanus immunization is to reduce fertility. Because MNT prevention campaigns specifically target women of childbearing age, local populations are particularly vulnerable to these rumors if distrust of government or international agencies already exists. In areas where the fervor of public accusations has been extreme—leading to court injunctions or political posturing—the level of disruption has greatly affected women's, and even children's, immunization.[816–820] These accusations continue to circulate and must be combated with vigorous education campaigns involving credible community leaders in anticipation of problems.

Use of TT to prevent NT is highly cost-effective both when delivered as part of routine prenatal packages and when given in the context of SIAs.[243,821–827] It costs approximately

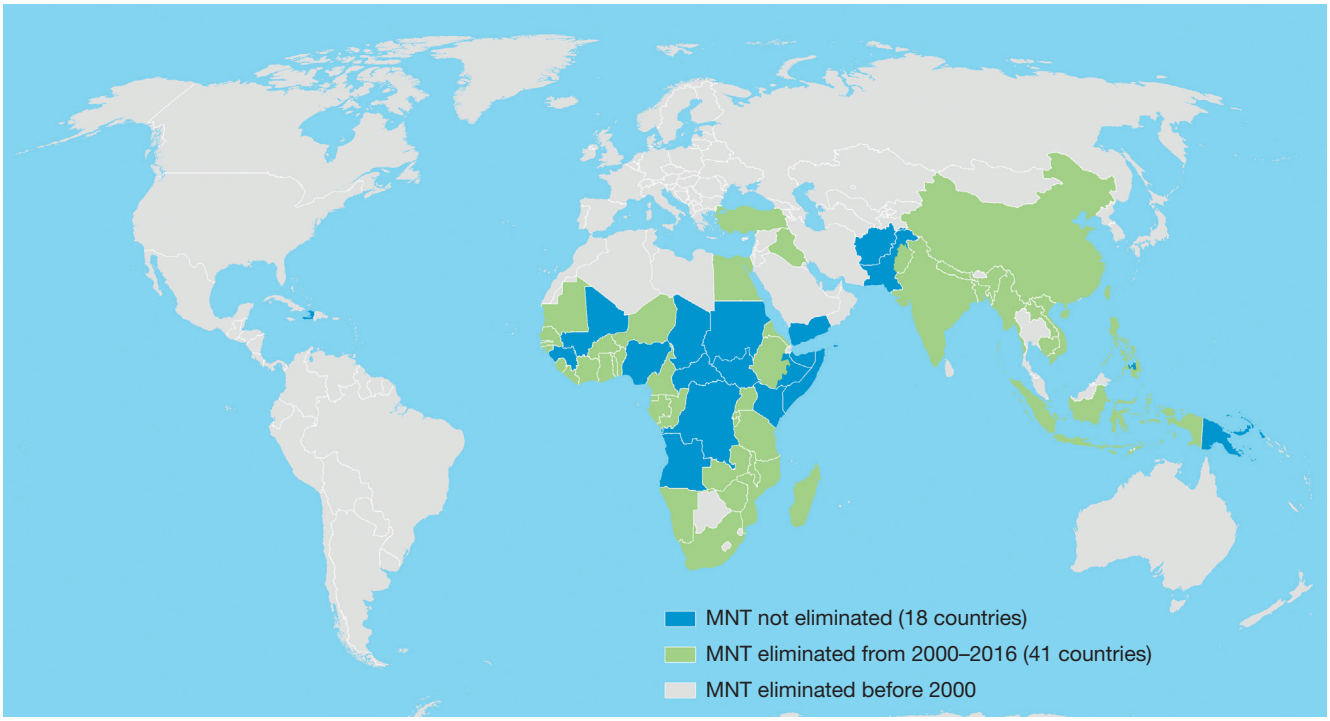


Figure 58.8. Global maternal and neonatal tetanus (MNT) elimination status by country, October 2016. MNT elimination is defined as <1 case of neonatal tetanus (NT) per 1000 live births in each district (third administrative unit) of a country. Formal validation of apparent elimination has been underway since 2000 (see text); most of the 18 countries that have not yet achieved elimination status are nearing that goal. Also shown are that 16 of 17 regions of the Philippines have validated MNT elimination, as has Ethiopia except for the Somali region, and Punjab province of Pakistan. *(From the World Health Organization. Maternal and Neonatal Tetanus (MNT) Elimination: The Initiative and Challenges. Available at: http://www.who.int/immunization/diseases/MNTE_initiative/en/.)*

US$2.00 to deliver three doses of toxoid by supplementary immunization.[534] A 2004 study using data from TT SIAs conducted in a rural district of Pakistan with a high NT burden confirmed current cost-effectiveness: the cost of three rounds of supplementary immunization was US$117 per averted death.[825]

Once countries achieve MNT elimination, ongoing support to health infrastructures is essential to continue and expand access to clean deliveries, to maintain high routine immunization coverage with TT for women and DTP for infants, and to introduce appropriate booster doses of TT-containing vaccine.[42,243,449,828] Use of school-based immunization strategies is proving to be an effective approach for delivering booster doses in many countries.[243,829,830] Enhanced NT surveillance and ongoing monitoring of immunization program quality indicators are necessary to identify areas of increased risk for MNT requiring targeted interventions to maintain elimination status.

References for this chapter are available at ExpertConsult.com.

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59 Tickborne Encephalitis Vaccines

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Tickborne encephalitis virus (TBEV) is a member of the genus *Flavivirus*, family Flaviviridae, which comprises approximately 70 viruses that cause many serious diseases, including dengue, Japanese encephalitis, West Nile virus neuroinvasive disease, and yellow fever. TBEV is one of the major human pathogenic flaviviruses, with disease being caused by three subtypes termed European or Central European (TBEV-Eu), Far Eastern or Russian Spring Summer encephalitis (TBEV-Fe), and Siberian (TBEV-Sib).[1] TBEV is the prototype member of the mammalian tickborne virus complex (previously known as the tickborne encephalitis [TBE] serocomplex), a group of genetically and serologically related viruses including Omsk hemorrhagic fever virus (OHFV), louping ill virus, Langat virus (LGTV), Kyasanur Forest disease virus (KFDV), Powassan virus, and Alkhumra virus. OHFV, KFDV, and Alkhumra virus are also associated with hemorrhagic fever, while Powassan virus and louping ill virus mostly cause subclinical disease with occasional cases of encephalitis. LGTV does not typically infect humans and appears to be naturally attenuated.

The manifestations of the European subtype were first described in 1931 by Schneider,[2] who reported a seasonal outbreak of meningitis in the district of Neunkirchen in Lower Austria. Shortly afterward, a similar outbreak was reported from the far eastern region of Russia. Beginning in 1939, a number of outbreaks were also reported in the European region. More recently, disease outbreaks in Siberia were related to the viral subtype, TBEV-Sib. In summary, the disease is now seen throughout most of Europe, except in the Southern regions, and in Russia, and Asia, corresponding to the distribution of Ixodid tick species, the chief vector of TBEV. The disease has also been referred to as spring-summer meningoencephalitis, central European encephalitis, Far Eastern encephalitis, Taiga encephalitis, Russian spring-summer encephalitis, biundulating meningoencephalitis, diphasic milk fever, Kumlinge disease, and Schneider disease.

TBEV is a major cause of neurological disease in humans living in endemic areas. According to a study conducted in 1958 prior to use of vaccine, 56% of all viral central nervous system (CNS) infections in Austria were caused by TBEV.[3,4] Before the start of the vaccination program, it was the most frequent CNS infection in adults, with several hundred hospitalizations reported each year.

During the last 25 years between 5000 and 12,000 clinical TBE cases have been reported annually worldwide. However, the number of cases of TBE is considered to be underreported, both in countries where the disease is well known and in regions where TBE is unrecognized. In addition, the increasing exposure of the mobility in people to the outdoor environment increases the risk of infection. Although there are several safe and effective vaccines available, TBE remains a growing public health problem in Europe and parts of Asia.

BACKGROUND

Clinical Description

Neurologic symptoms seen with TBE are similar to other forms of acute viral meningoencephalitis.[5] The clinical course of TBE can be either monophasic or biphasic. If biphasic, the disease starts with an influenza-like illness, followed by a few days of well-being, and then a second phase with neurological symptoms. While the biphasic course is typical for TBEV-Eu (72–87%) and TBEV-Sib (21%), the TBEV-Fe subtype is primarily a monophasic neurologic illness.[6–8]

After infection by an infected tick, there is an asymptomatic incubation period that lasts between 4 and 28 days, with most cases ranging between 7 and 14 days. The first stage of clinical illness, lasting for 1 to 8 days and corresponding to the viremic phase, is characterized by nonspecific influenza-like symptoms such as fatigue, headache, aching back and limbs, nausea, and general malaise. Temperatures generally rise to 38°C or higher. Very high initial temperatures have been reported, particularly in children.[9,10]

An afebrile interval then follows the first stage of TBE, lasting 1 to 20 days, with most patients being asymptomatic during this time. Then another sudden rise of temperature marks the beginning of the second stage of the disease. The clinical manifestations during this second stage are far more serious than during the first. In approximately 50% of cases there is CNS involvement in the form of meningitis with pleocytosis. Approximately 40% of cases have more-severe disease with signs of encephalitis, including paralysis, stupor, and pyramidal tract signs. Meningoencephalomyelitis, defined as neurological symptoms including spinal nerves, the most-severe form of the disease, occurs in approximately 10% of patients and is more common in older individuals.[10] In the paralytic forms of the disease, often seen in the region of the shoulder girdle, paralysis develops within 5 to 10 days after the remission of fever in the course of the second phase. Paralysis may progress for up to 2 weeks, followed by a tendency toward improvement. The overall risk of long-term sequelae increases with the severity of the neurologic symptoms.[10] One study showed that 80% of TBE patients with meningoencephalomyelitis did not fully recover within a 10-year follow-up period.[11]

Approximately 5% to 12% of patients require intensive care secondary to respiratory paralysis or serious disorders of consciousness.[9] Hospitalizations last between 3 and 40 weeks,[10] depending on the severity of the illness and long-term rehabilitation is required for approximately 20% to 30% of hospitalized patients.[11]

Not all persons infected with TBEV experience the monophasic or full biphasic spectrum of clinical disease. Seroconversion without obvious disease is common. On average, approximately 25% of infected persons develop clinical symptoms.[5] Differences in proportions have been reported by viral subtype and age. In children, the proportion of inapparent infection may be higher and normally results in seroconversion and establishment of immunity.[6,12]

Among those who develop clinical illness, 3% to 8% of TBEV-Fe patients, 21% of TBEV-Sib patients, and 72% to 87% of TBEV-Eu patients will experience the full biphasic course of disease; all other patients will experience the first phase of influenza-like illness only. In a minority of cases, the infection is inapparent during the first stage and the onset of clinical illness shows the characteristics of the second phase of the disease.[5]

Disease manifestations vary by age.[5,7–9,13] Older patients tend to have more-severe disease and suffer more neurologic sequelae, whereas in children asymptomatic infection is more common.[10] However, there are scattered reports of severe, even fatal cases in very young children.[14–19] In children and adolescents, meningitis rather than encephalitis is the predominant form of the disease. However, between 35% and 58% of hospitalized patients younger than age 15 years have long-term cognitive or neuropsychiatric sequelae, including sensory disturbances, ataxia, dysphasia, spinal nerve paralysis, hearing loss, concentration difficulties, memory impairment, and emotional instability.[5,7,13] Patients older than 40 years tend to have the encephalitic form of the disease, and those older than 60 years tend to have the most-severe disease, including paralysis and seizures, with the highest case-fatality rate.[5,10,20] In Austria, Germany, Sweden, Switzerland, and Lithuania, more than 50% of TBE cases are reported in people 50 years of age or older.[20] Thus, the elderly are a special target group for immunization.[12] In children younger than age 7 years, TBE tends to be less severe, with a lower probability of permanent sequelae.[10] However, a recent small-scale study suggests that children also have a significant risk of incomplete recovery.[21]

Although assessing clinical severity can be difficult when based on case reports from healthcare systems that differ in access to healthcare, case definitions, and diagnostic procedures, disease manifestations appear to differ by virus subtype.[5] The clinical course of TBEV-Sib in children is more severe than TBEV-Eu, with half experiencing encephalitis.[14] However, complete recovery from TBEV-Sib disease is reported in 80% of cases.[15]

The clinical presentation of TBEV-Fe differs from TBEV-Eu. TBEV-Fe presents more gradually, with a prodromal phase that includes fever, headache, anorexia, nausea, vomiting, and photophobia. These symptoms are followed by stiff neck, visual disturbances, and variable neurologic dysfunctions, including paresis, paralysis, sensory loss, and seizures. In a TBEV-Fe outbreak in Novosibirsk hemorrhagic manifestations in the form of gastrointestinal bleeding and local hemorrhages on mucosa and skin were observed.[16] It has been reported that only 25% of patients with TBEV-Fe will recover without sequelae.[15] Occasional chronic infections have also been reported.[22]

Besides age, the case-fatality rate of TBE appears to depend on the virus subtype. The case-fatality rates have been reported to be 1% to 2% in TBEV-Eu, 6% to 8% in TBEV-Sib, and up to 40% in TBEV-Fe.[5,8,12,17,18] However, the higher rates reported for TBE-Sib and TBE-Fe may be impacted by limited access to medical care or different healthcare seeking behavior of patients in the eastern TBE-endemic regions.[18]

A postencephalitic syndrome, with symptoms persisting beyond 3 months, has been described for all viral subtypes.[19,23] Chronic forms of TBE occur in 1% of cases caused by TBEV-Sib and have been verified by viral isolation.[5,22] Additionally, years after initial disease caused by TBEV-Fe or TBEV-Sib, rare cases of progressive neuritis and epileptic seizures have been described, but definitive proof of an etiological link to TBEV is lacking.[24]

Virology and Pathogenesis

The TBEV particle is icosahedral, approximately 50 nm in diameter, and consists of an electron-dense spherical nucleocapsid of approximately 30 nm in diameter, surrounded by a lipid bilayer. In sucrose density gradients, purified virus sediments at about 200S and bands after equilibrium centrifugation at a density of about 1.19 g/cm.[21] The viral genome is single-stranded, positive-sense RNA of approximately 11,000 nucleotides in length. The many strains of all three subtypes have been sequenced (including vaccine strains TBEV-Eu–Neudörfl, K23, and TBEV-Fe–Sofjin, TBEV-Fe–205, and TBEV-Fe–Senzhang).[25,26] There is 16% or less nucleotide variation between TBEV-Eu and TBEV-Fe subtypes and only 4% or less amino acid variation between the subtypes, including less than 2.2% amino acid variation between the different TBEV-Eu strains. Consequently, the three subtypes of TBEV are serologically closely related and vaccines against one subtype induce cross-protective immunity to all three subtypes (see below). Although initial studies suggested that the three TBEV subtypes were restricted to geographically distinct locations, phylogenetic studies demonstrate that the three subtypes of TBEV overlap geographically.

Mature virions are composed of three structural proteins: envelope (E), core (C), and membrane (M) proteins, with molecular weights of 55,15, and 8 kDa, respectively. The E protein is glycosylated and exists as 90 copies of a dimer orientated parallel to the viral surface as shown by x-ray crystallography.[27] A glycosylated precursor of the membrane protein (prM) is present in immature virus particles. The mature nonglycosylated M protein is derived by a furin-mediated cleavage from the precursor protein prM. C is the only protein constituent of the isometric nucleocapsid that contains the virion RNA. The virus RNA also encodes seven nonstructural (NS) proteins. The coding sequence of the positively stranded RNA is 5′-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3′. All viral proteins are encoded within a single open reading frame and the individual proteins are released from a precursor polyprotein by co-and posttranslational cleavage.[28] Structural elements of the E protein are involved in the binding of virions to cell receptors, receptor-mediated endocytosis, and low-pH–dependent membrane-fusion. The E protein contains the important antigenic determinants responsible for hemagglutination inhibition (HI) and neutralization. E protein epitopes induce protective immune responses in the infected host and monoclonal antibodies to these epitopes have been extensively studied.[29,30]

After the bite of an infected tick, the virus replicates in the dermal cells at the site of the bite. Then the virus is transferred through afferent lymphatic vessels to the regional lymph nodes where further replication occurs. The virus then spreads via the lymphatic system and the bloodstream, and invades susceptible tissues, including the reticuloendothelial system. There extensive viral replication occurs, leading to seeding of the CNS. High viral loads are required for the virus to cross the blood–brain barrier since the capillary endothelium is not easily infected. To evade host defenses, TBEV rearranges intracellular membranes to prevent recognition of double-stranded RNA (formed during virus replication) by cytoplasmic pathogen recognition receptors,[31] allowing an approximate 24-hour delay in interferon induction. The interferon response is further reduced by the NS TBEV protein NS5 that blocks JAK/STAT (Janus kinase–signal transducer and activator of transcription) signaling.[32,33] How TBEV enters the CNS is poorly understood but likely involves either crossing the blood–brain barrier via olfactory or autonomic neurons.[34] Animal models suggest that $CD4^+$ T cells may have a role in limiting the severity of TBE, while $CD8^+$ T cells seem to be mediators of immunopathology.[35,36]

Diagnosis

Because the clinical manifestations of TBE are nonspecific, TBE must be confirmed by laboratory techniques. In the initial viremic phase of the disease, the virus can be identified through inoculation of serum in a suitable cell line or in suckling mice.[37] However, polymerase chain reaction (PCR) technology has largely replaced such culture technologies for

virus identification.[38] With the onset of the second phase of the disease, the virus has been cleared from the blood and is also difficult to detect in cerebrospinal fluid.[39] Because the symptoms of CNS involvement are usually not observed until 2 to 4 weeks after the tick bite, antiviral antibodies are nearly always present at the time of admission to a hospital and can be detected readily by standard serologic tests. A recent TBEV infection can be confirmed by an increase in the HI titer, the neutralization titer (NT), or complement fixation titer, and by a decrease in HI titer after 2-mercaptoethanol treatment, suggesting the presence of immunoglobulin (Ig) M antibodies.[39] These tests are now used mainly for confirmatory purposes and have been largely replaced by rapid, sensitive, and reliable enzyme-linked immunosorbent assays (ELISAs) directed at the detection of IgM antibodies in the early phase of TBE. A four-layer ELISA system for the detection of TBEV-specific IgM has been developed that is extremely sensitive and prevents interference when high-titer, virus-reactive IgG antibodies for TBEV are present.[40] At an early stage after the onset of illness, TBEV-specific IgM can be detected in serum dilutions up to 1 : 10,000. However, in cases of other flavivirus exposures (e.g., vaccinations against yellow fever or Japanese encephalitis or following dengue virus infections), the use of a NT assay (e.g., rapid fluorescent focus inhibition test) is necessary to eliminate interference of flavivirus cross-reactive antibodies in ELISA and HI testing.[39,41] NT tests are only conducted in specialized laboratories with biosafety level 3 or 4 (depending on national requirements) and are not routinely available.[40] An alternative approach to diagnosis is the comparison of ELISA antibody titers in paired sera collected 14 days apart, where a fourfold or greater increase in titer is considered a confirmatory diagnosis. A high correlation has been demonstrated between the ELISA IgG units and the HI and NT titers, provided there was no other exposures to flavivirus antigens by natural infection or vaccination.[42,43] Standardized methods for serological and reverse-transcription PCR assays for European TBE have been published.[38,40]

Treatment

No specific therapy for TBE exists. Although reports of treatment with ribonuclease obtained from bovine pancreas[44] or with emetine[45] have been published, they are not generally accepted. Corticosteroids appear to hasten defervescence and improve subjective symptoms,[46] but also prolong the period of hospitalization.

Symptomatic treatment includes maintenance of water and electrolyte balance, provision of sufficient caloric intake, and the administration of analgesics, vitamins, antipyretics, and antiepileptics, as needed. Physiotherapy of paralyzed limbs is essential to prevent muscle atrophy.[10] Because person-to-person transmission of the virus has never been observed, there is no need to isolate patients with TBE. It is hoped that the improved knowledge of the structure of flavivirus proteins will soon lead to the development of antiviral agents.

EPIDEMIOLOGY

Incidence and Prevalence Data

TBEV is exclusively restricted to Europe and Asia, with the distribution of TBEV including almost the entire southern part of the nontropical Eurasian forest belt, from Alsace-Lorraine in the west to Vladivostok, and the northern and eastern regions of China to Hokkaido in Japan and some regions of the Republic of Korea (Figs. 59.1 and 59.2).[18,47] In the peak-year of 1996, more than 12,000 cases of TBE were reported from 19 European countries including Russia (Fig. 59.3 and Table 59.1). Although TBEV is found in valleys and nearby rivers with adequate habitats for ticks[47] rather than in higher altitudes, in recent years it has been reported from altitudes up to 2100 meters above sea level.[48–50] Geographically restricted foci with very high TBEV activity have also been reported.[47,51] TBEV prevalence varies from year to year,[52] possibly depending on microecological conditions such as humidity and temperature.[12]

In Europe, eight species of ticks have been identified that can transmit TBEV. *Ixodes ricinus*, the common castor bean tick, is the chief vector for the TBEV-Eu subtype and *Ixodes persulcatus* is the chief vector for the TBEV-Sib and TBEV-Fe subtypes. Other tick species have been associated with local TBE outbreaks in Siberia and the Far East. *I. ricinus* is found in much of Europe, extending to Turkey, northern Iran, and the Caucasus. *I. persulcatus* occurs mainly in eastern Europe, Russia, China, and Japan. The prevalence of TBEV in ticks has been shown to be as high as 21% in the District of Regen and 18% in the District of Freyung-Grafenau in southern Germany.[52] Other studies have reported the TBEV prevalence in ticks to be as high as 39%.[1,18,53–55] The distribution of TBEV subtypes is correlated to the distribution of tick species. In regions where both *I. ricinus* and *I. persulcatus* are present, TBEV subtypes cocirculate. However, the TBEV-Sib subtype has also been isolated from *I. ricinus*,[56] and the TBEV-Eu subtype has been found in *I. persulcatus*.[57]

The TBEV-Eu subtype is most prevalent in western, northern, and eastern Europe and European parts of Russia, but it has also been detected in Asian regions of Russia, such as Altai, eastern Siberia, and the northern Caucasus.[24,51,56,58] The TBEV-Sib subtype is prevalent in regions between Japan, the Baltic states, and Bosnia,[18,51,56,59] and may have replaced the TBEV-Fe subtype in several regions of Russia.[1,56] The TBEV-Fe subtype has been detected in regions between the easternmost Japanese and Russian islands and the Baltic states, including regions in China and Mongolia.[18,51,56]

Given the highly focal and variable proportion of infected ticks, there is no internationally accepted definition for when a region is considered endemic for TBE. According to Suess, regions with presence of TBEV or with locally acquired TBE cases (laboratory confirmed) in a 20-year period can be regarded endemic for TBE.[1] Dobler and associates expanded this definition to include TBE cases in animals and the presence of specific TBEV antibodies in wild animals and domestic animals with habitat fidelity.[47] Regions are suggested to be at high risk for TBE (e.g., Austria, Czech Republic) if more than five cases of TBE per 100,000 people occurred before widespread use of TBE vaccines.[47,18,60]

TBE vaccination coverage in Austria has been around 88% since 2007. However in the unvaccinated Austrian population, incidence rates of six per 100,000 population have been described, meaning Austria is highly endemic for TBE according to the definitions mentioned above.[61] Reporting systems often register the place where a person is diagnosed with TBE, which may differ from the place of infection. Therefore, reported cases may not always mirror the distribution of TBEV[47]; consequently, a travel history should be obtained from TBE patients.

Disease Surveillance and Reporting

TBE is an "emerging disease" because it is spreading to regions where TBEV had not been previously detected.[51] In the last few years, new foci of TBE cases have been identified in Austria, China, Denmark (mainland), Finland, Germany, Kazakhstan, Kirgizstan, Mongolia, Norway, Sweden, Switzerland, and Uzbekistan.[1,47,51] This may be the result of many different factors, including climate change, migration of populations to

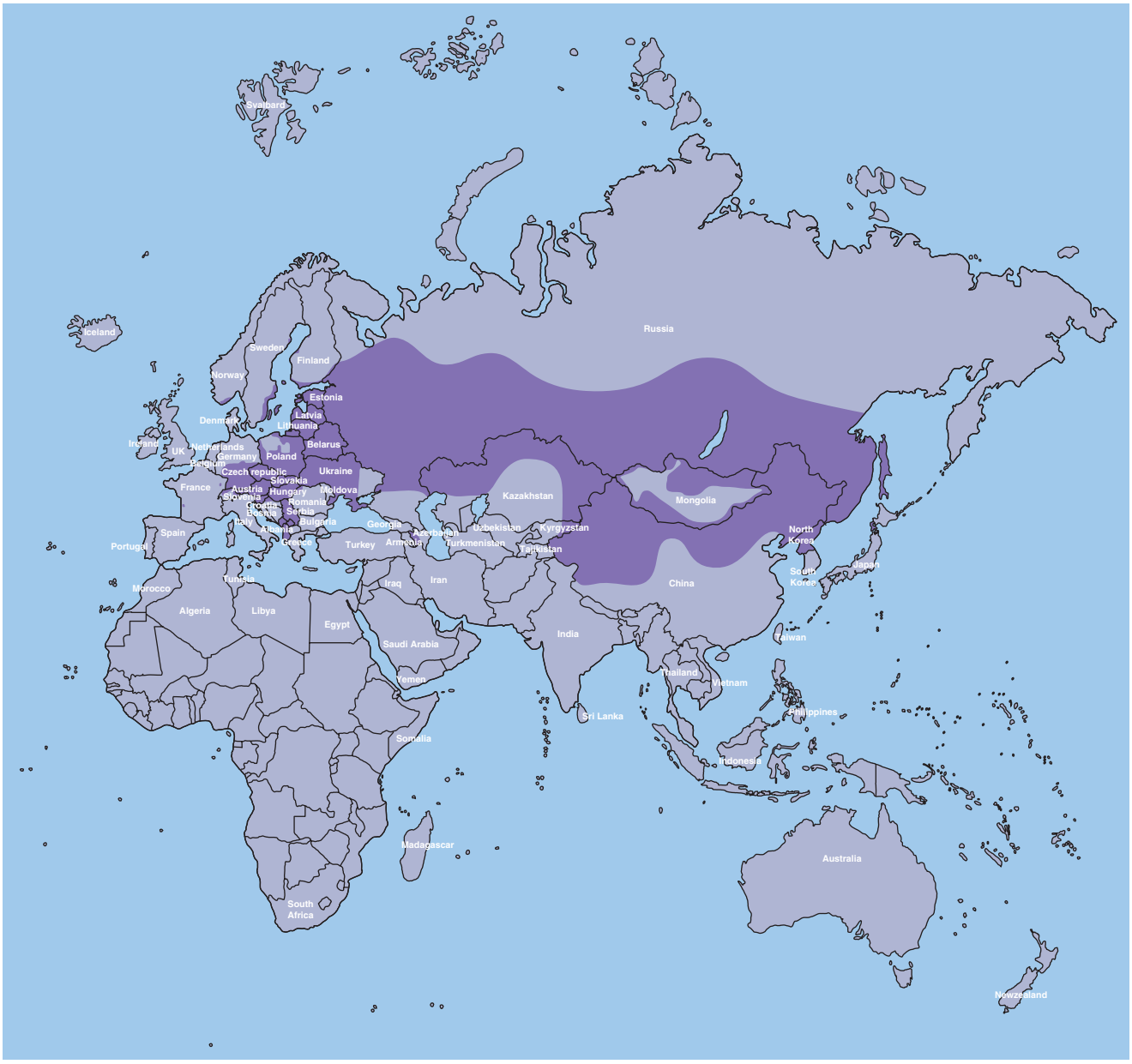


Figure 59.1. Geographic occurrence of tickborne encephalitis viruses in ticks, animals, and humans. *(From Dobler G, Gniel D, Petermann R, et al. Epidemiology and distribution of tick-borne encephalitis. Wien Med Wochenschr. 2012;162[11-12]:230–238.)*

suburban areas, changes in leisure habits, changes in agricultural practices, increased reforestation, and increased awareness and reporting.[18,51,62–65] Overall, awareness is limited in low-endemic countries and cases may not be diagnosed unless there is a clustering. Also, the disease is still not notifiable in countries without a dedicated surveillance system.[66] Furthermore, surveillance and reporting are hampered by the lack of an internationally harmonized case definition for TBE,[1,67] limiting the comparison of data from different countries. Data on TBE are collected by national institutions, reference laboratories, regional institutions, and the European Center for Disease Prevention and Control.[67,68] TBE surveillance is implemented in 20 European countries, 16 have mandatory reporting (see Table 59.1), and a case definition is in place in 10 countries (see Table 59.1).[47,68] Case definitions that include clinical symptoms and a laboratory confirmation are applied in the Czech Republic, Germany, and Hungary.[67] Since 1950 TBE is also a notifiable disease in Russia, although without a uniform case definition.[60] Table 59.1 lists the numbers of reported cases of TBE.

Risk Groups

A high percentage of detectable TBEV antibodies has been observed among high-risk groups, including persons working in agriculture and forestry, hikers, people engaged in outdoor sports, and collectors of mushrooms and berries. An increasing proportion of the population seems to be part of such recognized high-risk groups. In addition, increased tourism of nonvaccinated persons to central and eastern Europe exposes large numbers of people to significant risk of TBEV infection.[69] It is estimated that around 50 million travelers

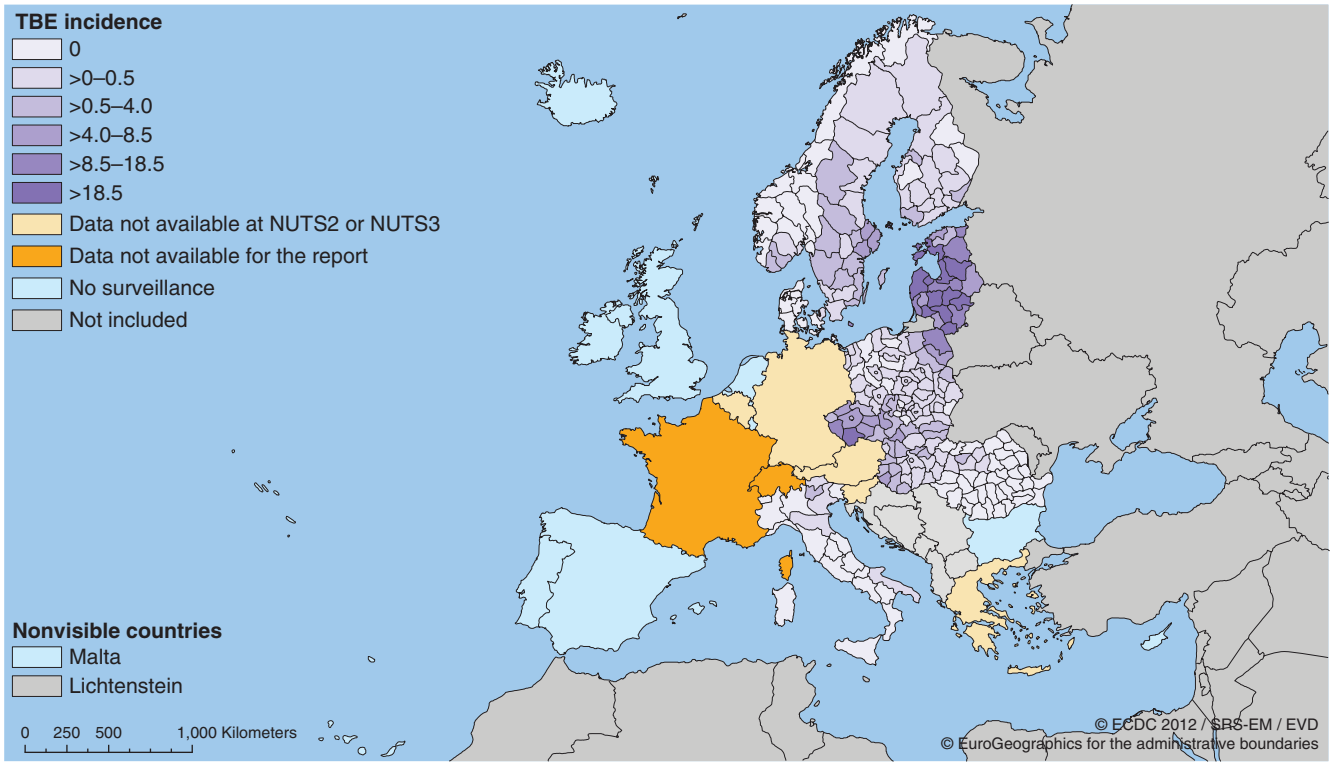


Figure 59.2. Tickborne encephalitis (TBE) average annual incidence rate per 100,000 inhabitants in the European Union (EU)/European Free Trade Association (EFTA) at lower administrative level NUTS (Nomenclature of Territorial Units for Statistics) 2 (Italy) or NUTS3 according to the European Centre for Disease Prevention and Control 2012. *(From European Centre for Disease Prevention and Control. Epidemiological Situation of Tick-Borne Encephalitis in the European Union and European Free Trade Association Countries. Stockholm, Sweden: ECDC, 2012.)*

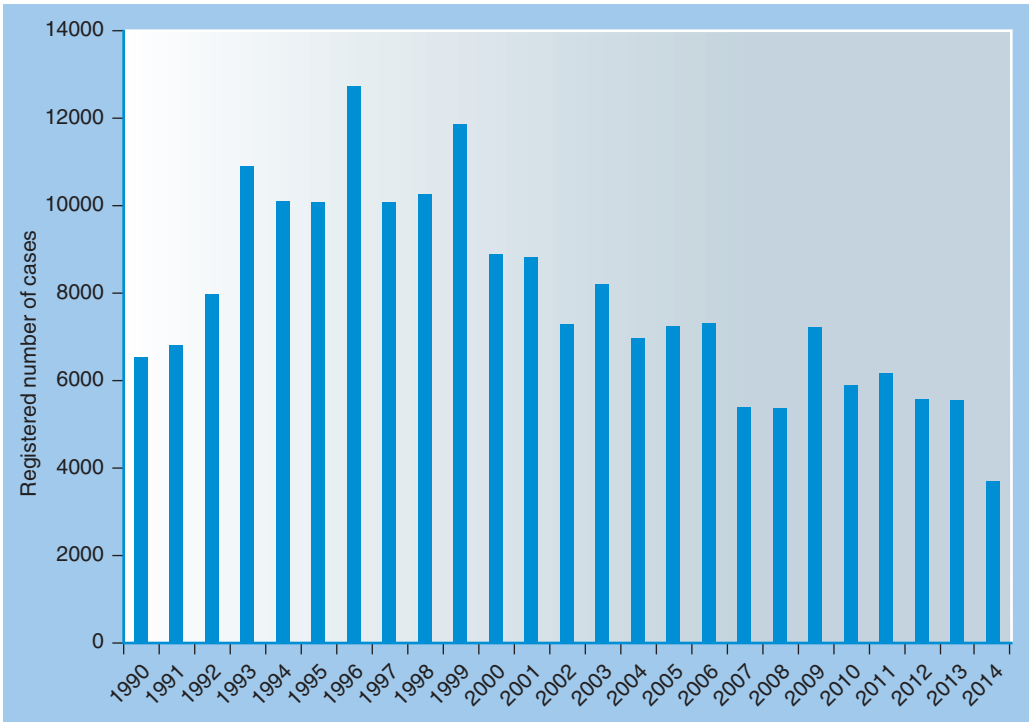


Figure 59.3. Registered number of cases of tickborne encephalitis (TBE) in Austria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Lithuania, Norway, Poland, Russia, Slovakia, Slovenia, Sweden, and Switzerland between 1990 and 2014 (2014: preliminary data) as of March 2015. *(Data courtesy Prof. J. Suess, Friedrich-Löffler-Institute, National Reference Laboratory for Tick-borne Diseases, Jena, Germany.)*

visit TBE-endemic regions every year.[70] The risk of contracting TBE in a highly endemic region has been calculated at one per 10,000 human-months during TBE season (April to October).[69] This risk of TBEV exposure was also highlighted in case studies of UK and U.S. citizens who contracted TBE during visits to Europe, Russia, and China.[71,72] The United States Centers for Disease Control and Prevention (CDC)[73] and the World Health Organization (WHO)[74] have on their respective websites recommendations for vaccination of travelers. However, implementation of such recommendations is limited by the availability of the TBE vaccines outside endemic regions.

Genetic factors have also been implicated in infection rates with persons having a mutation in the *CCR5* gene (CCR5Δ32)[75] and a wild-type *TLR3* gene reported to have an increased risk of TBE.[76]

Modes of Transmission and Reservoirs of Infection

TBE is a zoonotic disease, and ticks are the main vectors and reservoir hosts of TBEV in nature. Ticks become active at temperatures above 8°C and a relative humidity of 70% to 80%, explaining the seasonality of the disease and the suitability of Europe and Asia forested areas as habitats. Vertebrates serve as amplifying reservoir hosts of TBEV and the primary source of infection for ticks, thus forming a cycle of transmission. Small rodents, such as mice and voles, are believed to be prominent reservoirs, but also large mammals, such as roe, deer, and goat, may be involved.[8,77,78] Transovarial transmission of the virus as well as transstadial transmission have been described, when nymphs and larvae cofeed on the same host. Once infected, ticks carry the virus for life. TBEV is usually transferred to the host through the saliva of the infected tick. The virus can be transmitted to humans or other hosts by larvae, nymphs, and adult ticks. On humans, ticks attach themselves to the hair-covered portion of the head, ears, and the extremities. The epidermis is punctured with the chelicerae, and the hypostome is inserted. Because of the anesthetizing effect of the tick's saliva, the bite causes no pain and often passes unnoticed by the host, leading the victim to not recall having been bitten by a tick.

While the main route of TBEV transmission occurs through tick bites, there are reports from several European countries of occasional transmission through the alimentary route by means of ingestion of unpasteurized dairy products.[1] Goat, sheep, and cattle that are hosts for *I. ricinus* can be infected by TBEV, and raw milk from these animals collected during the viremic phase can be infectious to humans. More than 50 cases of TBE have been reported in Slovakia after consumption of cheese made from raw sheep milk,[79] and in Estonia an epidemic of TBE was linked to raw goat milk.[80] Unpasteurized milk and cheese were reported to be responsible for 64 cases of TBE in the Czech Republic between 1997 and 2008.[81] In an endemic region of Poland, reverse transcriptase–PCR demonstrated the presence of TBEV in the milk of more than 10% of the cows and more than 20% of the sheep and goats.[82] Although it has not been observed, human-to-human transmission is a theoretical possibility when blood from a viremic donor is transfused or when infected human milk is consumed by an infant.[12]

Significance as a Public Health Problem

In endemic areas, TBEV is one of the most important causes of viral meningitis/encephalitis and constitutes a major public health problem. As endemic regions are expanding, public health authorities should carefully monitor the situation in adjacent areas. Endemic locations are also expanding in altitude, up to 2100 meters above sea level.[50,83] On average, some 8000 cases of TBE are reported from European countries and Russia (see Table 59.1 and Fig. 59.3), although with significant year-to-year variation. Despite being a notifiable disease in the European Union since 2012,[66] the disease burden is believed to be significantly underestimated. Of note, even in the most severely affected areas, the disease is confined to certain foci, making it difficult to define at risk areas. Overall, more cases are being reported from males than females, which may reflect different risks of exposure. While TBE can affect all age groups, the age distribution of cases varies between endemic regions. For example, in the highly endemic region of western Siberia, most cases were reported in adults (20 to 49 years of age), with only 20% to 30% of cases reported in children younger than 14 years.[14] In contrast, among German TBE patients only 12% were children and a substantial proportion of the cases occurred in the elderly.[10] These epidemiological patterns may further change as life style and recreational activities evolve.

The burden of disease, including long-term sequelae, is not well defined. The disease tends to be relatively severe in adults and long-term sequelae from the disease are quite common in adult age groups. However, a significant risk of incomplete recovery also exists in children.

Ecological studies show that vaccination can have a profound impact on disease incidence. The WHO recently published a position paper on TBE vaccines,[18,84] recommending vaccination for whole populations in highly endemic areas (>5 cases/100,000/year) and vaccination of risk groups in low to moderate endemic areas (>0.5 cases/100,000/year).

Passive Immunization

An Ig concentrate containing specific γ-globulin against TBEV was previously used prophylactically or as a postexposure treatment; today, this approach is only used in Russia. As a number of cases of suspected antibody-mediated enhancement of infection were reported after use of TBE Ig in children,[85] these products were withdrawn from the European markets in the late 1990s. Although there was no conclusive evidence of enhancement of TBEV infection after use of Ig in humans and no such enhancement occurred in a mouse model,[86] postexposure treatment using TBE Ig is no longer recommended in most European countries.[87]

In Russia, on the other hand, passive immunization after tick bites is current practice in TBE endemic areas. Russian studies have reported that early application of TBE-specific Ig after tick bites can prevent clinical disease in approximately 80% of cases. This level of protection is achieved by timely administration of a single dose of 0.05 mL/kg body weight of TBE Ig with a titer of no less than 1 : 80.[88,89] Increasing the dose to 0.1 mL/kg or readministration of Ig provided no additional protective effect. Concerns have been raised in relation to the content of neutralizing antibody of some commercial preparations.[89]

ACTIVE IMMUNIZATION

History of Vaccine Development and Manufacture

In 1937, TBEV was discovered in the Far Eastern sections of the Union of Soviet and Socialist Republics (USSR) and the first candidate vaccine was generated as early as 1938.[90] Smorodintsev and coworkers developed a formalin-inactivated vaccine based on mouse brain infected with the strain Sofjin and administered it to forest workers in endemic regions.[91] Although mouse brain–derived vaccines were immunogenic

TABLE 59.1 Number of Reported Cases of Tickborne Encephalitis From Various European Countries and Russia as of March 2015

	Incidence According to Dobler et al.[47]	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
Austria[a,b]	High (nonvaccinated)	89	128	84	102	178	109	128	99	62	41
Croatia	Low	23	60	27	76	87	59	57	25	24	26
Czech Republic[a,b]	High	193	356	338	629	613	744	571	415	422	490
Denmark	Regional/sporadic	–	–	–	–	–				1	4
Estonia[a,b]	High	37	68	163	166	177	175	177	404	387	185
Finland[a,b]	Low	9	?	14	25	16	23	10	19	17	12
France	Low	2	1	2	5	4	6	1	1	2	5
Germany[a,b]	Low	?	44	142	118	306	226	114	211	148	115
Hungary[a,b]	Low	222	288	206	329	258	234	224	99	84	51
Italy	Regional/sporadic	0	0	2	2	8	6	8	8	11	5
Latvia[a]	High	122	227	287	791	1366	1341	716	874	1029	350
Lithuania[a]	High	9	14	17	198	284	426	309	645	548	171
Norway[a,b]	Low	0	0	0	0	0	0	0	0	1	1
Poland[a, b]	Low	8	4	8	249	181	267	257	201	209	101
Russia[a]	Intermediate	5486	5225	6301	7893	5596	5982	9548	6539	6987	9955
Slovakia[a]	Intermediate	14	24	16	51	60	89	101	76	54	57
Slovenia[a,b]	High	235	245	210	194	762	260	406	274	136	150
Sweden[a]	Intermediate	54	75	83	51	116	68	44	76	64	53
Switzerland[a]	Intermediate	26	37	66	44	97	60	62	123	68	112
Total (without Russia)		1043	1571	1665	3030	4513	4093	3185	3550	3267	1929
Overall Total		6529	6798	7966	10923	10109	10075	12733	10089	10254	11884

[a]Registration of tick-borne encephalitis case in these countries is mandatory (Greece is not listed but also mandatory).
[b]Case definition in place (Greece is not listed, but also has a case definition in place).
Data courtesy Prof. J. Suess, Friedrich-Loeffler-Institute, National Reference Laboratory for Tick-borne Diseases, Jena, Germany.

and effective, they frequently caused allergic reactions. Subsequently, in the 1970s the naturally attenuated mammalian tickborne flavivirus LGT virus was evaluated as a vaccine in clinical trials involving more than 600,000 volunteers in the former USSR. A high rate of adverse events involving the CNS (one in 18,570 volunteers) was observed and the clinical trials were stopped.[92]

Initial attempts to develop a vaccine against TBEV-Eu were made in Czechoslovakia in the 1960s.[93,94] The virus was grown in primary avian fibroblast cultures and formalin inactivated. It was shown to be an effective vaccine in a variety of laboratory animals and human volunteers.[95] In 1971, the Institute of Virology in Vienna, Austria, and the Microbiological Research Establishment in Porton Down, England collaborated to develop a formalin-inactivated vaccine based on the Austrian TBE strain Neudörfl. This strain was passaged in the brains of specific pathogen-free baby mice and grown for vaccine production on suspensions of primary specific pathogen-free chicken embryo cells. The vaccine virus was concentrated through centrifugation followed by inactivation with formalin and purification using hydroxylapatite chromatography. Aluminum hydroxide was added as an adjuvant. More than 400,000 people were vaccinated in Austria, and seroconversion rates of more than 90% after two vaccinations, as measured by the HI test, were documented.[96] However, despite its immunogenicity, local and systemic adverse events were common. Consequently, in a collaboration with Immuno AG, another purification step using continuous-flow zonal density-gradient centrifugation was added.[97] This resulted in a 90-fold increase in vaccine purity and a marked reduction in vaccine-associated side effects.[96] In 1999, the preservative thimerosal was removed from the final formulation, and in 2000 a production virus seed was generated that was free of both potential contaminating mouse brain protein and human serum albumin (HSA). Unexpectedly, the removal of HSA from the final formulation led to an increase in the rate of high fever in infants and small children, and a number of cases of febrile seizures were observed in children up to the age of 24 months. Subsequent animal studies suggested that HSA has an inhibitory effect on the production of tumor necrosis factor-α.[98] The HSA was reintroduced into the vaccine formulation in 2001, and thereafter there was a dramatic reduction in the rate of fever (FSME-IMMUN).[99] Immuno AG was purchased by Baxter Healthcare in 1996, and subsequently, the FSME-IMMUN vaccine was acquired by Pfizer in 2015.

A second Western European TBE vaccine, similar to FSME-IMMUN, was licensed in Germany in 1991 (Encepur, Chiron Behring, Marburg, Germany) and was subsequently introduced in a number of other European countries.[100] Chiron's vaccines were purchased by Novartis in 2005, and Novartis

2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014 (Preliminary)
60	54	60	82	54	100	84	45	86	79	63	106	52	98	80
18	27	30	36	38	28	20	12	20	?	?	?	?	?	?
719	411	647	606	500	642	1029	542	630	816	589	762	611	624	410
3	1	1	4	8	4		2	1	1	12	1	0	0	0
272	215	90	237	182	164	171	140	90	179	201	250	178	114	?
41	33	38	16	31	17	18	20	23	26	44	62	39	38	48
0	0	2	6	7	0	6	7	10	2	2	10	3	3	7
133	253	226	278	274	431	546	238	285	313	260	400	195	442	277
45	76	80	114	89	52	56	62	70	64	50	43	49	?	?
15	19	6	14	23	22	14	4	34	32	23	?	?	?	?
544	303	153	365	251	142	170	171	181	328	494	429	376	265	172
419	298	168	763	425	242	462	234	220	617	612	365	495	501	?
1	0	2	1	2	3	3	13	9	8	11	15	6	5	12
170	205	126	339	262	174	316	233	202	335	294	221	217	221	195
5931	6339	5150	4770	4235	4551	3510	3098	2817	3721	2691	3077	2716	2336	1969
92	76	62	74	70	28	91	46	77	71	90	110	95	164	121
190	260	262	282	204	297	373	199	246	307	166	247	164	309	102
133	128	105	105	160	130	163	190	224	211	174	284	287	215	180
91	107	53	116	138	206	259	113	127	118	96	153	94	203	114
2947	2467	2111	3438	2718	2682	3781	2271	2535	3507	3181	3458	2861	3202	1718
8878	8806	7261	8208	6953	7233	7291	5369	5352	7228	5872	6149	5577	5538	3687

vaccines were acquired by GlaxoSmithKline in 2015. The vaccine is based on the K23 TBEV-Eu virus strain, isolated in southern Germany. This strain, which has a nucleic acid sequence very similar to that of the Neudörfl strain,[25] was grown on primary chick embryo cells, inactivated by formaldehyde, and purified by continuous-flow density-gradient centrifugation. The vaccine then was stabilized with processed bovine gelatin and adsorbed onto aluminum hydroxide.[101] Formulations were available for both adults and children.[102] However, the first pediatric version of Encepur resulted in cases of immediate allergic reactions in rare cases.[103] A detailed evaluation suggested that these adverse events were most likely IgE-mediated reactions to the vaccine's polygeline stabilizer, a total of 38 cases were registered in the postmarketing surveillance between 1994 and 1997, a rate of approximately 2×10^{-5} doses distributed.[104] As a consequence, the first pediatric TBE vaccine was voluntarily withdrawn from all markets in early 1998. A new TBE vaccine formulation for children and adults was developed that contained an increased amount of sucrose, making the use of an additional protein-derived stabilizer unnecessary, and was commercialized as of 1999.[103,105]

Two chick embryo cell–derived, formalin-inactivated TBE vaccines have been developed and marketed in Russia. The vaccine TBE-Moscow is manufactured by the Federal State Enterprise of Chumakov Institute of Poliomyelitis and Viral Encephalitides (IPVE), using the TBEV-Fe prototype strain, Sofjin.[106] The second Russian vaccine, EnceVir, manufactured by Microgen, Tomsk, is based on the TBEV-Fe subtype strain 205.[107]

A TBE vaccine derived from the TBEV-Fe strain Senzhang is manufactured and marketed domestically in China by the Changchun Institute of Biological Products (CIBP).[108] This vaccine is produced in primary hamster kidney cells and then formalin inactivated. It uses HSA as stabilizer and aluminium hydroxide as adjuvant. Table 59.2 provides summary information on current TBE vaccines.

Producers

TBE vaccines are produced commercially by five manufacturers: Pfizer (Vienna, Austria), GlaxoSmithKline Vaccines (Marburg, Germany), IPVE (Moscow, Russia), Microgen (Tomsk, Russia), and CIBP (Changchun, China). GlaxoSmithKline and Pfizer use essentially the same process to produce the vaccine, the major differences being the use of different strains and the addition of different stabilizers. They also both provide adult (Pfizer, FSME-IMMUN; GlaxoSmithKline, Encepur) and pediatric (Pfizer, FSME-IMMUN Junior; GlaxoSmithKline, Encepur-Children) formulations for children older than 1 year. FSME-IMMUN is marketed as TicoVac in some

TABLE 59.2 Antigen Details, Excipients, and Presentation of Tickborne Encephalitis Vaccines

Name and Producer	FSME-IMMUN; Pfizer	Encepur; GSK	TBE-Moscow; Federal State Enterprise of Chumakov Institute	EnceVir; Microgen	Changchun Institute of Biological Products
ANTIGEN DETAILS					
Strain	TBEV-Eu Neudörfl	TBEV-Eu K23	TBEV-Fe Sofjin	TBEV-Fe Strain 205	TBEV-Fe Senzhang
Passages	PCEC	PCEC	Mouse brain	NK	NK
Production	PCEC	PCEC	PCEC	PCEC	PHKC
Amount of antigen	2.4 µg/1.2 µg children	1.5 µg/0.75 µg children	0.5–0.75 µg	2.0–2.5 µg	NK
EXCIPIENTS					
Adjuvant	$Al(OH)_3$	$Al(OH)_3$	$Al(OH)_3$	$Al(OH)_3$	$Al(OH)_3$
Preservative	No	No	No	No	NK
Stabilizer	HSA	Sucrose	Sucrose, HSA	Sucrose, HSA	HSA
PRESENTATION					
Formulation	0.5/0.25 mL liquid	0.5 mL liquid	0.5 mL liquid	0.5 mL liquid	NK
Packaging	Prefilled syringe	Prefilled syringe	Prefilled syringe	Prefilled syringe	NK
Shelf life	30 months (2–8°C)	30 months (2–8°C)	3 years (2–8°C)	2 years (2–8°C)	NK

$Al(OH)_3$, Aluminium hydroxide; HAS, human serum albumin; NK, not known; PCEC, primary chicken embryonic cells; PHKC, primary hamster kidney cells; TBEV, tickborne encephalitis virus.

From Il'chenko TE, et al., Organization of Public Health. Siberian J. Med. *2009;2:50–55; Vorob'eva MS, Rasshchepkina MN, Ladyzhenskaia IP. [Vaccines, immunoglobulins, and test systems for the prevention and diagnosis of tick-borne encephalitis] [in Russian].* Vopr Virusol. *2007;52(6):30–36; and Kollaritsch H, et al.* Background Paper on Vaccines and Vaccination Against Tick-borne Encephalitis (TBE). *Geneva, Switzerland: WHO Strategic Advisory Group of Experts on Immunization; 2011.*

countries. Table 59.2 provides an overview of the excipients of the Western European and Russian vaccines. HSA is used as a stabilizer by Pfizer, IPVE, CIBP, and Microgen, whereas GlaxoSmithKline uses an increased amount of sucrose as a stabilizer. IPVE's TBE-Moscow was approved for use in adults in 1982 and has been administered to more than 25 million people in Russia and neighboring countries.[106] Microgen's EnceVir was licensed in the Russian Federation in 2001. Russian manufacturers do not offer specific pediatric formulations, and neither vaccine is licensed for use in children younger than 3 years. TBE-Moscow has been approved for use in children age 3 years or older since 1999. EnceVir had been administered to children 3 to 16 years of age, but in 2010 this was temporarily stopped by the Russian Ministry of Healthcare because of increased rates of febrile reactions. The Chinese CIBP vaccine is approved for those older than 3 years.

Vaccine Stability and Storage

FSME-IMMUN may be stored at 2°C to 8°C for 24 months (for prefilled syringes without needle attached) or for 30 months (for prefilled syringes with needle attached). Encepur and TBE-Moscow have shelf lives of 24 months and EnceVir of 36 months at this temperature. No stability data are available for the Chinese vaccine.

Vaccine Immunogenicity

All vaccines have been registered on the basis of their quality, immunogenicity, and safety, but no controlled trials with clinical efficacy end points have been conducted with any of the current TBE vaccines. Immunogenicity studies consistently show strong antibody induction after primary vaccination. A Cochrane Collaboration review published in 2009 summarized 11 randomized or quasi-randomized clinical trials involving a total of 8184 participants, including both children and adults. All vaccines (FSME-IMMUN and Encepur) gave seroconversion rates of more than 87%.[109]

Data on the immunogenicity of Encepur from eight clinical trials and postmarketing studies of 7500 subjects showed that following primary immunization, all subjects seroconverted or showed a fourfold increase in anti-TBEV antibodies.[103] The seroconversion rate (ELISA) of FSME-IMMUN was 98.5% and 100% after primary immunization of 412 children ages 1 to 15 years (pediatric dose); 96% in 64 vaccinees 12 to 15 years old (pediatric dose); and 98.2% in 57 vaccinees ages 16 to 35 years (adult dose).[110]

However, immunogenicity studies are not always directly comparable as different assay systems have been used for the various trials. Published FSME-IMMUN immunogenicity studies frequently measure TBEV-neutralizing antibodies or total IgG neutralizing antibodies against the homologous TBEV Neudörfl strain according to Adner and colleagues[111] or Holzmann and colleagues[42] or a commercially available ELISA kit (Immunozym FSME [TBE]-IgG, Progen Biotechnik, Heidelberg, Germany). Reported studies with Encepur measure neutralizing antibodies (NT assay) and total IgG antibodies (Enzygnost TBE, Dade Behring, Marburg, Germany) against the homologous K23 strain. Immunogenicity studies of the Russian vaccines have primarily used HI tests. While neutralizing antibodies against the E protein of the virion are believed to confer protection, no formal correlate of protection has been established for TBE and no internal reference reagents are available.[61]

All vaccines, except TBE-Moscow, have been tested using both conventional and accelerated schedules (Table 59.3). In the Cochrane review[109] four randomized controlled trials (RCTs) of the currently licensed western vaccines included 5063 children and adults who were followed up for 6 to 12 months for both serologic and safety data. All vaccines induced

TABLE 59.3 Immunization Schedules for Tickborne Encephalitis Vaccines According to WHO Recommendations[a]

	Basic Immunization: Conventional Schedule (Dose 1 on Day 0)		Basic Immunization Rapid Schedule (Dose 1 on Day 0)				
	2nd Dose (Mo)	3rd Dose (Mo)	2nd Dose	3rd Dose (Mo)	4th Dose (Mo)	First Booster (yr)	Subsequent Boosters (yr)
FSME-IMMUN	1–3	5–12	14 days	5–12	–	3	5[b]
Encepur	1–3 (14 days)	9–12	7 days	21 days	12–18[c]	3	5[b]
TBE-Moscow vaccine	1–7	12				3	3
EnceVir	5–7	12	21–35 days[d]	42–70 days[d]	6–12[c]	3	3
CIBP	½ (14 days)	None				1[e]	

[a]Intervals given in months unless indicated.[27]
[b]In persons aged ≥50 years interval of 3 years (Austria: persons aged ≥60 years).
[c]Considered as first booster.
[d]Double dose of total 1.0 mL.
[e]"Booster before endemic season."

seroconversion rates in the range of 92% to 100%, as measured by ELISA or NT, with seroconversion being defined by a NT of 1 : 10 or higher or a corresponding ELISA bridged to NT.[112–114] In a subsequent RCT, more than 95% of the enrolled 334 children achieved NTs of 1 : 10 or higher following two doses of either Encepur or FSME-Junior vaccine.[115] Similar immunogenicity was achieved with either conventional or rapid immunization schedules.[114]

The vaccines TBE-Moscow and EnceVir have been evaluated in two RCTs. In 2001–02, two studies of the Russian vaccines involving 200 adults each given two doses of vaccines showed that antibody titers of 1 : 80 or higher were detected in 84% and 93% of subjects, and with EnceVir, in 82% and 89% of the vaccinees following two doses, 2 or 5 months apart, respectively.[116,117] Similarly, an evaluation involving 325 individuals stratified into the age groups of 3 to 6 years, 7 to 14 years, and 15 to 18 years, after TBE-Moscow vaccine showed a fourfold or greater increase of HI-antibody titers in 96%, 93%, and 89% of subjects, respectively, compared to 84%, 97%, and 92% with EnceVir vaccine.[115] A newer randomized study compared the immunogenicity in adults of TBE-Moscow, EnceVir, FSME-IMMUN (new), and Encepur adults to TBEV-Fe.[118] Immunogenicity was measured at 2 to 5 months and again at 2 years following the administration of three doses of the respective vaccines. All vaccines induced neutralizing antibody against the TBEV-Fe subtype strain P-73. With TBE-Moscow, antibody was detected in 100% and 94% of the vaccinees after 2 to 5 months and 2 years, respectively. With EnceVir the corresponding figures were 88% and 84%, with FSME-IMMUN the figures were 88.2% and 78.1%, and with Encepur the figures were 100% and 100%.

No immunogenicity data have been reported for the Chinese vaccine.

Because different virus subtypes exist and sometimes coexist in the same TBE-endemic region, TBE vaccines need to provide cross-protective antibody responses. Owing to the high degree of antigenic similarity between TBEV subtypes, all currently marketed TBE vaccines are expected to provide good cross-protection against all TBE subtype viruses. Various studies with TBEV-Eu vaccines demonstrate cross-neutralization against TBEV-Eu, TBEV-Fe, and TBEV-Sib subtypes.[116,117,119] A systematic review of the immunogenicity of TBEV-Eu vaccines against TBEV-Fe and TBEV-Sib subtype viruses has been published[120] supporting robust cross-neutralization, except with one strain (TBEV-Fe P-69) showing a significantly lower level of neutralization.

It is well-documented that the capacity to mount immune responses against vaccines decreases with age, commonly labeled as immunosenescence, and this has been shown for TBE vaccines as well. Decreased postprimary and anamnestic antibody responses to TBE vaccine have been documented in individuals older than 50 years.[121] In contrast, there is no indication that antibody avidity decreases with age.[122] The need for booster doses will be addressed below.

Administration and Vaccination Schedule

Dosage and Route

Table 59.3 provides an overview of dosing schedules for Western European and Russian vaccines according to the respective product leaflets and WHO recommendations.[51] The standard immunization schedule for both Western European vaccines is similar, consisting of three immunizations administered intramuscularly, starting on day 0, the second dose given 1 to 3 months later, and a third dose is administered 5 to 12 months (FSME-IMMUN) or 9 to 12 months (Encepur) after the second one. The first booster is often recommended to be administered within 3 years, but there is overall little information available on the duration of protection post primary series. Consequently, national recommendations for booster vaccinations differ. In most countries, subsequent booster doses are recommended every 5 years for persons younger than 60 years and every 3 years for persons 60 years of age or older.[20,51,123] In Germany, booster doses every 3 years are recommended for persons 50 years of age or older. Booster doses are also recommended for Encepur recipients 50 years of age or older in some other countries by the respective national authorities. However, the Summary of Product Characteristics for Encepur recommends boosters every 3 years beginning at age 60 years. In Austria, TBE vaccination is routinely recommended for all individuals at least 1 year of age, and, in highly endemic regions such as parts of Carinthia, Styria or Upper Austria, children are vaccinated as early as 6 months of age as an off-label use.[51,124] Switzerland recommends a booster 10 years after primary immunization for all age groups.[125]

Accelerated immunization schedules are also available for persons requiring more rapid protection (e.g., when traveling to TBE-endemic areas or when the tick season has already begun). The rapid immunization schedule for Encepur consists of three immunizations administered on days 0, 7, and 21,[105,114,126] with a booster after 12 to 18 months. The rapid

immunization schedule for FSME-IMMUN allows a shortened interval for the administration of the second dose, that is, 2 weeks after the first dose, and a third dose to complete the primary vaccination program after 5 to 12 months.[127] Encepur is also licensed for a schedule with doses administered on days 0 and 14, with a third dose after 9 to 12 months.

The standard primary immunization schedule for both Russian vaccines is two doses administered 1 to 7 months apart with a booster 12 months after the second dose and every 3 years thereafter. A rapid immunization schedule for the emergency use of EnceVir prescribes the administration of the second dose 5 to 7 days after the first dose.[18]

For the Chinese TBE vaccine the vaccination schedule consists of two doses given 14 days apart and a booster that should be given right before the endemic season; no data, however, are available on boosting intervals.

Irregular Vaccination

The effectiveness of TBE vaccines are unaffected even by prolonged intervals between doses in the primary immunization schedule.[61,128] In a cohort study with more than 1100 persons who were vaccinated but deviated from the recommended schedule, the participants were administered a single booster up to 20 years after one, two, or three primary vaccinations.[129] The results of this study demonstrated that, independent of age of the vaccine recipient and of the interval to the last vaccination, an effective booster response was seen if two or three primary vaccinations were administered previously, even if the time intervals between doses were very prolonged.[129,130] Hence, there is no need to restart a primary vaccination series after an incomplete primary schedule or markedly delayed booster vaccination.

Duration of Immunity and Protection

A large number of studies have investigated the kinetics and seropersistence of antibodies after primary and booster doses of TBE vaccines.[131–136] Based on these data, immunization schedules have been proposed; however, there is no common practice on boosting intervals.[18] Table 59.3 lists the current dose schedules for each vaccine.

Several studies evaluated booster recommendations for FSME-IMMUN and Encepur and assessed the interchangeability of the two TBEV-Eu vaccines. The seropersistence of TBEV antibodies in adults 18 to 67 years of age was evaluated 2 and 3 years after primary vaccination (first two doses, FSME-IMMUN or Encepur; third dose, FSME-IMMUN) in a multicenter prospective study ($n = 347$).[137] Seropositivity rates 2 and 3 years after the primary vaccination were 96.8% and 95.4%, respectively, as determined by neutralizing antibody titers. Following the booster vaccination, seropositivity rates increased to 100%. Another study analyzed antibody titers before and after booster vaccination in subjects who had received their last FSME-IMMUN vaccination 3 to 7 years previously.[121] Before booster vaccination, antibody titers were significantly higher in subjects younger than 30 years compared with subjects 50 years of age or older. In addition, the response to a booster vaccination was significantly higher in the group younger than 30 years compared with subjects 50 years of age or older. However, there was no difference in booster responses in any of the age groups 50 years of age or older. NT were measured in 430 adults 2 to 10 years after the last vaccination to determine the seroprotection rate (SPR; defined as NT ≥1 : 10) over a longer period and to indicate age-specific differences in antibody persistence.[133,134,138] Subjects from this series of studies were given a booster dose of Encepur after having received a regular primary course of FSME-IMMUN at least 3 years before,[130,139] and SPR was evaluated from 2 to 10 years after the booster. Seroprotection was detected in 95.9%, 96.7%, and 93.8% of subjects after 2, 3, and 4 years, respectively. Geometric mean titers after 3 years were significantly lower in subjects 50 years of age or older, and were also significantly lower after 4 years in subjects 60 years of age or older. After 4 years, the proportion of subjects seroprotected dropped to 93.0% and 91.7% for people 50 to 60 years of age and 60 years of age, respectively. After 5 and 6 years, seroprotection was detected in 96% and 94%, respectively, of subjects younger than 60 years. For the group 60 years of age or older, the SPR dropped to 89% and 86% at 5 and 6 years, respectively, after the booster. Finally, 8 and 10 years after booster SPR was 86.8% and 77.3%, respectively, but age dependency became even more apparent: after 10 years the SPR was 83.9% in subjects younger than 50 years, but dropped to 66% in subjects older than 50 years.[133] These results indicate that seroprotection lasts considerably longer than the recommended boosting interval of 5 years in healthy volunteers, but there are variable responses by age. Data from a 5-year follow up of vaccinees 15 years of age or older after different (conventional, modified conventional, or accelerated) primary vaccination schedules also demonstrate long-term antibody persistence for 5 years.[140]

Most seroprotection data include subjects who were primed with TBE vaccines below the age of 50 years.[133,140] Data are limited for those older than age 50 years who are vaccinated for the first time, and only one study investigated the comparative primary immune response in older and younger subjects.[141] This study showed lower titers and earlier loss of neutralizing antibodies in those primed after the age of 50 years. However, the ability to boost was similar in both groups, indicating an adequate B-memory response, even in the older group of subjects.

During clinical development and the postlicensure period a number of studies were performed to evaluate differences between the licensed vaccines with respect to immunogenicity and seropersistence. However, head-to-head studies with Encepur and FSME-IMMUN are hampered by using different antibody assays and by the fact that the studies were sponsored by the manufacturers.[109,115,127,135,140,142,143] However, published data do not suggest relevant differences between the two TBEV-Eu vaccines, and they are regarded as equivalent.[51] For the two Russian vaccines data on immunogenicity and efficacy are limited and do not allow an assessment of equivalence.[118,144]

Cellular Immune Responses

Few data are available on the generation or role of T-cell responses in immunized persons, but it has been reported that immunization with inactivated TBE vaccine induces primarily a $CD4^+$ T-cell response with a very low induction of $CD8^+$ cells.[141,145] Recent immunological investigations of low-responders after TBE vaccination show that low cellular, humoral, and cytokine responses levels (interleukin-2 and interferon-γ) are correlated with each other.[146]

Correlates of Protection

Human antibody responses after vaccination differ from those seen after natural infection,[147] but in both situations, neutralizing antibodies against protein E are induced.[147]

According to the WHO, these neutralizing antibodies can be used as a surrogate marker for immunity.[18] As mentioned before, although a titer of 1 : 10 or higher is generally used to define seroprotection,[42] during the vaccine licensure procedure titers of 1 : 2 or greater were accepted as a correlate of

immunity.[123] A formal correlate of protection has not been established for TBE vaccines, and international reference reagents are lacking. Using ELISA titers as marker of protection, introduces a number of pitfalls, including cross-reactivity with other flaviviruses (antibodies deriving from infection or vaccination) and lack of a quantitative correlate of protection ("cutoff"). Thus, ELISA measurements are useful in screening but not in definitive assessments of immunity. HI tests do not offer advantages and are rarely used today.

Efficacy and Effectiveness of Vaccine

Although no controlled clinical efficacy trials have been performed, the public health impact of vaccination programs is well documented. In Austria, where TBE vaccines have been in use for more than 30 years, the protection rate can be calculated from both clinical and epidemiologic data (Fig. 59.4). The protection rate of the vaccines for the years 2000–06, when approximately 90% to 95% of TBE vaccines administered in Austria were FSME-IMMUN, was estimated to be approximately 99% in regularly vaccinated persons, with no statistically significant difference between age groups.[61] During this period, approximately 90% to 95% of TBE vaccines administered in Austria were FSME-IMMUN. A second analysis of effectiveness during the years 2010–11, when Encepur comprised approximately one-third of the vaccine used in Austria, showed very similar data: for regularly vaccinated persons effectiveness was between 98.7% (confidence interval [CI] 98.2–99.0; best case scenario for those with known vaccination status) and 96.3% (CI 95.5–97.0; worst case scenario for those with unknown vaccination status) and for subjects vaccinated with nonstandard schedules 92.5% (CI 90.3–94.3; best case scenario) and 91.3% (CI 88.9–93.3; worst case scenario), respectively, indicating a similar effectiveness over the years and for both vaccines.[128] Sensitivity analysis of best and worst case scenarios reflect the assumptions of vaccination status of those TBE with cases with unknown vaccination history.

Vaccine effectiveness after the second dose was high with no vaccine breakthroughs recorded in this group within the first year. However, a significantly lower effectiveness was calculated (≈96%) in persons not receiving the recommended schedule.[61,128] The risk of acquiring TBE was calculated to be increased three- to eightfold in persons receiving a nonstandard schedule, but potential confounders cannot be excluded. Nevertheless, the findings highlight the importance of adhering to the recommended vaccination schedule, in particular in relation to the primary series. Studies on breakthrough infections despite complete TBE vaccination[148,149] do not provide conclusive data if vaccine failures tend to increase with time after the last booster vaccination. Data also do not exclude the possibility that reported breakthroughs actually represent primary vaccination failures.[146]

It is estimated that approximately 4000 TBE cases and more than 20 deaths were prevented by vaccination in Austria between 2000 and 2011.[61,128] Since vaccine uptake is highest in endemic regions, exposure to TBEV is likely to be higher in the vaccinated population; therefore, effectiveness may, in fact, be even higher than calculated based on national coverage estimates. Impressively, whereas the incidence of TBE in Austria has declined markedly since the advent of generalized vaccination, that of neighboring Czech Republic, where only a small percentage of the population is immunized (16% in 2009) has actually increased.[128] A similar situation exists in Slovenia, with an incidence of 9.90 per 100,000 in 2007 and vaccination rates of only 12% in 2009.[83] The most affected region of Slovenia, which borders on Austria, reported a TBE incidence of 47 per 100,000 in 2006,[150] whereas the mean annual incidence rate in Austria from 2003 to 2007 was only 0.82 per 100,000, again suggesting that vaccination has an important impact on disease burden.[61]

A mass immunization program initiated in 1996 in the Sverdlovsk Region in Russia demonstrated the impact of the Russian TBE vaccines (TBE-Moscow, 80%; EnceVir, 6%; FSME-IMMUN, 12%; and Encepur, 2%). Using available data, this suggested that the incidence of TBE decreased from 42.1 per 100,000 in 1996, to 9.7 per 100,000 in 2000 and to 5.1 per 100,000 in 2006. Vaccine effectiveness was estimated to range from 62% in 2000 to 89% in 2006.[144]

Although vaccination is highly effective, rare cases of TBE have been reported after partial or complete primary

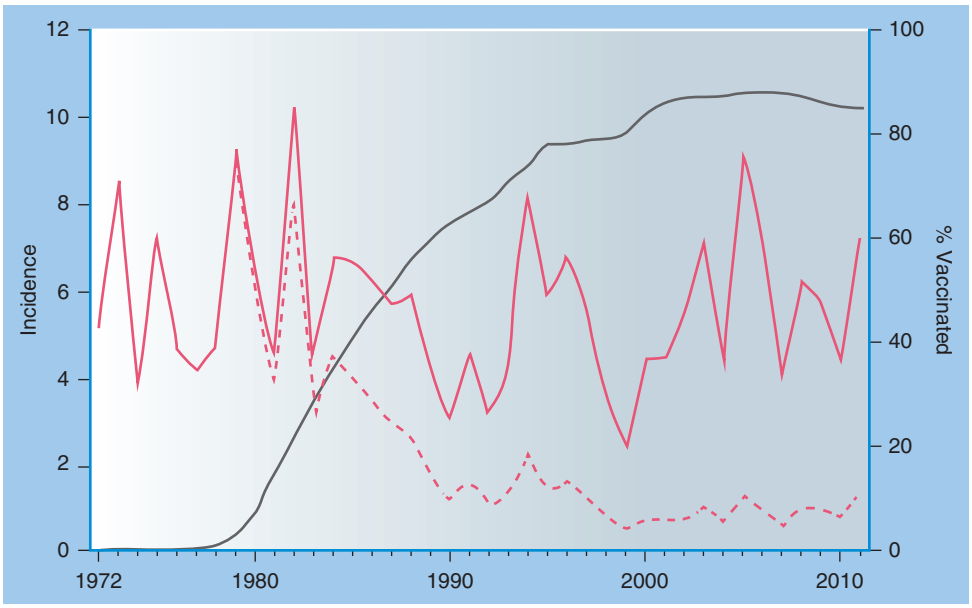


Figure 59.4. Tickborne encephalitis (TBE) incidence rate (cases/100,000 population/year) in Austria among vaccinated *(dashed red line)* and nonvaccinated populations *(solid red line)*, juxtaposed to the population vaccination coverage *(gray line)*. *(From Heinz FX, Stiasny K, Holzmann H, et al. Vaccination and tick-borne encephalitis, central Europe. Emerg Infect Dis. 2013;19[1]:69–76.)*

TBE vaccination, but not following booster immunization.[148,149,151,152] The majority of these breakthroughs occurred in persons older than age 50 years. Recent immunological investigations identified insufficient T-cell response as a possible mechanism of vaccine failure in a small proportion of primary vaccinees.[146]

Cross-Protection

There is good evidence that TBE vaccines protect not only against the homologous subtype, but also heterologous subtypes.[153] In vitro and in vivo studies demonstrate that vaccine-induced neutralizing antibodies are broadly cross-reactive and cross-protective.[118,154,155] There is no evidence that antibodies evoked by TBE vaccines provide cross-protection against other flaviviruses, although TBE prevaccinated subjects developed a significantly higher antibody response against Japanese encephalitis after vaccination with an inactivated Japanese encephalitis vaccine based on the attenuated SA-14-14-2 strain.[156]

Safety and Tolerability

Over the past decades, TBE vaccine formulations have been refined, significantly reducing vaccine reactogenicity. The currently available vaccines are considered to be safe with no serious adverse events reported.[18,109] Occasionally, however, fever and allergic reactions can occur. Following the standards of good clinical practice and extensive pharmacovigilance the excellent safety records of the two TBEV-Eu vaccines, FSME-IMMUN and Encepur, have been well documented. Little published data are available on the safety of the two Russian vaccines and almost no such information is available on the Chinese vaccine.[51] As with all intramuscularly administered vaccines, mild local reactions, such as redness, swelling, and local pain, may occur following TBE vaccination. Systemic adverse events after TBE vaccines include headache, fatigue, malaise, muscle pain, joint pain, and fever, and are more frequent after the first vaccination.

Safety data on FSME-IMMUN, after vaccination of more than 13,500 subjects of all age groups (>1 year of age) in both Phase II and Phase III clinical studies and postmarketing studies, show no safety concerns.[110,112,113,127,137,143] Typically, mild local reactions include redness, itching, and local injection site pain. Table 59.4 summarizes the relevant data from the Summary of Product Characteristics. There is evidence that HSA, when used as a stabilizer in the vaccine, is influencing the frequency of fever reactions. Experimental data,[98] as well as clinical reports of a HSA-depleted vaccine formulation,[124] suggest that HSA is associated with decreased fever reactions. HSA is hypothesized to suppress tumor necrosis factor-α production after vaccination.[98] However, it remains unclear why Encepur (see "Encepur" below), which does not contain HSA, is not associated with febrile reactions.

Postmarketing surveillance of FSME-IMMUN, including almost 42 million doses administered, indicates a rate of severe adverse events of 1.6 per 10,000 doses administered (spontaneous reports, not checked for causality).[123] An additional postmarketing study of general practitioners conducted by the Institute for Vaccine Safety of the Austrian Green Cross evaluated more than 25,000 vaccinations (85% vaccinated with FSME-IMMUN). A total of 107 adverse events of any severity were reported, including 69 (64.5%) in toddlers up to 2 years of age. In this study, 63 cases of fever were reported, 75.8% of which were in children after the first vaccination. The fever exceeded 40°C in only three cases.[157]

Encepur

Data from Encepur reveal a very similar picture for product safety based on RCTs, postmarketing studies, and regulatory documents in subjects ages 1 to 76 years (see Table 59.4).[105,114,158,159] Transient pain at the injection site, general malaise, myalgia, and headache were most commonly reported (>10% of vaccinees); redness and/or swelling at the injection site, flu-like symptoms, nausea, and arthralgia were observed in 1% to 10% of vaccinees. Transient fever, mostly low grade, was comparable to the rates after FSME-IMMUN administration.

Based on reports after the administration of more than 30 million doses of the licensed formulation of Encepur between 2002 and 2009, including pediatric and adult doses, the rate of serious adverse events was 1.9 per 10,0000 doses, a comparable safety profile to that of FSME-IMMUN.[51]

TABLE 59.4 Safety and Reactogenicity of Encepur and FSME-IMMUN

Probability	≥1/10	≥1/100 <1/10	≥1/1000 <1/100	≥1/10.000 <1/1000	Not Known
FSME-IMMUN First: *n* = 3512 Second: *n* = 3477 Third: *n* = 3277	Local reaction at injection site: redness, swelling, induration	Headache, nausea, myalgia, arthralgia, malaise, fatigue	Lymphadenopathy, vertigo, vomiting, fever (only exceptionally >39°C)	Acute allergic reactions; somnolence, diarrhea, abdominal pain	Aggravation of autoimmune disease; visual impairment, photophobia, meningismus, epilepsy, encephalitis, neuritis; tachycardia; urticaria, pruritus, exanthema; flu-like symptoms, weakness, edema
Encepur (pooled data from clinical studies and postmarketing surveillance)	Transient pain at injection site; general malaise, myalgia, headache	Redness, swelling at injection site; flu-like symptoms; nausea, arthralgia	Arthralgia and myalgia (neck)	Granuloma at injection site; lymphadenopathy, neuritis-like symptoms; diarrhea; systemic allergic reactions like urticaria, dyspnea, bronchospasm, hypotension	Extremely rare: Guillain-Barré syndrome

Data from summary of product characteristics.

Thus, both clinical studies and pharmacovigilance from the vaccine manufacturers indicate that the TBEV-Eu vaccines have an acceptable safety and tolerability profile. According to safety grading of the WHO, there is no causal association of these vaccines with serious adverse reactions.[160]

For the vaccines EnceVir and TBE-Moscow, there are no published large-scale, randomized, controlled trials from which to assess safety. Small-scale studies report low rates of adverse events and reactogenicity in children and adults.[99,106,161] Comparing the safety of immunization with TBE-Moscow vaccine and FSME-IMMUN (old formulation used before 2000) in children between 7 and 17 years of age, fever was reported more frequently with TBE-Moscow vaccine, but the differences were not significant.[107] Available data from post-marketing surveillance of the two Russian vaccines did not identify any serious adverse events.[162,163] According to the Russian National Regulatory Authority, TBE-Moscow vaccine and EnceVir are both safe and well tolerated, and their manufacturing is in accordance with WHO requirements.[18,123] However, no documentation on the quality assessment and control of the two Russian vaccines has been found. More recently, some lots of EnceVir were associated with high fever and acute allergic reactions in up to 19% of the vaccinees, particularly in children. These lots were withdrawn by the manufacturer, and the vaccine is currently not indicated for immunization of individuals younger than 17 years.[51]

No safety data have been found for the Chinese vaccine.

Contraindications and Precautions

Persons with acute inflammatory disorders that require treatment should not be vaccinated until at least 2 weeks after full recovery. Allergies to components of the vaccines constitute contraindications (listed in Table 59.2). Nonsevere allergy to egg protein does not usually constitute a contraindication to TBE vaccination. However, persons with such allergies should be vaccinated only under appropriate supervision. It has been speculated that previous exposure to other flaviviruses could affect TBE vaccination. Such problems are difficult to study because of cross-reactive flavivirus antibodies. Interestingly, although vaccination with TBE vaccine also enhances the immune response to an inactivated Japanese encephalitis vaccine,[156] there is no evidence of cross-protection between Japanese encephalitis and TBE. Pregnancy does not constitute a contraindication for the two TBEV-Eu vaccines.

Public Health Considerations

Even though no formal vaccine efficacy or effectiveness studies have been conducted for TBE vaccines, there is ample evidence of the public health impact of immunization, as discussed previously. The most striking data have been obtained from Austria, where TBE vaccination campaigns were initiated in the 1980s and reliable epidemiological data are available since 1972. Reported disease incidence dropped from 5.7 per 100,000 cases (range, 3.9–9.0) to 0.9 per 100,000 cases (range, 0.6–1.3) in parallel with increase in vaccination coverage with 85% to 88% of the population having received at least one dose of vaccine (see Fig. 59.4).[128] The findings are corroborated by the higher disease incidence in a neighboring country that did not introduce vaccine at population level.

Very few studies have been published on the economic burden of TBE disease. However, it is obvious that in regions with high disease prevalence, the disease has high costs to both individuals and society, particularly in those with long-term neurological sequelae. One study suggested that, as a result of widespread vaccination from 1991 to 2000, savings related to patient care, lost productivity, and early retirement amounted to 828 million Austrian Schilling or US$80 million.[164] These estimates would depend on the vaccine price, disease incidence, vaccine uptake, and booster vaccine policies.[18]

With the exception of Austria, no country currently has a universal vaccination recommendation (Table 59.5). The majority of endemic countries have issued recommendations targeting populations at risk, including those in endemic regions and with occupational exposure. As pediatric TBE cases tend to be less severe, some countries limit their recommendations to older age groups. Reimbursement regulations vary, but in many countries TBE vaccination is an out-of-pocket expenditure. In addition, many countries have issued vaccination recommendations for travelers to endemic regions.

In 2011, the WHO published a position paper on TBE vaccination.[18] The recommendation acknowledged the complexity of risk assessment, given highly variable risk patterns. The WHO recommends vaccination of all age groups in areas of high prevaccination disease incidence, defined as an incidence of 5 or more cases per 100,000 population per year. In lower-incidence areas, TBE vaccination should be confined to populations with a particular risk of exposure. The WHO also notes the significant knowledge gaps that exist in relation

TABLE 59.5 Vaccination Recommendations

Country	Recommendation Status
Austria	Universal recommendation older than age 1 yr
Bulgaria	No policy
Croatia	For highly endemic areas and occupational exposure
Czech Republic	For highly endemic areas and occupational exposure
Estonia	Recommended, but reimbursed only if occupational exposure
Germany	Recommended for endemic districts
Finland	Recommended for endemic region
Hungary	Recommended and reimbursed
Latvia	Recommended, mostly not reimbursed
Lithuania	Recommended, but not reimbursed
Norway	Recommended for high-risk populations, not reimbursed
Poland	Recommended for high-risk populations, not reimbursed
Romania	No policy
Russia	Recommended at regional level
Slovenia	For highly endemic areas and occupational exposure
Slovakia	For highly endemic areas and occupational exposure
Sweden	Recommended for highly (>1 year of age) and moderately (>3 years of age) endemic districts
Switzerland	Recommended for all endemic districts, >6 years of age
Turkey	No policy

From Zavadska D, Anca I, André F, et al., Recommendations for tick-borne encephalitis vaccination from the Central European Vaccination Awareness Group (CEVAG). Hum Vaccin Immunother. *2013;9(2):362–374; and European Centre for Disease Prevention and Control (ECDC).* Epidemiological Situation of Tick-Borne Encephalitis in the European Union and European Free Trade Association Countries. *Stockholm, Sweden: ECDC, 2012.*

to booster requirements,[165] and encourages research to better define booster needs and cost-effectiveness of immunization programs. The WHO also recommends the vaccine for travelers planning on outdoor activities in endemic areas during the period April to November.[74]

Other Disease-Control Strategies

Adequate clothing should be used to make skin access more difficult for ticks. Repellents containing 20% or more of *N*,*N*-diethyl-3-methylbenzamide (DEET) provide protection for several hours, and clothes can be treated with permethrin.[167] Skin should be checked daily for the presence of ticks, and ticks should be removed using fine-tipped tweezers. It is important to note that ticks may transmit other diseases, particularly Lyme disease (also termed borreliosis). Swiss authorities estimate that some 9000 cases of borreliosis and 105 cases of TBE occurred in Switzerland in the year 2014.[168] No vaccines are available against these bacterial infections, but they can be treated with antibiotics, although long-term sequelae have been described.

Related and Future Vaccines

In addition to the TBE vaccines described above, there is also a vaccine against KFDV that is manufactured and licensed in India. Originally, this was a formalin-inactivated mouse brain vaccine, but this was replaced in approximately 1990 with a formalin-inactivated chick embryo fibroblast-derived vaccine given as two doses, 28 days apart, with an initial booster at 6 to 9 months and subsequent boosters every 5 years.[169] The effectiveness of the two-dose vaccine was estimated as 93.5%; however, the duration of protective immunity is unclear.

Although there are licensed vaccines against TBE and KFDV, other members of the mammalian TBE serogroup are pathogenic and the question of cross-protection of vaccines is important. To date there have been no clinical trials to evaluate cross-protection against Omsk hemorrhagic fever, Kyasanur Forest disease, and anaplastic lymphoma kinase viruses. However, there have been some mouse studies investigating cross-protection. Neutralization tests performed on sera from OHFV-and TBEV-infected mice showed that neutralizing antibodies are cross-protective. The geometric mean titers of antibodies against TBEV and OHFV from TBEV-Fe strain Sofjin-infected mice were similar. The FSME-IMMUN TBE vaccine provided 100% protection against OHFV infection. Furthermore, 86% of FSME-IMMUN or Encepur TBE vaccinees seroconverted against OHFV following a three-dose vaccination regimen, and the geometric mean titers of neutralizing antibodies against OHFV were comparable to those against TBEV.[170]

All current TBE vaccines consist of purified, inactivated, whole-virus particles. A number of candidate TBE vaccines using recombinant or live attenuated technology are at various stages of development. Candidate chimeric live attenuated TBEV vaccines have been developed by replacing the prM and E genes of dengue type 4 (DENV-4) virus with the corresponding genes of TBEV-Fe strain Sofjin.[171] Another chimeric candidate TBEV vaccine was based on the antigenically distinct LGTV (LGTV prM and E genes in a DENV-4 backbone). A single dose of either of these chimeric vaccines was efficacious in protecting monkeys against wild-type LGTV challenge.[172] The chimeric LGT/DEN-4 vaccine candidate was subsequently shown to be safe in a Phase I clinical trial.[173] However, low levels of viremia were detected in one subject, and antibody responses to TBEV were poor. In a later report, this vaccine candidate was shown to replicate to moderate titers in the CNS of monkeys, causing pathological lesions in two and illness in one of four animals.[174] Further studies on a TBEV/DEN-4 vaccine candidate reported the introduction of defined mutations in several regions of the viral genome (E, NS5, and 3′NCR) that have led to improved attenuation in mice and arthropods, which represent the natural vector for the two wild-type viruses.[175,176] It was also shown that neurovirulence of the TBEV/DEN-4 chimera could be reduced by insertion into the viral genome of target sequences for cellular microRNAs expressed in the CNS.[177]

A number of promising experimental recombinant subunit and nucleic acid vaccines were reported in the 1990s and in the first half of the last decade,[30,145,178–181] but there have been no published follow-up data or registered clinical trials for the majority of these proposed vaccine candidates. Finally, a novel TBE vaccine candidate, RepliVAX-TBE, is currently being developed,[182] using a replication-defective flavivirus platform (or single-cycle replicating vaccine), RepliVAX. Attenuation is achieved by a deletion in the capsid gene that prevents replication; thus, the vaccine is generated in helper cells that supply this protein in *trans*.[183] RepliVAX-TBE based on a West Nile virus backbone (RV-WN/TBE; the prM and E genes were of TBEV-Eu strain Hypr) multiplied efficiently, was highly immunogenic and efficacious in a mouse model, and highly immunogenic in Rhesus macaques after a single dose, inducing a significantly higher humoral immune response when compared with three doses of the licensed FSME inactivated TBE vaccine. This study also showed that candidate live chimeric yellow fever vaccine strain 17D/TBE, dengue 2/TBE, and LGTV strain E5/TBE vaccines were not sufficiently attenuated to be vaccine candidates.

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60 Tuberculosis Vaccines

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Bacille Calmette-Guérin (BCG) is currently the only licensed vaccine available against tuberculosis (TB). The vaccine has been given to over 4 billion people; yet, TB still poses a major public health threat globally.[1] One-third of the world's population is thought to be infected with *Mycobacterium tuberculosis*, while 10.4 million new cases of TB disease, causing 1.4 million deaths, occurred in 2015.[1] Drug-resistant forms of disease, difficult and expensive to treat, are emerging and are associated with high morbidity and mortality.[1] Intervention in the TB pandemic requires optimal delivery of BCG, antimicrobials, and diagnostics. In settings where these current tools could be delivered optimally, the decline in TB incidence has been approximately 5% per year, compared to the global average decline of 1.5% per year.[2,3] Even at decline of 5% per year, the World Health Organization (WHO) Stop TB Partnership goal of achieving a disease incidence of 20 cases per 100,000 per year by 2030 is not achievable.[4] New tools are needed; among these, a vaccine that is effective in preventing adult forms of pulmonary TB is likely to have the greatest impact.[5,6] The ability of the current TB vaccine, BCG, to prevent pulmonary disease is variable, as the vaccine mainly protects infants and young children against disseminated forms of TB (see below).

TUBERCULOSIS—A BRIEF OVERVIEW

History and Epidemiology

TB has probably been the leading cause of death in Europe and the United States in recorded history.[7] The earliest known cases of TB were discovered in Egyptian mummies dating from 4000 to 2000 years BC where evidence of spinal disease was uncovered.[8] Recent studies analyzing global distribution and variability among *M. tuberculosis* strains suggest that the pathogen migrated out of Africa with humans during the Neolithic period and may have coexisted with humans for up to 70,000 years.[9,10]

In 1882, Koch identified the tubercle bacillus as the cause of TB.[11] The discovery that TB is an infectious disease led to four successive disease control approaches.[7] First, a sanatorium movement was established in Europe in 1854 and in the United States in 1882, and remained in place until after the advent of antimicrobial therapy against TB. This intervention included exposure to fresh air and sunlight in a community setting. Other intervention during this period included ordinances to improve sanitation and housing. The second development was pasteurization of cow's milk, which virtually eliminated human disease caused by *Mycobacterium bovis*, the most common cause of TB in animals. Third, the BCG vaccine was developed in the early 20th century in France (see below). Finally, antimicrobials against TB were developed, including streptomycin, first used in the 1940s, followed by isoniazid and rifampin, which were introduced in the 1950s and 1970s, respectively. These anti-TB drugs would be used both to treat established disease and to prevent progression of latent *M. tuberculosis* infection to active disease. The cornerstone of TB control for the past 5 decades in the United States has been a combination of targeted screening, chemotherapy, and contact investigation of persons potentially exposed to patients with TB disease.

It is worth noting that TB disease incidence declined in Europe and the United States long before the availability of chemotherapy and without use of BCG.[12] The decrease in incidence was associated with industrialization, urbanization, and an accompanying rise in socioeconomic status.

Today, the global burden of TB disease remains high, with an estimated 10.4 million incident cases and 1.4 million deaths in 2015 (Fig. 60.1).[1] Despite these numbers, substantial progress in TB control has been made in all regions of the world over the past 20 years. The United Nations Millennium Development Goal target of halting and reversing the rise in TB incidence globally by 2015 was met: between 2000 and 2013, global incidence was reduced by 1.5% per year.[1,13] The STOP TB Partnership's target of halving mortality attributable to TB from 1990 to 2015 was reached in three of six WHO regions; globally, mortality fell by 45% in the period up to 2013.[1]

Adolescents and adults between the ages of 15 and 54 years account for the vast majority of cases of TB disease,[1] and those with pulmonary disease are responsible for the transmission of TB. Modeling studies show that interruption of transmission would be the most effective intervention in the TB epidemic.[5] In contrast to adolescents and adults, children with TB do not commonly spread the pathogen, a consequence of the paucibacillary nature of their disease. Therefore, many TB vaccine developers are now focusing efforts on developing vaccines for delivery to adolescents and adults as a priority.[6] In contrast, the current TB vaccine, BCG, targets infants and works best in infant and childhood populations (see below). Ultimately, novel TB vaccine strategies should address all populations at risk, including, for example, the elderly, whose contribution to the Western Pacific Rim region burden of TB disease is increasing.[1]

The HIV epidemic has compounded challenges of meeting TB control targets: globally, approximately 13% of TB cases occur in HIV-infected persons.[1] HIV infection leads to increased susceptibility to TB disease, and complicates treatment, because of drug interactions between TB and HIV medications, and immune reconstitution inflammatory syndrome.[14] Novel vaccines would also need ultimately to be effective in HIV-infected persons if the burden of disease is to be prevented.

The rise of multidrug resistant (MDR) TB poses a global health threat. The WHO estimated that there were 480,000 new cases of MDR TB globally in 2013.[15] Extensively drug-resistant and totally drug-resistant strains of *M. tuberculosis* have now been isolated from patients, compounding efforts to control TB even more.[16] Drug-resistant disease needs to be treated for prolonged periods, and the total cost of care is manyfold higher than for drug-sensitive TB. Even in countries where drug-resistance rates are low, managing these forms of the disease may absorb a major portion of TB control budgets.[17] The situation clearly calls for new tools, of which novel, better vaccines would be particularly attractive. *M. tuberculosis* drug-resistance patterns are not likely to be a factor in vaccine efficacy.

BACTERIOLOGY

All members of the genus *Mycobacterium* share the property of acid-fastness, which is related to mycolic acid components of

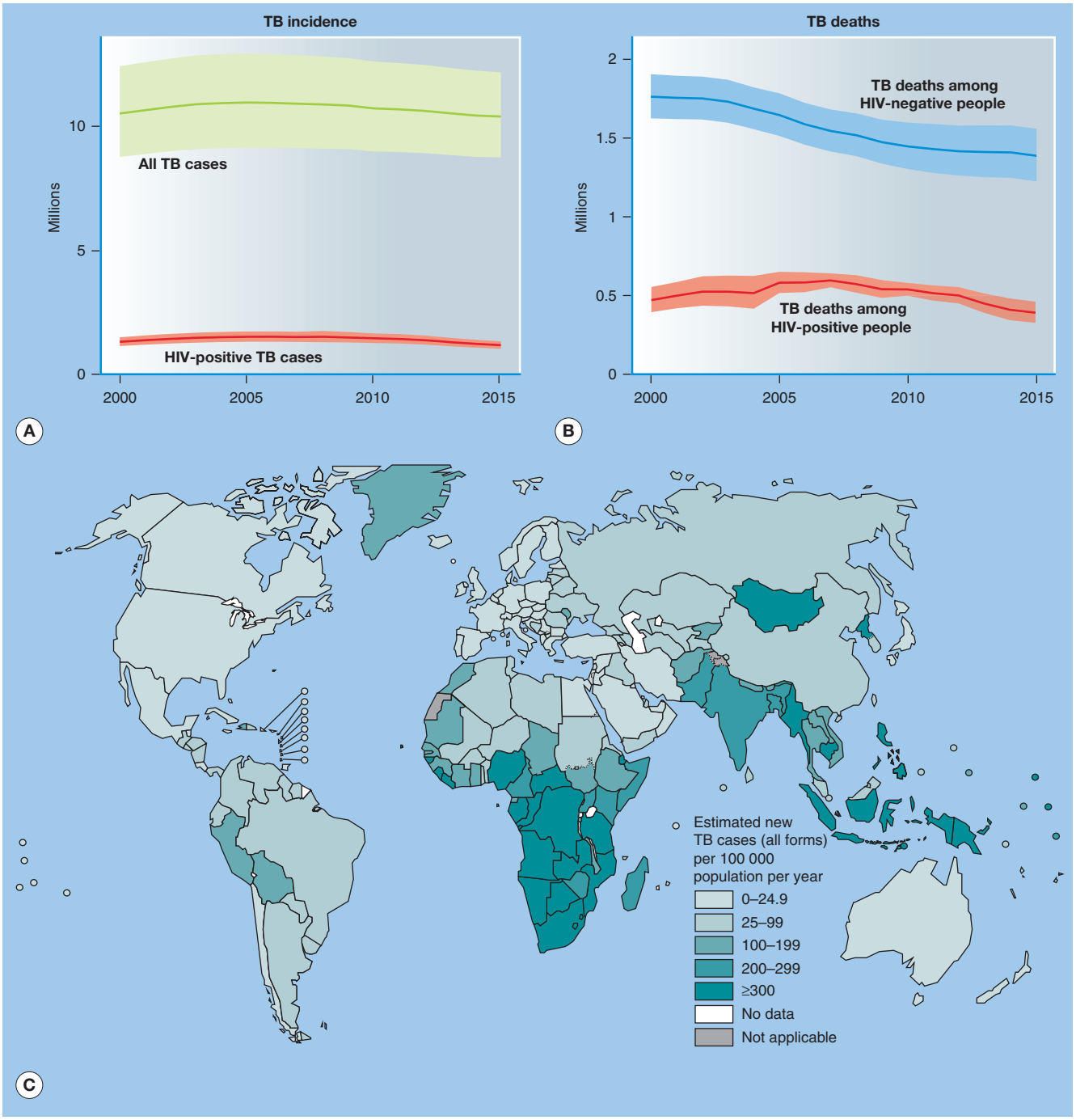


Figure 60.1. Changes in (A) global incidence of and (B) mortality from tuberculosis (TB). C, Incidence rates of TB disease in 2013 in countries from around the globe. *(From World Health Organization. Global Tuberculosis Report 2016. Geneva, Switzerland: WHO; 2016.)*

a complex cell wall. Each species has a unique pattern of mycolic acid composition.[18] Nine species—*M. tuberculosis*, *Mycobacterium africanum*, *Mycobacterium canetti*, *M. bovis*, *Mycobacterium caprae*, *Mycobacterium pinnipedii*, *Mycobacterium microti*, *Mycobacterium mungi*, and *Mycobacterium orygis*—share 99.9% similarity at the nucleotide level and have identical 16S ribosomal RNA sequences, and are classified together as the *M. tuberculosis* complex. These strains share growth characteristics and biochemical reactions, and can each cause clinical TB disease in humans and other mammals.[19,20] *M. bovis* causes TB in cattle and other animals, primarily, but can cause disease in humans who have extensive contact with infected animals or who drink animal milk that contain the pathogen. Veterinary control programs have all but eliminated *M. bovis* disease in the United States, but human infection still occurs in some countries.[21]

Approximately half of the approximate 3,800 open reading frames of the *M. tuberculosis* genome are involved in fatty acid metabolism. A major effort is ongoing to determine whether lipid components could be used in future vaccines against TB[22] (see below). Most new vaccines in clinical testing contain immunodominant protein antigens, including early secretory antigen target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) (see below). These antigens belong to the so-called RD1

region, which also contains the secretory apparatus for transport of ESAT-6 and CFP-10 to the cell surface to mediate host cell membrane disruption and bacterial release, thereby promoting cell-to-cell infection.[23,24] Importantly, the RD1 region is missing in BCG. Other important protein families, some of which have been incorporated into novel vaccines, include mycolyl transferases (Ag85 family), which play a role in building and stabilizing the cell wall by anchoring units of mycolic acid, and major chaperonins such as DnaK, GroEL, GoeES, and α crystalline. Recent studies have expanded our understanding of the breadth of antigens that stimulate T-cell responses in humans[25]; many novel CD4 and CD8 immunodominant epitopes[26] have been discovered with screening strategies. Interestingly, many of these belong to the PE/PPE group of antigens, which appear to have immunomodulatory and virulence properties.[27] Functional attributes of many *M. tuberculosis* proteins remain unknown. In addition, it is not known which particular antigens are critical for protection against TB. Rather, the lack of variation of immunodominant antigens in *M. tuberculosis* strains across the globe in the face of possibly tens of thousands of years of immune pressure suggest that responses to these components may even hold evolutionary advantage to the pathogen.[28]

Nontuberculous mycobacteria (NTM) were discovered shortly after Koch's identification of the tubercle bacillus, but were recognized as human pathogens only in the 1940s, approximately 20 years after the first use of BCG.[29] NTMs are a group of more than 140 species and are found worldwide, often in soil and water reservoirs.[30,31] The most ubiquitous species are those of the *Mycobacterium avium–intracellulare* complex. Although environmental mycobacteria rarely cause disease, immunologic effects on humans may modulate the course of *M. tuberculosis* infection. In addition, NTM interactions may partially explain variability in efficacy induced by BCG (see below).

PATHOGENESIS

TB is spread by adolescents and adults with cavitary lung disease who release the pathogens into the air, largely through coughing. Approximately 35% of contacts of a patient will become infected following inhalation of *M. tuberculosis*; proximity and duration of exposure are important determinants of infection. Only a small fraction of exposed persons resist infection, as demonstrated by their having persistently negative tuberculin skin tests (TSTs) or interferon-γ release assays (IGRAs).[32] These individuals may have an innate alveolar macrophage response to *M. tuberculosis* that resists initial infection or rapidly clears the bacillus before a T-cell response develops.

Upon inhalation, once in the lung, the bacilli bind to receptors on macrophages and other myeloid cells, such as monocytes, dendritic cells, and neutrophils, resulting in detection by the innate immune system.[33,34] Several phagocyte receptors mediate this detection, including toll-like receptors (TLRs: TLR1/2/6/8/9), Nod-like receptors (NLRs: NOD2), C-type lectin receptors (CLRs: CLEC4E or Mincle), mannose receptor (MR), dendritic cell-specific intracellular adhesion molecule-3 (ICAM-3)-grabbing non-integrin (DC-SIGN or CD209), and DNA sensors (STING).[35–38] In animal models, inoculation with tubercle bacilli first causes inflammation of the lung interstitium, with local tissue damage and histamine and prostaglandin release. Infected macrophages secrete tumor necrosis factor, which causes local tissue cells to release chemokines. Leukocytes are attracted, including neutrophils and T cells. T cells migrate to the lung following sensitization in regional lymph nodes by dendritic cells that have migrated to this compartment. A so-called TB primary complex visible on roentgenography consists of a local infiltrate in the lung as well as enlarged regional lymph nodes, both caused by cellular accumulation.

The onset of specific T-cell immunity greatly alters the pathogenic picture. The incubation period between when tubercle bacilli enter the body and the development of cutaneous delayed type hypersensitivity, a hallmark of the T-cell response, is most often between 3 and 8 weeks. During this time, a granuloma is formed at the site of infection. This granuloma, the pathognomonic histopathologic feature of the infection, contains a center of macrophage-derived cells with an outer ring of lymphocytes. The granuloma may contain the infection through encapsulation, or may break down leading to caseous necrosis with the subsequent local spread of mycobacteria. It is also thought that systemic spread of the tubercle bacilli to multiple organs occurs early in the pathogenesis of the infection.

Only 10% of untreated infected persons will develop TB disease, with approximately 50% of these individuals developing disease within 2 years of infection—so-called primary progressive disease. TB disease may also occur much later in life, by reactivation and growth of live bacilli contained within contained granulomas. This so-called secondary, postprimary, or adult-type disease is often associated with a temporary decline in T-cell immune status, associated with aging, cancers, poor nutrition, or other forms of immune compromise. In settings where TB is endemic, primary disease drives the epidemic. In this setting, exogenous reinfection may occur in persons previously infected and play a role in manifestation of clinical disease. As epidemic disease control advances, the relative contribution of postprimary, endogenous reactivation TB becomes greater.

There is evidence that a primary *M. tuberculosis* infection imparts a degree of protection from disease resulting from secondary exogenous infection. Studies from the preantibiotic era in areas with high TB rates compared active TB disease rates in those with or without original primary *M. tuberculosis* infection (as indicated by positive skin test reactivity). A metaanalysis of 18 studies with an aggregate sample size of 19,886 individuals estimated that individuals with latent *M. tuberculosis* infection (positive skin test reaction) had a 79% (95% confidence interval [CI], 70–86%) lower risk of developing active TB disease after reinfection when compared with uninfected individuals.[39] Other studies have had similar findings.[40–42] Together, these studies suggest that humans can acquire partial immunity to TB disease from natural exposure, and that understanding this protection may hold clues to successful vaccination against TB.

The immunologic mechanisms that control *M. tuberculosis* disease remain incompletely understood.[43] CD4 T cells are indispensable and act by producing T-helper type 1 (Th1) cytokines, including interleukin (IL)-2 and interferon (IFN)-γ, to activate infected macrophages for control of the pathogen. Animal studies show that CD8 T cells may also contribute, either through cytokine production or cytotoxic activity. In addition to effector responses, a host response characterized by immune mechanisms to control excessive inflammation may be important.[44] An optimal vaccination response would therefore not necessarily be a quantitatively greater T-cell response. To date, the immunological correlates of protection against *M. tuberculosis* infection and/or TB disease have not been identified.[45] Although identification of correlates of protection against TB is not possible until completion of a placebo-controlled trial of a protective vaccine, some clues have emerged from prospective studies of BCG-vaccinated infants or of other persons at risk of TB disease. A prospective study of infants vaccinated with BCG at birth, where a control arm of unvaccinated infants was not possible, showed that classical CD4 and CD8 T-cell responses induced by BCG and

measured 10 weeks after vaccination did not differ between infants who ultimately developed TB disease and whose who did not develop TB disease in the first 2 years of life.[46] These results suggest that host responses that are essential may not be sufficient for protection against TB. In another study, *M. tuberculosis*–infected South African adolescents were enrolled and followed for 2 years to identify those who developed TB disease and those who did not.[47] Examination of blood collected prior to development of disease revealed a 16-gene expression signature of risk. The signature predicted tuberculosis progression with a sensitivity of 66.1% (95% CI, 63.2–68.9%) and a specificity of 80.6% (79.2–82.0%) in the 12 months preceding tuberculosis diagnosis. The risk signature was validated in various independent African cohorts. Although further analysis of the character of the host response associated with disease risk is needed, these results represent a step toward better understanding of correlates of protection against TB.

During thousands of years of coevolution with humans, *M. tuberculosis* has evolved multiple strategies to evade or modulate a host response. The bacillus employs phagolysosome modification and inhibition of apoptosis (and promotion of bacillus survival) of innate cells,[48] for example. The pathogen also induces cellular trafficking to the granuloma to allow expansion of the number of cells available for infection to increase its survival.[34,49–51] After infection, T-cell responses to the pathogen are delayed; a positive skin reaction does not develop for approximately 6 weeks.[52,53] A similar delay in T-cell immune responses is observed in mice, which has been associated with delayed transport of bacilli from the lung to the draining lymph node.[54,55] Antigen-specific T cells often fail to recognize and eradicate *M. tuberculosis*-infected macrophages.[34,56] There are several possible mechanisms underlying impaired killing of bacilli, including a protected cellular niche for the pathogen during the first week that prevents recognition,[57] inhibition of IFN-γ activated pathways in macrophages,[58–60] and the development of excessive regulatory T-cell response,[61] which may dampen effective immune responses.

Successful vaccination against TB will require induction of immunity that is critical for protection, while, at the time of exposure to *M. tuberculosis*, circumvent the pathogen's ability to inhibit innate and adaptive host responses, and allow inflammation of an optimal character and magnitude. One possible approach would be to pursue a vaccine goal of prevention of *M. tuberculosis* infection, rather than disease.[62] Advantages would include circumvention of delayed T-cell induction by the pathogen, if CD4 T-cell or other effector cell responses were present at the time of exposure. Second, the kinetics of killing of *M. tuberculosis*-infected macrophages may be altered in acutely infected cells, compared with those with chronic infection. If T cells produced IFN-γ to activate macrophages immediately after infection, clearance of the bacillus may be more likely because of less time for development of *M. tuberculosis* modification of the macrophage. If vaccine-induced T cells were qualitatively different than those from chronic infection and present in higher quantities in the lung at the time of exposure, protection may be possible. Understanding why Th1 effector cells are ineffective during the early phases of infection and identification of markers of efficacy or of an optimal balance between effector and regulatory or exhaustion host responses may be critical for successful development of a TB vaccine.

CLINICAL FEATURES

The result of exposure to *M. tuberculosis* is no infection, transient infection,[63] or established infection. Established infection is so-called latent TB infection (LTBI). Individuals with LTBI have a positive TST or IGRA, but no clinical or radiographic evidence of active disease. TB disease occurs when manifestations of pulmonary or extrapulmonary TB become apparent, either clinically or on imaging studies.

The clinical presentation of pulmonary TB disease in childhood differs from that in adolescents and adults. The signs and symptoms of primary intrathoracic TB in children are often surprisingly mild, a result of the paucibacillary nature of the disease.[64] Nonproductive cough, mild dyspnea, and low-grade fever are the most common symptoms in infants. Chest roentgenograms may show hilar or mediastinal lymph node enlargement, a hallmark of childhood disease, and mild infiltrates. "Progressive primary disease" is characterized by lobular or lobar spread of TB and pulmonary dissemination is characterized by the appearance of miliary lesions. Complications of lymph node compression on the airway, such as segmental collapse, are relatively common, while parenchymal breakdown is uncommon.[65]

In contrast, adolescents and adults manifest more significant symptoms and signs because of the multibacillary nature of their disease.[66] The wide spectrum of clinical manifestations include chronic cough, low-grade fever, night sweats, fatigue, and weight loss. The disease occurs most commonly in the apical or posterior segment of the upper lobes, or the superior segment of the lower lobes. The typical pattern is airspace consolidation of a patchy or confluent nature, with cavitation occurring frequently. The development of fibrosis leads to volume loss in the involved lung. Older adults are more prone to having unusual radiographic manifestations of TB, including lower lung field disease.[67] At all ages, early pulmonary disease, identifiable by radiology, may be asymptomatic.

Extrapulmonary manifestations of disease occur in approximately 15% of immunocompetent adults and 25% of children.[68] Virtually any organ of the body can be involved. Superficial lymph node disease in the cervical or supraclavicular regions is the most common manifestation. Pleural TB accounts for almost 25% of the cases of extrapulmonary TB, but it is uncommon in children and rare in infants. Other sites of TB disease include the genitourinary system, the bones and joints, the peritoneum, and the pericardium.

Two forms of TB are life threatening: disseminated or miliary disease, and meningitis. The early clinical manifestations of miliary TB are protean, depending on the organs involved, including lungs, spleen, liver, bone marrow, meninges, or abdomen.[69] Central nervous system TB is fatal without effective treatment.[70] A meningeal exudate usually concentrates near the brainstem, while inflamed cortical or meningeal blood vessels lead to infarction of the cerebral cortex. Cranial nerve involvement is common, as is noncommunicating hydrocephalus. Death or profound handicap is a common outcome.

Adults with uncontrolled HIV infection who develop pulmonary TB often have a clinical and radiographic presentation that differs from that found in HIV-negative persons.[71] In advanced HIV infection, the radiographic presentation often includes hilar or mediastinal adenopathy, and diffuse or miliary pulmonary infiltrates, while cavitation is uncommon. Lower lung field involvement and endobronchial TB are much more common in patients with HIV infection. Disseminated or extrapulmonary TB occurs commonly. Patients with advanced HIV infection and TB who are started on both anti-TB drugs and antiretrovirals are prone to developing immune reconstitution inflammatory syndrome, which manifests as worsening of the original focus of infection or onset of new findings related to *M. tuberculosis* infection at other body sites.[72] The pathophysiology is dominated by a proinflammatory innate and adaptive immune response directed toward *M. tuberculosis* antigens.[73,74] The main treatment is corticosteroids to modulate the inflammation.

Diagnosis

Tuberculin Skin Test

A positive TST is the hallmark of latent infection with *M. tuberculosis* and remains positive for an individual's lifetime, even after chemotherapy.[75,76] The gold standard TST is the Mantoux test—the intradermal injection of purified protein derivative by use of a needle and syringe. The test should be read 48 to 72 hours after administration. Induration, not erythema, should be measured.

The appropriate cutoff size for a positive TST varies according to the clinical and epidemiological setting.[77,78] For adults and children at the highest risk for TB, a Mantoux induration of 5 mm is regarded as positive. For other groups at high risk for TB, induration of 10 mm is considered positive. In settings where TB is rare, the cutoff may be increased to 15 mm for individuals with no other risk factors for infection or disease.

Ten percent of adults and children with TB disease have a negative TST.[79,80] A variety of host-related factors such as young or old age, poor nutrition, immunosuppression by disease or drugs, viral infections, and overwhelming TB can lower TST reactivity. False-positive reactions may be the result of environmental mycobacterial exposure or BCG vaccination. The size of the skin test reaction after BCG vaccination varies with the strain and dose of the vaccine,[81,82] the route of administration,[83,84] the age of the person,[85–87] the nutritional status of the patient, the number of years since vaccination,[85,87,88] and the frequency of skin testing. There are conflicting findings about whether the TST reaction increases with repeated BCG vaccination or not.[89,90]

In a large number of studies of children who received BCG vaccine, the mean reaction to a TST ranged from 0 to 19 mm, although many experts believe that reactions larger than 10 to 15 mm that result from vaccination are unusual. Tuberculin reactivity wanes rapidly during the first few years after vaccination. Lifschitz found that approximately 50% of infants given BCG vaccine shortly after birth were tuberculin-negative at 6 months of age, and almost all children were tuberculin-negative at 1 year after vaccination.[85] Only 18% of Sri Lankan children vaccinated with BCG at birth had significant reactions at 1 year of age, and none was significant at 5 years of age.[91] A study from Canada investigated older children and adults who had received BCG vaccine by the scarification method on the lower back either in infancy or as older children.[92] Among children with a mean age of 11 years at skin testing, 4.9% vaccinated in infancy had a positive TST, compared with 12.5% of those vaccinated as older children. Among young adults with a mean age of 23 years at skin testing, 10.3% vaccinated in infancy had a positive TST compared with 25.5% of those vaccinated as older children. A similar study found that only 16% of U.S. naval recruits who had received BCG vaccine 8 to 15 years previously had a positive TST reaction at 10 mm or more.[93]

In summary, in BCG-vaccinated persons, the probability that a skin test reaction has resulted from infection by *M. tuberculosis* increases (1) with the size of the reaction, (2) when a patient had been in contact with a patient with infectious TB, (3) if the person is in a high-risk group for TB, (4) when the person's country of origin has a high prevalence of TB, and (5) when the interval between vaccination and skin testing is longer.[90]

Interferon-Gamma Release Assays

The newer IGRAs are also used to diagnose latent tuberculosis and contain antigens that are specific to *M. tuberculosis,* absent in BCG, and variably present in NTMs.[94] The QuantiFERON-TB Gold In-tube test (Qiagen, Germany) is based on a whole-blood enzyme-linked immunosorbent assay using three antigens: ESAT-6, CFP-10, and TB7.7. QuantiFERON-TB Gold Plus is the latest version of this test and includes additional ESAT-6 and CFP-10 epitopes that are targets of $CD8^+$ T cells. The test was designed to improve sensitivity and perform better in HIV-infected individuals with low CD4 T-cell counts. T-SPOT. TB (Oxford Immunotec, Oxford, UK) is also based on measurement of IFN-γ released by T cells stimulated with ESAT-6 and CFP-10, using an overnight enzyme-linked immunospot assay. These tests and other new tests may become important in clinical trials for new TB vaccines, addressing some of the methodologic problems encountered with previous BCG vaccine trials.

Microbiologic Diagnosis

The most rapid and widely available procedure to detect mycobacteria is microscopic examination of sputum or other body fluid with an acid-fast stain. Fluorochrome methods are more sensitive than carbolfuchsin techniques, but at least 10^4 acid-fast bacilli per milliliter of specimen are required for detection with either method. An acid-fast stain of sputum can identify 40% to 75% of adults with pulmonary TB.[95,96] Although environmental mycobacteria can be present in sputum and create a false-positive smear, the specificity of the acid-fast smear of sputum is high, especially when TB is endemic.[97] Most children with pulmonary TB do not spontaneously produce sputum and early morning gastric aspirates are used for diagnosis. As a consequence of the paucibacillary nature of childhood disease, sensitivity of acid-fast smears from these samples is low: usually less than 10% even in highly endemic areas.

Mycobacterial culture is commonly regarded as the gold standard for diagnosis. The best culture specimen is freshly expectorated sputum, from which *M. tuberculosis* can be isolated in approximately 80% of cases of pulmonary TB in adults.[98] Childhood gastric aspirate and induced sputum-derived cultures are positive in less than 50% of cases.[99]

Newer nucleic acid amplification techniques for direct detection of *M. tuberculosis* in sputum include Xpert MTB/RIF. In addition to rapid detection of presence of the pathogen, rifampin resistance may be evaluated simultaneously. A recent Cochrane review of 15 studies showed that Xpert had a pooled sensitivity and specificity of 88% and 98%, respectively, for replacement of smear microscopy.[100]

Newer Diagnostic Tests

In 2014, a consensus group convened by the WHO recommended the highest priority indications for TB diagnostic test development for impact.[101] First, a non–sputum-based, point-of-care test able to detect all forms of TB. Progress toward such a test, performed using blood or urine, is in early stages of development, and focuses on detection of specific host signatures or bacterial components like lipoarabinomannan. The recently marketed urine lipoarabinomannan detection test displays limited sensitivity, but may be useful for diagnosing TB in certain populations, such as HIV-infected persons.[102] Second, a simple, low-cost point-of-care triage test to be used by first-contact healthcare providers to rule out TB. Third, a point-of-care sputum-based test to be used as a replacement for smear microscopy. In some settings, such as South Africa, Xpert MTB/RIF is already used at a primary care level. Next-generation nucleic amplification tests hold the promise of being more compact and less expensive, and possibly more applicable to a primary care setting globally.[103] Finally, a rapid drug susceptibility test, which is likely to be incorporated into next-generation nuclear amplification tests[103] remains a priority.

Clinical Diagnosis

In both highly resourced and lesser-resourced environments, many cases of childhood TB are diagnosed on clinical grounds with the use of one of several scoring systems incorporating symptoms, signs, and, when available, radiographic findings.[104,105] Although helpful, these systems have low sensitivity and specificity.

TREATMENT AND INFECTION CONTROL

TB is treatable and preventable with antimicrobials. Guidelines for treatment are published by many expert groups, including the American Thoracic Society,[106,107] the American Academy of Pediatrics,[108] the Centers for Disease Control and Prevention,[109] and the WHO.[110] In general, TB disease should be treated with three or four drugs—isoniazid, rifampin, pyrazinamide, and ethambutol—for 2 months, followed by 4 months of isoniazid and rifampin.

Drug-resistant strains of *M. tuberculosis* are more difficult to treat. Strains are classified as extensively drug resistant when additional fluoroquinolone and injectable aminoglycoside resistance are present. Totally drug resistant strains of *M. tuberculosis* have also been described.[111] Treatment of MDR-TB is challenging because of lower efficacy, longer durations, and more adverse effects. With rising levels of drug resistance worldwide, the impact of an efficacious vaccine will be heightened.

The goals of infection control programs are to detect TB disease promptly, to isolate those who have or are suspected of having TB disease by using airborne precautions, and to treat people who have or who are suspected of having TB disease. Factors associated with infectiousness in adults include presence of a cough; lung cavities; acid-fast bacilli on sputum smear; disease of the lungs, airway, or larynx; and less than 2 weeks of adherent treatment.[112]

Bacille Calmette-Guérin Vaccine

BCG is a live attenuated strain of *M. bovis*. Two French scientists, Calmette, a physician, and Guérin, a veterinarian, selected a *M. bovis* strain isolated from a cow with tuberculous mastitis in 1908.[113] The isolate was cultured in a medium that contained glycerol, potato slices, and beef bile, and subcultured every 3 weeks for a total of 13 years or 239 cycles. Loss of virulence was shown first in animal models and phenotypic changes from rough, dry, and granular colonies to viscous, moist, and smooth ones were noted.

The vaccine strain was first used in 1921 in Paris, when it was given orally to infants.[114] In 1928, the League of Nations declared that oral BCG vaccine was safe. Unfortunately, from 1929 to 1930, 72 of 250 children vaccinated in Lübeck, Germany, died of TB, as the oral preparation was contaminated by virulent *M. tuberculosis*.[115] Despite this tragedy, BCG vaccination continued, with introduction of new methods of administration: intradermal in 1927, multiple puncture in 1939, and scarification in 1947.[116]

In 1948, the First International BCG Congress in Paris concluded that BCG was effective and safe, even though no controlled clinical trials or retrospective case-control studies of the intervention had been performed. After World War II, the WHO and the United Nations International Children's Emergency Fund (UNICEF) organized campaigns to promote vaccination with BCG in several countries. The seed-lot system for BCG was established in 1956,[113] and the WHO developed requirements for freeze-dried BCG vaccine in 1966.[117] Since 1974, BCG vaccination has been included in the WHO Expanded Programme on Immunization, which aims to strengthen the fight against infectious diseases among children in developing countries. Rates of BCG vaccination have increased dramatically; more than 4 billion persons have received the vaccine, and more than 100 million infants receive BCG annually at present.

Bacteriology of Bacille Calmette-Guérin Strains and Vaccine Constituents

In the decades since the first BCG created by Calmette and Guérin, multiple substrains have been generated using diverse culture methods that are best classified by genomic sequencing (Fig. 60.2).[118–122] A genealogy of BCG vaccine strains and a timeline for specific polymorphisms have been suggested.[118–121] BCG substrains differ in colony morphology, growth characteristics, biochemistry, immunogenicity, and virulence in animals.[123–134]

The variability among BCG strains is of concern for two reasons. First, extensive serial passage may have unintentionally resulted in overattenuation, compared to the original BCG strain.[135] Second, variability in substrains may be responsible, in part, for variability in BCG efficacy demonstrated in clinical trials (see below). The consequences of strain variation on human vaccine efficacy and adverse effects remain to be fully elucidated; multiple studies have now shown that different strains induce differential host responses in humans.[136–138] To prevent further accumulation of genotypic and phenotypic differences during vaccine production, the WHO and many national regulatory authorities have instituted standardized procedures for manufacture (see below).

Manufacturing, Quality Assurance, and Supply

BCG vaccine is produced from seed lots of lyophilized bacilli. A master seed lot is defined as a bacterial suspension of a single substrain originating from the bacillus of Calmette and Guérin that has been processed as a single lot and is of uniform composition.[139] The seed-lot system was adopted in the mid-1950s in an attempt to standardize production and vaccine characteristics. In the 1960s, the WHO recommended stabilization of the biologic characteristics of daughter strains by lyophilization and cryostorage of BCG.[140] Seed lots of the various BCG strains were originally maintained by the WHO and the Statens Serum Institut in Copenhagen.[117] Currently, BCG manufacture is the responsibility of individual suppliers, overseen by national regulatory authorities.

The classical method of manufacturing BCG involves inoculation into liquid medium for growth as a pellicle on the surface. Alternatively, organisms are dispersed through the liquid medium, which results in a slightly different colony morphology. Regardless, the WHO recommends, to avoid variation, that the total number of passages from master seed lot to final product should not exceed 12.[139,141] Early harvest of bacilli after 6 to 9 days of growth is essential for ultimate viability of the product. After filtering and pressing, the semidry mycobacterial mass is homogenized at controlled temperatures, diluted, and then freeze-dried. A stabilizer is added to the preparation.

Culture conditions can have profound effects on the final product.[123,142,143] For example, the Tice strain is grown in a medium containing glycerin, asparagine, citric acid, potassium phosphate, magnesium sulfate, and iron ammonium citrate. Lactose is added to the final preparation prior to freeze-drying.[144] In contrast, the Danish strain contains sodium glutamate, magnesium sulfate heptahydrate, dipotassium phosphate, citric acid monohydrate, L-asparagine monohydrate, ferric ammonium citrate, glycerol and water for

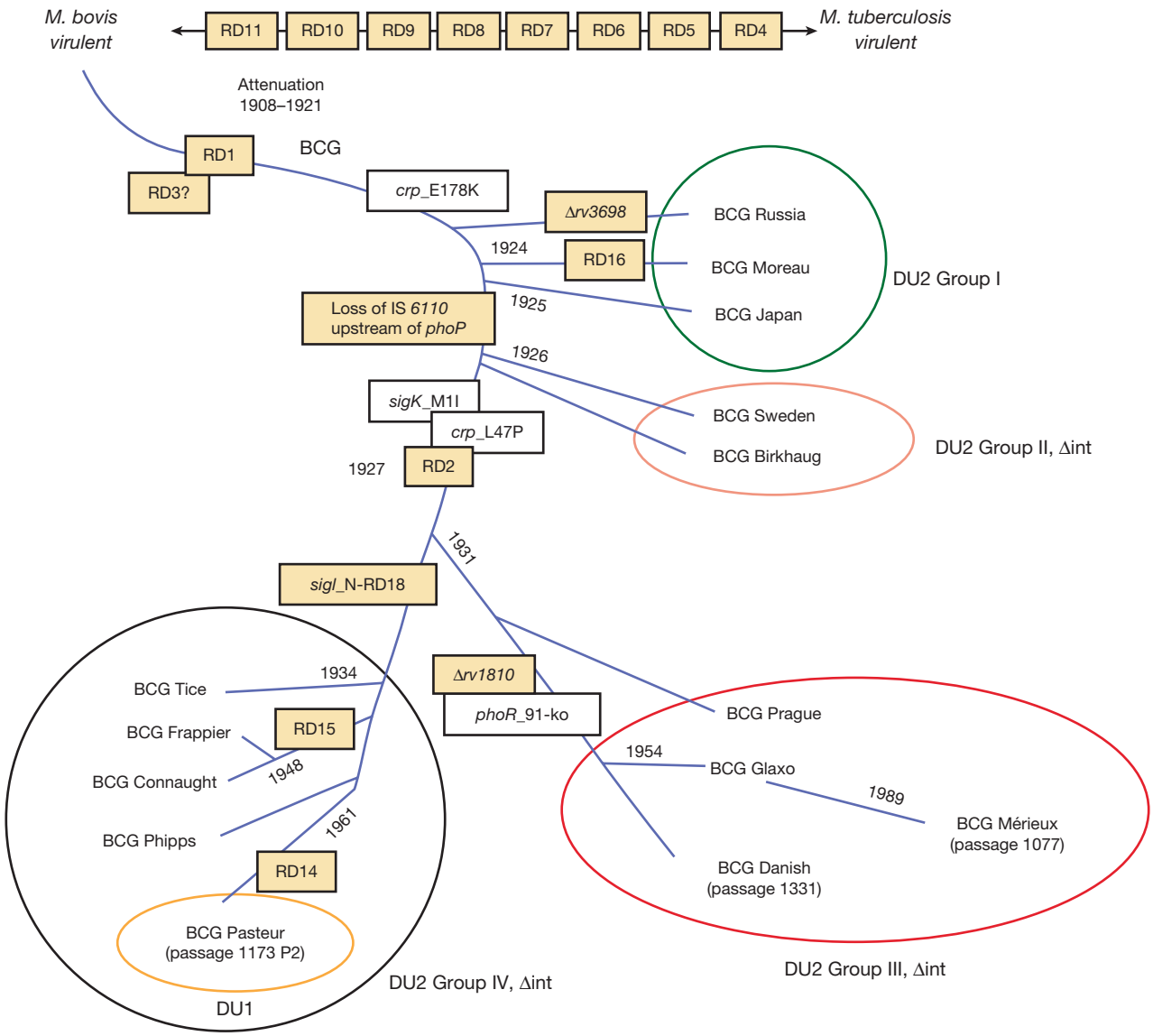


Figure 60.2. Classification of the bacille Calmette-Guérin (BCG) strains currently in clinical use. Of the 3954 protein-coding genes of BCG, 58 are present in two copies as a result of two independent tandem duplications, DU1 and DU2. Strains can be classified according to variation in DU1 and DU2. Genealogy is demonstrated by year in which deletions of groups of genes (RD [region of differentiation]) or individual genes, both shown in boxes, occurred. *(From Brosch R, Gordon SV, Garnier T, et al. Genome plasticity of BCG and impact on vaccine efficacy.* Proc Natl Acad Sci U S A. *2007;104[13]:5596–5601.)*

injection.[145] The Connaught strain contains monosodium glutamate, sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, polysorbate 80, and water for injection.[146] It is conceivable that these differences may ultimately affect vaccine efficacy.

The most recent WHO recommendations to assure quality, safety, and efficacy of BCG were published in 2013.[147] WHO recommendations focus on the manufacture of lots that do not differ significantly from prior lots shown to be safe and effective in humans and do not state a preference of one vaccine over another.[147] WHO Reference Reagent substrains are available as comparators for validity and consistency monitoring in viability assays[147]; the Danish 1331, Tokyo 172-1, Russian BCG-I, and Moreau RDJ strains of BCG are available[148] (see "Products Available" below).

Viability is assessed either by determining the number of culturable particles using a national regulatory authority–approved method or by a bioluminescence or other biochemical method that has been validated, such as the adenosine triphosphate method.[147] As the lyophilization and reconstitution process results in cell death, the final product contains both live and dead bacilli. The final total bacterial mass of the vaccine appears to correlate with local reactogenicity, whereas the number of culturable organisms appears to correlate with tuberculin sensitivity.[117] The number of culturable bacilli from some vaccines may also correlate with the rate of complications in newborns (see "Safety" below).

Other routine quality-control procedures include a test of identity, a test for contamination, and tests for dermal reactivity and safety in guinea pigs. Thermal stability testing requirements are considered to be at the discretion of the national regulatory authority, and are generally based on determining the number of culturable particles before and after samples are maintained at appropriate temperatures for designated

periods, with the ratios of before-to-after values and the absolute value approved by the national regulatory authority.[147]

All freeze-dried BCG vaccines are sensitive to ultraviolet and fluorescent light and heat and should not be exposed to direct sunlight; BCG vaccines are supplied in dark brown glass vials or ampoules and are typically supplied in multidose containers.[150] After reconstitution of the multidose vial, the vaccine should be used as soon as possible, while the remaining vaccine should be stored at 2°C to 8°C for a total expiration time determined by individual stability studies: typically a few hours.[147] As a result, a substantial amount of BCG vaccine is wasted during routine use, contributing to periodic supply shortages.

United Nations agencies supply more than 120 million doses of BCG per year. UNICEF purchases vaccines through a WHO prequalification process, which determines eligibility of different vaccines for clinical use. Currently, there are four WHO prequalified producers and distributors of BCG vaccine: BB-NCIPD/Intervax, BCG Japan, Serum Institute of India (SII), and Statens Serum Institut (SSI) in Denmark.[151] However, a number of other manufacturers produce vaccine for local use.

Products Available. The various strains of BCG are generally known by the name of the country or laboratory in which they are kept. The following account for more than 90% of the vaccines in use worldwide: French (Pasteur) strain 1173 P2, Danish (SSI) strain 1331, Glaxo strain 1077, Tokyo strain 172-1, Russian strain BCG-I, and Moreau RDJ.[152] Other commonly used strains include the Montreal strain (Canada) and the Tice strain (United States). The Tice strain, originally derived from the Pasteur strain 1173 P2 and produced by Organon Teknika (now owned by Merck) is the only product approved for use in the United States.

Dosage and Route of Administration

Worldwide, BCG is most commonly administered by intradermal injection with a syringe and needle. The only alternate route used currently is percutaneous administration with a multipuncture device.[153–155] A Phase IV trial conducted in South Africa compared multipuncture (percutaneous) and intradermal administration of the Japanese BCG strain and demonstrated equivalence between the two routes, in terms of both efficacy to prevent pulmonary TB and adverse events.[156]

Intradermal injection can be technically difficult in newborns, but other methods of administration have not been as successful. Subcutaneous injection induces tuberculin sensitivity, but may result in abscesses and unsightly, retracted scars.[157] Other techniques, such as scarification, jet injection, and use of bifurcated needles, have yielded variable results.[157–159]

The recommended dose of BCG vaccine differs by vaccine strain and age of the recipient. Similarly, the number of colony-forming units per dose varies by vaccine strain.[117] Although many body sites can be used for vaccination, the most common site is the deltoid region of the arm, usually on the right.

A wide range in BCG dosing schedules exists globally.[160] The WHO recommends a single dose given in infancy.[161] The effect of delaying BCG vaccination beyond the immediate newborn period has been studied, with variable results in different settings (see "Immunogenicity" below). BCG can be given to low-birth-weight newborns, as these infants develop a specific immune response.[162] When vaccination of preterm infants at 34 to 35 weeks' gestation was compared with delaying the vaccination to 38 to 40 weeks' gestation, no difference in tuberculin reactivity, scar formation, or immune response could be shown.[163] Safety and immunogenicity has been shown following administration to infants as preterm as 31 weeks.[164] Mangtani and colleagues investigated the question of age of vaccine administration in their metaregression analysis of published BCG studies[165] and reported that BCG is most effective when given at younger ages. In addition, school-age children who received BCG were better protected if their prevaccination TST reaction was small, which suggests that vaccination prior to sensitization by NTM or even *M. tuberculosis* may be a more important determinant of success (see below).Despite the WHO recommendation to give a single dose to newborns, some countries continue to give the first dose of BCG in infancy followed by one or multiple vaccinations throughout childhood.[166] Although this is an area of active investigation, evidence to support revaccination of older children and adults does not exist currently (see below). In some settings, repeating BCG vaccination is based on lack of tuberculin sensitivity, the absence of a typical BCG scar, or an individual's risk of exposure.[167] Regardless, neither absence of a scar after BCG vaccination nor tuberculin sensitivity correlates with risk of TB disease after BCG vaccination.[168,169]

Immunogenicity

The qualitative or quantitative determinants of BCG-induced immunity required for protection against TB are not known. Furthermore, knowledge of natural host protection against *M. tuberculosis* infection and TB disease is incomplete (see above). It is known that a CD4 T-cell response is essential for protection, as illustrated by markedly increased risk of mycobacterial disease in the presence of genetic mutations of the IL-12/IFN-γ pathway,[170,171] and by increased TB disease risk in untreated, advanced HIV disease.[172] For this reason, TB vaccinology efforts have relied heavily on the paradigm that a CD4 T-cell response should be induced, with production of so-called type 1 cytokines, IFN-γ, IL-2. and tumor necrosis factor–α.

Multiple studies of human newborns vaccinated with BCG have shown potent induction of CD4 T cells that produce type 1 cytokines (Th1 response).[136,137,173–178] However, there is great variability in the magnitude and character of the Th1 immune response in infants. In a study of nearly 6000 infants, the magnitude and character of the T-cell response to BCG did not correlate with risk of TB disease in the first 2 years of life,[46] suggesting that a Th1 response may not be sufficient for protection against TB.

BCG-specific T cells are able to proliferate in vitro and may have cytotoxic potential.[176,179] CD8 T cells, which may contribute to protection against TB, are also induced by newborn BCG vaccination, although at much lower frequency and with lesser diverse cytokine production profiles, compared with CD4 T cells.[46,175,180] Induced CD8 T cells may be cytotoxic. The phenotype of CD4 T cells induced by vaccination of human newborns is a mixture of effector memory and central memory cells; that is, a mixture of shorter-lived, ready-for-action (effector) cells and longer-lived (memory) cells that require additional immune stimuli to become effector cells again.[176,177]

After newborn BCG vaccination, the immune response appears to peak between 6 and 10 weeks of age, after which there is a gradual decline in frequency of specific cells in peripheral blood.[176] The reduction in detectable peripheral blood cells may be a consequence of homing to specific organs.

The effect of HIV exposure and infection of the newborn on the BCG induced immune response has also been studied.[181–185] Overall, HIV exposure in infants who do not become infected appears to have little effect on the

BCG-induced immune response, measured over the first year of life. A dramatically different scenario occurs in infants who are HIV infected. In the HIV-infected group, when not on antiretroviral therapy, the magnitude of the peak specific response is much reduced, and virtually no specific immune response is detectable at 1 year of age.[181]

The host response to BCG vaccination of infants beyond the immediate newborn period has also been defined.[175,177,186] Various projects have assessed whether delaying vaccination to 6 weeks of age, or even later, would influence the BCG-induced immune response. The results have been conflicting—in some instances, a delay in vaccine administration was associated with enhanced immunogenicity, whereas in others there has been no enhancement.

The importance of the epidemiological setting in BCG-induced immunity has been evaluated systematically.[178,187–189] Malawian infants vaccinated with BCG demonstrate quantitative and qualitative characteristics of a Th1 response that are distinct from those of UK infants vaccinated with BCG. These results may also be the consequence of genetic or other differences between populations.

When considering prior mycobacterial exposure, infants born to mothers who are latently infected with *M. tuberculosis* appear to have a lower host response to BCG vaccination compared to infants born to mothers who are uninfected.[190] Similarly, environmental mycobacterial exposure prior to BCG vaccination in children and adults vaccinated beyond the immediate newborn period may result in lower host responses.[191] This may translate to lesser protection, as a negative TST at the time of vaccination correlates with BCG-induced protection against TB (see below). These studies suggest that preexisting mycobacterial infection may result in masking or blocking of host responses to BCG. The effect of non–mycobacterial infections, particularly helminth infections, on the BCG-induced response has been studied extensively.[192–194] These results are inconsistent—in some cases, lower host responses have been reported and in others there has been no effect. Again, these results may reflect study design and epidemiological and genetic diversity.

BCG vaccination of adults does induce an immune response that is similar in character to that of newborn vaccination, although the induced immune response may peak earlier in adults.[84,186] One study shows that BCG may safely be given to persons latently infected with *M. tuberculosis* with the baseline immune responses not impacting the ultimate vaccine response (Thomas J. Scriba, personal communication, 2016).

Data on animal host responses induced by BCG—and protection afforded by vaccination—should be interpreted with caution, as none of these completely replicate human disease. For example, TB in the mouse is a chronic disease, with the histological appearance in the lung differing from humans. In mice, the breakdown of granulomas is unusual, whereas this is common in adults with TB disease.[195] In the mouse, BCG induces a predominance of effector memory T cells, whereas in humans, both effector and central memory responses are induced. Nonhuman primate species, such as rhesus and cynomolgus macaques, may best model human TB where immune responses to BCG vaccination have been very similar to that described in humans.[196,197]

Conversion of the TST has been used widely to assess whether a person has responded to BCG vaccination; in the past, revaccination was often advised if no response was detectable. Unfortunately, neither the presence nor size of the postvaccination TST reaction reliably predicts protection afforded by BCG.[198,199] Results from the Medical Research Council's BCG trial in Great Britain demonstrate a high level of protection against TB and show that the degree of tuberculin sensitivity is independent of the degree of protection conferred by vaccination.[200] Other studies, such as the Chingleput trial in southern India, demonstrated poor protective efficacy while high levels of vaccine-induced tuberculin sensitivity were shown.[201,202]

Bacille Calmette-Guérin Efficacy and Effectiveness

Overall, BCG appears most protective against disseminated forms of childhood TB, such as miliary TB and TB meningitis, with an overall efficacy of approximately 80% (see below). Protective efficacy against active pulmonary disease has been variable, ranging from 0% to 80%; protection is greater early in childhood, in persons who are TST-negative prior to vaccination, and in persons who live further from the equator—the latter two variables may be related to environmental mycobacterial exposure (see below). BCG appears to have a modest effect in preventing *M. tuberculosis* infection: overall, approximately 20% efficacy (see below). Finally, BCG may protect against other mycobacterial disease: the vaccine is highly effective at preventing leprosy,[203] but has limited protective efficacy against Buruli ulcer.[204]

Selected Controlled Trials of Bacille Calmette-Guérin Efficacy

Fig. 60.3 and Table 60.1 summarize the efficacy of BCG as demonstrated in major case-control, cohort, and household-contact studies. Comparison of the trials is difficult because of differences in eligibility criteria, methods of disease surveillance, diagnostic/outcome criteria, vaccine strain and administration, trial setting and environmental factors. Comstock published a comprehensive comparison of the major BCG vaccine studies.[205] Extensive discussion comparing the controlled trials can also be found in other reviews and in previous editions of this textbook.[206–209]

The largest controlled clinical trials, each involving more than 10,000 participant years of follow up, are discussed here. Three trials demonstrated excellent protection against pulmonary and other forms of TB.[200,210,211] The first was conducted among Native Americans in the 1930s and involved school-age children. The estimated efficacy in preventing pulmonary TB in this trial was 74% (95% CI, 67–80%).[210] The second study was also conducted in the 1930s, in Chicago, among infants living in high-risk areas. The protective efficacy against pulmonary TB was 66% (95% CI, 40–81%).[211] A third trial was initiated in 1950 among British schoolchildren and showed 78% (95% CI, 69–84%) efficacy against pulmonary TB.[200] These three clinical trials had three characteristics in common. First, they were conducted in northern geographic areas with a low prevalence of environmental mycobacteria (see below). Second, infants or children were enrolled. Third, in subsequent critical evaluation of all controlled trials, the methodology and statistical precision of these three trials have been judged to be superior to those of the other major BCG trials—for example, the protective efficacy of each had narrow 95% CIs, which was not the case in the majority of other trials.[165,209,212]

Other controlled trials have demonstrated moderate to poor protective efficacy. The first was sponsored by the U.S. Public Health Service and conducted in Puerto Rico starting in 1949.[213] The efficacy in preventing TB disease in school-age children was 28%, with a wide 95% CI of 10% to 43%. A study conducted in southern India (Madanapalle) enrolled school-age children between 1950 and 1955.[214,215] After 15 years of follow-up, the protective effect had decreased to 19% (95% CI, −26% to 48%). A study conducted in Muscogee County,

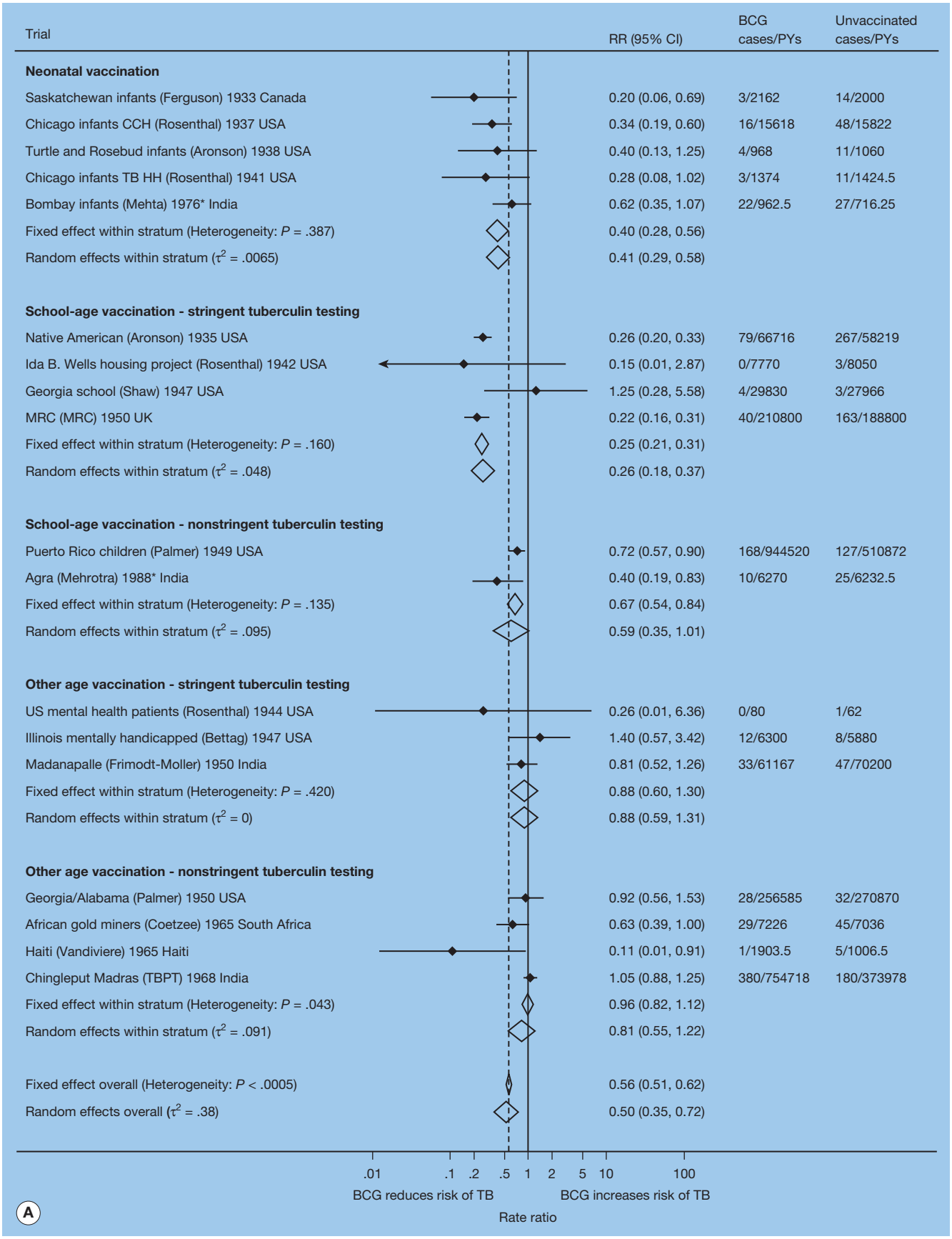


Figure 60.3. A, Rate ratios and 95% confidence intervals (CIs) for bacille Calmette-Guérin (BCG)'s efficacy in preventing pulmonary tuberculosis, stratified by age vaccinated and stringency of prevaccination tuberculin testing. Trials are ordered by year of study start (see Table 60.1 for details). The "other" age group includes studies in which older persons were vaccinated, as well as those in which BCG was given at any age. Both fixed effects and random effects metaanalyses were applied to determine summary results (for methods, see reference 165). *Date of study publication was used if study start date was not available.

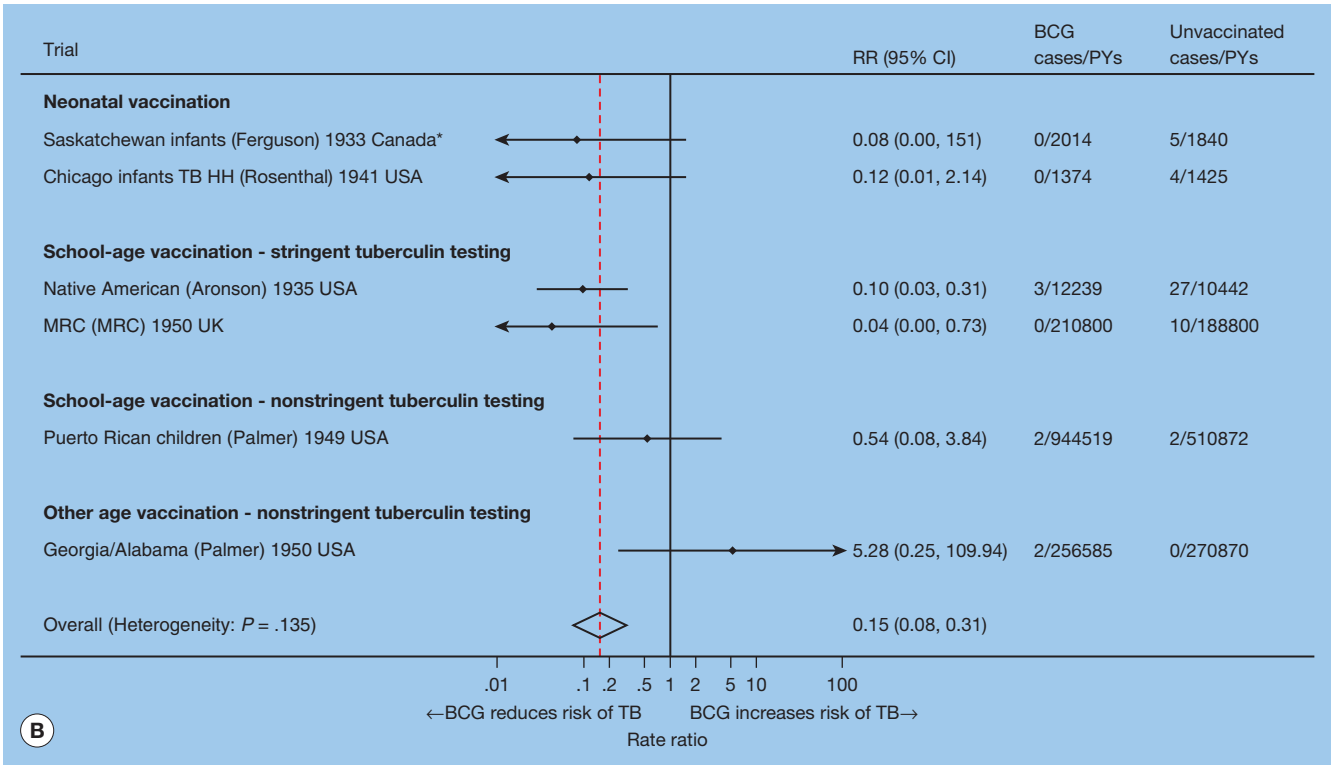


Figure 60.3, cont'd B, Rate ratios and 95% CIs for BCG's efficacy in preventing meningeal and/or miliary tuberculosis, stratified by age at vaccination and tuberculin testing stringency. Pooled results from only fixed effects metaanalysis is shown as the numbers of studies were small; these are ordered by year of study onset. *Outcome is miliary tuberculosis only. MRC, Medical Research Council; PY, person-years; TB, tuberculosis. *(Modified from Mangtani P, Abubakar I, Ariti C, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials.* Clin Infect Dis*. 2014;58:470–480.)*

Georgia, beginning in 1947 had three cases of TB in the control group and five in the vaccinated group, resulting in a negative protective efficacy with wide CIs.[216,217] Similarly, no protective efficacy (efficacy, 8%; 95% CI, −53% to 44%) was shown in Georgia and Alabama in the 1950s.[218] All these studies have in common a high prevalence of environmental mycobacteria (see below), and multiple problems with methodology.[165,209,212]

The largest BCG field trial ever conducted commenced in 1968 in the Chingleput district of southern India, and involved 260,000 participants.[201,202] Persons of all ages except infants younger than 1 month of age, and regardless of skin test reaction, were randomized to receive one of two vaccines at one of two dosages, or to receive placebo. Follow-up continued for 10 years, with active study visits every 2.5 years. The study was confounded by bias; for example, follow-up varied among groups depending on size of the enrollment skin test reaction, age, and perception of risk. Furthermore, microbiological tests were completed on a fraction of the TB cases only; regardless, culture confirmation was the finally agreed outcome, because other outcome measures were not applied uniformly. Because of these and other shortcomings, cases among young children were probably missed. It is noteworthy that the study was conducted in a geographic area in which environmental mycobacteria are endemic. The first results were published in 1979, after 7.5 years of follow-up. Neither of the two vaccines studied, in full or reduced dosage, showed any evidence of protection against pulmonary TB, compared with that of placebo (efficacy, −5%; 95% CI, −25% to 12%).

Metaanalyses and Other Evidence of Bacille Calmette-Guérin Efficacy and Effectiveness

Colditz published the first metaanalysis of the efficacy of BCG in 1994.[219] Fourteen prospective trials and 12 case-control studies were included in the analysis. A random-effects model estimated a weighted average relative risk or odds ratio, from which protective efficacy was deducted. In prospective trials, the protective efficacy of BCG to prevent any form of TB was 51% (95% CI, 30–66%), and in case-control studies, 50% (95% CI, 36–61%). Seven trials reported on death as an end point: BCG had a protective efficacy of 71% (95% CI, 47–84%), while five studies reported meningitis and showed a protective effect of 64% (95% CI, 30–82%). BCG appeared more protective in children, especially when including only case-control studies where a positive culture or histology was used for diagnosis (83% protective; 95% CI, 38–93%).

Mangtani and associates published a very comprehensive assessment of 18 randomized trials reporting on BCG induced protection against TB, including six that reported meningitis as an outcome.[165] Both fixed-effect and random-effect models were applied in their systematic review and metaanalysis and metaregression. The overall protective efficacy of BCG against pulmonary TB was 50% (95% CI, 28–65%). Protection was highest in infants, at 69% (95% CI, 42–71%), compared with older ages. Protection against meningeal and miliary tuberculosis was 90% in infants (95% CI, 23–99%).

Roy focused on 14 studies, most with a retrospective case-control design, that addressed whether BCG could protect against *M. tuberculosis* infection in childhood, in a

TABLE 60.1 Characteristics of Trials of Bacille Calmette-Guérin Vaccination Against Pulmonary and Miliary or Meningeal Tuberculosis

Trial	Years, Start of Entry to End of Follow-Up	No. BCG Vaccinated/ No. Unvaccinated	Age at Vaccination and Tuberculin Testing Stringency, Where Applicable	Vaccine Strain
Saskatchewan Infants (Ferguson[228])[a]	1933–48	306/303	Neonatal	Frappier/Pasteur 450-S1, 468-S1
Native American (Aronson[265])[a]	1935–98	1551/1457	School age, stringent	Phipps/Pasteur 317 used at U.S. sites; Pasteur 575 used at Alaskan sites
Chicago Infants CCH (Rosenthal[211,389])[a]	1937–60	5426/4128	Neonatal	Pasteur, Tice
Turtle and Rosebud Infants (Aronson[390])	1938–46	123/139	Neonatal	Phipps
Chicago Infants (TB HH) (Rosenthal[211,389])	1941–53	311/250	Neonatal	Pasteur, Tice
Ida B. Wells Housing Project (Rosenthal[391])	1942–56	699/625	School age, stringent	Pasteur, Tice
U.S. Mental Health Patients (Rosenthal[391])	1944–48	20/15	Other age, stringent	Pasteur, Tice
Illinois Mentally Handicapped (Bettag[392])	1947–59	531/494	Other age, stringent	Not specified
Georgia (School) (Shaw[393])	1947–67	2498/2341	School age, stringent	Tice 811K, 811L, 812E, 812L, 813E
Puerto Rican Children (Palmer[213])[a]	1949–68	50,634/27,338	School age, nonstringent	Phipps
Madanapelle (Frimodt-Moller[214,215,394])	1950–71	5069/5803	Other age, stringent	Danish/Copenhagen
Georgia/Alabama (Palmer[213])[a]	1950–70	16,913/17 854	Other age, nonstringent	Tice
MRC (MRC[200])[a]	1950–70	20,800/13,300	School age, stringent	Danish/Copenhagen
African Gold Miners (Coetzee[395])	1965–68	8317/7997	Other age, nonstringent	Glaxo
Haiti (Vandiviere[396])	1965–68	641/340	Other age, nonstringent	Frappier/Montreal, 1202
Chingleput (Baily[397])	1968–83	73,459/36,404	Other age, nonstringent	Danish/Copenhagen/1331, Paris/Pasteur-1173 P2
Bombay Infants (Mehta[398])	1976[b]	396/300	Neonatal	Danish/Copenhagen
Agra (Mehrotra[399])	1988[b]	1259/1259	School age, nonstringent	Not specified

[a]Miliary and/or meningeal outcomes reported as well as pulmonary disease outcomes.
[b]Date of study publication was used if study start date was not available.
BCG, bacille Calmette-Guérin; CCH, Cook County Hospital; CI, confidence interval; MRC, Medical Research Council; PY, person-years; RR, rate ratio; TB HH, tuberculosis households; TBPT, Tuberculosis Prevention Trial.
Modified from Mangtani P, Abubakar I, Ariti C, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. Clin Infect Dis. *2014;58:470–480.*

systematic review and metaanalysis.[220] Infection was defined by a positive result using one of two types of IGRAs (enzyme-linked immunosorbent spot or QuantiFERON). The overall protective efficacy was 19% (95% CI, 8–69%). When the analysis was restricted to six studies with information on progression to active TB, BCG had a 27% effect in protection against infection (95% CI, 13–59%) and 71% against active disease (95% CI, 42–85%). Among those infected, protection against progression to disease was 58% (95% CI, 23–77%).

In the 1980s, the WHO initiated a global study using a standardized case-contact protocol to evaluate children who were household contacts of TB cases with infectious disease. The evaluation allowed an estimation of BCG efficacy; results were similar to those of the major controlled trials, with efficacy ranging from 0% to greater than 80%.[221–238] Studies that evaluated meningitis or miliary disease in children as outcomes demonstrated that BCG offers good protection.[153,198,238–243] Case-control and contact studies have the advantages of ease of conduct and lesser expense, but the potential for bias is increased.

Reasons for Variable Efficacy

The protection demonstrated in the major controlled BCG field trials ranged from 0% to 80%. In this section, variables that could have influenced the outcomes of these vaccine trials and of current vaccination practice are discussed.

Trial Methodology. It is a given that substantial variation will result from different trial designs, conducted in different time periods (between 1935 and 1975), in nine different geographic areas in three countries, by different investigators and various organizations. The role of methodologic and statistical variation among the eight major BCG trials has been evaluated critically.[205,212,244] Some authors argue that methodologic differences may have biased some of the trials and account for the variability in outcomes, whereas others believe that none

of the shortcomings could have appreciably influenced conclusions. Mangtani and associates evaluated bias in execution of studies systematically in a metaregression analysis.[165] The authors demonstrated that a lack of efficacy of BCG shown in many studies may have been the result of some sources of bias, such as diagnostic detection bias, although this finding was not consistent for all sources of bias.[165]

Vaccine Strain. At least six different vaccines were used in the trials. Thawed vaccine preparations were used in all studies except for the Chingleput trial, which used vaccine from reconstituted lyophilized vials. The differences in vaccine strains (see "Bacteriology of Bacille Calmette-Guérin Strains and Vaccine Constituents" above) are likely to have had an effect on outcome.

Environmental Mycobacteria, *Mycobacterium tuberculosis*, and Helminthic Exposure. Exposure to environmental mycobacteria may lead to tuberculin sensitization and immunity against *M. tuberculosis* and *M. bovis* BCG; environmental mycobacteria have many antigens in common with these organisms.[245] Environmental mycobacteria are endemic in tropical and subtropical regions of the world, including the southern areas of the United States, whereas human exposure to these organisms is less in Europe and northern parts of North America. Overall, BCG appears to be more protective in latitudes farther from the equator.[165] Three of the controlled trials were conducted in geographic areas where the prevalence of environmental mycobacteria was low, and these studies demonstrated the highest vaccine efficacy. In contrast, the five trials conducted in the southern United States and southern India, where the prevalence of environmental mycobacteria was high, demonstrated low vaccine efficacy.

Environmental mycobacteria may either block induction of immunity by BCG as immunity induced by the environmental mycobacteria may kill the live organism in the vaccine, or mask protection induced by BCG because protection may have been induced by the environmental organisms themselves.[246] For example, BCG-induced host responses, measured by a whole-blood IFN-γ assay or a purified protein derivative skin test, differ following BCG vaccination in the United Kingdom and Malawi; Malawi children have quantitatively greater baseline responses, whereas children from the United Kingdom demonstrate a greater increase in responses after vaccination.[189,247] This result suggests that sensitization by nontuberculous mycobacteria could inhibit immune responses to BCG vaccination.

An observer-blinded, cluster-randomized trial conducted in Brazil showed that efficacy of BCG vaccination at school-age was higher in Salvador, a coastal city where environmental mycobacterial exposure is thought to be low (efficacy, 34%; 95% CI, 7–53%), compared with Manaus, a city in the Amazon with presumed high environmental endemicity (efficacy, 8%; 95% CI, −39% to 40%).[248] In contrast, vaccine efficacy for neonatal vaccination was similar in Salvador (efficacy, 40%; 95% CI, 22–54%) and Manaus (efficacy, 36%; 95% CI, 11–53%). These findings concur with a blocking mechanism, as suggested by the Malawi/UK studies.

Although several studies suggest a possible effect of heterologous immunity from NTMs, there are major knowledge gaps in these studies. There is no qualified or reliable immunologic assay available to measure prior exposure or current infection with NTMs. Although intriguing, these previous studies were small and underpowered, focused on a limited number of immune responses—blood IGRAs or delayed-type hypersensitivity in skin—and employed whole-cell reagents rather than single proteins or peptide pools, while evaluating a limited number of NTMs in only a few regions of the world. With ongoing efforts to develop new vaccines for TB, understanding the role of NTM exposure and heterologous immune responses may be critical to success. Development of validated NTM-specific immunologic assays will be required to assess this important issue.

Evidence from animal and human studies indicates that sensitization to environmental mycobacteria provides protection against TB that is similar to that provided by BCG. In a study of 625,000 Navy recruits with serial skin testing using purified protein derivatives from *M. tuberculosis* and *Mycobacterium intracellulare*,[249] the incidence of active TB disease was lower in individuals with an *M. intracellulare* skin test result that was greater in magnitude than the *M. tuberculosis* skin test. As stated above, even *M. tuberculosis* infection appears to have a protective effect—a metaanalysis of the protective effect of latent infection against reinfection-mediated TB disease, incorporating studies from the preantibiotic era, showed a protective efficacy of approximately 80%.[39] The caveat from this observation is that many persons would have died from TB disease during the preantibiotic era, therefore skewing the results to show a positive effect on a population basis.

Other infections may also influence the BCG-induced response. For example, Ethiopian adults with latent *M. tuberculosis* and helminthic coinfection who are treated with albendazole prior to BCG vaccination demonstrated increased specific immune responses to the vaccine.[193] However, a large study in Uganda showed that treating maternal helminthic infection during pregnancy had little effect on the infant BCG-induced immune response[194] or on mycobacteria-specific immune responses in BCG-vaccinated 5-year-olds.[250] As mentioned before, the immune correlates of BCG-induced protection against TB disease are not known; consequently, results should be interpreted with caution. Interestingly, BCG vaccination may be associated with a lower helminthic infection rate.[251]

Host Genetic, Microbiotic, and Environmental Factors. The BCG trials were completed in populations that were genetically diverse. Human and animal evidence suggest that genetic factors may determine the host's response to BCG vaccines,[252–255] and therefore likely also protective efficacy. The role of genetic variation on susceptibility to mycobacterial disease and to infant immune responses to vaccination has been reviewed.[171,256,257]

The human microbiome has a significant influence of multiple health indicators; limited evidence suggests that infant microbiome constituents may also affect the BCG-induced immune response.[258]

Experimental research has demonstrated that nutritional deficiencies affect the magnitude and character of the immune response to BCG vaccination.[259–262] However, studies in humans remain controversial.[218,263]

Regional Differences in *Mycobacterium tuberculosis* Strains. There is marked heterogeneity among seven major families of *M. tuberculosis* that occur in different regions of the world.[10,264] This heterogeneity manifests in differential host responses to the pathogen, and epidemiological evidence suggests that BCG might have skewed the epidemic toward greater prominence of certain *M. tuberculosis* strains. It is therefore a reasonable hypothesis that variation among strains that occurred in different setting could have confounded results of clinical trials of BCG.

Duration of Protection and Revaccination

When Native American participants of the 1935 BCG trial were followed up for 60 years, an efficacy of 52% (95% CI,

27–69%) in protection against disease was found.[265] The results suggest that a long duration of protection from a single dose of BCG is possible. In contrast, a case-control study investigating efficacy of neonatal BCG vaccination over the first 20 years of life found 82% protection in children younger than 15 years of age, 67% protection in the 15- to 24-year-old age group, and 20% protection in persons 25 to 34 years of age, indicating better protection in younger age groups and waning immunity over time.[266] Furthermore, the kinetics of the host immune response to newborn BCG vaccination suggest that immunity is likely to be relatively short-lived.[176,181]

The practice of BCG revaccination continues in many places of the world (see above). The only large-scale efficacy trial of BCG revaccination was completed in Brazil, where school-age children received a second dose of BCG, following newborn BCG vaccination.[248] Interestingly, revaccination was poorly effective in preventing TB disease in the coastal region of Brazil, and not at all effective in the Amazon. In the coastal region, children without evidence of previous BCG vaccination showed greater protective efficacy than those who had received BCG before; the respective efficacies were 19% (95% CI, 7–53%) and 1% (95% CI, −27% to 33%). The results concur with those from a metaanalysis of BCG efficacy that suggests that prior exposure to environmental mycobacteria—presumedly high in the Amazon and low in the coastal region—and in the case of the Brazil study, prior exposure to BCG itself, results in lesser efficacy of BCG.[165]

Safety

BCG has been administered safely to billions of people throughout the world. Complications are rare, but rates of these vary depending on the skill and method of administration, the strain, dose, gender, age, and immune status of the recipient.[117,267,268] The Pasteur 1173 P2 and Danish 1331 strains induce more adverse reactions than the Glaxo 10777, Tokyo 172-1, or Moreau RDJ strains.[117] The concentration of live particles in BCG vaccines ranges from approximately 50,000 to 3 million per dose, depending on the strain; vaccines with relatively low numbers of live bacilli per dose are generally less reactogenic.[269] Women and older vaccinees are generally more likely to develop local reactions and abscesses, and vaccination at less than 6 months of age is better tolerated than after that age.[270]

Common Adverse Events in Nonimmunocompromised Persons

Local adverse reactions are common after BCG vaccination, but serious long-term complications from these are rare (Table 60.2). In summary, up to 95% of persons vaccinated with BCG develop a local reaction (Fig. 60.4). Erythema, induration, and tenderness are common. A papule that is often red, tender,

TABLE 60.2 Estimated Age-Specific Risks for Complications After Administration of Bacille Calmette-Guérin Vaccine

	Incidence per 1 Million Vaccinations	
Complication	**Age <1 y**	**Age 1–20 y**
Local subcutaneous abscess, regional lymphadenopathy	387	25
Musculoskeletal lesions	0.39–0.89	0.06
Multiple lymphadenitis, nonfatal disseminated lesions	0.31–0.39	0.36
Fatal disseminated lesions	0.19–1.56	0.06–0.72

From Lotte A, Wasz-Hockert O, Poisson N, et al. Second IUATLD study on complications induced by intradermal BCG vaccination. Bull Int Union Tuberc. *1988;63:47–59.*



Figure 60.4. Characteristic evolution of the injection sites after administration of intradermal bacille Calmette-Guérin. *(Courtesy Gregory D. Hussey.)*

and indurated may emerge 2 or more weeks after vaccination. Ulceration may follow, and if this occurs, healing takes 2 to 5 months, followed by scar formation. Ipsilateral lymphadenopathy may also occur and is usually less than 1.5 cm in greatest dimension and seen in the axilla.

A small U.S. study of healthy TST-negative adults vaccinated with BCG intradermally found that all patients developed a local reaction with erythema, induration, and tenderness at the vaccination site; 75% developed muscle soreness; and 70% had local ulceration with drainage.[271] Only 2% developed tender regional adenopathy. Overall, local ulceration and regional lymphadenitis occur in less than 1% of immunocompetent recipients who receive intradermal administration.[272] Certain BCG strains, such as the Tokyo and Moreau strains, are rarely associated with lymphadenitis, whereas this complication appears more often when the Pasteur strain is used.[272–274] Outbreaks of lymphadenitis have been reported following introduction of new BCG strains into vaccination programs[275,276]; altered administration technique may have contributed. The risk of suppurative lymphadenitis is greater among newborns than among older infants and children, especially when a full dose of vaccine is given.

A lasting cutaneous scar develops in most patients, although the likelihood is lower when vaccination is given in early infancy.[277]

The treatment of local adenitis as a complication of BCG vaccination is controversial and ranges from observation, to surgical drainage, to the administration of antimycobacterial drugs, to a combination of surgical management and medications.[278] Nonsuppurative lymph nodes usually improve spontaneously, although resolution may take several months. Several studies have shown that some children with lymphadenitis respond to a course of isoniazid of several weeks' or months' duration.[279–281] However, a metaanalysis of four randomized controlled trials revealed no significant difference in the frequency of suppuration between the treatment and control groups for the erythromycin and isoniazid treatments; the conclusion was that medical treatment does not reduce the frequency of suppuration.[282] Furthermore, a Cochrane evaluation concluded there was no evidence that antimicrobial therapy or aspiration was associated with an improved outcome.[283]

Persons with latent *M. tuberculosis* infection appear to have an accelerated adverse effect response to BCG vaccination, without any obvious difference in severity.[284] In a study of 82 South African adults, injection-site erythema (68%) and induration (86%) peaked 1 week after revaccination, whereas ulceration (76%) peaked at 2 weeks, and resolved by 3 months in all but three subjects. A residual scar was common (85%).

Rare Adverse Events

The risk of osteitis after BCG vaccination has varied from 0.01 to 300 per million doses.[267,285–288] In a controlled study of 132 former BCG osteitis patients, variant genotypes of the TLR2 and TLR6 genes were associated with a higher risk of BCG osteitis whereas TLR1 variant genotypes were associated with a decreased risk.[289]

Generalized BCG infection is extremely rare in immunocompetent patients.[290–294] Autopsy studies of children who died of unrelated causes show granulomas in various organs of vaccinated infants with apparently intact immune systems, suggesting that generalized nonfatal dissemination may occur in normal hosts.[292,295,296]

A report from Thailand described four immunocompetent neonates with fever and *M. tuberculosis* complex isolated from blood within 72 hours of BCG vaccination.[297] All infants recovered and remained healthy without treatment. One isolate was genotyped as *M. bovis* BCG, and all four infants were presumed to have had BCG bacteremia.

Treatment of extremely rare complications, including lupus vulgaris, erythema nodosum, iritis, osteomyelitis, and disseminated BCG disease, should include systemic anti-TB drugs; pyrazinamide should not be included because all BCG strains are resistant to this drug.[298]

Adverse Events in Immunocompromised Individuals

Fatal disseminated BCG disease has been reported at a rate of 0.19 to 1.56 cases per 1 million persons vaccinated.[272] Invariably, cases have occurred in patients with severe defects in cell-mediated immunity, such as those with HIV, severe combined immunodeficiency, and complete DiGeorge syndrome, but cases have also been associated with chronic granulomatous disease, malnutrition, cancer, and abnormalities of the IL-12–IFN-γ axis (genetic defects identified in IL-12p40, IL-12R, IFN-γR1,IFN-γR2, and STAT1).

Since the advent of the HIV era, it has become clear that BCG vaccination of untreated HIV-infected children places them at a dramatically increased risk of potentially severe and fatal adverse events; rates of disseminated BCG disease in this population have approached 1%.[299–301] These complications may also occur in BCG-vaccinated HIV-infected infants who are on antiretroviral therapy,[302] although early therapy reduces the risk dramatically.[303] BCG disease has also been described in a person who became infected with HIV as an adult and who had received BCG 30 years prior to becoming ill.[304]

All cases of BCG disease in HIV-infected children have followed vaccination in the neonatal period and there appears to be no clear association with particular BCG strains.[299–301] The median age of onset was 8 months (range, 3 to 35 months). Manifestations included local or regional lymphadenopathy in the majority of infants. Many cases of disseminated disease, often involving bone, nonregional lymph nodes, and multiple other organs, have been described. Disease was mostly associated with very low CD4 T-cell counts, and often occurred with other opportunistic infections. Outcome has been poor in the absence of antiretroviral therapy, even if antimycobacterial therapy is given: more than 80% of infants with disseminated disease and more than 50% of infants with local or regional disease will succumb to the disseminated infection. Antiretroviral therapy is associated with a vastly improved outcome.

HIV-exposed but uninfected infants may be at greater risk of other infectious diseases in the first year of life,[305] but no evidence of an increase of BCG-related adverse events have been reported in these infants. Variable results of immunogenicity of newborn BCG vaccination in this population has been reported[181,184]; regardless, delaying BCG vaccination to 8 weeks of age does not compromise ability of this vaccine to induce a robust immune response.[183]

Recommendations for Use

In the United States

The United States has never had a universal BCG vaccination program.[166] In the United States, TB control involves treatment of patients with disease, contact investigation, and prophylaxis for latent infection.[306,307] In this setting, BCG vaccination is considered only in special circumstances involving unavoidable continued exposure and failure to employ other methods of control. The Centers for Disease Control and Prevention's Advisory Council for the Elimination of Tuberculosis and

Advisory Committee on Immunization Practices have published guidelines for the use of BCG vaccination in young children and healthcare workers.[308] In the United States, BCG immunization should be considered for all persons with continuous exposure to MDR TB who cannot be removed from the exposure, but only if the person has a negative TST and is not infected with HIV; infants younger than 2 months of age with such exposure do not need a TST prior to vaccination. BCG should also be considered for children with continuous exposure to contagious drug-sensitive TB who cannot be removed from the exposure or treated with anti-TB therapy.

Vaccination with BCG should be considered for healthcare workers only in areas that have a high risk of transmission of MDR TB in which aggressive infection-control measures have been implemented and have failed.[308] Even in high-risk settings, BCG should not be the major method of control or protection, because the vaccine's efficacy is variable and does not protect unvaccinated patients or visitors, yet confounds TST interpretation, thereby also confounding surveillance and preventive treatment measures.

In the United States, it is recommended that local TB control authorities be contacted when BCG vaccination is considered.[309]

In Other Countries

Zwerling and associates published an atlas of global BCG vaccination policy and practices from 180 countries.[166,310] Like the United States, Canada, Italy, Belgium, and The Netherlands have never had universal BCG vaccination programs, and recommend use of the vaccine only in select cases. Ecuador, Australia, New Zealand, and Western European countries other than Belgium and The Netherlands had universal programs before, but now recommend vaccination for only select populations. The only European countries that practice universal BCG vaccination are Portugal and Ireland, and the vaccine is usually given to newborn babies.

The International Union against Tuberculosis and Lung Disease (IUATLD) has suggested criteria for countries to consider when shifting from routine universal BCG vaccination to selective vaccination of high-risk groups.[311] The IUATLD recommends that BCG be discontinued only if an efficient notification system is in place, and if either the average annual notification rate of smear-positive pulmonary TB is less than five per 100,000 population per year, or the average annual notification rate of TB meningitis in children younger than 5 years of age is less than one per 10 million population over the previous 5 years, or the average annual risk of TB infection is less than 0.1%.

It is known that BCG vaccine has some efficacy against leprosy[204] and that some countries, such as Brazil, Cuba, and Venezuela, recommend BCG vaccination for contacts of leprosy patients.[160] Widespread BCG vaccination may have contributed to the decline of leprosy in certain populations.[312,313] BCG vaccine also has a transient protective effect against *Mycobacterium ulcerans* disease, or Buruli ulcer, which manifests with skin lesions and osteomyelitis.[204] Finally, one of the most frequent indications for use of BCG in high-income countries today is for the treatment of bladder cancer by intravesicular administration, and here the vaccine has a nonspecific immunostimulant effect; it is worth noting that the supply and dose for this indication differs from that for prevention of TB.[314]

In the Setting of HIV Infection

HIV-infected persons are at increased risk of complications of BCG vaccination; this risk is mitigated by antiretroviral therapy, although not completely averted. The issues become prominent in the setting of infants born to HIV-infected mothers, where the majority of infants will be HIV-uninfected. However, the status will not be known immediately after birth, the time that BCG is given routinely in most countries. Additionally, in lesser-resourced environments, follow-up HIV testing and BCG vaccination of uninfected babies often cannot be guaranteed. Not vaccinating a significant portion of babies in countries where maternal HIV infection and TB disease is common, such as South Africa, could precipitate an epidemic of disseminated and pulmonary TB in infants.

The latest WHO recommendation and a follow-up position statement have addressed this dilemma.[315,316] BCG vaccination is now contraindicated in HIV-infected persons, including infants. The approach to HIV exposed infants depends on the existing public health structures. BCG administration should be delayed, if certain conditions are met, until HIV infection has been excluded in the baby. These conditions include a high uptake of maternal HIV testing coupled with effective prevention of mother-to-child HIV transmission strategies; early virological diagnosis of HIV infection coupled with early initiation of antiretroviral therapy; and coordination of prevention of mother-to-child HIV transmission, BCG vaccination, and TB control programs to minimize loss to follow up, implementation of alternative TB preventive strategies, and delivery of BCG vaccination following selective nonvaccination at birth. Although there have been significant advances in introduction of these programs globally, in many regions of the world, HIV-infected infants continue to receive BCG at birth.

Contraindications to Bacille Calmette-Guérin Vaccination

Contraindications vary among countries and often reflect different healthcare delivery resources available in the locale. In the United States, BCG vaccine is contraindicated for persons with impaired immunity, including patients with HIV infection, congenital immunodeficiency, leukemia, lymphoma, generalized malignant disease, and who are on suppressive corticosteroids, alkylating agents, antimetabolites, or radiation, as well as those who are pregnant.[308] Caution should also be used when administrating BCG to persons at high risk for HIV infection.

Nonspecific Effects of Bacille Calmette-Guérin

The major controlled trials that evaluated BCG-induced protection against TB in the United Kingdom and the United States in the first half of the 20th century showed an unanticipated effect: a reduction in nonaccidental deaths by 25% (95% CI, 6–41%).[317] Subsequently, observational studies, mainly conducted in West Africa, showed that BCG vaccination is associated with a 40% to 60% reduction in all-cause mortality, when compared with unvaccinated children.[318–326] The effects appear particularly prominent in the neonatal period, with a major effect on neonatal sepsis and respiratory infections.[327,328] In a randomized trial of immediate versus delayed BCG in 2343 infants in Guinea Bissau, there was no difference in the primary outcome of mortality at 6 months. Secondary analyses suggested possible mortality benefits of immediate BCG,[328] particularly in low-birth-weight infants. These results are controversial because the mortality trends were observed as early as 3 days after vaccination, there was no placebo injection, and there was a risk of allocation bias.[329] Some plausibility to the nonspecific effects of BCG has been provided by a finding that BCG induces epigenetic modifications of monocytes—the vaccine appears to "train" innate cells, which may result in protection against other pathogens.[330]

BCG may also enhance antibody responses and, in some cases, T-cell responses to other childhood vaccines, such as hepatitis B, tetanus toxoid, pertussis, and pneumococcal vaccines.[241,331–334] The mechanism of this beneficial effect is unclear.

Incomplete data suggest that BCG may also have nonspecific effects that extend to cancer and other chronic inflammatory disorders. BCG vaccination of newborns might reduce incidence of certain childhood cancers.[335] A cohort study of 542 malignant melanoma patients showed previous vaccination with BCG or vaccinia in childhood was associated with significantly longer life.[336] A large Danish case-cohort study found a beneficial effect of BCG vaccination on the risk of lymphomas.[337] Multiple observational studies have shown either a protective or no effect of newborn BCG vaccination on development of asthma. Subsequent metaanalyses show that an effect is unlikely, and if present, likely to be transient.[338,339] Furthermore, a systematic review and metaanalysis showed no association between childhood vaccination and emergence of inflammatory bowel disease,[340] although a Danish study suggested that BCG vaccination in infants younger than 4 months of age is associated with lower occurrence of the disease[341]; one of the hypothesized causes of inflammatory bowel disease is chronic mycobacterial infection.[342]

NEW TUBERCULOSIS VACCINES

Multiple modeling studies show that the global TB epidemic would be impacted significantly by interrupting transmission of *M. tuberculosis*. This may be achieved either by preventing infection or by preventing disease; preventing disease will have earlier and marginally greater impact in high-prevalence settings.[5,62,343] Although the ideal vaccine should have both effects, we do not currently know whether this is possible; regardless, TB vaccine development is shifting to add the goal of prevention of infection to that of prevention of disease.

The primary target populations should be adolescents and younger adults, and not infants; the first modern Phase IIb trial of a new TB vaccine was conducted in infants.[344] The bulk of global TB disease occurs in adolescents and younger adults.[1] These populations transmit *M. tuberculosis* and thus should be targeted for impact. In contrast, infants and children generally do not transmit the pathogen. Modeling shows that, compared with an infant vaccine, an adult vaccine would have considerably greater early impact on the epidemic and would be cost-effective, even with a relatively low efficacy and short protection duration.[6] Infants, HIV-infected persons, persons with comorbidities (e.g., diabetes mellitus), and the elderly should be included in the secondary testing of new candidates.

New Vaccines in Clinical Development

The last 15 years has seen a concerted effort toward developing novel TB vaccines that would be safe and more effective than BCG, while still being affordable and accessible to high-risk populations globally. Some of this research has been aimed at replacing BCG in infants with an improved vaccine, although it is now thought that this approach would have a limited impact on the global epidemic.[6] Rather, the focus has shifted to developing vaccines that would be delivered to adolescents and adults.

At the time of this writing, there are 16 TB vaccine candidates in clinical development globally (Fig. 60.5). The candidates include whole, live mycobacteria such as recombinant BCGs and recombinant *M. tuberculosis*, lysates of whole mycobacteria, subunit vaccines (adjuvanted recombinant proteins),

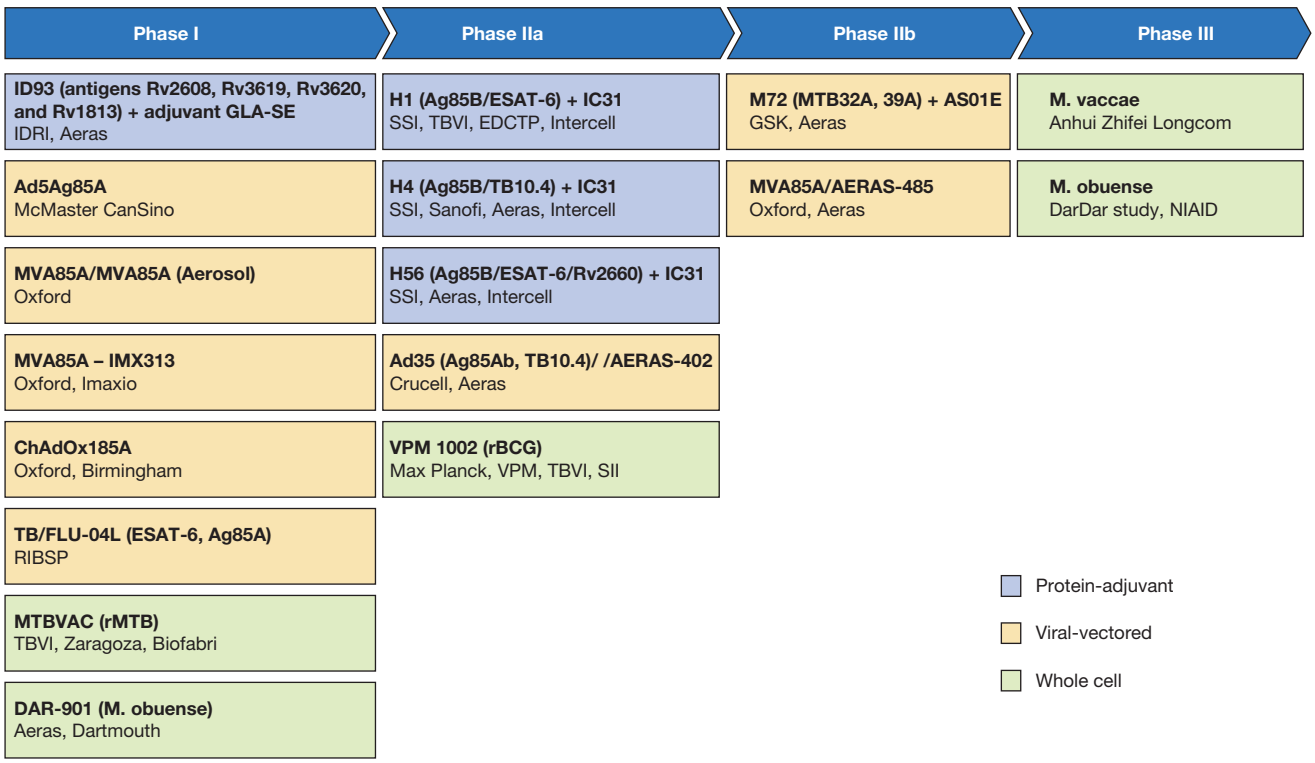


Figure 60.5. Tuberculosis vaccine candidates currently in clinical development. EDCTP, European and Developing Countries Clinical Trials Partnership; GSK, GlaxoSmithKline; IDRI, Infectious Disease Research Institute; NIAID, National Institute of Allergy and Infectious Diseases; RIBSP, Research Institute for Biological Safety Problems; SSI, Statens Seruminstitut; TBVI, Tuberculosis Vaccine Initiative.

and viral-vectored candidates.[345–348] Some efforts are also ongoing to evaluate combinations of these vaccine approaches. Two candidates have completed or are currently in Phase III testing, two candidates have completed or are currently in Phase II proof-of-concept trials, and multiple candidates are in earlier testing. Many more candidates are in preclinical product development.

Whole-Cell Vaccines

Mycobacteria other than *M. bovis* have been considered TB vaccine candidates for many years. A killed *M. tuberculosis* vaccine was studied in the 1930s and was found to have a protective efficacy of approximately 50% against disease in humans.[349] Until the modern era, only BCG and the vole bacillus (*M. microti*) have been considered seriously as candidates for vaccination. Whole mycobacterial vaccines, or vaccines derived from whole mycobacteria, are attractive since knowledge of specific antigens involved in a protective host response against TB remains limited.[350]

One whole-cell vaccine candidate, SRL172, has been tested in a Phase III trial. SRL172, thought at the time to be the environmental mycobacterium *Mycobacterium vaccae*, but more recently determined by 16S ribosomal RNA sequence to be *Mycobacterium obuense*, a close relative of *M. vaccae*, demonstrated limited efficacy against microbiologically confirmed TB in HIV-infected adults in Tanzania, when delivered as a series of five intradermal doses[351]: protection against microbiologically confirmed TB was 49% (95% CI, 4–61%). No protection was shown against disseminated TB or non–microbiologically confirmed TB. The vaccine was manufactured by a nonscalable process, but a liquid fermentation based manufacturing process was developed for this candidate. The new product, DAR901, is heat-inactivated and currently in first-in-human testing.

A heat-inactivated version of *M. vaccae*, already licensed as an immunotherapy in China (Vaccae), is currently in Phase III evaluation in China for prevention of TB in persons who are strongly TST positive, that is, with LTBI. Results are expected in 2017.

A live, attenuated *M. tuberculosis* candidate, known as MTBVAC, is currently in a Phase I trial in South Africa, after successful first-in-human testing in Europe. The candidate has two mutations—of phoP and fadD26—rendering it less virulent than BCG in immunocompromised animals.[352] Although initially developed as a replacement for BCG in infants, a shift in strategy now has this vaccine also targeted for use in adolescents and adults.

Multiple recombinant BCG candidates have emerged. The candidate in the most advanced stages of development is VPM1002, which is engineered to allow phagosomal acidification and lysosomal escape in the macrophages or dendritic cells that take up the organism, and as such potentially enhance induction of both CD4 and CD8 T-cell immunity.[353] The vaccine is currently in Phase II testing in India, on a track for BCG replacement. AERAS-422 was another recombinant BCG with a lysosomal escape mechanism, but its development was halted during its first-in-human trial because of a safety signal—two participants developed herpes zoster (Tom Evans, personal communication, 2016). rBCG30, a recombinant BCG that overexpresses antigen 85B (a 30-kDa antigen), completed a first-in-human study that demonstrated immunogenicity and an acceptable safety profile.[354] To date, the vaccine has not progressed further in development.

RUTI is a preparation of detoxified fragments of *M. tuberculosis* cells, delivered in liposomes, and being developed to prevent TB disease in persons with LTBI.[355–357] The vaccine is currently in Phase II testing.

Subunit Vaccines

The subunit vaccines currently in development contain a limited number of immunodominant *M. tuberculosis* antigens, typically as fusion proteins for ease of production, and formulated in Th1 cell-inducing adjuvants like AS01E,[358] GLA-SE,[359,360] CAF01,[361] or IC31.[362] Each of these candidates has demonstrated an acceptable safety profile, immunogenicity, and some degree of protection against *M. tuberculosis* challenge in animal models. The most advanced candidate in this category is M72/AS01E,[363–367] which is currently in a proof-of-concept Phase IIb trial, testing whether TB disease can be prevented in latently infected, HIV-negative adults in Africa. Another subunit vaccine, H4:IC31,[368] is currently in a proof-of-concept Phase II trial, testing whether *M. tuberculosis* infection can be prevented in adolescents without LTBI.

Virally Vectored Candidates

Viral vectors being developed as platforms for antigen delivery in TB vaccines include modified vaccinia Ankara (in the vaccine MVA85A),[344,369–375] influenza (TB/FLU-04L), and several human and chimpanzee adenoviruses (Ad5/Ag85[376] and Ad35/AERAS-402).[377–380] Most platforms contain attenuated, live, and replication-deficient viruses, and each of the clinical candidates express an antigen from the immunodominant *M. tuberculosis* antigen-85 complex, either as the sole antigen or in combination with one or more additional *M. tuberculosis* antigens.

MVA85A was evaluated recently in a Phase IIb trial in infants in South Africa, testing whether TB disease could be prevented if the vaccine is given as a boost at 4 months of age, following BCG vaccination at birth.[344] MVA85A induced a modest immune response in the infants and failed to show efficacy beyond that of BCG alone, leaving open the question of whether it was the nature or the magnitude of the induced immune response that was inadequate for protection. MVA has also been evaluated in a Phase II trial of HIV-infected persons, primarily assessing safety and immunogenicity, with disease prevention as an underpowered secondary end point. Although the safety profile was acceptable, and immunogenicity was shown, no protection was demonstrated. More recently, a combination approach of one or two doses of an adenovirus-vectored antigen-85A–expressing candidate followed by a single dose of MVA85A demonstrated robust $CD4^+$ and $CD8^+$ immune responses (Tom Evans, personal communication, 2016).

New Concepts in Vaccine Development

Targeting Different Immunological Compartments

The current portfolio of new TB vaccines in clinical trials relies on induction of a relatively narrow immune repertoire–classical major histocompatibility complex (MHC) class II–restricted Th1 CD4 cells, and in some cases, classical MHC class I–restricted CD8 T cells—so-called conventional T cells. (Whole mycobacterial candidates may be the exception.) This paradigm could be challenged, as one possible explanation of the MVA85A trial results is that CD4 T-cell immunity to 85A was insufficient to confer protection in newborns, compared with BCG alone (see above). However, it is not clear whether it was the quantity or the nature of the induced immune response that was inadequate. Furthermore, the immunodominant antigens/epitopes in current vaccines appear conserved across global TB strains, suggesting there may be benefit of the vaccine-induced T-cell responses for the TB pathogen.

In a paradigm shift, the field is moving toward developing vaccines that address immunological hypotheses other than that a conventional T-cell response is essential for protection.[381,382] The new approaches focus on eliciting a response in immunological compartments other than conventional T cells, or inducing responses to diverse classes of antigens. The immunity induced by new approaches could differ from that which occurs in the natural setting; many examples of vaccines that induce "unnatural immunity" already exist (e.g., natural infection with *Clostridium tetani* does not induce long-lived protection, whereas vaccination with tetanus toxoid does, and protein conjugation of various polysaccharide vaccines affords immune memory that does not occur when children younger than 2 years of age are immunized with the polysaccharide alone).

Induction of nonconventional immunity includes induction of antibodies, traditionally not thought to play a major role in protection against intracellular bacteria such as *M. tuberculosis*, but which may play a role in prevention of infection, rather than disease.[62] Other examples of induction of nonconventional immunity include exploitation of antigen presentation that is less polymorphic, involving molecules such as CD1, HLA-E, and MR1, or use of novel antigen classes, such as glycolipids or phosphoantigens. The cells targeted include CD1-restricted T cells, γδ T cells, and mucosal-associated invariant T cells.[381,382] Finally, preliminary results using an attenuated live replicating cytomegalovirus vector suggest that novel classes of T cells (e.g., HLA-E restricted CD8 T cells and MHCII restricted CD8 T cells) are elicited and that these may contribute to protection against TB, as has been suggested for HIV (Louis Picker, personal communication, 2016).

Antigen Selection

It is not known which antigens in the *M. tuberculosis* genome are responsible for host responses associated with protection. *M. tuberculosis* may hyperconserve immunodominant antigens, presumably to its own pathogenic advantage,[383,384] which has motivated the selection of nondominant antigens for a number of next-generation TB vaccine candidates. This is an area of active research.

Targeting the Lung in Vaccination

Preclinical data suggest that mucosal vaccination against TB elicits lung and systemic immunity that differs from that induced by parenteral vaccination, and in some cases, elicits enhanced protection. Furthermore, knowledge is emerging about so-called tissue-resident memory T (T_{RM}) cells,[385,386] which express receptors that allow homing to the lung, where they live for extended periods. (Classical central memory or long-lived T cells have receptors that allow preferential homing to lymph nodes.[387]) T_{RM} cells are thought to be particularly important for protection against pulmonary pathogens, and may be induced preferentially through mucosal vaccination approaches. New approaches in the field aim at defining the role of these cells in protection against TB. Overall, a strong rationale is proposed for developing aerosol vaccination approaches, which are currently being tested.[370,388]

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61 Typhoid Fever Vaccines

Myron M. Levine

HISTORY OF THE DISEASE

Typhoid fever is an acute generalized infection of the mononuclear phagocyte system, intestinal lymphoid tissue, and gallbladder caused by *Salmonella enterica* serovar Typhi. A broad spectrum of clinical illness can ensue, with more severe forms being characterized by persisting high fever, abdominal discomfort, malaise, and headache. In the preantibiotic era, the disease ran its course over several weeks and was accompanied by a case-fatality rate of approximately 10% to 20%. Before the first quarter of the 19th century, typhoid fever was not recognized as a distinct clinical entity and was often confused with other prolonged febrile syndromes, particularly typhus fever of rickettsial origin. There is considerable debate over who first clearly differentiated typhus fever from typhoid (i.e., typhus-like) fever. Medical historians have argued over who should receive credit for this clinical clarification, and it has been variously bestowed on Huxham (1782),[1] Louis (1829),[2] Gerhard (1837),[3] and Schoenlein (1839).[4] Gerhard, in Philadelphia, argued that two similar, yet distinct, febrile illnesses existed that were clearly discernible from one another on the basis of pathologic findings; one (typhoid) manifested marked intestinal lesions. Schoenlein referred to two distinct forms of typhus: "exanthematicus" and "abdominalis." However, William Jenner is responsible for definitively dispelling controversy on the subject.[5,6] He provided precise clinical descriptions and observations of pathologic conditions from postmortem examinations that allowed a clear-cut differentiation between the two illnesses. Jenner argued that the pathologic lesions in Peyer patches and mesenteric lymph nodes were peculiar to typhoid and were never seen with typhus. In 1847, the term *enteric fever* was introduced in an attempt to replace "typhoid fever" and avoid confusion with "typhus."[7] Although this term is frequently used, it has by no means replaced the appellations typhoid fever and paratyphoid fever in common usage.

William Budd's book, *Typhoid Fever: Its Nature, Mode of Spreading and Prevention*, published in 1873,[8] was a milestone in epidemiology because it clearly described the contagious nature of the disease and incriminated transmission via fecally contaminated water sources years before the causative organism was identified. Eberth (1880)[9] visualized the causative bacilli in tissue sections from infected patients, and Gaffky (1884)[10] grew it in pure culture. Known in earlier years as *Bacillus typhosus, Eberthella typhosa, Salmonella typhi*, and *Salmonella typhosa*, it is currently referred to as *S. enterica* serovar Typhi (*Salmonella* Typhi).[11,12] *S. enterica* Paratyphi A and Paratyphi B (and uncommonly Paratyphi C) also cause enteric fever (paratyphoid fever), which is clinically indistinguishable from typhoid fever.

Inactivated (heat-killed, phenol-preserved) *S.* Typhi was used as a parenteral vaccine as far back as 1896 by Pfeiffer and Kolle[13] in Germany and by Wright in England.[14] Wright administered his vaccine (three doses 2 weeks apart) to two medical officers in the Indian Army, one of whom thereafter ingested wild typhoid bacilli without developing illness.

Wright then evaluated his vaccine in 2835 volunteers in the Indian Army.[15] Although local and generalized adverse reactions were common, his results were considered to be sufficiently encouraging for a decision to be made to vaccinate troops embarking for the Boer War in South Africa. Outcry over the frequency of adverse reactions led to a suspension of vaccination. However, on Wright's insistence, a board of inquiry was established to review data on the reactogenicity and efficacy of the vaccine. The committee concluded that the vaccine was efficacious and that its value in preventing typhoid fever exceeded the price paid in adverse reactions. By World War I, typhoid vaccination became virtually routine in the British Army.

WHY TYPHOID FEVER IS IMPORTANT

The transmission of typhoid fever is fostered where sanitation is primitive and water supplies are not treated, and where there exist chronic or short-term carriers of *S.* Typhi who serve as a reservoir of infection. In such situations, human fecal material can contaminate water supplies. In most endemic areas, the peak incidence of clinically recognizable, bacteriologically confirmed typhoid fever detected by passive surveillance is typically observed among school-age children. Typhoid constitutes the main enteric disease threat faced by children in developing countries after they have survived the gauntlet of diarrheal and dysenteric infections encountered during their first 5 years of life.[16]

Because the case-fatality rate in the preantibiotic era was 10% to 20%, typhoid fever was a much-feared disease. Following the discovery in 1948 that chloramphenicol (and subsequently certain other antibiotics) can successfully treat typhoid fever,[17] dropping the case-fatality rate to well below 1%, the interest in typhoid vaccines became a weathervane for the prevalence of antibiotic-resistant strains of *S.* Typhi. Interest in vaccines waned as long as effective and inexpensive oral antibiotics were available, only to resurge when resistant strains appeared and rendered treatment ineffectual. Beginning about 1990, strains of *S.* Typhi that exhibited plasmid-encoded resistance to all the oral antibiotics that were the mainstays of therapy in the 1970s and 1980s (chloramphenicol, trimethoprim-sulfamethoxazole, and amoxicillin) began to disseminate throughout Asia and northeast Africa.[16,18–22] This emergence of multiple-antibiotic–resistant typhoid during the last decade of the 20th century led to an increase in the incidence of severe cases, hospitalizations, and complications, and to an increase in typhoid mortality.[16,18–20,22]

CLINICAL DESCRIPTION OF TYPHOID FEVER

Typhoid fever exhibits a wide range of clinical severity. Classic full-blown cases begin with malaise, anorexia, myalgia, fever that increases in stepwise fashion to reach 39°C to 40°C, abdominal discomfort, and headaches.[23–25] A bronchitic cough is common in the early stage of illness. The fever often follows three stages. Initially, the temperature rises gradually, in stepwise fashion, by daily increments of 0.5°C to 1°C until, after 5 to 7 days, a sustained temperature of 39°C to 41°C is present. Without appropriate antimicrobial therapy, the fever remains at this level for approximately 10 to 14 days. With convalescence, the fever diminishes, also in stepwise fashion, over several days. During the period of sustained fever,

approximately 20% of white patients manifest an exanthem (so-called rose spots) consisting of subtle, salmon-colored macules, 2 to 4 mm in diameter, which blanch with pressure. Rose spots are seen most often on the chest, abdomen, and back; *S.* Typhi can be cultured from rose spots.[26] Constipation or loose stools may occur in older children and adults, whereas diarrhea is the more common bowel pattern alteration in young children with typhoid fever.

The peripheral leukocyte count in typhoid fever is often below 4500/mm^3, which helps in the differential diagnosis. Thrombocytopenia is also common, with platelets dropping to less than 80,000/mm^3. Liver dysfunction, as detected by mildly elevated serum transaminase values, is observed in most patients. Before the availability of fluoroquinolone antibiotics such as ciprofloxacin, approximately 10% of patients treated with earlier antimicrobials of choice, such as chloramphenicol, trimethoprim-sulfamethoxazole, or amoxicillin, manifested clinical relapses; notably, the clinical illness in relapse is much milder.

A particularly severe form of typhoid fever is occasionally encountered in which cerebral dysfunction—including obtundation, delirium, and coma—and shock are present.[27] In the 1970s and 1980s, this severe form of typhoid was encountered not infrequently on the Indonesian island of Java. Unless steroid therapy accompanies appropriate antimicrobial therapy in patients suffering from this severe form of the disease, the case-fatality rate can exceed 20%.[27]

Although infants may manifest severe clinical forms of typhoid fever, bacteremic *S.* Typhi infection in children younger than 2 years of age is often remarkably mild and is not recognized clinically as enteric fever but rather as a nondescript febrile syndrome.[28,29]

COMPLICATIONS OF TYPHOID FEVER

Complications may affect virtually any organ system. Two feared complications of typhoid fever, intestinal perforation and hemorrhage, are a consequence of the intestinal lesions that are prominent in the pathology of *S.* Typhi infection. These complications occur in approximately 0.5% to 1.0% of cases and are more common in persons who have been ill for several weeks without proper antibiotic therapy. Other uncommon complications of typhoid fever include typhoid hepatitis, empyema, osteomyelitis, and psychosis. More rarely, arthritis, meningitis, myocarditis, and empyema of the gallbladder can occur. Approximately 2% to 5% of patients with typhoid fever, depending on age and sex, become chronic gallbladder carriers of the organism.[30,31] More rarely, chronic renal or bladder carriers occur, the latter in patients harboring *Schistosoma haematobium* or *Schistosoma mansoni* bladder coinfection.

BACTERIOLOGY

Taxonomy in the genus *Salmonella* continues to evolve and can be confusing. Originally, speciation was based on association with distinct clinical syndromes. With the Kauffman-White serologic classification, each distinct O:H serotype was given species status, a situation that rather quickly became ponderous. In 1980, the Approved Lists described five species: *Salmonella enteritidis, S. typhi, Salmonella* Typhimurium, *Salmonella choleraesuis,* and *Salmonella arizonae*.[32] The most recent classification and speciation, based on DNA relatedness and molecular analysis, reduces the genus *Salmonella* to two species, *S. enterica* and *Salmonella bongori*. *S. enterica* is further subdivided into subspecies designated with Roman numerals.[11,12,33] Serotypes in *S. enterica* subspecies I are still almost always referred to by their previous genus and species designations. Thus, for example, to avoid confusion, *S. enterica* serovar Typhi continues to be referred to in most international journals of microbiology, infectious diseases, epidemiology, and vaccinology as *Salmonella* Typhi; use of a capital letter without italics indicates that Typhi designates a serovar, not a species. Following the most current taxonomy, *Salmonella* Typhi (abbreviated *S.* Typhi) is the terminology that will be used in this chapter.

Serologically, *S.* Typhi falls into group D *Salmonella* on the basis of its immunodominant lipopolysaccharide (LPS) O antigen 9.[34] *S.* Typhi is motile, and its Phase I peritrichous flagella bear flagellar (H) antigen d, which is also encountered in approximately 80 other bioserotypes of *Salmonella*.[34] Occasional isolates from Indonesia have flagella that bear other antigens (j and z66).[35,36] Strains freshly isolated from patients express on their surface a polysaccharide capsule, the Vi (for virulence) antigen.[34,37–40] Vi consists of a homopolymer of repeating unit (1 → 4) α-D-galacturonic acid that is 40% to 80% O-acetylated at the C3 position[41,42]; the presence of Vi prevents O antibody from binding to the O antigen.[38] There is evidence that each of the antigens O, Vi, and H, plays a protective role with certain typhoid vaccines.

At the level of the clinical microbiology laboratory, *S.* Typhi exhibits a remarkable degree of homogeneity, in comparison with the other species of *Salmonella*. *S.* Typhi rarely exhibits biochemical or serologic variability. One exception is found in Indonesia, where a few percent of isolates bear flagellar antigen j or z66 rather than d. Previously, only phage typing using Vi phages, pulsed-field gel electrophoresis, and ribotyping were helpful in differentiating strains from different geographic areas.[43–46] Currently, partial- and full-genome high-throughput sequencing have revolutionized the ability to differentiate *S.* Typhi strains from diverse sources.[47–52] *S.* Typhi does not ferment lactose; it produces hydrogen sulfide (H_2S), but does not produce gas. As a consequence, suspect colonies are evident on usual lactose-containing media such as *Salmonella-Shigella* agar or MacConkey agar as lactose-negative colonies. The biochemical pattern in triple-sugar iron agar is rather characteristic, manifested by an acid butt without gas, an alkaline slant, and obvious H_2S production. Fresh isolates typically agglutinate with Vi but not necessarily with group D antiserum. However, if the bacteria are boiled to remove the Vi capsule that overlies the LPS O antigen, a reaction with group D antiserum is readily seen. Fig. 61.1 is a diagrammatic representation of the relationships among the O (cell wall), Vi (capsular polysaccharide), and H (flagellar) antigens of *S.* Typhi.

The complete sequences of the approximately 4.8 million base-pair (bp) genomes of multiple-antibiotic–resistant *S.* Typhi strain CT18[47] and of antibiotic-sensitive strain Ty2[49] have been reported. Approximately 70% to 80% of the genome shares a backbone containing genes similar in sequence and order to those in *Escherichia coli* and other salmonellae. Intermittently along this backbone are clusters of *S. enterica*–specific and *S.* Typhi–specific genes (i.e., genes not found in *S.* Typhimurium or other *S. enterica* serovars) and more than 200 pseudogenes (including several that play a role in virulence in *S.* Typhimurium). In addition to the 218,150-bp plasmid encoding antibiotic resistance in CT18, another 106,516-bp plasmid was found that shows common derivation with the pFra virulence plasmid of *Yersinia pestis*.[47,48]

PATHOGENESIS

Pathogenesis of Acute Typhoid Fever

S. Typhi and *S.* Paratyphi A and *S.* Paratyphi B are highly invasive bacteria that pass through the intestinal mucosa of humans rapidly and efficiently to eventually reach the

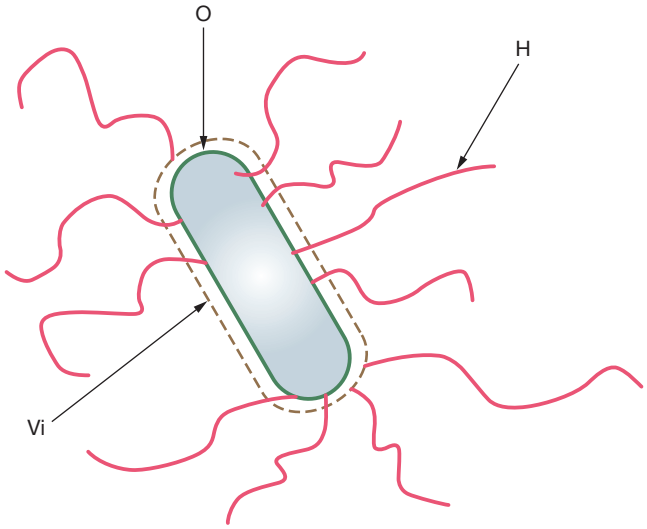


Figure 61.1. Demonstration of the locations of the O, Vi, and H antigens of *Salmonella* Typhi. The specific O antigen immunodominant epitope (epitope 9) that characterizes *S.* Typhi as falling in *Salmonella* group D consists of a tetrasaccharide repeat, wherein the dideoxyhexose sugar tyvelose is linked to a mannosyl-rhamnosyl-galactose backbone. Further internally, these O tetrasaccharide repeats are linked to core sugars, which are further linked to cell wall lipid A components. A capsular polysaccharide, the Vi antigen, overlies the O antigen. Peritrichous flagella emanating from the bacteria carry protein H antigen d in the case of *S.* Typhi (with a few exceptions from Indonesia that exhibit flagellar antigen z66).

reticuloendothelial system, where, after an 8- to 14-day incubation period, they precipitate a systemic illness.[53] *S.* Typhi is a highly host-adapted pathogen; humans comprise the only natural host and reservoir of this infection.

Comprehension of the steps involved in the pathogenesis of typhoid fever comes from four sources: (1) clinicopathologic observations in humans,[54,55] (2) volunteer studies,[56,56a–c] (3) studies of a chimpanzee model,[57,58] and (4) analogies drawn from *S.* Typhimurium and *Salmonella* Enteritidis infection in mice (the "mouse typhoid" model).[59,60] The following is a summary of the probable steps in the pathogenesis of *S.* Typhi infection in humans.

Susceptible human hosts ingest the causative organisms in contaminated food and water. The inoculum size and the type of vehicle in which it is ingested greatly influence the attack rate for typhoid fever and also affect the incubation period. Dosages of 10^9 and 10^8 pathogenic *S.* Typhi ingested by volunteers in 45 mL of skim milk induced clinical illness in 98% and 89% of persons, respectively; dosages of 10^5 organisms caused typhoid fever in 28% to 55% of volunteers, whereas none of 14 subjects who ingested 10^3 organisms without buffer developed clinical illness.[56]

A modern typhoid model was able to lower the inoculum of *S.* Typhi needed to cause clinical illness and bacteremia by administering the organisms with sodium bicarbonate to neutralize gastric acid.[61] Inocula of 10^3 or 10^4 typhoid bacilli evoked illness in 55% and 65% of volunteers, respectively.[61] When the ingested typhoid bacilli pass through the pylorus and reach the small intestine, they rapidly penetrate the mucosal epithelium by one of two mechanisms to arrive in the lamina propria. One mechanism of invasion involves typhoid bacilli being actively taken up by M cells,[62–64] the dome-like epithelial cells that cover Peyer's patches and other organized gut lymphoid tissue. From here they enter mononuclear and dendritic cells in the underlying lymphoid tissue. In a distinct second invasive mechanism, bacilli are believed to be internalized by enterocytes, wherein they enter membrane-bound vacuoles, which pass through the cell and ultimately release the organisms at the basal portion of the cell without destroying the enterocyte. Takeuchi provided a highly descriptive electron-photomicrographic documentation of the analogous passage of *S.* Typhimurium through intestinal mucosa.[65] A third paracellular mode of entry also has been proposed.[66]

On reaching the lamina propria in the nonimmune host, typhoid bacilli elicit an influx of macrophages that ingest the organisms but are generally unable to kill them. Some bacilli apparently remain in macrophages of the small intestinal lymphoid tissue. Other typhoid bacilli are drained into mesenteric lymph nodes, where further multiplication and ingestion by macrophages take place. Shortly after invasion of the intestinal mucosa, a primary bacteremia is believed to take place in which *S.* Typhi organisms are filtered from the circulation by fixed phagocytic cells of the mononuclear phagocyte system. It is believed that the main route by which typhoid bacilli reach the bloodstream in this early stage is by lymph that drains from mesenteric nodes to eventually reach the thoracic duct and then the general blood circulation. It is conceivable that ingestion of a massive inoculum followed by widespread invasion of the intestinal mucosa could result in rapid and direct invasion of the bloodstream. As a result of this primary bacteremia, the pathogen rapidly attains an intracellular haven throughout the organs of the reticuloendothelial system, where it resides during the incubation period (usually 8 to 14 days) until the onset of clinical typhoid fever.

Clinical illness is accompanied by a fairly sustained secondary bacteremia. In their report of experimental *S.* Typhi challenge studies in volunteers, Hornick and colleagues described one volunteer who began a 7-day course of oral chloramphenicol only 1 day after ingesting pathogenic *S.* Typhi, and who developed clinical typhoid fever 9 days after the antibiotic was discontinued.[56] This report provides evidence that the typhoid bacilli have attained their intracellular haven within 24 hours after ingestion.[56]

The Vi antigen is a virulence property.[37,40,67] Felix and Pitt, who originally described and named the antigen, showed that Vi antigen enhanced the pathogenicity of *S.* Typhi for mice.[37,67] Virtually all strains freshly isolated from patients possess this polysaccharide capsule. Both epidemiologic observations and studies in volunteers support the contention that *S.* Typhi strains that possess Vi are more virulent than strains lacking this polysaccharide.[56] Nevertheless, Vi-negative strains have caused typhoid fever when fed to volunteers.

A *S.* Typhi toxin has been described, but its role in pathogenesis is not yet defined.[68,68a]

Pathogenesis of the Chronic Typhoid Carrier State

During the primary bacteremia that follows ingestion of typhoid bacilli and seeds the mononuclear phagocyte system, organisms also reach the gallbladder, an organ for which *S.* Typhi has a remarkable predilection. After intravenous inoculation, *S.* Typhi rapidly appear in the gallbladder of rabbits.[69,70] *S.* Typhi can be readily cultured from bile or from bile-stained duodenal fluid in patients with acute typhoid fever.[71–74] In approximately 2% to 5% of patients, the gallbladder infection becomes chronic.[30,31] The proclivity to become a chronic carrier is greater in female patients and increases with age at the time of acute *S.* Typhi infection, thereby resembling the epidemiology of gallbladder disease. The infection tends to become chronic in persons who have a preexisting pathologic gallbladder condition at the time of acute *S.* Typhi infection.

DIAGNOSIS

Bacteriologic Diagnosis

Confirmation of the diagnosis of typhoid fever requires recovery of *S.* Typhi from a suitable clinical specimen. Because of practicality and relative ease of access, multiple blood cultures should be obtained from patients in whom the diagnosis is suspected clinically. The rate of recovery of *S.* Typhi in blood cultures depends on many factors, including the volume cultured, the ratio of the volume of blood to the volume of culture broth in which it is inoculated (ideally, the ratio should be at least 1:8), the inclusion of anticomplementary substances in the medium (such as sodium polyanetholsulfonate or bile), and whether the patient has already received antibiotics to which the *S.* Typhi is sensitive. With three 5-mL blood cultures, *S.* Typhi can be recovered from the blood in approximately 70% of suspected cases.

The gold standard of bacteriologic confirmation of typhoid fever is the bone marrow culture, which is positive in 85% to 96% of cases, even when the patient has received antibiotics.[26,75] In the 1980s, great interest was shown in the use of duodenal string devices to obtain bile-stained duodenal fluid for culture.[70–72,74] The combination of a duodenal string and two blood cultures provides a sensitivity of bacteriologic confirmation equal to that achieved with bone marrow cultures but without the invasiveness of the latter.[71] Stool cultures lead to recovery of the organism in only 45% to 65% of cases, with a somewhat higher yield in children.[72]

Serodiagnosis of Typhoid Fever

Serodiagnosis of typhoid fever has been attempted since the late 19th century, when Widal and Sicard (1896),[76] among others,[77,78] showed that the serum from patients with typhoid fever agglutinated typhoid bacilli. The Widal test, still practiced today in many areas, involves the search for agglutinins in the patient's serum and may be performed with antigen in tubes or on slides; the former is generally more accurate. By careful choice of antigen, both O and H antibodies can be selectively measured. By use of *S.* Typhi strain 0901, which lacks flagellar and Vi antigens, *S.* Typhi O antibody can be selectively measured. To detect antibodies against the appropriate H antigen (d), a strain such as *Salmonella* Virginia is selected that possesses the identical Phase I flagellar antigen (d) but shares no O somatic antigens with *S.* Typhi.[79] Most patients with typhoid fever have elevated levels of O and H antibody at the time of onset of clinical illness.[79,80] Anderson[81,82] and Anderson and Gunnell[83] emphasized the importance and usefulness of H titers in serodiagnosis of typhoid fever. However, others reported that the prevalence of H antibodies in adults living in endemic areas is too high for the test to be useful in that age group.[79] Nevertheless, it can be helpful in diagnosing children younger than 10 years of age in endemic areas and persons of any age from nonendemic areas.[79] A history of inoculation with parenteral killed whole-cell vaccines invalidates the Widal test. One report showed optimistic results for use of the slide test for O agglutinins of *S.* Typhi, even for adults in endemic areas.[84] Several relatively simple commercial serodiagnostic kits exist for detecting antibodies to *S.* Typhi O antigen and to a 50-kDa outer membrane protein antigen.[85–88]

Serologic tests to measure Vi antibody using highly purified Vi antigen are available.[89–91,91a] Although measuring the titer of serum Vi antibody is practical for the detection of chronic *S.* Typhi carriers, most of whom have highly elevated titers of Vi[91] antibody, it is of little help in diagnosing acute typhoid fever because only a minority of patients with acute infection manifest detectable Vi antibody.

Rapid Immunoassays

Many attempts have been made to develop tests that detect *S.* Typhi antigens or nucleic acid in blood, urine, or body fluids, thereby providing a rapid diagnostic test for typhoid fever.[92–104] With few exceptions, these tests have failed to warrant the enthusiasm of the initial reports. The immunoassays are based on the detection of the O, Vi, or other antigens of *S.* Typhi in blood or urine using coagglutination, enzyme-linked immunosorbent assay (ELISA), or countercurrent immunoelectrophoresis. The polymerase chain reaction methods attempt to amplify *S.* Typhi genes. These assays aim to be more sensitive, practical, economical, and rapid than bacteriologic culture, yet comparably specific. So far, no assay has adequately accomplished these objectives, and a satisfactory test that might replace bacteriologic culture remains a laudable but elusive goal.

TREATMENT WITH ANTIMICROBIALS

Treatment of Acute Typhoid Fever

The hallmark report of Woodward and colleagues[17] in the late 1940s first showed that the antibiotic chloramphenicol could drastically ameliorate the severity of clinical illness and drop the case-fatality rate to less than 1% (with early initiation of therapy). Several reviews summarize current treatment.[105–107] If it is known, or is likely, that strains are sensitive, oral ciprofloxacin is highly effective. In developing-country situations, where the cost of therapy is a critical factor, amoxicillin and trimethoprim-sulfamethoxazole remain useful oral drugs if prevalent strains are sensitive. In the face of resistance to ciprofloxacin and other oral fluoroquinolones, oral azithromycin and parenteral ceftriaxone are highly effective.

Treatment of the Chronic Carrier State

Before the appearance of fluoroquinolone antibiotics, to achieve a high cure rate in the treatment of chronic biliary carriers of *S.* Typhi, it was necessary to perform cholecystectomy followed by several weeks of intravenous ampicillin or oral amoxicillin plus probenecid. When ciprofloxacin and norfloxacin became available, it was found that 4 weeks of oral therapy without surgical removal of the gallbladder could successfully treat 90% of chronic carriers despite the presence of gallstones.[108,109] The fact that these oral bactericidal antibiotics are highly concentrated in bile led to these hallmark clinical results.

EPIDEMIOLOGY

Incidence and Prevalence Rates

In endemic areas, using passive surveillance that detects patients sufficiently ill to seek healthcare, a characteristic age-specific incidence of typhoid fever is typically seen with a very low incidence in infants younger than 1 year of age, a low or moderate incidence in preschool-age children 2 to 4 years of age, a peak incidence in school-age children (5 to 19 years), and a low incidence in adults older than 35 years. The apparent low incidence in infants relates in part to decreased exposure to vehicles of transmission, and the clinical consequence of *S.* Typhi infection in the infant host is often mild or atypical illness, even in the face of bacteremic infection.[28,29] Recent evidence based on systematic collection of blood cultures from febrile children (mainly detected during active household surveillance visits or when children seek care at ambulatory healthcare facilities) suggests that in some countries in

Asia, bacteremic *S.* Typhi infection in children 1 to 4 years of age may be much more common than previously appreciated.[110–115] Moreover, in some areas of South and Southeast Asia, high incidences of typhoid fever in preschool children have been recorded through passive surveillance.[116,117] It is assumed that in developing countries with primitive conditions of human waste disposal and widespread contamination of water supplies, water represents the most common vehicle of transmission, and that the number of organisms ingested is usually small. Thus, multiple subclinical and mild infections are believed to occur for each full-blown clinical case. In contrast, in more-developed countries with good sanitation, typhoid is transmitted when chronic carriers contaminate food vehicles through a breakdown in proper practices of personal and food hygiene. In these common-source foodborne outbreaks, the inocula ingested are presumably often relatively large, and high attack rates ensue; under these conditions, fewer subclinical cases occur.

It is difficult to quantify the magnitude of the typhoid fever problem worldwide because the clinical picture is confused with that of many other febrile infections (including paratyphoid), and the capacity for routine bacteriologic confirmation is absent in most areas of the less-developed world.[118–120] Nevertheless, incidence data are available from two sources. First, in the course of several vaccine field trials carried out from 1980 to 2011 in Latin America, Asia, and Africa, it was possible to confirm the incidence of typhoid fever that occurred in the unimmunized control subjects during several years of surveillance. In these trials, high annual typhoid fever incidence rates per 105 person-years of observation (PYO) were recorded, including 810/105 PYO in Indonesia,[121] 653/105 PYO in Nepal,[122] 470/105 PYO in South Africa,[123] 227/105 PYO in Chile,[124] 375/105 PYO in Vietnam,[125,126] 255/105 PYO in India,[127] and 210/105 PYO in Pakistan.[128] The method of surveillance in Chile, Indonesia, India, and Pakistan was passive and detected only those patients ill enough to seek attention at healthcare facilities. In contrast, the surveillance methods in the Nepal, South Africa, and Vietnam field trials included active case detection with household visits to look for febrile patients (in South Africa this was in addition to a passive surveillance system); subsequent follow-up (months 28 to 46) in the Vietnam trial involved passive surveillance.[126] Second, population-based studies to measure the incidence of bacteriologically confirmed typhoid fever, based on passive treatment facility–based surveillance, have been carried out in the last decade of the 20th and the first decade of the 21st century in Karachi, Pakistan[113,116]; Kolkata, India[116,129]; Mekong Delta, Vietnam[117]; North Jakarta, Indonesia[116]; and China.[116,130]

Seroepidemiologic studies that measure the prevalence of *S.* Typhi H antibody have been very useful in quantifying the prevalence of typhoid fever in different geographic areas.[79,118,131] However, this seroepidemiologic technique has not been widely applied.

The emergence in South Asia of paratyphoid fever caused by multiple-antibiotic–resistant *S.* Paratyphi A poses a challenge for both indigenous populations[132,133] and travelers[134] to this region from industrialized countries, because currently there are no licensed paratyphoid vaccines, and licensed oral Ty21a and parenteral Vi typhoid vaccines do not offer cross-protection.

RISK GROUPS

Three populations are at particularly high risk of developing typhoid fever and would benefit from immunoprophylaxis with a safe, effective, inexpensive, and practical vaccine. These include children in endemic areas,[28,79,110,112,113, 115–117,129,135–137] travelers and military personnel from industrialized countries who visit endemic areas in less-developed countries,[138–142] and clinical microbiology technicians.[143–145]

Seroepidemiologic studies in Peru and Chile in the 1970s (when typhoid was highly endemic in those countries) showed that by 15 to 19 years of age, 50% to 80% of teenagers had serologic evidence of past infection with *S.* Typhi.[79,118,131] In endemic areas, typhoid fever is a major cause of absenteeism from school and from employment. Direct expenditures for hospitalization and medication further raise the public health costs of this disease.[111,146] For areas in which it is unlikely that improved sanitation and treated water supplies will become a reality in the near future, a safe vaccine that confers long-term protection (e.g., Ty21a[147] or Vi conjugate[126]) would be particularly beneficial in relation to its cost, because an initial investment in immunization would provide many years of protection. Because typhoid fever exhibits its peak incidence in school-age children—a "captive" population in many countries—a school immunization program could target the high-risk population of schoolchildren.[148–151] For some urban slum and other populations in Asia where the incidence of typhoid fever may be known to be high in preschool children, the increasing use of a second measles-containing vaccine in the second year of life offers an opportunity to administer a two-dose regimen of Vi conjugate at approximately 9 and approximately 15 months of age, or to administer a single dose to toddlers (or older infants) if a single dose of the vaccine is highly immunogenic in those age groups.[152,153] Travelers from industrialized countries who visit less-developed countries in which typhoid fever is endemic are at particular risk of developing the disease.[138–142] Travelers are probably at special risk in endemic areas because they do not have the background immunity that much of the indigenous population has acquired as a consequence of multiple subclinical infections. For U.S. travelers, the Indian subcontinent (India, Pakistan, and Bangladesh), Mexico, Philippines, and Haiti are the areas of highest risk.[142] Since 1990, isolates from Asia and northeast Africa have shown increasing resistance to many clinically relevant antibiotics, including chloramphenicol, amoxicillin, and trimethoprim-sulfamethoxazole.[21,105,119,154] Partial resistance to ciprofloxacin and other fluoroquinolones is also prevalent in Southeast Asia and some other regions.[155–157]

Microbiology technicians in laboratories, particularly clinical laboratories, also constitute a high-risk group for the development of typhoid fever. The Centers for Disease Control and Prevention (CDC)[143] reported that 11.2% of the sporadic (i.e., not outbreak-associated) cases of typhoid fever in the United States in a 33-month period occurred in laboratory technicians. In the course of their work, these people process stool or blood cultures containing *S.* Typhi before it is recognized that the pathogen is present. The predilection for laboratory workers to develop typhoid fever suggests that, under these special conditions, the disease may be spread by aerosol or by direct contact. This observation is in contrast to the lack of contact spread of this infection under more natural conditions and may be related to the organism existing in pure culture in the laboratory.

MODES OF TRANSMISSION AND RESERVOIRS OF INFECTION

Humans are the sole reservoir of *S.* Typhi infection as well as the only natural host. The infection is transmitted when susceptible hosts ingest food or water that has been contaminated by fecal matter. In contrast, transmission from person to person by direct contact is exceedingly uncommon. As a consequence of these epidemiologic features, typhoid fever represents the quintessential infectious disease for which

transmission is related to levels of sanitation and quality of water supply. Typhoid fever can abound wherever sanitation and food hygiene are primitive. The highest incidence usually occurs where water supplies serving large populations are contaminated by fecal matter. This situation existed at the end of the 19th century in most large cities in the United States and Western Europe, where piped water supplies were available but the water was usually untreated.[158] The water sources (usually rivers) were also the repository for the discharged sewage of the cities. In this manner, the transmission of typhoid fever was amplified, causing the disease to be highly endemic in large cities throughout the United States and Europe. With the introduction of water treatment at the turn of the 20th century, including sand filtration and chlorination, the incidence of typhoid fever plummeted precipitously in the large cities of the United States despite the continued existence in those cities of many chronic carriers of *S.* Typhi.[158,159] Typhoid fever remains endemic in most of the less-developed areas of the world, where fecal contamination of water sources still occurs. This includes many countries in Africa, Asia, and Latin America.

Significance as a Public Health Problem

It has been estimated that each year, around 22 to 33 million cases and 216,000 to 500,000 deaths occur worldwide as a result of typhoid fever.[118–120,160] Data quantifying the incidence of bacteriologically confirmed typhoid fever are available from control (usually placebo recipient) groups participating in large-scale field trials of typhoid vaccines, or from populations in sites being prepared for field trials. It may be argued that such populations represent hot spots of typhoid and that the incidence rates from such areas cannot be readily extrapolated to other developing regions. Nevertheless, from sites being prepared for field trial evaluations of typhoid vaccines and from field trials carried out in the late 1970s through 2009 in South America (Chile), Africa (South Africa), and Asia (Nepal, Indonesia, India, Pakistan), annual incidence rates of 198 to 810 cases per 10^5 PYO were recorded (school-age children were the main age group under surveillance).[113,117,121–124,127–129]

PASSIVE IMMUNIZATION

Passive protection by means of antiserum or immunoglobulin is not used to prevent typhoid or paratyphoid fever.

ACTIVE IMMUNIZATION

History of Vaccine Development

For historical as well as practical purposes, the various typhoid vaccines that have been evaluated in clinical trials and the few that have been used as licensed vaccine products can be grouped into three categories:

- Vaccines no longer in use or no longer under investigation;
- Vaccines currently available in various countries as licensed products; and
- Future vaccines under active clinical evaluation.

The various past, present, and future typhoid vaccines can be considered to fall into five broad groups for review:

- Inactivated whole-cell parenteral vaccines;
- Subunit parenteral (which includes purified Vi polysaccharide) or aerosolized vaccines;
- Inactivated whole-cell oral vaccine;
- Attenuated *S.* Typhi strains used as live oral vaccines; and
- Parenteral polysaccharide-carrier protein conjugate vaccines.

Early Vaccine Approaches That Were Abandoned

For a more detailed review of the early vaccines, readers are referred to the chapters on typhoid vaccine in the first two editions of this book.[161,162] A few of the vaccines mentioned here were licensed products that at one time were in fairly widespread use. Others among the cited vaccines did not progress beyond early clinical trials or large-scale field trials because of unsatisfactory safety profiles, poor immunogenicity, disappointing efficacy relative to other typhoid vaccines, or difficulty in manufacture (e.g., loss of potency after lyophilization).

Alcohol-Inactivated Whole-Cell Parenteral Vaccine

The prototype alcohol-inactivated vaccine was prepared in the 1940s by Felix, who published experimental evidence contending that alcohol treatment of *S.* Typhi was superior in preserving the Vi antigen because it resulted in a vaccine that outperformed the heat-inactivated, phenol-preserved vaccine in the mouse protection assay.[163,164] For some years, this vaccine replaced the heat-inactivated, phenol-preserved vaccine in routine use by the British Armed Forces.

Formalin-Inactivated, Phenol-Preserved, Whole-Cell Parenteral Vaccine

This vaccine, which was evaluated for efficacy in a controlled field trial,[165] was prepared by inactivating *S.* Typhi with formalin and then preserving the vaccine in phenol.

Acetone-Inactivated and Dried Whole-Cell Parenteral Vaccine

This type of vaccine was prepared because of evidence showing that acetone inactivation preserves the Vi antigen (thereby increasing potency in active mouse protection assays) and improves the stability of the vaccine on long-term storage.[166–168] In preparing acetone-inactivated and dried vaccine, *S.* Typhi bacteria are precipitated and inactivated with acetone and then air dried or lyophilized.[167] The manufacturing process required to prepare acetone-inactivated, dried vaccine was much more demanding than for making heat-phenolized vaccine. Therefore, although there were some manufacturers of this vaccine until the 1990s (including Wyeth, which made a lyophilized formulation for use by the U.S. military), further production was discontinued.

Heat-Inactivated, Phenol-Preserved, Whole-Cell Parenteral Vaccine

Parenteral heat-inactivated, phenol-preserved, whole-cell vaccines to prevent typhoid fever were used since the end of the 19th century[14,15,169] and the experience with the early killed *S.* Typhi parenteral vaccines has been reviewed.[170] These vaccines, which were relatively easy to prepare and standardize, were the first typhoid vaccines to be widely used, particularly by the British and U.S. military in the first quarter of the 20th century. Use continued into the early 1990s of both the liquid and lyophilized formulations (and occasionally adsorbed to alum adjuvant). There are no current manufacturers of this vaccine. Readers are referred to previous editions of this book for technical information on this type of typhoid vaccine.

Oral-Inactivated, Whole-Cell Oral Vaccines

As early as the 1920s, Besredka[171,172] promulgated the use of killed *S*. Typhi or attenuated strains as oral vaccines to elicit "local immunity." In the 1960s and 1970s, several large-scale field trials[173–178] and experimental challenge studies in volunteers[179] were carried out to assess the efficacy of oral inactivated whole-cell vaccines, which at that time were widely sold and used in Europe. The oral killed vaccines evaluated in field trials or volunteer studies include acetone-inactivated vaccine and formalin-inactivated vaccine.

Parenteral Subunit (Including Early Attempts to Purify Vi Polysaccharide) or Aerosolized Subunit Vaccines

Many attempts were made to prepare extracts and sonicates of *S*. Typhi and to purify antigens for use as parenteral vaccines. The various subunit immunizing agents (which in the 1960s were referred to as chemical vaccines) evaluated for efficacy in controlled field trials (or in volunteer studies) included: freeze-and-thaw extract vaccines,[180] trypsinized extract vaccines,[181] purified LPS vaccines (hot water-phenol extraction method),[182] and purified Vi polysaccharide vaccine (prepared by a method that unwittingly denatured the polysaccharide).[183,184] One trypsinized extract chemical vaccine administered by aerosol was evaluated for efficacy in a controlled field trial.[173]

A parenteral freeze and thaw extract vaccine prepared by the method of Grasset[180] was assessed for efficacy in a controlled field trial in Poland.[185] Several trypsinized extract vaccines, prepared according to Topley and associates,[181] were subjected to field trials of efficacy in the Soviet Union and Poland.[165,185,186] Two parenteral purified LPS vaccines consisting of *S*. Typhi LPS O antigen prepared by the hot water–phenol extraction method[182] were evaluated in controlled field trials in Poland[185] and the Soviet Union.[165] In the early 1950s, Landy and coworkers[183,187] isolated Vi antigen from *Citrobacter freundii* (previous designations of this bacterium include *Paracolon ballerup*, *Bethesda ballerup*, and *Escherichia coli* 5396/38) and *S*. Typhi for use as a parenteral vaccine. Organisms grown on solid agar were inactivated with acetone, after which the dried, acetone-killed bacteria were submitted to multiple extractions with saline, ethanol, and acetic acid to separate Vi from protein, LPS, and nucleic acid. This early method of preparation of purified Vi antigen apparently denatured the polysaccharide, resulting in a complete loss of *O*-acetyl and a diminution of *N*-acetyl moieties.[40,188,189] Landy's denatured Vi vaccine was never evaluated for efficacy in field trials. However, in experimental challenge studies in volunteers, a single 25-μg parenteral dose conferred only modest (25% vaccine efficacy) insignificant protection.[56]

Attenuated Strains as Live Oral Vaccines

Attenuated *S*. Typhi strains evaluated in Phase I and Phase II clinical trials but abandoned from further development include: streptomycin-dependent strains[190–192]; Vi-positive variant of *galE* mutant Ty21a[193]; *galE,via* (Vi-negative) recombinant mutant strain EX642 derived from wild-type strain Ty2[194]; G 2260 hybrid strain (carrying DNA from *E. coli* K-12 and *Shigella flexneri*)[195]; Δ*aroA*, Δ*purA* auxotrophic strain 541Ty derived from wild-type strain CDC 10-80[196]; 543Ty, Vi-negative derivative of auxotrophic strain 541Ty[196]; Δ*aroC*, Δ*aroD* double-mutant recombinant strain CVD 906 (derived from wild-type strain ISP 1820, phage type 46)[197,198]; Δ*cya*, Δ*crp* mutant strain χ3927 (derived from wild-type strain Ty2)[199,200]; Δ*aroA*, Δ*phoP*/*phoQ* strain Ty445 (derived from wild-type strain CDC10–80)[201]; Δ*aroA*, Δ*aroD* strain PBCC211 (derived from wild-type strain CDC10–80)[202]; Δ*aroA*, Δ*aroD*, Δ*htrA* strain PBCC222 (Δ*htrA* derivative of PBCC211).[202]

Readers interested in details about these strains and their clinical evaluation are referred to earlier editions of this book.

Currently Licensed, Available Vaccines

Currently licensed, used, and commercially available typhoid vaccines include the following, which are described in Table 61.1:

- Purified Vi polysaccharide parenteral vaccine;
- Attenuated *galE*, *via* (Vi-negative) strain Ty21a, used as a live oral vaccine;
- Purified Vi polysaccharide conjugated to tetanus toxoid as a parenteral vaccine (two conjugates, so far, have been licensed in India, one is becoming widely used).[153,203–206,206a]

Results of the clinical trials and field trials that established the safety, immunogenicity, and efficacy of the available typhoid vaccines are described in other sections of this chapter.

Purified Vi Polysaccharide Vaccine

To purify Vi under nondenaturing conditions, Wong and colleagues[207] and Robbins and Robbins[40] treated *S*. Typhi with hexadecyltrimethylammonium bromide.[208] This extraction method results in nondenatured purified Vi that preserves the *O*- and *N*-acetyl moieties, as opposed to the methods used by Landy and coworkers that yielded denatured Vi. Fig. 61.2 shows the chemical structure of Vi polysaccharide.

TABLE 61.1 Currently Licensed Typhoid Vaccines

Type of Vaccine	Product Name	Producer	Site of Manufacture
Parenteral purified Vi polysaccharide	Typhim Vi	Sanofi Pasteur	France
	Typherix	GlaxoSmithKline	Belgium
	TypBar	Bharat Biotech	India
	Shantyph	Shanta Biotech	India
	Typho-Vi	BioMed	India
	Zerotyph inj	Boryung	South Korea
	Typhevac inj	Shanghai Institute of Biological Products	China
Live oral Ty21a	Vivotif	PaxVax Berna	Switzerland
	Zerotyph caps	Boryung	South Korea
Parenteral Vi conjugate	Typbar-TCV	Bharat Biotech International	India
	PedaTyph	Bio-Med	India

Attenuated *S.* Typhi Strain Ty21a as a Live Oral Vaccine

Ty21a was derived from wild-type strain Ty2 by treatment with the mutagenic agent nitrosoguanidine.[209] A mutant was selected that exhibited a complete absence of activity of the enzyme uridine diphosphate (UDP)-galactose-4-epimerase and a reduction of approximately 80% in the activity of two other Leloir enzymes, galactokinase and galactose-1-phosphate uridyl transferase. A further mutant was then selected that lacked the Vi antigen. This strain was designated Ty21a.[209]

For many years, it was thought that the *galE* and Vi mutations together accounted for the impressive safety of Ty21a.[210] It is now recognized that the *galE* and Vi mutations in Ty21a do not by themselves explain the attenuation of this strain.[194] Other mutations that also were induced by the nonspecific chemical mutagenesis contribute to the safety of this oral vaccine strain.[211] One such mutation may be in *rpoS* (*katF*), which encodes an RNA polymerase sigma factor; this mutation diminishes the ability of the bacteria to survive under stress, including nutrient deprivation.[212]

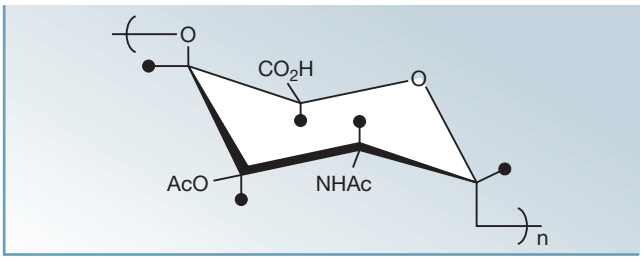


Figure 61.2. Chemical composition of Vi polysaccharide, which is a homopolymer of (1→4)-α-*D*-GalpANA, which is variably acetylated at carbon 3.

Purified Vi Polysaccharide Conjugate Vaccine

Two Vi polysaccharide conjugate vaccines, Typbar-TCV (Bharat Biotech) and PedaTyph (BioMed P), each consisting of Vi covalently linked to tetanus toxoid, have been licensed in India based on multisite clinical trial results that demonstrated safety and immunogenicity (serum anti-Vi antibodies) of each vaccine in infants and children[153,203,205,206]; Typbar-TCV was also tested in older children and adults up to age 45 years.[153]

Other conjugates that are in development but are not yet licensed are discussed later in this chapter (Table 61.2).

TABLE 61.2 Characteristics and Status of the Vi Conjugates Licensed by National Regulatory Agencies or in Clinical Development

Product	Developer (Manufacturer)	Source of Vi CPS	Carrier Protein	Conjugation Strategy	Amount of Conjugated Vi in Each Dose	Status of Licensure by National Regulatory Authority	WHO Prequalification
Typbar-TCV	Bharat Biotech International, Hyderabad, India	*S.* Typhi	Tetanus toxoid (TT)	TT is modified by attaching the homobifunctional linker adipic acid dihydrazide (ADH). Vi is then linked to TT-ADH using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) coupling chemistry	25 µg	Licensed by the Drug Controller General of India	Application in preparation
PedaTyph	BioMed	*S.* Typhi	Tetanus toxoid	Has not been described	5 µg	Licensed by the Drug Controller General of India	No
Vi-rEPA	NIH (Lanzhou Institute of Biological Products, Lanzhou, China)	*S.* Typhi	Recombinant exotoxin A of *Pseudomonas aeruginosa*	rEPA is modified by attaching the homobifunctional linker ADH. Vi is then linked to rEPA-ADH using EDC coupling chemistry	25 µg	In advanced clinical development	
Vi-CRM_{197}	Sclavo Behring Vaccines Institute of Global Health (BioE, Hyderabad, India)	*Citrobacter freundii*	CRM_{197}	CRM_{197} is modified by attaching the homobifunctional linker ADH. Vi is then linked to CRM_{197}-ADH using EDC coupling chemistry	5 µg		

CPS, capsular polysaccharide; WHO, World Health Organization.

VACCINE CONSTITUENTS

Purified Vi Polysaccharide Parenteral Vaccine

Purified Vi is available as a solution containing 25 µg of Vi polysaccharide in 0.5 mL of phenolic isotonic buffer. Each immunizing dose contains 25 µg of Vi polysaccharide, less than 1.25 mg of phenol in Typhim Vi and 1.1 mg in Typherix, and 0.5 mL (or as much as will suffice) of isotonic buffer (4.15 mg sodium chloride; 0.065 mg sodium dibasic phosphate, $2H_2O$; 0.023 mg sodium monobasic phosphate, $2H_2O$; and 0.5 mL [or as much as will suffice] of water for injection). The Vi vaccine manufactured by the Shanghai Institute of Biological Products contains 30 µg of Vi polysaccharide.

Ty21a Live Oral Vaccine

The enteric-coated capsule formulation of Ty21a consists of gelatin capsules coated with phthalate to render them resistant to acid. Each coated capsule contains 2 to 6×10^9 colony-forming units (CFU) of Ty21a, 5 to 50×10^9 nonviable Ty21a, 26 to 130 mg of sucrose, 1 to 5 mg of ascorbic acid, 1.4 to 7.0 mg of an amino acid mixture, 100 to 180 mg of lactose, and 3.6 to 4.4 mg of magnesium stearate (U.S. specifications). The sucrose, ascorbic acid, and the amino acid mixture are used as stabilizers for the lyophilization process. The lyophilized bacteria are mixed with lactose to achieve a defined potency of the lyophilate. Magnesium stearate is added to the lyophilized bacteria for a defined fluidity of the lyophilate.

The liquid suspension formulation of Ty21a consists of a vaccine sachet that contains 2 to 10×10^9 CFU of Ty21a, 5 to 60×10^9 nonviable Ty21a, 15 to 250 mg of sucrose, 0.6 to 10 mg of ascorbic acid, 0.8 to 15 mg of an amino acid mixture, 1.5 g of lactose, and 20 to 30 mg of aspartame. The accompanying sachet of buffer contains 2.4 to 2.9 g of sodium bicarbonate, 1.5 to 1.8 g of ascorbic acid, and 0.18 to 0.22 g of lactose. To prepare the liquid suspension, the contents of the two sachets are suspended in 100 mL of water.

Purified Vi Polysaccharide-Tetanus Toxoid Conjugate Parenteral Vaccine

One dose (0.5 mL) of Typbar-TCV contains 25 µg of Vi polysaccharide from *S.* Typhi linked to tetanus toxoid protein in isotonic saline.

One dose (0.5 mL) of the PedaTyph conjugate contains 5 µg of Vi polysaccharide from *S.* Typhi and 5 µg of tetanus toxoid protein in isotonic saline.

MANUFACTURE

Purified Vi Polysaccharide Parenteral Vaccine

Scale-up of production of Vi to an industrial manufacturing process was initially worked out by scientists from the laboratory of John B. Robbins at the National Institute of Child Health and Human Development and Sanofi Pasteur.[213] The Ty2 strain of *S.* Typhi is cultured in 1000-L fermenters, after which the bacteria are fixed with formaldehyde and Vi polysaccharide is extracted from supernates using cetrimonium bromide. The Vi is further purified, dried, dissolved in a buffer solution, and filter sterilized. After appropriate controls of this material, it is filled into single-dose syringes.

Ty21a Live Oral Vaccine

Galactose residues are an important component of the smooth LPS O antigen in wild-type *S.* Typhi. The enzyme encoded by *galE*, UDP-galactose-4-epimerase, isomerizes UDP-glucose to UDP-galactose, and vice versa (Fig. 61.3). UDP-galactose provides galactose residues that can be incorporated into the smooth LPS O antigen of *S.* Typhi. When grown in the absence of galactose, Ty21a does not express smooth O antigen, because it has no source of UDP-galactose; in this state it is not immunogenic.[214] In contrast, when *galE* mutant Ty21a is grown in the presence of exogenous galactose, the two other Leloir pathway enzymes (Fig. 61.3) allow this monosaccharide to be assimilated to UDP-galactose and used to synthesize smooth O antigen. However, because of the lack of epimerase, strain Ty21a (like other *galE* mutants)

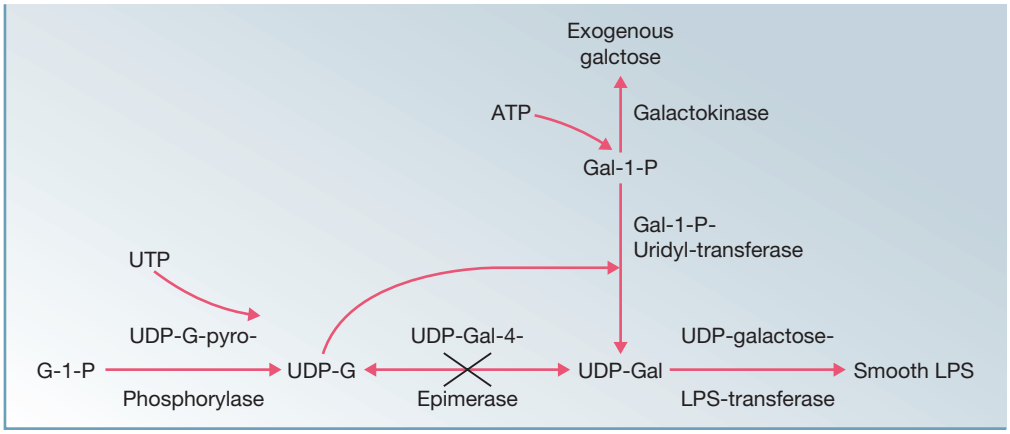


Figure 61.3. Scheme of incorporation of exogenous galactose by *galE* mutant Ty21a; attenuated *Salmonella* Typhi vaccine strain is shown. When grown in the absence of galactose, Ty21a does not produce smooth lipopolysaccharide (LPS) O antigen and is nonimmunogenic. Grown in the presence of exogenous galactose, this hexose is sequentially processed to become galactose-1-phosphate (Gal-1-P) and then uridine diphosphate-galactose (UDP-Gal). Because of the complete lack of UDP-Gal-4-epimerase in Ty21a, UDP-Gal cannot be converted to UDP-glucose (UDP-G), but it can be further incorporated into smooth LPS O antigen. However, the accumulation of UDP-Gal and Gal-1-P consequent to the block of UDP-Gal-4-epimerase activity results in bacteriolysis. *(Modified from Germanier R, Furer E. Immunity in experimental salmonellosis, II: basis for the avirulence and protective capacity of galE mutants of Salmonella* Typhimurium. Infect Immun. *1971;4:663–673.)*

accumulates galactose-1-phosphate and UDP-galactose when grown in the presence of exogenous galactose (Fig. 61.3). If the concentration of galactose reaches a certain level, accumulation of these intermediate products leads to bacterial death by lysis.[209] An appropriate concentration of galactose is included in the medium used in an early step of manufacture to ensure expression of smooth O antigen.

Purified Vi Polysaccharide–Tetanus Toxoid Conjugate Parenteral Vaccine

To manufacture Typbar-TCV, Bharat Biotech International uses Vi polysaccharide purified as is done for Typbar, their unconjugated Vi vaccine. The carrier protein, tetanus toxoid (TT), is modified by attaching the homobifunctional linker adipic acid dihydrazide (ADH). Vi is then linked to TT-ADH using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) coupling chemistry.

For PedaTyph no detailed description is available for the specific method of purification of the Vi polysaccharide component or of conjugation to the TT carrier protein. The manufacturer claims to have developed a "unique process of conjugation."

Producers of Currently Available Typhoid Vaccines

Purified Vi Polysaccharide Parenteral Vaccine

There are presently two licensed manufacturers of purified Vi among industrialized countries, Sanofi Pasteur Vaccines (Typhim Vi, available in the United States and many European and other countries) and GSK Biologics (Typherix, available in many European and other countries). In addition, several vaccine manufacturers in Asia (China, India, Vietnam),[151,215,216] Russia, and Cuba make Vi products for local and regional consumption.[217]

Ty21a Live Oral Vaccine

Ty21a is currently manufactured by two companies: PaxVax Berna, of Berne, Switzerland (Vivotif) and Boryung Pharmaceutical Co., Ltd., of Seoul, Korea (Zerotyph). Under license, Ty21a is also distributed by a number of other vaccine distributors.

Purified Vi Polysaccharide–Tetanus Toxoid Conjugate Parenteral Vaccine

Typbar-TCV is manufactured by Bharat Biotech International of Hyderabad, India.[153] PedaTyph vaccine is manufactured by Bio-Med Private Limited, located in Ghaziabad, India.[205,206]

Preparations Available, Including Combinations

Purified Vi Polysaccharide Parenteral Vaccine

Purified Vi is available as a solution containing 25 µg of Vi polysaccharide in 0.5 mL of phenolic isotonic buffer. Single-dose syringes are the usual formulation. By special order, 20-dose and 50-dose vials can be made available. The Vi vaccine formulation manufactured by the Shanghai Institute of Biological Products contains 30 µg of Vi polysaccharide.

Ty21a Live Oral Vaccine

There were three successive commercial formulations of Ty21a, each representing a notable improvement over the earlier version. The initial commercial formulation of Ty21a (which was available for only a very short time) consisted of gelatin-coated capsules containing either $NaHCO_3$ (0.4 to 0.5 g) or lyophilized vaccine (2 to 6×10^9 CFU/dose).[148,218–220] Vaccination with this initial formulation involved the ingestion of two gelatin capsules, each containing bicarbonate, followed by a third capsule containing vaccine; doses were ingested every other day for a total of three vaccine doses.

Field trials in Santiago, Chile, compared the efficacy of the gelatin capsule–$NaHCO_3$ formulation with a formulation consisting of lyophilized vaccine in enteric-coated capsules (2 to 6×10^9 CFU/capsule) that requires no pretreatment with buffer to neutralize gastric acid.[149,218] After results of controlled field trials demonstrated the unequivocal superiority of the enteric-coated capsule formulation over the gelatin-coated capsule formulation, the latter was withdrawn and replaced with the enteric-coated capsule formulation. Enteric-coated capsules should be ingested on a fasting stomach with a little water. The enteric-coated capsule formulation is the only one that is currently manufactured and available.

In 1997, the liquid suspension formulation of Ty21a was licensed in several countries with a three-dose immunization regimen that recommended that the doses be given every other day. Unfortunately, this useful formulation is no longer manufactured. In the late 1990s, when this formulation was available, it came as a double sachet, with one sachet containing 2 to 10×10^9 CFU of lyophilized vaccine and the other containing buffer. The contents of the two sachets were added to 100 mL of water with stirring, resulting in the vaccine suspension. Some authorities interested in controlling typhoid fever in endemic areas have urged that this formulation be manufactured once again for immunizing young children with Ty21a oral vaccine.[220a,281] At present, enteric-coated capsules, the formulation that is preferred by adult and older children because of its practicality, is the only formulation that is currently manufactured and available worldwide. This oral vaccine is also the only vaccine intended for self-administration.

Vi Combination Vaccines

Combination typhoid–hepatitis A vaccines (mainly for use by travelers) have been developed by GSK Biologics (Hepatyrix)[221–223] and Sanofi Pasteur MSD (Viatim).[223–226] Hepatyrix combines 25 µg of Vi and 1440 units of inactivated hepatitis A virus antigen adsorbed to 0.5 mg of alum adjuvant as a 1 mL liquid suspension in a prefilled syringe. Viatim comes formulated as a dual-chamber syringe in which one 0.5-mL chamber contains 25 µg of Vi polysaccharide and the other 0.5-mL chamber holds 160 antigen units of inactivated hepatitis A virus; a 25-gauge needle is attached to the dual-chamber syringe. As the plunger of the dual-chamber syringe is depressed, the vaccines from the two chambers mix and emerge as a combination.[223] Viatim uses the dual-chamber because the manufacturer's preliminary data suggested that a premixed combination diminished the response to the Vi component compared with antigen administered alone. Each of these combination vaccines stimulates seroconversion to the component vaccine antigens in a manner equivalent to monovalent Vi and hepatitis A vaccines.

Purified Vi Polysaccharide–Tetanus Toxoid Conjugate Parenteral Vaccine

One single-dose (0.5 mL) vial of Typbar-TCV Vi-TT conjugate vaccine contains 25 µg of purified Vi linked to TT, suspended in isotonic saline. Multidose (2.5 mL) vials contain five doses of Typbar-TCV in isotonic saline with 5 mg of 2-phenoxyethanol preservative. One vial of PedaTyph contains a single dose

(0.5 mL) of conjugate consisting of 5 μg of *S.* Typhi Vi polysaccharide conjugated to 5 μg of TT protein, in isotonic saline.

DOSAGE AND ROUTE

Purified Vi Polysaccharide Parenteral Vaccine

The modern nondenatured Vi vaccines are administered as a single subcutaneous or intramuscular injection generally containing 25 μg of purified polysaccharide.[122,123,213,227,228] Single doses that contain 30 μg and 50 μg of purified polysaccharide were also studied in Phase II, Phase III, and Phase IV clinical trials.[122,151,215,216,229]

Ty21a Live Oral Vaccine

Irrespective of the formulation or whether a three-dose or four-dose oral immunization schedule is followed, each dose (capsule or sachet of lyophilized vaccine) contains 2 to 6 × 10^9 CFU of Ty21a. With the enteric-coated capsule formulation, a four-dose oral immunization schedule is recommended in the United States and Canada, whereas in other countries a three-dose regimen is used. Ty21a vaccine is administered with an interval of 2 days between doses. Increasing the interval between doses of enteric-coated vaccine to 21 days does not enhance protection.[149,218] One of the field trials with Ty21a carried out in Santiago, Chile, showed that immunization with four doses within an 8-day period provides significantly greater protection than three doses.[150] Based on results of this field trial, the U.S. Food and Drug Administration (FDA) and the Canadian Drug Board licensed Ty21a as a four-dose immunization schedule, with a dose given every other day.

Two field trials, carried out in Chile[230] and Indonesia,[121] each directly compared the relative efficacies of three doses of Ty21a given in enteric-coated capsules versus vaccine administered as a liquid suspension (which is reconstituted by emptying a sachet containing lyophilized vaccine and a sachet containing buffer into 100 mL of water). The doses of vaccine were given every other day in the trial in Chile, whereas in Indonesia the doses were administered 1 week apart. In each of these trials, three doses of Ty21a in the liquid formulation provided better protection than the enteric-coated capsules. In the Chilean field trial, the difference in the level of efficacy conferred by the two formulations was highly significant.[230] In 1997, the liquid formulation of Ty21a was licensed by a number of countries with a three-dose immunization regimen that recommends an interval of 2 days between doses. However, because this formulation did not gain popularity among travelers, its manufacture was discontinued.

A practical question arises when a subject fails to complete the full course of immunization with three to four doses of Ty21a: Does one need simply to complete the number of doses, or must one complete the full schedule? A definitive answer is not available. However, as a rule of thumb, if less than 3 weeks have passed since the last dose, one may continue to complete the immunization with the missing doses. If more than 3 weeks have passed, the full regimen of this well-tolerated vaccine should be administered de novo.

Purified Vi Polysaccharide–Tetanus Toxoid Conjugate Parenteral Vaccine

A 0.5-mL dose of Typbar-TCV is administered intramuscularly to infants 6 months of age and older, toddlers, children, or adults. A single dose suffices for all age groups including infants age 6 months and older.[153] The manufacturer indicates that a booster may be administered after 3 years. However, the longevity of the serologic responses is not yet fully elucidated, so it is possible that a much longer interval may suffice before a recall booster is required. In subjects older than 2 years of age, PedaTyph Vi conjugate vaccine is administered intramuscularly in a two-dose schedule with 4 to 8 weeks between the doses. A booster is recommended every 10 years, although no data are available to support that recommendation. For children 3 to 23 months old, two doses of vaccine are recommended spaced 4 to 8 weeks apart followed by a booster at 24 to 30 months of age. The manufacturer also recommends a booster every 10 years thereafter in persons immunized at this young age.

VACCINE STABILITY

Purified Vi Polysaccharide Parenteral Vaccine

The Vi polysaccharide vaccine is highly stable and, in theory, should not require a cold chain even in tropical conditions, as it retains its physicochemical characteristics after 6 months' storage at 37°C and after 2 years at 22°C. Nevertheless, the manufacturers' labels recommend that the vaccine be stored in a refrigerator at 2°C to 8°C for up to 18 months, and they caution against freezing the products.

Ty21a Live Oral Vaccine

Long-term storage of Ty21a should be at 4°C. The shelf life of lyophilized Ty21a depends on residual moisture content and maintenance of a cold chain.[231,232] The potency requirements of Ty21a dictate that the viable counts in each dose (enteric-coated capsule or packet of lyophilized vaccine) exceed 2 × 10^9 CFU. Prolonged storage for 7 days at room temperature (20°C to 25°C) resulted in progressively lower viable counts over time. Nevertheless, after a 7-day test period, all 10 lots tested still met the minimum potency requirements.[233] Similarly, three separate lots of Ty21a maintained potency when stored at 37°C for 12 hours.[233]

Purified Vi Polysaccharide–Tetanus Toxoid Conjugate Parenteral Vaccine

Typbar-TCV conjugate is stored at 2°C to 8°C and has a 3-year shelf life at this temperature. If one or more doses are withdrawn from a multidose vial, the vial with remaining doses should be stored at 2°C to 8°C for further use but discarded after 6 hours.

The manufacturer of PedaTyph recommends that this vaccine be stored at 2°C to 8°C and that it must not be frozen. No stability data have been published.

IMMUNOGENICITY

Parenteral Inactivated Whole-Cell Vaccine

Although these vaccines, which conferred moderate protection, are no longer available, the immune responses that they elicited are described to provide background against which immune responses to newer vaccines can be compared.

Serum Antibody Response

Typically, in assessing the seroconversion after vaccination with a parenteral inactivated whole-cell typhoid vaccine, serum antibodies are measured to the O, H (d), and Vi antigens. O antibodies were formerly assayed by bacterial agglutination (Widal test),[234] but ELISA using purified LPS eventually replaced agglutination.[198,200,235–240] H antibody can be measured by agglutination using an appropriate whole-cell antigen

such as *S.* Virginia (which has the same d flagellar antigen as *S.* Typhi but lacks Vi and has distinct O antigens) or by ELISA using purified *S.* Typhi flagella.[235–238] Vi antibody can be measured by passive hemagglutination,[89,90] radioimmunoassay,[40,122,229,241] or ELISA.[91,123,153,242] It is important to use highly purified undenatured Vi antigen to avoid cross-reactions. For this reason, only since the 1980s has Vi serology become reliably specific.

Some evidence suggests that H antibodies may play a role in protection. In Yugoslavia, Poland, the Soviet Union, and Guyana, field trials of efficacy were carried out with several different well-characterized parenteral killed whole-cell vaccines, including alcohol-inactivated, heat-phenolized, and acetone-inactivated vaccines. Measurement of serologic responses to these vaccines in groups of vaccinees and in immunized laboratory animals allowed protective efficacy to be correlated with antibody response. Those killed whole-cell parenteral vaccines that were most protective in the field stimulated the highest levels of H antibody. A similar correlation between H titer and protection against typhoid fever was found in healthy young adult volunteers who in the 1960s and 1970s participated in experimental challenge studies to assess the efficacy of typhoid vaccines.[214,243,244] Control volunteers who had elevated H titers (presumably derived by natural infection or vaccination many years earlier during military service) were significantly protected against development of typhoid fever.

However, the most convincing evidence that H antibody is important comes from the field trial by Wahdan and colleagues[245] of an acetone-killed and dried vaccine prepared from a strain of *S.* Typhi that lacks H antigen. This vaccine, which did not elicit H antibodies, failed to confer significant protection.

O antibody that appears after inoculation with parenteral inactivated whole-cell typhoid vaccines is largely immunoglobulin (Ig) M, whereas the H antibody response is initially IgM and then becomes IgG.[246–251]

Mucosal Immune Response

Not surprisingly, the few studies that examined mucosal immunity after administration of parenteral inactivated whole-cell vaccine reported minimal secretory IgA (sIgA) antibody or gut-derived IgA antibody-secreting cell (ASC) responses.[246,252–254]

Cell-Mediated Immune Response

Some measurements of cell-mediated immune responses were reported after vaccination of humans with parenteral killed whole-cell vaccines.[255–258] The cell-mediated response after administration of these vaccines was not prominent.

Purified Vi Polysaccharide Parenteral Vaccine

Serum Antibody Response

Parenteral Vi polysaccharide vaccine elicits serum IgG Vi antibody responses in approximately 85% to 95% of adults and children older than 2 years of age. Table 61.3 shows the serum Vi and O antibody responses reported by Tacket and colleagues[229] after vaccination of young adults with one of two purified Vi antigen vaccines prepared from *S.* Typhi. The vaccines differed in their degree of purity: one Vi vaccine had 5% residual contamination with LPS, and the other was 99.8% pure. This difference was reflected in the serologic response. Although similar Vi antibody seroconversions occurred in approximately 90% of recipients of either preparation, those who received the more purified preparation had only a 26% seroconversion of O antibody, whereas 83% seroconverted to O antigen after inoculation with the less-purified vaccine.

The serologic responses of other groups of adults and children in nonendemic and endemic areas following immunization with a 25-μg dose of Vi polysaccharide vaccine and with antibodies measured by radioimmunoassay or ELISA are shown in Table 61.4.[122, 153,241,258,260,261] High rates of seroconversion (76% to 95%) were generally observed in adults in industrialized countries and children in developing countries, while a lower seroconversion rate (62.5%) was observed in Nepalese adults. Whereas at 1 month after immunization 87% of U.S. adults had serum anti-Vi titers of 1.0 μg/mL or greater, by 12 months only 57% and by 24 months only 46% still had this level of anti-Vi.[260]

High seroconversion rates measured by ELISA have been reported among preschool-age children 2 to 4 years of age (99%),[262] school-age children 4 to 14 years of age (99.5%),[227]

TABLE 61.3 Immune Response to Two *Salmonella* Typhi Vi Polysaccharide Vaccine Candidates

	Vi Antibody (μg/mL)[a]			S. Typhi Lipopolysaccharide Antibody (ELISA)		
	Geometric Mean Titer			Geometric Mean Titer		
	Pre	Post	Seroconversion[b] (%)	Pre	Post	Seroconversion[c] (%)
Vi Lot 53226[d]						
Maryland students	0.17	2.57	100	0.11	0.78	83
Chilean recruits	–	–	–	0.15	0.77	83
Vi Lot IMS1569[e]						
French volunteers	0.07	2.73	95	0.12	0.22	26[f]

[a]Measured by radioimmunoassay.
[b]Increase in antibody titer of 0.15 μg/mL.
[c]Increase in net optical density of 0.15.
[d]Vi Lot 53226 had 5% residual contamination with LPS.
[e]Vi lot IMS 1569 was 99.8% pure.
[f]$X^2 = 28.3$; $P < .0001$ vs. recipients of lot 53226.
ELISA, enzyme-linked immunosorbent assay.
Data from Tacket CO, Ferreccio C, Robbins JB, et al. Safety and characterization of the immune response to two Salmonella typhi *Vi capsular polysaccharide vaccine candidates.* J Infect Dis. *1986;154:342–345.*

TABLE 61.4 Serum Vi Antibody Responses Measured by Radioimmunoassay or ELISA in Adults and Children Immunized With 25-μg Doses of Liquid Formulations of Purified Vi Polysaccharide Vaccine

			Vi Antibody				
			Geometric Mean Titer (μg/mL)				
Location	Age Group(y)	N	Pre	4–6 Weeks Post	Rate of Seroconversion (%)[a]	% Seropositive	Reference
USA[b]	18–55	37	0.2[c]	2.8[c]	–	87[d]	258
USA[b]	18–40	182	0.2[c]	3.2[c]	93	79[e]	234
Belgium[f]	18–40	24	3.8[g]	52[g]	88	–	307[a]
Belgium[f]	18–40	19	2.2[g]	37[g]	95	–	307[a]
Nepal[b]	15–44	43	0.38[c]	3.68[c]	79.1	–	118
Nepal[b]	45–55	8	0.51[c]	4.44[c]	62.5	–	118
Nepal[b]	5–14	25	0.24[c]	1.89[c]	76.9	–	118
India[h]	2–4	90	10[g]	454[g]	94.4	–	200[c]
Kenya[b]	5–15	97	0.3[c]	2.0[c]	76.2	–	252

[a]Fourfold or greater rise in titer.
[b]Prelicensure lots or postlicensure lots of Typhim Vi (Sanofi Pasteur).
[c]Anti-Vi antibodies measured by radioimmunoassay.
[d]Percentage of subjects who attained an antibody concentration of ≥1.0 μg/mL of serum.
[e]Percent of subjects who attained an antibody concentration of ≥1.5 μg/mL of serum.
[f]Typherix (GSK Biologics).
[g]Anti-Vi antibodies measured by ELISA.
[h]Typbar (Bharat Biotech International).
ELISA, enzyme-linked immunosorbent assay.

and adolescents 11 to 18 years of age (99%)[228] immunized with the Typherix vaccine. Agglutinating antibody responses were also seen in 96% of adults.[263] A report compared the ELISA serologic responses in Belgian adults after a dose of Typhim Vi or a dose of Typherix.[264] At 28 days after immunization, 94% of Typhim Vi and 95% of Typherix recipients had serum Vi antibodies. Measured again at 12 months after immunization, 74% and 67%, respectively, still had elevated Vi titers; the geometric mean titers (GMTs) of recipients of the two vaccines were very similar at 28 days and at 12 months after immunization.[264]

Purified Vi polysaccharide behaves like a T-lymphocyte–independent antigen. As with other T-cell–independent purified polysaccharide vaccines, Vi is not a good immunogen in infants. Many do not respond and among those who do, the responses are short lived. More importantly, in neither older children nor adults is the serum antibody response boosted by the administration of additional doses of Vi vaccine and, as with other purified polysaccharide vaccines, there is no evidence of immunologic memory.[241,265] Vi polysaccharide, like other purified polysaccharide vaccines, presumably stimulates short-lived B-cell responses by crosslinking the B-cell receptor.[266] This drives the B cells to differentiate to plasma cells that produce Vi antibody, and it results in a depletion of the pool of memory B cells without the generation of new memory B cells.[266] Thus, without the induction of immunologic memory, whatever serum antibody levels are reached after an initial parenteral inoculation with Vi cannot be boosted further by the administration of additional doses. Titers of serum Vi antibody after a dose of vaccine progressively fall over time.[241] In a large proportion of vaccinated subjects, antibody levels fall to putatively nonprotective levels after 2 years and drop even lower by 3 years.[260]

The depletion of memory B cells implies that the administration of subsequent doses of the T-independent polysaccharide vaccine may be accompanied by lower serologic responses than were observed after the initial vaccination (hyporesponsiveness). This phenomenon has been described with meningococcal polysaccharide[267,268] and pneumococcal polysaccharide[269,270] vaccines. There are only a few studies with limited data to be analyzed to address the question of whether hyporesponsiveness occurs with Vi vaccine and the data are conflicting.[226,241,264,271,272] Some data indicate that the response to a reimmunization with Vi 2 to 3 years after the initial immunization, when antibody titers have fallen, does not fully restore the titers to the peak level observed after the initial immunization.[226,264] By contrast, the study of Keitel and colleagues[241] showed that the titers after a reimmunization with Vi 27 months after the initial immunization did restore serum anti-Vi to the same peak titer recorded after the initial immunization with Vi more than 2 years earlier, and Roggelin and colleagues did not see evidence of a diminished responses in travelers who had previously received Vi vaccine.[271] A study with locally produced Vi vaccine was carried out in China to address the safety and immune responses of revaccination of school-age children 3 years after an initial Vi vaccination versus response to a primary immunization with Vi.[272] The GMT of serum Vi antibodies measured by passive hemagglutination was 1:40 in primary vaccinees and 1:29 in revaccinated children ($P = .24$); the rise in anti-Vi was 7.7-fold in primary vaccinees but was 3.1-fold in revaccinated children ($P < .001$).[272]

Relevant to the hyporesponsiveness question, there is only one report of a search for B memory (B_M) cells among peripheral blood mononuclear cells (PBMCs) in recipients of parenteral Vi vaccine. None of eight recipients of Vi had detectable IgG B_M for Vi but two vaccinees had low levels of Vi-specific IgA B_M cells.[273] Different levels of serum Vi antibody titers after administration of Vi polysaccharide vaccine have been proposed as constituting a threshold for protection at a given point in time, including 1.0 μg/mL[274] and 1.5 μg/mL.[275] The scarce published data indicate that most of the serum IgG antibody stimulated by Vi vaccine falls within the IgG_1 subtype.[153,276]

A randomized clinical trial in British adults compared responses to Vi and hepatitis A antigens after immunization with either Viatim (Sanofi Pasteur) or Hepatyrix (GSK Biologics) combination typhoid–hepatitis A vaccines. Viatim elicited stronger serologic responses to both typhoid and hepatitis A vaccine antigens but also caused significantly more, albeit mild, adverse reactions.

Mucosal Antibody Responses

There are no reports of mucosal sIgA anti-Vi antibody appearing in gut fluids or fecal extracts following parenteral vaccination with Vi polysaccharide. However, it has been hypothesized that in the small intestine of persons in developing countries serum IgG transudes onto the mucosal surface in quantities that may have protective biological consequences.[277] Because the small intestinal mucosa of children and adults residing in fecally contaminated environments in developing countries often exhibit evidence of environmental enteropathy,[278] such transudation of IgG may indeed be common.

Cell-Mediated Immune Responses

There are no reports of T-cell studies following immunization with T-independent Vi polysaccharide. However, there is one report of an attempt to detect IgG and IgA Vi-specific B_M cells in eight recipients of Vi. No Vi-specific IgG cells were detected but two of eight exhibited Vi-specific IgA B_M cells.

Ty21a Live Oral Vaccine

Serum Antibody Response

The serum antibody response has been extensively studied with Ty21a. Gilman and colleagues[214] noted that Ty21a vaccine grown in the presence of galactose (which leads to organisms bearing smooth LPS O antigen) was highly protective, whereas vaccine grown in the absence of galactose (resulting in rough organisms) was not. These investigators reported that recipients of vaccine grown in the presence of galactose experienced a significantly greater seroconversion of O antibody.

Using serum IgG O antibody measured by ELISA in Chilean 15- to 19-year-olds, Levine and associates[149] showed a correlation between seroconversion to various dosage schedules and formulations and protective efficacy in field trials (Table 61.5). With the currently licensed enteric-coated capsule formulation, there is a stepwise increase in the proportion of vaccinees who manifest significant rises in serum IgG O antibody depending on whether one, two, or three doses of vaccine are administered within 1 week. Although serum O antibody is not believed to be the operative mechanism of immunity elicited by attenuated strains, it clearly correlates with protection. Because measurement of serum IgG ELISA antibody to *S.* Typhi O antigen is a simple technique, it provides investigators with a practical tool for comparing immunization schedules and formulations and for evaluating new candidate live oral vaccines.

Two studies documented high rates of serum IgG O antibody seroconversion following immunization of children 2 to 6 years of age in Thailand with three liquid formulation doses of Ty21a (every-other-day schedule).[279,280] Investigators in Bangladesh also immunized children 2 to 5 years of age with three doses of a liquid formulation of Ty21a and recorded robust plasma IgA and IgG responses to a Ty21a membrane preparation.[281] Several large clinical trials examined the safety and immunogenicity of coadministering the first or the third dose of the liquid suspension formulation of Ty21a in a combination oral vaccine cocktail along with single-dose live oral cholera vaccine CVD 103-HgR.[282–284] These studies showed that there was no diminution in either the serum IgG *S.* Typhi O antibody response or the vibriocidal antibody response when these vaccines were coadministered as a combined oral vaccine cocktail compared with when they were administered alone.[282–284]

TABLE 61.5 Rates of Seroconversion of IgG-ELISA *Salmonella* Typhi O Antibody After One to Three Oral Doses of Ty21a Live Oral Typhoid Vaccine Given Within 1 Week

Formulation	Doses (N)	Seroconversion Rate *N/N* (%)	Efficacy in Field Trials (%)
Enteric-coated capsules	3	61/96 (64)	67
	2	22/50 (44)	47
	1	9/50 (18)	18
Vaccine and $NaHCO_3$ in gelatin capsules	3	99/195 (50)	21

ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G.
Serologic data from Levine MM, Ferreccio C, Black RE, et al. Progress in vaccines to prevent typhoid fever. Rev Infect Dis. *1989;11(Suppl 3):S552–S567. Field trial surveillance data are from the first 36 months of follow-up in field trials in Area Norte (Black RE, Levine MM, Ferreccio C, et al. Efficacy of one or two doses of Ty21a* Salmonella *Typhi vaccine in enteric-coated capsules in a controlled field trial.* Vaccine. *1990;8:81–84) and Area Occidente (Levine MM, Ferreccio C, Black RE, et al. Large-scale field trial of Ty21a live oral typhoid vaccine in enteric-coated capsule formulation.* Lancet. *1987;1:1049–1052), Santiago, Chile.*

Mucosal Immune Responses

During the past decade, the intestinal mucosal immune response to Ty21a and several new live oral vaccines has been studied rather extensively. Most recipients of the usual three-dose oral regimen of Ty21a develop local antibody responses to O antigen.[246,253,254,285–294] Forrest[295] reported that the propensity to develop a significant rise in intestinal sIgA O antibody after immunization with Ty21a is inversely correlated with the preimmunization baseline level of intestinal antibody. Subjects who have elevated baseline titers of sIgA O antibody mount significantly lower-fold rises than do those vaccinees with absent or low titers. This inverse correlation between baseline titer and propensity to seroconvert has also been reported for the serum vibriocidal antibody response after immunization with live oral cholera vaccines[296,297] and with Ty21a expressing *Vibrio cholerae* 01 antigen.[298]

After oral administration of antigen, activated lymphocytes in the Peyer patches and other gut-associated lymphoid tissue migrate to local lymph nodes to mature. After maturation, they return to the lamina propria of the intestine, as well as to other organs of the mucosal immune system, such as the salivary glands, respiratory tract, genitourinary tract, and mammary glands. Kantele and coworkers[246,285–288,292,294] and Forrest[299] have shown that such gut-derived migrating cells can be detected in peripheral blood after oral immunization with Ty21a, and that the ability of these cells to secrete specific IgA antibody in the presence of specific antigen can be quantified by means of the enzyme-linked immunospot assay[300] or similar techniques.[299] These IgA-producing migrating cells are only detectable during a few days after immunization. The peak detection of gut-derived IgA ASCs in peripheral blood

TABLE 61.6 Magnitude of Trafficking, Gut-Derived IgA Antibody-Secreting Cell Responses to *Salmonella* Typhi O Antigen After Immunization With Different Formulations and Dosage Schedules of Live Oral Typhoid Vaccine Ty21a

Formulation of Ty21a	Doses (N)	Viable Organisms/ Dose (N)	IgA ASC Response	
			Responders[a] N (%)	GMN[b]
Gelatin capsule/ $NaHCO_3$	3[c]	2×10^9	7/10[c] (70)	6
Enteric-coated capsules	3[c]	2×10^9	18/20 (90)	23
Liquid suspension	3[c]	2×10^9	19/20 (95)	63
Liquid suspension	2[c]	2×10^9	16/20 (75)	12
Liquid suspension	1	2×10^9	10/20 (50)	3

[a]Number of responders/number of subjects vaccinated.
[b]GMN of IgA ASCs per 10^6 peripheral blood mononuclear cells.
[c]Every-other-day schedule.
ASC, antibody-secreting cell; GMN, geometric mean number; IgA, immunoglobulin A.
Data from Kantele A. Antibody-secreting cells in the evaluation of the immunogenicity of an oral vaccine. Vaccine. *1990;8:321–326.*

after oral immunization with Ty21a occurs approximately 7 days after vaccination.[246,285–288,292]

Kantele[285] immunized adult Finnish volunteers with different formulations and immunization schedules of Ty21a, attempting to parallel the different regimens that were used in field trials of the efficacy of Ty21a in Chile and Indonesia. Kantele's results demonstrate that the gut-derived IgA ASC response closely correlates with the efficacy results recorded in field trials (Table 61.6). Thus three doses (taken every other day) of Ty21a are markedly more immunogenic than one dose, and Ty21a in a liquid suspension is more immunogenic than vaccine in enteric-coated capsules.

Forrest and associates[301] studied the mucosal immune response when three doses of Ty21a are administered per rectum on days 0, 2, and 5. Each dose of vaccine contained 2×10^{11} CFU, a 100-fold larger dose than is contained in the commercial Ty21a preparation. These vaccinees showed a significant increase in sIgA anti-*S.* Typhi O antibody in jejunal fluid, serum, and saliva and in gut-derived IgA ASCs.[301]

Wahid and colleagues[273] showed that oral immunization of North Americans with the U.S. four-dose regimen of Ty21a induced significant IgG and IgA B_M cell responses to flagella, which were detected among PBMCs. Notably, B_M cells specific for T-independent (LPS), as well as for T-dependent (flagella) *S.* Typhi antigens were documented after oral immunization with live oral Ty21a. B_M cells generally express high levels of CD27, and the phenotype of the IgG and IgA B_M cells responding to *S.* Typhi flagella was $CD19^+$ IgD^- $CD27^+$, concordant with other descriptions of B_M cells specific for bacterial proteins. The IgA B_M cells detected specifically responding to T-independent LPS also exhibited the classical $CD19^+$ IgD^- $CD27^+$ B_M phenotype.

Bhuiyan and colleagues[281] measured mucosal immune responses to Ty21a membrane antigen including IgA antibody in stool extracts and antibody in lymphocyte secretions following the ingestion by 2- to 5-year-old Bangladeshi children of three doses of a liquid formulation of Ty21a.

Cell-Mediated Immune Responses

Cell-mediated immune responses have been measured after vaccination with Ty21a.[254–256,302–311,311a] Oral Ty21a vaccine stimulates both $CD4^+$ T-helper cell and $CD8^+$ cytotoxic T-cell responses, both of which are believed to play roles in defense against *S.* Typhi. The cell-mediated immune responses that follow oral immunization of subjects with Ty21a include lymphoproliferative responses; secretion of T-helper type-1 cytokines (e.g., interferon-γ [IFN-γ]); secretion of tumor necrosis factor-α; appearance of classical class Ia–restricted $CD8^+$ cytotoxic T lymphocytes (CTLs),[305,312] and novel, nonclassical class Ib human leukocyte antigen-E–restricted $CD8^+$ CTLs[307,312] and antibody-dependent mononuclear cell killing of *S.* Typhi.[254–256,302] Newer live oral vaccines stimulate fairly potent cell-mediated immune responses.[312–317]

In their studies of the lymphocyte replication response to various *S.* Typhi antigens after oral immunization with Ty21a, Murphy and coworkers[303,304] observed that the most sensitive and specific antigen was heat-inactivated, phenol-preserved particulate *S.* Typhi. Sztein and coworkers[313,314,318,319] found purified flagella from *S.* Typhi to be an excellent stimulator of immune lymphocytes.

Tagliabue and coworkers[254–256,302] described a potent anti-*Salmonella* immune response after oral immunization with Ty21a that involves PBMCs and immune serum. Mixing PBMCs from a neutral donor with postimmunization sera from vaccinees results in marked inhibition of growth of *S.* Typhi. Neither mononuclear cells by themselves nor postvaccination serum alone had this effect. These investigators reported that the serum antibodies conferring specificity are of the IgA class and that intestinal sIgA could substitute for serum IgA.

Salerno-Goncalves and colleagues[305,307] observed that immunization with Ty21a induced specific CTLs that could lyse *S.* Typhi–infected cells and secrete IFN-γ, an important effector molecule against intracellular pathogens. Most Ty21a vaccinees exhibited consistently increased CD8-mediated lysis of target cells by postimmunization PBMCs compared with preimmunization levels. Using an IFN-γ enzyme-linked immunospot assay that they developed to quantify the frequency of IFN-γ spot-forming cells in PBMCs from Ty21a vaccinees, Salerno-Goncalves and coworkers[305] also detected significant increases in IFN-γ spot-forming cells after immunization compared with preimmunization levels. IFN-γ was secreted predominantly by $CD8^+$ T cells. A very strong correlation was recorded between the cytolytic activity of CTL lines and the frequency of IFN-γ spot-forming cell.[305]

Lundin and colleagues[320] corroborated that oral vaccination with three doses of Ty21a induces both $CD4^+$ and $CD8^+$ IFN-γ–producing cells and antigen-specific $CD4^+$ and $CD8^+$ memory T cells. Moreover, they studied the homing characteristics of T-cell responses and found that almost all the IFN-γ producing memory T cells express the gut-homing integrin β_7.

Based on their expression of homing molecules (CD62L, CCR7), CD45RO or CD45RA, two main populations of memory T cells are recognized: T central memory and T effector memory (T_{EM}).[321,322] Although T_{EM} populations appear ready for immediate effector action, T central memory cells are important in the generation of a new wave of effector T cells. Flow cytometric analysis has shown that T-cell clones derived from Ty21a vaccinees exhibit a T_{EM} phenotype—that is, CCR7(−), CD27(−), CD45RO(+), CD62L(−)—and coexpress gut-homing molecules (i.e., high levels of integrin $\alpha_4\beta_7$, intermediate levels of CCR9, and low levels of CD103).[307] The long-term T-cell responses to Ty21a are oligoclonal and involve multiple T-cell receptor Vbeta families. The *S.* Typhi–specific $CD8^+$ T cells bearing defined Vbeta specificities that appear after oral immunization with Ty21a are phenotypically and functionally consistent with T_{EM} cells with gut-homing potential.[307] Some of these key findings were confirmed by other investigators.[290,320] Two important recent observations

following immunization with Ty21a that help explain the longevity of protection are the appearance of $CD8^+$ T cells that produce interleukin (IL)-17A and the identification of multifunctional $CD8^+$ T_{memory} subsets that simultaneously produce combinations of IL-10, IL-17A, IL-2, IFN-γ, tumor necrosis factor-α, and macrophage inflammatory protein-1β.[310] These responses are likely to be important in resistance to *S.* Typhi by several mechanisms, including increased macrophage bactericidal activity, enhancement of antigen presentation, and lysis of *S.* Typhi–infected targets. Notably, no correlations were observed between cell-mediated immune and serum antibody responses in multiple studies.[313,314,323]

Purified Vi Polysaccharide–Protein Conjugate Parenteral Vaccines

There have been no reports monitoring B_M cells or T-cell responses in recipients of Vi conjugate vaccines.

Serum Antibody Response

Purified Vi Polysaccharide–Protein Conjugate Parenteral Vaccines. There remain impediments to the interpretation of serum anti-Vi antibody responses following immunization with Vi conjugate vaccines and in comparing the relative immunogenicity of one conjugate versus another. The following factors create the dilemma: (1) there does not exist a standardized ELISA method that all Vi conjugate vaccine developers, evaluators, and regulators agree should be the common accepted method for measuring antibodies; (2) only recently has a proposed human serum standard become available[324]; key stakeholders have not yet agreed upon a threshold titer that signifies protection at the time the specimen was collected or on a postimmunization titer that predicts long-term protection, with both being measured using an agreed upon ELISA method and with an accepted serum standard.[325] Because of this, while intrastudy interpretations of serological data from clinical trials with Vi conjugates are possible (e.g., titers achieved by the Vi-protein conjugate vaccine vs an unconjugated Vi polysaccharide vaccine within the same study[153,242,326]), comparisons of different conjugates cannot readily be made because so far two different conjugates have not been used in the same randomized study for direct comparison. These points need to be taken into consideration as one reviews the serological data from immunogenicity trials of several Vi conjugate vaccine, which trials are summarized in Table 61.7.

Typbar-TCV. Table 61.7 summarizes the immunogenicity of Typbar-TCV in stimulating serum Vi antibodies in individuals of different age groups.[153] In participants 2 years of age or older, unconjugated Vi served as the comparator. The Vi antibody response following a dose of Typbar-TCV was significantly stronger than following a dose of unconjugated Vi polysaccharide (Typbar) vaccine. There were also important differences in the quality of the antibody elicited. Typbar-TCV stimulated antibodies of higher avidity than the antibodies induced by unconjugated Vi vaccine.[153] The conjugate also primed the vaccinees to mount strong booster responses when a second dose of Vi conjugate was administered 720 days after the initial dose. The response to the booster included components of all IgG subclasses with significantly enhanced IgG_4 and IgG_3 compared to the antibodies elicited by a second dose of unconjugated Vi administered 720 days after an initial dose of unconjugated Vi vaccine.[153]

Vi-CRM_{197}. Table 61.7 shows the immune responses in adults, children, older infants, and younger infants with the Vi-CRM_{197} conjugate that is in clinical development by BioE (Hyderabad, India).[242,261,327] Shown are the results of clinical trials with early good manufacturing practices pilot lots undertaken by the developer, the Sclavo Behring Vaccines Institute of Global Health. Young infants (i.e., immunized within the first 6 months of life) mounted modest anti-Vi responses to this conjugate despite three spaced doses and it was not obvious that the subsequent two doses boosted antibody titers elicited by the first dose. All the Vi conjugates summarized in Tables 61.2 and 61.7 contain 25 μg of Vi polysaccharide as the saccharide component except for the PedaTyph and Vi-CRM_{197} conjugates that contain only 5 μg. This formulation was selected based on results of a Phase I trial in European adults that examined serum anti-Vi responses following administration of three concentrations of Vi-CRM_{197} containing 1.25 μg, 5.0 μg, or 12.5 μg of Vi antigen.

PedaTyph. At the time of its licensure by the Drug Controller General of India, there were no peer review publications that documented the clinical safety profile and immunogenicity of PedaTyph,[204] the first licensed (in India) Vi conjugate vaccine. Only limited serologic results were found on the manufacturer's website.[328] That a new vaccine can become licensed for use in infants on the basis of very limited safety and immunogenicity data, and without efficacy data, is a function of the differences in countries' criteria for licensure and use of the vaccine in that country.[203,204,327,329] In India, the initial paucity of published or otherwise public prelicensure data on the safety, immunogenicity, and efficacy of this vaccine generated active controversy.[203,204,329,330]

Subsequently, two postlicensure publications appeared that summarized the safety and immunogenicity results following immunization of Indian children younger than 5 years of age with PedaTyph.[205,206] Additional larger postlicensure trials intended to generate additional clinical data are underway with this Vi conjugate.

Vi-rEPA. Extensive serologic data demonstrate the serum Vi antibody responses to prelicensure lots of a still unlicensed Vi conjugate that uses recombinant exoprotein A of *Pseudomonas aeruginosa* as the carrier protein (see Table 61.7).[125,326,331,332] Table 61.7 summarizes the anti-Vi antibody responses in a subset of Vietnamese preschool-age children who participated in the efficacy trial of this vaccine. The GMT at 24 months after the two-dose regimen remained approximately 97-fold above baseline. Young infants given doses of Vi-rEPA at 2, 4, and 6 months of age and then boosted at 12 months of age exhibited prominent rises in anti-Vi at 13 months of age, 1 month after the booster.

Comparison With Natural Infection

The circulating, secretory, and cell-mediated immune responses are relatively strong after natural infection and include prominent serum and cell-mediated components.[56,79,333–339] Parenteral killed whole-cell vaccines elicit a serum response equal to that of natural infection but not a comparable cell-mediated response. With live oral vaccines, the opposite is true.

Murphy and associates[303,304] observed that the PBMCs from healthy adults living in typhoid-endemic areas who have no known history of acute typhoid fever often specifically proliferate when exposed to *S.* Typhi antigens. This corroborates results of antibody prevalence studies that indicate that considerable mild or subclinical infection occurs in such areas.

High titers of serum IgG antibodies directed at the capsular Vi polysaccharide antigen that covers *S.* Typhi are found in approximately 80% of chronic biliary carriers of *S.* Typhi[89,91] in endemic areas, but only rarely in healthy

TABLE 61.7 Immunogenicity of Vi Conjugate Vaccines Licensed or in Clinical Development

Product			Age Group								
			24 mo–45 y			24 mo–4 y			5–15 y		
	Multisite Study in India	Timing of Serum Collection	Typbar-TCV[b]	Typbar[b]	*P* Value	Typbar-TCV[b]	Typbar[b]	*P* Value	Typbar-TCV[b]	Typbar[b]	*P* Value
Typbar-TCV (Bharat Biotech International, Hyderabad, India)[153]	GMT, EU/mL[a] (95% CI) [N]	Day 0	10.4 (9.6–11.3) [332]	11.6 (10.5–12.9) [305]	>.05	8.8 (8.0–9.6) [100]	10.0 (8.5–11.7) [90]	>.05	10.2 (9.1–11.3) [146]	11.1 (9.5–12.9) [146]	>.05
		Day 42	1293 (1153–1449) [332]	411 (359–471) [305]	.001	1334 1081–1648) [100]	454 356–578) [90]	.001	1701 (1473–1965) [146]	409 (334–499) [126]	.001
		Day 720	81.7 (73–92) [243]	45.8 (40–53) [197]	.001	64.1 (58–99) [56]	31.7 (23–44) [39]	.001	84.4 (71–101) [114]	47.6 (39–58) [87]	.001
		Day 762 (42 days after a booster)	1685 (1468–1797) [175]	446 (323–615 [57]	–	–	–		–	–	–
	Seroconversion, ≥4-fold rise over day 0 baseline (95% CI) [N]	Day 42	97.3% (94.8–98.6) [332]	93.1% (89.6–95.5) [305]	.01	99.0% (94.0–99.9) [100]	94.4% (87.3–97.9) [90]	.08	99.3% (95.8–99.9) [146]	95.2% (89.8–98.0) [126]	.039
		Day 720	74.1% (68.2–79.2) [243]	53.3% (46.3–60.1) [197]	.001	76.8% (64.1–86.0) [100]	48.7% 33.9–63.8) [39]	.001	74.6% (65.8–81.7) [114]	56.3% (45.8–66.2) [87]	.001
	Fold-rise over baseline GMT	Day 42	124	35	.001	152	46	.001	168	37	.001
		Day 720	7.8	3.8	.001	7.5	3.0	.001	8.8	4.6	.001
			6–23 mo			**6–11 mo**			**12–23 mo**		
	GMT, EU/mL[a] (95% CI) [N]	Day 0	9.5 (8.7–10.3) [307]			9.6 (8.4–10.9) [139]			9.4 (8.3–10.5) [168]		
		Day 42	1937 (1785–2103) [307]			1851 (1628–2104) [139]			2012 (1811–2236) [168]		
		Day 720	48.3 (42–55) [220]			45.3 (37–55) [105]			50.9 (42–61) [115]		
		Day 762 (42 days after a booster)	1722 (1503–1972) [187]			–			–		
	Seroconversion, ≥4-fold rise over day 0 baseline (95% CI) [N]	Day 42	98.1% (95.7–99.2) [307]			97.8% (93.6–99.5) [139]			98.2% (94.6–99.6) [115]		
		Day 720	59.5% (52.9–99.2) [220]			59.0% (49.5–67.9) [105]			60.0% (50.8–68.5) [115]		

	Multisite Study in India	**Timing of Serum Collection**	**3 mo–5 y**	**≤2 y**	**2–5 y**
PedaTyph (BioMed, Ghaziabad, India)[205,206]	GMT, µg/mL[c] (95% CI) [N]	Day 0 Day 56 Day 890	0.22 (0.20–0.25) [163] 2.08 (1.76–2.48) [163] 14 (4.8–29.8) [20][g]	0.24 (0.20–0.27) [101] 2.72 (2.22–3.31) [101] –	0.21 (0.18–0.24) [62] 1.35 (1.02–1.79) [62] –
	Seroconversion, ≥4-fold rise over day 0 baseline (95% CI) [N]	Day 56	83%	89%	73%
	Fold-rise over baseline GMT	Day 56	9-Fold	11-Fold	6-Fold
	Vietnam			**2–5 y**	**Young Infants (Three Primary Doses Given at 2, 4, & 6 mo and a Booster at 12 mo)**
Vi-rEPA[d] (Lanzhou Institute of Biological Products, Lanzhou, China)[125,126,332]	GMT, EU/mL[c] (25th–75th percentile), [N]	Cord blood		–	0.66 (0.30–1.46) [100]
		Day 0		0.11 (0.06–0.18) [36]	–
		Day 28 after 2nd dose (~ day 70)		72.9 (50.7–124) [36]	–
		6 mo after 2nd dose		22.5 (13.8–47.3) [~48]	–
		12 mo after 2nd dose		18.7 (10.3–32.6) [~48]	–
		24 mo after 2nd dose		10.7, (6.4–24.8) [~48]	–
		Day 28 after 3rd dose (age 7 mo)			17.42, (7.33–47.25) [90]
		6 mo after 3rd dose (age 12 mo)			4.76 (2.20–12.71), [85]
		Day 28 after 4th dose (age 13 mo)			50.07 (22.33–133.6), [80]

Continued on following page

TABLE 61.7 Immunogenicity of Vi Conjugate Vaccines Licensed or in Clinical Development *(Continued)*

Product			Age Group			
				24–59 mo[e]		
	Pakistan Site		**9–12 mo[e], Vi-CRM$_{197}$**	**Vi-CRM$_{197}$**	**Vi**	**6–8 wk[f], Vi-CRM$_{197}$**
Vi-CRM$_{197}$ (BioE, Hyderabad, India)[242]	GMT, EU/ml[c] (95% CI) [N]	Day 0	1.65 (1.27–2.14) [20]	1.65 (1.27–2.14) [20]	2.23 (1.72–2.9) [20]	2.77 (2.1–3.66) [20]
		Day 28	201 (138–294) [20]	201 (138–294) [20]	93 (64–135) [20]	–
		Day 56	–	–	–	28 (20–38) [20]
	Seroconversion, ≥4-fold rise over day 0 baseline [n/N] % (95% CI)	Day 28 after 1st dose	20/20 100% (83–100)	20/20 100% (83–100)	18/20 90% (68–99)	–
	Seroconversion, ≥4-fold rise over day 0 baseline, n/N % (95% CI)	Day 28 after 2nd dose	19/20 95% (75–100)	19/19 100% (82–100%)	17/19 89% (67–99)	16/20 80% (56–94)
		Day 28 after 3rd dose	–	–	–	17/20 85% (62–97)
		6 mo after 2nd dose	16/18 89% (65–99)	19/20 95% (75–100)	13/19 68% (43–87)	–
		6 mo after 3rd dose	–	–	–	5/20 25% (9–49)
	Philippines		**9–12 mo[e]**	**24–59 mo[e]**		**6–8 wk[f]**
	GMT, EU/mL[c] (95% CI) [N]	Day 0	2.33 (1.64–3.32) [20]	3.62 (2.6–5.04) [20]	2.66 (1.91–3.71) [20]	2.88 (1.95–4.25) [20]
		Day 28	249 (130–477) [20]	368 (234–580) [84]	193 (123–304) [19]	–
		Day 84	–			103 (67–159) [20]
	Seroconversion ≥4-fold rise over day 0 baseline, n/N % (95% CI)	Day 28 after 1st dose	20/20 100% (83–100)	20/20 100% (83–100)	20/20 100% (83–100)	–
		Day 28 after 2nd dose	20/20 100% (83–100)	20/20 100% (83–100)	19/19 100% (82–100)	20/20 100% (83–100)
		Day 28 after 3rd dose	–	–	–	20/20 100% (83–100)
		6 mo after 2nd dose	17/20 85% (46–88)	18/20 90% (68–99)	18/19 95% (74–100)	–
		6 mo after 3rd dose	–	–	–	14/20 70% (46–88)

[a]Vi antibodies measured by both a commercial ELISA (Vacczyme, *S.* Typhi Vi IgG ELISA kit, Binding Site Ltd., Birmingham, UK) and by an in-house ELISA.
[b]A single dose was administered.
[c]Vi antibodies measured by an in-house ELISA.
[d]Two doses, 6 weeks apart.
[e]Two doses, 8 weeks apart.
[f]Three doses, 4 weeks apart.
[g]It is not clear if this sample of participants was selected randomly or a convenience sample.
Note: Typbar refers to the unconjugated purified polysaccharide vaccine of Bharat Biotech International and Typbar-TCV refers to their Vi conjugate vaccine.
CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; EU/mL, enzyme-linked immunosorbent assay unit/milliliter; GMT, geometric mean titer; Ig, immunoglobulin; N, number.

persons[89,91,259]; 12% to 38% of persons with acute typhoid fever manifest elevated Vi titers.[89,91] Therefore, the measurement of Vi antibodies has been applied with success as a screening test to detect chronic carriers in nonendemic and endemic areas.[89–91,340–342] In endemic areas, a variable proportion of healthy subjects exhibit low titers of Vi antibody, and the prevalence increases with age.[122,343] The low titers of serum Vi antibody consequent to wild-type *S*. Typhi infection among persons living in endemic areas are mainly in the IgG_2 subclass.[343]

Using decomplemented human sera from cord bloods and persons of different age groups living in a typhoid hyperendemic area (Kathmandu, Nepal) versus in a nonendemic area (United Kingdom), Pulickal and coworkers[343] described a serum bactericidal assay that uses infant rabbit complement. Bactericidal antibodies were low in all UK age groups. Among the Nepali sera, GMTs of bactericidal antibody were high in cord blood, dropped drastically during infancy and childhood, and reached "adult" levels by the time the children were 9 to 15 years old. A correlation between bactericidal titers and anti-Vi in the Nepali sera was not observed.

EFFICACY AND EFFECTIVENESS OF TYPHOID VACCINES

Results of Controlled Field Trials

Parenteral Inactivated Whole-Cell Vaccines

Although the killed whole-cell vaccines are no longer available, a summary of their efficacy in controlled field trials provides helpful context when considering the performance of the main licensed vaccines that have been their successors (Vi polysaccharide and Ty21a). From the mid-1950s to the early 1970s, the World Health Organization (WHO) sponsored a series of well-designed, randomized, controlled field trials in countries with endemic typhoid to assess the absolute and relative efficacy of various typhoid vaccines and their duration of protection. In these studies, only culture-confirmed cases were used in calculating incidence rates and vaccine efficacy. In the first trial, in Yugoslavia, the efficacy of two doses of alcohol-killed vaccine was compared with that of heat-inactivated, phenol-preserved vaccine, with TT serving as the control vaccine.[344–346] Both vaccines gave significant protection, but the heat-inactivated, phenol-preserved vaccine proved to be superior to the alcohol-killed vaccine.

In the early 1950s, laboratory studies demonstrated that acetone-inactivated typhoid vaccine resulted in better preservation of the Vi antigen,[166] thus raising the question of whether such a vaccine might be superior to the heat-inactivated, phenol-preserved vaccine. The Walter Reed Army Institute of Research prepared for the WHO large reference lots of acetone-inactivated and heat-inactivated, phenol-preserved vaccines,[167,168] designated vaccines K and L, respectively, for large-scale field trials to be carried out in Yugoslavia,[347] Guyana,[348,349] Poland,[185,350] and the Soviet Union.[165] The K and L vaccines and the methods for their production also served as international standards to prepare future lots of vaccines of these types for subsequent field trials. For example, in addition to the K and L reference vaccines themselves, two additional K-type vaccines were tested in controlled field trials and provided critical information. These include a K-type vaccine used in a randomized, controlled field trial in Tonga to directly compare the efficacy of one versus two doses of vaccine[351]; a K-type vaccine made from a nonflagellated *S*. Typhi strain, which was field tested for efficacy in Alexandria, Egypt[352]; and a K-type vaccine evaluated in a single dose against an alum-adsorbed, heat-inactivated, phenol-preserved vaccine.[353,354]

The major conclusions that can be drawn from these field trials are the following:

- Both acetone-inactivated and heat-inactivated, phenol-preserved typhoid vaccines provided significant protection against typhoid fever after two subcutaneous doses, but the acetone-killed vaccine was somewhat better (79% to 88% protection for the K vaccine vs 51% to 66% for the L vaccine).[165,185,347,349]
- The efficacy of the reference K and L vaccines varied from one geographic site to another.
- When directly compared in a randomized trial (Tonga), two doses of an acetone-inactivated vaccine gave significantly better protection than a single dose.[351] Previously in nonrandomized comparisons, little difference had been noted between the efficacy conferred by one or two doses.[349]
- A K-type vaccine prepared from a nonflagellated *S*. Typhi strain failed to provide significant protection.[352]
- A single dose of a K-type vaccine, without adjuvant, provided protection comparable to that of a single dose of an alum-adsorbed, heat-inactivated, phenol-preserved vaccine.[353,354]

The K and L vaccines were also evaluated for efficacy in experimental challenge studies in North American volunteers.[56] Perhaps the most important insight to come from these studies was the observation that protection conferred by the vaccines was relative to the number of pathogenic *S*. Typhi administered in the experimental challenge. When the challenge inoculum was 10^5 pathogenic *S*. Typhi, the K and L vaccines provided approximately 70% protection. However, when the challenge inoculum contained 10^7 bacilli, there was virtually no protection demonstrable (0% to 14% vaccine efficacy). In nature, differences in inoculum size may be responsible for some of the differences of vaccine efficacy encountered among different field sites and over time.

Purified Vi Polysaccharide Parenteral Vaccine

Two pioneering randomized, controlled prelicensure field trials to assess the efficacy of the first nondenatured purified Vi vaccine were carried out in Nepal[122] and South Africa.[123] A single 25-μg dose conferred 72% protection against typhoid fever in Nepal during 17 months of follow-up, and 64% efficacy in South Africa over 21 months of surveillance. The Nepal trial included all ages from preschool to adults, whereas the South African trial was performed in schoolchildren (Table 61.8). A subsequent report from the South African trial showed that over 3 years of follow-up, vaccine efficacy was 55%.[274]

Subsequently, a Vi vaccine manufactured by the Shanghai Institute of Biological Products was evaluated in a randomized, placebo-controlled, double-blind trial in Guangxi, China.[215] In total, 65,287 subjects received a single 30-μg dose of Vi and 65,984 controls got a dose of saline; 92% of subjects were children 5 to 19 years of age at the time of vaccination.[215] During 19 months of follow-up, seven cases of blood culture–confirmed typhoid fever were detected among the vaccinees, versus 23 confirmed cases among the controls, demonstrating a vaccine efficacy of 69% (95% confidence interval [CI], 28% to 87%) (see Table 61.8).[215] Confirmatory data on the effectiveness of this vaccine from its use in relation to an outbreak of typhoid have been reported.[216]

Vi-negative strains of *S*. Typhi are rare in nature but such variants are capable of causing clinical typhoid fever.[56,355] Some have argued that because the Vi vaccine is unlikely to protect against such strains, the widespread use of Vi may select for such strains.[356–358] This hypothesis has biological plausibility because pathogenicity island 7 in the *S*. Typhi

TABLE 61.8 Randomized, Controlled, Double-Blind Field Trials[a] Assessing the Efficacy of a Single Dose of Nondenatured Purified Vi Polysaccharide Subunit Vaccine in Preventing Culture-Confirmed Typhoid Fever

	Period of Follow-Up	Vi Vaccine	Control Vaccine
Nepal trial[b]	17 mo		
No. of subjects		3457	3450
Cases		9	32
Incidence/10^5		260	928
Efficacy		72%	–
(95% CI)		(42–86%)	–
South Africa trial[c]	21 mo		
No. of subjects		5692	5692
Cases		16	44
Incidence 10^5		281	773
Efficacy		64%	–
(95% CI)		(36–79%)	–
	36 mo		
Cases		30	66
Incidence/10^5		527	1160
Efficacy		55%	–
China trial[d]	19 mo		
No. of subjects		65,287	65,984
Cases		7	23
Incidence/10^5		10.7	34.9
Efficacy		69%	–
(95% CI)		(28–87%)	–

[a]Two trials (in Nepal and South Africa) evaluated 25-μg doses of a Vi vaccine, and one trial (in China) evaluated a 30-μg dose of another Vi vaccine.
[b]Participants were randomized to receive a 0.5-mL intramuscular inoculation containing either 25-μg of purified Vi or 23-valent pneumococcal polysaccharide. (Data from Acharya VL, Shrestha MB, Cadoz M, et al. Prevention of typhoid fever in Nepal with the Vi polysaccharide of *Salmonella* Typhi: a preliminary report. *N Engl J Med*. 1987;317:1101–1104.)
[c]Participants were randomized to receive a single 25-μg intramuscular dose of Vi or a 50-μg dose of meningococcal polysaccharide vaccine. (Data from Klugman K, Gilbertson IT, Koornhof HJ, et al. Protective activity of Vi polysaccharide vaccine against typhoid fever. *Lancet*. 1987;2:1165–1169; and Klugman KP, Koornhof HJ, Robbins JB, et al. Immunogenicity, efficacy and serological correlate of protection of *Salmonella* Typhi Vi capsular polysaccharide vaccine three years after immunization. *Vaccine*. 1996;14:435–438.)
[d]Participants were randomized to receive a single 0.5-mL intramuscular dose containing either 30 μg of Vi or saline. (Data from Yang HH, Wu CG, Xie GZ, et al. Efficacy trial of Vi polysaccharide vaccine against typhoid fever in south-western China. *Bull World Health Organ*. 2001;79:625–631.)
CI, confidence interval.

TABLE 61.9 Field Trial of Efficacy of Three Doses of a Liquid Formulation of Ty21a Vaccine Given With $NaHCO_3$ to 6- and 7-Year-Old Schoolchildren in Alexandria, Egypt

Group[a]	Subjects (N)	Confirmed Cases of Typhoid Fever	Incidence per 105	Vaccine Efficacy (%)
Vaccinees	16,486	1	6.1	96 (77–99)[b]
Placebo	15,902	22	138.3	–

[a]Period of observation, 1978–81.
[b]95% Confidence interval.
Modified from Wahdan MH, Serie C, Cerisier Y, et al. A controlled field trial of live Salmonella *Typhi strain Ty21a oral vaccine against typhoid: three-year results.* J Infect Dis. *1982;145;292–296.*

chromosome, which contains the genes encoding the expression of Vi, is a relatively unstable region.[47] Heretofore, there has been no convincing evidence of the emergence of Vi-negative isolates of *S.* Typhi. However, arguably, there has not been sufficiently widespread and sustained use of Vi in an area of high endemicity to have created the selective pressure for emergence of Vi-negative strains that are known to exist in some populations.[355]

Ty21a Live Oral Vaccine

In early studies in adult volunteers, multiple doses of freshly harvested Ty21a organisms were found to be safe and to confer significant protection against experimental challenge.[214] Thereafter, a field trial of efficacy was carried out in Alexandria, Egypt, where approximately 16,000 schoolchildren 6 and 7 years of age were given three 10^9-organism doses of vaccine on Monday, Wednesday, and Friday of one week.[245,359] Lyophilized vaccine in glass vials was reconstituted with 30 mL of diluent and ingested by the children approximately 1 to 3 minutes after they chewed a tablet containing 1.0 g of $NaHCO_3$. Approximately 16,000 other children received placebo in this randomized, double-blind, controlled field trial. During 3 years of epidemiologic surveillance, only one culture-confirmed case of typhoid fever occurred among the vaccinees, as opposed to 22 cases among the controls (96% protection) (Table 61.9).

Although this preliminary field trial with Ty21a provided highly encouraging results, considerable further work needed to be done to ascertain whether Ty21a could be a practical public health tool. The liquid formulation used in Egypt was not readily amenable to mass production, so alternative formulations had to be prepared and evaluated. It was necessary to determine whether fewer (one or two) doses could protect, to assess the duration of protection, to investigate the effect of increased spacing between vaccine doses, and to ascertain whether infants and young children could be immunized safely and successfully. Many of these questions were answered in a series of five randomized, controlled field trials carried out in Santiago, Chile (four trials), and Indonesia (one trial) under the auspices of the WHO and the Pan American Health Organization.[124,147–150,218,230]

The four field trials in Chile involved approximately 532,000 children ages 6 to 19 years who were vaccinated in school-based programs, after which epidemiologic surveillance was maintained through the health centers of the National Health Service. Only culture-confirmed cases were used in the computation of incidence rates and vaccine efficacy.

In the field trial in the Western (Occidente) administrative area of Santiago,[218] the efficacy conferred by three doses of vaccine in enteric-coated capsules was compared with that provided by three doses of vaccine in the gelatin capsule–$NaHCO_3$ formulation. As seen in Table 61.10, over 3 years of surveillance the enteric-coated vaccine provided significantly superior protection. Over 7 years of follow-up, the regimen of three doses of Ty21a in enteric-coated capsules (every-other-day dosing) conferred 62% protection.[147]

TABLE 61.10 Comparison* of the Efficacy of Two Different Formulations of Ty21a Live Oral Vaccine Given By Two Different Immunization Schedules in Area Occidente, Santiago, Chile

		Enteric-Coated Capsules		Gelatin Capsules With $NaHCO_3$	
	Placebo	Long Interval†	Short Interval‡	Long Interval	Short Interval
Study population	21,906	21,598	22,170	21,541	22,379
No. of cases	68	34	23	46	56
Incidence§	310.4[e]	157.4[a]	103.7[b]	213.5[c]	250.3[d]
Efficacy	–	49%	67%	31%	19%
95% CI		(24–66%)	(47–79%)	(0–52%)	(0–43%)

*Results of 36 months of follow-up (September 1983 to August 1986).
†Three doses, 21 days between doses.
‡Three doses, 1 to 2 days between doses.
§Significance: a vs e, $P = .0006$; b vs e, $P < .00001$; c vs e, $P = .0023$; d vs e, $P = .21$; a vs c, $P = .23$; b vs d, $P = .00052$; a + b vs c + d; $P = .001$.

TABLE 61.11 Comparison of the Efficacy of One Versus Two Doses of Ty21a Live Oral Typhoid Vaccine Given in Enteric-Coated Capsule Formulation From a Large-Scale, Randomized, Placebo-Controlled, Double-Blind Field Trial in Area Norte, Santiago, Chile

	One Dose ($N = 27,618$)	Two Doses* ($N = 27,620$)	Placebo ($N = 27,305$)
YEAR 1 (JULY 1982–JUNE 1983)			
No. of cases	47	30	62
Incidence/10^5†	170.2[a]	108.6[b]	227.1[c]
Efficacy	25%	52%	–
YEAR 2 (JULY 1983–JUNE 1984)			
No. of cases	25	11	38
Incidence/10^5†	90.5[d]	39.8[e]	139.2[f]
Efficacy	35%	71%	–
YEAR 3 (JULY 1984–JUNE 1985)			
No. of cases	19	15	19
Incidence/10^5	68.8	54.3	69.6
Efficacy	0%	22%	–
YEAR 4 (JULY 1985–JUNE 1986)			
No. of cases	30	23	28
Incidence/10^5	108.6	83.3	102.5
Efficacy	–6%	19%	–

*The two doses were administered 1 week apart.
†Significance: a vs c, $P = .15$; b vs c, $P < .001$; d vs f, $P = .10$; e vs f, $P < .001$.
Note that the vaccine efficacy of the two-dose regimen over the first 2 years of follow-up was 71% (95% confidence interval [CI], 41% to 71%; $P < .001$) and the efficacy with the one-dose regimen was 29% (95% CI, 4% to 47%; $p = .03$).
Data from Black RE, Levine MM, Ferreccio C, et al. Efficacy of one or two doses of Ty21a Salmonella *Typhi vaccine in enteric-coated capsules in a controlled field trial.* Vaccine. *1990;8:81–84.*

TABLE 61.12 Comparison* of the Efficacy of Two, Three, and Four Doses of Ty21a Vaccine in Enteric-Coated Formulation

Surveillance From November 1984 to October 1987	Two Doses	Three Doses	Four Doses
No. of vaccinees	66,615	64,783	58,421
No. of cases	123	104	56
Incidence/10^5†	184.6[a]	160.5[b]	95.8[c]
95% Confidence interval	152–271	130–191	71–121

*Results of a randomized field trial in Area Sur and Area Central, Santiago, Chile. Doses of vaccine were administered on an every-other-day schedule.
†Significance: a vs c, $P = .0004$; b vs c, $P = .002$; a vs b, $P = .32$.
Data from Ferreccio C, Levine MM, Rodriguez H, et al. Comparative efficacy of two, three, or four doses of Ty21a live oral typhoid vaccine in enteric-coated capsules: a field trial in an endemic area. J Infect Dis. *1989;159:766–769.*

A randomized, controlled, double-blind field trial in the Northern (Norte) area of Santiago showed that immunization with only one or two doses (given 1 week apart) of Ty21a in enteric-coated capsules resulted in a moderate level of protection that was short lived (Table 61.11).[124] Although two doses of vaccine conferred 60% protection during the first 2 years of surveillance, efficacy dropped to insignificant levels during the third year and virtually disappeared by the fourth year of surveillance.

A very large trial involving more than 200,000 schoolchildren was carried out in the Southern (Sur) and Central administrative areas of Santiago to compare directly the protective effects of two, three, or four doses of Ty21a vaccine in enteric-coated capsules and to assess the use of Ty21a as a public health tool.[150] No placebo group was included in this trial. The salient feature of this trial is the observation that ingestion of four doses of vaccine (administered on an every-other-day schedule) resulted in a significantly lower incidence of typhoid than three doses (Table 61.12). The results of this trial formed the basis for the recommended four-dose immunization schedule following licensure of Ty21a in the United States and Canada (a three-dose regimen is used elsewhere).

In the mid-1980s, the Swiss Serum and Vaccine Institute succeeded in preparing a liquid suspension formulation of Ty21a for large-scale field trials that was amenable to large-scale manufacture. The new formulation consisted of two packets, one containing a dose of lyophilized vaccine and the other containing buffer. Contents of the two packets were mixed in a cup containing 100 mL of water, and the suspension was then ingested by the vaccinee. Field trials were initiated in Santiago, Chile,[230] and in Plaju, Indonesia,[121] to directly compare this new liquid formulation of Ty21a (which somewhat resembles what was used in the Alexandria, Egypt, field trial) with the enteric-coated capsule formulation. Table 61.13

TABLE 61.13 Comparison[a] of the Efficacy of Three Doses of Ty21a Administered in Enteric-Coated Capsules or as a Liquid Suspension of Vaccine Organisms

	Santiago, Chile			Plaju, Indonesia		
	Enteric-Coated Capsules	Liquid Suspension	Placebo	Enteric-Coated Capsules	Liquid Suspension	Placebo
No. of subjects	34,696	36,623	10,302	5209	5066	10,268
No. of cases of typhoid	63	23	28	61	48	208
Incidence/10^5	182	63	272	468	379	810
Efficacy	33%	77%	–	42%	53%	–
95% CI	0–57%	60–87%	–	23–57%	36–66%	–

[a]Results of randomized, placebo-controlled field trials in Santiago, Chile, and Plaju, Indonesia.
Data from Levine M, Ferreccio C, Cryz S, et al. Comparison of enteric-coated capsules and liquid formulation of Ty21a typhoid vaccine in a randomized controlled field trial. Lancet. *1990;336:891–894; and Simanjuntak C, Paleologo F, Punjabi N, et al. Oral immunisation against typhoid fever in Indonesia with Ty21a vaccine.* Lancet. *1991;338:1055–1059.*

summarizes the results of these two trials. Vaccine administered as a liquid suspension was superior to vaccine in enteric-coated capsules. In the Santiago trial, the difference was highly significant. Ty21a given as a liquid suspension protected young children as well as older children.[230] In previous trials with enteric-coated vaccine, young children were not as well protected as older children.[218]

Purified Vi Polysaccharide–Tetanus Toxoid Conjugate Parenteral Vaccine

The two Vi conjugate vaccines that have been licensed (in India), Typbar-TCV and PedaTyph, were approved on the basis of clinical data supporting their safety, clinical tolerability, and immunogenicity. There have been no reports of these conjugates having been evaluated for efficacy in randomized, controlled, double-blind field trials. However, in 2016 there was a report of a quasirandomized, open-label trial of Ped-Typh that included 905 Kolkata children age 6 months to 12 years who received two doses of conjugate, 6 weeks apart, and were followed with active surveillance (weekly telephone calls plus monthly school visits) for 1 year, along with 860 unvaccinated control children.[206a] Overall, among the vaccinees, 61.8% were older than 5 years, 29.1% were age 3 to 5 years, and 9.1% were age 6 months to 2 years. The control children had a similar age distribution, as 61.8% were older than 5 years, 29.1% were age 3 to 5 years, and 12.7% were age 6 months to 2 years. Notably, no cases of blood culture–confirmed typhoid fever were recorded among the 905 vaccinees during the 1 year of surveillance versus 11 confirmed cases among the 860 unvaccinated control children, indicating a vaccine efficacy of 100% (95% CI, 97.7–100) ($P < .001$).

Correlates of Protection

Because of the complex nature of the pathogenesis of *S.* Typhi clinical infection, a protective role is probably played by secretory intestinal antibody (in preventing mucosal invasion), circulating antibody (against bacteremic organisms), and cell-mediated immunity (to eliminate intracellular bacilli). With parenteral vaccines, the circulating antibody response is substantial and presumably provides the predominant protective effect. In contrast, with live attenuated oral vaccines the circulating antibody response may be modest but the vigorous secretory intestinal IgA and cell-mediated immune responses evoked are believed to be responsible for the protection conferred by that type of vaccine.

Unfortunately, with *S.* Typhi the critical antigens responsible for protection are not agreed on, and data are somewhat contradictory. With parenteral whole-cell vaccines, elicitation of serum H (flagellar) antibodies in humans correlated with protection observed in field trials of efficacy and in volunteer challenge studies, whereas stimulation of O and Vi antibodies (measured in an era when highly purified, nondenatured Vi was not available for use as antigen) did not.[234,344,348,352] In contrast, with live oral vaccines the mucosal IgA and systemic cell-mediated immune responses appear in large part to be directed toward the O and H antigens, but not to the Vi antigen.[149,198,200,235–239,246,255,256,285–290,302,313,314,318–320,360–362] In fact, one attenuated strain (Ty21a) lacks Vi antigen[209] yet provides significant protection.[121,124,147,150,218,230,359]

Parenteral Inactivated Whole-Cell Vaccine

H antibodies appear to contribute to long-term protection. Killed whole-cell parenteral vaccines that were most protective in field trials elicited the highest levels of H antibody. Serum H antibody titers also correlated with protection against typhoid fever among young adult volunteers who participated in experimental challenge studies that assessed the efficacy of typhoid vaccines.[214,243,244] Control volunteers with elevated H titers (presumably derived by natural infection or vaccination many years earlier during military service) were significantly protected against development of typhoid fever compared with those who had no H antibody. The most convincing evidence that H antibody is important comes from the field trial by Wahdan and colleagues[245] of an acetone-killed and dried vaccine prepared from a strain of *S.* Typhi that lacks H antigen. This vaccine, which did not elicit H antibodies, failed to confer significant protection.

K-type whole-cell vaccine that stimulated higher levels of Vi (as well as H) antibody conferred better protection than L-type whole-cell vaccines that elicited lower titers of Vi (and H) antibodies.[40]

Purified Vi Polysaccharide Parenteral Vaccine

The protection conferred by parenteral Vi polysaccharide vaccine is clearly correlated with the ability to stimulate serum Vi antibodies.[122,123] The relatively short-lived nature (~2 years) of the antibody response[343] aligns with the length of protection conferred by unconjugated Vi polysaccharide.

Ty21a Live Oral Vaccine

Using serum IgG O antibody measured by ELISA in Chilean 15- to 19-year-olds, Levine and associates[149] showed a correlation between seroconversion to various dosage schedules and

formulations and protective efficacy in field trials (see Table 61.5). With the currently licensed enteric-coated capsule formulation, there is a stepwise increase in the proportion of vaccinees who manifest significant rises in serum IgG O antibody depending on whether one, two, or three doses of vaccine are administered within 1 week. Although serum O antibody is not believed to be the operative mechanism of immunity elicited by attenuated strains, it clearly correlates with protection. Because ELISA measurement of serum IgG antibody to *S.* Typhi O antigen is a simple technique, it provides investigators with a practical tool for comparing immunization schedules and formulations and for evaluating new candidate live oral vaccines.

Kantele and coworkers[246,285–288,292,294] showed that gut-derived migrating IgA O ASCs detected in the peripheral blood after oral immunization of adult Finnish volunteers with different formulations and immunization schedules of Ty21a closely correlated with the efficacy results recorded in field trials (see Table 61.6). Thus three doses (taken every other day) of Ty21a in enteric-coated capsules are markedly more immunogenic than one dose, and Ty21a in a liquid suspension is more immunogenic than vaccine in enteric-coated capsules.

Purified Vi Polysaccharide–Tetanus Toxoid Conjugate Parenteral Vaccine

Ample serological data document the immunogenicity of Typbar-TCV Vi conjugate vaccine, which is licensed in India. Although still unlicensed, extensive data demonstrate the serum Vi antibody responses to the extensively tested Vi-rEPA conjugate which has shown efficacy in a randomized controlled field trial.[125,326,331,332] Typbar-TCV has demonstrated robust immunogenicity following the administration of a single dose of vaccine in subjects age 6 months and above. No pre- or postlicensure trials have been undertaken to assess the level of protection conferred by Typbar-TCV. Limited published postlicensure data document the immunogenicity of PedaTyph and one small randomized, open-label trial comparing 905 children who received two doses of this Vi conjugate 6 weeks apart versus 860 unvaccinated children followed for 1 year found 100% efficacy. There were no confirmed cases among the 905 vaccinees versus 11 confirmed typhoid cases among the 860 similarly-aged controls.[206a]

Summary of Correlates of Protection

In summary, the correlate of protection of Vi-based parenteral vaccines (including unconjugated Vi polysaccharide and Vi conjugates) is serum IgG antibody measured by ELISA or by radioimmunoassay. However, there is not yet agreement on the titer that serves as a threshold indicating protection, nor is there yet a specific assay nor a standard reference serum that is accepted by all key stakeholders. Finally, there is ongoing research to try and identify a biological activity (e.g., bactericidal or opsophagocytic activity) of the antibodies that may provide a mechanistic correlate of protection.

For Ty21a live oral vaccine, mucosal IgA response to *S.* Typhi LPS, in particular IgA ASCs that make antibody to LPS, constitutes one correlate of protection. The rate of seroconversion of serum IgG antibodies directed against LPS also correlated with results of field trials of Ty21a. Formulations and regimens that elicited the strongest serum anti-O responses correlated with better efficacy in field trials. Finally, the elicitation of long-lived major histocompatibility complex I–restricted CD8 cytotoxic T-cell responses to targets infected with *S.* Typhi constitutes the most likely immune response that explains the long duration of protection stimulated by that live oral vaccine. However, a more formal quantitative correlation of cell-mediated immunity responses with efficacy has not been established. Regrettably, these modern cell-mediated immunity methods were not available when the large-scale efficacy trials of Ty21a were taking place.

Duration of Vaccine-Derived Immunity

Parenteral Inactivated Whole-Cell Vaccines

The longest periods of surveillance (7 years) for efficacy of the parenteral killed whole-cell vaccines were carried out in the Guyana and Tonga field trials. In Guyana, two doses of the acetone-killed K vaccine conferred a high level of protection (88%) for 7 years.[349] In contrast, in Tonga, two doses of a K-type vaccine provided moderate protection for a period of only 5 years, after which protection was no longer demonstrable.[351] Two doses of the heat-inactivated, phenol-preserved vaccine (L) tested in Guyana showed moderate (77%) protection during the first 3 years, but this level fell to 47% protection during the last 4 years of surveillance. In several other field trials in which the period of surveillance was only 2.5 years, the acetone-inactivated and the heat-inactivated, phenol-preserved vaccines conferred significant protection for at least 30 months.[165,185,347]

Purified Vi Polysaccharide Parenteral Vaccine

In the South African field trial, surveillance was maintained for 3 years. During this 3-year period, the efficacy of the Vi vaccine was 55% (see Table 61.8).[274] Similarly, in a field trial in Southern China, protective efficacy lasted only 2 years. Additional data showing that protection from Vi does not endure beyond 3 years came from an epidemiologic investigation of an outbreak of typhoid fever in French military personnel in Ivory Coast. The outbreak investigation revealed that soldiers immunized with Vi vaccine more than 3 years previously had a twofold higher risk of developing typhoid fever than soldiers immunized less than 3 years earlier.[363]

Ty21a Live Oral Vaccine

In the field trial in Alexandria, Egypt, three doses of a liquid formulation of vaccine conferred a high level of protection (96%) that persisted for 3 years, the point at which surveillance was discontinued.[359] Three doses of vaccine in enteric-coated capsules given at an interval of every-other-day conferred 67% protection over 3 years[218] and 62% protection over 7 years of follow-up in a field trial in Santiago, Chile[147]; fewer (one or two) doses of vaccine (given 1 week apart) in enteric-coated capsules, also in Santiago, conferred significant protection for 2 years but not longer.[124]

Three doses of Ty21a in a liquid formulation (administered at an every-other-day interval) provided 77% protection for 3 years[230] and 79% protection during a fourth and fifth year of follow-up (78% protection over the 5 years of follow-up) in a field trial in Santiago.[147]

Purified Vi Polysaccharide–Tetanus Toxoid Conjugate Parenteral Vaccine

The only data on the duration of efficacy of the Vi-TT conjugates licensed in India is the 1-year assessment of efficacy of Peda-Typh that showed no cases among 905 vaccinees during that short follow-up. Consequently, there are no data on the duration of efficacy conferred by these two licensed Vi conjugate vaccines. A field trial of efficacy of a prototype Vi-rEPA conjugate administered to preschool-age children in Vietnam

provided a point estimate of 89% efficacy over 46 months of follow-up (that included 27 months of active household visit surveillance and 19 months of passive health center-based surveillance).

Comparison of Vaccine-Derived Immunity With Natural Immunity

Several sources of data suggest that the immunity that follows clinical infection with pathogenic *S*. Typhi is relative and can be overcome. Marmion and colleagues[364] and others reported successive outbreaks of typhoid fever in soldiers who, in the space of a few months, experienced two separate bouts of typhoid fever. In the volunteer studies carried out by Hornick and colleagues[56] and DuPont and associates,[179] two relevant observations were reported. The first is that immunity to typhoid fever is relative and can be overcome if a large infecting dose is ingested. Second, the protective effect of a prior clinical typhoid infection was only 33%; that is, five of 22 volunteers (23%) who recovered from an induced *S*. Typhi infection developed typhoid fever when rechallenged with pathogenic organisms, as opposed to 11 of 34 (32%) control volunteers ($P > .05$).

Based on these observations, it can be argued that Vi polysaccharide vaccine, and Ty21a, as well as the no longer available parenteral killed whole-cell vaccines, stimulate rather credible immunity compared with natural immunity elicited by infection with wild-type *S*. Typhi. The same is likely to be true for Vi conjugate vaccines assuming that recently licensed Vi-TT conjugates confer similar levels and duration of protection as Vi-rEPA. Undoubtedly, in endemic areas most persons experience multiple subclinical *S*. Typhi infections, each serving to further boost the state of immunity; persons who develop overt disease represent a minority of all infected persons.

Postexposure Prophylaxis and Therapeutic Vaccination

Postexposure prophylaxis is not practiced with typhoid vaccines because the incubation after ingestion of a large inoculum may be less than 1 week, and the organisms rapidly gain their intracellular niche (within 24 hours). Thus, after exposure, there would be insufficient time after administration of the currently licensed vaccines to mount an effective immune response. There have been anecdotal reports of unsuccessful attempts to immunize chronic gallbladder carriers with the intent to interrupt chronic carriage.

Adverse Events

Parenteral Inactivated Whole-Cell Vaccines

Common. Although the parenteral killed whole-cell vaccines provided moderate to good protection, the high rates of systemic and local adverse reactions that were associated with their use made them unsatisfactory public health tools.[165,347,348,365–367]

Rare. Rarely, more significant reactions were attributed to vaccination with parenteral killed whole-cell typhoid vaccines. These included thrombocytopenic purpura,[368,369] acute renal disease,[370–374] dermatomyositis,[375] appendicitis,[376] erythema nodosum,[377] multiple sclerosis,[378] and a syndrome of high fever, severe malaise, and toxemia,[366,367] sometimes accompanied by coagulopathy, thrombocytopenia, hepatitis, and renal insufficiency.[379] Rarely, sudden death occurred after parenteral inoculation with inactivated whole-cell typhoid vaccine.[379] Among these rare severe adverse events, the syndrome of high fever and toxemia and the occurrence of sudden death have biologic plausibility based on abnormally potent responses of certain persons to *S*. Typhi endotoxin inoculated parenterally via whole-cell vaccine, leading to cytokine release and a cascade of events ultimately resulting in shock and perhaps death.

Purified Vi Polysaccharide Parenteral Vaccine

When Vi vaccine is highly purified, it is well tolerated.[213,227–229,241,272,380] As little as 5% impurity with LPS results in systemic adverse reactions in a proportion of recipients, emphasizing the importance of manufacturing highly purified Vi vaccine.[229] In controlled Phase II trials in U.S. adults, local reactions including pain and tenderness were the most common adverse events.[229,241] Passive surveillance carried out during field trials showed the Vi vaccine to be as well tolerated as the licensed (meningococcal and pneumococcal) polysaccharide vaccines that served as the control preparations in these trials.[122,123]

In one Belgian study, adults were randomly allocated to be inoculated with Typhim Vi or one of three lots of Typherix, and local and systemic reactogenicities were compared.[264] This was not a double-blind study. None of the recipients of either vaccine had high fever (≥39°C); 0% of Typhim Vi and 2% of Typherix recipients reported low-grade fever (<39°C). Local erythema, soreness, and swelling were reported significantly more often in the 100 recipients of Typhim Vi (21%, 33%, and 17%, respectively) than among the 300 subjects who received the lots of Typherix (3%, 8%, and 2%, respectively).

The results of postmarketing surveillance for Vi vaccine from the Vaccine Adverse Event Reporting System (VAERS) maintained by the CDC and the FDA were reported for the period 1994 (time of licensure) through June 2002.[381] A total of 321 adverse event reports were received in relation to administration of Vi vaccine, alone or in conjunction with other vaccines. The overall rate was 4.5 adverse events per 100,000 doses of Vi vaccine distributed, with a rate of serious adverse events of 0.34 per 100,000 doses distributed. Unexpected commonly reported symptoms included dizziness and pruritus.

Ty21a Live Oral Vaccine

Ty21a provides significant protection and appears to be quite safe.[121,149,245,279,280,382] Results of three double-blind, placebo-controlled studies that used active surveillance methods to assess the reactogenicity of Ty21a in adults and children are shown in Table 61.14. Surveillance methods to assess the reactogenicity of Ty21a in adults and children are shown in Table 61.14. The rates of adverse reactions in the vaccine recipients were not significantly higher than those for the placebo group for any symptom or sign. In large-scale field trials with Ty21a, involving approximately 550,000 schoolchildren in Chile and 32,000 schoolchildren in Egypt, and approximately 20,000 subjects ranging in age from 3 years to adulthood in Indonesia, passive surveillance failed to identify vaccine-related adverse reactions.[121,124,218,230,245,383]

The results of postmarketing surveillance for Ty21a vaccine from VAERS were reported from 1990 (immediately after licensure in December 1989) through June 2002.[381] A total of 345 reports of adverse event occurring in persons 18 to 65 years of age were received in relation to administration of Ty21a vaccine, alone or in conjunction with other vaccines. The overall rate was 9.7 adverse events per 100,000 doses of Ty21a vaccine distributed, with a rate of serious adverse events of 0.59 per 100,000 doses distributed. Unexpected commonly

TABLE 61.14 Randomized, Placebo-Controlled, Double-Blind Clinical Trials of Three Doses of Ty21a in Enteric-Coated Capsules or in Buffer Suspension to Assess Reactogenicity of the Vaccine in Adults, School-Age Children, and Preschool-Age Children

	Adults, Chile		6- and 7-Year-Olds, Chile		All Ages, Indonesia			
Adverse Reaction[a]	Enteric-Coated Vaccine (*n* = 385)	Enteric-Coated Placebo (*n* = 367)	Enteric-Coated Vaccine (*n* = 172)	Enteric-Coated Placebo (*n* = 172)	Enteric-Coated Vaccine (*n* = 311)	Enteric-Coated Placebo (*n* = 291)	Liquid Suspension Vaccine (*n* = 333)	Liquid Suspension Placebo (*n* = 255)
Diarrhea	1.8[b]	1.1	1.2	9.9	3.9	3.1	3.8	5.5
Vomiting	0.5	0.3	2.3	11.0	1.0	1.7	1.5	0.8
Fever	0.3	0.5	0.6	0.6	4.8	1.7	4.8	3.5
Rash	0.5	0.5	ND	ND	1.0	0.3	1.2	0.4

[a]No adverse reactions occurred significantly more frequently in vaccinees than in placebo controls in these clinical trials, all of which utilized active surveillance methods to detect adverse reactions.
[b]Percent of total subjects in the group with reactions.
ND, not determined.
Data from Levine MM, Black RE, Ferreccio C, et al. The efficacy of attenuated Salmonella typhi *oral vaccine strain Ty2 evaluated in controlled field trials. In: Holmgren J, Lindberg A, Molly R, eds.* Development of Vaccines and Drugs Against Diarrhea. *Lund, Sweden: Studentlitteratur; 1986:90–101; Black RE, Levine MM, Young C, et al. Immunogenicity of Ty21a attenuated* Salmonella typhi *given with sodium bicarbonate or in enteric-coated capsules.* Dev Biol Stand. *1983;53:9–14; and Simanjuntak C, Paleologo F, Punjabi N, et al. Oral immunisation against typhoid fever in Indonesia with Ty21a vaccine.* Lancet. *1991;338:1055–1059.*

reported symptoms included fatigue and myalgias.[381] There is one report of two cases of reactive arthritis possibly associated with immunization with Ty21a[384]; interestingly, the affected persons were human leukocyte antigen B27–negative.

Purified Vi Polysaccharide–Tetanus Toxoid Conjugate Parenteral Vaccine

Most data establishing the safety of the Vi conjugate vaccines derive from prelicensure clinical trials,[153,205,206,242,326,331,332] except for one small postlicensure trial of one Indian conjugate. The collective experience with the two licensed Vi-TT conjugates and with the various other conjugates in clinical development reveal them to be similarly well tolerated as other conjugate vaccines in children and adults.

Indications

Populations for whom vaccine is indicated include the following:

- Travelers to less-developed areas where typhoid is known or believed to be endemic;
- Military personnel (who represent a special group of travelers);
- School-age children in areas in which typhoid is endemic, particularly where multiple antibiotic-resistant strains are prevalent;
- Preschool-age children where epidemiologic data indicate there is a notable disease burden (e.g., some urban slums in South Asia); and
- Microbiology technicians in clinical microbiology laboratories or in research laboratories in which *S.* Typhi is handled.

Table 61.15 summarizes the immunization schedules and dosages, for different age groups, for the oral Ty21a vaccine, the parenteral Vi vaccine, and one licensed Vi conjugate vaccine.[148,385]

Typhoid vaccine is not generally indicated after floods, earthquakes, or other natural disasters during which the water and sewage systems may suffer structural damage.[386,387] Resources should rather be directed toward repairing the contaminated water sources, which, it is hoped, could be achieved long before a mass vaccination could be completed and the protective effect of vaccine initiated. Nevertheless, there are situations in which typhoid vaccines may serve as a helpful adjunct to other control measures. For example, if a major upheaval occurs in a region of a country where typhoid is endemic, and where formidable economic or political obstacles are expected to impede timely improvements in water supply quality and sanitation infrastructure, selective vaccination of high-risk groups may be helpful.[388,389]

It was suggested that a period of increased risk for acquisition of typhoid fever occurred for approximately 10 days after the administration of the first dose of parenteral killed whole-cell vaccine; this was referred to as the negative phase after vaccination.[390–393] Such a phenomenon has not been described for Vi, Ty21a, or Vi conjugate vaccines.

Contraindications

There are no contraindications to immunization with parenteral Vi polysaccharide vaccine other than known hypersensitivity to any component of the vaccine. Because hyporesponsiveness has emerged as a concern after the repetitive administration of meningococcal[267,268] and pneumococcal[269,270] polysaccharide vaccines, this is a theoretical concern for reimmunizing persons who in recent years received multiple prior immunizations with Vi vaccine. There are no robust data to indicate that hyporesponsiveness frequently follows repetitive immunization with Vi, or that if it does occur it has any clinical consequences.[226,241,271,272] Thus, a history of having been immunized on multiple previous occasions with Vi, several years apart, is not a contraindication to administering another dose of the vaccine.

As a general rule, Ty21a should not be given to pregnant women, although adverse effects on the pregnant woman or fetus have not been reported. Similarly, caution should be taken before anyone with a known depression of cell-mediated immunity is given Ty21a. However, there would appear to be no risk for immunocompromised household contacts of Ty21a vaccinees, because excretion of Ty21a has never been detected in any subject given the available formulations that contain 2 to 6×10^9 CFU per dose.

TABLE 61.15 Immunization Schedules for Ty21a, Vi Polysaccharide, and Vi Conjugate Typhoid Vaccines

Vaccine	Formulation	Route	Age	No. of Doses	Interval Between Doses	Interval Until Next Booster
TY21A LIVE STRAIN						
Primary	Enteric-coated capsules	Oral	≥6 y	3 or 4[a]	2 days (~48 h)	5 y
	Reconstituted liquid suspension[b]	Oral	≥2 y	3	2 days (~48 h)	5 y
Booster	Enteric-coated capsules	Oral	≥6 y	3 or 4	2 days (~48 h)	5 y
	Reconstituted liquid suspension[b]	Oral	≥6 y	3	2 days (~48 h)	5 y
VI-CAPSULAR POLYSACCHARIDE						
Primary	Liquid	Intramuscular (0.5 mL)	≥2 y	1	–	3 y
Booster	Liquid	Intramuscular (0.5 mL)	≥2 y	1	–	3 y
VI CONJUGATE VACCINE[c]						
Primary	Liquid	Intramuscular (0.5 mL)	Adults and children > age 3 y	1	–	3 y[d]
Booster[d]	Liquid	Intramuscular (0.5 mL)	Adults and children > age 3 y	1	–	–
Primary	Liquid	Intramuscular (0.5 mL)	9–12 mo[e]	1	–	
Booster	Liquid	Intramuscular (0.5 mL)	24–36 mo	1	–	3 y

[a]Four doses in the United States and Canada; three doses in all other countries.
[b]The liquid suspension formulation is licensed in several countries but is not presently available commercially.
[c]The information given in the table is for the Bharat Biotech International Vi-TT conjugate (Typbar-TCV), as this is the only Vi conjugate vaccine that is currently both licensed (India) and has recommendations for its use from an immunization advisory committee (the Indian Academy of Pediatrics Advisory Committee on Vaccines and Immunization Practices).
[d]Only limited clinical and immunologic data are available to support the timing of the booster immunization schedule proposed by the manufacturer.
[e]Typbar-TCV has been licensed to be given as a single dose to infants as young as age 6 months. However, the Advisory Committee on Vaccines and Immunization Practices of the Indian Academy of Pediatrics currently recommends that Typbar-TCV be administered to infants at 9–12 months of age, either 1 month before or 1 month after the infants receive their first dose of measles-mumps-rubella (MMR) vaccine and that a second dose of the Vi conjugate vaccine be given between 24 and 35 months of age.[152] Should data become available showing that Typbar-TCV does not interfere with the magnitude of the immune responses to MMR vaccine, and vice versa, it is anticipated that in the future Typbar-TCV will be approved to be administered concomitantly with MMR1.

If immunocompromised persons, including those with HIV infection, must travel to endemic areas, Vi vaccine should be administered. A study of HIV-infected persons in Peru indicated that they were at greatly increased risk for developing typhoid fever,[394] whereas HIV positivity appeared to be protective against typhoid fever in Tanzania.[395]

Ty21a should not be administered to persons who are taking antibiotics. Certain antimalarials, particularly mefloquine, exhibit activity against Ty21a.[233,396,397] In clinical trials, coadministration of chloroquine, mefloquine, or chloroquine plus pyrimethamine-sulfadoxine did not significantly suppress the IgG O antibody response after immunization with Ty21a,[282,398] whereas coadministration of proguanil did.[282] On the basis of these data, it has been proposed that Ty21a should not be taken with proguanil. However, it has since been shown in a randomized, double-blind, placebo-controlled clinical trial that daily administration of Malarone (atovaquone plus proguanil) for 12 weeks did not diminish the serum IgG *S.* Typhi O antibody response in children (4 to 16 years of age) given three doses of the liquid formulation of Ty21a; notably, the first dose of Ty21a was coadministered with a dose of CVD 103-HgR live oral cholera vaccine.[399] Ty21a may be taken with chloroquine, but one should wait 8 to 24 hours after administration of mefloquine before initiating immunization with Ty21a.

PUBLIC HEALTH ASPECTS

Epidemiologic Results of Vaccination

Because of their reactogenicity, parenteral killed whole-cell vaccines were rarely used in a systematic fashion in public health programs in endemic areas. The one exception was Thailand, where in the 1980s school-based immunization programs used parenteral heat-inactivated, phenol-preserved vaccine to control endemic typhoid. A retrospective review suggested that the Thai control program was highly successful.[400]

The lack or rarity of frequent or severe adverse reactions associated with live oral vaccine Ty21a or with parenteral Vi polysaccharide vaccine renders them particularly suitable for public health use in school-based immunization programs. In a large effectiveness trial involving immunization of 216,692 schoolchildren in Santiago, Chile, with the enteric-coated capsule formulation of Ty21a, the vaccine was found to be quite practical for large-scale use in schools.[150] Similarly, in southern China, mass vaccination with parenteral Vi vaccine proved to be logistically feasible and practical in a targeted population of 118,000 persons, although it was concluded that great attention had to be paid to injection safety issues.[151] Large-scale feasibility projects of school-based immunization with Vi vaccine were also carried out in North Jakarta, Indonesia,[401] and in Hue, Vietnam.[402] These projects demonstrated the logistical feasibility, safety, and practicality of delivering Vi vaccine through school-based immunization programs.

A large-scale, cluster-randomized, postlicensure trial of the effectiveness of Vi vaccine was carried out in an urban slum in Kolkata, India.[127] Two new observations were generated during this trial. First, there was evidence of significant indirect protection whereby nonvaccinated subjects in the clusters that were allocated to receive Vi vaccine had significantly lower incidence rates of confirmed typhoid fever (16 cases among 12,206 contacts; 0.19 cases per 10^5 person-days of observation) than unvaccinated subjects in the hepatitis A vaccine clusters (31 cases among 12,877 subjects; 0.35 cases per 10^5

person-days of observation).[127] This translates to 45% indirect effectiveness (95% CI, 1% to 70%; $P = .04$). The second notable observation was that the point estimate of effectiveness of Vi vaccine in the Kolkata trial appeared to be higher among preschool-age children from 2 to 5 years of age (82% effectiveness; 95% CI, 58% to 92%) than among older children from 5 to 15 years of age (59% effectiveness; 95% CI, 18% to 79%) or subjects older than 15 years of age (48% vaccine effectiveness; 95% CI, 44% to 81%).[127] This was unusual in that with other typhoid vaccines, such as parenteral killed whole-cell and oral Ty21a, and with earlier trials of Vi vaccine, the level of protection was always lower in younger children than in older subjects.[218,349,403] Indeed, in contrast to the Kolkata results, another postlicensure effectiveness trial with a similar cluster-randomized design (with the same Vi vaccine [Typherix] as was used in Kolkata) that was carried out in subjects 2 to 16 years of age in poor urban squatter settlements in Karachi, Pakistan,[128] showed no evidence that Vi vaccine protected preschool-age children younger than 5 years of age, whereas it did confer significant protection to the older subjects. Nor was evidence of indirect protection observed in the Karachi effectiveness trial.[128] Differences in the level of immunization coverage between Kolkata and Karachi and inclusion of adults in the Kolkata trial could help explain the divergent results on indirect protection. There is no clear explanation for the unusually high level of protection of preschool-age children in the Kolkata trial and lack of protection of preschoolers in Karachi.

There is epidemiologic evidence that the large-scale field trials in Santiago, Chile, with Ty21a produced a herd immunity effect.[149] In children in the placebo group in the first field trial of Ty21a in Area Norte, Santiago, the incidence of typhoid fell progressively as each of three field trials was initiated in subsequent years in other administrative areas of the city.[149] The incidence rate in this group diminished by approximately 70% from the mean incidence in the 3 years before the field trials. These data suggest that systematic application of live oral typhoid vaccine, even with a formulation that provides only approximately 60% to 70% efficacy, can notably diminish the incidence of the disease in endemic areas.

Table 61.16 summarizes the salient features of the two typhoid vaccines that have been available in recent years, oral Ty21a and parenteral Vi polysaccharide, and for two Vi-TT conjugates licensed in India. One report of 905 children vaccinated with two doses of Peda-Typh and of 860 unvaccinated controls who were actively followed for 1 year provides the first efficacy data for that Indian Vi-TT conjugate.[206a] It is expected that planned postlicensure evaluations of Typbar-TCV and perhaps of other (future-licensed) Vi conjugates will generate additional more robust effectiveness data.

Disease Control Strategies

Because the peak incidence of clinically recognizable typhoid fever in most endemic areas (based on passive, health facility–based surveillance that detects cases severe enough to seek healthcare) occurs in school-age children 5 to 19 years of age, and because this is a captive population, in theory it should be possible to design control programs to incorporate school-based immunization with well-tolerated typhoid vaccines.[148,149] Field trials have clearly demonstrated that both Ty21a and Vi confer a moderate level of protection that endures for several years. Moreover, field experiences with both Ty21a and Vi support the logistical practicality of such an approach.[150,151,401,402] Ferreccio and colleagues[150] reported the practicality of school-based immunization in an effectiveness trial in 216,692 schoolchildren that compared two-dose, three-dose, and four-dose regimens (all doses given within 8 days) of Ty21a. Further encouragement for programmatic use of typhoid vaccines comes from the observation of Levine and coworkers[149] that a herd immunity effect was evident in geographically separate areas of Santiago after the large-scale use of typhoid vaccine in other areas. Similar positive experiences of logistical feasibility of school-based immunization have been demonstrated for Vi vaccine,[401,402] and evidence that Vi can confer indirect protection on unvaccinated contacts has also been reported.[127] There is also a report of a school-based immunization of 441 students in the face of an epidemic of typhoid in Guangxi, China, in which the vaccine showed 71% effectiveness (although with wide confidence limits).[216]

TABLE 61.16 Comparison of the Characteristics of Live Oral Vaccine Ty21a and Parenteral Vi Polysaccharide Vaccine

Characteristic	Ty21a		Vi	Vi Conjugate[a]
Formulation	**Enteric-Coated Capsules**	**Liquid Suspension[b]**	**Liquid**	**Liquid**
Type of vaccine	Live	Live	Subunit	Subunit
Route of administration	Oral	Oral	Parenteral	Parenteral
Immunization schedule	3 or 4 doses[c]	3 doses[c]	1 dose	1 or 2 doses
Cold chain required by manufacturer	Yes	Yes	Yes	Yes
Well tolerated	Yes	Yes	Yes	Yes
Range of efficacy	35–67%	55–96%	64–72%	89–100%
Duration of efficacy	62% for ≥7 y	78% for ≥5 y	55% for 3 y	89% for 46 mo
Herd immunity effect	Yes	Yes	Yes	?
Can interfere with use of serum Vi antibody as a screening test to detect chronic typhoid carriers	No	No	Yes	Yes

[a]The information given in this table on the route of administration, immunization schedule, and clinical tolerability are for the Bharat Biotech International Vi-TT conjugate (Typbar-TCV), as this is the only Vi conjugate vaccine that is currently both licensed (India) and has recommendations for its use from an immunization advisory committee (the Indian Academy of Pediatrics Advisory Committee on Vaccines and Immunization Practices).[152] Information on the level and duration of efficacy are based on prelicensure data from a field trial of Vi-rEPA,[125,126] and from a postlicensure efficacy evaluation of Peda-Typh limited to 1 year of follow-up.[206a]

[b]Lyophilized vaccine from a sachet is added to 100 mL of water, along with a buffer powder and stirred, resulting in a vaccine suspension.

[c]Given every other day (i.e., ~48 hours between doses).

Unfortunately, heretofore, with only a few exceptions, public health authorities in endemic areas have not enthusiastically embraced the use of Ty21a or Vi in school-based immunization programs. One reason that health authorities declined to institute school-based immunization with Ty21a or Vi is the concern that such a program would deflect scarce resources from the Expanded Programme on Immunization (EPI), which, in developing countries, focuses mainly on infants younger than 12 months of age. For this reason, as an alternative strategy, several national health authorities have expressed interest in the possible inclusion of a typhoid vaccine in the EPI for infants. Such a strategy would allow typhoid fever control to proceed without deflecting resources from the traditional infant-targeted EPI. Unfortunately, no data are currently available to demonstrate that, if either Ty21a or Vi were given to infants, the immunity elicited would endure and protect years later when the children reach the high-risk school-age years. The commercial liquid formulation of Ty21a was shown to be immunogenic in toddlers and preschool-age children 2 to 5 years of age,[279,280] but that formulation has not been tested in infants younger than 12 months of age and is not currently commercially available. Unconjugated Vi polysaccharide vaccine is not recommended for administration to infants.[213] If public health authorities in endemic areas desire typhoid vaccines that are immunogenic and protective in infants so that they can be added to the EPI, those vaccines are becoming available through the licensure of Vi conjugates (Typbar-TCV and PedaTyph, have already been licensed in India and the Indian Academy of Pediatrics has made specific recommendations on the pediatric target populations for Typbar-TCV). In future years the improved attenuated live oral vaccine strains that have been in clinical trials may also become available and they are expected to confer long-term protection as does Ty21a.

Cost–Benefit Information

Several studies have looked at cost–benefit, cost-effectiveness, and other health economic assessments of using typhoid immunization as a measure to control endemic disease.[111,146,404–407] The results of such analyses are very context-specific. The data of Ferreccio and colleagues[146] suggest that generically the two most important factors that drive the analysis in favor of cost-effectiveness are the magnitude of the disease burden (disease incidence) and the duration of protection conferred by the vaccine.

Eradication or Elimination

Whereas chronic carriers constitute the reservoir of *S*. Typhi, the maintenance of a high incidence of typhoid fever requires conditions that permit amplified transmission of *S*. Typhi to susceptible persons. Usually, this involves fecal contamination of water sources consumed by large numbers of persons. In the late 19th and early 20th centuries, it was demonstrated in Europe and the United States that treatment of municipal water supplies caused the incidence of typhoid fever to plummet, despite the continued existence in the population of large numbers of chronic carriers. Over one to two decades this led to near-elimination of typhoid fever from many areas.[158]

It is not known if programmatic use of Ty21a or Vi conjugate could accomplish the same near-elimination of typhoid fever as a public health problem, as have improvements of water and sanitation. Unconjugated Vi vaccine with its relatively short-term protection and Ty21a with its requirement for three spaced doses are not likely to be lynchpins of elimination campaigns. On the other hand, some Vi conjugates and a few attenuated strains that function as single-dose oral vaccines are much more amenable for this purpose. If these recently licensed and future vaccines are well tolerated, immunogenic in infants,[332] and confer a high level of long-lasting protection, they could serve as the basis of typhoid fever–control programs. Aggressive control programs that achieve a high level of coverage with such vaccines might nearly eliminate typhoid fever in certain populations as remaining chronic typhoid carriers would eventually die off and few new chronic carriers would replace them.

It is epidemiologically feasible that typhoid fever can someday be eradicated from the world. This would require a combination of treatment of water supplies and provision of sanitation to diminish transmission, systematic screening to detect chronic typhoid carriers and treatment of the carriers to diminish the reservoir of infection, and, finally, programmatic use of well-tolerated, highly effective typhoid vaccines that confer enduring protection. However, other pressing global priorities and the enormous costs involved so far keep typhoid eradication to the level of a theoretical concept. Even if typhoid fever were eliminated in large regions of the world where that disease is now endemic, the endemicity of paratyphoid A fever caused by *S*. Paratyphi A, which exhibits a clinically indistinguishable syndrome, would not be affected by Vi conjugate vaccines or live *S*. Typhi vaccines.

RECENTLY LICENSED AND FUTURE VACCINES

Vi Conjugates

Because purified Vi polysaccharide acts like a T-lymphocyte–independent antigen, the serum antibody response cannot be readily boosted by administration of additional doses of Vi vaccine.[241] In contrast, when Vi is conjugated to carrier proteins, such as tetanus or diphtheria toxoids, mutant diphtheria toxin CRM_{197}, cholera toxin, cholera toxin B subunit, or recombinant exotoxin A of *P. aeruginosa*, it behaves like a T-lymphocyte–dependent antigen.[42,265,408,409] In animal models, subsequent inoculations with Vi conjugate vaccine clearly boost the serum Vi antibody titer.[42,265,408,409] Clinical studies carried out in adults, school-age children, and preschool-age children in Vietnam compared the immunogenicity of two formulations of Vi–exoprotein A conjugate prepared using two different methods of conjugation. The conjugate prepared by treating the carrier protein with ADH and binding to Vi in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide elicited significantly higher titers of serum IgG Vi antibody than the other Vi conjugate (and also higher than Vi polysaccharide alone), yet was well tolerated. The superior conjugate showed a high rate of seroconversion in all ages after a single injection (containing 24 μg of Vi and 21.5 μg of exoprotein A) but a gradation in GMT that decreased progressively from adults to toddlers. The group of subjects given a second dose of this conjugate 6 weeks later exhibited a clear booster response in IgG Vi antibody titers.

Szu and colleagues[265,326] pursued a stepwise program to develop an optimal Vi conjugate vaccine that culminated in a candidate for clinical trials consisting of Vi polysaccharide conjugated to recombinant *P. aeruginosa* exotoxin A (Vi-rEPA). After demonstration of the safety and immunogenicity of this conjugate,[326] the efficacy conferred by a two-dose (6 weeks apart) immunization schedule of the vaccine was evaluated in a large-scale, randomized, controlled field trial in Vietnam in children 2 to 5 years of age.[125] The conjugate used in the Phase III field trial of efficacy in Vietnam contained 22.5 μg of Vi and 22 μg of exoprotein A per 0.5-mL dose of vaccine.[125]

In total, 5525 children received two doses of Vi conjugate and 5566 received placebo in a field trial in Vietnam that

TABLE 61.17 Cases of Typhoid Fever in Pediatric Recipients[a] of Parenteral Vi Conjugate Vaccine or Placebo in a Randomized, Double-Blind, Controlled Field Trial in Vietnam

	Vi-Recombinant Exoprotein A Conjugate	Placebo	Vaccine Efficacy (95% CI)	*P* Value
27 Months of Active Surveillance				
Two-dose recipients	5525	5566		
Typhoid fever cases[b]	4	47	91.5% (77.1–96.6%)	<.001
Cases/10^3 children	0.72	8.44		
All children (1 or 2 doses)	5991	6017		
Typhoid fever cases	5	56	91.1% (78.6–96.5%)	<.001
19 Months of Passive Surveillance				
Typhoid fever cases[c]	3	17	82.4% (22.3–99.1%)	.001
Cases/10^3 children	0.50	3.05		
Total 46 Months of Follow-up (27 Months of Active Plus 19 Months of Passive Surveillance)				
Typhoid fever cases[c]	8	73	89.0% (76.0–96.9%)	<.001
Cases/10^3 children	1.45	13.1		

[a]Children were 2, 3, 4, or 5 years of age at the time of vaccination and were followed for 27 months thereafter.
[b]Per protocol analysis.
[c]Intent-to-treat analysis.
CI, confidence interval.
Data from Lin FYC, Ho VA, Khiem HB, et al. The efficacy of a Salmonella *Typhi Vi conjugate vaccine in two-to five-year-old children.* N Engl J Med. *2001;344:1263–1269; and Mai NL, Phan VB, Vo AH, et al. Persistent efficacy of Vi conjugate vaccine against typhoid fever in young children.* N Engl J Med. *2003;349:1390–1391.*

evaluated the efficacy of Vi-rEPA conjugate. During 27 months of follow-up, active surveillance was carried out to detect cases of typhoid fever by visiting children weekly, eliciting a symptom history, and recording their axillary temperature. Subjects who had a temperature of 37.5°C or higher for at least 3 days were referred to a health center, where 5 mL of blood was drawn for bacteriologic culture. Typhoid fever was diagnosed in four of 5525 vaccinees and 47 of 5566 controls, demonstrating an efficacy of 91.5% (95% CI, 77.1% to 96.6%) (Table 61.17).[125] The level of efficacy did not vary by year of age among these 2- to 5-year-old children, nor did it appear to wane during the second year of surveillance.

After the code was broken, an additional 19 months of follow-up were instituted by means of passive surveillance. During these 19 months, three cases of confirmed typhoid fever were detected in vaccinees and 17 cases in placebo recipients who sought healthcare (82.4% efficacy; 95% CI, 22.3% to 99.1%). Over the full 46 months of follow-up (27 months using active and 19 months using passive surveillance methods), overall vaccine efficacy was 89.0% (95% CI, 76.0% to 96.9%) (see Table 61.17).[125,126] Subsequently, the prototype Vi-rEPA conjugate demonstrated satisfactory immunogenicity when young Vietnamese infants were immunized according to an EPI schedule (priming immunization with three doses at 2, 4, and 6 months of age followed by a reinforcing dose at age 12 months), and there was no significant interference with other EPI vaccines.[332]

Besides the two Vi conjugates licensed in India (Typbar-TCV and PedaTyph) and Vi-rEPA, Vi conjugate vaccines in clinical development include a Vi-CRM_{197} conjugate[327,410] manufactured by BioE (Hyderabad, India). Other Asian manufacturers are developing diphtheria toxoid–based conjugates that are slated for clinical trials in 2017 and 2018.

New Recombinant *Salmonella* Typhi Strains as Live Oral Vaccines

Various investigators have applied recombinant DNA technology to engineer new candidate vaccine strains of *S.* Typhi that will be as well tolerated as Ty21a but much more immunogenic, so that protective immunity can be elicited with a single dose. Toward that ambitious goal, candidate attenuated vaccine strains have been prepared by inactivating genes encoding various biochemical pathways,[194,196,197,411] global regulatory systems,[199,412] stress proteins,[413] other regulatory genes,[414,415] and putative virulence properties.[411]

Among these various strains, based on results of completed Phase I and Phase II clinical trials, three strains remain viable vaccine candidates and are in various stages of clinical development as live oral typhoid vaccines or as live vectors. The status of these vaccine strains is briefly summarized below.

***ΔaroC,ΔssaV* Strain M01ZH09.** The SsaV protein forms part of the *S. enterica* type III secretion system that plays a role in virulence, including affecting systemic invasiveness (in a mouse model) and replication in macrophages.[411] Hindle and coworkers[411] reported results of a small Phase I clinical trial with strain M01ZH09, a Δ*aroC,*Δ*ssaV* mutant of wild-type strain Ty2. In this small trial, the vaccine was well tolerated, minimally excreted, and moderately immunogenic.[411] A Phase II dose-ranging trial in U.S. adults compared the reactogenicity and immunogenicity of a single 5×10^7, 5×10^8, or 5×10^9 CFU dose of vaccine administered with bicarbonate buffer (16 subjects per vaccine dosage level, and a total of 12 placebo recipients).[362] Five recipients of the two higher dosage levels developed mild fevers. Immune responses were most prominent in recipients of the highest dosage level. Another Phase II trial showed that a single 5×10^9 CFU dose was immunogenic whether administered alone or after bicarbonate buffer (to neutralize gastric acid).[239] Single doses between 5×10^9 CFU and 1.7×10^{10} CFU were evaluated for clinical acceptability and immunogenicity in 187 healthy U.S. adults (150 vaccinees and 37 placebo recipients).[416] The vaccine was well tolerated and satisfactorily immunogenic. M01ZH09 was then evaluated in a randomized, placebo-controlled Phase II trial in Vietnamese children, 5 to 14 years of age, who ingested a 5×10^9 CFU dose of vaccine ($N = 101$) or placebo ($N = 50$).[417] The vaccine was well tolerated and

immunogenic; the magnitude of the serologic responses was modest.

Darton et al.[214] randomly allocated young Oxford adults to receive a single oral dose of M01ZH09 (n = 33) or a dose of placebo (n = 33) or (open label) three doses of Ty21a (n = 33). Twenty-eight days after vaccination the volunteers participated in an experimental challenge with wild-type *S.* Typhi. In a secondary analysis that involved comparing the number of cases in each randomized group that had fever (≥38°C) as a clinical endpoint plus blood culture confirmation, the efficacy of M01ZH09 was 52% (95% CI, −25 to 81%) and for Ty21a was 80% (95% CI, 16–95%). This analysis is relevant for two reasons. First, the definition of a case was similar to the early volunteer challenge studies of Ty21a at the University of Maryland in the early 1970s (with the identical Quailes challenge strain) that showed 87% vaccine efficacy for Ty21a.[214] Thus, this analysis provides a "bridge" between the somewhat different old Maryland and new Oxford challenge models. Second, the analysis that defines a case as having both clinical illness and a positive blood culture mimics the follow-up that would occur in a field trial of vaccine efficacy where blood cultures would only be obtained from enrolled subjects who have a febrile illness suggestive of typhoid fever. This analysis suggests that M01ZH09 is insufficiently protective when given as a single dose, even as it corroborates the efficacy of a three-dose regimen of Ty21a.

***ΔaroC,ΔaroD,ΔhtrA* Strain CVD 908-*htrA*.** Chatfield and coworkers[413] observed that inactivation of *htrA*, a gene encoding a stress protein that functions as a serine protease, attenuates wild-type *S.* Typhimurium for mice and protects orally immunized mice against challenge with a lethal dose of wild-type *S.* Typhimurium. Based on these observations, a deletion in *htrA* was introduced into Δ*aroC*,Δ*aroD S.* Typhi strain CVD 908 to yield the further derivative CVD 908-*htrA*. In Phase I clinical trials, single doses of CVD 908-*htrA* ranging from 5×10^7 to 5×10^9 CFU were as well tolerated as the CVD 908 parent,[237] although two of the 22 subjects developed loose stools[237]; mild diarrhea had not been observed in any recipients of CVD 908 during Phase I studies.[200,236] CVD 908-*htrA* stimulated significant rises in serum IgG O antibody, gut-derived IgA antibody ASCs, and cell-mediated immune responses in 90% to 100% of vaccinees. CVD 908-*htrA* was subsequently tested in a Phase II trial in which 79 subjects ingested either a 10^7 or a 10^8 CFU dose reconstituted from a lyophilate.[314,323] In this larger trial, the vaccine was again well tolerated and immunogenic.[314,323]

Strain CVD 909. A higher level of protection may be attainable if a live oral vaccine can stimulate Vi antibody (as Vi and Vi conjugate vaccines do), in addition to the other humoral and cellular responses elicited by live oral vaccines such as Ty21a.[149,252,255,285,299,307] Disappointingly, otherwise promising live oral vaccine strains CVD 908-*htrA*, Ty800, and M01ZH09, although they express Vi, have failed to consistently stimulate serum Vi antibodies or IgA Vi ASCs when administered as oral vaccines.[237,323,360] This is not surprising, because only 20% of patients with acute typhoid fever develop serum Vi antibodies.[89,91] On the other hand, chronic gallbladder carriers of *S.* Typhi typically manifest high titers of serum Vi antibody, demonstrating that infection with *S.* Typhi can stimulate serum Vi antibodies.[91] The fact that expression of Vi is highly regulated in relation to environmental signals such as osmolarity may explain these somewhat contradictory observations.[418] Two separate two-component systems are involved in this regulation.[414,418] Vi expression ensues when typhoid bacilli reside in certain extracellular environments such as blood and bile to protect them from complement-mediated, O antibody–dependent bacterial killing,[40,67,418,419] but it is apparently turned off when the bacteria gain their intracellular niche in macrophages. It was hypothesized that if Vi expression by a live oral vaccine can be rendered constitutive so that it is expressed continuously, this may allow the stimulation of serum IgG and mucosal IgA Vi antibodies in orally vaccinated subjects. The *viaB* locus of *S.* Typhi contains the genes required for synthesis, surface transport, and anchoring of Vi.[420,421] Wang and colleagues[422] replaced the promoter of *tviA*, the most upstream gene in the viaB locus of CVD 908-*htrA*, with a strong constitutive promoter to derive strain CVD 909, which expresses Vi constitutively. Phase I and Phase II clinical trials showed CVD 909 to be well tolerated.[273,423,424] Disappointingly, few subjects developed serum IgG Vi antibody after ingestion of CVD 909. In contrast, 75% of the recipients of a single dose containing 10^8 or 10^9 CFU did develop strong gut-derived IgA O antibody ASC responses, detected in PBMCs 1 week after oral vaccination and serum opsonophagocytic antibody.[423,425] Oral immunization with a single dose of CVD 909 has been shown to elicit powerful T-cell-mediated immune responses, including homing responses of T cells to the gut.[315,316] CVD 909 also stimulates strong IgA B_M cell responses.[273]

Attenuated Recombinant *Salmonella* Typhi Strains as Live Vector Vaccines

Attenuated *S.* Typhi strains can function as so-called live vectors to express critical genes of other organisms and deliver them to the host immune system.[426–428] The features of *Salmonella* strains that make them attractive as live vectors include the following: (1) the vaccines can be given via mucosal (oral or nasal) immunization; (2) *Salmonella* strains elicit a broad immune response that includes serum antibodies, mucosal sIgA antibodies, and different types of cell-mediated immune responses; and (3) considerable experience has been gained in recent years in genetically manipulating *Salmonella*. Only a few clinical trials with the new generation of attenuated *S.* Typhi carrying foreign antigens as live vectors have been published.[429–432] In animal models, *S.* Typhi can serve as a mucosal delivery system for DNA vaccines.[433–435]

***Salmonella* Paratyphi A Vaccines.** The emergence of multiple-antibiotic–resistant *S.* Paratyphi A as an increasingly important cause of endemic enteric fever in countries in South Asia[132,133] and among travelers[134] to endemic areas has stimulated efforts to develop vaccines to prevent paratyphoid A fever.

Salmonella *Paratyphi A Conjugate Vaccines.* Several companies and research institutes are developing Paratyphi A conjugate vaccines consisting of the O polysaccharide of *S.* Paratyphi A linked to a carrier protein such as CRM_{197} (GSK Vaccines Institute of Global Health and BioE), TT (Bharat Biotech International), diphtheria toxoid (International Vaccine Institute), and Paratyphi A flagellin (H:a) subunits (the Center for Vaccine Development [CVD] of the University of Maryland). These conjugates, which are in various preclinical stages, are slated for clinical trials in late 2017 and 2018.

Attenuated Recombinant* Salmonella *Paratyphi Strains as Live Vector Vaccines. Two attenuated *S.* Paratyphi strains that have been developed are Δ*phoPQ* mutant MGN 10028[436] and Δ*guaBA*,*clpX* mutant CVD 1902.[437] MGN 10028 has been shown to be immunogenic after oral immunization of rabbits, whereas CVD 1902 has been tested in a dosage-escalating Phase I clinical trial, where it was found to be well tolerated and immunogenic and is ready to enter Phase II trials.

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62 Varicella Vaccines

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Varicella-zoster virus (VZV) causes two diseases, varicella (chickenpox) and herpes zoster (HZ, shingles). Infection with VZV (varicella) in temperate climates where vaccination is not used approaches 100% by the fourth decade of life. Whereas varicella may be mild in some people, epidemiologic studies indicate that there is significant morbidity and some mortality with primary infection in previously healthy persons. Takahashi developed the live attenuated varicella vaccine (Oka strain) in 1974,[1] and it was licensed in Japan in 1986. Varicella vaccine was licensed for routine use in childhood in the United States in 1995. Monovalent varicella vaccines are licensed and available throughout the world for the prevention of infection in healthy children, adolescents, and adults. A combination vaccine for the prevention of measles, mumps, rubella, and varicella was first licensed in the United States in 2005. Progress has been made in understanding the pathogenesis of HZ, and a large collaborative clinical trial has demonstrated the effectiveness of vaccination of the elderly for preventing HZ and its major complication, postherpetic neuralgia. Except for the varicella vaccine licensed in South Korea, all VZV vaccines available worldwide are based on the Oka strain; the live vaccine used to prevent HZ contains at least 14 times the amount of virus as the live attenuated varicella vaccine.[2] This chapter focuses on varicella vaccine; Chapter 63 discusses HZ vaccines.

HISTORICAL ASPECTS

Historically, varicella and smallpox were often confused; in 1767, Heberden clinically differentiated them. Numerous studies followed in which investigators induced varicella in volunteers by inoculation with vesicular fluid or exposure to patients with either varicella or HZ. They showed that varicella is an infectious disease (1875),[3] the two diseases are caused by the same agent,[4–6] and HZ is the result of reactivation of the virus acquired earlier in life.[7] The virus was first isolated in cell culture by Weller and Stoddard in 1952,[8] from vesicular fluid from varicella patients. Later studies indicated that the viruses isolated from subjects with varicella and HZ were morphologically and serologically identical, and the virus was named VZV.[9–11]

Molecular studies showed the restriction endonuclease patterns of viral genomes from a subject with varicella and subsequent HZ,[12] as well from vaccine recipients with subsequent HZ,[13,14] to be identical, proving that HZ is caused by reactivation of latent VZV.

CLINICAL DESCRIPTION

Varicella (Chickenpox)

VZV is transmitted by the airborne route.[15–17] Although it is less readily transmitted than measles, VZV is highly contagious, with secondary attack rates in susceptible household contacts of patients with varicella ranging from 61% to 100%.[18–21] VZV also can be transmitted to susceptible persons from patients with HZ, although studies suggest that the risk of viral transmission is considerably less from HZ than from varicella. In a household study, 16% of 71 susceptible children younger than 15 years exposed to HZ developed varicella, a risk approximately five times lower than that from varicella.[22] In a small daycare study, a 3-year-old with HZ transmitted varicella to approximately 30% of susceptible children.[23]

Immunocompetent Children and Adults

Patients with varicella typically have a generalized vesicular rash concentrated on the head and trunk, and fever. In the immunocompetent person, malaise and fever may occur 1 or 2 days before rash onset, but more commonly these symptoms occur concurrently with the appearance of the rash, a major differentiating feature from smallpox (Fig. 62.1).[24–26] The rash appears in crops; each crop usually progresses within less than 24 hours from macules to papules, vesicles, pustules, and, finally, crusts. New lesions occur in crops over the next few days, with various stages of healing. The lesions are pruritic and may scar.

The average number of skin vesicles ranges from 250 to 500 in otherwise healthy children.[20,27] The height of the fever usually parallels the extent of rash, and the subject is usually ill for 5 to 7 days. The rash has a central distribution, with a concentration of lesions on the trunk, scalp, and face. Second cases of varicella can occur in immunocompetent persons, but their incidence is unknown.[28–32] It is hypothesized that the avidity of VZV antibodies is lower in persons who develop second cases than in those who do not.[31] Another possibility is that VZV temporarily subverts the immune system by immune evasion.[33–35] Subclinical reinfection with VZV also occurs.[36–38]

Varicella in otherwise healthy children is usually not severe, but the disease has a wide variety of infrequent extracutaneous manifestations or complications.[39] These include pneumonia, encephalitis, cerebellar ataxia, arthritis, appendicitis, hepatitis, glomerulonephritis, pericarditis, and orchitis.[40–43] The most common complication in children is secondary bacterial infection.[40,42] Staphylococci or Group A β-hemolytic streptococci are the usual causative pathogens. Group A streptococcal infections may be unusually severe and even fatal after varicella.[44–47] The pathogenesis of severe group A streptococcal infections following varicella is probably multifactorial and could include breakdown of skin providing a portal of entry for streptococci, the type of streptococci, and possibly transient effects of the virus on white cell function.

Acute cerebellar ataxia may develop before rash onset or up to 10 days afterward, with truncal ataxia often the only neurologic sign. Cerebellar ataxia occurs in approximately 1 in 4000 varicella cases among children younger than 15 years old; the prognosis is usually good.[48] A more recent study from The Netherlands showed a risk of cerebellar ataxia after varicella, requiring hospitalization of 1 in 20,000 varicella cases in children younger than 5 years of age.[49] Varicella encephalitis is a more serious and less common complication in children (1 in 33,000 to 1 in 50,000 cases) than cerebellar ataxia and carries a more guarded prognosis.[48,50] Adults with varicella have significantly higher case morbidity and mortality with primary VZV infection than children.[42,51–54]

In adults, the height and duration of the febrile response are greater and rash is frequently more severe, with a greater

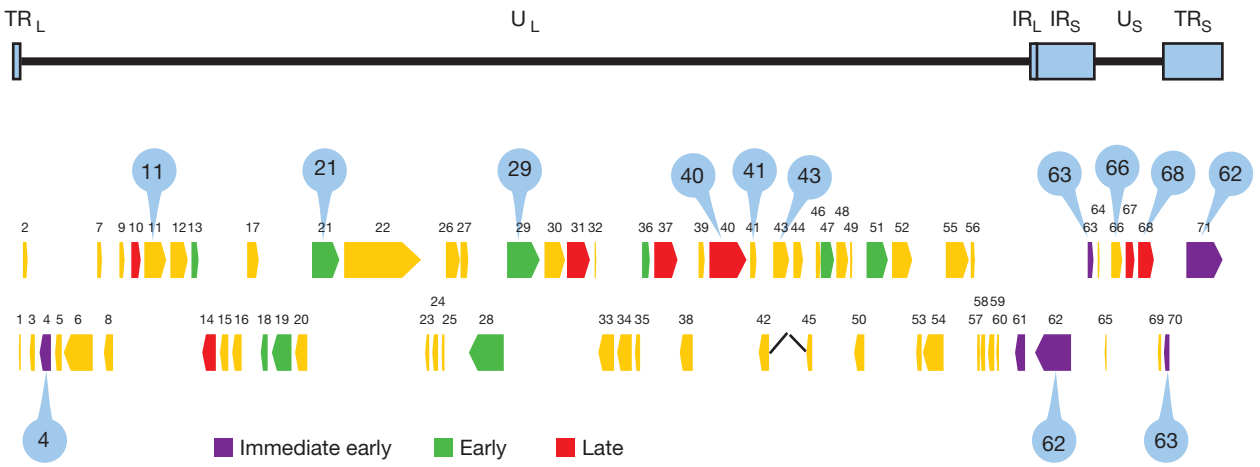


Figure 62.1. Varicella-zoster virus (VZV) transcripts detected in human trigeminal ganglia. The 125,000–base-pair VZV genome is composed of unique long (U_L) and unique short (U_S) segments each bound by respective inverted terminal repeats ($TR_{L/S}$) and internal repeats ($IR_{L/S}$). A total of 71 open reading frames have been annotated on the virus genome. Three open reading frames (ORFs)—62, 63, and 64—are located within the IR_S and are thus also present in the TR_S as ORFs 71, 70, and 69, respectively. Herpesvirus genes are classified as immediate-early, early, or late based on the time following infection of their transcription and their dependency on de novo protein or VZV DNA synthesis; however, for VZV this classification is based on homology to herpes simplex virus type 1. Multiple analyses of human trigeminal and dorsal root ganglia have identified transcripts mapping to VZV ORFs located throughout the virus genome *(blue balloons)*. *(Courtesy Randall Cohrs, PhD.)*

number of lesions and increased time for clearing.[55,56] Constitutional signs and symptoms and a prodrome are of greater intensity in adults.[25]

Pregnant Women and Newborns

Varicella may be more severe in pregnant women (especially in the last trimester) than in other adults.[57,58] Fetal morbidity is increased in pregnant women with varicella.[57,59] Varicella during pregnancy may damage the fetal central nervous system, resulting in permanent scarring of the skin, aplasia of extremities, chorioretinitis, microphthalmia, optic atrophy, cataract, Horner syndrome, blindness, mental retardation, fetal demise, and a high incidence of HZ and death in infancy. This constellation of problems in infants whose mothers had varicella in pregnancy is clinically diagnosed as the congenital varicella syndrome. There is a high correlation between the presence of limb abnormalities and serious central nervous system damage.[57] Various studies have indicated rates of the syndrome after maternal varicella in the range of 0.4% to 2.0%. Harger and colleagues noted that 0.4% of offspring of 347 women with laboratory-confirmed varicella in pregnancy had the congenital varicella syndrome.[60] A prospective case-control study of 106 pregnant women with varicella, including a metaanalysis combining the results of this study with those of other published prospective studies, estimated a 2% risk of varicella embryopathy if infection occurred during the first trimester of gestation.[61] Enders and associates prospectively studied 1373 women with varicella and 366 with HZ during pregnancy.[59] After maternal varicella, the risk of the fetal syndrome was 0.4% in weeks 1 through 12, and 2.0% in weeks 13 through 20. Although no cases occurred in pregnant women infected after 24 weeks of gestation in this study (346 pregnancies affected between 25 and 36 weeks were followed up), isolated cases with birth defects consistent with congenital varicella syndrome have been described after infections in the third trimester, with the latest occurring at 28 weeks of gestation.[62,63] These varying rates may not be significantly different; the syndrome is real but rare.

The study by Enders and associates is also the largest prospective study of consequences of HZ in pregnancy (366 women).[59] No cases of congenital varicella syndrome were reported. There are rare case reports of fetal abnormalities after maternal HZ,[64,65] but whether they represent the congenital varicella syndrome is unclear.[63] If it does occur, the congenital varicella syndrome acquired when a pregnant woman has HZ is exceedingly rare.[57] Infection at any time during fetal life may result in latent infection that is poorly controlled by the host and subsequently reactivates as HZ in infancy or early childhood.[57,59,66,67]

Maternal varicella that develops within 5 days before or 2 days after delivery is potentially very serious for the newborn infant.[57] Maternal antibodies to VZV may not have formed or crossed the placenta, and VZV may infect the baby before or after delivery. In addition, because of the immaturity of the infant's cellular immunity, it is at risk for severe varicella especially if there is no maternal antibody to VZV. The infected infant may develop hemorrhagic skin lesions and primary varicella pneumonia. Severe varicella can usually be avoided with prophylactic administration of passive immunization and acyclovir therapy.[57,68–71] Vaccination of women before pregnancy is the preferable strategy.[72]

Immunocompromised Persons

Varicella in immunodeficient patients can become very serious.[73] Those receiving chemotherapy or radiotherapy (or both) or high dosages of steroids for any reason (transplantation, severe asthma), and those with congenital deficits in cell-mediated immunity, are at greatest risk of developing severe varicella disease. The association between cancer therapy and severe varicella was noted in the early 1950s.[74] By 1975, 30% of children with leukemia who developed varicella manifested dissemination, with 7% mortality.[75] In the era of antiviral therapy, the prognosis improved with treatment administered early in the course of illness,[76] but deaths continued to occur. Severe varicella became especially common as greater numbers of children were treated successfully for malignant disease,[75] transplantation,[77] and asthma.[78,79] Although severe varicella occurred in children with HIV infection, the risk of severe varicella was not as great as for leukemic children.[80]

Herpes Zoster (Shingles)

During the primary infection with VZV, the virus reaches sensory ganglia by way of viremia and sensory nerves. Latency is then established in neurons of the dorsal roots and cranial nerves as well as in the myenteric neurons of the intestine.[81–86] Waning VZV cell-mediated immunity later in life or during immunosuppression enables reactivated VZV to cause HZ, usually a unilateral, painful vesicular rash. Compared with healthy persons, HZ in immunocompromised persons is more likely to be severe and disseminated. Additional information on HZ is found in Chapter 63.

VIROLOGY

VZV is an alpha herpesvirus.[73] Infection is confined to humans and some higher primates; there is one serotype. The virion has approximately 125,000 base pairs. The genome has been sequenced.[87] The linear genome is $(80 \pm 3) \times 10^6$ Da with internal inverted terminal sequences resulting in two predominant isomeric DNA molecules.[88–90] Virions are round or polygonal with a central double-stranded DNA core. The nucleocapsid is approximately 100 nm and formed of 162 hexagonal capsomeres organized into an icosahedron (20 sides).[87] The capsid is surrounded by a tegument and an envelope derived in part from cellular membranes. The diameter of the VZV particle is 180 to 200 nm in diameter. Seventy-four open reading frames (ORFs) encode at least 71 viral gene products (Fig. 62.1). VZV DNA is synthesized in a cascade of expression of immediate early (IE) or alpha regulatory genes, followed by expression of early (E) or beta genes that encode regulatory and structural proteins, followed by expression of late (L) or gamma genes that encode structural proteins. Interruption of the cascade, particularly at the IE stage, may result in failure to synthesize infectious virus.[91]

At least 30 polypeptides have been detected in VZV; at least nine are glycosylated.[91] The known glycoproteins (gps) are termed B, C, E, H, I, K, L, M, and N (Fig. 62.2), corresponding to those of herpes simplex virus (HSV). There is, however, no equivalent of the major gp of HSV, gD, in VZV.[87] Glycoproteins are expressed on the virion and on the surface of infected cells; they facilitate infection of other cells and are the target of neutralizing antibodies.[73] The major gp of VZV is gE, which is the most abundant gp and highly immunogenic. It is linked to gI and with gI is an Fc receptor on infected cells.[92] It also binds to mannose 6-phosphate receptors, along with other gps in the virus, which are critical to VZV infection. gE provides trafficking sequences that mediate assembly of viral proteins and envelopment in the *trans*-Golgi network.[93,94] gE and gI are thought to function as navigator gps, directing additional gps to the cell surface and *trans*-Golgi network, where final envelopment of virions takes place.[95–98] Mannose 6-phosphate receptors are receptors for VZV gps, and the interactions between these receptors, and VZV play critical roles in viral entry and egress from cells. gH plays a role in spread of VZV, and monoclonal antibodies to it prevent spread.[99,100] The insulin-degrading enzyme is also a receptor for VZV gE, and it is important for cell-to-cell spread and infectivity.[101–103]

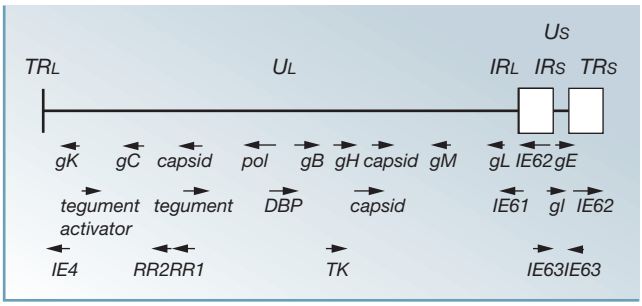


Figure 62.2. Structure of the varicella-zoster virus (VZV) genome. The linear double-stranded DNA consists of a long unique segment (U_L) of approximately 199 kbp, flanked by terminal and internal repeats (TR_L and IR_L), and a short unique sequence (U_S) of approximately 5.4 kbp, flanked by terminal and internal repeats of 6.8 kbp (TR_S and IR_S). The directions of transcription *(arrows)* of some of the important gene products are indicated below the map. *(From Gershon A, Silverstein S. Varicella-zoster virus. In: Richman D, Whitley R, Hayden F, eds.* Clinical Virology. *Washington, DC: ASM Press; 2009:451–473.)*

A new variant VZV virus with a mutated gE has been isolated from six persons in the United States, Canada, and Europe.[104–106] These viruses may be escape mutants; their biological significance remains to be defined. An immunocompetent 15-year-old boy with fatal hepatitis caused by a VZV with this mutation was described.[107] Because this is only one patient, and deaths from varicella occur without this mutation, the pathogenic potential of this mutated virus remains unknown. In addition to glycosylated proteins, some IE gene products of VZV are also immunogenic.[108] IE62 protein, which is encoded by VZV ORF62, is closely related to the regulatory polypeptide ICP4 of HSV 1. This protein was at one time thought to be purely nonstructural, as is ICP4 for HSV.[109] However, IE62 protein has been identified as a major component of the VZV tegument[110] and is highly immunogenic.[111] IE62 protein is the initial transactivating protein of VZV. Other regulatory proteins of VZV are encoded by ORFs 4, 10, 61, and 63.[112–116] It has been reported that during latency, six genes are expressed in sensory ganglia: IE genes (ORFs 4, 62, 63, and 66) and E genes (ORFs 21, 29); L genes are not expressed.[91,117–124] However, a study of specimens of human ganglia obtained at autopsy suggests that there may be some gene expression and that latency is incomplete.[125] False-positive results are proposed to be the result of cross-reacting antigens in patients with type A blood,[126] and neuromelanin may be mistaken for immunologic evidence of VZV proteins.[127] Studies of gastrointestinal neurons from asymptomatic persons undergoing surgery, however, are not subject to all these possibilities, and reveal that the same suite of six RNA transcripts mentioned above are found in human enteric ganglia.[86,128] Thus VZV gene expression during latency remains an unsettled issue.

Latent VZV occurs only in neurons.[129–133] Patients with impaired cell-mediated immunity have an increased incidence of HZ, consistent with the hypothesis that suppression of VZV reactivation is under immunologic control.[73] Rodent models of latent VZV infection may help clarify how latent infection with VZV is maintained.[121,134–136] There is an in vitro model of VZV latency in isolated myenteric sensory ganglia from guinea pigs that can be manipulated to reactivate in vitro,[81,117,137,138] as well as models that use the fetal human dorsal root ganglion.[85]

Growth of VZV in cell monolayer cultures is slow, with poor infectious yields and an absence of infectious virions in supernatant media. Viral spread occurs directly from an infected cell to another uninfected cell. In the infected person, VZV also spreads almost entirely from cell-to-cell, which is why cellular immunity is crucial in host defence. To obtain cell-free VZV in vitro, infectious virions must be released artificially by disruption of cells by methods such as sonication.[73] The cell-associated nature of the virus has tended to impede research on VZV and has played a role in slowing vaccine development.

Although there is only one VZV serotype, different genotypes have been identified.[139–154] Thus far, five major genotypes (clades 1 to 5) of wild-type VZV have been described, with an additional two putative clades (6 and 7) that remain to be

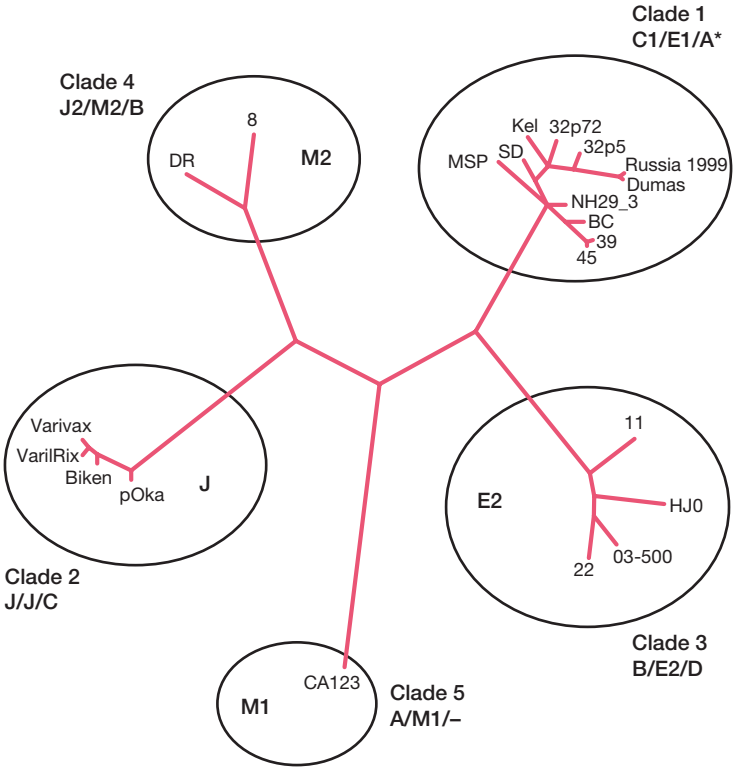


Figure 62.3. Phylogenetic tree showing five major varicella-zoster virus (VZV) clades. J, Japanese; E, European; M, mosaic. *(Modified from Loparev VN, Gonzalez A, Deleon-Carnes M, et al. Global identification of three major genotypes of varicella-zoster virus: longitudinal clustering and strategies for genotyping.* J Virol. *2004;78:8349–8358.)*

confirmed by whole-genome sequencing (Fig. 62.3).[145,147,150,153] The VZV clades show a distinctive geographic distribution,[141,146] although all clades circulate at low levels outside the regions where they predominate. For example, occasional clade 2 Asian strains have been identified in the United States and Australia,[147,154] and clade 5 African strains have been identified in the United Kingdom.[148] However, only the clade 1 and clade 3 strains most commonly isolated in temperate climates have been observed in most countries in Europe and in New Zealand.[144,147] This may reflect an ongoing shift in strain distribution and could be related to immigration and travel patterns. All seven clades have been observed in the United States, although clade 1 and clade 3 isolates predominate (approximately 80% clade 1 and clade 3). Clade 2 strains are the most prevalent genotypes in Japan and China[146,149]; the Oka vaccine is derived from a clade 2 strain bearing a genomic marker present in 10% of wild clade 2 strains (Pst 1 negative in Japan). Rare clade 2 wild-type strains bearing this marker have now been identified in the United States, Australia, and China.[142,147,154] Recent analyses of whole-genome sequences of VZV revealed the presence of small numbers of strain-specific markers that may prove useful for subgenotyping VZV clades[150,152]; phylogenetic analysis suggests that circulating genotypes arose through limited mutation and interstrain recombination.[151]

PATHOGENESIS AS IT RELATES TO PREVENTION

Varicella is a highly contagious disease.[155] Infectivity is postulated to occur by aerosol spread of virions from vesicular skin lesions,[156,157] and possibly to a lesser extent from respiratory secretions.[158] Airborne transmission has been described in hospital settings.[15,16] Studies of transmission of vaccine-type VZV in leukemic vaccinees have implicated skin lesions as the source of infectious virus.[157] In patients with varicella, virus isolation in cell culture from skin is easily accomplished, but it is extremely difficult to isolate VZV from respiratory secretions.[159] As a result of the loss of mannose 6-phosphate receptor in the superficial epidermis, high concentrations of cell-free VZV develop in skin vesicles; these virions may be aerosolized and transmit the virus to others from the skin.[156] Polymerase chain reaction (PCR) studies of respiratory secretions have yielded different results and have not clarified the issue of whether respiratory spread of VZV is significant.[160,161] In one study, VZV DNA was detected in the respiratory tract of 45 patients with varicella, in 28 patients (62%) on day 1, in 50% on day 6, and in 22% after day 6.[162] VZV DNA was detected in the saliva of 100% of 54 HZ patients.[163] Different rates of PCR positivity may reflect differences in primers and differences in respiratory secretion collection methods. Demonstration of VZV DNA by PCR does not necessarily indicate the presence of infectious virus. Prolonged presence of VZV DNA in secretions or dust has not been associated with transmission of this labile virus.

The pathogenesis of varicella was conceptualized by Grose in 1981,[164] based on the pathogenesis of mousepox, outlined by Fenner in 1948.[165] Host invasion of VZV is thought to occur in the conjunctivae or the mucosa of the upper respiratory tract (including tonsils). It was hypothesized that, in primary infection, VZV replicates locally in the lymph nodes for several days, causing a low-level primary viremia that delivers the virus to the viscera, where further multiplication occurs. A demonstrable secondary viremia of greater magnitude subsequently occurs. Culture of mononuclear cells and PCR testing of peripheral blood mononuclear cells from 5 days before to 2 days after appearance of rash in patients with natural varicella

yielded VZV.[162,166–171] Viremia has also been demonstrated in patients with HZ using either virus isolation or PCR.[172–175] The presence of VZV in $CD4^+$ and $CD8^+$ T lymphocytes also has been demonstrated by in situ hybridization during early varicella.[176] In a model of VZV infection in severe combined immunodeficient mice with human skin grafts (SCID-hu), VZV could also be detected in human T lymphocytes.[177]

Experiments with the SCID-hu mouse model, in which human skin was implanted into immunodeficient mice, suggested additional events in the pathogenesis of varicella.[178] VZV-infected human mononuclear cells injected into SCID-hu mice were found to infect the skin implant within 24 hours. Innate immune mechanisms were downregulated in infected keratinocytes, but not in neighboring ones. These observations suggested that the skin may be infected early after VZV enters a host, and that VZV circulates in memory $CD4^+$ T cells to establish focal infection of additional keratinocytes. The long incubation period of varicella is attributed to the time needed for VZV to overcome innate antiviral responses of cutaneous cells.[178] Exactly how this scenario relates to the Fenner model with infection of viscera is not yet clear.

IMMUNE RESPONSES TO VARICELLA-ZOSTER VIRUS

The precise roles of humoral immunity and cell-mediated immunity in protection against VZV infection are not entirely understood but are summarized as follows.[41,179]

Humoral Immunity

Serum Immunoglobulin M Antibody Response

After natural varicella and HZ, serum VZV immunoglobulin (Ig) M, if it occurs, may be detectable for days to weeks. As with many IgM tests, false-negative and false-positive reactions occur.[180] Fig. 62.4 shows the kinetics of humoral, nasopharyngeal, and cellular immunity in patients with clinical varicella from 10 days before rash onset to 20 days after onset.[181]

Serum Immunoglobulin G Antibody Response

After natural infection, VZV-specific IgG antibody, as measured by the fluorescent antibody to membrane antigen (FAMA) test and which correlates with neutralizing antibodies to VZV,[182] is detected in most patients within the first 4 days after the onset of rash.[180] Peak IgG levels are attained at 4 to 8 weeks, and levels usually remain high for up to 6 to 8 months, after which titers decline twofold to threefold. Positive VZV IgG FAMA titers have been detected decades after clinical varicella.[38,183–186]

Serum Immunoglobulin A Antibody Responses

Serum IgA antibody responses to natural infection are detectable early in the course of varicella.[181] Serum IgA antibody was detected in 44% of subjects up to 14 months after varicella.[181]

Nasopharyngeal Immunoglobulin A Antibody Responses

Nasopharyngeal VZV–specific IgA antibody responses are detectable at the onset of symptoms in patients with natural infections.[187] Maximal titers are attained at the third week of illness.[41]

Cell-Mediated Immunity

Cell-mediated immunity appears to be the response of major importance in control of VZV by the host. Structural and regulatory proteins of VZV are recognized during varicella by T lymphocytes, which induce protection from further VZV infection. Immunity is usually maintained for decades and is mediated by both $CD4^+$ and $CD8^+$ T lymphocytes. Memory T lymphocytes belong to the $CD45RO^+CCR7^-$ phenotype.[188] Memory responses may be maintained in part because of periodic exogenous reexposure to others with either varicella or HZ, as well as by possible endogenous reexposure to the virus during subclinical reactivation of VZV. T lymphocytes from varicella-immune persons produce cytokines of the T-helper cell type 1, such as interleukin-2 and interferon-γ, which potentiate clonal expansion of virus-specific T cells on

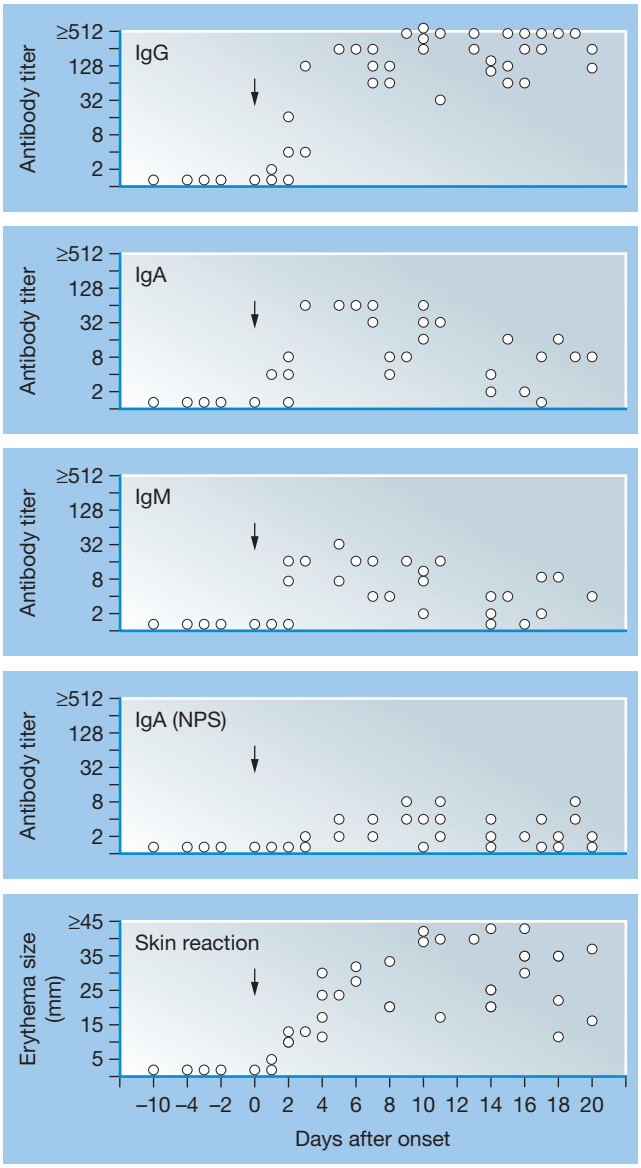


Figure 62.4. Kinetics of appearance of humoral (blood), nasopharyngeal (NPS, secretory), and cellular (skin reaction) immunity in patients with clinical varicella. Antibody titers were measured by fluorescent antibody to membrane antibody. Arrows at day 0 indicate the onset of varicella infection. *(From Baba K, Yabuuchi H, Takahashi M, et al. Seroepidemiologic behavior of varicella zoster virus infection in semiclosed community after introduction of VZV vaccine.* J Pediatr. *1984;105:712–716.)*

exposure to VZV antigens.[41] Natural killer cells, which are part of innate immunity, are another source of interferon-γ and play a role in host defense against VZV.[189,190] $CD4^+$ T lymphocytes provide help so that humoral responses to VZV antigens develop and are maintained after varicella.[191] gE-specific $CD4^+$ lymphocytes may play a role in long-term control of multiplication of VZV.[192] ORF4 protein is a target antigen for persistent VZV-specific $CD4^+$ T cells.[193]

Cell-mediated immune responses are promptly detected after natural infection; peak activity occurs within 1 to 2 weeks and then gradually decreases.[181,194–197] Cell-mediated immunity to VZV can be detected by stimulation of lymphocytes in vitro with VZV antigens,[198,199,200–203] and by lysis of histocompatible target cells by cytotoxic T cells stimulated with VZV antigens.[204–206] An enzyme-linked immunospot assay indicates synthesis of interferon-γ by T cells in response to antigenic stimulation by VZV that can be boosted by immunization.[207] Cellular immunity can also be assessed by an intradermal skin test composed of VZV antigens.[196,197,208] Using tetramer analysis, the importance of the CD4 response and cellular immunity to gE in recovery from varicella, a role for IE 63 in control of viral latency, and subclinical reactivation of VZV have been demonstrated.[191,192,209–211] Cell-mediated immune responses remain positive for many years; however, declining cell-mediated immunity occurs with advancing age, beginning at age 50.[202,212,213] The strongest cell-mediated immune responses to VZV appear to be in early adulthood.[214,215]

Clinical observations indicate that patients with isolated agammaglobulinemia have normal courses of varicella and become immune to the disease, whereas those with defects in cell-mediated immunity are at risk to develop disseminated and fatal VZV infections.[198] Cell-mediated immunity is probably crucial during VZV disease because spread in the body is by the intracellular route, rather than by release of cell-free virus as occurs in vesicular fluid. That cell-mediated immunity is required to maintain the balance between the host and latent VZV is also suggested by the correlation between diminished VZV cell-mediated immunity and an increased risk for HZ.[198,199] In contrast, elderly adults usually have high VZV antibody levels and are not particularly subject to second attacks of varicella. Thus, both antibodies and cellular immunity have roles in protection, suggesting that redundancy in the immune system provides the best protection against disease.

Subclinical Reactivation

Subclinical or clinical reactivation results in both antibody and cellular immune responses. Antibody responses to VZV in healthy subjects with past varicella are characterized by occasional high activity without symptoms, suggesting either exposure to VZV with exogenous boosting of immunity, or subclinical reactivation of VZV. Increases in cell-mediated immunity usually occur after HZ, even in immunocompromised patients.[216] Subclinical reactivation of VZV has been demonstrated by PCR in healthy and in immunocompromised persons.[163,217–220]

DIAGNOSIS

The diagnosis of VZV infection can usually be made clinically by the characteristic rash. In vaccinees who have breakthrough varicella, both clinical and laboratory diagnosis may be more difficult because of mild rashes indicative of partial immunity. For varicella, epidemiologic information, such as age of the patient, lack of history of the illness, and exposure to persons with varicella or HZ in the preceding 2 to 3 weeks, is useful for diagnosis. Unusual skin manifestations of varicella have been described in areas of chronic dermatitis or after sunburn.[221] HZ has a characteristic unilateral, painful, and sometimes pruritic, dermatomal rash. HZ may be confused with recurrent HSV infection.[222] HZ can also occur in the absence of a skin rash, which can make diagnosis extremely difficult.[223]

When it is deemed necessary to prove either varicella or HZ, laboratory diagnosis is best made by demonstration of viral antigens or DNA by PCR in vesicles or body fluids.[180] Virus isolation of VZV is expensive, insensitive, and time consuming, so it is little used today. Rapid diagnosis can also be accomplished by direct immunofluorescence. The direct immunofluorescence test is done by swabbing a suspicious skin lesion, making a smear on a glass slide, and staining with fluorescein-tagged monoclonal antibody to VZV gE.[180]

Vesicular fluid and swabs from maculopapular lesions, respiratory secretions, saliva or buccal secretions, cerebrospinal fluid (CSF), urine, and tissue specimens at autopsy may be tested for VZV by PCR.[180,224–230] It is possible to detect VZV DNA in saliva in patients with varicella and HZ, so in the absence of skin lesions, PCR testing of saliva can be diagnostic.[224,231] For patients with neurologic symptoms, PCR on CSF may reveal VZV infection.[154]

PCR is more sensitive than direct immunofluorescence for diagnosis.[224,230] Identification of VZV DNA by PCR, however, does not necessarily indicate infectivity of the virus.

Serologic methods are less useful than antigen detection methods for laboratory diagnosis of varicella and have limited value for diagnosis of HZ.[224] Varicella can be diagnosed by demonstration of IgM antibodies to VZV in serum, but a negative IgM test does not rule out the diagnosis.[224] Further studies of the kinetics of the IgM response are needed for accurate confirmation of varicella infections. Serologic tests are of limited value for the diagnosis of HZ, because heterologous rises in VZV antibody titer may occur when HSV reactivates in a person immune to varicella.[180] A transient IgM response may also follow HZ, as well as varicella.

Serologic tests to detect VZV IgG antibodies are used to assess immunity to varicella, most commonly in testing of healthcare workers, ideally before employment. Unfortunately, these tests often lack sensitivity and may yield false negative results, especially in vaccinated individuals. Alternatively, a documented history of at least two doses of live attenuated varicella vaccine or a history of varicella is indicative of immunity to varicella. The FAMA assay is considered the gold standard for determining VZV antibodies; this test, however, is rarely available. The major serologic assay in commercial use is the enzyme-linked immunosorbent assay (ELISA) for measurement of IgG antibodies to VZV. ELISA assays are fairly specific, but as many as 10% false-positive reactions in truly susceptible persons have been recorded.[232] Commercially available ELISA tests are also less sensitive than the FAMA test,[180,233] especially for assessing immunity after immunization. An ELISA that uses VZV gp as the antigen has been reported to be sensitive,[234] but it is not commercially available. Positive antibody titers detected by this method in young unvaccinated children, moreover indicate that the assay may be overly sensitive.[235] This assay has shown reproducibility when different batches of vaccine were used for immunization, and there was a linear concordance when neutralizing antibody levels and gp-ELISA titers were compared.[236] Specific gp-ELISA units, however, used as end points were not always consistent in published studies.

A latex agglutination assay based on the clumping of latex particles coated with VZV gps, in the presence of VZV antibody, has sensitivity similar to that of FAMA but is no longer commercially available.[184,185,237] A time-resolved fluorescence immunoassay for measuring antibodies to VZV is being evaluated in the United Kingdom for sensitivity and specificity.[238–240]

While the VZV skin test appears to be sensitive and specific for demonstrating immunity to varicella,[196,232,241] it is cumbersome to perform on children and is not currently available for clinical use. A serologic assay (termed "line assay") using five recombinant VZV antigens was described in which vaccinated children had weaker IgG responses to certain antigens (proteins 4, 14, and 49) than children who had experienced natural varicella. If extended and confirmed, this technique might provide a practical means to distinguish between varicella immunes and susceptibles as well as to identify individuals who had been vaccinated.[242]

A reliable, sensitive, rapid, available means of identifying people with immunity to varicella, particularly after immunization, continues to be very much needed. In summary, the most reliable indication of immunity to varicella today is medically diagnosed varicella or a written documentation of two doses of live attenuated varicella vaccine.

TREATMENT AND PROPHYLAXIS

Effective antiviral therapy for varicella and HZ first became available in the early 1970s. Antiviral therapy is helpful to speed recovery, but it does not terminate virus shedding or prevent latent infection. Vidarabine, the first successful antiviral drug, was supplanted by acyclovir, a DNA chain terminator and an inhibitor of DNA polymerase that is less toxic.[243–245] Intravenous acyclovir speeds healing of varicella and HZ in immunocompromised patients, and orally administered acyclovir, at high dosages, may be used to treat patients with HZ.[246,247] Orally administered acyclovir may be used to treat otherwise healthy children with varicella, but the antiviral effect on varicella is minimal, in part because of the poor absorption of the drug when given orally and the lower sensitivity of VZV to acyclovir, compared with that of HSV.

A double-blind placebo-controlled study in which 102 healthy children were given acyclovir (40 to 80 mg/kg) or placebo daily for 5 days, beginning within 24 hours of rash onset, revealed that the mean number of skin lesions was significantly reduced from over 500 to 336.[27] On average, there was 1 day less of fever. A multicenter collaborative study involving 815 similarly treated children given 80 mg/kg of acyclovir daily yielded similar results.[248] The benefit to secondary household cases (which are typically more severe than the first case in the household) was not different from that of primary cases. The modest benefit conferred by acyclovir therapy is not surprising in view of the typical self-limited nature of chickenpox. Studies in adolescents[249] and adults[250] have not indicated more striking antiviral effects, although acyclovir is recommended for these groups because they are at higher risk of developing severe varicella than healthy children. A study in otherwise healthy children with varicella indicated that 5 days of oral acyclovir is as effective as 7 days.[251]

The oral antiviral prodrug valacyclovir, which is well absorbed from the gastrointestinal tract and is rapidly converted to acyclovir in the body, results in blood levels of acyclovir that are significantly higher than those from oral acyclovir. Although valacyclovir has efficacy for treatment of HZ,[252] no treatment studies of varicella have been performed. One study, however, addresses dosage of valacyclovir for children.[253] Currently, it is acceptable to use valacyclovir to treat children older than age 2 years who have VZV.[254]

Famciclovir, which is also administered orally, is rapidly converted to penciclovir in the body; penciclovir has an action similar to that of acyclovir. At present, either valacyclovir or famciclovir is preferred treatment for HZ in nonimmunocompromised adolescents or adults. Famciclovir has not been studied for treatment of varicella.[255,256] The antiviral drug sorivudine[257] was not approved by the U.S. Food and Drug Administration (FDA) because of the lack of significant benefit over acyclovir and the potential for toxicity in patients being treated for cancer. This drug is available in countries other than the United States.

Postexposure prophylaxis with antiviral therapy for prevention of varicella has been tried and has met with some success in small studies. Acyclovir administered to 25 children after household exposure mostly prevented disease.[258] Immunity appears to persist for several years even with no evidence of clinical disease.[259] Unfortunately, however, less than 85% of patients may develop antibodies to VZV after this prophylaxis,[260] and adequate future protection cannot be ensured. Therefore, two doses of varicella vaccine should be administered subsequently, if possible, to children who did not develop varicella. Although dosages and timing have varied, administration of acyclovir at a dosage of 10 to 20 mg/kg four times a day between days 7 and 14 after exposure was most often used. Prophylaxis administered earlier than 1 week after exposure does not seem to provide as good protection.[261] There are no published studies in which immunization and antiviral therapy have been combined. Postexposure antiviral prophylaxis has not been studied in immunocompromised persons, for whom passive immunization with immunoglobulin is preferable. If passive immunization is not available, an alternative would be to treat immunocompromised patients who develop varicella with acyclovir at the very first sign of illness, but this approach may not be successful in all patients. For healthy nonpregnant persons, postexposure vaccination is preferred to preventive antiviral therapy (see "Passive Immunization" later).

EPIDEMIOLOGY

Varicella is a highly contagious disease that occurs worldwide and, in the absence of a vaccination program, affects nearly every person by mid adulthood. HZ cases represent a method for regular exposure and introduction of VZV into communities that otherwise may not be large enough to sustain VZV endemic transmission. The epidemiology of varicella in temperate climates is different from that in tropical climates; the differences may relate to properties of VZV, climate, population density, and the risk of exposure.[262–266] In temperate climates, most persons are infected by young adulthood, with the highest incidence of disease occurring among preschool-age children or children in early elementary school.[266–274]

Varicella shows a strong seasonal pattern in temperate and some tropical climates, with a peak incidence during winter and spring. Periodic large outbreaks may occur, with an interepidemic cycle of 2 to 5 years.[265,268–270,275–278] Outbreaks occur in settings where children congregate, such as childcare centers and schools, but have also been described in hospitals, facilities for institutionalized children and adults, refugee camps, and adult settings, including among healthcare workers, military personnel, and correctional facilities.[279–291]

Prior to the varicella vaccination program in the United States, the annual average varicella incidence measured from national household survey data was 15 to 16 cases per 1000 population.[265,267] Reported incidence from other countries has generally been lower than U.S. survey rates, most likely reflecting less-complete case ascertainment as a result of differences in study methods.[265,268–270,275–278] Apart from age, factors associated with differences in varicella incidence or seroprevalence include urban (as opposed to rural) residence, attendance in childcare facilities, attendance at and size of school, and presence of older siblings in the household, all of which are likely to increase the risk of exposure.[265,292–294] In countries where universal varicella vaccination programs are in place, not being vaccinated is a risk factor for varicella, and in the United

States, where high vaccine coverage has been achieved, one study highlighted that children of parents who refused varicella immunizations have nine times the risk of requiring a medical visit for varicella than did children whose parents accepted varicella vaccine for their children.[295]

VZV seroprevalence data reflect age-specific disease incidence. National data from the United States before use of varicella vaccine (1988 to 1994) demonstrated high population VZV seroprevalence: 86% for 6- to 11-year-olds, 93% for 12- to 19-year-olds, 95.5% for 20- to 29-year-olds, and 99% or greater for adults 30 years of age and older.[296] Similarly, more than 90% of adolescents (10 to 15 years of age) or young adults are VZV seropositive in many countries around the world where seroprevalence has been measured.[277,297–306] A comparative study of prevaccine VZV seroprevalence in 11 European countries using sera from 1995 to 2003 demonstrated that although the vast majority of children acquire VZV during childhood, the rate of transmission of VZV varied considerably between countries; VZV seroprevalence by 5 years of age was 97% in The Netherlands, approximately 70% in Spain and Germany, and 38% in Italy.[305] This study also highlighted that in some European countries, 10% or more of adolescents 10 to 14 years of age are susceptible to varicella (e.g., 18% in Italy); findings in Australia were similar.[307] Another survey of varicella occurrence among 70,226 school children in Greece showed that 21.4% of sixth grade children lacked a history of varicella and were probably susceptible as they entered adolescence.[308]

Differences in seroprevalence have been described by race, sex, country of origin, and number of siblings in the household.[296,298,309–313] Measuring VZV seroprevalence in countries that have implemented varicella vaccine programs presents new challenges because of the low sensitivity of commercially available VZV IgG tests to measure vaccine-induced immunity.[314] Using the gp-ELISA assay that has good sensitivity for detection of vaccine-induced immunity, the most recent serosurvey in the United States demonstrated that among children 6 to 11 years of age seroprevalence increased from 86% in the prevaccine era to 98% in 2009–10, attributed to the childhood vaccination program, while other age-group-specific estimates were similar to prevaccine estimates.[315]

Population-based epidemiology data are less complete for countries in tropical climates but VZV seroprevalence reflects a higher mean age of infection than in temperate climates,[266,316–321] with higher susceptibility among adults. In such countries, seroprevalence among adolescents or young adults has ranged from 10% to 20% in St. Lucia (an island community) to 80% to 100% in Taiwan and urban Calcutta, India, with rural-versus-urban and other differences described.[262–265,283,318,320,322–333] Similarly low VZV seroprevalence has been reported in isolated tribal adults in Eritrea (44%) and in rural Bengali adults 17 to 25 years of age (42%), compared with much higher VZV seroprevalences among similar-age nonisolated adults in Eritrea (91–96%) and among Bengali adults 17 to 25 years of age in Calcutta (96%).[264,334] Studies in adult subgroups who could be considered for screening and vaccination programs have demonstrated levels of seronegativity among healthcare workers ranging from 11% in Egypt, to 14% to 19% in Malaysia and Saudi Arabia, to 22% or higher in Thailand, to as high as 51% among first-year medical and engineering students in Sri Lanka.[290,335–337] Studies among women of childbearing age demonstrated that 27% lacked VZV antibodies in Iran and 56% in Sri Lanka, whereas 24% of Singapore military recruits were VZV seronegative.[338–340]

In island populations that may not have sufficient size to support continuous endemic transmission, seroprevalence can also vary with the timing of the last epidemic year in relation to the serologic study. For a number of countries the highest incidence was described in the driest, coolest months.[263,341] A study examining risk factors for susceptibility among newly arrived migrants in Canada found the highest varicella susceptibility among those originating from climates with the highest temperature and the most months of humidity per year (tropical rainforest).[342] Crowding in densely populated cities or in households may overcome VZV's diminished ability to spread in tropical climates. In several South American countries higher seropositivity rates in children and lower adult susceptibility were reported in Brazil, Argentina, Mexico, or Bolivia.[343–346]

Significance as Public Health Problems

Some countries have achieved control or elimination of other vaccine-preventable diseases using vaccines recommended for routine use in childhood vaccine programs.[347] Understanding the varicella and HZ health burden and costs as well as the costs and benefits of vaccination strategies is important when countries consider a varicella vaccination program. In the immediate prevaccine era in the United States, an average of 4 million varicella cases occurred each year, which resulted in an average of 11,000 to 13,500 hospitalizations (4.1 to 5.0 hospitalizations per 100,000 population) and 100 to 150 deaths annually (0.4 to 0.6 per million population).[52,53,267,348–350] Varicella also contributes significantly to the disease burden from invasive group A streptococcal infections in children.[44–46,351]

Risk factors for severe varicella include older and younger ages, cellular immune dysfunction caused by illness (e.g., acute leukemia) or medications, and intensity of exposure.[52,75,267,352] In developed countries where data on mortality have been reported, overall varicella mortality has ranged from 0.20 to 0.65 per million population in the United States, Australia, and the United Kingdom.[52,353–355] Population-based case-fatality data have been described only from developed countries with overall case-fatality rates of approximately 2.6 per 100,000 cases as described in the United States in the prevaccine era. The highest case-fatality rate (20 per 100,000 cases; 20 to 25 times higher than for preschool-age children) occurs among adults.[52,354,355] Second to mortality in developed countries, hospitalizations caused by varicella provide the next best estimate of severe disease burden. Various studies have described annual rates of varicella hospitalizations in the range of 4.0 to 4.5 per 100,000 population,[53,354] with some studies reporting lower or higher rates (2.6 to 9.9 per 100,000 population).[268,356] Reflecting the high incidence among children, the majority of varicella hospitalizations also occur among children; however, infants and adults are at significantly increased risk of severe disease and hospitalization compared with children 1 to 9 years of age.[48,53,267,354,357,358] Varicella also leads to a considerable number of school days missed for children as well as workdays missed for adult patients and for parents looking after sick children. These data are important for describing morbidity and for calculating the economic and societal costs of varicella, which is now vaccine preventable.

There are few data on the health burden of varicella in developing countries, in countries with high HIV seroprevalence, and in countries with tropical climates where a higher proportion of varicella cases may occur among adults. In these situations, varicella morbidity and mortality may be higher than described in developed countries. When comparing results across countries, however, differences in methods that may affect ascertainment of hospitalization, number of patients, and access to care must be considered. A large prospective household study in Guinea-Bissau in 2000–01 identified 1539 cases of varicella and found almost 80% of cases

occurred among children 1 to 9 years of age and only 4% of cases occurred among those older than 15 years of age.[352] However, 12% of cases occurred in infants and two patients died, so the case-fatality rate in this small series was 130 in 100,000 cases, approximately 50 times higher than in the United States.[52] Several studies in Sri Lanka have highlighted the high median age of varicella infection—14.5 years—and the associated morbidity.[340,359] One study of all patients admitted to the main infectious disease hospital in Sri Lanka from 2000 to 2001 demonstrated that 58% of the total 1690 hospitalizations resulted from varicella. Varicella was the most common disease treated at the infectious disease hospital and a significant cause of morbidity and mortality among adults.[359]

The estimated global annual varicella disease burden would include 140 million cases with approximately 6200 deaths.[360]

PASSIVE IMMUNIZATION

The first report indicating that varicella could be modified by human immunoglobulin (Ig) after exposure to VZV was in 1962.[20] It was demonstrated that varicella could be attenuated by giving Ig to varicella-susceptible children within 72 hours after household exposure. To try to improve passive immunization, globulin was prepared only from serum of patients recovering from HZ who had high VZV antibody titers. When given to healthy children who had been exposed to varicella, chickenpox was prevented.[361] This material, termed zoster immunoglobulin (ZIG) (ZIG), was then tested in a double-blind, controlled fashion in high-risk children with underlying leukemia, in whom it was found that that ZIG modified varicella.[362]

Subsequently, to increase the production of this product, which was in short supply, a high-potency globulin with titers equal to those in ZIG was prepared using plasma with high VZV antibody titers from persons who had not necessarily had HZ, increasing the potential donor pool.[363] Termed varicella-zoster immunoglobulin (VZIG), it was licensed and recommended for use in the United States in the early 1980s by the Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention (CDC).[364] It was estimated that VZIG contained more than 10 times the amount of antibody to VZV as ordinary Ig. After licensure of varicella vaccine in the United States, however, use of VZIG declined dramatically, and its production was discontinued. It was supplanted by a Canadian product, VariZIG, available initially in the United States under an investigational new drug protocol and licensed in 2012. VariZIG can be obtained by calling the 24-hour telephone number of FFF Enterprises (800–843–7477) or ASD Healthcare (800–746–6273).[365]

Passive immunization is recommended for persons without evidence of varicella immunity who are at risk for severe disease and complications, and for whom varicella vaccine is contraindicated, including immunocompromised patients, neonates whose mothers develop varicella around the time of delivery (5 days before to 2 days after), hospitalized premature infants born at 28 weeks or more of gestation whose mothers do not have evidence of immunity, hospitalized premature infants born after fewer than 28 weeks of gestation or who weigh less than 1000 g at birth regardless of maternal immunity, and pregnant women.[365] If there is uncertainty about immunity to varicella in an immunocompromised person, many experts recommend that it is safest to administer passive immunization upon a close exposure to VZV. Results of serologic tests regarding immunity to VZV cannot be relied upon, especially in this situation. VariZIG prophylaxis should be administered as soon as possible after an exposure to VZV in high-risk patients but within 10 days of that exposure.[365]

Zoster immune plasma, rarely used today, has efficacy in preventing or modifying varicella in immunocompromised patients,[366,367] but treatment with zoster immune plasma of immunocompromised patients with disseminated HZ is not effective.[368] Although passive immunization is usually effective against varicella, on occasion, disease may be severe in passively immunized immunocompromised children.[76,362,368,369]

ACTIVE IMMUNIZATION: THE OKA VACCINE STRAIN

The Oka strain of VZV is the only vaccine strain now available other than a vaccine produced in Korea (SuduVax) in 1988, the molecular profile of which closely resembles that of the Oka strain.[370]

SuduVax contains the MAV/06 varicella strain that was isolated from a 33-month-old South Korean boy with chickenpox in 1989, in Seoul, South Korea.[371] Prior to clinical trials with Oka in the 1970s, the American KMcC strain underwent a few clinical trials; it proved inferior in attenuation compared with the Oka strain.[372–375]

To develop the Oka strain of varicella vaccine,[1,376] fluid was taken from the vesicles of an otherwise healthy 3-year-old boy (whose family name was Oka) who had typical chickenpox, caused by wild type (WT) VZV. His VZV was isolated in primary human embryo lung fibroblast cell cultures. The virus was serially propagated 11 times at 34°C in human fibroblast cell cultures, followed by 12 passages in guinea pig fibroblast cells to further attenuate the virus. Finally, there were two passages in WI-38 human diploid cells (HDCs) to prepare the master seed virus, followed by three passages in MRC-5 HDCs to prepare the working seed virus. Vaccine pools were usually made after two or three additional passages in MRC-5 cells.

For vaccine preparation, infected tissue cultures were washed with phosphate-buffered saline, and infected cells were harvested in the presence of ethylenediaminetetraacetic acid. The cell suspension in vaccine medium was then sonicated to obtain cell-free virus; a titer of 1500 to 5000 plaque-forming units (PFU) per milliliter of VZV was usually obtained. Initial safety testing of the vaccine included demonstration of lack of pathogenicity after parenteral and intracerebral inoculation of small animals and monkeys. The absence of C-type particles and latent viruses was confirmed morphologically and biochemically.

Vaccine Virus Attributes and Varicella-Zoster Virus Strain Identification

The Oka vaccine virus has various biological, biophysical, and molecular attributes that distinguish it from WT viruses.[13,377] The vaccine strain was cold-adapted to grow at 34°C, and it grows less well at 39°C than WT strains.[13] It also grows better in guinea pig cells and is more immunogenic for guinea pigs than WT VZV.[378–380] All varicella vaccines contain a mixture of Oka vaccine strain variants, even with some sequences resembling WT VZV, which might rarely impact virulence in recipients or laboratory identification of vaccine strains.[381–385]

The Oka strain (parental/wild and vaccine) and Dumas WT VZV have been sequenced (Fig. 62.5). Over the entire genome of 125,000 base pairs, 42 individual single nucleotide polymorphisms (SNPs) have been identified, only a third of which result in an amino acid substitution. Deep sequencing of the vaccine has identified additional putative polymorphisms, some of which have been confirmed.[385–387] Most of these differences are in ORF62 and its duplication, ORF71.[383,388,389] It is not known which, if any, of these mutations are responsible for attenuation of the vaccine strain.[381,390–394] Two independent laboratories have shown that four vaccine point

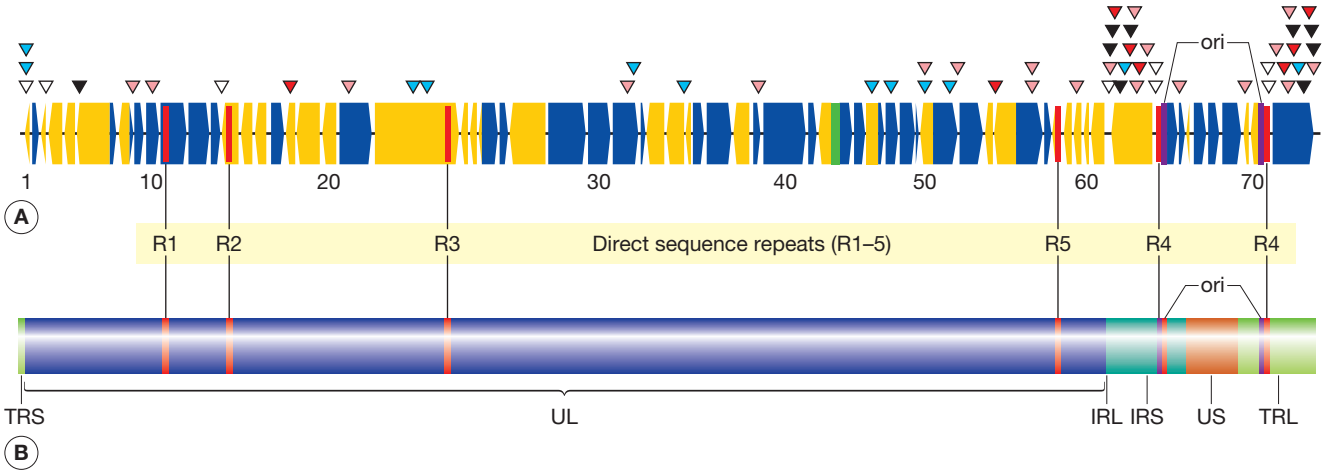


Figure 62.5. Molecular differences between wild-type and vaccine-type varicella-zoster virus (VZV). A, Model of the VZV genome illustrating the single-base differences between pOka (parental-strain Oka) and vOka (vaccine-strain Oka). *White triangles*, single nucleotide polymorphism (SNP) located in noncoding region; *black triangles*, SNP present uniformly among vaccine strains leading to amino acid substitutions; *red triangles*, SNP present uniformly among vaccine strains that do not lead to amino acid substitutions; *pink triangles*, SNP present as a mixed base in vaccine preparations and vaccine-associated SNP that leads to amino acid substitutions; *blue triangles*, SNP present as a mixed base in vaccine preparation that does not lead to amino acid substitutions. Dark blue open reading frames (ORFs) are transcribed from the sense strand; yellow ORFs are transcribed from the antisense strand. **B,** Display of VZV genome architecture. IRL, internal repeat long; IRS, internal repeat short; ori, origin of replication; TLR, terminal repeat long; TRS, terminal repeat short; UL, unique long; US, unique short. *(From Schmid DS, Jumaan AO. Impact of varicella vaccine on varicella-zoster virus dynamics.* Clin Microbiol Rev. *2010;23:202–217.)*

mutations are universally present in all strains isolated from documented vaccine adverse events, all of which are located in ORF62.[383,386,388,389] These findings provide a logical starting point for evaluating the contribution of individual, elevated-risk point mutations to VZV pathogenicity. Analysis of vaccine-associated SNPs among more than 100 documented cases of vaccine varicella and HZ revealed two loci in ORF62 at which selection of the WT allele has occurred. The data suggest that the WT allele at this locus in vaccine virus contributes to rash formation, although the mechanism for this remains unknown.[386]

Differences in the migration patterns of DNA fragments of the vaccine-type virus and WT strains were demonstrated after cleavage of purified DNA obtained from viruses propagated in cell cultures, using restriction endonucleases. In a comparison of DNA from the vaccine-type and WT viruses, different cleavage patterns were seen using HpaI, BamHI, BglI, and PstI enzymes.[376,395–397] In early U.S. studies using BglI, a novel restriction site in vaccine-type virus was demonstrated.[396] It was later found that approximately 20% of U.S. WT strains are also BglI-positive. With PstI, an additional cleavage site was demonstrated in WT virus circulating in the United States (PstI-positive) that is not found in vaccine-type virus.[395,398] Oka VZV is PstI-negative, and, with rare exceptions, circulating WT VZV in the United States is PstI-positive.[154] In Japan, however, approximately 10% of circulating WT VZVs are PstI-negative, as is the vOka strain and its parental strain pOka.

Historically, the first practical approach that did not require virus isolation to distinguish between vaccine-type and WT VZV used PCR-amplified DNA fragments followed by their digestion by specific restriction endonucleases.[398] This method is based on the differences (noted earlier) between WT VZV and the Oka strain. In one method, two primer pairs are used, one that generates a fragment flanking the BglI restriction site in vaccine-type virus (gene 54), and another that generates a fragment that is cleaved in all WT VZVs by PstI (gene 38).[398,399] All Oka strains lack a PstI restriction site and carry a BglI restriction site. Approximately 80% of WT VZVs in the United States lack a BglI restriction site, and until recently all had a PstI restriction site. Early studies of viruses obtained from New York, California, and Australia (113 specimens) did not identify circulating viruses with the DNA profile of the Oka strain; although a number of circulating Japanese genotype viruses (clade 2) were found, none lacked a PstI restriction site.[147] In 2001–02, 3% of strains from the West Coast of the United States tested by CDC laboratories ($N = 130$) were clade 2, but they were Pst1-positive.[143] A study of VZV isolates from West Coast cases of HZ meningitis/encephalitis, however, for the first time, identified three WT Oka-like clade 2 VZV strains lacking a Pst1 restriction site in the United States, indicating that vaccine-like WT strains have been introduced here.[154] In countries where the Japanese WT VZV is not circulating, a useful marker for the vaccine strain is the absence of a PstI restriction site; however, rare identifications in the United States,[54] Australia,[147] and China[142] of circulating Japanese WT strains that are Pst1-negative highlight the limitation of using the PstI marker alone as a vaccine strain marker. In Japan, PCR amplification of a portion of gene 14 and analysis of the PstI site was used to distinguish between vOka and other clinical isolates.[400] SmaI and NaeI restriction sites in ORF62, which clearly discriminate between the vOka strain and all WT strains,[390,401] have been used in rapid real-time PCR assays that are useful for identifying adverse events associated with varicella vaccine.[226,402]

Currently, it is recommended that for determining whether adverse events are attributed to vaccine type VZVs, markers in genes 38 (PstI), 54 (BglI), and 62 (especially at positions 106262, 108011) be used.[377] The marker at position 106262 in gene 62 results from the substitution of a C for T in the vaccine strain, resulting in a SmaI restriction site in vOka. In one instance, a marker at position 107252 was found in a WT VZV from an elderly patient with HZ, so this marker may not be useful for identifying vaccine-type VZV.[403] Nevertheless, it is now recommended that for identification of VZVs causing adverse events after vaccination that markers at the following positions be determined: 105705, 106262, 107252, and 108111



A

position(+100,000)		5169	5310	5356	5544	5705	6262	6262	7136	7252	7599	7797	8111	8838	9137	9200
Oka parental	all 9 clones	A	A(Leu)	T(Ile)	A(Val)	T(Ala)	T(Arg)	A(Ala)	T(Ala)	T(Ser)	A(Val)	A(Leu)	T(Pro)	A(Met)	A	A
Oka vaccine	clone 1	A	A	C(Val)	G(Ala)	C(Ala)	C(Gly)	G(Ala)	C(Ala)	C(Gly)	G(Ala)	A	C(Pro)	A	A	G
	clone 2	A	A	C	G	C	C	A	C	C	G	A	C	A	A	A
	clone 3	G	G(Ser)	C	G	C	C	A	C	C	A	G(Pro)	C	A	A	G
	clone 4	A	G	C	G	C	C	G	C	C	G	A	C	A	A	G
	clone 5	G	A	C	G	C	C	A	C	C	G	A	C	A	A	A
	clone 6	A	A	C	G	C	C	T(Ala)	C	C	A	A	C	A	A	G
	clone 7	G	A	C	G	C	C	A	C	C	G	A	C	A	A	A
	clone 8	G	A	C	G	C	C	A	C	C	G	A	C	G(Thr)	G	A
	clone 9	G	G	C	G	C	C	A	C	C	G	G	C	G	G	A

B

Figure 62.6. Summary of sequence analysis of the distinct nine clones isolated from Oka vaccine virus and the nine clones from Oka paternal virus. A, Structure of gene 62. Amino acid residues (aa) are numbered from 1 to 1310 moving from the N- to the C-terminus. Vertical lines indicate the positions of nucleotide differences between Oka vaccine and its parental viruses. **B,** Sequence analysis of clones from Oka vaccine and its parental virus. All nine clones of paternal virus had the same DNA sequence, whereas the vaccine virus was a mixture of at least eight different clones that had a variety of mutations at 15 positions. Nucleotide changes at eight positions caused amino acid conversions. *(From Gomi Y, Ozaki T, Nishimura N, et al. DNA sequence analysis of varicella-zoster virus gene 62 from subclinical infections in healthy children immunized with the Oka varicella vaccine.* Vaccine. *2008;26:5627–5632.)*

(Fig. 62.6).[385] Genotyping of ORFs 21, 22, and 50 has also been used to differentiate between U.S. WTs and Oka vaccine strains.[141,143,147]

It is now considered medically relevant to distinguish vaccine and wild strains of VZV in vaccinated patients with severe rash, neurologic events, or other suspected serious adverse events to determine if they are related to the Oka strain or not, and also to determine if HZ in a patient who was vaccinated in the past is caused by the Oka or the WT strain. The ability to distinguish between vaccine and WT VZV, as well as different genotypes, has led to the discovery that patients may (rarely) have acute or latent infection with more than one type of VZV.[404,405] Detailed methodologies for clinical microbiology laboratories have been published.[180]

Molecular Evidence of Attenuation

Remarkable mutations were identified in IE62 when the nucleotide sequences of the Oka vaccine and its parental VZV were compared.[381,391,392] As shown in Fig. 62.6, the vaccine virus gene 62 contains a mixture of different sequences that have variations at 15 nucleotide positions, whereas only one sequence is present for the Oka parental virus gene 62. There were no nucleotide changes in genes 4, 10, 61, or 63, all of whose products are known to transregulate VZV genes. Nucleotide changes in gene 62 of the Oka vaccine strain resulted in the creation of new restriction sites that were recognized by BssHII, SmaI, and NaeI, whereas that of the Oka parental virus and more than 200 other clinical isolates had none of these cleavage sites. Thus, the Oka vaccine virus can also be distinguished from the parental virus and other clinical isolates using a simplified restriction-enzyme fragment length polymorphism analysis using NaeI, SmaI, and BssHII.[392,406,407]

VZV gene 62 is present in large quantities in virion tegument,[110] and the yield of infectious virus generated by four overlapping cosmid clones is greatly improved when a gene 62–expressing plasmid is transfected.[408] Thus, the mutation in Oka gene 62 may influence the replication of the virus and play a role in the attenuation of VZV. Studies of the regulatory activities of gene 62 in a transient transfection assay indicate that this gene in the vaccine virus has weaker transactivation activity than that of the WT virus in activating IE gene promotors (such as gene 4), E gene promotors (such as gene 28 for DNA polymerase and gene 29 for the major DNA-binding protein), and L gene promoters (such as gene 68 for gE) (Fig. 62.7).[381] An ORF0 transitional stop codon mutation has recently been proposed as contributing to attenuation of Oka VZV.[382] Although there are clear molecular differences between WT VZV and the Oka strain, the exact molecular mechanisms of attenuation remain to be further identified.

Clinical Evidence of Attenuation

Clinical evidence of the attenuation of the Oka strain of VZV is as follows. First, the incidence of rash after subcutaneous injection and inhalational methods of delivering varicella vaccine is far lower than the incidence of rash and other symptoms of natural infection.[409] Second, when rare, inadvertent transmission of vaccine virus to a healthy susceptible contact occurs, the disease is invariably mild or subclinical.[157,229,410–414] The rate of transmissibility of the Oka strain in a household setting in leukemic vaccinees was four to five times lower than that of WT VZV,[157] and its transmissibility from healthy vaccinees is almost negligible. Clinical evidence of reversion of the Oka strain to virulence has not been observed. Additional evidence suggestive of attenuation is that, in contrast to natural

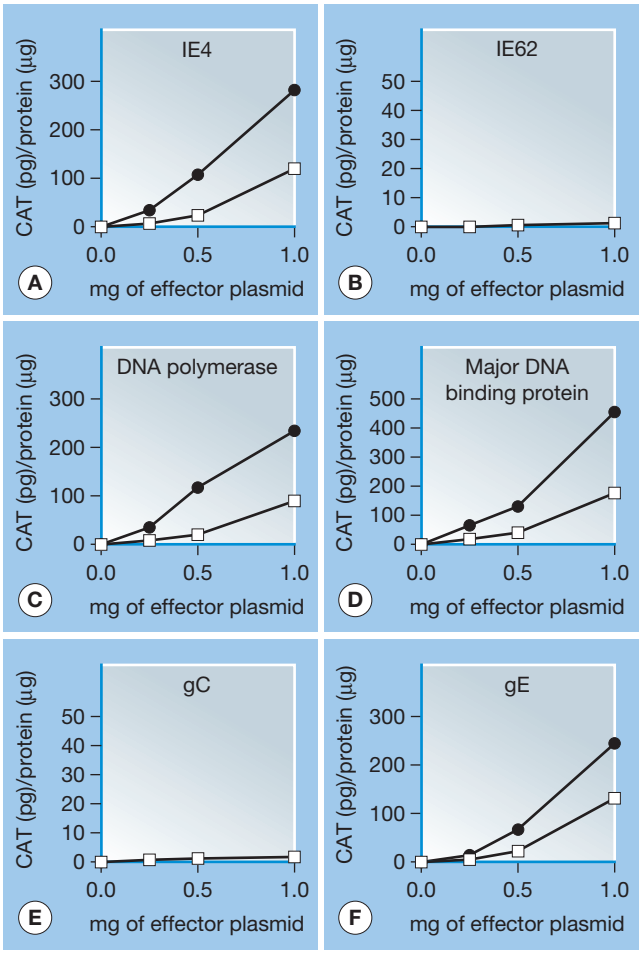


Figure 62.7. Comparison of transactivation activity by the Oka vaccine (squares) and its parental (circles) IE62 on varicella-zoster virus gene expression, including immediate early (IE) genes, E genes (DNA polymerase and DNA binding protein), and late genes (glycoproteins C and E). CV1 cells were cotransfected with 0.25 µg of a reporter plasmid and the same amounts of either pVac-IE62 or pPar-IE62. The CAT concentrations were standardized to each protein concentration. All experiments were repeated at least three times independently. *(From Gomi Y, Imagawa T, Takahashi M, et al. Comparison of DNA sequence and transactivation activity of open reading frame 62 of Oka varicella vaccine and its parental viruses.* Arch Virol Suppl. *2001;17:49–56.)*

infection, vOka uncommonly induces a viremia after immunization. Infectious viremia was not detected in 18 healthy or 10 immunocompromised vaccinees who had no clinical symptoms.[415] Viremia after vaccination was demonstrated using PCR, however, in five (3%) of 166 vaccinated Japanese children who were asymptomatic.[416] Viremia from vaccine virus was found in two leukemic children in remission for 5 and 6 months when they were immunized, one of whom died. They had vesicles and fever 32 and 20 days, respectively, after vaccination.[417,418] Thus, viremia may occur after vaccination but at a lower frequency than that seen with WT varicella. Viremia has also been documented after HZ and vaccination against HZ (reviewed in Levin[419]). Viremia can lead to latent VZV infection of neurons and explains why varicella vaccinees can develop HZ from vaccine-type virus although they never experienced a VZV rash.

Production and Constituents of Vaccine

For vaccine preparation, VZV-infected cells are harvested, suspended in the vaccine medium, and sonicated or otherwise ruptured to obtain cell-free virus. Clinical studies suggest that the ratio of viral antigen to infectious virus may be important in eliciting the immune response to vaccination.[420] Vaccine medium can vary according to the manufacturer; however, it generally contains sucrose and buffering salts. The vaccine is marketed in a lyophilized form to improve stability. Reconstitution is accomplished with sterile distilled water according to the manufacturer's directions. Manufacturers provide reconstitution fluid with the vaccine.

Oka varicella vaccine is produced in the United States (Varivax; ProQuad; Merck & Co.), Belgium (Varilrix; Priorix-tetra; GlaxoSmithKline), Japan (Okavax; Biken, distributed by Sanofi Pasteur), and China (four manufacturers: Shanghai Institute of Biologic Products, Changchun Keygen Biological Products Co., Changchun BCHT Biotechnology Co. [Baike], Changchun Changsheng Life Sciences Ltd.). SuduVax is produced in South Korea (Green Cross). The vaccines vary in passage number in HDCs, trace antibiotics added to ensure sterility, stabilizers, and other minor constituents. Inactive ingredients in Merck vaccines include: sucrose, hydrolyzed gelatin, NaCl, monosodium glutamate, sodium phosphate dibasic, potassium phosphate monobasic, KCl, urea, neomycin, and bovine serum. GlaxoSmithKline vaccines contain the following inactive substances: amino acids for injection, lactose, mannitol, neomycin sulfate, sorbitol, and water for injections.

Seed lots of Oka vaccine were prepared from virus passed 11 times in human embryo lung fibroblast cells, 12 times in guinea pig fibroblast cells, and five times in HDC. Manufacturers add from three to nine additional passages in HDC to prepare enough vaccine to meet marketing needs. Varicella vaccine is stored at refrigerator temperatures (2°C to 8°C) or at freezer temperature (less than −15°C), according to the manufacturer's instructions.

Vaccine Immunogenicity, Efficacy, and Safety

Takahashi initiated clinical trials with the Oka vaccine in Japan in 1974.[1] The vaccine was first given to 70 healthy children at dosages ranging from 100 to 2000 PFU. The vOka was immunogenic at dosages greater than 200 PFU, and no significant reactions were noted. Initial protective efficacy was demonstrated by vaccinating susceptible household contacts immediately after exposure.[421] When healthy children were vaccinated within 3 days of exposure, none of 18 developed varicella, whereas all the 19 unvaccinated contacts did. Additional early studies in Japan included vaccination of children with serious underlying diseases including malignancies where the potential severity of natural varicella was great. The Oka strain was licensed to several other pharmaceutical companies, each of which later conducted clinical trials in Europe, Asia, the United States, and Canada.[422,423] In the United States, there is ample evidence of the effectiveness and safety of vaccine, as it has been administered to healthy children since its licensure in 1995 (see reviews in references 424 to 426).

VARICELLA-ZOSTER VIRUS VACCINE IN HEALTHY CHILDREN

Dosage and Route of Administration

Currently licensed monovalent vaccines include Varivax (Merck), manufactured to guarantee greater than 1350 PFUs in a 0.5-mL dose at expiration and released across a dosage

range of 3000 to 17,000 PFU/dose; Varilrix (GlaxoSmithKline), 2000 PFU/dose; Okavax (Biken), not less than 1000 PFU/dose; four vaccines licensed in China with at least 2000 PFU/dose (0.5 mL) (manufacturers: Shanghai Institute of Biologic Products, Changchun Keygen Biological Products Co., Changchun BCHT Biotechnology Co. [Baike], Changchun Changsheng Life Sciences Ltd); and SuduVax (Green Cross, South Korea) with greater than 1350 PFUs/dose.

The Oka vaccine is administered subcutaneously. Although data on the intramuscular route are limited, it, too, appears to be safe and effective.[427] All manufactured varicella vaccines are lyophilized; both refrigerator-stable and frozen vaccine formulations are available. In 2006, a Merck process-upgrade varicella vaccine was developed for successful storage of varicella vaccine at refrigerator temperatures; it contains 50,000 PFU of VZV and was tested in 1366 children older than 13 years of age. Injection-site reactions were 10% more common than after Varivax, but not severe. Two doses were safely administered; immunogenicity was similar to that of Varivax.[428,429] In the United States, six formulations of Merck vaccines that contain vOka are licensed: monovalent, measles-mumps-rubella-varicella (MMRV), and HZ vaccines (both lyophilized) in refrigerator-stable and frozen formulations, but only the frozen formulations are available.

The MMRV vaccine (ProQuad, Merck), licensed for use in children 12 months to 12 years of age, contains the same measles, mumps, and rubella (MMR) components as those in MMRII, but it has a higher concentration of varicella vaccine virus (approximately 10,000 PFU at expiration) than does Varivax (1350 PFUs at expiration). This high dose of VZV in MMRV was required to achieve acceptable immune responses to the varicella component of the vaccine. The reason for the apparent interference with VZV immunity was not proven but assumed to be related to the measles virus component. A formulation of MMRV manufactured by GlaxoSmithKline (Priorix-Tetra) is also licensed for use in a number of countries. The titer of VZV is the same as that of Varilrix.[428,430] GlaxoSmithKline-manufactured varicella vaccines are lyophilized and stored at refrigerator temperatures.[431–433]

A number of dosage-range studies of vaccine administered subcutaneously were done before licensure of varicella vaccines. Children in Japan were given varying amounts of the vOka strain to determine the minimal effective dosage.[434–441] It is now clear, however, that the higher the vaccine dosage, the higher the resultant immune response.

Alternative methods of administration of varicella vaccine have been studied, such as inhalation. The vaccines were well tolerated and immunogenic but licensure for those methods of administration was not pursued.[442,443]

Immune Responses to Vaccination

Because prelicensure and postlicensure studies of safety and immunogenicity of varicella vaccine used varying dosages of the Oka strain and vaccines from several manufacturers, comparisons across studies are difficult. Additionally, different serologic tests have been used to assess immunogenicity, including neutralization, FAMA, gp-ELISA, and other immunofluorescent antibody tests. Finally, cutoff levels to define seroconversion with the gp-ELISA test have varied, with initial studies using greater than 0.6 gp-ELISA U/mL and newer studies using a higher cutoff level or serologic response rate of greater than 5 gp-ELISA U/mL.

After immunization with at least 500 PFU of the Oka strain, serum IgG antibody responses are easily detected within 1 month after vaccination, and antibody can then be detected for months to years thereafter. After immunization, however, individual and mean IgG antibody titers may be 10 to 30 times lower than those after natural infection, depending on the dosage of vaccine virus, the number of doses given, and the patient's age at immunization.[38,420,444,445] Early studies of duration of vaccine-induced immunity observed increasing titers of VZV antibodies with time. Presumably as a result of exposure to the WT virus and possibly subclinical reactivation of latent VZV[218,446] subclinical boosting of the immune response occurred, eventually resulting in IgG titers that were similar to those after natural infection.[447,448] An early study demonstrated asymptomatic increases in VZV antibody titers in vaccinated children, but it is unclear if this boosting was a consequence of exposure to WT VZV or subclinical reactivation of the vaccine virus or both.[449] Long-term studies are needed in countries with universal vaccine programs to assess duration of the immune response and protection from varicella in the absence of external boosting from varicella exposures. Internal boosting from silent reactivation of latent Oka VZV may also aid in boosting immunity.

Serum IgA antibody is detectable only occasionally and at low levels in vaccinees immunized by inhalation, and it is virtually undetectable in subjects immunized subcutaneously.[409] In contrast to the response after varicella, secretory IgA antibody responses to VZV have not been demonstrated after immunization, regardless of the route of administration or dosage of vaccine.[409] It is not known if IgA at mucosal sites offers protection against infection.

One study suggested that genetic factors play a role in humoral immune responses to varicella vaccine.[450] A study of 141 families with multiple children found a clustering of robust and less-vigorous humoral immune responses among siblings in certain families.

Immune Correlates of Protection Against Varicella

Immune correlates of protection against varicella have been studied but need further clarification and development. The partial efficacy of passive immunization shows that antibody is at least partly responsible for protection, but cell-mediated killing is also important, particularly for control of disease. It is probable that B- and T-cell functions induced by vaccination collaborate in protection. During prelicensure clinical trials conducted by Merck from 1982 to 1991 in the United States, a correlation between the dosage of vaccine administered and the antibody level measured by gp-ELISA was observed.[448] In 3303 children immunized in U.S. clinical trials during the late 1980s, VZV antibody titers were determined with the VZV gp-ELISA assay 6 weeks after immunization.[451] Their seroconversion rate was 96%, with a geometric mean titer (GMT) of 1 : 12 gp-ELISA U/mL. The cutoff point considered positive for seroconversion using the gp-ELISA test, however, was not given. In a subsequent analysis conducted by Merck in 2004, a cutoff of 5 gp-ELISA U/mL was used as a positive humoral immune response. After one dose of Varivax in 1104 vaccinees, the seroconversion rate was 87.3%, with a GMT of 12.8 gp-ELISA U/mL.[452] As part of this study, 1017 vaccinees received a second dose of varicella vaccine 3 months after the first. The seroconversion rate increased to 99.5% and the GMT to 141.5 gp-ELISA U/mL, indicating a marked booster response after the second dose.

A gp-ELISA titer of 5 U/mL or greater 6 weeks after vaccination is reported to be an approximate correlate of protection for individual vaccinees. Rates of breakthrough disease (defined as development of chickenpox resulting from WT VZV infection despite vaccination at least 6 weeks previously) were significantly lower among children with varicella antibody titers of greater than gp-ELISA 5 U/mL, compared with children with titers of less than gp-ELISA 5 U/mL.[235,453–455] After one dose of varicella vaccine, 85% to 89% of children

achieve this antibody level, and after two doses, more than 99% achieve this level. A second dose, either after 3 months or in 3 to 5 years, results in a marked boost in humoral and cellular immunity.[452,456–466] (See also Varivax and ProQuad package inserts.) The gp-ELISA assay, however, is not commercially available and, as has been mentioned, false-positive values may occur.

A positive FAMA titer or skin test after natural varicella, at the time of close exposure to VZV, is highly predictive of protection against disease.[196,232,241,467] A positive FAMA after vaccination, at the time of intimate exposure to the virus, also correlates strongly with protection.[232,467] In 2006, a seroprevalence study, using the FAMA assay, of toddlers vaccinated from 1998 to 2003 indicated a seroprevalence rate of 76% (FAMA >1:4) 6 to 16 weeks after one dose of Varivax.[467] A similar study in Korean infants who were vaccinated at 12 months of age and in whom VZV antibodies were measured by FAMA (cutoff: 1:4 or greater) a median of 6 months after vaccination showed a seroprevalence rate of 84%.[468] Consequently, it was postulated that many cases of varicella in vaccine recipients who received one dose might be the result of primary vaccine failure.

In prelicensure clinical trials, it was noted[451] that the seroconversion measured by gp-ELISA in 13- to 17-year-old adolescents was only 79%; moreover, their GMT was 1:6, half the level seen in healthy children in this study. These results led to the early recommendation in the United States of two doses of vaccine for persons 13 years of age and older. Since 2007, two doses of varicella vaccine have been recommended for all healthy children in the United States.

There are few published data on the use of commercially available VZV antibody tests to determine seroconversion rates in healthy children. Demonstrating the lower sensitivity of commercial assays compared with the gp-ELISA or FAMA assays, a study in Korea documented that only 45% of healthy children tested positive for VZV IgG antibodies by a commercial ELISA test (Dade Behring, Marburg, Germany) a median of 6 months after vaccination at 12 months of age, compared with 84% when FAMA was used.[468] One study suggests that a commercial ELISA test manufactured in Germany (Serion) may be useful and superior to the Dade Behring ELISA.[469]

The immunogenicity of Merck MMRV (ProQuad), licensed in the United States and a number of countries, is excellent for all four viral components. This combination vaccine was difficult to develop in the United States, because antibody titers to the varicella component were at first unacceptably low.[470–473] In prelicensure clinical trials, three dosages were tested in 1559 healthy children 12 to 23 months of age; they contained 3.48 $\log_{10}$ (approximately 3019), 3.97 $\log_{10}$ (approximately 9333), and 4.25 $\log_{10}$ (approximately 17,738) PFU of VZV. Because the safety and immunogenicity profiles were optimal in the children who received the higher dosage, a formulation close to this titer was chosen for licensure—not less than 3.99 $\log_{10}$.[457,458,462] Table 62.1 shows the antibody responses against all four virus components with the licensed MMRV formulation containing 3.99 $\log_{10}$ (approximately 10,000 PFU) of VZV.[474]

In another study of 2925 children in the United States and Canada, in which three consistency lots of ProQuad vaccine were tested, a response rate of greater than 5 gp-ELISA U/mL was reported in 93.5% after one dose.[460] Immunogenicity against VZV compared favorably with the values obtained in 1012 children who were given MMR and Varivax at separate sites.

In evaluating the MMRV vaccine produced by GlaxoSmithKline (Priorix-Tetra), antibody titers to VZV IgG were determined by indirect immunofluorescence (Virgo) in 371 children. The seroconversion rate after two doses 6 to 8 weeks apart was 100%.[430] Excellent responses to the MMR vaccine components also occurred.

Cell-Mediated Immune Responses

In vaccinated healthy children, the skin test became positive as early as 4 days after immunization in about half the children, 7 to 9 days before detection of neutralizing antibodies.[194] Similarly, in another study, lymphocyte transformation became positive approximately 1 week before neutralizing antibodies were detected.[475] The stimulation index against VZV antigen in 74 healthy U.S. children 6 weeks after immunization was 58.6 (±6.5) (Table 62.2).[459,476]

In a study conducted at Stanford University, 39 healthy children received one dose of varicella vaccine and a similar number received two doses given 3 months apart. The mean VZV stimulation index at 1 year after one dose was 9.3 (±1.3) and after two doses it was 22.2 (±6.42).[463] In a similar study, a second dose of varicella vaccine was given 4 to 6 years after the first dose to 419 healthy children. Before receipt of the second dose, the mean VZV stimulation index was 40.3 (±5), and 3 months later it was 61.4 (±6).[459]

Persistence of Varicella-Zoster Virus Antibodies and Cellular Immunity

Several studies indicate that humoral immunity persists for many years after immunization of healthy children with one

TABLE 62.1 Antibody Responses to One Dose or Two Doses of ProQuad in Clinical Trials

Antigen	Time	Subjects *(N)*	Response Rate[a] % (95% CI)	GMT Titer[b] (95% CI)
Measles	After 1 dose	943	97.8% (96.6%–98.6%)	2966.0 (2793.4–3149.2)
	After 2 doses	943	99.4% (98.6%–99.8%)	5919.3 (5486.2–6386.6)
Mumps	After 1 dose	920	98.7% (97.7%–99.3%)	106.7 (99.1–114.8)
	After 2 doses	920	99.9% (99.4%–100%)	253.1 (237.9–269.2)
Rubella	After 1 dose	937	97.7% (96.5%–98.5%)	91.1 (85.9–96.6)
	After 2 doses	937	98.3% (97.2%–99.0%)	158.8 (149.1–169.2)
Varicella-zoster virus	After 1 dose	864	86.6% (84.1%–88.8%)	11.6 (10.9–12.3)
	After 2 doses	864	99.4% (98.7%–99.8%)	477.5 (437–520.7)

[a]Seroconversion: ≤1.25 to ≥5 gp-ELISA U/mL.
[b]GMTs reported in mIU/mL for measles; Ab U/mL for mumps; IU/mL for rubella; and gp-ELISA U/mL for VZV.
95% CI, 95% confidence interval; Ab, antibody; GMT, geometric mean titer; gp-ELISA, glycoprotein enzyme-linked immunosorbent assay; VZV, varicella-zoster virus.
Modified from Kuter BJ, Brown ML, Hartzel J, et al. Safety and immunogenicity of a combination measles, mumps, rubella and varicella vaccine (ProQuad). Hum Vaccin. *2006;2:205–214.*

TABLE 62.2 Immune Response to One and Two Doses of Varicella Vaccine (Varivax) and MMRV (ProQuad) in Children 1 to 12 Years Old

Immune Response	6 wk After Dose 1		6 wk After Dose 2 (3-mo Interval Between Doses)		6 wk After Dose 2 (4- to 6-y Interval Between Doses)	
	Varicella Vaccine (Varivax)	MMRV (ProQuad)	Varicella Vaccine (Varivax)	MMRV (ProQuad)	Varicella Vaccine (Varivax)	MMRV (ProQuad)
VZV IgG gp-ELISA (% seroconverted)	85–92[452,458]	90–94[458,460,462]	99.6[452]	99.2[458]	99.4[456]	99.4[61]
GMT VZV IgG gp-ELISA (U/mL)	12–13[456,458]	13–15[452,453,458]	145[456]	588[458]	212–219[459,461]	317[456]
Mean stimulation index[a]	Group A 28.6 ± 6.21[463] Group B 22.1 ± 3.84[463]	—	Group B 36.9 ± 9.3[463]	—	Group B 58.6 ± 6.52[b,476]	—

[a]Mean stimulation indices from different laboratories or from different studies should not be directly compared.
[b]Mean stimulation index immediately prior to second dose of varicella vaccine was 40.3 ± 5.
GMT, geometric mean titer; gp-ELISA, glycoprotein enzyme-linked immunosorbent assay; IgG, immunoglobulin G; MMRV, measles-mumps-rubella-varicella; VZV, varicella-zoster virus.

vaccine dose, particularly when circulation of WT VZV continues. In Japan, a 5-year follow-up study of 26 healthy immunized children revealed that 100% had detectable neutralizing antibodies and 96% had positive FAMA titers.[447] None of these children developed breakthrough chickenpox, although many were exposed to VZV. In a subsequent study of 106 Japanese children, 14 of whom had been receiving steroid therapy when immunized up to 10 years previously, there were five cases of breakthrough chickenpox.[477] Four of the cases developed in year 1 after immunization. There were 147 recognized occasions when these children were exposed to VZV. Serum specimens were available from 38 children: FAMA antibody titers were 1 : 4 or greater in 37 (97%), with a GMT of 1 : 9. In 29 control children who had experienced natural varicella, FAMA titers were 1 : 4 or greater in 100%, with a GMT of 1 : 10. The VZV skin test was positive in 97% in each group. Because the average VZV antibody titer after immunization is lower than that after natural varicella, these results indicate that a boost in antibody titer had occurred in vaccinees, possibly as a result of subclinical reinfection after exposure to exogenous virus.

A 10-year follow-up as well as a subsequent 20-year follow-up after immunization in Japan showed that 25 of 25 young adults immunized as children remained seropositive.[477–479]

A follow-up of 87 U.S. vaccinees who were given approximately 17,000 PFU of vaccine revealed that 97% retained positive VZV immune responses (measured by gp-ELISA) 5 to 6 years after immunization.[480] In other U.S. studies, in which most of 127 vaccinees received dosages of vaccine of roughly 1000 to 3000 PFU, 95% remained seropositive for up to 6 years.[480] Persistence of antibodies for 2 years after vaccination was 94% in a study of 36 immunized toddlers.[481] Of more than 500 healthy additional children who were immunized in various studies in the United States, for whom there is a follow-up of as long as 6 years, greater than 95% remained seropositive by gp-ELISA.[453,482,483] Another group of 603 vaccinated children showed greater than 89% persistence of varicella antibodies 6 years after vaccination.[484] In a study of 53 vaccinated children whose FAMA antibody titers were measured for as long as 10 years after immunization, most remained VZV antibody positive.[485] Humoral immune responses against VZV, as measured by FAMA, appear to be more durable in healthy children than in immunized adults or leukemic children.[232,486]

A study of the persistence of antibodies after immunization with ProQuad in 3928 healthy children indicated that 1 year after vaccination, antibodies (measured for VZV by gp-ELISA) were detectable in 95% of children against all four viruses.[460] This study also measured antibody levels among study children 4 to 6 years old who had received Varivax 3 to 5 years earlier, before receiving MMRV as a second dose of the varicella vaccine component. Of these children, 88% had VZV gp-ELISA antibody levels of more than 5 U/mL before the second dose of varicella vaccine.[461]

A major question is whether vaccine-induced immunity to VZV will remain positive when exposures to VZV become unusual in the postvaccine era. It was possible to assess whether exposure and consequent boosting are required to maintain positive VZV antibody titers measured by FAMA after vaccination in a small group of handicapped institutionalized Japanese children with no exposures to VZV for 5 years after immunization.[487] The 16 vaccinated children, as well as seven children who had experienced natural varicella, maintained positive VZV antibody titers for 5 years. Skin test reactions also remained positive in 14 of 16 vaccinees. Six VZV-seronegative children remained seronegative during this 5-year period, indicating that no VZV was circulating in this isolated population. Thus, humoral and cellular immunity conferred by vaccine lasted at least 5 years, without boosting by natural infection. It is possible that subclinical reactivation of latent VZV may serve to stimulate immunity, making exogenous exposure to VZV alone less necessary to preserve immunologic memory.

Positive cell-mediated immunity, as measured by lymphocyte stimulation or skin testing, was shown to be durable for many years in a number of other studies in the United States and Japan, all of which, however, were done while there was continuing circulation of WT VZV.[477–480]

In a California study involving 11,356 children followed by active surveillance, the possibility of waning immunity after a single dose of varicella vaccine was explored. Breakthrough varicella, however, was not verified by laboratory testing; clinical illness was twice as likely to be moderate/severe (defined as manifesting over 50 skin lesions) in children who became ill more than 5 years after vaccination compared with those who developed breakthrough disease after a shorter interval. The annual breakthrough rate of disease was higher in children vaccinated 9 years earlier compared with those vaccinated 5 years previously.[488] Although this study suggests waning immunity, primary vaccine failure in 16% to 24% of children after one dose of vaccine also plays a significant role in the failure of a single dose of vaccine to provide complete protection against varicella.[467,489] The possibility of waning immunity after one dose of varicella was reexamined in a case-control study; it was found that development of breakthrough varicella was directly related to the interval of time after immunity. The study was

however retrospective and laboratory confirmation of varicella was not obtained.[490] Importantly, newer studies have not identified waning immunity after varicella vaccination in children who had had either one or two doses of varicella vaccine.[491,492] Additional studies regarding whether or not waning immunity follows varicella vaccine are indicated.

Measures of Protection

Prelicensure Efficacy

Before vaccine licensure in Japan, the United States, and Europe, a number of open-label (dosage-ranging) studies were conducted in Japan and elsewhere; two double-blind, placebo-controlled studies were conducted in the United States and Europe. The vaccine dosages used varied from less than 500 to 17,500 PFUs, limiting the ability to make comparisons across studies. In the original study conducted by Takahashi and colleagues, immunization was used to terminate a potential outbreak of nosocomial chickenpox.[1] All the immunized healthy children developed specific antibodies, and no further cases of varicella occurred. The vaccinated children were protected after four subsequent hospital exposures to varicella.[493] Although this was not a classic efficacy study, these data strongly suggested that live attenuated varicella vaccine would be effective in preventing disease.

Three double-blind, placebo-controlled efficacy studies have been done in healthy children. The first was conducted in the early 1980s using Merck's Oka vaccine in suburban Philadelphia.[494] In this study, 468 children were immunized with one dose of varicella vaccine containing approximately 17,000 PFU (the original report of 8700 PFU was erroneous), and 446 were given placebo. Over the following 9 months, there were 39 cases of varicella, all in the placebo recipients, with a calculated vaccine efficacy of 100%. Of 33 vaccine recipients exposed to varicella cases in their households, none developed varicella, so the vaccine was 100% efficacious after household exposure. During the second year of follow-up, one vaccinated child developed modified varicella consisting of 17 lesions after exposure to varicella, resulting in an efficacy of 98% overall and 92% after household exposures. During a 7-year follow-up, 95% of these vaccinees were estimated to have remained free of varicella.[482] These data cannot be compared with those of subsequent studies in the United States, however, because these children received the highest dosage of vaccine used in the United States as a monovalent vaccine.

A second double-blind, placebo-controlled study was performed in Finland, using vaccine produced by SmithKline Beecham (now GlaxoSmithKline). This study included 513 healthy children, ages 10 to 30 months, who were divided into three groups, given a high-dosage vaccine (10,000 or 15,850 PFU), a low-dosage vaccine (630 or 1260 PFU), or placebo. Eight children who were initially seropositive were excluded from analysis. Children were observed for 29 months on average. There were 65 serologically confirmed cases of varicella: five in the high-dosage group (3% attack rate; calculated vaccine efficacy: 88%), 19 in the low-dosage group (11.4% attack rate; calculated vaccine efficacy: 55%), and 41 (25.5% attack rate) in the placebo group. Breakthrough varicella in vaccinees was a minor illness with an average of less than 30 skin lesions.[495]

The third double-blind, placebo-controlled study was conducted in China using a live attenuated vOka strain vaccine (10,000 PFUs) manufactured by Changchun Keygen Biological Products Co.[496] The study was conducted among 5000 children 3 to 7 years of age with no history of varicella or varicella vaccine. Mumps vaccine was the placebo; the follow-up period was 12 months. Based on varicella incidence rates of 0.8 per thousand in the vaccine group (2/2476) and 8.7 per thousand in the control group (22/2534), vaccine efficacy was 90.8% (95% confidence interval [CI], 88.7–95%).

A 10-year follow-up of 2216 healthy children 1 to 12 years of age who were randomized to receive one or two doses of varicella vaccine (five different lots of vaccine ranging from 2900 to 9000 PFUs) (the licensed vaccine is 1350 PFUs at expiration) 3 months apart, yielded an estimated vaccine efficacy of varicella vaccine of 94.4% (one dose) versus 98.3% (two doses) ($P < .001$).[452] After household exposures, efficacy of one dose was 90.2%, versus 96.4% for two doses ($P = .112$).[452]

Postlicensure Vaccine Effectiveness for Prevention of Varicella

In the postlicensure period, monitoring vaccine performance is essential to assess effectiveness of the licensed vaccine product under conditions of community use. Considerable postlicensure experience has been gained from more than 20 years of use of varicella vaccine in the United States and from use in universal childhood vaccination programs in other countries. Vaccine effectiveness studies have used a variety of methods, including prospective and retrospective cohort, case cohort (screening method), case-control, and household secondary attack rate studies; they have been performed in schools, communities, clinical practices, and households. Case definitions for varicella and for moderate and severe disease vary between studies and may influence estimated vaccine effectiveness. Studies using a clinical case definition tend to underestimate vaccine effectiveness; those requiring laboratory confirmation may overestimate effectiveness.[497]

One Dose. A review of 17 postlicensure vaccine effectiveness evaluations in the United States in 2008 showed that one dose of varicella vaccine (Varivax) was 84.5% (median; range, 44–100%) effective in preventing varicella, and 100% effective (mean and median) in preventing severe varicella.[230,425,498–513] A systematic review that assessed the effectiveness of varicella vaccines identified 42 publications worldwide through the end of 2014.[230,425,498–514,515–538] This review reported that after one dose of monovalent vaccine there were 58 estimates of vaccine effectiveness for prevention of all varicella, 34 estimates for prevention of combined moderate and severe varicella (including two studies that provided estimates for moderate varicella alone), and 25 estimates for prevention of severe varicella.[514] The majority of the studies examined performance of varicella vaccine during outbreaks of varicella in schools and child-care centers; others were case-control, cohort, and household secondary-attack-rate studies. The metaanalysis of the reported estimates indicated that one dose of varicella vaccine was moderately effective at preventing all varicella (81%; 95% CI, 78–84%) and highly effective in preventing combined moderate to severe varicella (98%; 95% CI, 97–99%); effectiveness against severe disease was 100%.[514] Data are for effectiveness within the first decade after vaccination, with a median time since vaccination likely less than 10 years. Vaccine effectiveness estimates were similar whether the analysis was stratified by individual vaccines or study design (Table 62.3); pooled vaccine effectiveness estimates were 82% (95% CI, 79–85%) for Varivax, 77% (95% CI, 62–85%) for Varilrix, 86% (95% CI, 78–91%) for other vaccines (that included Okavax, Keygen, Shanghai, Baike, and Changsheng vaccines), and 81% (95% CI, 76–85%) for unspecified or more than one vaccine used. Most of the reported one-dose vaccine effectiveness estimates for prevention of all varicella were close to the pooled estimate. Two estimates, however, were below 50%, one each for Varivax (44%) and Varilrix (20%).

TABLE 62.3 One-Dose Varicella Vaccine Effectiveness Estimates by Type of Vaccine and Varicella Severity

Vaccine	Prevention of All Varicella			Prevention of Combined Moderate and Severe Varicella			Prevention of Severe Varicella		
	Estimates (N)	Pooled Estimate (95% CI)	Range of Reported Estimates	Estimates (N)	Pooled Estimate (95% CI)	Range of Reported Estimates	Estimates (N)	Median	Range
Varivax	26	82% (79–85%)	44–100%	18	98% (95–99%)	86–100%	10	100%	100%
Varilrix	10	77% (62–85%)	20–92%	6	98% (89–100%)	86[a]–100%	7	100%	100%
Other vaccines	5	86% (78–91%)	77–93%	1	100% (91–100%)	–	1	100%	–
Unspecified/ >1 vaccine used	17	81% (76–85%)	60–100%	9	99% (95–100%)	84–100%	7	100%	85[b]–100%

[a]86% is for prevention of moderate disease only.
[b]Severe disease defined as hospitalizations.
Moderate, 50–500 lesions, no complications; Severe: (a) >500 lesions or a complication requiring physician visit, or any complication (most studies); (b) any hospitalization regardless of the number of lesions (1 study); (c) >250, >200, and >150 lesions (3 studies, respectively); (d) disease severity scale used in clinical trials: number of lesions, fever, systemic signs, and subjective assessment of illness or other combination of clinical criteria (5 studies); (e) parental assessment of severity (2 studies).
Data from Marin M, Marti M, Kambhampati A, et al. Varicella vaccine effectiveness worldwide: a systematic review and meta-analysis. Pediatrics. *2016;137(3):e20153741.*

One possible explanation for low vaccine effectiveness estimates from some outbreak investigations is that outbreaks that come to public health attention are more likely to represent situations in which the vaccine failed and may therefore represent extreme estimates of vaccine effectiveness.[539] Only one study compared directly the effectiveness of one dose of Varivax and Varilrix and found lower effectiveness for Varilrix (56% vs 86%), but the overlapping CIs suggest that the values were not significantly different.[517] The same study provided the only estimate on VE for one dose of MMRV (55%), which was on the lower end of the range reported for monovalent vaccines but with wide CIs that overlapped those of the monovalent vaccines.

A long-term case-control effectiveness study has been carried out in private practices in the United States (Connecticut) since 1999.[230,511] Initial results showed that one dose of the vaccine was 85% (95% CI, 78–90%) effective in preventing PCR-confirmed varicella, and 97% effective in preventing severe varicella (95% CI, 93–99%). In vaccinated children, 86% had mild disease, compared with 48% of unvaccinated children ($P < .001$). A continuation of this study through 2004 indicated a drop in vaccine effectiveness from 97% in year 1 to 85% in year 2, with continued follow-up for as long as 8 years, during which time there was no further loss in vaccine effectiveness.[511] A follow-up analysis evaluating one- and two-dose vaccine effectiveness demonstrated that one-dose vaccine effectiveness through 8 years of follow-up was 86%.[518] Another U.S. study reported a significant decline in one-dose vaccine effectiveness, from 94% within 5 years after vaccination to 88% for 5 to 9 years and 82% for 10 years or longer after vaccination[519]; four other studies also reported a decline in vaccine effectiveness with time since vaccination, but the differences did not reach statistical significance.[422,520–522]

In summary, available data support similar performances of monovalent varicella vaccines in preventing varicella (see Table 62.3). The duration of protection after one dose is not fully understood or studied, especially in settings of low varicella incidence. Further studies could be beneficial, especially regarding effectiveness of MMRV vaccines, but instituting a second routine dose for children is a better way to increase effectiveness and improve herd immunity.

Risk Factors for Vaccine Failure. Several studies have identified potential risk factors for lower vaccine effectiveness (or vaccine failure), including asthma, eczema, early age at vaccination, time since vaccination, receipt of varicella vaccine within 28 days of MMR vaccine, and oral steroids around the time of acquisition of breakthrough disease.[499,502–506,510,540,541] Many other vaccine effectiveness studies that examined possible reasons for lower vaccine effectiveness did not find these or other factors, such as vaccine storage and handling problems, to be associated with vaccine failure. The relatively small number of cases in many studies limits the ability of researchers to conduct multivariate analyses and, thus, to examine the independent effect of each risk factor. Consistent with the findings described by Vazquez and associates of higher vaccine effectiveness during the first year after vaccination,[511] data from a large case-control study from China (1000 cases) also suggested lower vaccine effectiveness after the first year after vaccination; however, the differences did not reach statistical significance.[422] It was projected that a routine two-dose regimen of varicella vaccine for all vaccine recipients will decrease the incidence of breakthrough varicella.

Two Doses. Fewer studies assessed vaccine effectiveness after two doses of monovalent varicella vaccine in children because a routine two-dose regimen was a newer recommendation (e.g., in 2007 in the United States) and fewer other countries/regions adopted it. In the metaanalysis study, overall two doses of vaccine provided 10% or better protection than one dose.[514] The pooled two-dose vaccine effectiveness for prevention of all varicella, based on eight estimates reported through 2014, was 92% (95% CI, 88–95%)[515,516,518–521,532]; two of the estimates, both from outbreak investigations, were below 90%, which may have lowered the pooled estimate to the lower 90s.[515,516] A study published subsequently reported a vaccine effectiveness of 94%.[532a] The only vaccine effectiveness reported for two doses of MMRV/Priorix-Tetra was 91% (95% CI, 65–95%).[517]

The routine two-dose varicella vaccine recommendation for children in the United States since 2007 has provided opportunities to study the effectiveness of this policy. By individual studies, a follow-up of the aforementioned Connecticut case-control study through 2010 demonstrated that two doses of vaccine provided better protection than one dose; vaccine effectiveness for prevention of PCR-confirmed varicella was 98% after two doses, compared with 86% for one dose ($P < .001$). Of the 71 cases of laboratory-confirmed varicella in this

case-control study, none occurred in children who received two doses of vaccine.[518] A subsequent study confirmed the superiority of the two-dose regimen.[299] An earlier investigation of a varicella outbreak of 84 cases affecting a prekindergarten through elementary school complex of 880 children where greater than 97% of children had received at least one dose of varicella vaccine and 39% had received two doses, suggested that two doses did not improve protection compared with one dose (88.1% vs 83.4%, respectively).[515] Cases of putative varicella were subject to laboratory investigation, but in only two of 25 children who had received two doses was the diagnosis confirmed. The second estimate for two-dose vaccine effectiveness lower than 90% was also from an outbreak investigation; outbreaks in two schools were investigated and considering only students in classrooms with cases vaccine effectiveness was 84% in one school and 95% in the other.[516] In this study, laboratory testing could not confirm varicella as the etiology of the outbreak but clinical and epidemiologic data were suggestive. In a study during a varicella outbreak in an elementary school, the incremental benefit of the second dose during outbreak control efforts was 76% (95% CI, 44–90%) for students with classroom exposure. Students who received a second dose were classified as two-dose recipients 4 days after vaccination, thus assuming a rapid anamnestic immune response. The incremental effectiveness increased to 94% when a 10-day delay between the receipt of second dose and the date the immune status was assessed.[532] Although not calculated in the publication, using data provided in it (crude incidence rates for one dose, 4.9 cases per 1000 person-days; for two doses, 1.4 cases per 1000 person-days; and for unvaccinated children, 25.8 cases per 1000 person-days), one- and two-dose vaccine effectiveness estimates are 79% and 95%, respectively. A case-control study that enrolled 133 cases and 365 controls in all West Virginia public schools reported a two-dose vaccine effectiveness of 94%.[519] This study also examined two-dose vaccine effectiveness by time since vaccination and found no difference through 5 years since the second dose; there were too few subjects to confidently examine effectiveness for more than 5 years after the second dose. Another study conducted in the two varicella active surveillance sites during 2009–11 reported a two-dose vaccine effectiveness of 94% among children age 4 years and older (45 cases and 247 controls). In this study, about half of the cases were laboratory confirmed (26 cases); when only laboratory-confirmed cases were considered, two-dose vaccine effectiveness for prevention of all varicella was 96%.[532a] Outside the United States, one study each in Germany and Spain evaluated two-dose vaccine effectiveness both against PCR-confirmed cases and using a matched case-control study design; the reported estimates were 95% and 97%, higher than the one-dose estimates of 72% and 87%, respectively.[520,521]

Postexposure Effectiveness

In the 1970s and early 1980s, postexposure use of different formulations of varicella vaccine was extensively studied by Japanese researchers, who administered vaccine from 1 to more than 5 days after exposure, to children in hospital, community, and household settings (Fig. 62.8).[1,18,435,493,542–544] In two postexposure studies of healthy seronegative children comparing those vaccinated within 3 days of household exposure and those unvaccinated, vaccine effectiveness for prevention was 94.2% in one study and 100% in the other.[421,545] The vaccine was 100% effective in preventing moderate or severe disease if administered within 3 days of exposure.

A double-blind, placebo-controlled trial after exposure was conducted in the United States with an Oka/Merck vaccine (4350 PFU) given to healthy seronegative children 1 to 5 days after exposure in their households.[546] The vaccine was 90% effective in preventing disease if administered within 3 days of exposure, and 67% effective if administered within 5 days. It was 100% effective in preventing moderate or severe disease.

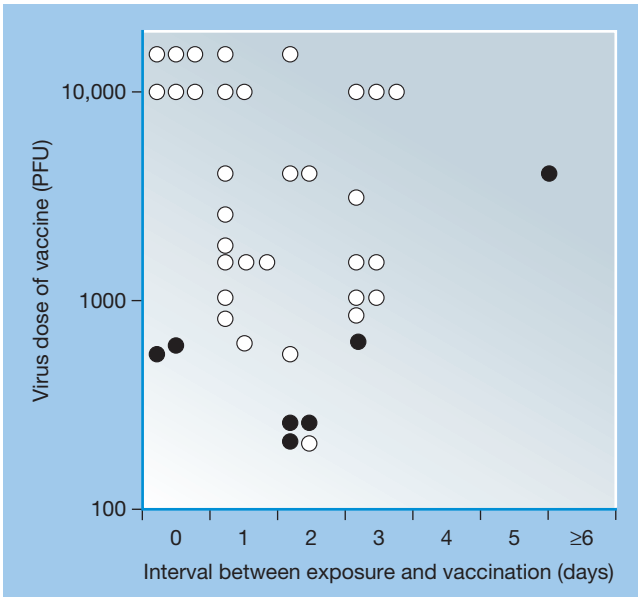


Figure 62.8. Postexposure immunization against varicella-zoster virus. The protective effect of inoculation with live varicella vaccine in household contacts with regard to the dose of virus and the time between exposure and vaccination. Solid circles, varicella; open circles, no varicella. *(Modified from Asano Y, Hirose S, Iwayama S, et al. Protective effect of immediate inoculation of a live varicella vaccine in household contacts in relation to the viral dose and interval between exposure and vaccination.* Biken J. *1982;25:43–45.)*

Studies conducted using licensed varicella vaccines after exposure have shown inconsistent results for prevention of disease of any severity but consistently high effectiveness for prevention of moderate and severe disease. A double-blind, placebo-controlled trial conducted in Israel among 30 families with 42 exposed children who were vaccinated within 3 days of exposure found that the vaccine was not effective in preventing disease (secondary attack rate of 42% of vaccinated children, compared with 45% of placebo recipients), but postexposure effectiveness was 86% in preventing moderate to severe disease.[547] The results of the three studies that used a comparison group were summarized in a 2014 Cochrane collaboration review.[548] These studies involved a combined total of 110 healthy children who were siblings of household contacts with varicella. The authors concluded that evidence suggests that varicella vaccine administered within 3 days to children following household contact with a case reduces infection rates and severity of cases.

Other uncontrolled observational studies have demonstrated high postexposure vaccine effectiveness in preventing either varicella or severe varicella disease when vaccination occurred within 3 to 5 days of exposure.[549–552] Postexposure use of varicella vaccine is currently recommended in the United States.[553]

Breakthrough Varicella

Breakthrough varicella is defined as varicella developing more than 42 days after immunization. Most published studies with long-term follow-up of healthy children vaccinated with one

dose report that despite high rates of seroconversion (measured by gp-ELISA), from 1% to 3% developed breakthrough chickenpox annually.[235,451,453,482,554] Studies of the rate of breakthrough varicella in vaccinated U.S. children for as long as 10 years after vaccination in prelicensure clinical trials indicated a rate of breakthrough varicella from 0% to 4% per year; lower rates of illness were associated with higher dosages of vaccine.[448] In a study in which 17,000 PFU was given, 95% of children remained varicella free 7 years after vaccination.[480,482] Protection after this dose of varicella vaccine was higher than that seen in other studies, in which lower dosages were administered. For example, in the study by Johnson and associates ($N = 280$; dosage: 950 to 3250 PFU), 17% developed varicella after 10 years[485]; in of the study by Clements and associates ($N = 465$; dosage: 950 to 3250 PFU), 19% had varicella after 5 years[453]; and in of the study by Takayama and colleagues ($N = 459$; dosage: 1000 PFU),[555] 34% had varicella after 7 years.

Breakthrough varicella in vaccinated children is typically a modified illness.[451] In U.S. clinical trials, where 12% of healthy children vaccinated with 1000 to 1625 PFU Oka (Merck) vaccine developed breakthrough disease after household exposure, these breakthrough infections were very mild, with one-tenth the number of expected skin lesions.[451] These observations are consistent with those of many other investigators.[374,447,453,481,485,499,554]

Most cases of breakthrough varicella manifest a skin rash of fewer than 50 lesions, unaccompanied by fever. Lesions may be vesicular but are more commonly atypical, with papules that do not progress to vesicles. In a prelicensure clinical survey, natural and breakthrough varicella were compared; children with varicella were examined by clinicians unaware of their vaccination status, using a standard examination. Breakthrough varicella was milder than primary infection. Vaccinated children were less likely to have fever (temperature >37.8°C, or >38.9°C; 12% and 3%, respectively) compared with 57% and 15% for unvaccinated children ($P < .001$ for both comparisons). Vaccinated children also had a lower median number of lesions (53 compared with 300 [$P < .001$]), a lower proportion had more than 500 lesions (1.7% compared with 17.4% [$P < .001$]), and they had a shorter duration of illness (6 days vs 7 days [$P < .001$]).[554] Considering household exposures only, breakthrough varicella cases had a higher number of lesions (median: 118) but still fewer than natural varicella (median: 326). Other than fever, associated constitutional symptoms were not different between the two groups—pruritus occurred in 89% with natural varicella and in 84% of the breakthrough cases.[554]

Postlicensure data on clinical presentation of breakthrough cases confirms that they are milder than cases in unvaccinated persons.[229,499,501,504,509,546,556,557]

In a 9-year summary of population-based varicella active surveillance data, clinical and epidemiologic characteristics of varicella were compared between 1671 one-dose vaccinated and 5609 unvaccinated cases among children 1 to 14 years old.[557] As seen in the prelicensure findings, vaccinated cases, compared with unvaccinated cases, had fewer lesions (75% vs 34% with fewer than 50 lesions), were less likely to have high and prolonged fever, or moderate or severe illness (38% vs 89%), or presence of a complication (5% vs 12%). Nevertheless, approximately 25% of vaccinated cases had more than 50 lesions, and some were associated with serious complications similar to those occurring in unvaccinated cases. Presumably, unmodified varicella disease and complications occur in persons who do not develop an adequate primary immune response from vaccination. Serious complications in vaccinated persons have included pneumonia, sepsis and secondary infection resulting in bacteremia, encephalitis, and aseptic meningitis, the last confirmed to be caused by WT varicella.[558,559,563a] Only rarely have varicella hospitalizations or deaths among persons who had received one dose of varicella vaccine been reported to the Vaccine Adverse Event Reporting System (VAERS), and when reported, have mostly been among patients who, at the time of vaccination, were unknowingly immunocompromised by a medical condition or medications (e.g., systemic steroids for asthma).[229,560–563] Six fatal cases of breakthrough varicella have been reported.[563a]

Several postlicensure studies examined the clinical presentation of breakthrough varicella in one- versus two-dose vaccinated children and did not identify a significant difference in rash severity even though two-dose recipients with varicella appear to present with fewer lesions than one-dose recipients, and no severe rash or complications involving disseminated VSV infection with other organ involvement in addition to skin had been reported to date in two-dose vaccine recipients.[491,519,563a,564] It is unclear if breakthrough varicella severity is truly similar among one- and two-dose recipients, or if only the more-severe spectrum of two-dose varicella is diagnosed.

The mild and atypical nature of breakthrough varicella makes clinical and laboratory diagnosis challenging.[179,224,557,565,566] Healthcare providers should seek history of exposure to varicella or HZ, and in countries that have instituted routine childhood vaccination programs, laboratory confirmation may be needed as disease declines to low levels. Experience with PCR confirmation of breakthrough disease was gained in a study of postlicensure vaccine effectiveness in clinical practice, although the aim of this study was not to evaluate the accuracy of PCR in breakthrough disease. In this study, the rate of confirmation in vaccinated cases was lower (46%) than in unvaccinated cases (90%), possibly as a result of increased PCR testing of children with minor rashes; atypical rashes are more likely to be examined for VZV in vaccinees than in children who have not been immunized.[567] In another study of 33 suspected varicella cases in vaccinated children, VZV was detected by PCR in 58% of cases overall and in 76% of those with adequate tissue specimens.[565] Nevertheless, PCR of skin rashes remains the best approach to diagnosis of breakthrough varicella.[224]

In contrast to the rarity of person-to-person transmission of the Oka strain, vaccinated persons with breakthrough varicella may transmit the WT virus to varicella-susceptible persons. The rate of spread of WT VZV from children with breakthrough disease to their *vaccinated* household contacts was reported to be 12%.[556] The contagiousness of WT VZV in healthy vaccinees was studied in a large household-contact postlicensure study where data were available on the vaccination status of both primary household cases and their unvaccinated and vaccinated sibling contacts. The transmission rate from vaccinated children with breakthrough infections to unvaccinated contacts was 37.1% (26 of 70), which was half the transmission rate from unvaccinated varicella cases to their unvaccinated siblings (71.5%; 1071 of 1499).[509] Vaccinated cases with fewer than 50 skin lesions were only one-third as contagious as unvaccinated cases. When vaccinees with breakthrough infections had more than 50 skin lesions, however, their transmission rate was similar to that seen in unvaccinated children with chickenpox.[509] Some varicella outbreaks in the United States have started with vaccinated cases, and outbreaks have been sustained in highly vaccinated school populations.[502,510,513,515] Administering two doses to all children may greatly decrease the number of outbreaks of varicella in schools and transmission of WT VZV in highly vaccinated populations.

Theoretically, reasons to decrease the rate of breakthrough varicella are to decrease transmission of VZV and to prevent the WT virus from establishing latent infection, which has the

potential to reactivate subsequently and cause HZ. Development of a VZV rash, either WT or caused by Oka, may be associated with subsequent development of HZ but is not required.[199,568] The rate of HZ after vaccination is lower in healthy children than after natural infection.[568–570,787] It is possible that development of HZ is also under genetic control.[571] During the period when only one dose of varicella vaccine was routine, approximately 50% of the reported cases of HZ after vaccination were caused by WT VZV, presumably resulting from asymptomatic as well as symptomatic reinfection by the WT virus.[561,562,570] This proportion will decrease as circulation of WT VZV continues to decrease during the varicella vaccination program. It is anticipated that in populations where two doses are routinely given, not only will the incidence of varicella fall, but so will the occurrence of HZ caused by WT VZV.

VARICELLA-ZOSTER VIRUS VACCINE IN ADOLESCENTS AND ADULTS

Safety and Immunogenicity

Varicella-susceptible adults benefit from vaccination.[54,572,573] Immunized adults have lower seroconversion rates than vaccinated healthy children. Early studies suggested that the vaccine was more immunogenic in adults than it is now recognized to be. In 1985, in Switzerland, 32 healthy seronegative adults, mainly medical personnel, were given an Oka strain vaccine manufactured by RIT, with a reported 90% seroconversion rate.[574] At a similar time, in the United Kingdom, 34 seronegative nurses were immunized with the same Oka strain (RIT), and 94% seroconverted as determined by several serologic tests, including ELISA and immunofluorescence.[575] In early U.S. studies,[546] 89% to 94% of 53 adults seroconverted after one dose of vaccine, as determined by FAMA.

In the National Institute of Allergy and Infectious Diseases (NIAID) collaborative study, from roughly 1980 to 1990, more than 350 healthy adults whose average age was 27 years were immunized in an open-label study.[38,486,576] Most were given two doses of vaccine 3 months apart; their FAMA seroconversion rate was 82% after one dose and 90% after two doses. This experience is also consistent with rates of gp-ELISA seroconversion in 79 vaccinated adolescents after one dose of vaccine.[451] In the NIAID study, the rate of mild vaccine-associated rash (average of nine skin lesions) was 10%, but there was no comparison group. Although it was possible to isolate vaccine-type VZV from skin lesions from a few vaccinees, there was no recognized spread to others.

Larger studies in adults using Varivax have indicated that adults require two doses of vaccine to achieve a seroconversion rate by gp-ELISA of greater than 90%.[577] As for children, there are scant data on seroconversions in healthy adults and those data are based only on commercially available tests. It would not be surprising, however, given the insensitivity of commercial tests, to find that many real seroconversions in vaccinated adults cannot be detected by standard ELISA assays. In one comparative study using latex agglutination, FAMA, and a commercial ELISA in 48 adults 1 to 3 years after immunization, positive VZV antibody levels were detected in 52%, 69%, and 36%, respectively.[184]

A study in Australia, in which two doses of GlaxoSmithKline varicella vaccine were administered to 100 susceptible healthcare workers 17 to 49 years of age, found that 95% of subjects had detectable antibodies using a commercial immunoassay 2 months after the first dose, and 100% of vaccinees had antibodies 6 weeks after the second dose.[578] Reports of complications in adults after vaccination are rare. A healthcare worker was reported to experience prolonged varicella viremia, streptococcal infection, and toxic shock after vaccination.[579]

Cytotoxic T lymphocytes directed against four proteins of VZV were observed in vaccinated adults in U.S. studies.[206] The cytotoxic T-cell response in 23 adult vaccinees was positive and similar to that observed in adults with past natural varicella. In other studies, the magnitude of the cell-mediated immune responses of adults to varicella vaccine were, however, lower than those observed in children.[463,486]

Duration of Immunity

Studies of U.S. adults vaccinated in the NIAID collaborative study indicated that 60% to 90% are seropositive by FAMA or latex agglutination antibody tests as long as 13 years after vaccination (Table 62.4).[486,572] In subsequent follow-up studies of a subset of these vaccinees (120 healthcare workers), 31% lost detectable FAMA antibodies after, on average, 8 years. Twelve (10%) developed varicella; all had mild infections despite loss of detectable VZV antibodies.[232] In another analysis of this study, 9% of almost 500 adult vaccinees developed chickenpox, with follow-up for some persons as long as 21 years. Most of these persons had received at least two doses of vaccine. Eighteen (21%) developed varicella after household exposure ($n = 85$). Neither the severity of breakthrough varicella nor the attack rate after household exposure appeared to increase with time (Fig. 62.9).[573]

A recent study found that 12% of 101 healthcare personnel with documentation of two doses of varicella vaccine were seronegative a median of 4 years after vaccination using a sensitive gp-ELISA test; seven seronegative healthcare workers received a third dose of varicella vaccine and all seroconverted; however, three of seven produced low avidity antibodies, suggesting that they may have mounted a primary immune response to the third dose.[580] Among 61 healthcare personnel with detectable vaccine-induced antibodies and specimens available for testing of antibody avidity, 14% had low avidity antibody, indicating that T-cell immunity may have been incompletely engaged by vaccination. Interpretation of antibody levels after two doses of vaccine is challenging and it is unclear whether absence of vaccine-induced antibodies represents lack of protection.

Follow-up of 100 healthcare workers who received two doses of GlaxoSmithKline varicella vaccine showed that three (4%) of 81 who had sera tested 12 months after the second dose (using an indirect immunofluorescent technique [Virgo]) had lost detectable antibody.[578]

TABLE 62.4 Follow-Up[a] of Healthy Adults After Varicella Vaccination

Follow-up Year	Patients *(N)*	Patients With Varicella *(N* [%])	Patients Seronegative After Vaccination (%)
1	343	8 (2)	34
2	234	8 (3)	33
3	174	2 (1)	40
4	115	5 (4)	10
5	68	4 (6)	30
6	45	2 (4)	10
7–13	40	2 (5)	18

[a]Long-term follow-up focused on breakthrough illness and antibody loss with time. During 13 years of follow-up, breakthrough varicella developed in 9%.

From Gershon A. Varicella-zoster virus: prospects for control. Adv Pediatr Infect Dis. *1995;101:93–104.*

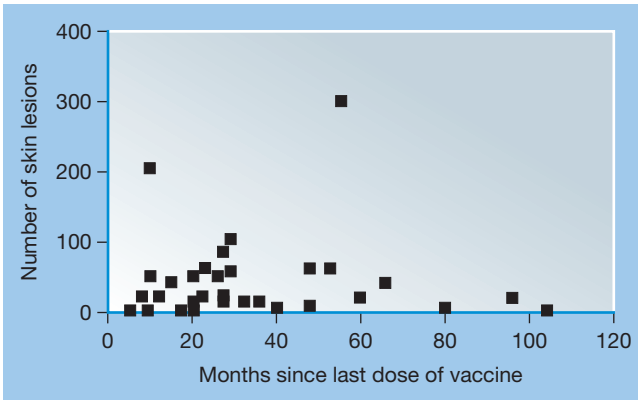


Figure 62.9. Number of skin lesions in 31 vaccinated adults with breakthrough varicella months to years later with respect to the time since the last dose of varicella vaccine was given. Note that the severity of illness did not increase with an increasing interval (in months) since immunization. The average number of skin lesions was 43. *(From Gershon A. Varicella-zoster virus: prospects for control.* Adv Pediatr Infect Dis. *1995;10:93–124.)*

Efficacy and Effectiveness

In the NIAID collaborative study, 57 adults were reported to have experienced household exposures to varicella, with 15 resultant breakthrough cases of chickenpox and an attack rate of 26%. An attack rate of 80% would have been expected had they been fully susceptible. This attack rate of 26% appeared to be higher than that seen in children who had been given similar lots and dosages of vaccine. Only adults who lost detectable VZV antibodies by FAMA or latex agglutination developed breakthrough varicella. However, only 50% of adults who had undetectable antibodies within the previous year developed clinical varicella after a household exposure. Most experienced a modified form of varicella.[38,576,581]

IMMUNOCOMPROMISED PATIENTS

Safety and Immunogenicity in Immunocompromised Children

In Japan in the mid-1970s, a group of 39 children with underlying diseases such as nephrosis, nephritis, asthma, and hepatitis, a third of whom were receiving steroids, were immunized. They were given a dosage of 1000 to 2000 PFU of the Oka vaccine manufactured by Biken. All developed VZV antibodies as determined by complement fixation.[1,376]

A major departure in immunization with live virus vaccines occurred when children with leukemia or other malignancies were vaccinated.[582] Before this, live vaccines were contraindicated in immunocompromised children. Given the high risk from natural varicella, however, 11 children with leukemia and six with solid tumors who were in remission were immunized in Japan. Their chemotherapy, 6-mercaptopurine and methotrexate, was withheld for 1 week before and 1 week after immunization.[582–586] In these studies, the seroconversion rate for antibodies against VZV, measured by complement fixation or immune adherence hemagglutination, approached 100%. Subsequently, a report of 326 leukemic children who were immunized in Japan was published, confirming and extending these data.[437] Most of these children seroconverted to VZV after immunization as determined by FAMA or immune adherence hemagglutination.

In the early 1970s, a chickenpox infection rate of 30% with 7% mortality was reported in U.S. children with leukemia.[75] Based on the successful studies on immunization of leukemic children in Japan, open-label clinical trials in children with acute lymphoblastic leukemia in remission were begun in the United States and Canada in 1979. It was these studies that brought varicella vaccine to the United States.

The largest clinical trial in leukemic children was conducted by the Varicella Vaccine Collaborative Study Group, which was organized to conduct these trials and was sponsored by NIAID.[199,201,411,486,581,587–590] At the time of immunization, children had to meet the following criteria: being in continuous remission from leukemia for at least 1 year, having no detectable antibodies to VZV by FAMA, having a positive response to mitogens in vitro, and having more than 700/mm^3 circulating lymphocytes. Over one decade, 64 children whose chemotherapy for leukemia had been completed and 511 children whose chemotherapy was suspended for 2 weeks (3 weeks for prednisone[591]) were immunized. Chemotherapy was mostly daily 6-mercaptopurine, weekly methotrexate, and monthly pulses of prednisone and vincristine, regimens that were more intense than those given to Japanese children in earlier studies. By the late 1980s in the United States, higher doses of methotrexate, prednisone, and cyclophosphamide were being administered. Although it was initially planned to administer one dose of vaccine, because of a failure of seroconversion as measured by FAMA after one dose in approximately 15% of the vaccinees with leukemia, and a loss of detectable VZV FAMA antibody after 1 year in approximately 5% of the vaccinees with leukemia, two doses of vaccine were finally recommended, given 3 months apart. In this study, a seroconversion to VZV occurred in 82% of leukemic children after one dose of vaccine and in 95% of leukemic children after two doses. In general, approximately 80% of vaccinees tested developed positive cell-mediated immune responses after one dose of vaccine and 90% after two doses, mirroring the experience with humoral immune responses.

Rash developed in 5% of leukemic children no longer receiving chemotherapy, and in approximately 50% of those still receiving maintenance chemotherapy, within 1 month after the first dose of vaccine.[486] Rashes were less common after the second dose, occurring in only 10% of children still receiving maintenance chemotherapy. The rash after vaccination in children with acute lymphoblastic leukemia is usually maculopapular and vesicular and can resemble a mild form of varicella. Approximately 40% of children receiving chemotherapy who developed rash were treated with high oral doses (900 mg/m^2 four times a day) of acyclovir (particularly those with more than 50 skin lesions or with rash that lasted more than 7 days). Children with more-severe rashes (usually those with more than 200 lesions) were treated with intravenous acyclovir at a standard dosage. Oka-strain VZV is sensitive to acyclovir, and acyclovir therapy did not appear to interfere with the development of the immune response.[486] There was no increase in the rate of relapse of leukemia in vaccinated leukemic children compared with children who had natural varicella.[587,592] In other, smaller studies, 84 leukemic children were immunized.[593,594] The seroconversion rates were similar to those observed in the collaborative study. Twenty-two children with solid tumors (Wilms tumor and rhabdomyosarcoma) also were immunized in the collaborative study (Gershon and colleagues, unpublished results, 1980–90). After two doses of vaccine, 77% seroconverted as determined by FAMA assay; 32% had minor vaccine-associated rashes.

In Japan, five cases of severe reactions to varicella vaccine, consisting of extensive skin rash and high fever, were reported.[437] These children were receiving high dosages of immunosuppressive medications that were not withheld before immunization. None suffered permanent sequelae; all recovered. In the United States, a few severe reactions to

vaccine were reported in leukemic children who received a vaccine lot that is no longer being used.[588,595] In postlicensure safety studies, several children with immunodeficiency diseases (discussed later) were diagnosed when they developed complications from Varivax.[229]

There are additional reports of immunization of small numbers of immunocompromised children. In a study of 23 French uremic children, vaccine was given before renal transplantation. It was well tolerated, with 87% seroconversion after one dose.[596] In Spain, 34 children, half on dialysis and half after transplantation, were immunized. Their ELISA seroconversion rate was 85% after one dose; 10% had a mild rash.[597] An additional 212 French children with renal failure were immunized before renal transplantation between 1980 and 1994. One year after immunization, 62% were VZV antibody positive by FAMA and ELISA.[598] There are additional reported safety and immunogenicity trials in children with renal failure[599] and after renal and liver transplantation.[600]

Several additional studies by the Pediatric AIDS Clinical Trials Group evaluated varicella vaccination in children with HIV infection. In a clinical trial involving 41 HIV-infected children whose $CD4^+$ T-lymphocyte levels were relatively normal (>25%) and who were at most mildly symptomatic (CDC stage N1 or A1) from HIV, 85% developed immune responses to VZV after two vaccine doses. Antibodies were detectable by FAMA in 60%, and a positive stimulation index developed in 83%. The rate of vaccine-associated rash was 5% and, unlike leukemic vaccinees, none required treatment with an antiviral drug to control rash from the vaccine.[601] In a Pediatric AIDS Clinical Trials Group study of 44 HIV-infected children with CD4 levels as low as 15%, vaccinated children also tolerated varicella vaccine well, with 80% developing an immune response after two doses.[602] Additional small studies in HIV-infected children have shown varicella vaccine to be immunogenic and safe.[603,604]

There is interest in boosting VZV immunity in HIV-infected persons who have had varicella, in hopes of protecting them from developing HZ by stimulating their cellular immune responses to VZV. Studies in children and adults with immunity to varicella are ongoing, using varying dosages of vaccine.[605,606] It appears that Zostavax is safe for HIV-infected adults with CD4 counts greater than 200/mm^3 (ACTG 5247).[607] Details can be found in Chapter 63.

Further studies in varicella-susceptible immunocompromised children are sparse. In Thailand, 29 children with chronic liver disease were safely immunized, with 100% seroconversion.[608] A clinical trial in which 11 children, ranging from 6 months to 8 years of age (median: 10 months of age), were given one dose of varicella vaccine approximately 3 months before liver transplantation was reported. Only three of 11 seroconverted, and after an exposure, passive immunization was still given.[609] A study in Switzerland that included children 6 months to 18 years of age (median: 7.8 years of age) who were at least 1 year after liver transplantation and on low immunosuppressive therapy reported that all 36 seronegative children reached antibody levels defined as protective and 97% remained seropositive a median of 1.7 years after vaccination. No breakthrough disease was reported a median of 4.1 years of follow-up.[610] In this study, two doses of GlaxoSmithKline varicella vaccine were administered but 22% of children required a third dose to sustain antibody levels defined as protective (≥50 IU/L). Varicella vaccine was administered to 21 Canadian children before solid-organ transplantation, with excellent persistence of antibody titers and no cases of breakthrough varicella.[611]

A study on safety and immunogenicity of Varivax after liver and intestinal transplantation included 16 varicella-susceptible children 13 to 76 months of age who were vaccinated 257 to 2045 days after transplantation. Five developed mild fever and non–injection-site rashes. Humoral and cellular immunity developed in 86% to 87%. No chickenpox occurred after five exposures to varicella.[612] There are additional reports on small numbers of children immunized after liver transplantation.[613,615,616] A small study of varicella vaccine in children with juvenile rheumatic arthritis receiving methotrexate and steroids showed no significant safety concerns and a 50% antibody response rate, measured by commercial ELISA, to one dose of varicella vaccine (IgG titers were detected in 72% of controls).[617] A small study in children with atopic dermatitis given GlaxoSmithKline varicella vaccine reported that the vaccine was well tolerated and immunogenic.[618] Another study in pediatric patients (12 months to 8 years of age) with asthma evaluated whether budesonide inhalation suspension reduces the immunogenicity of Merck varicella vaccine. There was no difference in the proportion of patients achieving a VZV antibody level, as measured by gp-ELISA, of 5 or greater gp U/mL (85%) (129/151) in the budesonide inhalation suspension group and 90% (83/92) in the nonsteroidal conventional therapy group (relative risk [RR] = 0.95; 95% CI, 0.86–1.04).[619] A small number of adults ($n = 45$) who had undergone stem cell transplantation were given Oka-RIT vaccine 4.5 and 6.5 months after transplantation. Many of these adults were seropositive before vaccination and immune responses to VZV in all patients were rather weak. There were no serious adverse events after vaccination, but during the follow up several patients developed a varicella-like illness (from Oka or WT VZV) and several developed clinical HZ. It was concluded that alternative strategies were necessary to prevent VZV infections in stem cell transplant patients.[620]

Duration of Immunity in Immunocompromised Patients

There has been a high degree of persistence of antibodies to VZV in vaccinated leukemics, although the rate of persistence is lower than that in healthy children. Over 11 years, 13% of vaccinees who originally seroconverted become seronegative by FAMA or latex agglutination.[486] Many were exposed to varicella but did not become ill. After a reseroconversion, titers in leukemic children usually remain detectable, and, in a number of patients who reseroconverted without symptoms, the Western blot antibody pattern was characteristic of an anamnestic response.[588,621] Furthermore, the attack rate of clinical varicella among leukemic vaccinees who had again become seronegative and who had household exposures was only 30%, not the 80% that would be expected in varicella-susceptible patients.[588] Neither the incidence nor the severity of breakthrough varicella in leukemic vaccinees increased with time, even as many of these vaccinees became young adults.[486] The meaning of loss of a detectable antibody titer years after immunization is difficult to interpret, but it may represent some waning of immunity. At present, however, no booster immunizations are recommended for these persons.

The only other study of immunocompromised patients for whom there is long-term follow-up involved antibody persistence in French children immunized before renal transplantation. After 10 years, 42% were seropositive by ELISA, a test that usually has low sensitivity.[598] It is possible that had a more sensitive test been used, the rate of antibody persistence would have been greater.

Prelicensure and Postlicensure Vaccine Performance in Immunocompromised Vaccinees

Significant information on efficacy in immunocompromised leukemic children in the United States and Canada was

gathered from the NIAID collaborative study. In this group, there were 123 household exposures to varicella over a decade, with 17 cases of breakthrough chickenpox, a rate of protection of 86%.[201,411,486,581] Varicella occurred in 39 vaccinated children, most of whom did not have household exposures to chickenpox; this was generally a modified illness, with an average of 96 skin lesions (range, 1 to 640). None of these leukemic children with breakthrough varicella required treatment with antiviral drugs.

The protective efficacy of vaccine was examined in 212 French children immunized prior to renal transplantation. They were compared with 49 similar children with no vaccination or chickenpox and 415 similar children who had previous varicella. The incidence of varicella was significantly lower in vaccinees (26 of 212 [12%]) than in those who were not vaccinated (22 of 49 [45%]). The disease was also less severe in the vaccinated children, with no deaths; there were three deaths from varicella in the unvaccinated group. Varicella occurred only in vaccinees who lost detectable VZV antibodies after vaccination; no cases of varicella were observed in those who remained antibody positive. Four (1%) of the 415 patients with a history of past varicella developed a second attack, which was similar to the attack rate in vaccinees.[598] The calculated vaccine efficacy in this study was 73%.

Attempts have been made to determine how effective live attenuated varicella vaccine is for HIV-infected children. One study of 356 HIV-infected children suggested that the vaccine was effective in preventing HZ, but it was not possible to distinguish between the protective effects of highly active antiretroviral therapy (HAART) on their immune systems in preventing zoster and those of the vaccine itself.[622] A subsequent collaborative study determined that the vaccine was 85% effective in protection against any varicella and 100% effective in preventing zoster, based on comparative disease incidence per 1000 person-years (PY) of observation, in 77 vaccinated HIV-infected children.[623] In this study, in which unvaccinated children served as controls, there was also no evidence that HAART alone was responsible for the high degree of prevention of VZV disease; the excellent degree of protection from both varicella and HZ was attributable to the vaccine itself.

There is no published information on the protective efficacy of varicella vaccine in children after liver transplantation or those with solid tumors.

VACCINE ADVERSE EVENTS

Prelicensure Studies

In two placebo-controlled studies, conducted by Merck[494] and GlaxoSmithKline,[495] the most common adverse events reported after varicella vaccination of healthy children were minor and included mild tenderness and redness at the injection site, fever (reported in one trial but not the other), and mild rash.[494,495] In the U.S. double-blind, placebo-controlled clinical trial,[494] the only complaints that occurred significantly more often in vaccinated children than in placebo recipients were pain (26.4% vs 17.5%) and redness (5% vs 2.5%) at the injection site ($P < .05$).[494] Nonspecific rash within 43 days of vaccination occurred among 2% of placebo and 4% of vaccine recipients, although the few generalized varicella-like rashes occurred in vaccine recipients. There was a similar rate of rash at the injection site, usually consisting of two to four lesions.

Among persons 13 years of age and older, a higher percentage of injection-site complaints was reported after both one and two doses of vaccine: 24.4% and 32.5%, respectively (Varivax package insert). Varicella-like rash at the injection site occurred in 3% of one-dose vaccine recipients and 1% of two-dose recipients. A nonlocalized rash occurred in 5.5% of vaccine recipients after the first injection and in 0.9% of vaccine recipients after the second, at a peak of 7 to 21 and 0 to 23 days postvaccination, respectively. There was no comparison group that received a placebo injection.

A study of the MMRV vaccine that is now licensed in the United States (ProQuad, Merck) was conducted among 480 healthy 12- to 23-month-old children. The children were randomized to receive either MMRV and placebo or MMRII and Varivax. The subjects who received MMRV and placebo received a second dose of MMRV 3 months later. Measles-like rash and fever (temperature higher than 38.9°C) during days 5 to 12 were more common after the first dose of MMRV (rash: 5.9%; fever: 27.7%) than after MMR and Varivax (rash: 1.9%; fever: 18.7%). Measles-like rashes in both groups averaged 6 days and were mild to moderate in intensity. Among the subjects with measles-like rashes, 30% had concurrent fever. The rate of varicella-like rashes after a single dose of MMRV (2.2%) was comparable with the rate in the group that received Varivax. The rate of adverse events was generally lower after the second MMRV dose than after the first.[458] Another study administering MMRV (ProQuad) for a second MMR or varicella vaccine dose at 4 to 6 years of age showed no differences between the children in systemic reactions, including fever, occurring within 43 days of vaccination.[461] Analysis of fever occurring 5 to 12 days after vaccination was not provided. Adverse reactions at the injection site, including pain/tenderness/soreness, erythema, and swelling were more common with MMRV vaccine than with MMR or Varivax, alone or in combination. However, there was no substantial increase in the number of injection-site reactions for MMRV. The reason for the increased rate of measles-like rashes after MMRV compared to MMR and monovalent varicella vaccine is not fully understood, but might suggest a more vigorous immune response in response to an increase in measles virus replication; the measles GMTs measured 6 weeks after vaccination were higher among children who received the first dose of MMRV vaccine than among children who received the first dose of MMR and varicella vaccine administered at the same visit, whereas the varicella GMTs were similar.[458] Statistical modeling indicated that the level of the measles antibody titer after receipt of MMRV vaccine was associated positively with the rate of fever and the rate of measles-like rashes.[474]

An MMRV vaccine (Priorix-Tetra) produced by GlaxoSmithKline is licensed in Canada, Australia, and some countries in Europe. The safety profile of this combination vaccine given as a first or second dose 6 to 8 weeks after the first dose was studied in children 12 to 18 months old; 371 were given MMRV and 123 were given MMR plus varicella vaccine.[430] After the first dose, a higher incidence of fever was observed within 15 days after MMRV vaccine compared with MMR plus varicella vaccine (67.7% vs 48.8%, $P < .05$). However, the incidence of grade 3 fever (temperature >39.5°C rectal, or >39.0°C axillary) did not differ between the two groups (11.6% vs 10.6%, $P = .87$). The incidence of local reactions, pain, and redness within 4 days of vaccination was higher in second-dose MMRV recipients than in children who received MMR plus varicella vaccine. This tendency was also present after dose one, although the differences were not significant.[430] Studies of safety and immunogenicity of the GlaxoSmithKline MMRV vaccine yielded similar findings.[624,625]

Postlicensure Studies

Monovalent Varicella Vaccine

In a clinical trial of two doses versus one dose conducted postlicensure among approximately 900 children ages 12 months to 12 years, the safety profile of the two-dose regimen

was comparable to that of the one-dose regimen. Incidence of injection-site complaints observed 3 or fewer days after vaccination was slightly higher after dose two (25%) than after dose one (21%) (P = .015). Incidence of systemic clinical complaints was lower after dose two; fever incidence from days 7 to 21 postvaccination was 7% after dose one and 4% after dose two (P = .009), and varicelliform rash incidence after dose one was 3%, compared with 1% after dose two (P = .008), with peak occurrence 8 to 21 days postvaccination.[456]

Postlicensure safety monitoring of Merck varicella vaccine in the United States, now reflecting more than 20 years' use and 150 million doses of vaccine distributed, has been reassuring. An early postlicensure study of almost 90,000 children and adults immunized in a northern California large health maintenance organization (vaccine safety datalink site) between 1995 and 1996 found that the vaccine was safe and free of rare complications. Clinical conditions were examined for elevated risk within 30 days of varicella vaccination. Cerebellar ataxia, reported to occur in one in 4000 persons after natural varicella, did not occur after varicella vaccination in this vaccinated cohort. There were no occurrences of encephalitis or bleeding disorders, although the study size was not sufficient to draw conclusions about prevention of these rarer complications of varicella potentially resulting from vaccination.[626]

Serious adverse events after varicella vaccination continue to be rare. Considering the approximately 48 million vaccine doses distributed in the United States from 1995 to 2005, there were 25,306 adverse events reported (52.7 per 100,000 doses distributed); 5% were classified as serious (2.6 per 100,000 doses distributed). The most common adverse events, accounting for 67% of all reports, have consistently been rash, fever, and injection-site reactions.[562] Similarly, postmarketing surveillance over the first 5 years of Varivax use in Europe (approximately 3.3 million doses distributed) found that most (88%) adverse events reported after vaccination were nonserious and the rate of reporting was 30 reports per 100,000 doses distributed.[627]

Serious adverse events that have been reported as temporally related to varicella vaccination include death, urticaria (including some cases of recurrent papular urticaria), ataxia (median: 16 days; range, 1 to 61 days), thrombocytopenia, pneumonia, anaphylaxis, encephalitis (median: 20 days; range, 4 to 365 days), erythema multiforme, stroke, and transverse myeltitis (see also the Varivax package insert).[229,561,628–631] Most of these events were not found to be caused by the vaccine virus (see later). In one study, no association between varicella vaccine and subsequent ischemic stroke was found[632]; stroke is a rare complication of natural varicella.[633] In Japan, a gelatin-free varicella vaccine with 1,300,000 doses distributed in 1999 and 2000 was associated with a marked reduction in reported anaphylactic reactions (no reports of serious reactions and five nonserious reactions) compared with 28 reports of serious anaphylactic reactions and 139 reports of nonserious reactions when gelatin-containing vaccine was in use, with 1,410,000 doses distributed from 1994 to 1999.[634]

For reported adverse events after vaccination that are plausibly biologically linked to the vaccine, laboratory testing of submitted specimens for VZV strain identification is critical. Early in the postlicensure period in the United States, a safety study linking reported serious adverse events with laboratory testing was undertaken by investigators at Merck and Columbia University using Merck's Worldwide Adverse Experience System.[229] This study is ongoing. Clinical data are reported, and coded laboratory specimens are tested in a blinded fashion, and the results are then analyzed together.[229,399] In particular, the study was probing for the following events: rashes of more than 50 lesions detected within 42 days after immunization; possible instances of secondary transmission of the Oka strain to others; varicella and HZ occurring after vaccination; and neurologic, dermatologic, and other possible complications of VZV infection after immunization. Analysis of data after approximately 48 million distributed doses of varicella vaccine in the United States showed that rashes caused by Oka strain occurred from 5 to 42 days after vaccination (median: 21 days) and ranged from one to 500 skin lesions (median: 51). In previously vaccinated persons, rashes caused by WT VZV occurred between 1 and 20 days after immunization (median: 8 days), with 10 to 1000 reported skin lesions (median: 100).[561] In the United States, strain identification to support vaccine safety evaluations is also available through the national VZV laboratory at the CDC, and in other countries (e.g., Canada, Australia, Germany) as well as regions (European) where similar programs for testing have been established.[627] HZ caused by vaccine strain VZV may occur in both healthy and in immunocompromised persons.[229,561,562,570] (For further details, see "Herpes Zoster in Vaccinated Persons" later.)

An Institute of Medicine systematic review of the epidemiologic, clinical, and biological evidence for adverse events associated with varicella vaccines through 2010 concluded that evidence supports causality in five adverse events: (1) disseminated vaccine strain virus without organ involvement (e.g., varicella-like rash extending to dermatomes beyond the initial injection); (2) disseminated vaccine strain virus with organ involvement (e.g., pneumonia, meningitis) in individuals with demonstrated immunodeficiencies; (3) vaccine strain reactivation (HZ) without organ involvement; (4) vaccine strain reactivation (HZ) with organ involvement; and (5) anaphylaxis.[635] These events are unusual or rare. A follow-up review of the evidence from the end of the Institute of Medicine report through 2012, identified evidence consistent with the Institute of Medicine report. Notable rare complications that have also been proven to be caused by VZV Oka strain include pneumonia, hepatitis, HZ meningitis, recurrent HZ, severe rash, secondary transmission, retinal necrosis,[636] vasculopathy of the central nervous system with immunodeficiency,[637] and, extremely rarely, death (see below). Treatment with an antiviral was often given, usually with recovery from VZV complications. Most of these patients were immunocompromised or had other serious medical conditions that were undiagnosed at the time of immunization, but others were basically healthy.[154,229,561,562,638] Two children with disseminated Oka disease were found to have an abnormality in invariant natural killer cells.[639,640] Two children and one adult with undiagnosed HIV infection who had very few $CD4^+$ lymphocytes developed disseminated varicella (as their presenting sign of HIV infection) from VZV Oka.[641–643] A vaccinee developed severe Oka varicella and was subsequently diagnosed with severe combined immunodeficiency.[644] Two children with neuroblastoma developed chronic HZ as a result of Oka VZV, which, with time and antiviral therapy, became drug resistant; they were then successfully treated with foscarnet.[645,646] Recurrent Oka HZ in a 2-year-old healthy boy was reported.[647] HZ and transmission of vaccine strain VZV are described in more detail in following sections.

Five fatal cases of Oka VZV infection have been described, all in immunocompromised patients. One was a child with leukemia from Germany who was vaccinated only 5 months after being in remission.[418] Another was a 15-month-old American child with undiagnosed symptoms of immunodeficiency who died after inadvertent vaccination against varicella.[648] Both of these children had pneumonia and characteristics of varicella. A third fatality was reported in an adult with a history of varicella in childhood, who was vaccinated against varicella following stem cell transplantation for lymphoma. He developed

persistent zoster involving skin and liver, despite treatment with valacyclovir. The VZV with which he was infected was found to be resistant to acyclovir.[649] A third death was reported in an elderly patient with chronic lymphocytic leukemia who had no longer been under immunosuppressive treatment for 6 months when he received the zoster vaccine.[649a] He developed disseminated VZV infection with multiorgan failure. A fourth death occurred in a child with a rare immunodeficiency (hypomorphic RAG2 deficiency) with late onset that was undiagnosed before vaccination. The child experienced autoimmune hemolytic anemia, hepatic and renal failure, and disseminated intravascular coagulation.[649b,649c] Additionally a child with DiGeorge syndrome and one diagnosed with severe combined immunodeficiency after vaccination died of pneumonia following MMRV and separate MMR and varicella immunizations, respectively, but it was unclear if the deaths were caused by varicella.[650,651]

Meningitis and encephalitis in association with HZ caused by VZV Oka strain has been confirmed by PCR in CSF in at least nine instances: one in a child with localized ophthalmic zoster on chemotherapy for cancer,[561,562] two in children with neuroblastoma who were receiving chemotherapy and developed disseminated HZ,[645,646] and six in otherwise healthy children, including one case of encephalitis.[154,405,559,627,652–654] All these patients recovered; most were treated with acyclovir. Other cases of HZ and meningitis in healthy vaccinated children with PCR-confirmed VZV in CSF have been reported.[559,561,562] These children all recovered, but without strain typing, it is not possible to assess vOka causality. Interestingly, WT VZV was identified as causal in an older woman given HZ vaccine who developed meningitis 1 day after immunization,[655] and in a few children who had been vaccinated.[154,562,656] These experiences illustrate the importance of typing of VZVs that are implicated as causing severe adverse events in vaccinated persons; not all are caused by the Oka strain, and those that are need to be identified as such, in the interest of assessing vaccine safety.

A pregnancy registry to track outcomes of inadvertent varicella vaccination 3 months before or during pregnancy was established in 1995 by Merck in collaboration with the CDC. A review of data reported after 10 years from the United States and Canada showed no features consistent with congenital varicella syndrome among the 531 live births of women prospectively enrolled in the registry, including among the 131 offspring of varicella-susceptible women (rate = 0; 95% CI, 0–6.7).[657] An updated report with 17 years of registry data (through 2012) continues to show no risk of congenital varicella syndrome among 788 live births of women prospectively enrolled in the registry including among the 160 offspring of seronegative women (rate = 0; 95% CI, 0–2.4).[658] Interestingly, the incidence of the congenital varicella syndrome significantly decreased in Australia after institution of their varicella immunization program.[659] No cases of the congenital varicella syndrome caused by Oka vaccine have been reported from any source. Because of a decrease in reporting and no data to indicate risk, the Merck/CDC varicella vaccine pregnancy registry was closed in 2013.[660]

There has been concern about the safety of various vaccines in children with defects in intermediary metabolism such as those with abnormal mitochondrial function. A retrospective study of varicella disease in 64 such children, however, revealed that 8% required hospitalization during clinical varicella. The conclusion of the study was that a clinical trial of varicella vaccine should be done in such children to determine whether vaccination is safer than the natural disease.[661]

Molecular studies of VZV DNA amplified from rashes in vaccinees with vesicular lesions have provided insight into possible virulence of the Oka strain, which is known to represent a mixture of VZVs with different SNPs.[662] Some studies have identified VZV in vesicles as clonal or monomorphic,[383,389] whereas others have found predominantly mixtures of the vaccine strain variants.[663] When mixtures are found, it is possible that more than one vesicle was sampled and incorporated into one specimen. Both research groups have primarily studied vesicular fluid that was not subjected to culture, making viral propagation in vitro an unlikely explanation for the described mixtures. One study suggested that there are specific VZV WT-like SNPs in the vaccine that are commonly associated with development of rash and thus may be associated with virulence.[386] Currently, there is considerable interest in whether recombinant VZVs (between WT and Oka) have emerged in the vaccine era.

Differing SNPs in vaccines prepared by Merck and GlaxoSmithKline have been identified. Merck's varicella vaccine is composed of 20% more WT SNPs than GlaxoSmithKline vaccines, which might relate to protection rates against varicella and occurrence of HZ after these vaccines.[393] There appear to be four core sets of SNPS that distinguish between wild and vaccine types of VZV.[385,393]

Combination Varicella (Measles-Mumps-Rubella-Varicella) Vaccines

Because of the higher rate of fever seen in prelicensure studies after the first dose of MMRV vaccine compared with MMR and varicella vaccines, postlicensure studies to evaluate the risk for febrile seizures were conducted by the vaccine manufacturer in the United States (Merck) and by the FDA and the CDC.[664,665] Compared with MMR and monovalent varicella vaccines, both studies demonstrated a twice-higher incidence of febrile seizures in vaccinated children (predominantly 12 to 23 months of age), 5 to 12 (RR, 2.0; 95% CI, 1.4–2.9%), or 7 to 10 (RR, 2.2; 95% CI, 1.0–4.7%) days after the first dose of MMRV, with one extra febrile seizure for every 2500 children vaccinated. No increased risk for febrile seizures was identified among children 4 to 6 years of age who received the second dose of MMRV compared with same-age children who received MMR and monovalent varicella vaccine at the same visit.[666] After consideration of the postlicensure data and other evidence, the ACIP adopted, in 2010, revised recommendations regarding the use of MMRV vaccine for the first dose and identified a personal or family (i.e., sibling or parent) history of seizure as a precaution for use of MMRV vaccine[667] (see "Routine Vaccination Schedule" later). Similar risk for febrile seizures after first dose Priorix-Tetra (GlaxoSmithKline MMRV) was identified in a study conducted in Germany.[668]

Transmission of Vaccine Virus

In leukemic vaccinees, the risk of transmission was directly proportional to the number of skin lesions in the vaccinee. Of 93 unimmunized and susceptible siblings exposed to a leukemic vaccinee with a vaccine-associated rash, 21 (23%) seroconverted. Of these contact children, five of 21 (24%) never developed a rash.[157] In comparison, the normal expected subclinical attack rate of WT varicella is approximately 5%.[20] In exposed infected siblings who developed rash, the median number of skin lesions was 12 (range, 1 to 200).[157] The greater the number of skin lesions in vaccinees, the greater the chance of transmission there was of the Oka strain. There was only one report of tertiary spread, further suggesting that the vaccine virus has limited ability for transmission.[157] No spread was found in 112 siblings exposed to a leukemic vaccinee who did not have a vaccine-associated rash.[157]

Transmission of the Oka strain was addressed in two clinical studies in healthy children using the Merck vaccine before

licensure.[439,669] In one, children in a household were randomized to receive either vaccine or placebo.[439] In that study, there was no transmission of clinical disease. However, three of the 439 initially seronegative placebo recipients exposed to vaccinees seroconverted to VZV without symptoms. No rash was reported in the vaccine recipients to whom they had been exposed. In view of the importance of rash in transmission, it seems that these results are more likely to be the result of serologic errors rather than actual transmission of VZV. In the second study, siblings of susceptible immunocompromised children (most with a diagnosis of leukemia) were vaccinated.[669] There was no evidence of clinical or serologic transmission of the Oka virus after vaccination in the approximately 30 families studied.

In contrast to data involving leukemic vaccinees in research studies,[201] in healthy vaccinees, transmission of Oka has been exceedingly rare, with only 11 documented occurrences from nine vaccinees (two vaccinees transmitted the virus to two other people each[412,414]), most commonly after household exposures. The exceptions are transmissions occurring in institutional and school settings. A 15-year-old institutionalized boy developed more than 500 lesions after vaccination and transmitted Oka strain to two other persons in his facility, including to an unvaccinated healthcare worker.[412] A 23-year-old kindergarten teacher with no history of varicella or varicella vaccination developed 50 to 100 vesicular lesions, confirmed as vOka, after exposure to a 5-year-old boy in her class 17 days after he developed HZ. He had received varicella vaccine (Varilrix) 13 months previously.[670] Thus, despite the distribution of more than 150 million doses of vaccine worldwide between 1995 and 2015, only these 11 cases of transmission have been reported. Although other cases of transmission have been suspected, they were not documented by laboratory testing, or perhaps they remain unreported in the literature. In contrast, the WT virus under similar circumstances, such as family contacts, is highly transmissible to susceptible persons from unvaccinated persons with a secondary attack rate as high as 87%,[20] and moderately transmissible from cases of natural varicella in vaccinees (secondary attack rate: approximately 37%).[509] In each instance of confirmed transmission of vaccine-type VZV, there was a rash caused by the Oka strain in the vaccinee. Among the secondary cases, seven were acquired from vaccinees with varicella-like rashes after vaccination, and four others were from children with HZ caused by the Oka strain.[229,410,412,414,627,670–673] The seven persons to whom transmission of Oka virus occurred directly from a healthy person soon after vaccination were a 4-month-old sibling, a healthy father, a pregnant mother, two healthy children of the vaccinee, an institutionalized adolescent, and a healthcare worker.[229,412,414,671] All these contact cases were mild. In addition, there was a report of neonatal varicella with vaccine-strain VZV diagnosed 22 days after maternal postpartum vaccination; the mother did not have a rash but the newborn was in the room when the mother was vaccinated and the most plausible mode of transmission was deemed aerosolization when the vaccine-filed syringe was cleared of air bubbles rather than transmission from the mother.[672]

The low transmission rate of the Oka strain is of importance because of the potential for spread of vaccine-type VZV from healthy vaccinees, such as healthcare workers, to other susceptibles. If a healthy vaccinee has no rash, the opportunity for viral spread of the virus may be close to nonexistent.

Herpes Zoster in Vaccinated Persons

During prelicensure clinical trials, a major question concerning the varicella vaccine was whether the vaccine virus could establish latency, potentially resulting in later development of HZ. It has now been demonstrated that the vaccine virus *can* become latent, and can later, reactivate to cause HZ in both healthy and immunocompromised persons.[649,674,675] In the postlicensure period, HZ after vaccination has been laboratory confirmed as both wild- and vaccine-strain VZV.[229,561,562,570] Some vaccine-strain HZ cases have required hospitalization and, as stated earlier, nine HZ cases had concurrent meningitis or encephalitis confirmed to be vaccine-strain VZV.[154,405,561,562,627,645,646,652–654] An analysis of data on HZ in vaccinated persons reported passively to the vaccine manufacturer found that HZ occurred a median of 362 days after vaccination; 30% to 40% of these cases were caused by WT VZV.[229,561,562] However, these data are not population based and are subject to reporting bias. In a cohort study of 172,163 vaccinated children from a U.S. health maintenance organization who were followed up for a median of 2.6 years, the incidence of HZ was 0.27 in 1000 PY. Most of the children were vaccinated between 12 and 18 months of age. The average age of children who contracted HZ was 4.1 years.[676] No comparative data are available on the risk of HZ after varicella acquired at this age. In a retrospective analysis of children in a large managed care practice, the incidence of laboratory-confirmed HZ was 0.48 per 1000 PY in vaccinated children and 2.30 per 1000 PY in unvaccinated children; the incidence of HZ in 118 vaccinated children was 79% lower than in unvaccinated children. Fifty percent of vaccinees had HZ caused by WT VZV.[570] Long-term follow-up of vaccinated, healthy children is required to provide detailed data of risks and severity of vaccine-strain HZ throughout the life span. It remains unclear whether the morbidity associated with Oka zoster is significantly less than that associated with the WT infection.[569,570] Only one case of HZ in a vaccinated adult (Varivax) resulting from the Oka strain has been recorded,[649] but the numbers of vaccinated adults is very small compared with numbers of children vaccinated. This case was fatal and occurred in an immunocompromised middle-aged man.[649] Finally, one case of proven Oka HZ in an immunocompetent 68-year-old woman who received Zostavax 9 months previously has been reported.[677] The effect of VZV vaccines on the occurrence of HZ is discussed in detail in Chapter 63.

INDICATIONS FOR VACCINATION

Single-antigen (monovalent) varicella vaccines are now widely available throughout the world, and MMRV vaccines are becoming more available. Varicella vaccines are licensed for use in healthy children 12 months of age and older, except for the GlaxoSmithKline varicella vaccine, which can be administered to children as young as 9 months in some countries. Okavax is licensed for use in certain groups of mildly immunocompromised persons (see package insert). In the United States, monovalent varicella vaccine (later Varivax) was initially available under a special protocol for use in children with acute lymphoblastic leukemia that was in remission; the protocol is no longer operative.[678] MMRV (ProQuad) is licensed in the United States for healthy children 1 to 12 years old (see package insert). Four domestic varicella vaccines are licensed and available in China.

Depending on the goals of a varicella vaccination program, indications for use of varicella vaccine vary from country to country. Possible vaccine strategies include universal vaccination of children after 9 or 12 months of age, preferably with catch-up vaccination of older children or adolescents to prevent development of a susceptible cohort that was neither vaccinated nor exposed to wild VZV. Other strategies include vaccination of adolescents and adults, including women of childbearing age, or targeted vaccination of high-risk groups, including children with HIV infection and candidates for

organ transplantation, healthcare workers, and family contacts of immunocompromised persons. Children being treated for leukemia or other malignancies or are otherwise immunocompromised should not be vaccinated; they are less likely to develop varicella than they used to be as a consequence of decreasing transmission of VZV in countries that use universal vaccination. If they are exposed to varicella or HZ, VariZIG is recommended for postexposure prophylaxis. Should varicella occur, however, immediate treatment with acyclovir should be given.

In 1995, the United States became the first country to recommend a national universal childhood vaccination program. Others that now implement universal childhood vaccination programs include Australia, Canada, Costa Rica, Germany, Greece, Israel, Japan, Qatar, Latvia, Luxembourg, Taiwan, Saudi Arabia, Turkey, South Korea, United Arab Emirates, Uruguay, and some areas of Italy and Spain. Targeted vaccination programs, most commonly for susceptible healthcare workers and immunocompromised persons for whom the vaccine is licensed, have been adopted in a number of countries in Europe.[679,680]

In countries with one-dose childhood vaccination programs, varicella vaccine is recommended for children 9 or 12 months through 12 years of age; two doses, 4 to 8 weeks apart, are recommended for persons aged 13 years or older. A routine two-dose vaccine policy for varicella-containing vaccines for children, similar to the policy recommendations for MMR vaccines, was recommended in 2007 in the United States by the ACIP and the American Academy of Pediatrics,[553,681] and in Germany in 2009.[682] Vaccine policy recommendations in the United States evolved between 1996 and 2007.[553] In 1995 and 1996, varicella vaccine was recommended for routine use in healthy children 12 to 18 months of age; for healthy, susceptible children 19 months through 12 years of age; and for persons in close contact with those at high risk for serious varicella.[683,684] Updated recommendations in 1999–2000 and in 2007 progressively expanded these recommendations to cover the entire susceptible population (Box 62.1).[553]

The following factors led to the change to a routine two-dose recommendation for children: instances of primary and possibly secondary vaccine failure, outbreaks of varicella in schools despite high rates of vaccination, costs for investigation and control of outbreaks, contagiousness of breakthrough varicella, boosting of both humoral and cell-mediated immunity after a second dose of vaccine, and the projected higher vaccine efficacy with two doses.[553,681] Catch-up programs for children who received only one dose were recommended. Without a second dose, it was predicted that there would be an accumulation of vaccinated adolescents who either had not seroconverted or had developed a completely protective antibody response after one dose and were therefore at risk of becoming varicella-susceptible young adults, potentially at high risk of developing severe varicella. Potentially, this would occur particularly when there was less opportunity for immune boosting because there was less circulation of WT VZV. Although it was not predicted that there would be complete protection against varicella after two routine doses of varicella vaccine, it was projected that numbers of breakthrough cases would fall significantly, with a subsequent decrease in transmission of the virus. Current U.S. recommendations are provided in detail in the next section.

BOX 62.1 Recommendations for Varicella Vaccine in the United States, 2007

Routine childhood schedule: two doses (at least 3 months apart)[a]

Preschool and school children:

- First dose at 12 to 15 months
- Second dose at 4 to 6 years,[a,b] or before the child enters school

Persons ≥13 years:

- Two doses (4 to 8 weeks apart)[a]

[a]Unless other evidence of immunity; see Box 62.2.
[b]Or at least 3 months after first dose.

Routine Vaccination Schedule

Children Younger Than 13 Years of Age

A routine two-dose varicella vaccine policy was recommended in the United States in 2007 (see Box 62.1).[553] All healthy children should receive two 0.5-mL doses of a varicella-containing vaccine administered subcutaneously. The recommended ages for administering the first (12 to 15 months) and the second (4 to 6 years) doses of MMR and varicella vaccines are harmonized; however, the second varicella vaccine dose may be administered at an earlier age if the interval between the first and second doses is at least 3 months. For the first dose vaccination at age 12 to 47 months, MMR vaccine and monovalent varicella vaccine or MMRV vaccine may be used. Because of the increased risk of febrile seizures following MMRV vaccine compared with MMR and monovalent varicella vaccines administered at the same visit (see "Vaccine Adverse Events" earlier), the CDC recommends that MMR vaccine and monovalent varicella vaccine be administered for the first dose in this age group, unless the parent or caregiver expresses a preference for MMRV vaccine. If the first dose is administered at age 48 months or older, and also for the second dose at any age, use of MMRV vaccine is preferred over separate injections of its equivalent component vaccines (i.e., MMR and varicella vaccines).[667]

Adults and Adolescents 13 Years of Age and Older

Because varicella infection is more severe among adolescents and adults, vaccination is important for them. MMRV vaccine is not licensed for use in this age group. Persons 13 years of age and older without evidence of immunity should receive two doses of monovalent varicella vaccine, 4 to 8 weeks apart. If an interval longer than 8 weeks elapses after the first dose, the second dose can be administered without restarting the schedule. Vaccinating adult groups at high risk of exposure/transmission or close contact with persons at high risk of severe varicella should be a priority. These groups include students and staff in schools, colleges, and other post–high school institutions, inmates and staff members of correctional institutions, and military personnel, international travelers, household contacts of immunocompromised persons, and healthcare workers. Because a fetus may be affected if a pregnant woman acquires varicella, women of childbearing age should be assessed for evidence of varicella immunity; if not pregnant, those without evidence of immunity may be immunized. If already pregnant, they should be vaccinated on completion or termination of the pregnancy, and they should receive the first dose of vaccine before discharge from the healthcare facility.

Healthcare Workers

Nosocomial transmission of VZV is a well-recognized medical and public health problem.[580,685–687] Because of their high risk of exposure to varicella or HZ and close contact with persons at high risk for serious complications, healthcare workers should be routinely vaccinated with two doses of varicella

vaccine unless they have other evidence of immunity. Birth in the United States before 1980 is not considered evidence of immunity in this group. In healthcare settings, serologic screening before vaccination of personnel with a negative or uncertain history of varicella is likely to be cost-effective because approximately 80% are likely to be immune. Some institutions may elect to perform serologic testing on all workers before vaccination because a small proportion of persons with a positive disease history may be susceptible.[688–692] Serologic testing after vaccination *is not recommended* unless a sensitive and specific test can be used. Available commercial assays are not sensitive enough to detect low levels of antibody after vaccination.[238–240,553]

Varicella vaccine is recommended in the United States for postexposure vaccination, for outbreak control, and for certain groups in whom there are adequate data on vaccine safety and immunogenicity or efficacy.[553] These include selected HIV-infected persons and certain groups of immunocompromised persons (see later).

Nursing Mothers

Varicella vaccine can be administered to nursing mothers. A small study of 12 seronegative women who received varicella vaccine after delivery and who breastfed their infants found no evidence of vaccine VZV excretion in breast milk.[693] VZV DNA was not detected by PCR in any of 217 breastmilk specimens. None of the infants were seropositive, and serum samples from six of the infants were all negative for VZV DNA by PCR.

Persons Infected With Human Immunodeficiency Virus

In small clinical trials, two doses of monovalent varicella vaccine were administered 3 months apart to HIV-infected children 1 to 8 years of age. The $CD4^+$ T-lymphocyte levels were higher than 25% (in 41 children) and 15% or higher (in 44 children), and more than 80% of the children had subsequent detectable immune responses (antibody and/or CMI) to VZV at 1 year after vaccination.[601,602] Because children infected with HIV are at greater risk than healthy children for morbidity from varicella and HZ, the ACIP recommended that, after weighing potential risks and benefits, monovalent varicella vaccine be considered for HIV-infected children with $CD4^+$ T-lymphocyte levels higher than 15%. Eligible children should receive two doses of monovalent varicella vaccine 3 months apart. Data on the use of varicella vaccine in HIV-infected adolescents and adults are lacking. Although the immunogenicity of vaccination might be lower and the safety profile different in older HIV-infected children, adolescents, and adults than in children 1 to 8 years of age, the risk for severe disease from wild VZV and the potential benefit of vaccination must be recognized. Expert opinion is that vaccination may be considered (two doses administered 3 months apart) for HIV-infected persons with $CD4^+$ T-lymphocyte levels higher than 200 cells/μL in these age groups. If vaccination of HIV-infected persons results in clinical disease, the use of acyclovir would be expected to modify the severity of disease. Monovalent varicella vaccine is highly effective in preventing both varicella and HZ in HIV-infected children.[623] *Because data are not available on safety, immunogenicity, or efficacy of MMRV vaccine in HIV-infected children, MMRV vaccine should not be substituted for the component vaccines when vaccinating these children.*

Evidence of Immunity

Box 62.2 lists the criteria used to evaluate evidence of immunity to varicella. Evidence of immunity includes documentation of age-appropriate vaccination; laboratory evidence of immunity or laboratory confirmation of disease; being born in the United States before 1980; a healthcare provider diagnosis of varicella or HZ; or verification of a history of varicella disease or of HZ. *Note: Birth in the United States before 1980 is not considered evidence of immunity for healthcare personnel, pregnant women, or immunocompromised persons.*

BOX 62.2 Evidence of Immunity

1. Documentation of age-appropriate vaccination:
   - Preschool-age children, 12 months or older: one dose
   - School-age children, adolescents, and adults: two doses[a,b]
2. Laboratory evidence of immunity[c] or laboratory confirmation of disease
3. Born in the United States before 1980[b,d]
4. A healthcare provider diagnosis of varicella or verification of history of varicella disease[c,e]
5. A healthcare provider diagnosis of herpes zoster or verification of history of herpes zoster

[a]Unless other evidence of immunity.
[b]For children who have received their first dose after 12 months of age and before age 13 years, and when the interval between the two doses was at least 28 days, the second dose is considered valid.
[c]Commercial assays can be used to assess disease-induced immunity, but they lack sensitivity to detect vaccine-induced immunity in all instances (may yield false-negative results).
[d]For healthcare workers, pregnant women, and immunocompromised persons, birth before 1980 should not be considered evidence of immunity.
[e]Verification can be done by any clinician (e.g., school or occupational clinic nurse, physician assistant, physician). For persons reporting or presenting with atypical or mild cases, assessment by a physician or designee is recommended, and either of the following should be sought: (1) an epidemiologic link to a typical varicella case, or (2) evidence of laboratory confirmation, if it was performed at the time of acute disease. When such documentation is lacking, persons should not be considered as having a valid history of disease, because other diseases may mimic mild atypical varicella.

Contraindications and Precautions

Information in package inserts should be reviewed carefully before administration of a varicella-containing vaccine. For contraindications and precautions to MMR components of MMRV vaccine, see Chapters 37, 40, and 53. Although no infants with congenital varicella syndrome secondary to vaccine-type virus have been reported,[657,658,660,694] vaccination in pregnancy is contraindicated.

PUBLIC HEALTH CONSIDERATIONS

Goals and Cost-Effectiveness of Vaccination

The goals of varicella vaccination programs vary. It may be prevention of varicella and its complications, prevention of severe disease and death, or disease prevention and control in selected target groups such as adolescents, adults, or healthcare workers. The goal of the U.S. vaccination program is a greater than 90% reduction in varicella disease. The United States adopted a strategy of universal childhood vaccination to achieve this goal.

The World Health Organization (WHO), in its 2014 position paper on varicella vaccines, recognized the strong scientific evidence that in immunocompetent individuals, varicella vaccine is safe and effective for preventing morbidity and

mortality from varicella.[695] The WHO recommended consideration of routine immunization of children against varicella in countries where varicella has an important public health impact. The WHO also recommended that in such countries, resources should be sufficient to support sustained vaccine coverage of 80% or greater, because when varicella vaccine coverage levels are less than 80%, there is a risk of shifting the infection to older ages in some settings, which may result in an increase in morbidity and mortality, despite a reduction in the total number of cases, because of the increased risk of severe disease in adults. The WHO recommended one dose if the goal of the vaccination program is to reduce severe morbidity and mortality from varicella, and two doses if the goal also includes reduction of the number of varicella cases and outbreaks. Healthcare workers constitute a special group who should receive two doses, particularly if they have contact with immunocompromised patients.

Several published studies on the significance of varicella as a public health problem were considered when varicella vaccine was added to the childhood immunization schedule in the United States.[40,696–698] An updated 2008 analysis examined the economic costs and benefits of a one- and two-dose vaccination program for children using estimates of varicella morbidity and mortality in the United States that accounted for the declines reported during the one-dose program, an assumption of neutrality regarding the potential effects of vaccination on HZ (similar to previous analyses), and direct and indirect costs at the time of the update. Compared with no vaccination program, a one-dose program was cost-saving from the healthcare payer and societal perspective, and a two-dose program was cost-saving from a societal perspective; the incremental cost of the second dose if a one-dose program was already in place was $109,000 per quality-adjusted life-year saved.[699] Several analyses in other countries have focused on costs of a two-dose varicella vaccination program during early childhood, adolescence, or both. These programs are cost-effective from the societal perspective, but not from the direct payer perspective.[700,701] Additional studies providing data on costs of varicella, as well as cost–benefit and cost-effectiveness studies providing economic evaluations of childhood vaccination programs and selective vaccination programs among adolescents, adults, women of childbearing age, military recruits, healthcare workers, seafarers, and pediatric liver and kidney transplant recipients, have since been published from many countries and in review articles.[280,614,686,687,690,702–721] Most studies did not include dynamic models that account for community protection effects and are likely to have underestimated the impact of varicella vaccination. There is general agreement that universal vaccination of children with one or two doses of varicella vaccine in the second year of life is beneficial from a societal perspective mainly because of savings from prevented lost workdays for the parents. From the healthcare payer perspective, most studies show that universal childhood vaccination programs will not provide savings at the current price of the vaccine. Exceptions are a study from Israel and the 2008 analysis from the United States that found that a national varicella vaccination program was cost saving or cost neutral (break-even), respectively, from the health service perspective.[699,707] No data on cost-effectiveness of routine childhood varicella vaccination are currently available from low- and middle-income countries.

Because of modeled predictions of an increase in HZ as a possible short- and medium-term effect of a varicella vaccination program, and the significant described health burden resulting from HZ, some economists have argued for including these costs in economic analyses, which in the short-to-medium term may make a varicella vaccination program much less cost-effective or even non–cost-effective. However, in the long term, as HZ incidence declines to levels lower than currently experienced, models predict the net health savings from varicella vaccination to increase substantially.[307,722]

A recent study modeling the combined effects of varicella (two doses in childhood) and HZ vaccination programs concluded that varicella vaccination will lead to a net increase in HZ for 40 years, and the effectiveness of this combined strategy is limited, as much of the increase in HZ is expected to occur in adults too young to be vaccinated against HZ in existing vaccine programs.[723] Varicella vaccination of susceptible adolescents would be expected to have a smaller potential effect on HZ epidemiology, although such a program would also be expected to have limited impact on varicella morbidity and mortality. These analyses, however, do not consider the potentially complex interactions that may be involved in maintenance of immunity to VZV after varicella or varicella vaccine. Both subclinical reactivation and external boosting may play important roles in maintaining VZV immunity, and it is plausible that a decline in external boosting may be compensated for by an increase in subclinical reactivation. This analysis in addition does not recognize that the age for HZ vaccination can be adjusted as epidemiologically necessary.

The costs and public health impact of varicella disease in hospital settings have been well documented with regard to such factors as work furloughs, serologic testing, patient isolation, administration of passive immunization, wages paid to replacement healthcare workers, and infection control expenses.[280,686,687,709,716] Most studies of vaccination programs aimed at preventing nosocomial VZV exposure and transmission to high-risk patients by vaccinating healthcare workers have found that these programs generate savings from an employer's perspective.[280,686,687,709,716,724,725,725a] Factors that affect the results the most are vaccine price and accuracy of screening of disease history as it relates to true susceptibility. In the United States, healthcare workers were designated as a priority group for vaccination at the start of the vaccination program because of their risk for close contact with persons at high risk for severe disease. Because nosocomial VZV problems are universal and the problem may be greater in populations where a higher proportion of adult healthcare workers are susceptible, protection of healthcare workers through vaccination should be strongly considered even in countries where a childhood vaccination program is not a high priority.[685]

Postvaccine Epidemiology

United States: One-Dose Varicella Program

In the United States, substantial reductions in varicella morbidity and mortality have occurred since implementation of the one-dose varicella vaccination program. By 2005, before implementation of the two-dose program, vaccine coverage among children 19 to 35 months of age was 88%.[553] Coverage reached 90% in 2007 and has stayed at or above that level since. Monitoring occurrence of infectious diseases in the United States is most commonly conducted using a nationally notifiable disease surveillance system. However, because varicella was not a nationally notifiable disease at the start of the vaccination program, active varicella surveillance was established in 1995 in three communities with a combined population of 1.2 million persons. Initial impact of the vaccination program was described in these communities; active surveillance continued through 2010 in two of them. Compared with 1995, vaccine coverage among children 19 to 35 months of age in the three communities had reached 74% to 84% by 2000, and reported varicella cases declined by 71%, 84%, and 79%.[509] Varicella-related hospitalizations had declined by approximately 80%.

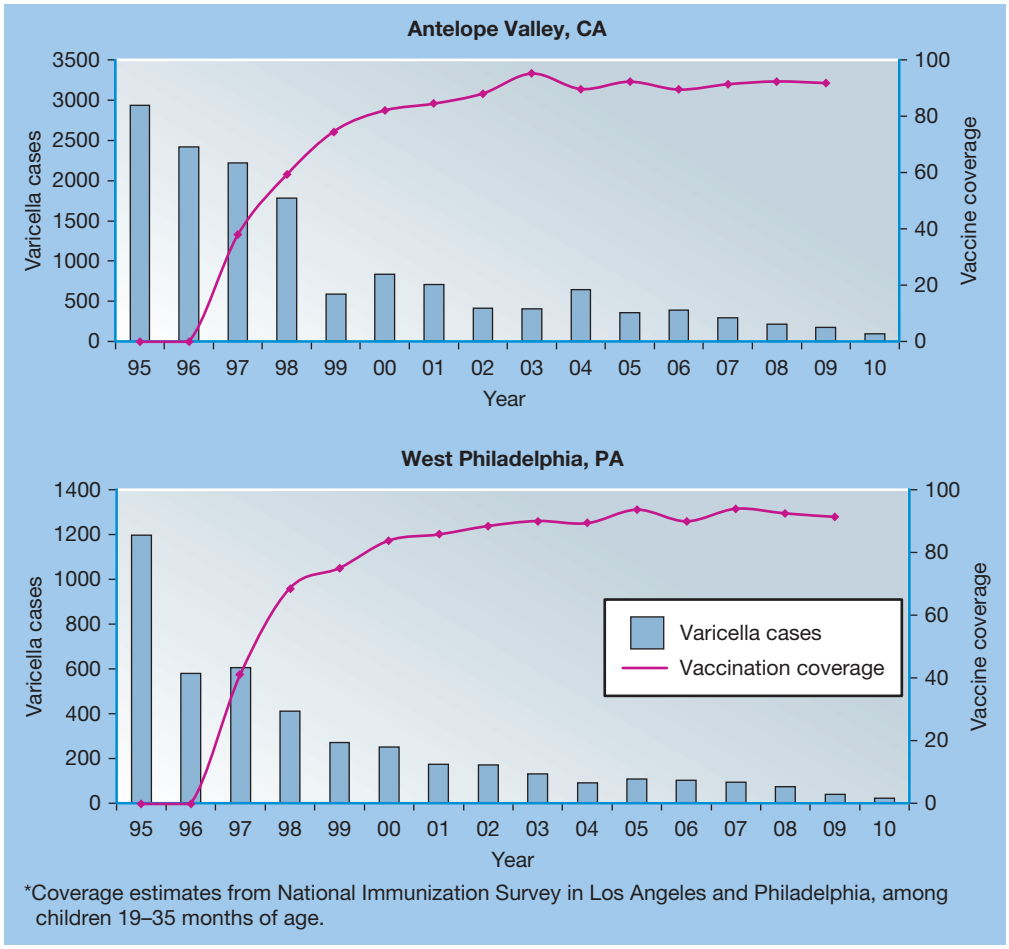


Figure 62.10. Reported varicella cases from 1995 to 2010 in two sentinel counties with vaccination coverage. *(From Guris D, Jumaan AO, Mascola L, et al. Changing varicella epidemiology in active surveillance sites: US, 1995–2005.* J Infect Dis. *2008;19[Suppl 2]:S71–S75; and Bialek SR, Perella D, Zhang J, et al. Impact of a routine two-dose varicella vaccination program on varicella epidemiology.* Pediatrics. *2013;132[5]:e1134–e1140.)*

By 2005, vaccine coverage had reached approximately 92% and 94% in two surveillance areas, Antelope Valley, California, and West Philadelphia, Pennsylvania, respectively, and varicella incidence had declined 90% in both sites, with declines in incidence in all age groups.[726] The greatest declines (>90%) occurred in children 1 to 9 years of age, but an approximately 80% decline in incidence also occurred in infants not eligible for vaccination (Fig. 62.10).[726] In the context of declines in varicella incidence in all age groups, the peak age of varicella infection increased from 3 to 6 years in 1995 to 9 to 11 years in 2005, and the proportion of persons who were vaccinated increased from less than 1% to 60% over the same time period.[726] From 1995 to 2005, the declines in incidence were mirrored by declines in the number, size, and duration of varicella outbreaks in childcare centers and schools.[727]

The effect of varicella vaccination on varicella incidence has also been noted from four states with consistent varicella reporting to the passive national notifiable disease surveillance system (Illinois, Michigan, Texas, and West Virginia), and from statewide survey or surveillance data in the states of Massachusetts and Connecticut.[424,728,730,731]

On the national level, significant declines in varicella-related deaths, hospitalizations, ambulatory visits, and health expenditures were noted initially within 5 to 6 years of program implementation (see Figs. 62.11 and 62.12).[349,350,732–735] Considering all ages and both underlying and contributing

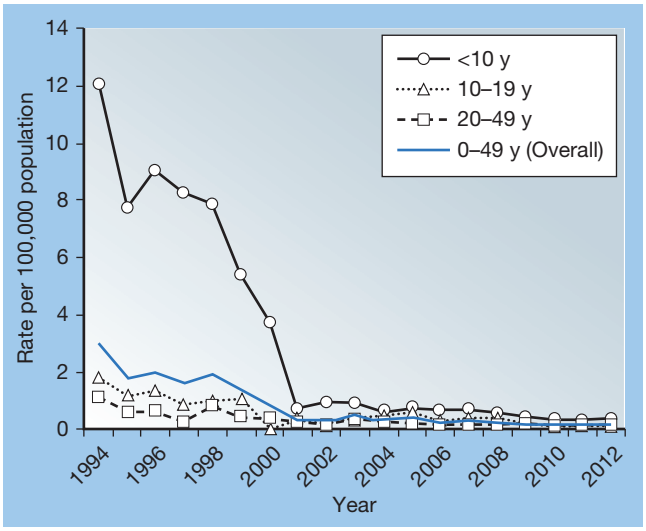


Figure 62.11. Reduction in varicella hospitalizations for different age groups after a vaccination program. *(From Leung J, Harpaz R. Impact of the maturing varicella vaccination program on varicella and related outcomes in the United States: 1994–2012.* J Pediatric Infect Dis Soc. *2016;5[4]:395–402.)*

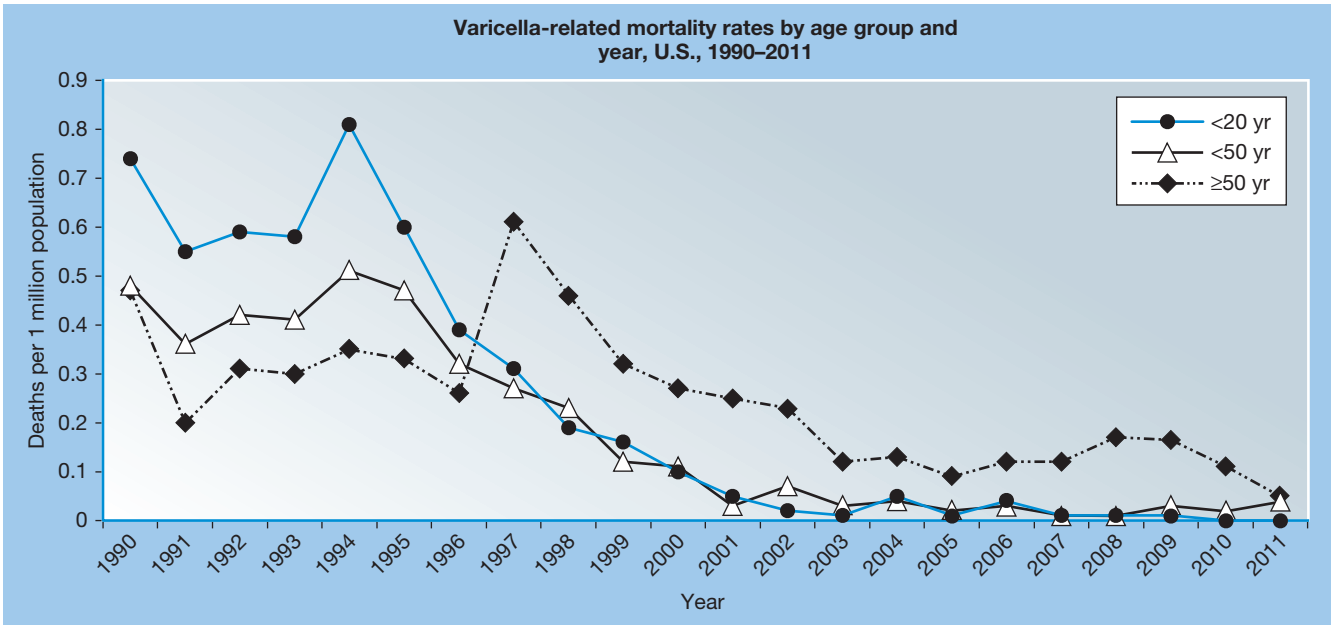


Figure 62.12. Annual age-specific mortality rates for varicella listed as the underlying cause, United States, 1990 to 2011. *(From Leung J, Bialek SR, Marin M. Trends in varicella mortality in the United States: data from vital statistics and national surveillance.* Hum Vaccin Immunother. *2015;11[3]:662–668.)*

causes of death, by 1999 to 2001, compared with the 5 years preceding the vaccination program (1990 to 1994), national varicella deaths declined from an average of 145 deaths a year to 66 deaths a year. Considering varicella as the underlying cause of death, over this time period only, mortality rates declined 92% in children 1 to 4 years old, and 74% to 89% in infants younger than 1 year and persons 5 to 49 years.[350] By 2005 to 2007, which still primarily reflected the impact of the one-dose vaccination program in children, the average age-adjusted mortality rate with varicella as an underlying cause of death declined 88% to 0.05 per million population from 0.41 per million in 1990 to 1994, and the age-specific mortality rates declined 97% among children and adolescents younger than 20 years of age, 90% among adults 20 to 49 years of age, and 67% among adults 50 years of age and older (Fig. 62.12).[732] During 2002 to 2007, there continued to be no racial differences in mortality rates, as achieved for the first time in 2001. Varicella hospitalizations declined significantly as well (Fig. 62.11). By 2002, compared with 1994 to 1995, hospitalizations for varicella declined by 88% and ambulatory visits by 59%, with more than 90% of the declines in children younger than 10 years of age and adolescents 10 to 19 years old. Direct medical expenditures for varicella hospitalizations and ambulatory visits decreased by 74%.[734]

Updated analyses showed continued declines in varicella hospitalizations in all age groups from 2000 to 2006, with an estimated 50,000 hospitalizations prevented during this time period, and a 98% reduction in varicella hospitalizations by 2004 among patients 0 to 4 years old.[735,736] Additional studies have documented the impact of the varicella vaccination program at local levels on hospitalizations, including reducing differences in varicella hospitalization rates between African American and white children emergency room visits, among Native American and Alaska Native populations, and, additionally, on invasive group A streptococcal infections.[564,737,740,742] In the latter study, no shift in the burden of varicella to older age groups was found in hospitalized patients.[742]

United States: Two-Dose Varicella Program

Despite these considerable successes in control of varicella disease and its severe complications in the United States, varicella outbreaks, albeit with milder cases and of shorter duration, have occurred in highly vaccinated school children, as mentioned previously. This, coupled with evidence that one dose of vaccine for children may not provide a sufficient immunologic "burst" to provide adequate and long-lasting immune memory, which may lead to cohorts of partially or fully susceptible vaccinated children aging into adolescence and adulthood,[743] resulted in adoption of a routine two-dose policy in the United States for children in 2007.

During the first 5 years after introduction of the two-dose program, the reported varicella incidence was the lowest since the start of the vaccine program compared with the fairly stable incidence from 2000 to 2006 with fewer outbreaks and milder disease.[564] In 2010, in the two varicella active surveillance sites, incidence declined 76% and 67% compared with 2006 and 98% in both sites since the vaccination program implementation in 1995.[564] Age-specific incidence rates were lower for all age groups in 2010 compared with 2006, and the decline was particularly pronounced among children 5 to 9 years of age (79% and 88% decline), the target age group for the two-dose vaccination program, with the next greatest declines in adolescents 10 to 14 years old (75% and 91% decline) and children 1 to 4 years of age (54% and 73% decline).[564]

By 2010, two-dose coverage among 4 to 6 year olds were 84% and 59% in the catchment area of the active surveillance sites. The median age of varicella infection increased from 6 to 8 years to 7 to 9 years among vaccinated patients and from 12 to 15 years to 16 to 20 years among unvaccinated patients, but the number of cases was significantly lower in each age group. An analysis of varicella outbreaks reported to the CDC from six states indicated that the routine two-dose program significantly reduced the number, size and

duration of outbreaks; the number of outbreaks declined 78%, from 147 in 2005 to 33 in 2012, and the median size from 12 to 7 cases.[744] Similar impact of the two-dose program was reported in Connecticut, a state with good varicella surveillance.[745] Analyses of passive national notifiable disease surveillance system data documented an 85% decline in varicella incidence from 2005–06 (the end of the one-dose varicella vaccination program) to 2013–14. In the four states with consistent reporting since before implementation of the vaccination program, incidence declined an average of 97% from 1993–95 to 2013–14 (range, 93–98%).[745a]

Additional significant declines in varicella-related hospitalizations and outpatient visits have been documented since the implementation of the two-dose varicella vaccination program.[746] Between 2006 and 2012, outpatient visits and hospitalizations declined 60% and 38%, respectively, for an overall decline of 84% and 93% compared with prevaccine years (1994–95).[746]

As far as mortality, while during 2008–11, the overall rate for varicella as the underlying cause of death remained unchanged from the end of the one-dose era, a decline in deaths in persons younger than age 20 years was seen, and there have been no deaths among 1 to 9 year olds in 2008–11 or among 10 to 19 year olds in 2010–11.[563]

Laboratory-confirmed varicella cases in two-dose vaccinated children have been confirmed, both in outbreak settings and in active surveillance sites, but are unusual.

Other Countries

As other countries have implemented one-dose childhood varicella vaccination programs, additional data on the impact of these programs have become available. In Germany, where a one-dose national vaccination program was implemented in 2004, a decrease in the incidence of varicella and its complications was observed using data from physician-based sentinel surveillance.[682,748] Reported varicella cases declined 55% from April 2005 to March 2009, with a 63% reduction among children 0 to 4 years of age. Comparing varicella seasons in 2005 and 2009, a 63% decline in cases and an 81% decline in varicella complications was apparent.[748] In a surveillance project in Munich and its surrounding districts, aside from reductions in incidence, a 43% decline in varicella hospitalizations in children younger than 17 years of age was reported during 2005 and 2009 with 78% decline among children younger than 5 years of age; coverage reached 53% in 2008–09.[747] Because of varicella cases occurring in vaccinated children and the experience of the United States, a two-dose schedule was formally recommended in Germany in July 2009.[682]

In Italy's Veneto region, varicella incidence rates decreased significantly 2.5 years after the universal vaccination program was introduced and greater than 70% vaccine coverage reached.[749]

In Canada, where the varicella vaccination program was recommended in 1999, declines of 81% to 88% in the number of hospitalized varicella cases were reported between 2000 and 2008, with effects of the vaccination program being noted beginning 1 to 2 years after the start of the program; indirect protection of persons outside the vaccinated cohorts was also documented.[750] Vaccination coverage was available for a few provinces and ranged from 74% to 91% in 2007–08.

The impact of a one-dose vaccination program on varicella and its complications has also been described from Taiwan, Uruguay, Sicily, and Australia.[738,741,751–755] Studies from Australia have indicated a significant decrease in both the congenital varicella syndrome (100%) and neonatal varicella (85%) as a result of the national vaccination program begun in 2005.[659]

In the Navarra region, Spain, where a two-dose routine childhood program was introduced from the beginning and high coverage was achieved early in the program, declines of 98.5% in incidence in children and 89% in hospitalizations were achieved within a period of only 5 years.[756]

A two-dose European study of GlaxoSmithKline MMRV vaccine that was randomized and controlled demonstrated an efficacy of 94.9% (97.5% CI, 92.4–96.6) against all varicella compared to 65.4% (97.5% CI, 57.2–72.1) efficacy after one dose.[757] In this study, the interval between the first and second dose of MMRV was 6 weeks. The follow-up lasted for 3 years.

Varicella Vaccine and Zoster

Because Oka VZV has the capacity to reactivate to cause HZ, understanding the effect of varicella vaccine on HZ epidemiology is important. This includes the risk of HZ after receiving the Oka strain in vaccinated persons, as well as the potential effect that the declining incidence of varicella disease from decreased viral transmission may have on HZ in the general population. Potential effects might either reduce or increase the risk of HZ. The risk of acquiring HZ is related to loss of cell-mediated immunity to VZV. Hope-Simpson first hypothesized that immunity to VZV may be maintained by periodic internal reactivation of VZV, and external boosting of immunity through exposures to varicella or HZ, or both.[446] The next section summarizes prelicensure and postlicensure studies of HZ in vaccinated immunocompromised and healthy persons, studies examining the effect of external and internal boosting of VZV immunity through varicella exposures on HZ incidence and risk, and HZ epidemiology in different populations in the prevaccine and postvaccine eras.

Varicella Vaccination and Risk (Incidence) of Zoster

Immunocompromised Persons. Prelicensure and postlicensure clinical studies have demonstrated a considerably lower risk of HZ in vaccinated immunocompromised children with leukemia, children undergoing renal transplantation, and HIV-infected children, compared with those with a history of natural varicella.

Because of the severity of HZ in immunocompromised populations, the effect of varicella vaccination on the risk of HZ was studied intensively in the prelicensure period. These persons were a pragmatic cohort to study because patients who have had natural chickenpox develop HZ at a much higher rate than healthy children, so it was possible to study the incidence of HZ in a relatively short time frame.[758] Vaccinated leukemic children were therefore followed closely for development of HZ and compared with leukemic children who had natural varicella. In Japan, one study found HZ in eight (15%) of 52 vaccinated leukemic children and in 11 (18%) of 63 leukemic children with a history of varicella.[759] In the United States, none of 34 vaccinated leukemic children, but 15 (21%) of 73 leukemic children with a history of varicella, developed HZ ($P = .017$), suggesting that HZ was less common after immunization than natural infection.[760] In the NIAID collaborative study, the rate of HZ was 2% in vaccinees and 15% in controls with a history of varicella. Members of a subset of these vaccinees were prospectively matched, according to chemotherapeutic protocol, with 96 leukemic children who had experienced natural varicella. A life-table analysis revealed that the incidence of HZ was about three times lower in vaccinees (8 per 1000 PY) than in the matched leukemic children who had experienced natural varicella (24.6 per 1000 PY; $P = .01$) (Fig. 62.13).[199,592] Thus, varicella vaccine was approximately 67% effective in this cohort in preventing HZ. Similar results were found in vaccinated children who

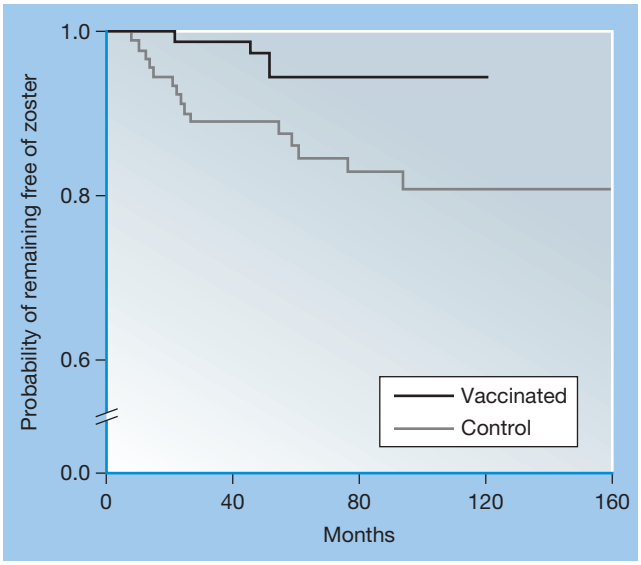


Figure 62.13. Zoster after vaccination in leukemic children after natural disease and immunization. Kaplan-Meier product-limit analysis of the probability of remaining free of zoster in 96 children with leukemia who received varicella vaccine and 96 children with leukemia who had naturally acquired chickenpox before or after the diagnosis of leukemia. *(From Hardy IB, Gershon A, Steinberg S, et al. The incidence of zoster after immunization with live attenuated varicella vaccine: a study in children with leukemia.* N Engl J Med. *1991;325:1545–1550.)*

underwent renal transplantation. In a study of French children undergoing renal transplantation, the rate of HZ over 10 years was 7% in vaccinees, 13% in those with varicella before transplantation, and 38% in those who developed varicella after grafting.[598]

In the postlicensure period, a study of HIV-infected children, after adjusting for receipt of HAART and excluding HZ occurring within 6 weeks of initiation of HAART (which could have resulted from immune reconstitution inflammatory syndrome), found varicella vaccine to be 100% effective (95% CI, 67–100%) in preventing HZ for a median of 4 years of follow-up after immunization.[623] Another study, from 1989 to 2006, reported a decline in HZ in HIV-positive children after the year 2000, coincidental with varicella vaccination and introduction of HAART therapy. The authors speculated that the decline probably represented the combined effect of immunization and HAART.[622]

Healthy Persons. Several prelicensure studies with long-term follow-up reported rates of HZ in healthy vaccinated children and adults. In one study, HZ was reported in six of 7000 vaccinated children 1 to 17 years of age, with 42,884 PY of follow-up, which represents 0.14 cases per 1000 PY of observation.[761] In the prevaccine era, the incidence of HZ in children 5 to 9 years of age was 0.3 cases per 1000 PY.[762] However, a number of these children would not have experienced varicella, so this comparison should be viewed with caution. The incidence rate among children of this age group would be higher if only children with a history of varicella were considered.

Data on HZ risk in healthy vaccinated adults are limited by small numbers.[763,764] In the NIAID collaborative study where healthy adults were vaccinated, two of 363 adults developed HZ with active follow-up of 1 to 27 years (mean: 5 years). Both of these adults had experienced breakthrough varicella, and in one instance HZ caused by the WT virus was documented; the other patient was not available for VZV genotyping.[764] The incidence of HZ in this group of adults was one per 1000 PY (95% CI, 0.12–3.62), which is low for middle-aged adults. Follow-up of these and additional adult vaccinees over additional time is critical to further evaluate possible effects of varicella vaccination on the incidence of HZ.[763] As has been mentioned, only one case of HZ caused by the Oka strain in an adult who was given Varivax has been recorded; this occurred in an immunocompromised patient and was fatal.[649]

The Postlicensure Period

Incidence and Trends of Herpes Zoster in Vaccinated Healthy Children. In the postlicensure period, it has become feasible to assess the risk of HZ in healthy vaccinated children, although there continue to be challenges because of the rarity of HZ in children and the availability of data on a comparable comparison group of children with a history of varicella. A cohort study of 172,163 vaccinated children from a health maintenance organization in Southern California who were followed up for a median duration of 2.6 years (25th percentile, 0.9 years; 75th percentile, 4.0 years) reported an incidence of HZ of 0.27 per 1000 PY. The average age of children who contracted HZ was 4.1 years.[676] No comparative data on HZ risk of similarly aged children with a history of varicella were then available.

Another study examining the risk of HZ in vaccinated healthy children compared with similarly aged children with a history of varicella was conducted in a population-based varicella and HZ active surveillance project site. From 2000 to 2006, starting 4 to 5 years after initiation of the varicella vaccine program in the United States and as varicella incidence was declining, HZ incidence declined 55% among children younger than 10 years. HZ incidence in vaccinated children during this time was 0.19 per 1000 PY; it was four to 12 times lower than among children with a history of varicella when two different methods were used to calculate the incidence of HZ.[569] These risks could translate into reductions of HZ incidence in vaccinated children on an order of magnitude of 77% to 92%. The clinical presentation of HZ also tended to be milder in vaccinated children than in children with varicella history, with less pain (45% vs 77%; $P < .001$), and when pain was reported, it was reported as less intense ($P = .03$). The vaccinated patients were also less likely to have vesicular lesions, and the rash was smaller, but these characteristics were not statistically significantly different by vaccination status. These data provide convincing evidence that the risk of HZ, at least in children for 5 to 10 years after vaccination, is lower in healthy vaccinated children than in those with a history of varicella.[569]

As has been mentioned, a third population-based study of 144,000 children and adolescents younger than 18 years of age, from 2005 to 2009, in a health maintenance organization in Portland, Oregon, investigated and laboratory-confirmed all suspected HZ cases in vaccinated and unvaccinated children. HZ incidence was 0.48 per 1000 PY in vaccinated children and 2.30 per 1000 PY in unvaccinated children, which translates into a 79% lower incidence in vaccinated than in unvaccinated children. Presumably, the rate in unvaccinated children is an underestimate, as the denominator for this group includes children who would not have been infected with VZV, as well as those with a varicella history.[570] Half of the HZ cases in the vaccinated children were WT VZV. In contrast, 96% of the HZ cases in unvaccinated children had a history of varicella. In contrast to the study mentioned above,[569] here there were no significant differences in clinical presentation by VZV strain.

A study in Israel reported a 36% lower incidence of HZ among vaccinated than among unvaccinated children in a population in which varicella vaccination in the pediatric population was estimated to be 28%.[765]

There are several possibilities, which are not mutually exclusive, about why HZ may be less common after vaccination than after natural infection. One is that the virus is attenuated and less able to reactivate than the WT virus. Another is that the vaccine strain may have less frequent access to sensory nerves to establish latency because there is a lower incidence of infection of the skin after vaccination than with the natural infection.[156] Finally, the latency burden of virus may be lower after vaccination than after natural infection. Not all vaccinees who develop zoster, however, have a history of previous skin rash; viremia (some of which may have been subclinical breakthrough infections after one dose of vaccine) undoubtedly also plays a role in establishment of latency. In cases of HZ in vaccinees in 2005–09 in the United States, up to half of cases in vaccinated children were caused by WT VZV, and these cases occurred in children with no history of clinical varicella.[561,562,570]

It was observed in Japan that HZ occurred more frequently in immunocompromised children who had a vaccine-associated rash (16% of 83) than in those with no rash after immunization (2% of 249).[568] This observation was confirmed and extended in the NIAID collaborative study.[199,592] Of 13 vaccinated leukemic children who developed HZ, 11 (85%) had a prior history of a VZV-related rash, either vaccine-associated (eight children) or breakthrough varicella (three children). The two children with no history of rash developed HZ at the injection site of the vaccine. In the 268 vaccinees who had any type of previous VZV-related rash, the chance of subsequent development of HZ was more than five times greater than in the 280 vaccinees who had no VZV-associated rash ($P \leq .02$). In the NIAID collaborative study, it was possible to type four of the viruses causing HZ. Two were caused by vaccine-type virus and two were caused by WT VZV.[486] It is now recognized, however, that there are two paths to latency of VZV, from the skin lesions by way of nerves to neurons and from viremia. In all probability WT HZ in vaccinees who have not experienced clinical varicella stems from VZV that reached neurons by asymptomatic viremia. As a result of better protection against breakthrough disease and decreased occurrence of viremia, the risk of HZ might be further reduced in children who have received two doses of varicella vaccine.

External Boosting of Immunity Through Exposures to Varicella and Risk of HZ. Several studies have either directly or indirectly examined the effect of exposure to varicella on the risk of HZ in both immunocompromised and healthy populations. Garnett and Grenfell examined weekly reported data in a time-series analysis and reported no association between the temporal occurrence of varicella and of HZ, although they reported some correlation with the annual data for some age groups.[317] Exposure to varicella disease does boost VZV-specific immunity; whether ongoing exposure throughout life is critical to maintenance of immunity to VZV through external boosting, especially as the population ages, is not certain, although some studies offer support to this hypothesis. A number of studies have examined the risk of HZ in occupational groups considered to have varied risk of exposure to children (as a surrogate of exposure to varicella). Two small studies reported that pediatricians had a lower incidence of HZ compared to the general population,[766] and to psychiatrists[767]; however, the studies had serious methodologic limitations. Terada and associates[766] based their findings on small numbers of cases, whereas the findings of Solomon and associates[767] were based on low response rates (31% of pediatricians) and found no differences between them and dermatologists and psychiatrists, groups considered to have different rates of exposure to varicella cases. There was no laboratory documentation of VZV infections in either study. A third epidemiologic study in Taiwan, using a nationwide insurance database, examined HZ incidence in healthcare workers compared with adults of similar age, in decade strata, and found a higher HZ incidence in the healthcare workers who might be expected to have a higher level of exposure to VZV.[768] In a case-control study in the United Kingdom, Thomas and associates studied the association of HZ risk and occupational exposure but also studied exposure to varicella specifically.[769] This study found a substantially lower risk of HZ among persons with more than five exposures to varicella in the past 10 years than among persons with no exposures (odds ratio, 0.29 [95% CI, 0.10–0.84]). In a subsequent analysis of the same data, Thomas reported that people who worked with young children (primary school teachers, nursery nurses, playgroup leaders, and other childcare providers) were significantly less likely to develop HZ after adjusting for age, sex, ethnicity, and a child living in the household (RR, 0.70; 95% CI, 0.58–0.85).[770] Several other studies found that living with or exposure to children (as a surrogate of varicella exposure) was associated with a lower HZ risk.[771,772] In the United States this potential effect disappeared over the first decade of the varicella vaccine program.[772] Studies in the United States, however, did not find any differences in HZ incidence comparing states with high and low varicella vaccine coverage. This grouping of states was considered to represent differing rates of varicella exposures in the population.[772] In addition, the results of the association between exposure to VZV and lower HZ risk were not duplicated in two other studies that were designed to examine these associations.[773,774] The first of these studies, a large case-control study in the United States, did not find any association with HZ and the number of varicella contacts; there was no trend, even at the highest exposure level (three or more contacts). Similarly, there was no association with exposure to persons with HZ or to children, or with workplace exposures.[773] In discussing potential explanations for the different findings between this and the Thomas study, Donahue and colleagues[773] noted that the proportion of cases and controls with various levels of varicella exposures were lower in the United States than in the United Kingdom, consistent with the vaccination program.

The second study examined data from a national random-digit dial telephone survey in the United States that obtained information on self-reported HZ, demographic characteristics, and exposure to children with chickenpox in the past decade. Previous exposure to chickenpox did not protect against HZ in this population. Seven percent of adults 65 years of age and older reported exposure to children with chickenpox in the past decade. The authors suggested that the potential contribution of exposure to chickenpox as a mechanism for maintaining cell-mediated immunity against HZ may be limited to a small percentage of the population.[774] This is consistent with the findings of Thomas and colleagues that only a small proportion of HZ cases reported the highest levels of exposure to varicella that were associated with decreased risk of HZ. On a total population basis, this may have limited impact on the overall risk of HZ. A number of studies, including a recent analysis by Leung and associates, have found a higher HZ incidence rate in women, including young women who are more likely than men to be primary caretakers.[769,772,775–777] As commented on by a number of investigators, this finding is not consistent with varicella exposures affecting the risk of HZ, at least on a population-based level. Recent research in Ontario, Canada, indicated that after introduction of a

publicly funded varicella vaccination program, there was no increase in the incidence of HZ in the general population although there was a decrease in incidence in children.[778] In the United States, a study that examined the influence of the varicella vaccination program on trends in HZ incidence in the population older than 65 years of age between 1992 and 2010 found that although HZ incidence has increased in elderly persons during this interval, introduction and widespread use of varicella vaccine did not seem to affect this increase—the increase in HZ incidence predates 1996 and the increase did not accelerate after implementation of varicella vaccination. Additionally, this study reported that state varicella vaccination coverage did not affect HZ incidence.[779] Finally, a study of secluded religious populations in Europe that had no contact with children indicated that their rate of HZ was similar to that of the general public over a 30-year period; the study concluded that boosting of immunity by contact with circulating VZV was not required for long-term protection against HZ.[780,781]

Mathematical modelers have used data from some of the aforementioned studies to predict the effect that a varicella vaccination program may have on HZ incidence. Using the assumption that exposure to varicella is an important mechanism for preventing reactivation of VZV, models predict that a short- to medium-term (more than 10 to 40 years in one study, and up to 70 years in another) increase in HZ cases may occur after implementation of varicella vaccination program, although, in the long term, a reduction in HZ cases is expected to occur, assuming that vaccine recipients have a lower risk of developing HZ than persons who acquire natural infection.[724] Considerable experience has now been gained with observing HZ epidemiology in countries implementing varicella vaccine programs, as well as in countries without a varicella vaccination program. The predictions of a short-term increase in HZ secondary to varicella vaccination to the levels predicted in the models have not been consistently observed, and it appears unlikely that increasing trends in HZ incidence can be attributable only to varicella vaccination. Studies in a number of countries (United States, United Kingdom, Canada, Spain, Japan) have documented increasing trends of HZ incidence in the absence of a vaccination program or increases that started years before use of varicella vaccine.[722,775,776,779,782–784]

In countries monitoring the effect of varicella vaccination on HZ, challenges include the following: (1) trends over time for HZ have not been described in detail, especially in the immediate prevaccine time period, so baseline data may not be available against which to interpret trends in HZ incidence; (2) studies have used different methodologies, making comparisons across studies difficult; (3) HZ is clinically diagnosed and rarely laboratory confirmed; (4) the age structures of populations under study may differ significantly, and calculating age-adjusted rates is essential to compare studies and to interpret trends over time within studies; and (5) few studies of the epidemiology of HZ have examined in detail other factors that may affect HZ, particularly immunocompromising medical conditions, medications, and certain other conditions (e.g., diabetes, stress).

A 2013 systematic multidisciplinary review of HZ risk reduction through exposure to varicella patients analyzed the peer-review publications on exogenous boosting studies: 13 observational studies on HZ incidence after widespread varicella vaccination; four longitudinal studies on VZV immunity after reexposure; nine epidemiological risk factors studies; seven mathematical modeling studies; and other studies. The authors concluded that exogenous boosting exists, although not for all persons nor in all situations, and that its magnitude is yet to be determined adequately in any field study.[785]

In addition to external boosting of VZV internal boosting occurs, by asymptomatic reactivation of the virus from latency. Asymptomatic reactivation of VZV has been described, initially by demonstration of increases in serum antibodies in the absence of symptoms,[786] and later by showing silent presence of VZV DNA in blood or saliva of immunocompromised and healthy individuals such as astronauts or stressed individuals.[217–220] The possibility of internal boosting does not seem to have been considered by computer modeling analyses.

Trends in Incidence of Herpes Zoster in the Population. The incidence of HZ is increasing in the United States and in other countries. The longest experience with monitoring the effect of the varicella vaccine program on HZ has been gained in the United States, where a childhood vaccine program has been in place for two decades. In the United States, HZ is not a nationally notifiable disease. Therefore, epidemiology of HZ has been studied using a variety of different methods and populations. Seven studies have reported on trends in HZ since vaccination in the overall population; some of these studies had baseline data available before the start of the varicella vaccination program for comparative purposes, and others did not. As previously described, two studies documented that trends in HZ in children younger than 10 years of age, and in children and adolescents younger than 18 years of age, are declining, consistent with a lower risk of HZ in vaccinated children.[569,570]

The first of these studies reported an increase in incidence of HZ in adolescents during 2000 and 2006.[569] A follow-up analysis that included 4 more years of data reported continued decreases in children younger than 10 years of age, while among older children and adolescents 10 to 19 years old the increase reported through 2006 did not continue further and lower rates were observed through 2010.[787] As the prevalence of WT VZV infection declines in these older children and an increasingly larger proportion in this cohort are children and adolescents who had received varicella vaccine and are at a lower risk for HZ, the earlier increases in HZ incidence in 10- to 19-year-olds would plausibly now be reversing. In the overall population, two other studies reported no change in age-adjusted rates of HZ over time periods spanning the first 7 to 8 years of the varicella vaccination program, as varicella incidence declined.[729,788] One of these studies found significant increases in HZ incidence rates among children 10 to 17 years of age that were attributable to increased use of oral steroids.[729]

Three additional studies showed increases of HZ in the overall population. The first study examined varicella and HZ trends in Massachusetts from 1999 to 2003, using data from a statewide phone survey, and noted that as varicella decreased by 66%, HZ increased significantly from an incidence of 2.77 to 5.25 per 1000 PY ($P \leq .001$).[730] The second study looked at HZ incidence among insured people in the United States from 1993 to 2006 from a MarketScan medical claims database and reported an overall incidence of HZ increasing from 1.9 per 1000 PY from 1994–95, to 3.4 per 1000 PY in the year 2000 (the period before significant vaccine impact), to 4.4 per 1000 PY in 2006. However, the incidence in the early years of the study was low compared with other population estimates. Increasing trends were observed in all age groups except children 0 to 17 years of age, where HZ rates stabilized after 1999, consistent with a decline in incidence in a subset of this population that had been vaccinated.[772]

A retrospective study of physician-diagnosed HZ among adults 22 years of age and older living in Olmstead County, Minnesota, showed continuous increases in age-adjusted HZ incidence over three time periods 1945–47, 1980–82, and

2005–07.[788a] Although methodologic differences complicate comparisons across studies, the rates described for 1945–47 in this study of adults only (0.76 per 1000 PY) were considerably lower than those described in most other studies of HZ incidence in the population (most commonly, 3 to 4 per 1000 PY) over the past 50 years, including from 1947 to 1962 in the United Kingdom, where Hope-Simpson described an incidence of HZ in his general practice of 3.4 per 1000 PY.[446] A study that examined HZ incidence among persons older than 65 years of age using Medicare claims data from 1992 through 2010 reported a 39% increase in age- and sex-standardized HZ incidence, from 10.0 per 1000 PY in 1992 to 13.9 per 1000 PY in 2010.[779] Also noteworthy is that the annual incidence of HZ in U.S. veterans almost doubled between 2000 and 2007, from 3.1 episodes per 1000 veterans to 5.2 episodes per 1000 veterans, but only in groups older than 40 years of age.[789]

Data are becoming available from other countries that assist in interpretation of HZ trends after vaccination. These countries are either not implementing vaccination programs or are in the early stages of implementing programs. In Australia, where a universal, publicly funded childhood varicella vaccination program was initiated in 2005, varicella decreased significantly between 1998–99 and 2008–09, with a significant rise in HZ.[790,791] The increase in HZ, however, was not necessarily attributed to vaccination programs. Three studies in Canada have documented increasing HZ trends either before or after initiation of a publicly funded vaccination program.[722,775,784] Brisson and colleagues[771,772] reported on trends in varicella and HZ in Canada and the United Kingdom between 1979 and 1997—over this time, the overall HZ consultation rates increased from 2.58 to 3.48 and from 3.15 to 3.82 per 1000 PY in Canada and the United Kingdom, respectively. Studies in Korea,[776] Madrid,[782] and Japan[783] examining HZ trends from 2003 to 2007, 1997 to 2004, and 1997 to 2006, respectively, have reported increasing HZ incidence, mainly reflecting increases among older adults.

In summary, although studies among vaccinated healthy children show declining rates of HZ, and some studies of population rates of HZ show stable incidence rates over time, the majority of studies examining overall population rates of HZ from around the world show evidence of increasing trends in HZ that are not explained by well-described risk factors for HZ, including age and immunocompromised conditions, nor to potential effects of the varicella vaccination program secondary to decline in varicella exposures. These increases preceded the use of varicella vaccine and have also been described in countries that are not implementing national varicella vaccine programs, as well as in countries with vaccination programs. Further studies are needed to understand factors that may explain these increases, including methodologic issues and improved ascertainment, changes in access to healthcare or health-seeking behaviors, and risk factors for HZ that have not been well described to date (e.g., chronic medical conditions like diabetes, stress, and possible immunocompromising effects of medications). The zoster vaccine, now licensed to prevent HZ (for persons 50 years of age and older) and postherpetic neuralgia (for persons 60 years of age and older), offers the opportunity to reduce the burden of illness caused by HZ in adults (see Chapter 63).

POTENTIAL FOR ELIMINATION OF ENDEMIC DISEASE TRANSMISSION AND ERADICATION

The varicella zoster virus is transmissible while the patient has varicella, and it is able to establish latency and cause HZ, which represents another potential source of introduction of VZV into communities. In addition, breakthrough varicella occurs in approximately 15% of exposed vaccinated children after they receive one dose of varicella vaccine. For these reasons, it is not likely that a universal, one-dose immunization program for varicella vaccine will eliminate circulation of the virus. Partial control was achieved in the United States with a one-dose vaccination program, and there is improved disease control with a routine two-dose program. Elimination of endemic disease transmission even with a two-dose policy may not completely eliminate varicella, and exposure to VZV from HZ patients will continue to occur. The widespread use of varicella vaccine, especially in a national vaccine program, however, has lowered virus transmission significantly and has improved the health of many children, adolescents, and adults, who no longer have to suffer with varicella and its serious complications and will experience less HZ. Complete eradication of diseases caused by VZV seems unlikely, but development of a highly effective subunit zoster vaccine that avoids latency may play a significant role in further decreasing transmission of VZV.[792]

Eventual availability of additional inactivated or subunit vaccines that avoid latency for prevention of varicella and HZ may offer additional possibilities for decreasing transmission of VZV in the future.

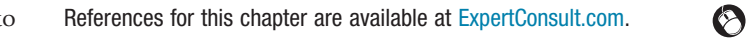
Acknowledgments

This chapter is dedicated to the memory of Prof. Dr. Michiaki Takahashi (1928–2013) who developed the live attenuated varicella vaccine.

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63 Yellow Fever Vaccines

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Yellow fever virus is the prototype member of the Flaviviridae (Latin *flavus*, "yellow," after the jaundice seen with the disease) family of viruses. The virus causes a viral hemorrhagic fever, a systemic illness characterized by high viremia, hepatic, renal, and myocardial injury; hemorrhage; and high lethality. A highly effective vaccine (17D), developed in 1936, is widely used to protect travelers to and residents of endemic regions of tropical South America and Africa. Although the vaccine provides long-lasting immunity, rare but serious side effects have emphasized the importance of determining the need for vaccination on a case-by-case basis and of continued exploration of new vaccines.

HISTORY OF DISEASE

The early history of yellow fever is uncertain owing to the inexactness of clinical and epidemiologic descriptions. Carter found the earliest record in a Mayan manuscript describing an epidemic with hematemesis (*xekik*, "black vomit") in Yucatan in 1648[1] and suggested that the virus and mosquito vector were introduced from West Africa during the slave trade 400 years ago.[2] The nosologic term "yellow fever" was first used in 1750 during an outbreak in Barbados.[3] Yellow fever became a major problem in the 18th century in colonial settlements in the Americas and West Africa.[4] It was introduced repeatedly into seaports in the United States and Europe via sailing vessels infested with *Aedes aegypti* mosquitoes that sustained transmission among the passengers and crew. In 1793, for example, yellow fever appeared in Philadelphia, the federal capital of the United States, killing 10% of the population.[5] Similar fates befell residents of other cities throughout the 18th and 19th centuries.[6,7] In one of the worst medical disasters in the early history of the United States, yellow fever caused more than 13,000 deaths in the lower Mississippi Valley in 1878.

Until the 20th century, yellow fever was widely believed to be an airborne miasma arising from filth, sewage, and rotting organic matter. Several physicians, most notably Carlos Finlay in Cuba, suggested that yellow fever was transmitted by mosquitoes.[8] Proof was not obtained until 1900, when Walter Reed and colleagues conducted experiments on human volunteers in Cuba and demonstrated that the agent was a filterable virus transmitted by *Ae. aegypti* mosquitoes.[9] This led to successes in preventing disease through mosquito abatement during the first 20 years of the 20th century. The last outbreak in the United States occurred in New Orleans in 1905, with 8399 cases and 908 deaths and the last human case of endemic yellow fever in the United States occurred in 1911 in Hawaii, prior to it becoming a state.[10]

In 1925, the Rockefeller Foundation's West African Yellow Fever Commission laboratory in Yaba (Lagos), Nigeria, set out to determine the etiology of yellow fever, using imported monkeys for isolation of the causative agent. On June 30, 1927, blood of a 28-year-old man called Asibi, a resident of the village of Kpeve, Ghana, was obtained 33 hours after the onset of mild yellow fever and was inoculated into a rhesus monkey at the field laboratory in Accra. The animal was moribund 4 days later and had hepatic lesions consistent with yellow fever. Blood from this monkey was inoculated intraperitoneally into a second animal, which was transported during the incubation period to Yaba, where it developed yellow fever the day after arrival. Stokes and associates established the Asibi strain by continuous direct passage in monkeys and indirect passage through *Ae. aegypti* mosquitoes.[11] Contemporary efforts at the Institut Pasteur in Dakar led to isolation of the French strain from a Syrian (François Mayali) with mild yellow fever.[12] Isolation of the Asibi and French strains in 1927 enabled the development of vaccines with research initiated immediately in England, the United States, West Africa, and Brazil. Many years later, comparison of the genomes of the Asibi and French viruses confirmed that despite differing passage histories, they were 99.8% identical at the sequence level, divergent at only 23 nucleotides and nine amino acids.[13]

Vaccine development was spurred, in part, by a growing number of laboratory infections. In the 5 years following isolation of yellow fever virus in 1927, 32 cases (five fatal) had occurred among laboratory workers.[14] In 1928, Edward Hindle of the Wellcome Research Laboratories in London described the first attempt to produce an inactivated vaccine.[15] This and other contemporary efforts on inactivated yellow fever vaccines were, however, unsuccessful. In 1931, Sawyer and associates at the Rockefeller Institute in New York first vaccinated humans with a live attenuated virus, the neuroadapted French strain, mixed with immune serum.[16]

In 1932, Sellards and Laigret tested the French strain passaged in mouse brain as a vaccine (French neurotropic vaccine) without immune serum in humans,[17] and in 1934, Mathis, Laigret, and Durieux described the first field trial of this vaccine.[18] Believing the mouse brain tissue virus to have safety concerns for use in humans, Theiler and Smith at the Rockefeller Foundation developed a live vaccine (17D) attenuated by serial passage of the Asibi strain in tissue cultures prepared from embryonated chicken eggs.[19–21] In 1936, the 17D vaccine was tested in a small number of human volunteers in New York,[22] and it entered field trials in Brazil the following year.[23] By 1939, more than 1 million Brazilians had received the 17D vaccine and more than 100,000 persons in French West Africa had received the French neurotropic vaccine.[24–26] During this era, important discoveries surrounding the epidemiology of yellow fever were made, first in South America and then in Africa, showing that yellow fever virus was transmitted between monkeys and forest mosquitoes. These findings suggested that eradication of yellow fever virus was unlikely and established a mandate for vaccinating human populations wherever there was a risk of exposure to the zoonotic cycle. During the 1940s, control of yellow fever at a population level was achieved in francophone Africa by a program of compulsory vaccination with the French neurotropic vaccine administered by scarification.[24] Immunization of laboratory workers, travelers, and military and expatriate residents in endemic areas removed the threat of acquiring the disease, and the disease faded from public view, having been transformed from a major human plague to a medical curiosity by the end of World War II.

DISEASE AND VACCINE IMPORTANCE

Despite the availability of a highly effective vaccine, full control of the disease has failed owing to incomplete implementation

of routine vaccination, the movement and migration of unvaccinated people into endemic areas, and the maintenance of the virus in an enzootic cycle.[27] Since the 1980s, there have been repeated resurgences of yellow fever, particularly in areas where the disease had not been reported for several decades.[28–32,32a,32b,32c] Yellow fever was the first disease for which the International Health Regulations required a certificate of vaccination for travelers entering a receptive (*Ae. aegypti*–infested) area from an endemic country.

In 2001, a previously unrecognized type of serious viscerotropic adverse event following 17D vaccine was first described. This reaction resembled wild-type yellow fever and has similar lethality, but it is, fortunately, rare. The awareness of this serious adverse event has led to the need for a more careful risk-to-benefit analysis for vaccine use in individual persons and populations and continued exploration into the vaccine attenuation and molecular determinants of both virulence as well as host factors involved in immune responses.

This chapter provides background information on the basic virology, virus transmission, clinical disease presentation, pathogenesis, immune response, and epidemiology of yellow fever before presenting detailed information on the development, immunogenicity, and reactogenicity of existing yellow fever virus vaccines. Finally, ongoing work to develop inactivated vaccines, to explore alternative delivery routes for dose sparing, and to use yellow fever virus vaccine in the development of chimeric vaccines is discussed.

BACKGROUND OF YELLOW FEVER VIRUS AND DISEASE

Virology

Yellow fever is the prototype member of the Flaviviridae (Latin *flavus,* "yellow") family, which includes approximately 60 positive-sense, enveloped, single-stranded RNA viruses, most of which are transmitted by mosquitoes or ticks.[33] This section focuses on the basic virology of yellow fever virus and the molecular basis for attenuation of yellow fever 17D vaccine. For reviews of flavivirus genome and protein structure, virus entry, and replication and assembly see Chambers and Monath,[34] Bollati and colleagues,[35] Kaufmann and Rossmann,[36] and Perera and Kuhn.[37]

Classification and Phylogenetic Relationships

Members of the *Flavivirus* genus are distinguished serologically by the neutralization test and were originally classified into eight antigenic complexes.[38,39] In the current classification that synthesizes epidemiologic, antigenic, and genetic phylogeny, yellow fever virus is considered the type species in a complex of *Aedes*-borne flaviviruses that includes *Wesselsbron, Sepik, Edge Hill, Bouboui, Uganda S, Banzi, Jugra, Saboya,* and *Potiskum* viruses.[40–42]

Yellow fever viruses belong to a single serotype. The relatively high genetic stability of wild-type yellow fever viruses compared to many other RNA viruses is critical to the effectiveness of a single virus strain (17D) as a vaccine. Only seven distinct genotypes have been found by sequencing wild-type yellow fever virus strains of different geographic origin.[2,43–59] African virus strains belong to five distinct genotypes, which differ in nucleotide sequence by 0% to 26% and in amino acid sequence by 0% to 9%.[49,51,62a] The five African genotypes are West African type I (represented mainly by strains from eastern areas of the region, e.g., Nigeria) and genotype II (represented mainly by strains from the western West Africa, e.g., Senegal, Guinea Bissau); the East and Central Africa genotype (Central African Republic, Ethiopia, Sudan, Zaire, Uganda); the East Africa genotype (Uganda, Kenya); and the Angola genotype (1971 and 2015–16 outbreaks). The Central African, East African, and Angolan genotypes are notably different from the West African genotypes (7% amino acid differences),[54] consistent with their ecological separation, namely different mosquito vectors. Recent molecular clock studies showed the origins of yellow fever virus in Central Africa, with evolution from a common ancestral virus within approximately the last 1000 years.[60] The introduction of the virus from West Africa to the New World during the slave trade approximately 400 years ago was also confirmed.[2]

South American viruses fall into two phylogenetic groups with respect to certain structural (envelope [E]), nonstructural ([NS]; NS4A-NS4B), and 3′ noncoding region sequences.[53,61] In contrast to the situation in Africa, only two genotypes (I and II) are found in South America. Genotype I is widely dispersed in South America, including Brazil, Bolivia, Colombia, Ecuador, Panama, Venezuela, and Trinidad, whereas the (apparently older) genotype II circulates in the western part of the continent (Peru, Bolivia, portions of western Brazil) divergent by 0% to 4.6% at the amino acid sequence level.

Biological differences between yellow fever strains are also considered in virus-specified factors. By cross absorption with polyclonal antisera, African and New World yellow fever viruses can be serologically distinguished.[62] Slight antigenic differences were discerned between East African and West African strains.[63] Wild-type strains vary with respect to reactivities with monoclonal antibodies[64] but have not been classified to geographic origin or genotype.[65]

Genome Structure and Gene Products

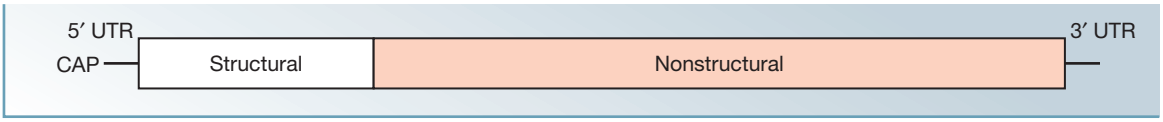
Flaviviruses are small (approximately 50 nm) spherical positive-sense, single-stranded RNA viruses with an envelope containing lipid and two envelope proteins and a single capsid protein. The genome of the prototype yellow fever virus strain 17D-204 contains 10,862 nucleotides, composed of a 5′-terminal type I cap structure, a short 5′ noncoding region, a single open reading frame of 10,233 nucleotides, and a 3′ noncoding region.[34,66] The 5′ and 3′ noncoding regions have conformational structure and interactive sequences important in cyclization of the viral genome during encapsidation and replication. The 3′ and 5′ noncoding regions function as promoters for negative and positive RNA strands, respectively, during replication, and mutations or deletions in the 3′ noncoding region affects replication and virulence.

The open reading frame encodes three structural proteins at the 5′ end (capsid [C], premembrane [prM], and E proteins), followed downstream by seven NS proteins. The proteins are encoded in the order C-prM/M-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5 (Fig. 63.1).

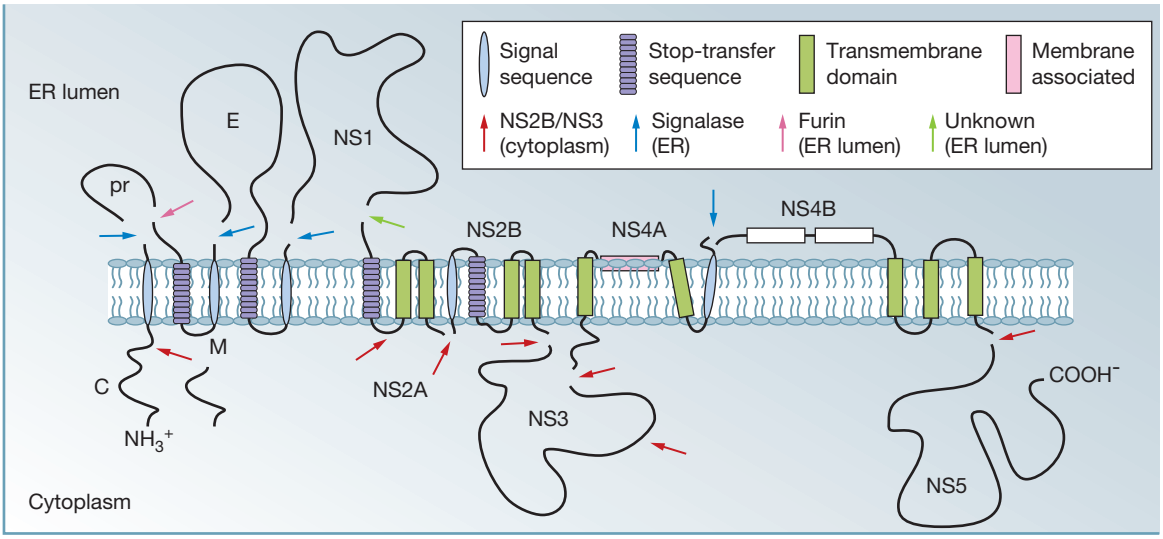
Molecular Determinants of Virulence

The entire genomes have been sequenced of both the 17D and the French neurotropic vaccines and their wild-type progenitors, Asibi and the French viscerotropic virus.[13,67,68] This includes examples of the two substrains of 17D (17D-204 and 17DD) used by many of the producers.[13,69–73] Because a large number of mutations occurred during the more than 230 passages that separate vaccines from their parental strains and the lack of availability of the initial derivatives, it is impossible to define those responsible for attenuation, nor is it clear which determinants encode viscerotropism and neurotropism.

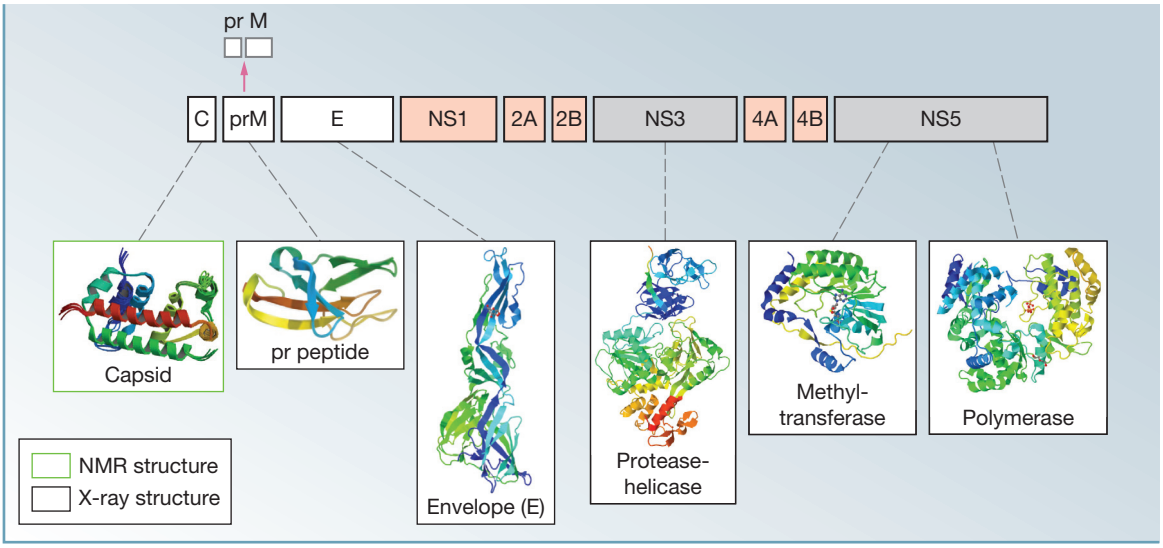
Sequence comparison of strains with different biological properties has reduced the number of possibilities and allowed some educated interpretations. Although some potentially



A



B



C

Figure 63.1. A, Organization of the flavivirus genome, a positive-sense RNA 11 kb in length. The structural proteins are encoded at the 5′ one third of genome, followed by the nonstructural proteins. **B,** The viral RNA is translated as a polyprotein and processed by host cell and viral proteases *(arrows)*. During virus maturation, premembrane M is cleaved by furin in the trans-Golgi network into premembrane and M protein. **C,** Structural proteome of a flavivirus. NMR and x-ray structures are shown for C, prM, E, NS3, and NS5 methyltransferase and polymerase domains. Structures are currently not available for the proteins denoted in red. E, envelope; ER, endoplasmic reticulum; NMR, nuclear magnetic resonance; NS, nonstructural; pr, premembrane; UTR, untranslated region. *(From Perera R, Kuhn RJ. Structural proteomics of dengue virus.* Curr Opin Microbiol. *2010;11[4]:369–377.)*

important mutations have been identified, it is clear that virulence is multigenic, determined by contributions of both nonstructural and structural genes of the virus. It is also important to note that nearly all studies on the molecular determinants of virulence have employed wild-type mouse models, which reveal only one of the two biological properties of the virus (neurotropism). Mice deficient in the interferon-alpha/beta (IFN-α/β) receptor (A129) or the STAT1 signaling molecule (STAT129) were found to be highly susceptible to infection and disease, succumbing within 6 to 7 days. Importantly, these transgenic mice developed viscerotropic disease reminiscent of human yellow fever, instead of the encephalitic signs

typically observed in wild-type mice.[74] Asibi virus caused a lethal infection in these transgenic models, but infection with 17D-204 vaccine virus was subclinical, suggesting that this model might have applications for investigating the molecular basis of attenuation and virulence of yellow fever virus. Hamsters were shown to be susceptible to a lethal disease with hepatic dysfunction and necrosis resembling wild-type yellow fever after infection with virus strains adapted by serial passage in hamster liver.[75,76] This model also permits dissection of the molecular determinants associated with viscerotropism (at least for the hamster).

The first comparison of nucleotide and amino acid differences between 17D-204 (ATCC) and Asibi virus was made by Hahn and associates.[68] Of a total of 10,862 nucleotides, 67 changes (0.62%) were identified, resulting in mutations in 31 (0.91%) of 3411 amino acids. The changes were not randomly distributed across the genome; the highest rates of change were in genes encoding E, NS2A, and NS2B, and in the 3′ noncoding region. As additional sequences of 17D-204 and 17DD substrain vaccines became available, the number of amino acid differences between parental Asibi virus and attenuated 17D viruses, and shared by vaccines from different sources, was reduced from 31 to 20, and the number of nucleotide differences in the 3′ noncoding region was reduced from six to four (Table 63.1).[69,73,77] Although the possibilities have been reduced to a limited number of mutations, it has not been possible to determine precisely which are responsible for the reduced virulence of the vaccine strain.

The E protein contains structures involved in cell receptor interactions and virus entry into cells. Therefore, the eight amino acid differences between Asibi and vaccine strains are suspected to play a role in attenuation. The role of the E protein in neurovirulence is illustrated by a study in which heterologous yellow fever chimeras were constructed having a yellow fever 17D backbone and prM and E genes from flaviviruses with different neurovirulence profiles.[78]

The N-terminal 400 amino acids of the E protein form what is termed the *ectodomain*, and the C-terminal region contains a stem-anchor region that anchors the E protein in the virus envelope. The ectodomain contains three domains (I, II, and III) (Fig. 63.2).[79] Domains I and II are discontinuous in primary amino acid sequence, and domain III consists of a linear region of 100 amino acids, approximately residues 295 to 395. Mutations in three principal areas of E protein alter virulence properties of yellow fever and other flaviviruses. These include the tip of the fusion peptide in domain II, the molecular hinge between domains I and II, and the upper lateral surface of domain III, containing the putative receptor-ligand.[79,80] Among the eight amino acids in the E protein that distinguish Asibi and 17D viruses (see Table 63.1), four are nonconservative changes: E52 Gly→Arg, E200 Lys→Thr, E305 Ser→Phe, and E380 Thr→Arg. Wild-type yellow fever strains (Asibi, the French viscerotropic virus, and Peruvian strain 1899/81) share these amino acid residues, suggesting that one or more of the mutations at these sites in the 17D vaccine strain may contribute to attenuation.[13,68,81]

Amino acid residues E52, E173, and E200 are in the molecular hinge region and are also located in domain II at the top of the virion, where they are exposed to interactions with cell receptors and binding moieties. Mutations in the hinge region could alter the acid-dependent conformational change in E protein required for virus entry. Neuroadaptation of yellow fever 17D virus resulted in an increase in neurovirulence of the virus for mice and was associated with a reversion (Ile→Thr) at residue E173.[78,82] Furthermore, mutations in the hinge region at E176 and E177 of a yellow fever–Japanese encephalitis chimera were associated with attenuation.[83] Moreover, residue E173 is implicated in an epitope binding to wild-type specific monoclonal antibody (MAb 117).[84] Reversion of this amino acid to the wild-type Thr was implicated in the increased neurovirulence phenotype of a variant [17D(wt$^+$)] recovered from a 17D-204 vaccine.[85]

A chimeric virus composed of Japanese encephalitis prM-E genes in the yellow fever 17D backbone having a single amino acid change to the wild-type Japanese encephalitis amino acid at E279 in the molecular hinge caused an increase in neurovirulence for mice but reduced viscerotropism for monkeys.[86] This result suggested that neurovirulence and viscerotropism of yellow fever virus strains might not be linked at the molecular level. This may explain why the French neurotropic vaccine, which was developed by adapting the virus during over 128 passages in mouse brains, was highly neurovirulent for mice but had lost the ability to cause hepatitis in monkeys and humans. Similarly, dengue-2 virus adapted by sequential mouse brain passages was also attenuated for humans.[87,88] The adapted virus had a critical nonconservative (Glu→Lys) mutation at E126, also in the hinge region.[87,89]

Vaccine-specific mutations at positions E305 and E380 are located in domain III, and the mutation at position E299 is located at the interface of domains I and III. E305 and E380

TABLE 63.1 Amino Acid Differences Between Asibi Virus and Attenuated 17D Vaccines

Nucleotide	Gene	Amino Acid	Asibi	17D-204 and 17DD Vaccines
854	M	36	Leu	Phe
1127	E	52	Gly	Arg
1482		170	Ala	Val
1491		173	Thr	Ile
1572		200	Lys	Thr
1870		299	Met	Ile
1887		305	Ser	Phe
2112		380	Thr	Arg
2193		407	Ala	Val
3371	NS1	307	Ile	Val
3860	NS2A	118	Met	Val
4007		167	Thr	Ala
4022		172	Thr	Ala
4056		183	Ser	Phe
4505	NS2B	109	Ile	Leu
6023	NS3	485	Asp	Asn
6876	NS4A	146	Val	Ala
7171	NS4B	95	Ile	Met
10,142	NS5	836	Glu	Lys
10,338		900	Pro	Leu
10,367	(3′ NCR)		U	C
10,418			U	C
10,800			G	A
10,847			A	C

Some authors[73,77] reported a mutation at E331 LYS in Asibi to ARG in 17D, but E331 is unique in Asibi compared to all other yellow fever genomes sequenced so it may be eliminated as relevant to the attenuation process.

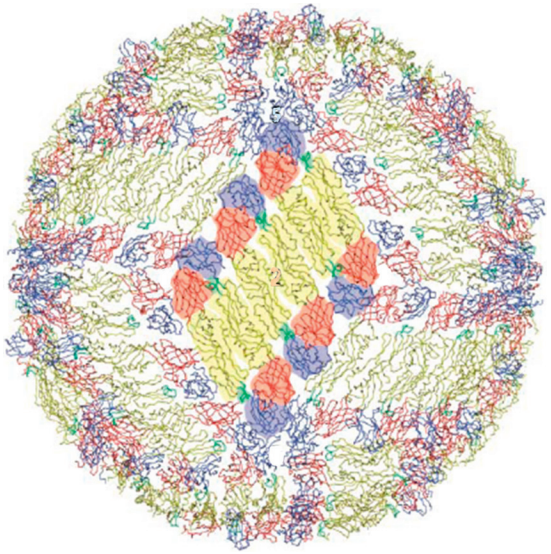


Figure 63.2. Crystal structure of the mature Flavivirus envelope. E protein monomers form head-to-tail dimers in a herringbone pattern. Each monomer consists of three domains dominated by b strands. Domains I, II, and III are colored red, yellow, and blue, respectively, and the fusion peptide is shown in green. The E protein dimers are organized as 30 rafts in a herringbone pattern. One of these rafts is highlighted in the figure. *(From Zhang Y, Corver J, Chipman PR, et al. Structures of immature flavivirus particles.* EMBO J. *2002;22: 2604–2613.)*

are located at the top surface of the virion in domain III. Because domain III contains ligands for cell-receptor interactions, these mutations could alter tropism of the vaccine virus. Yellow fever 17D strains that were neuroadapted and neurovirulent had reversions at E305, E325, and E380 (as well as the reversions to wild-type residues at E52 and 173 noted earlier).[78,82,90,91] Similarly, mutations in the region E308-E311 of tickborne encephalitis virus resulted in significant attenuation.[80] Moreover, the possible role of the E305 mutation was illustrated by sequence analysis of yellow fever 17D virus recovered from the brain of a child with a fatal yellow fever vaccine–associated neurotropic adverse event.[92,93] The 17D virus recovered had increased neurovirulence for mice and monkeys. The isolate differed from 17D vaccine at E303 Glu→Lys, located in domain III very near the 17D-204–substrain-specific E305 amino acid. However, two other mutations (at E155 and NS4B76) were also present in the brain isolate and could have played a role in reversion to virulence.

The mutation at amino acid E380 of 17D occurs in the moiety that is a putative integrin cell-receptor ligand; the sequence change in this motif is from Thr-Gly-Asp in Asibi to Arg-Gly-Asp (RGD) in 17D vaccine.[68] Although mutations in the RGD sequence predicted to alter integrin binding did not interfere with 17D virus replication,[94,95] studies with another flavivirus (Murray Valley encephalitis) also showed that RGD mutations attenuated neurovirulence for mice.[96,97] Multiplication of the Murray Valley encephalitis mutant viruses were also inhibited by heparin, suggesting that cell receptors other than integrin bind the viral ligands. The neuroinvasiveness of Japanese encephalitis and Murray Valley encephalitis viruses was attenuated by passage in adenocarcinoma cells, and the phenotypic changes were associated with mutations in the putative cell receptor-binding region of domain III (at E390 and E306, respectively).[98] The mutant viruses were inhibited by heparin and had enhanced affinity for glycoaminoglycan (GAG) receptors on nonneural cells, indicating that changes in charged amino acids with increased affinity for GAG receptors reduced viremia and neuroinvasion.

Collectively these studies suggest a role for similar mutations in domain III in the attenuation of yellow fever 17D, and this was demonstrated by two groups. The first investigated three amino acid substitutions (305 Phe→Val, 326 Lys→Glu, and 380 Arg→Thr) as critical determinants of mouse neuroinvasiveness in a severe combined immunodeficiency model. Virus containing vE326-Glu caused a rapid lethal encephalitis, but mutants with either E380-Thr or E305-Val did not independently affect neuroinvasiveness. Testing a panel of viruses with various amino acid substitutions at E326 revealed that attenuation of neuroinvasiveness required a positively charged residue (Lys or Arg) at this position. Molecular-modeling studies of protein domain III indicate that E326 and E380 contribute to charge clusters on the lateral surface of domain III that constitute putative heparin-binding sites.[91] The second group investigated E305, E325, and E380 and showed that these residues inhibit spread of 17D in extraneural tissues and attenuate virulence in types I/II IFN-deficient mice. One of these residues (E380-Arg) was a dominant GAG-binding determinant, which mediated more-rapid in vivo clearance of 17D from the bloodstream in comparison to 17D-derived variants with wild-type–like E protein.[95] However, these studies did not include wild-type strain Asibi for comparison.

The stem-anchor region of the E protein is involved in the reconfiguration of the E protein from dimeric to trimeric structure during low-pH–induced fusion and virus entry and also plays a role in localizing and retaining the envelope proteins in the ER during morphogenesis.[99–101] The Val→Ala mutation at amino acid E407 of Asibi virus occurs in the N-terminal stem-anchor region of the E protein. Mutations in this region can affect the structural integrity and three-dimensional structure of the prM-E heterodimer and are known to attenuate flaviviruses, including dengue, tickborne encephalitis group virus, Japanese encephalitis, and yellow fever–Japanese encephalitis chimeras.[83,87,102,103] In addition to the E protein, the M protein also contains a single amino acid substitution at residue 36 (L36F) and is found in a proapoptotic sequence described for dengue virus.[104]

The 17D vaccine is not fixed with respect to neurovirulence, so that sequential mouse-brain passage of the vaccine results in increasing mouse virulence.[105] The neuroadapted 17D virus reverted to the wild-type (Asibi) sequence at amino acid residues E52 and E173 and also accumulated mutations at the putative virulence determinant E305 (Ser→Val) and in nonstructural genes (NS1, NS2A, NS4A, NS4B, and NS5).[78,82] A chimeric virus with the original 17D backbone and the neuroadapted E sequence did not have increased neurovirulence compared to original 17D. In contrast, when all mutations in E and NS genes of the neuroadapted strain were introduced into the 17D infectious clone, neurovirulence was increased, illustrating that multiple genes were involved in virulence and implicating mutations in the NS proteins or the 3′ noncoding region of the virus. Studies with other flaviviruses also have shown that mutations in the NS coding region might reduce neurovirulence, probably by reducing the rate of replication.[73,106]

Eleven amino acid changes in the NS proteins of Asibi viruses occurred during the derivation of 17D vaccine (see Table 63.1): one in the NS1 protein; four in NS2A; one each in NS2B, NS3, NS4A, and NS4B; and two in NS5. Although the role of NS2A mutations are not known, this protein plays roles in viral RNA replication, and virion assembly and release.[107–109] The change in NS3 occurs at residue 485, in the region coding for the RNA helicase and triphosphatase enzymes for unwinding RNA during replication. The two mutations in the NS5 RNA dependent RNA polymerase potentially

influence replication efficiency and might contribute to attenuation of 17D. A plaque variant purified from a 17D vaccine had reduced mouse neurovirulence and differed at an amino acid residue in NS5 137 Pro→Ser.[110] This mutation is in a region encoding the methyltransferase activity of the NS5.

The 3′ noncoding region plays a critical role in replication, and changes in this sequence can contribute to attenuation. The 3′ noncoding proximal region is variable in length among yellow fever strains and contains one to three repeat sequence elements.[61] The 3′ terminal region contains a 90- to 120-nucleotide conserved region involved in folding of the stem-loop structure and serving as a promoter for minus strand synthesis during replication. Mutations in the stem-loop region can interfere with virus replication.[111,112] In the case of dengue-4 virus, the proximal region of the 3′ noncoding region does not appear to be critical to replication, but mutations or deletions in this region can nevertheless attenuate virulence.[113] It is clear that mutations in the 3′ noncoding region of 17D present in both the variable and the conserved proximal region might have contributed to attenuation of the vaccine virus. The change at nucleotide 10,367 might be an exception because one 17D vaccine contains a heterogeneous mixture including the wild-type sequence.[71]

Much less is known about the determinants of yellow fever virus viscerotropism, principally because of the difficulty of assessing this property in nonhuman primates. As noted earlier, the neurotropism and viscerotropism properties of yellow fever virus might reside in distinct regions, and thus one cannot conclude that attenuation of one feature correlates with attenuation of the other. However, the hinge region of E apparently contains residues implicated in both neurotropism and viscerotropism. A mutation at E279 in the hinge 4 region of a yellow fever–Japanese encephalitis chimeric virus reduced viscerotropism of the virus in nonhuman primates,[86] suggesting that mutations in the hinge region of the yellow fever genome during derivation of 17D virus could be responsible for attenuation of viscerotropism. The Met→Lys mutation at position E279 in the yellow fever–Japanese encephalitis chimera is within the beta-strand of the secondary structure, increases the net positive charge of the protein, and is predicted to result in a shortening of the beta-strand secondary structure.

Wang and colleagues compared the sequence of the French viscerotropic strain with that of the attenuated French neurotropic vaccine, which was derived by serial mouse brain adaptation.[13] French neurotropic vaccine is not viscerotropic for monkeys or humans, whereas it has enhanced neurovirulence for mice and monkeys. Comparison of the parental and vaccine strains revealed 35 (1%) amino acid changes, with the highest frequency of mutations in C, M, E, NS2A, and NS4B. The specific roles of these changes are unknown. Sequence comparison of French neurotropic vaccine with 17D vaccines showed only two shared differences from the parental strains and other wild-type yellow fever strains. These common differences, which evolved during the development of vaccine strains by adaptation to different hosts, were in the M protein (M36 Leu→Phe) and NS4B (95 Ile→Met). Thus, the molecular structures involved in viscerotropism remain uncertain.

Golden hamsters develop hepatitis resembling human yellow fever, and the model could potentially be used to identify molecular determinants of viscerotropism.[75] Unfortunately, to induce viscerotropic disease in the hamster, wild-type yellow fever virus must be adapted by serial liver–liver passage. Thus, Asibi virus became hamster-virulent between the sixth and seventh passage. The genome of the viscerotropic passage 7 virus contained seven amino acid mutations, of which five are in the E protein (positions E27 (Gln→His), E28 (Asp→Gly), E155 (Asp→Ala), E323 (Lys→Arg), and E331 (Lys→Arg)].[114] Two changes (E323 and E331) are in the part of domain III implicated as ligands for cell receptors, and thus they might be responsible for hepatotropism in hamsters. In addition, two amino acid mutations occurred in NS2A48 (Thr→Ala) and NS4B98 (Val→Ile). A mutation near the latter site in NS4B (at residue 95) is associated with attenuation of yellow fever 17D and French neurotropic vaccines. In addition the mutation at E155 in yellow fever 17D was associated with reversion to virulence (neurotropism) of yellow fever 17D.[92]

Further light was shed on the viscerotropism genes when McArthur and colleagues attempted to reproduce the serial passage experiment.[114,115] The second passage 7 virus was not lethal, although it still caused histopathologic changes in liver. The genome sequence of the first (lethal) and second (nonlethal) passage 7 viruses differed at two amino acids (E98 and E331). Thus, although these studies in hamsters do not identify individual amino acids involved in attenuation of 17D for humans, they do point to functional regions and specific determinants of the genome for further investigation. In contrast to the hamster, mice deficient in the IFN-α/β receptor (A129 mice) or the STAT1 signaling molecule (STAT129 mice) were susceptible to viscerotropic disease after infection with unadapted wild-type yellow fever virus and could serve as a useful model for future investigations of viral virulence determinants.[74] The 17D vaccine strain was attenuated in these mice and did not cause viscerotropic infection or injury.

Asibi and French viscerotropic viruses lost both neurotropism and viscerotropism after a few passages in HeLa (Henrietta Lacks) cells.[116,117] The HeLa cell–adapted virus contained 10 amino acid mutations, including five in the E protein, one in NS2A, and three in NS4B. The NS4B (95 Ile→Met) mutation is associated with attenuation of Asibi to 17D by passage in chick embryo tissue and of French viscerotropic virus to French neurotropic vaccine by passage in mouse brain; this mutation also appeared during HeLa cell adaptation, suggesting a role in attenuation of yellow fever vaccines. As pointed out earlier, a nearby mutation at NS4B98 was associated with viscerotropism for hamsters. HeLa-, chick embryo–, and mouse brain–adapted viruses share two phenotypic markers: loss of viscerotropism and loss of vector competence for *Ae. aegypti*. The role of NS4B95 is not known but NS4B plays a number of roles, including formation of the viral replication complex and inhibiting the host innate immune responses via blocking type I IFN signaling. The changes in the HeLa-passed virus E protein occur at E27 (Gln→His), E155 (Asp→Ala), E228 (Met→Lys), E331 (Lys→Arg), and E390 (His→Pro). Three mutations (at E27, E155, E331) were also associated with adaptation in the hamster, suggesting that they could be viscerotropism virulence factors.[114]

The interactions of yellow fever 17D virus with dendritic cells that are involved in early sites of replication after inoculation of the vaccine were studied by Palmer and colleagues. 17D virus replication in these cells was restricted, confined to the lysosomal compartment, and was rapidly processed with early expression of activation markers, suggesting a basis for attenuation and immunogenicity of the vaccine virus.[118] However, an earlier study found that 17D virus would replicate in both mature and immature dendritic cells.[119] Clearly, more work is needed in this area; however, it may be significant that that the two groups used 17D virus from different sources and so may differ in sequence. The ability to establish disseminated infection and cause viscerotropic disease in IFN-α/β-deficient (A129) mice correlated with infectivity for A129-derived, but not wild-type 129-derived, dendritic cells in vitro, indicating that these cells play an important gatekeeper role in pathogenesis.[74] As is pointed out later (see "Adverse Events"), yellow fever 17D vaccine is associated with rare neuro- and viscerotropic adverse events in patients with and without known

risk factors. The residual capacity to cause a disease in humans resembling wild-type yellow fever suggests that either attenuation of virulence is incomplete or there is a role for host genetic factors. Although many animals reveal the residual neurotropism of 17D virus, there is currently only one partially characterized mouse model of viscerotropic adverse events (IFN-receptor–deficient mice).[74] Such a model could be used to study the molecular basis of residual viscerotropism and to devise improved vaccines and treatment strategies.

In addition to attenuation of viscerotropism and neurotropism, a critical property of 17D vaccine is its inability to disseminate from the midgut to other tissues in the mosquito, and thus it is not transmissible from the mosquito to vertebrate hosts. Higgs and coworkers have used infectious clone technology to generate chimeras of Asibi and 17D viruses containing swapped residues in NS2A (four amino acids), NS4B (one amino acid), and the 3′ untranslated region (four nucleotides) to identify viral genes involved in dissemination in *Ae. aegypti*. Dissemination was found to be related to whether the chimera had an Asibi or 17D E protein domain III, but E domain III appeared not to be the only genetic factor controlling dissemination.[120,121]

While the precise molecular determinants of attenuation and virulence have not been elucidated, recent data have shed light on the molecular basis of attenuation of 17D vaccine. It is known that RNA viruses, including yellow fever virus, exist as a quasispecies, or a group of nucleotide sequences related by a similar mutation or mutations that compete within a viral population, because of the high error rate of the RNA-dependent RNA polymerase. Until recently, nucleotide sequences were obtained by "first-generation sequencing," such as Sanger sequencing (chain-termination method), where the nucleotide sequence of the dominant member of a population was obtained and the quasispecies was seen as a "consensus" sequence. However, "next-generation sequencing" (NGS) has enabled amplification of a single molecule to obtain nucleotide sequence of the quasispecies population. Comparison of a 17D-204 commercial lot with wild-type Asibi strain was undertaken by NGS and revealed that the wild-type virus population structure differs from that of the vaccine. Specifically, Asibi virus had the typical quasispecies structure of a RNA virus while in contrast the vaccine virus was relatively homogeneous suggesting that the lack of diversity in the 17D vaccine virus population may contribute to the attenuated phenotype.[122]

Molecular Identification of Antigenic Determinants in Yellow Fever Virus and Vaccine Strains and Their Relationship to Virulence

Monoclonal antibodies recognize structurally distinct regions in the E protein of yellow fever virus, including vaccine strain–specific epitopes and yellow fever virus–specific epitopes, as well as determinants cross reactive with specific heterologous flaviviruses and with broad flavivirus-group epitopes.[123–130] Antibodies against vaccine strain–specific, virus-specific, and flavivirus group–reactive epitopes neutralize virus, and many passively protect mice against intracerebral challenge. Interestingly, monoclonal antibodies generated following immunization with 17D virus neutralized wild-type (Asibi) virus but not 17D, and flavivirus group–reactive monoclonals generated after immunization with 17D, Asibi, or heterologous flaviviruses neutralized wild-type virus.[124,125,131] This multiplicity of neutralizing determinants helps explain the broad protective immunity afforded by 17D vaccine against wild virus strains and the partial cross-protection by heterologous flaviviruses against yellow fever.

Additional studies have defined epitopes that are substrain specific, differentiating 17D-204 vaccine from other yellow fever viruses and 17D-204 from 17DD vaccines and even distinguishing between vaccines of the same substrain from different manufacturers.[64,125] Plaque-size variants purified from 17D vaccine can also be distinguished in neutralization and hemagglutination inhibition tests.[64,123,132] Some monoclonals are specific for 17D and do not recognize wild-type virus.[126,133] The antigenic heterogeneity of 17D vaccine is due to the uncloned nature and different passage histories during manufacture and laboratory manipulation. At present, there is no recognized practical consequence, with respect to protective immunity, of the absence of some wild-type antigenic determinants in 17D vaccines.

Attenuation by passage of wild-type Asibi virus in HeLa cells was associated with acquisition of a vaccine-specific epitope recognized by MAb H5.[117] Gould and colleagues identified a plaque variant in 17D vaccine that reacted with a MAb specific for wild-type viruses and variants recovered from Asibi virus that reacted with a 17D-204 specific monoclonal, suggesting that 17D was derived by a process of selection of subpopulations during serial passage.[84]

Some neutralizing epitopes have been localized by sequencing escape mutants or wild-type antigenic variants recovered from 17D vaccine. An epitope at residue E173 is the only wild-type–specific antigenic determinant that has been localized[85]; this site is also a putative neurovirulence factor. A neutralization determinant in the E protein of both wild-type and 17D was identified at residues E71/72 in domain II of the E protein.[90,134] Ryman and colleagues found an additional neutralizing epitope at either E155 or 158 in domain I.[90] These data were confirmed using human MAb fragments that neutralized 17D and wild-type yellow fever viruses belonging to West African and to Central and East African genotypes.[135] This study suggested that amino acids E71 and E155 formed part of a single conformational epitope in the E protein dimer.

In a later analysis, Ryman and colleagues selected escape mutants from three 17D substrains with a monoclonal antibody (MAb 864) specific for 17D-204 virus that had a number of functional activities including neutralization, hemagglutination, and hemolytic and passive protection.[64,85,124] The escape mutants were characterized with respect to mouse neurovirulence and prM-E nucleotide sequence. One series of escape mutants had reduced neurovirulence for mice compared to the parental virus and had a Ser→Leu mutation at E325. E325 is the site of a Pro→Ser mutation that occurred in the derivation of 17D-204 from Asibi but is not present in the 17DD substrain and thus is not considered to be relevant to the attenuated vaccine phenotype.[68,69]

In contrast, another neutralization escape mutant was neurovirulent for mice and contained an amino acid mutation at E305 (Phe→Ser), which is a conserved substitution across all 17D strains (see Table 63.1). This mutation represents a reversion to the wild-type residue at E305. The E305 and E325 residues are spatially adjacent within domain III of the E glycoprotein and thus represent a conformational epitope recognized by the MAb 864 used to generate the different escape mutants.[85,136] The location of the epitope is consonant with the effector roles of antibody in blocking cell attachment and intracellular uncoating events. Clearly, this epitope also is critical to pathogenesis and neurovirulence. By Western blotting, the MAb 864 recognizes both the prM and E proteins,[84] suggesting that the E305-E325 epitope is involved in the prM-E interaction during virus assembly.[137]

Although an exhaustive search for neutralization and protective epitopes has not been made, it would appear that yellow fever viruses (like other flaviviruses) contain only

a few (but more than one) such determinants in the E protein, which are structurally diverse. These epitopes must be conserved across wild-type strains, consistent with the broad protective activity of yellow fever vaccine against wild virus strains.

Antigenic determinants involved in cell-mediated immunity have been localized in yellow fever 17D virus, dengue virus, and Murray Valley encephalitis viruses.[138–144] Yellow fever cytotoxic T-lymphocyte determinants are found in the E proteins and in multiple nonstructural proteins: NS1, NS2B, NS3, NS4B, and NS5.[145–147] These T-cell epitopes are highly conserved and probably contribute to the cross-protective activity of 17D vaccine against all geographic variants of wild-type yellow fever virus. In the case of yellow fever, cytotoxic T-cell epitopes were found on E, NS1, NS2B, and NS3, and these epitope sequences were conserved across multiple yellow fever strains.[145] T-cell responses tend to be cross-reactive.[143,144] Cytotoxic T-effector cells raised to Murray Valley encephalitis virus demonstrated significant cross-reactivity with target cells pulsed with yellow fever–derived NS3 peptides, despite the relative low sequence homology of the determinants.[148]

Modes of Transmission

The primary mode of transmission of yellow fever virus to humans is through the bite of an infected mosquito. Various mosquitoes are involved in the maintenance and transmission of yellow fever virus and the virus is maintained in an enzootic cycle by these mosquitoes and wild nonhuman primates. Other modes of transmissions, such as exposure to infected blood and aerosol, have occurred primarily among unvaccinated laboratory workers.

Transmission Cycles

The enzootic transmission cycle involves monkeys and diurnally active tree-hole–breeding mosquitoes (*Haemagogus* spp. in South America and *Aedes africanus* in Africa) (Fig. 63.3). Humans are sporadically exposed to infected mosquitoes when they encroach on this cycle during occupational or recreational activities ("jungle yellow fever"). This transmission cycle accounts for jungle yellow fever cases in South America and in the rainforest zone of Africa. In the moist savanna regions of Africa, tree-hole-breeding *Aedes* mosquitoes reach very high densities and are implicated in endemic and epidemic transmission, transferring virus from monkeys to humans and between humans. *Ae. aegypti*, a domestic mosquito that breeds in human-made containers and achieves highest densities in urban environments, can transmit yellow fever virus between humans ("urban yellow fever").

Many species of nonhuman primates are susceptible to yellow fever infection.[149] The majority of African species have viremic infections sufficient to infect mosquitoes without developing clinical illness, whereas some neotropical species (e.g., howler monkeys) develop fatal infections. Depletion of vertebrate hosts through natural immunization and death during epizootic waves is a factor in the cyclic appearance of yellow fever activity. In many areas, deforestation and hunting pressure have markedly reduced monkey populations, and human beings become the host in the yellow fever virus transmission cycle.[150,151] Although this point is still debated, there is little evidence that nonprimate vertebrates are involved in enzootic transmission.[150,152–154]

The pattern of yellow fever viral activity in South America is characterized by intermittent emergence around the edges of the Amazon region. These outbreaks are preceded by evidence for increased virus transmission between monkeys and

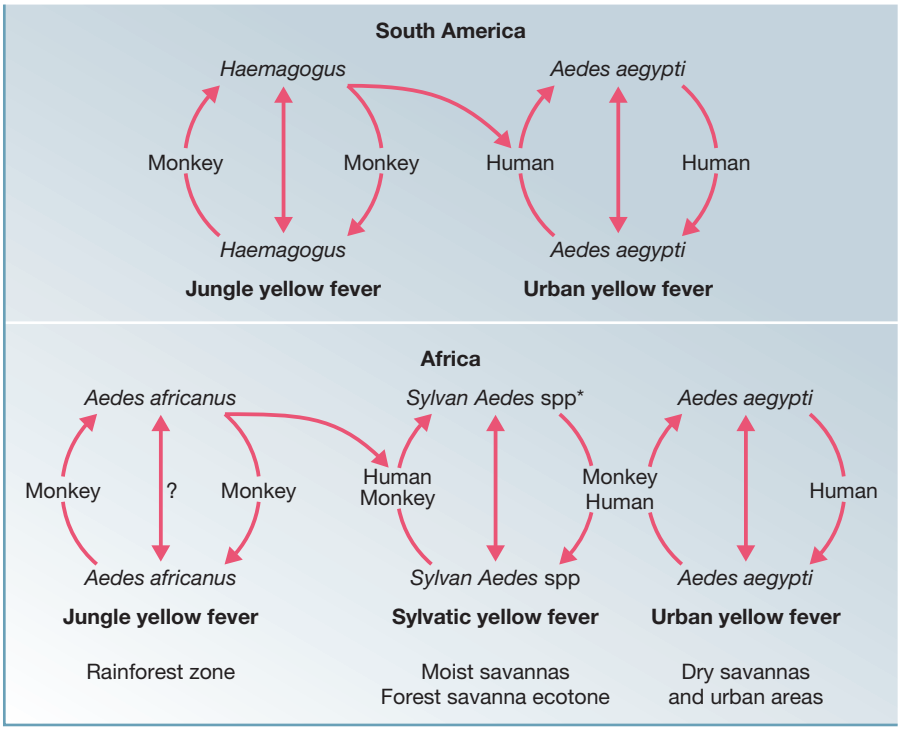


Figure 63.3. Transmission cycles of yellow fever virus in South America *(top)* and Africa *(bottom)*. In both continents, jungle yellow fever is transmitted through an enzootic cycle. Urban and sylvatic yellow fevers are transmitted in epidemic cycles. Transovarial transmission is indicated by vertical arrows showing virus transfer between mosquitoes, and this may be an important mechanism for virus survival over the long dry season. *Aedes* species found in West Africa include *Ae. furcifer*, *Ae. taylori*, *Ae. luteocephalus*, *Ae. africanus*, *Ae. opok*, *Ae. vittatus*, and *Ae. metallicus*. Species found in East Africa include *Ae. africanus* and *Ae. simpsoni* (complex).

Haemagogus spp. within the Amazon basin, which moves in a circular fashion within the forest or along gallery forests of river courses. A detailed analysis of the patterns of virus activity preceding and during epidemics in Brazil is provided by Mondet[155] and Vasconcelos and colleagues.[156]

The ecology of yellow fever virus differs in areas bordering the rainforest in Africa. A mosaic of savanna and forest in galleries along rivers characterizes this transitional vegetation zone. In this region and surrounding moist (Guinea) savanna, yellow fever virus transmission is effected by a wide variety of tree-hole–breeding mosquito vectors.[150,153,157–159] In West Africa, the principal vectors responsible for yellow fever virus transmission in the savanna zone include *Aedes furcifer, Aedes vittatus,* and *Aedes luteocephalus,* as well as *Ae. africanus*.[157] In East Africa, *Ae. africanus* and human-biting populations of the *Aedes simpsoni* species complex *(Ae. simpsoni, Aedes lilii, Aedes bromeliae)* play a similar role.[160–162] During the wet and early dry seasons, vector density reaches high levels. Vectors are active in plantation areas and in proximity to human dwellings and can enter houses.[158] Both humans and nonhuman primates may be involved as hosts in the transmission cycle, and the rate of virus transmission far exceeds that found in the rainforest zone. The savanna-forest ecotone and surrounding Guinea savanna has been described as the "zone of emergence" of yellow fever and represents the region principally affected by epidemics in Africa.[150,159]

An epidemiologically distinct transmission cycle involves *Ae. aegypti*, which breeds in containers used to store water or in artificial containers that collect rainwater around human habitations and predominantly feeds on humans. *Ae. aegypti* transmits yellow fever virus between humans, the sole viremic hosts in the cycle. The vector occurs in dry areas and heavily settled areas but is also widely dispersed in settlements in rural areas. Urban outbreaks have followed introduction by viremic persons from areas of jungle yellow fever activity.[163,164] In the Americas, urban yellow fever outbreaks were common prior to the successful eradication of the vector,[165–167] and outbreaks currently show signs of reoccurring owing to the repopulation of most urban areas with *Ae. aegypti*. The last documented urban outbreak in continental South America prior to efforts to control *Ae. aegypti* occurred in western Brazil in 1942. There was a long interval without urban yellow fever reports, and then *Ae. aegypti*–transmitted yellow fever was reported in Trinidad in 1954,[168] Santa Cruz, Bolivia in 1998,[169] and most recently, Asunción, Paraguay in 2008.[32]

Africa suffers many *Ae. aegypti*–borne epidemics, because the vector is prevalent in urban and rural areas. In dry areas (Sudan and Sahel savanna zones), where domiciliary *Ae. aegypti* might represent the only species capable of sustaining yellow fever virus transmission, outbreaks occur after introduction of the virus by viremic persons. *Ae. aegypti*–borne epidemics in the past 40 years include those in Senegal in 1965 and 2002; Angola in 1971, 2015–16; Ghana in 1969, 1977–80, and 1983; Côte d'Ivoire in 1982, 2001, and 2008; Nigeria between 1987 and 1991; and likely Democratic Republic of Congo (DRC) in 2016.[32a,170,171] Undoubtedly other outbreaks in Africa involved *Ae. aegypti*, but entomological investigations are infrequently performed or are too superficial to draw conclusions regarding the role of domestic vectors. In Africa, some feral populations of *Ae. aegypti* feed preferentially on mammals other than humans. *Aedes* spp. other than *Ae. aegypti* are responsible for interhuman yellow fever virus transmission, as noted earlier.

Maintenance of Yellow Fever Virus in Nature

The means of survival of yellow fever virus across the long dry season, when sylvatic mosquito vectors are virtually absent, remains incompletely understood. *Aedes* and *Haemagogus* eggs survive desiccation in tree holes and hatch with the return of rain. Experimental and field studies indicate that transovarial transmission is a means of virus survival across the dry season.[172–178] However, the filial infection rate of yellow fever virus by vertical transmission in mosquitoes is too low for this to maintain the virus indefinitely, and annual amplification in nonhuman primates is thus imperative. Low-level horizontal transmission by drought-resistant vectors and alternative horizontal and vertical transmission cycles involving ticks[179] have been suggested as ancillary mechanisms for survival across the dry season. Persistent infection of experimentally infected nonhuman primates has been documented,[180,181] but such infections are probably not accompanied by viremias sufficient to infect vectors.

Yellow Fever Virus in the Mosquito Vector

Infection of mosquitoes is initiated by ingestion of a blood meal containing a threshold concentration of virus (~3.5 $\log_{10}$/mL), resulting in infection of the midgut epithelium. The virus is released from the midgut into the hemolymph and spreads to other tissues, notably the reproductive tract and salivary glands. The time elapsed between ingestion of virus and secretion in saliva (the extrinsic incubation period) is temperature-dependent and has a median duration of 10 days at 25°C.[182] After the extrinsic incubation period, the vector is capable of transmitting virus when she refeeds on a susceptible host. Infection of reproductive tissues of the mosquito provides a mechanism for vertical transmission of yellow fever virus from the female mosquito to her progeny and from congenitally infected males to females during copulation.[172,177,178]

Transmission of Live Vaccine Viruses. The use of a live vaccine theoretically engenders a risk of secondary spread by mosquitoes, and passage of vaccine virus could result in a reversion to a more-virulent phenotype. This is unlikely for two reasons. First, viremia following 17D vaccination is very low and below the threshold of oral infection of the vector,[183,184] with a number of caveats including viremia may be higher in patients with viscerotropic adverse events, and viremia has not been measured in infants, who may sustain more-active infections than adults, or in immunosuppressed persons. Second, 17D virus is poorly infectious for mosquitoes. Whitman infected *Ae. aegypti* larvae with 17D virus after immersion in virus, but infected adult progeny were incapable of transmitting the virus.[185] Bhatt and colleagues reported that 17D vaccine virus inoculated by the intrathoracic route (thus bypassing the midgut barrier) replicated to a low level in *Ae. aegypti* and *Aedes albopictus*, but orally exposed mosquitoes contained no detectable virus after a 22-day extrinsic incubation period.[186] Jennings and colleagues showed that 45% of adult female *Ae. aegypti* that fed on a high concentration of 17D vaccine in an artificial blood meal developed midgut infections, but no virus was detected in head tissue.[92] In another study, only 1 of 32 *Ae. aegypti* orally exposed to 17D virus developed infection in head tissue, and none of the mosquitoes transmitted the virus.[187] Thus, yellow fever 17D virus has lost its ability to be transmitted by *Ae. aegypti*, possibly owing to inability of the virus to cross the midgut barrier.

At the molecular level, it has not been determined which mutations in 17D virus are responsible for restricted transmission by mosquitoes. However, it is of interest that Asibi virus passaged in HeLa cells lost mosquito competence and that this change may have been associated with a mutation at NS4B 95.[117,187] In support of a role for nonstructural genes in determining mosquito competence, studies of chimeric viruses in which the prM-E genes of Japanese encephalitis, West Nile,

and dengue were inserted into a 17D infectious clone showed retention of the 17D phenotype in mosquitoes.[186,188] The conclusion from these studies was that the inability to replicate efficiently in mosquitoes is determined by nonstructural genes of the virus and that the structural genes containing cell-receptor binding determinants were critical to dissemination.

An infectious clone of wild-type Asibi virus has been shown to infect and be transmitted by *Ae. aegypti*, allowing genetic modifications of the clone to define the molecular basis of virus infection in mosquitoes.[189] When the domain III of the envelope gene of 17D was inserted into the virulent wild-type (Asibi) infectious clone, the virus was markedly restricted for virus dissemination, indicating that the structural element of the vaccine strain containing putative cell attachment determinants was responsible for attenuation.[120] However, because the virus was still capable of escaping from the midgut barrier at a low rate, the study suggested that dissemination in the vector was also determined by the nonstructural genes.

Subsequent work substituting 17D nonstructural genes NS2A or NS4B into the Asibi backbone showed that the resulting viruses had substantially reduced ability to disseminate.[120] Moreover, when the Asibi prM-E structural genes were inserted into a 17D infectious clone, the resulting virus was attenuated for mosquitoes.[190] These studies showed that 17D virus, which contains multiple mutations in both structural and nonstructural genes, is attenuated for transmission by mosquitoes by virtue of multigenic properties.

Human Infection

Approximately 10^3 virions are inoculated during mosquito feeding.[191] Salivary virus is deposited mainly in the extravascular tissues of the skin of the host during probing, because saliva that is injected intravascularly is apparently reingested by the mosquito during blood feeding.[192] Virus replication is initiated in the epidermis and dermis at the site of inoculation, probably in Langerhans cells, and spreads through lymphatic channels to regional lymph nodes and then to other lymphoid organs and viscera via the bloodstream. In the immunized host, the small mosquito inoculum would encounter a vast excess of antibodies in extracellular transudate and lymph. This suggests that a low level of immunity is sufficient to protect the host against disease. It is not known whether immunity is sufficient to sterilize the mosquito inoculum. However, under conditions of artificial inoculation of 17D vaccine to previously vaccinated subjects or coadministration of vaccine viruses and immune serum, sufficient virus replication occurs for a booster or primary immune response, respectively.[16,183,193] It appears less likely that the amount of antigen inoculated (approximately 50,000 to 100,000 virions) would be capable of stimulating a booster response, with active replication.

The expression of neutralization epitopes (determined with monoclonal antibodies) can differ between yellow fever virus propagated in mosquito and in mammalian cells.[125] The evolutionary and functional relevance of this observation are uncertain, but it might represent a means whereby mosquitoes infected with yellow fever virus would avoid the effects of neutralizing antibodies ingested in a blood meal. In terms of flavivirus vaccine development, host specificity of neutralization determinants suggests that certain arthropod cells might not yield effective inactivated or subunit immunogens for vertebrates.

Other Modes of Human Infection

Laboratory infections with yellow fever virus were common in the prevaccine era and remain of concern today, particularly where unvaccinated clinical laboratory personnel encounter blood from patients during the early stage of illness. Some laboratory infections were probably acquired by the bite of mosquitoes experimentally infected in the laboratory or of wild mosquitoes infected after feeding on experimental animals; others were the result of direct contact with blood or aerosols of dried virus.[14,194–196] The stability of yellow fever virus is sufficient to permit transmission within short periods after generation of an infectious aerosol in the laboratory.[197] Under experimental conditions, a dose of only 10 mouse median lethal doses (MLD_{50}), equivalent to approximately 100 plaque-forming units (PFU), delivered by aerosol was sufficient to induce lethal infection in rhesus monkeys.[198] The absence of direct interhuman transmission indicates that virus is not shed in secreta or excreta at sufficiently high levels for transfer by contact or aerosols. There is no evidence that black vomit or urine is infectious. However, on numerous occasions, transmission has occurred between separately caged monkeys housed in a single room, possibly by aerosol spread.[199] Findlay and MacCallum[199] infected monkeys by a mucosal (intragastric) route and Bauer and Hudson[200] transmitted yellow fever virus to monkeys by rubbing virus on unbraided skin, a technique that might have exposed epidermal Langerhans cells to infection. Monkeys were efficiently infected with 17D vaccine applied to the skin by rubbing with a device that simply stripped the stratum corneum.[201] Some infections in laboratory workers may be explained by contact with viremic blood, but it remains unclear whether infection occurred via intact skin, via abrasions, or by contact with mucosal membranes.

There has been at least one case of perinatal transmission of wild-type yellow fever virus from a woman who developed her initial symptoms of yellow fever 3 days prior to giving birth.[202] The infant developed symptoms 3 days after birth and had yellow fever viral RNA in a blood sample obtained 5 days after illness onset. The infant unfortunately died of fulminant yellow fever on the twelfth day of life. In addition to perinatal transmission, the recent transmission of 17D vaccine to several infants via breastfeeding suggests that the natural virus may be spread the same way via either breast milk, in ingested blood, or possibly by inhalation.[203–205]

Yellow fever virus was extensively investigated in the U.S. Biological Weapons Program. Whereas the virus could infect by the aerosol route, it was considered too thermolabile for successful weaponization. Instead, extensive studies were undertaken on the use of *Ae. aegypti* as an entomologic weapon capable of delivering the virus.[206,207]

Clinical Description

The clinical spectrum of yellow fever is very broad, including subclinical infection, abortive infection with nonspecific flu-like illness, and potentially lethal hemorrhagic disease. This variability makes the clinical diagnosis of sporadic cases difficult and is responsible for the underestimation of morbidity and inflation of case-fatality rates when only cases of full-blown yellow fever are enumerated. As with many other infections, this variability in response is due to intrinsic and acquired host resistance factors and probably to differences in the pathogenicity of virus strains.

After an incubation period of 2 to 9 days (median, 4.3 days),[182] clinical disease begins abruptly with rigors and headache. The illness is often characterized by three stages.[14,208–210] The first stage, referred to as the period of infection, lasts 3 to 4 days and is marked by the virus being present in the blood.[14,211] Levels of the virus usually peak in the blood around days 2 to 3 of illness with titers of up to 5.6 $\log_{10}$ mouse intracerebral LD_{50}/mL, which may be equivalent to approximately 6.6 $\log_{10}$ PFU in cell culture.[211] This stage of illness is characterized by

fever, malaise, prostration, headache, photophobia, lumbosacral pain, pain in the lower extremities (particularly the knee joints), generalized myalgia, anorexia, nausea, vomiting, restlessness, irritability, and dizziness.[212] On physical examination the patient appears toxic, with hyperemia of the skin; congestion of the conjunctivae, gums, and face; epigastric tenderness; and tenderness and possible enlargement of the liver. The tongue is characteristically small and pointed and bright red at the tip and sides, with a white coating in the center. Early clinicians made much of this arcane sign, and it has stood the test of time. Initially, the pulse rate is high, but by the second day there is a bradycardia relative to fever (Faget sign). The average fever is 38.9°C to 39.4°C and lasts 3.3 days, but temperature can rise as high as 40.6°C. Young children can experience febrile convulsions. Laboratory abnormalities include leukopenia (1.5 to 2.5 $\times$ 10^9 cells/L) with a relative neutropenia. The leukopenia occurs precipitously, in concert with onset of illness.[14] Between 48 and 72 hours after onset, serum transaminase often are elevated and usually precede the appearance of jaundice.[213]

The period of infection may be followed by a distinct period of remission (also referred to as a period of calm), with abatement of fever and symptoms lasting up to 48 hours.[214] The remission is often not obvious or very brief. In cases of abortive infection, the patient simply recovers at this stage. Such cases typically remain anicteric, and the nonspecificity of the syndrome makes it impossible to diagnose yellow fever clinically except during an epidemic. A recent model that used data from previous outbreaks and serosurveys estimated that 55% of yellow fever virus infections are asymptomatic and 33% results in mild disease.[215]

The third stage of the disease, the period of intoxication, occurs on the third to sixth day after onset and is characterized by jaundice and the development of often life-threatening signs and symptoms. Approximately 12% of persons infected with yellow fever virus will enter the period of intoxication.[210,215,216] During this period, fever can reoccur, and there is relative bradycardia, nausea, vomiting, epigastric pain, jaundice, oliguria, and a hemorrhagic diathesis. Virus disappears from blood and antibodies appear. The subsequent course reflects dysfunction of multiple organ systems, including the liver, kidneys, and cardiovascular system. High levels of circulating proinflammatory cytokines are present, and the overall picture has the clinical characteristics of the systemic inflammatory response syndrome and multiple organ failure.

Serum aspartate aminotransferase and alanine aminotransferase peak early in the second week of illness and fall rapidly over a few days in patients who recover. Aspartate aminotransferase levels typically exceed alanine aminotransferase, probably owing to direct viral injury to myocardium and skeletal muscle. This distinguishes yellow fever from viral hepatitis, in which alanine aminotransferase levels typically exceed aspartate aminotransferase levels. Alkaline phosphatase levels are normal or only slightly elevated. Direct bilirubin levels are typically between 5 and 10 mg/dL.[217]

Kidney dysfunction is marked by an increase in albuminuria, reduction in urine output, and rising azotemia. Albumin levels in the urine typically range between 3 and 5 g/L but can reach 20 g/L. Serum creatinine levels are three to eight times normal. In some patients who survive the hepatitis phase, kidney failure predominates.[218] Death is preceded by virtually complete anuria. The hemorrhagic diathesis is manifested as coffee-grounds hematemesis, melena, hematuria, metrorrhagia, petechiae, ecchymoses, epistaxis, oozing of blood from the gums, and excessive bleeding at needle-puncture sites. Laboratory correlates include thrombocytopenia, prolonged clotting and prothrombin times, and reductions in clotting factors synthesized by the liver (factors II, V, VII, IX, and X). Some patients have clotting abnormalities that suggest disseminated intravascular coagulation (DIC), including diminished fibrinogen and factor VIII and the presence of fibrin split products.[219,220]

The clinical significance of myocardial injury remains poorly understood. The electrocardiogram can show sinus bradycardia without conduction defects, ST-T abnormalities, particularly elevated T waves, and extrasystoles,[14,221] presumably the result of virus replication and direct viral injury to the myocardium. Bradycardia can contribute to the physiologic decompensation associated with hypotension, reduced perfusion, and metabolic acidosis in severe cases. Acute cardiac enlargement can occur during the course of yellow fever infection.[14]

Central nervous system (CNS) signs include delirium, agitation, convulsions, stupor, and coma. In severe cases, the cerebrospinal fluid (CSF) is under increased pressure and might contain elevated protein but no cells. In one case, yellow fever virus was recovered from CSF after death.[222] In patients dying of yellow fever, CNS signs appear to result from cerebral edema or metabolic factors, based on the virtual absence of inflammatory changes in brain tissue. Pathologic changes include petechiae (perivascular hemorrhages) and edema.[223] True yellow fever viral encephalitis is exceedingly rare, with few extant clinical case reports of paralysis, optic neuritis, and cranial nerve palsy suggesting neurologic infection, but without substantiating virologic evidence to differentiate encephalitis from encephalopathy.[224,225]

The critical phase of the illness occurs between the fifth and 10th days, at which point the patient either dies or rapidly recovers.[214,217,226] A model incorporating data from several outbreaks estimated the case-fatality rate for severe yellow fever cases of 47%.[215] Characteristics noted to be potential risk factors for death include male gender, older age (>40 years), jaundice, leukocytosis, and higher levels of liver transaminases, bilirubin, and blood urea nitrogen.[209,211–213,217,226–228]

In one series of 103 hospitalized patients in Nigeria, the average stay in hospital for surviving patients was 14 days (range, 5 to 42 days) and the average duration of the acute illness was 17.8 days.[226] Although convalescence may be associated with weakness and fatigability lasting several weeks, healing of the liver and kidney is typically complete, without postnecrotic fibrosis. In some cases, jaundice and elevations in serum aminotransferases persist for months after onset.[213,217,229] It is uncertain whether patients with such atypical signs have had other underlying hematologic or hepatic diseases. In one study, the outcome of yellow fever in hepatitis B surface antigen–positive and –negative patients was similar.[230]

Complications of yellow fever include superimposed bacterial pneumonia, parotitis, and sepsis associated with recovery from renal tubular necrosis.[208,226] Late deaths during convalescence have been ascribed to myocarditis, arrhythmia, or heart failure, but documentation of these events is poor.[212]

Risk Factors for Developing Disease and Factors Impacting Disease Severity

Demographic and Occupational Risk Factors. The age, sex, and occupational distribution of yellow fever in South America and Africa differ. In South America, most disease is caused by the jungle transmission cycle where humans are infected by tree-top dwelling mosquitoes that previously fed on viremic monkeys.[153,165] Therefore, occupational activities, such as forest clearing, logging, and road construction, increase the risk of the disease.[165] Most of these activities are performed by young male adults.[231,232] The prevalence of immunity in male residents exceeds that in female residents by 2.5- to 7.5-fold.[233] The age and sex distribution of jungle yellow fever

cases differs from that observed in South America during *Ae. aegypti*–borne epidemics in the early 20th century.[233] In the urban transmission cycle, *Ae. aegypti* are breeding in and around houses and sustaining interhuman transmission of virus; thus a high prevalence of infection is often seen in children and women who are more likely to be in homes when the mosquitoes are active.

In Africa, the prevalence of natural immunity accumulates rapidly with age, so that children are at highest risk (Table 63.2).[163,209,212,234–251] A high attack rate in children (>70%) typically reflects an area where older persons are protected by previous yellow fever vaccination campaigns (e.g., Senegal, 1965; Burkina Faso and Ghana, 1983; Mali, 1987). However, in some epidemics affecting populations without prior vaccination, the attack rate has also been higher in children. In The Gambia, the disease incidence in persons 0 to 19 years old was significantly higher than in older adults ($P < .01$), possibly owing to naturally acquired yellow fever or heterologous flavivirus immunity in adults.[252] In Africa, a slight excess of cases among male residents has been observed during epidemics (Table 63.2). This pattern was seen regardless of the role of domiciliary *Ae. aegypti* or sylvatic vectors, and it is thus difficult to explain on the basis of differences in human behavior or exposure to mosquito bites. The higher percentage of male patients has been observed, not only among notified or hospitalized patients, but also in population-based surveys, indicating that it is not a result of reluctance of female patients to seek medical treatment. Serologic data have shown neither a consistently higher incidence of infection nor susceptibility to illness among male residents,[243,252] but limited sample size and sampling biases might preclude detection of small differences.

There are few data on the relationship between severity of illness and age. In the 1921 epidemic in Peru, the case-fatality rate was highest in infants and children 0 to 5 years old and in older adults (>50 years).[228] Other authors noted a higher case-fatality rate among the elderly,[209,211,227] but lethality in infants was not always assessed in these studies. Elderly persons have a higher risk than young adults of developing serious adverse reactions to yellow fever 17D vaccine,[253–255] and as discussed below in "Effects on Immune Response: Age," have enhanced viremia and delayed antibody responses after vaccination compared to younger persons.[256] Increased severity of illness in elderly persons is known to occur in other flaviviral infections, notably from Japanese encephalitis, St. Louis encephalitis, and West Nile viruses. In a mouse model, age-dependent defects in antibody response to West Nile virus were linked to delayed germinal center formation in lymph nodes draining the site of infection.[257]

Although data from some outbreaks and case series suggest that males are more likely than females to die of yellow fever,[227,258] this finding is not reported consistently in other studies. In fact, in the Kenyan outbreak from 1992–93, female patients were 10.9 times more likely to die than male patients.[236]

Race and Ethnicity. The older literature makes repeated reference to racial differences in the lethality of yellow fever, rates being lower in blacks than whites during outbreaks in West Africa, tropical America, and the United States.[209,259,260] It is uncertain whether the apparent increased resistance of blacks reflects acquired immunity or is a result of genetic factors. The question of racial differences in susceptibility to yellow fever will be resolved only by well-controlled epidemiologic and serologic studies in the setting of an outbreak affecting both races.

Genetic Factors. Resistance and susceptibility to flavivirus, including yellow fever virus, could be linked to genetic factors but, if so, they are likely to be multifactorial in humans.[261] In mice, flavivirus susceptibility has been mapped to a mutation in an allele in one of eight genes *(Oas1b)* encoding 2′5′ oligoadenylate synthetase (OAS), an enzyme important in type 1 IFN-mediated innate immunity.[262,263] A high degree of polymorphism in *Oas1* genes and mutations conferring susceptibility to flaviviruses might determine susceptibility to flaviviruses in humans[264]; in one fatal case of viscerotropic disease following 17D vaccine, a mutation in an *Oas1* allele was found that could have contributed to increased susceptibility.[265]

Genetic background also influences the immune response to flaviviruses in mice and to a limited degree in humans.[266,267] A case of viscerotropic disease following yellow fever 17D had wild-type OAS genes but polymorphisms in chemokine receptor 5 (CCR5) and its ligand RANTES (regulated upon activation, normal T-cell expressed and secreted).[266] The role of genetic factors in innate and adaptive immune responses to yellow fever infection has not been directly evaluated, and it is an area for future research with respect to both the natural disease and serious adverse events associated with 17D vaccine.

Virus-Specified Factors. Wild-type yellow fever virus strains in Africa and South America are classified into at least seven genotypes and there is microheterogeneity at the sequence level among strains within these groupings.[2,43,44,57] In animals, these wild-type yellow fever virus strains differ with respect to neurovirulence for mice or viscerotropism for monkeys.[268–270] Deubel and colleagues found that South American strains were neuroinvasive for 8-day-old mice,[65] whereas African viruses were not, and they suggested that the higher case-fatality rates in South America could be the result of strain differences in virulence. On the other hand, it was the impression of early workers that the South American viruses were less often lethal for rhesus monkeys.[271] Miller and colleagues showed that the mosquito responsible for epidemic transmission in Nigeria had a low vector capacity,[272] and they proposed that the vector served as a genetic bottleneck for selection of a virulent virus strain able to elicit high viremias in humans. No data exist on the potential virulence of the different wild-type yellow fever strains in humans. These data would be difficult to obtain because multiple strains within each genotype would have to be evaluated and because single mutations can affect the biological behavior of yellow fever virus.

Pathogenesis

Yellow fever virus has been found to be both viscerotropic, referring to the ability to cause viremia and to infect and damage liver, spleen, heart, and kidneys, and neurotropic, referring to the ability to infect the brain parenchyma and cause encephalitis. Wild-type yellow fever virus strains are predominantly viscerotropic in primates, including humans. With susceptible nonhuman primates, even if the virus is inoculated intracerebrally, death is from hepatitis rather than encephalitis. However, when a small amount of antibody is given to a monkey shortly before or at the same time as the virus is injected intracerebrally, the animal dies of encephalitis, presumably because the antibody is able to protect peripheral organs but not the brain.[225] This experiment reveals the essential duality of wild-type yellow fever virus (viscerotropism and neurotropism).

Antibody and (presumably) cellular responses occur coincident with the clinical crisis (period of intoxication), and both free virions and hemagglutinating, complement-fixing, or immune precipitating antigen, likely NS1, may be found in blood together with antibody at this time, suggesting that immune clearance of infected cells, associated with release of cytokines, might play a role in the pathogenesis of capillary

TABLE 63.2 Age and Sex Distribution of Yellow Fever Cases for Selected Epidemics in Africa, 1926–95[a]

Epidemic		Age Distribution					
Year	Country	Cases in Children (Age Range [y])	Total Cases	Children as Percentage of Total	Male/Female Ratio	Presumed Vectors (*Aedes* spp.)	Reference
1926–28	Ghana, Nigeria	32 (0–19)	122	26	2.3:1	*?Ae. aegypti*	Beeuwkes, 1936[209]
1940	Sudan (Nuba Mountains)	110 (0–19)	306	36	1.7:1	*Ae. aegypti, Ae. vittatus,* other sylvatic vectors	Kirk, 1941[212]
1965	Senegal (Diourbel)	86 (0–19)	89	97	—	*Ae. aegypti*	Brès et al, 1967[247]
1969	Nigeria (Jos Plateau area)	38 (0–19)	209	18	2.5:1	*Ae. luteocephalus*	Carey et al, 1972[238]
1969–1970	Ghana	99 (0–15)	164	60	—	*Ae. aegypti*	Addy et al, 1986[240]
1970	Nigeria (Okwoga District)	35 (0–19)	76	46	~ 1:1	*Ae. africanus*	Monath et al, 1973[239]
1977–1980	Ghana (Volta and Eastern Regions)	87 (0–15)	294	30	—	*Ae. aegypti*	Addy PA, Minami K, Agadzi VK, 1986[240]
1982	Côte d'Ivoire (Mbahiakro Subprefecture)	43 (0–15)	90	48	—	*Ae. aegypti*	Lhuillier et al, 1985[235]
1983	Burkina Faso (Manga and Fada N'Gourma regions)	40 (0–15)	45	89	~ 1:1	*Ae. furcifer*	Baudon et al, 1986[234] Roux et al, 1984[241]
1983	Ghana (Northern Region)	74 (0–15)	87	85	—	*Ae. aegypti*	World Health Organization, 1989[249]
1986	Nigeria (Oju LGA)	20 (0–19)	39	51	2:1	*Ae. africanus*	De Cock et al, 1988[244]
1987	Nigeria (Oyo State)	72 (0–19)	102	71	1.4:1	*Ae. aegypti*	Nasidi et al, 1989[163]
1987	Mali (Kati Cercle)	100 (0–15)	143	70	2.1:1	*Ae. furcifer, Ae. aegypti*	Kurz, 1988[248]
1990	Cameroon (Extreme North Province)	91 (0–9)	182	73	—	*Ae. aegypti*	Vicens et al, 1993[242]
1991	Nigeria	1209 (0–15)	2229	54	1.1:1	*Ae. aegypti*	WHO, 1993[245]
1992–1993	Kenya (Baringo and Elgeyo Marakwet Districts)	18 (0–19)	54	33	1.8:1	*Ae. africanus*	Sanders et al, 1998[236] WHO, 1993[246]
1993–1994	Ghana (Upper West Region)	47 (0–15)	69	68	2:1	Not known	WHO, 1994[250]
1994	Nigeria (Imo State)	37 (0–15)	116	32	1.3:1	*?Ae. africanus*	WHO, 1995[237]
1995	Senegal (Koungeheul District)	58 (0–19)	110	53	1.4:1	*Ae. aegypti; Ae. furcifer; Ae. metallicua,* and *Ae. luteocephalus*	Thonnon et al, 1998[251]

[a]Table includes data from outbreaks that have clearly documented the age and sex of yellow fever disease cases. For more recent outbreaks (e.g.., Uganda, Sudan, Ethiopia, Angola, and Democratic Republic of Congo), these data are not available.
WHO, World Health Organization.

leak and shock.[273,274] ter Meulen and colleagues found a pattern of elevated pro- and antiinflammatory cytokines (interleukin [IL]-6, tumor necrosis factor [TNF]-α, monocyte chemoattractant protein [MCP]-1, IL-1–receptor antagonist, IL-10), resembling bacterial sepsis in patients with fatal yellow fever, whereas patients with nonfatal yellow fever and without hemorrhagic diathesis only had antiinflammatory cytokine elevations.[275] Similar findings have been reported in cases of viscerotropic disease associated with 17D vaccine. Overall, these findings suggest that a cytokine storm, and in particular the vasoplegic response to the exuberant immune response, can contribute to lethality.

The cell types infected in vivo in viscerotropic infections are only partially defined. In the principal target organ, liver, Kupffer cells and hepatocytes are targets for replication. Human dendritic cells have been investigated in vitro following infection with 17D virus. A recent study that compared histopathologic features and immunohistochemical staining on tissues from persons who died following either wild-type yellow fever virus infection or viscerotropic disease secondary to 17DD vaccination found similar histopathology and immunohistochemical staining of hepatocytes and Kupffer cells for both conditions.[276] However, with viscerotropic disease cases, there was also staining of mesenchymal calls in the liver as well as other tissues, suggesting mesenchymal cell staining is unique and pathogenomic for viscerotropic disease caused by 17DD virus.

In rodents, yellow fever virus is principally neurotropic, and the only extraneural organ showing significant virus replication is the adrenal gland.[271] Mice develop encephalitis only after intracerebral, intraocular, or intranasal inoculation. The time to death is typically 7 to 10 days, depending on strain and brain passage history (neuroadaptation). Mice infected by the peripheral route develop encephalitis if the blood-brain barrier is not completely developed (as in suckling mice up to 5 to 7 days of age) or is compromised by sham intracerebral inoculation. In general, yellow fever virus is not neurotropic in humans. However, similar to what is seen with young mice, human infants are at increased risk of developing neurotropic disease following vaccination with yellow fever vaccine virus. Cases of encephalitis in young infants who acquired yellow fever 17D vaccine infection orally via breast milk from recently vaccinated lactating mothers emphasizes the susceptibility of very young infants to neurologic infection.[203–205] The pathway whereby virus invades the brain after parenteral inoculation is not established but is likely to be via the bloodstream, whereas in the case of exposure of infants through breastfeeding, the route is less clear. It could be via the olfactory apparatus or a mucosal route to the bloodstream. In experimental studies, monkeys were infected by virus inserted in the stomach via a catheter.[199] The basis for age-related susceptibility is uncertain. The blood–brain barrier is incomplete and matures during the first year of life. Zisman and colleagues suggested that development of resistance with age was related to maturation of macrophages involved in yellow fever virus clearance.[277]

The pathophysiology of yellow fever in rhesus monkeys and humans is characterized by liver dysfunction, kidney failure, coagulopathy, and shock[273,278,279]; monkeys develop a more-fulminating illness than humans, lasting only 3 to 4 days. Higher viral doses shorten the incubation period but do not alter the duration or outcome of illness,[280] implying that innate immune responses (e.g., IFN, cytokines, and natural killer [NK] cells) are downregulated and insufficient to clear even a minimal infection. Flaviviruses, including yellow fever virus, have multiple virus-specified mechanisms for evading innate immunity, including blocking of the IFN-induced JAK (Janus kinase)/STAT (signal transducer and activator of transcription) signaling pathway.[281] In the hamster model of viscerotropic yellow fever, proinflammatory cytokines (IFN-γ, IL-2, and TNF-α) were significantly reduced and the regulatory cytokine IL-10 significantly increased in the liver during the critical stage of virus replication, indicating a state of functional immune impairment.[282] The virus is rapidly internalized and cells are activated and serve to present antigens even though replication may be restricted.[118] Human vascular endothelial cells are susceptible to infection in vitro, with release of RANTES and IL-6, suggesting that endothelial infection and the resulting inflammatory response could mediate vascular leak in vivo.[283]

The course of infection following subcutaneous inoculation of wild-type yellow fever virus in the monkey model has not been investigated, but in the initial stage of infection it is likely to follow the same course as described for the 17D vaccine strain, which multiplies in skin at the site of inoculation and spreads to draining lymph nodes, then central lymph nodes, and subsequently to visceral organs.[284] After intraperitoneal inoculation of rhesus monkeys with wild-type virus, Kupffer cells in the liver are infected first (24 hours after inoculation). Virus is then detectable on day 2 in serum and kidney and on day 3 in bone marrow, spleen, and lymph nodes.[273] Early injury to Kupffer cells is also noted after subcutaneous infection of monkeys.[279,285] Infection and degeneration of hepatocytes is a relatively late event, occurring in the last 24 to 48 hours before death in the monkey and in the last phase of infection in humans. In fatal human cases, a median of 75% (range, 5% to 100%) of hepatocytes undergo coagulative necrosis.[286]

Infection with yellow fever virus induces pathologic changes in several organs, including liver, kidney, heart, and lymphoid tissues. The outcomes are a result of direct viral cytopathic effect, the necrosis and apoptosis of hepatocyte cells in the midzone, accompanied by minimal inflammatory response as well as low-flow hypoxia and cytokine overproduction. Quaresma and colleagues[287] review these events. Yellow fever virus infections typically affected the midzone of the liver lobule, with sparing of cells bordering the central vein and portal tracts. The reason for this peculiar distribution of hepatic injury is unknown. Midzonal necrosis has been described in low-flow hypoxia, owing to adenosine triphosphate depletion and oxidative stress of marginally oxygenated cells at the border between anoxic and normoxic cells,[288] and a similar mechanism might contribute to injury in yellow fever infection. However, yellow fever virus antigen and RNA have been observed principally in hepatocytes in the midzone,[289,290] suggesting a predilection of these cells to virus replication.

Injury to hepatocytes is characterized by eosinophilic degeneration with condensed nuclear chromatin (Councilman bodies), indicating apoptosis, rather than by ballooning and rarefaction necrosis seen in virus hepatitis and Labrea hepatitis.[291] Apoptosis has been confirmed in liver tissue of fatal human cases of dengue hemorrhagic fever, in dengue and yellow fever–induced cell death of human hepatocyte cells in culture, and in an immunocytochemical study of liver samples from fatal human cases of yellow fever showing staining for Fas ligand on cells in the hepatic midzone.[292–295] The apoptotic mode of cell death, rather than ballooning necrosis, might explain the minimal inflammation, principally characterized as $CD4^+$ T cells,[296] in hepatic tissue affected by yellow fever. Quaresma and colleagues showed that the midzonal cellular response associated with apoptosis included macrophages, NK cells, and $CD4^+$, $CD8^+$, $CD68^+$ Kupffer, and $CD20^+$ cells.[289,292,297] The portal tracts also showed evidence of immune activation. Although the local inflammatory responses were modest, they reflect the sequential involvement of innate and adaptive immune activation mechanisms related to yellow fever virus infection of the hepatic target tissue. Other hepatic

changes associated with yellow fever virus infection of the liver include microvesicular fat and ceroid or lipofuscin deposits and intranuclear (Torres) bodies. Because little inflammation occurs, the reticulin framework is preserved and complete healing occurs without residual fibrosis.

Kidney pathology is also characterized by eosinophilic degeneration and fatty change of renal tubular epithelium without inflammation. These changes might represent late-stage injury following shock. In monkeys, oliguria with maintenance of tubular function indicated prerenal failure associated with hypotension and the hepatorenal syndrome; acute tubular necrosis was a terminal event.[279] Yellow fever virus antigen was found in renal tubular cells of fatal human cases, suggesting that direct viral injury is responsible.[298] Glomerular lesions (Schiff-positive changes in basement membrane and degeneration of cells lining the Bowman capsule), and yellow fever antigen in glomerulae 2 to 3 days after infection have been observed in monkeys, implying that direct viral injury accounts for albuminuria observed in advance of kidney failure (Monath TP, unpublished observation, 1980).[299] The reports of finding yellow fever 17D virus (genomes) in the urine of 44% of vaccinated subjects, with samples taken as late as 25 days after vaccination, suggests that the kidney is a site of replication in humans, because viremia is generally cleared by the second week after vaccination.[300] However, in monkeys, 17D genomes were not detected in kidney tissue after 17D vaccination, but lymph tissues remained positive after clearance of virus from blood by antibody.[284] Thus, it is possible that shedding of genomes in urine reflects renal clearance of virus from other organs. Other flaviviruses, including dengue and members of the Japanese encephalitis complex, have also been found in urine.

Direct virus injury to myocardial fibers, which show cloudy swelling and fatty changes and viral antigen, can contribute to shock.[298,301,302] DIC and decreased synthesis of vitamin K–dependent coagulation factors by the liver contribute to the bleeding diathesis.[278] Platelet dysfunction, demonstrated by collagen and adenosine diphosphate–stimulated aggregation, has been reported in the monkey model (Fisher-Hoch S, et al., unpublished observations, 1985). Patients dying of yellow fever show cerebral edema at autopsy, probably the result of microvascular dysfunction.

Lymphocytic elements in the germinal centers of spleen, lymph nodes, tonsils, and Peyer patches are depleted, and large mononuclear or histiocytic cells accumulate in the splenic follicles.[279,286] Necrosis of germinal centers is more striking in monkeys than in humans. It is unknown to what extent the lymphoid injury is the direct result of virus replication.

Immune Response to Natural Infection

Most of our knowledge about the innate and adaptive immune response to yellow fever virus infection is derived from studies of patients receiving the live 17D vaccine (see "Immune Responses to Vaccination" later). However, what is known about the kinetics of the antibody response following wild-type infection is reviewed here.

The humoral response to wild-type yellow fever virus is characterized by the appearance of immunoglobulin (Ig) M antibodies, typically detected either with an enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence assay (IFA), during the first week of illness.[234,303,304] IgM levels peak during the second week after onset, and decline rapidly over 30 to 60 days. IgM antibodies have been found to persist for prolonged periods after 17D vaccination, and they might also persist following wild-type yellow fever, but this has not been thoroughly evaluated. The magnitude of the IgM response in cases of primary yellow fever infection is significantly greater than in patients with prior flavivirus exposure, in whom the ratio of IgM to IgG is low. Antibodies with biological activity (goose erythrocyte hemagglutination-inhibition and neutralization) appear rapidly, typically by the fifth day of illness.[17,305,306] However, hemagglutination-inhibiting (HI) and neutralizing antibody responses are not linked in all cases, reflecting different HI and neutralizing antigenic determinants on the viral envelope.[307] HI antibodies recognize antigen preparations of the virus and prevent the antigens from causing agglutination of erythrocytes, a common feature of many viruses. HI antibodies peak between 30 and 60 days after infection, and a significant decline in titer occurs during the succeeding 6 months. Neutralizing antibodies are able to neutralize live virus and prevent the virus from either infecting young mice or susceptible cell cultures. These antibodies persist for many years, if not lifelong, after natural yellow fever virus infection, and they provide complete protection against disease on reexposure to the virus. Neutralizing antibodies have been documented as long as 78 years after illness.[308,309] No documented case of a second clinical yellow fever infection has been reported.

Complement-fixing (CF) antibodies appear during the second week after onset, rise during the convalescent period, and decline between 4 and 12 months after onset. IgM and neutralizing antibody responses following 17D vaccine are generally similar (see "Immune Responses to Vaccination" later), except that CF antibodies are absent in flavivirus-naïve subjects. Antibody responses following primary yellow fever are specific for yellow fever antigen. With affinity maturation, specificity declines, and cross reactions with related flaviviruses appear during the second week after onset.[305] Patients with prior heterologous flavivirus immunity develop broadly cross-reactive antibody responses. The specificity of the immune response differs with the test used: the hemagglutination-inhibition test is the least specific, CF is intermediate, and neutralization is the most specific. The IgM antibody-capture ELISA is specific in cases of primary infection, but cross reactions develop over time and in cases with prior flavivirus experience. Because of the lower sensitivity of the IFA, IgM antibodies are specific in patients with secondary infections.[304]

Protein and Epitope Specificity and Functionality of Antibody Responses to Yellow Fever

The E protein of yellow fever viruses (and other flaviviruses) contains antigenic determinants responsible for neutralization and cytotoxic T-cell responses, and thus it plays the principal role in protective immunity.[130] This was demonstrated by active immunization of mice with recombinant vaccinia virus expressing the E protein[310] and by passive immunization of mice with anti-E monoclonal antibodies.[311,312] These studies demonstrate the role of neutralizing antibodies as a mediator of protective immunity against yellow fever.

Yellow fever–neutralizing epitopes are conformational (composed of discontinuous peptide sequences) or contiguous.[85,90,136] Yellow fever virus contains multiple neutralizing determinants, some of which are conserved across strains.[90,134,135] Three type-specific neutralization epitopes mapped at the genomic level by sequencing escape mutants are found at E-71/72 in domain II (recognized by yellow fever type-specific MAb 2E10), E155 in domain I (recognized by yellow fever type-specific MAb B39), and E305/325 in domain III (recognized by yellow fever substrain-specific MAb 864). Other epitopes show cross-reactive neutralizing activity, and some of these are strongly neutralizing (e.g., an epitope in the fusion loop of the E protein that blocks virus internalization).[313] (See Vaughan and colleagues for an inventory of

epitopes.[314]) The composite polyclonal immune response is therefore capable of protecting against multiple strains of virus that might differ at one or several, but not all, epitopes. This antigenic structure probably underlies the efficacy of vaccination with a single yellow fever strain (17D) against wild-type strains of yellow fever virus.

Neutralization is a multihit phenomenon, and the number of antibody molecules required to neutralize the virus depends on accessibility of the epitope (in general, epitopes on the external surface of domain III are strong neutralizers, and epitopes on domains I and II are less potent) and affinity of the antibody. The most potent neutralizing antibodies have the lowest occupancy requirements for neutralization.[315] The importance of accessibility of epitopes on the complex array of 180 E protein molecules on the intact virion surface has been demonstrated in a number of elegant studies with dengue and West Nile viruses.[315,316] In a study of the fine-specificity of the antibody response to yellow fever 17D, a high proportion of neutralizing activity was associated with antibodies directed to complex quaternary epitopes on the virion surface. Interestingly, domain III-specific antibodies (presumed to have the highest neutralizing activity) as well as broadly flavivirus cross-reactive antibodies were absent or present at very low titers.[317] Similar importance of quaternary epitopes in dengue virus neutralization has been emphasized.

Neutralization occurs both as an extracellular event (in which antibody bound to virus interferes with virion attachment to cell receptors) and intracellularly.[315] Intracellular neutralization is mediated by antibodies inhibiting acid-dependent fusion of virions to the endosomal membrane, thus preventing release of viral RNA into the cytoplasm.[313,315,318] The importance of the Fc domain of neutralizing antibody was demonstrated by comparing the in vitro and in vivo activities of $F(ab')_2$ fragments of monoclonal antibodies.[319] $F(ab')_2$ fragments neutralized strongly in vitro but did not protect against yellow fever virus infection, whereas the full IgG molecule was protective. Presumably, the mediator of protection is complement or Fc-mediated antibody-dependent cell-mediated cytotoxicity, but alteration in reactivity of the hypervariable domain with antigenic determinants could also be involved. The prM proteins of many flaviviruses have been shown to contain epitopes with weak neutralizing activity, but to date such epitopes have not been characterized for yellow fever virus.

The humoral immune responses to nonstructural yellow fever proteins have not been extensively studied in human infections, but their role has been partially explored experimentally. Antibody responses to natural infections with dengue, Japanese encephalitis, and West Nile virus nonstructural proteins NS1, NS3, and NS5 have been described.[320] These antibodies distinguish infection immunity from immunity to inactivated vaccines.[321] Antibody responses to natural infections analyzed by Western blot show that anti-NS1 antibodies are type-specific and thus have value in diagnosing infection with cross-reactive flaviviruses.[322] The NS1 protein is present in the cytoplasm and on the surface of infected cells, is secreted from infected cells, and contains determinants that elicit CF antibodies. MAb analysis of NS1 revealed the presence of both type-specific and cross-reactive epitopes, which are principally conformation-dependent.[323]

Mice and monkeys actively immunized with native or recombinant NS1 were protected against lethal yellow fever in the absence of neutralizing antibody,[324–326] the principal correlate of protection being complement-mediated cytolytic antibodies. Passive transfer of MAbs against NS1 having high complement fixation activity, but not antibodies with low or no complement fixation activity, afforded protection.[327] These studies suggested that antibodies recognizing cell membrane–bound NS1 might promote viral clearance by sensitizing infected cells to C1q-mediated cytolysis. An intact Fc portion of the immunoglobulin molecule is required for protection by anti-NS1, and antibody isotype-dependent differences in protective activities have been demonstrated.[319,327,328]

Natural infection with yellow fever virus is associated with the presence of soluble CF antigen (NS1) in blood during the acute phase of infection[273] and is followed by strong CF antibody responses (presumably directed against NS1). Detection of NS1 antigen early in the course of illness has emerged as an important diagnostic test for dengue, and it is likely that such a test would have value in yellow fever (in particular, for primary infections). Because CF antibody responses occur late after infection, and vaccination with 17D virus does not induce a CF antibody response,[329,330] it is uncertain to what extent complement-dependent anti-NS1 antibodies play a role in vivo in recovery or protection. Further studies in humans are needed to define the kinetics of the anti-NS1 response after natural infection.

Cross Protection

Previous infection with some flaviviruses might ameliorate the clinical severity of yellow fever though the evidence is conflicting. Observational studies going back to 1815 suggested that flavivirus immunity (e.g., to dengue) was the basis for resistance to yellow fever in long-term residents of endemic areas,[331–333] and it was later proposed as a barrier to introduction of yellow fever into Asia.[334–336] Early experiments indicated that passive transfer of dengue antibodies did not protect monkeys against challenge with yellow fever virus.[332] In contrast, monkeys *actively* immunized with dengue were relatively resistant to challenge with yellow fever virus, suggesting that cellular immunity played a role in cross protection.[337] Monkeys actively immunized with two African flaviviruses (Zika and Wesselsbron), but not with West Nile or Banzi viruses, resisted challenge with yellow fever virus.[338] In The Gambia (1978–79), the infection-to-illness (jaundice) ratio was higher in humans with prior flavivirus exposure.[252] However, in Nigeria (Oju Region, 1986), jaundice rates were not significantly different for patients with primary yellow fever virus infection (14/84 [17%]) and superinfections (13/53 [25%]) (odds ratio, 0.62; 95% confidence interval [CI], 0.24–1.56) (De Cock KM and Monath TP, unpublished data, 1987). The discrepancy could reflect differences in the specific heterologous flaviviruses responsible for prior immunity.

In retrospective studies, sera from dengue-immune subjects who had not been vaccinated against yellow fever neutralized yellow fever virus[339,340] (this finding needs to be confirmed in prospective analyses of seronegative patients who sustain dengue infections). A retrospective analysis of an outbreak of yellow fever among military recruits in Ecuador demonstrated that prior immunity to dengue type 2 significantly reduced the clinical severity of yellow fever and protected against death from the infection.[341]

Confirmation of the protective activity of dengue immunity is a matter of considerable importance because dengue occurs in the yellow fever–endemic regions in the Americas and Africa. In a limited retrospective analysis, yellow fever immunization did not provide cross protection of humans against West Nile fever.[342] The hypothetical suggestion has been made that prior yellow fever immunization could enhance dengue infections, leading to an increased incidence of dengue hemorrhagic fever and shock syndrome,[343] but there is no evidence to substantiate this suggestion. Similarly, prior dengue immunity could theoretically diminish the response to live attenuated yellow fever vaccine. This is discussed later

in "Heterologous Flavivirus Immunity" in the section "Results of Vaccination."

Antibody-Dependent Enhancement

Immune enhancement is believed to play a role in the pathogenesis of dengue hemorrhagic fever in patients with nonneutralizing antibodies from a previous dengue infection who are infected with a new dengue serotype. Although antibody-dependent enhancement of flavivirus replication in monocyte/macrophages has been demonstrated in vitro for a number of flaviviruses, including yellow fever,[344–347] the evidence for in vivo enhancement mediated by yellow fever antibodies is mixed. In one study, human volunteers previously immunized with yellow fever 17D had increased immune responses to live dengue-2 vaccine, possibly owing to antibody-mediated enhancement of dengue virus replication[348,349] or to rapid expansion of group-reactive memory T- and B-cell clones. An intriguing observation in a study of a live chimeric yellow fever and Japanese encephalitis vaccine in yellow fever–immune and –nonimmune subjects was a tendency toward higher viremias in the immune subjects, suggesting the possibility of immune enhancement.[193] However, the effect was slight, and the sample size was small. The chimeric virus system provides an ideal setting to test the immune enhancement hypothesis in humans.

Diagnosis

The preliminary diagnosis of yellow fever is based on the patient's clinical features, yellow fever vaccination status, and history of residence or recent travel to a yellow fever–endemic zone. Mild yellow fever cannot be distinguished clinically from a range of other infections and is unlikely to be recognized as yellow fever. Cases of yellow fever with jaundice must be differentiated from viral hepatitis, malaria, leptospirosis, louseborne relapsing fever (*Borrelia recurrentis*), Congo-Crimean hemorrhagic fever, Rift Valley fever, typhoid, Q fever, and typhus, as well as surgical drug-induced, and toxic causes of jaundice.[350] It also needs to be differentiated from other viral hemorrhagic fevers, which usually manifest without jaundice, include dengue hemorrhagic fever, Lassa fever, Marburg and Ebola virus diseases, and Bolivian, Argentinean, and Venezuelan hemorrhagic fevers.[351]

Specific laboratory diagnosis is made by detection of virus, viral RNA, or viral antigen in blood or by serology. Virus is readily isolated from blood during the first 4 days after onset, but isolations as late as 12 days or longer are recorded.[352] Of 90 cases confirmed during a yellow fever outbreak in Côte d'Ivoire in 1982, 27 (30%) were diagnosed by virus isolation from blood; all but three virus-positive patients were in the preicteric stage.[235] The virus can also be recovered from postmortem liver tissue.

Virus isolation was traditionally accomplished by intracerebral inoculation of suckling mice or intrathoracic inoculation of *Toxorhynchites* mosquitoes. Suckling mice develop encephalitis 7 to 14 days after inoculation, depending on the infectious dose; a virus-specific diagnosis may be made by immunofluorescence on impression slides of brain tissue or by serologic methods. Viral antigen is detected by immunofluorescence in head-squash preparations of inoculated mosquitoes after an incubation period of 10 to 12 days. Currently, cell culture is most often used to isolate the virus and is more sensitive than previous techniques.[353] *Aedes pseudoscutellaris* (AP61) cells are more sensitive than other in vitro methods for primary isolation of yellow fever virus[353,354] and show cytopathic effects within 5 to 7 days after inoculation and viral antigen detectable by immunofluorescence in advance of cytopathic effects (e.g., day 3 after inoculation). *Ae. albopictus* (C6/36) cells, *Toxorhynchites amboinensis* cells, and mammalian cells (e.g., Vero, SW13) may be used, particularly if combined with reverse-transcription polymerase chain reaction (RT-PCR) or detection of viral antigen.[355]

RT-PCR is now more commonly used than virus isolation to diagnose yellow fever disease in the acute period owing to its rapid turnaround time and the increased sensitivity it provides over viral isolation.[356] Sera that were negative by virus isolation can contain viral RNA detectable by RT-PCR.[357] A seminested polymerase chain reaction (PCR) analysis used to detect viremia in serum following 17D vaccination serum samples was sensitive enough to detect 1.15 PFU/mL.[358] Although RT-PCR testing has traditionally been available only in more advanced laboratories, recent work to develop more field-ready RT-PCR, more specifically isothermal protocol based on helicase-dependent amplification technology (RT-tHDA), has shown some promise to use this technology in more resource limited settings.[359] RT-PCR has been used to detect wild-type yellow fever viral genome and 17D viral genome in serum and human tissues (liver, spleen)[360–363] and a variety of tissues from nonhuman primates infected with 17D virus.[284] RT-PCR and sequencing is the preferred method for distinguishing wild-type yellow fever from a case of vaccine-associated viscerotropic disease. Using different primer sets along the length of the virus, RT-PCR can be used to sequence the infecting virus and thus help determine the origin of the virus and differentiate between natural infection and possible vaccine virus in the case of serious adverse events that occur in endemic areas. Although RT-PCR has been used to characterize the viremia seen following vaccination, only a limited number of yellow fever cases have been evaluated with RT-PCR. A recent quality assessment of laboratories performing RT-PCR testing for yellow fever viral RNA did suggest problems with sensitivity for wild-type infection with 38% (12/32) of laboratories not detecting wild-type yellow fever virus strains.[364] There was also problem with specificity as 78% (25/32) of laboratories had false-positive RT-PCR result for yellow fever. These data suggest noticeable variability between laboratories performing RT-PCR testing and emphasizes the need for confirmatory testing at reference laboratories.

Rapid, early diagnosis is also possible by measurement of yellow fever viral antigen in serum by immunoassay.[353,365] The sensitivity of the assay for detection of virus in serum is significantly lower than with RT-PCR, approximately 3 $\log_{10}$ PFU/0.1 mL. Under field conditions, antigen-detection ELISA had a sensitivity of 69% and specificity of 100% compared to virus isolation in AP61 cell culture.

Ideally, diagnostic samples should be kept cold or kept frozen on dry ice or liquid nitrogen to maintain stability of infectious virus and RNA. However, a suitable alternative may be to dry the sample on filter paper discs, a procedure commonly used for antibody samples. When maintained at room temperature, yellow fever virus infectivity and RNA was still detectable by culture and RT-PCR after 90 days.[366] Samples for RT-PCR and ELISA do not need to be handled in a way that preserves infectivity; specimens may also be intentionally inactivated as a safety precaution where the diagnosis of dangerous pathogens (e.g., other viral hemorrhagic fevers) is considered.

Identification of yellow fever virus has been accomplished by a highly sensitive microarray amplification and hybridization method as well as NGS.[367,368] Using microarrays with multiple probes or NGS could be useful for differential diagnosis, particularly when the etiology is unclear or their might be several pathogens cocirculating in an area at one time.

Examination of liver reveals the typical pathoanatomic features of yellow fever, including midzone necrosis.[369,370] *Liver*

biopsy should never be performed during the illness, because fatal hemorrhage can ensue. Histopathologic diagnosis may be difficult in patients who die after the second week of illness. Electron microscopy can reveal typical flavivirus particles in intracellular vacuoles.[371] Definitive postmortem diagnosis may be made by immunocytochemical staining for yellow fever viral antigen in liver, heart, or kidney,[290,298,372–374] even in specimens stored for years at ambient temperature. The distribution of virus in the liver is midzonal, suggesting that hepatocytes bordering the central veins and portal tracts undergo less-active virus replication. Viral genome may also be detected in formalin-fixed embedded tissues by hybridization.[290] RT-PCR can be used on RNA extracted from fixed tissues[375] to obtain yellow fever virus sequences from fatal cases as well as historical materials for epidemiologic and evolutionary studies; however, the quality of the formalin-fixation is critical.

Although older methods for serologic diagnosis (hemagglutination-inhibition and complement fixation) are useful, they have been replaced by the IgM-capture ELISA and newer IFAs.[376,377] The presence of IgM antibodies in a single sample provides a presumptive diagnosis, and confirmation is made using a plaque-reduction neutralization test to document a rise in virus-specific titers between paired acute and convalescent samples or a fall in titer between early and late convalescent samples. In general, the sensitivity of yellow fever IgM assays that are currently used is high for wild-type infection (though the sensitivity is lower for IgM formed following vaccination).[364] The specificity of the IgM ELISA is also high in primary infections and in many cases of secondary infection.[303] However, cross reactions complicate the diagnosis of yellow fever by all serologic methods, particularly in Africa, where multiple flaviviruses cocirculate. In a study conducted in Colombia, roughly 40% of both persons infected with wild-type yellow fever virus and those who received 17D vaccine were found to be dengue IgM–positive.[339] Furthermore, the phenomenon of "original antigenic sin" complicates flavivirus serologic investigations. Patients who have had prior heterologous infection and who develop yellow fever can have higher responses to the original virus, and those who have had prior yellow fever virus infection or vaccination and who are subsequently infected with another flavivirus can have higher responses to yellow fever.[342,378]

Detection of NS1 antigen in serum during the acute phase of illness is an important new method that has been successfully applied to the diagnosis of primary infections with dengue viruses.[379] However, antibodies to individual nonstructural proteins have not been investigated for the diagnosis of yellow fever but it should be evaluated with a high priority because it can be used as a bedside test-strip method.

An important diagnostic issue, in situations where yellow fever 17D vaccine is used in areas endemic for yellow fever, is the differentiation of yellow fever vaccine–associated viscerotropic disease (YEL-AVD) from wild-type yellow fever, because these conditions cannot be distinguished clinically. A case in point is the identification of a case of YEL-AVD during a retrospective study of genomic sequences of (supposedly) wild-type yellow fever strains.[380] The differential diagnosis can be made only by genomic sequencing of virus isolate or RT-PCR amplicon, or by MAb analysis of an isolate.[163]

Treatment

Although multiple drugs have been evaluated or used empirically to treat yellow fever disease, to date, none has demonstrated specific benefit.[381,382] Management is supportive and based on symptoms and the organ systems involved.

Supportive Care

In 1984, an expert panel recommended measures to improve the care and management of patients with yellow fever.[210] The recommendations included maintenance of nutrition and prevention of hypoglycemia; nasogastric suction to prevent gastric distention and aspiration; treatment of hypotension by fluid replacement and, if necessary, vasoactive drugs; administration of oxygen; correction of metabolic acidosis; treatment of bleeding with fresh-frozen plasma; dialysis if indicated by renal failure; and treatment of secondary infections with antibiotics. Use of heparin to reverse DIC is reserved for patients with documented consumption of clotting factors and activation of fibrinolytic mechanisms.

The final stage of yellow fever disease has features resembling a cytokine storm or septic shock.[279] Stress-dose corticosteroids (200 to 300 mg/day) have been shown to have therapeutic benefit in patients with septic shock, at least for those with adrenal insufficiency,[383] and appear to have also benefited patients with YEL-AVD.[384] Corticosteroids are indicated for treatment of selected patients with acute disseminated encephalomyelitis (ADEM) associated with yellow fever vaccine. There are no experimental or clinical data on other treatment approaches, such as monoclonal antibodies against cytokines or hemofiltration, for late-stage yellow fever disease. Finally, orthotropic liver transplantation theoretically might be considered as a clinical intervention in fulminant hepatitis caused by yellow fever virus as apoptotic cell death is central to the pathogenesis of yellow fever. However, the feasibility of transplantation should be explored first with a monkey model and even if successful, would have limited utility for the majority of yellow fever cases which often occur in locations where transplantation is not available.

Therapeutic Considerations

Treatment by administration of immune serum or by cross-circulation from an immune donor animal after clinical onset of yellow fever has minimal therapeutic effect.[381] One patient with YEL-AVD was treated with intravenous immunoglobulin (IVIg) during the acute illness and failed to survive suggesting no benefit of the treatment.[265] Furthermore, treatment with a mouse monoclonal neutralizing antibody that was given as a last resort to a yellow fever patient in late-stage hepatorenal failure did not provide any beneficial effect.[385] However, a human/mouse chimeric IgG monoclonal (but not IgM) was shown to protect IFN knockout mice when given before or up to 48 hours after lethal challenge with 17D virus.[386] Based on these available data, it is unlikely that antibody would be useful except in the setting of postexposure prophylaxis when given before onset of clinical disease or as treatment given during the earliest stages of illness or potentially to an immunocompromised individual.[386,387]

There are several reports in the literature on treatment of flavivirus infections of humans (dengue, Japanese encephalitis, and St. Louis encephalitis viruses) with IFN-α claiming improvement or reduced mortality, although the studies were small and not controlled.[388–390] IFNs have been investigated for the prevention and treatment of yellow fever in monkeys. Animals receiving 3 mg/kg of the IFN inducer polyinosinic:polycytidylic acid intravenously 8 hours before or 8 hours after viral challenge had low-level viremia and were significantly protected (71–75% survival) compared to untreated controls (0% survival), but those treated 24 hours after challenge were not protected.[391] IFN-γ administered to monkeys 24 hours in advance of challenge and at daily intervals for 4 days reduced yellow fever viremia and significantly delayed hepatic dysfunction and death.[392] IFN-γ knockout

C57BL/6J mice demonstrated deficient yellow fever viral clearance and reduced numbers of inflammatory cells after intracerebral inoculation when compared to normal C57BL/6J mice also undergoing intracerebral inoculation.[393] The requirement to administer IFNs very shortly before infection or during the incubation period likely suggests that it might not be a viable treatment option for wild-type yellow fever. In a hamster model of yellow fever, an adenovirus vector expressing IFN-α was found to be protective when administered intranasally 7 days before or up to 2 days after a lethal challenge.[394] This outcome appears to be similar to what can be achieved with exogenous IFN treatment.

Molecular targets for rational design and development of antiviral drugs against flaviviruses include both virus-specified functions (e.g., NS5 polymerase and methyltransferase)[395] and host cell enzymes.[396] This field is expanding owing to the interest in developing drugs against dengue and hepatitis C virus; a comprehensive review is not within the scope of this chapter. A partial list of compounds with antiviral activity against yellow fever in vitro, and in some cases in vivo, includes nucleosides and plant-derived alkaloids[397–403] that target inosine monophosphate dehydrogenase (ribavirin, EICAR [5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide], tiazofurin, selenazofurin, and mycophenolic acid), an adenosine inhibitor,[403] orotidine 5′-monophosphate decarboxylase (pyrazofurin and 6-azauridine), pyrazine carboxamine derivatives,[402] cytidine triphosphate synthetase (carbodine and cyclopentenyl cytosine), 2′-C-methylcytidine,[397] dihydrofolate reductase (methotrexate), and sulfated polymers (suramin, pentosan polysulfate, dextran sulfate, and PAVAS [sulfated copolymers of acrylic acid with vinyl alcohol]) that interfere with viral affinity for heparin sulfate on the cell surface.[400,404]

Ribavirin is active against yellow fever virus in vitro, and the mechanism of action appears to be inhibition of cellular inosine monophosphate dehydrogenase and the resulting depletion of intracellular guanosine triphosphate pools[405] but at concentrations that are too high (9–10 mg/mL) for safe and effective treatment in vivo. Monkeys treated with ribavirin were not significantly protected against a yellow fever virus challenge,[406] but dose and formulation might not have been optimized. In a hamster model of viscerotropic yellow fever, ribavirin dramatically reduced liver injury when administered as long as 5 days after infection (the time of peak liver injury in untreated controls).[407] The treatment effect may be explained by the high dose of ribavirin used in that study (80 mg/kg loading dose, followed by 40 mg/kg daily through day 7), which was nearly three times higher than that used in the treatment of human patients with hemorrhagic fevers (e.g., Lassa fever and Hantaan virus infection). If treatment is given earlier (e.g., initiated before or up to 2 days after challenge), a lower dose (50 mg/kg per day) was effective.[408] There are no clinical data to support safety of high doses of intravenous ribavirin that might be expected to ameliorate infection with yellow fever virus. Synergistic effects of ribavirin and related compounds, such as tiazofurin and selenazole, have been demonstrated in vitro but not investigated in vivo.

Interfering RNA targeting the E or NS1 genes has shown activity in yellow fever virus infections in vitro and in mice.[409] Protection was demonstrated in mice treated 12 hours before intracerebral virus challenge with short hairpin RNA-encoding plasmids, which were also injected intracerebrally; various controls showed that the effect was not a result of IFN induction. Formulation and delivery of RNA interference remains a challenge for development of interfering RNA. Other approaches to RNA therapeutics (e.g., RNA aptamers) have not been investigated in yellow fever.

EPIDEMIOLOGY

A detailed comprehension of the geography, seasonality, and incidence of yellow fever virus is critical to the proper use of yellow fever vaccine for both endemic and traveler populations. For more detail on risk factors for disease, see "Risk Factors for Developing Disease and Factors Impacting Disease Severity" under "Clinical Description" above.

Distribution of Yellow Fever Virus

Geographic Distribution

Yellow fever virus occurs in tropical South America and sub-Saharan Africa, where it is maintained in an enzootic transmission cycle involving tree-hole–breeding mosquitoes and nonhuman primates (see "Transmission Cycles" above for more details). The first map of endemic regions based on yellow fever serology surveys conducted in the 1930s[410,411] was prepared by the International Quarantine Commission and published by the United Nations Relief and Rehabilitation Administration (UNRRA) in 1946. This map has been modified from time to time based on new information and in 2008 there was a concerted effort by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) to homogenize yellow fever risk maps.[31,412–414] The current risk maps incorporated all available data to establish risk categories of endemic, transitional, low, and no risk (Fig. 63.4). However, much of the data used to determine areas of risk are out of date and in some areas based on scanty serologic data. Updated data, particularly in Africa, are needed to improve knowledge on yellow fever risk areas.

Seasonal Distribution and Climatic Factors Impacting Viral Activity

In South America, the incidence of yellow fever is highest during months of high rainfall, humidity, and temperature (January to May; peak incidence, February and March), corresponding to the activity of *Haemagogus* mosquitoes, which breed in tree holes and are thus dependent on rainwater. Human exposure during agricultural activities is also increased at this time of the year. In the savanna zone of West Africa, cases appear during the mid-rainy season (August) and peak during the early dry season (October), corresponding to the period of maximum longevity of sylvatic mosquito vectors.[415] The domiciliary vector *Ae. aegypti* breeds in receptacles used by humans for water storage and is thus less dependent on rainfall. Where this mosquito is involved in virus transmission, yellow fever can occur in the dry season in rural areas and in heavily settled urban areas. Thus, season is only a partially reliable guide to determining the risk of exposure and to making decisions on the need for immunization of travelers.

Fluctuations in rainfall profoundly affect the abundance of mosquito vectors and the potential for yellow fever epidemics.[150,158–160,416–418] The yellow fever outbreak in The Gambia in 1978 occurred after a period of two successive years of excess rainfall.[416] Similarly, excessive rainfall and prolongation of the rainy season reflected by vegetational indices in satellite images preceded the emergence of a sylvatic yellow fever epidemic in Nigeria in 1986.[418] In Brazil, excessive rainfall and abnormally high temperatures (a consequence of an El Niño event) occurred during December 1999 and the first quarter of 2000, coinciding with the emergence of a large jungle yellow fever outbreak.[419]

Temperature influences the transmission rates of several arboviruses.[420] The extrinsic incubation period of yellow fever

virus in the mosquito vector is very sensitive to temperature, and an increase of a few degrees can shorten the extrinsic incubation period by days, resulting in a significantly increased rate of transmission.[182,421] Even brief exposure to high temperatures (e.g., in a sunlit forest clearing) can have this effect. Warm temperature also increases biting and reproductive rates of *Ae. aegypti*.[422] Thus, long-term environmental change (global warming) might increase transmission rates of yellow fever virus.[423]

Incidence and Disease Occurrence

Endemic and Epidemic Areas

The last global disease estimate for the number of yellow fever cases and deaths was published by the WHO in the early 1990s. The WHO estimated that there were roughly 200,000 cases and 30,000 deaths occurring annually from yellow fever.[424] Since then, updated estimates of the disease burden in Africa have been published with an estimated 130,000 cases and 78,000 deaths occurring from yellow fever in 2013.[425] Overall, the 2013 estimates of disease were less than those from the 1990s, but the estimates of deaths were increased. The reduction in disease cases was attributed to the recent mass vaccination campaigns that have occurred predominantly in West Africa; the increase in deaths was likely the result of more precise estimates of disease occurrence and severity.[27,215,425,426] In the Americas, similar improvements in vaccination coverage have also occurred in recent years,[427] further suggesting that the global disease burden is less than previously estimated.

The disease burden estimates are substantially higher than the actual number of yellow fever disease cases and deaths reported from endemic regions on an annual basis. From 1995 through 2014, there has been a median of 273 yellow fever disease cases (range, 21 to 2794) and a median of 88 deaths (range, 12 to 287) reported annually (Table 63.3).[30,426,428–434] This notable difference between the estimated and reported disease burden is thought to be partly because of occurrence of the disease in remote areas, late recognition of outbreaks, and lack of diagnostic facilities. Where specific investigations have been undertaken, the ratio of actual cases to official notifications varies between 1 and 311 to 1 (Table 63.4).[163,214,236–240,244,247,249,252,435]

In Africa, there are 33 countries that are considered to have endemic transmission of yellow fever.[27] However, risk of

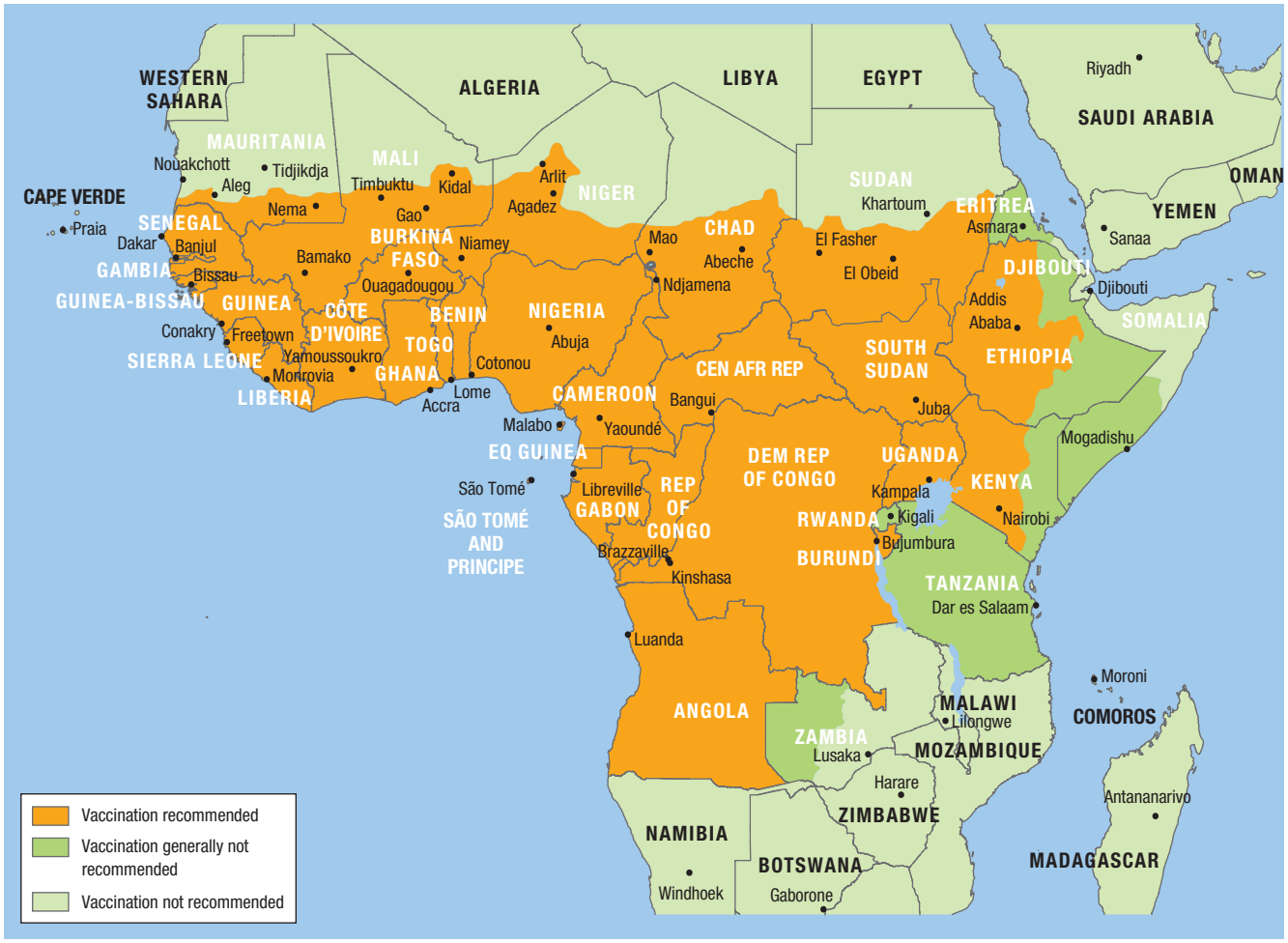


Figure 63.4. Regions of Africa and South America with yellow fever virus activity. Orange denotes areas considered to be endemic for yellow fever. Endemic areas have reported human disease regularly. The darker green denotes transitional areas; these areas have not reported human disease cases but have found antibodies against yellow fever virus and have the right vectors and nonhuman primate species to sustain virus transmission. Areas in light green are areas where there is no yellow fever virus transmission; some of these areas might be at risk for urban transmission as they have the right vectors to transmit the virus readily between humans. *(Modified from Centers for Disease Control and Prevention.* CDC Health Information for International Travel 2016. *New York: Oxford University Press; 2016.)*



Figure 63.4, cont'd

yellow fever disease varies among countries,[51,160] and often within a country, as many countries are not holoendemic.[412] The highest risk for disease occurs in West Africa where disease cases are reported on an annual basis (see Table 63.3).[436] Central and East African countries have a lower disease risk, often with no disease cases reported for several decades.[32a,51,160] For instance in Ethiopia, the first recognized outbreak of disease occurred in the early 1960s and no disease activity was reported again until 2013.[30] Another example is Angola, which experienced one of the largest disease outbreaks in recent history in 2015–16. Prior to this they last reported cases in 1988.[33] The differential risk of yellow fever, falling as one moves west to east and south in Africa, is thought to be potentially related to differences in the strains circulating in the region, in the frequency in which humans come into contact with sylvatic cycles, in population immunity, and in seasonal variations.[51,160]

From 1995 to 2014, there were 7714 cases reported from Africa countries. However, on an annual basis the number of cases reported varied between seven and 2677 cases (median, 165), suggesting inconsistency of reporting (Fig. 63.5 and see Table 63.3). During the years 1995 to 2014, there were 1062

TABLE 63.3 Cases of Yellow Fever Based on Official Reports to the World Health Organization, 1995–2014[a]

Country	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total
AFRICA																					
Benin		124	18	6		28															176
Burkina Faso			1	2	1	1	22		29	10	609		2	2							679
Cameroun									3	6	196	1	3	2	1	7	23		10		252
Central African Rep								1				1		5	4						11
Chad															1			139			140
Congo															1			1			2
Côte d'Ivoire			11			31	280	156	158	92	126	16		2	3		14				889
Dem Rep Congo										29		140					2		7	7	185
Ethiopia																			141		141
Gabon	16																				60
Gambia							2														2
Ghana		27	6				1	7	61	1	492	1					3				599
Guinea							651	172	20	60	6	263	1		25	9	9				1216
Guinee Bissau						1															1
Kenya	3																				64
Liberia	360		3	25		110	59		25	5	41			2	1						631
Mali										2	97	5	1								105
Mauritania																					21
Nigeria			7			22		20													49

Senegal	79	128					1	134	1	2	105					2	3					455
Sierra Leone	33	4							90		3			9			2					141
Sudan									222		605							847	48			1722
Togo							8				3	5										16
Uganda							5		5								273					283
Subtotal	*491*	*283*	*46*	*33*	*1*	*844*	*550*	*338*	*683*	*124*	*2677*	*28*	*11*	*47*	*20*	*20*	*318*	*987*	*206*	*7*	*7714*	
SOUTH AMERICA																						
Argentina														9								9
Bolivia	15	30	63	57	68	8	4	15	6	10	16	16	6	1		2	2	3				322
Brazil	4	15	3	34	76	85	41	15	64	5	3	2	13	46	42	2	2			1		453
Colombia	3	8	5	1	2	5	9	19	112	30	20	5	6	3	5				1			234
Ecuador	1	8	31	4	5	2											1					52
French Guiana				1																		1
Paraguay														28								28
Peru	499	86	44	165	58	6	28	35	26	61	66	63	23	15	8	18	13	11	22	13		1260
Venezuela	2			15	1		1	3	34	5	12											73
Subtotal	*524*	*147*	*146*	*277*	*210*	*106*	*83*	*87*	*242*	*111*	*117*	*86*	*48*	*102*	*55*	*22*	*18*	*14*	*23*	*14*	*2432*	
TOTAL	**1015**	**430**	**192**	**310**	**211**	**950**	**633**	**425**	**925**	**235**	**2794**	**114**	**59**	**149**	**75**	**42**	**336**	**1001**	**229**	**21**	**10,146**	

[a]Only countries reporting disease cases from 1995–2014 are listed in the table; consequently, several countries that previously reported yellow fever disease cases (e.g., Angola, Suriname) and which might be considered to be endemic for yellow fever virus activity are not listed.

Data from World Health Organization Yellow Fever Database (http://www.who.int/globalatlas/dataQuery) through 2004 and updated annual Weekly Epidemiological Record *of yellow fever disease in Africa and South America.*[30,426,428,429,431–434]

TABLE 63.4 Estimation of Number of Actual Cases Compared to Reported Cases During Yellow Fever Epidemics in Africa[a]

Epidemic Year	Country	Official Notification	Estimated by Direct Investigations	Ratio of Actual to Reported Cases	Reference
1960–62	Ethiopia	3,010	100,000	33:1	Serié et al, 1968[214]
1965	Senegal	243	2000–20,000	8–82:1	Brès et al, 1967[247]
1969	West Africa	322	>100,000	311:1	Carey et al, 1972[238]
1970	Nigeria	4	786	197:1	Monath et al, 1973[239]
1977–78	Ghana	713	2400	3:1	Addy et al, 1986[240]
1978–79	Gambia	30	8400	280:1	Monath et al, 1980[252]
1987	Mali	305	1500	5:1	World Health Organization, 1989[249]
1986	Nigeria	1289	9100	7:1	De Cock et al, 1988[244]
1987	Nigeria	2676	120,000	45:1	Nasidi et al, 1989[163]
1990	Cameroon	173	5000–20,000	29–116:1	World Health Organization, 1992[435]
1992–93	Kenya	54	55	1:1	Sanders et al, 1998[236]
1994	Nigeria (Orsu LGA)	120	775 in one community representing 22% of the total population	>6:1	World Health Organization, 1995[237]

[a]List includes outbreaks where an epidemiologic investigation was performed to estimate the actual number compared to the number of cases that were officially notified.

deaths reported from African countries, for a case-fatality ratio of 14%. The case-fatality rate in Africa is likely underestimated by official reports, because data are unavailable on the number of deaths. In addition, patients with severe yellow fever are often removed from hospitals by relatives, so that the outcome of disease is not recorded. The annual incidence varies by country and region, reflecting periodic expansions of yellow fever viral activity, but accuracy of reporting limits interpretation.

Major epidemics in Africa are a consequence of interhuman transmission by domestic *Ae. aegypti* and sylvatic *Aedes* spp. vectors present in high-density, high human populations, and low immunization coverage. During outbreaks of disease in Africa, it has been estimated through serosurveys that roughly 20% to 40% of a population will become infected with yellow fever.[163,214,237,241,242,244,247,252,437] In comparison, the burden of endemic yellow fever virus infection has been less-well characterized. In Nigeria (1970–71), a laboratory diagnosis of yellow fever was made in two of 205 patients (1%) hospitalized with jaundice in areas without epidemic activity.[438] Using data from serologic surveys in Nigeria and an estimated 7:1 infection-to-illness ratio, the annual incidence of overt infection with jaundice was estimated to be between 1.1 and 2.4 per 1000 population, and yellow fever deaths were estimated to be between 0.2 and 0.5 per 1000 population.[439] This estimate of endemic disease is likely lower as one moves eastward in Africa. Based on a serologic survey conducted in Central African Republic during 2009, the rate of disease with jaundice was 0.9 per 1000 population and death was 0.2 per 1000 population.[243] While indicating that endemic yellow fever may be a silent cause of significant morbidity, the incidence levels are 25- to 50-fold lower than those occurring during epidemics, and, thus, are below the threshold of detection by existing passive surveillance systems. It is likely that endemic yellow fever activity varies considerably from year to year and that it causes thousands of unrecorded deaths annually in West Africa. This provides a strong rationale for preventive immunization.

There are 13 countries in South and Central America that are considered endemic for yellow fever disease. Although only sporadic cases and small outbreaks are reported in these regions, nearly all major urban areas in the American tropics have been reinfested with *A. aegypti* and many persons living in these regions have not been vaccinated.[32b] Improvements in roads, increased settlement within the Amazon region, and more ready movements between these areas have led to more unvaccinated people moving in and out of endemic zones.

Between 1995 and 2014, 2432 cases (median, 95 cases; range, 14–542 cases/year) and 1164 deaths (case fatality ratio of 48%) were reported from South American countries (see Table 63.3 and Fig. 63.5). The annual incidence varies by country owing to fluctuating wandering epizootic activity that depends on herd immunity levels in monkey populations. The lower case numbers and incidence of yellow fever in South America than in Africa is a consequence of transmission by enzootic vectors (principally from monkey to human), low densities of vectors, susceptible monkeys and human hosts, and relatively high vaccination coverage. The higher case-fatality rate in South America probably reflects hospitalization of severe cases and surveillance based on death reports and the postmortem examination of livers rather than the South American virus strains being more virulent than those in Africa.[65]

Increased virus transmission, particularly in the endemic areas of South America, occurs with a periodicity of 7 to 10 years, in part because immunologically susceptible monkey populations are replenished at a slow rate after epizootics. Larger expansions of the disease into areas on the edge of the endemic region, such as Argentina, Paraguay, Trinidad, and coastal Brazil occur less frequently often at intervals of more than 30 to 40 years and was last seen from late 2016 to early 2017.[32,32b]

In South America, disease cases are reported routinely from Bolivia, Brazil, Colombia, and Peru. In comparison, Guyana, Panama, Suriname, and Trinidad have not reported cases for several decades. The last report of yellow fever laboratory-confirmed cases from French Guyana was in 1998, from Venezuela in 2005, and from Ecuador in 2011. Argentina and Paraguay last reported cases in 2008, which was the first disease activity reported since the 1960s and 1970s, respectively.[440]

For additional reviews on the epidemiology of yellow fever epidemiology see references 28, 170, 441, and 442.

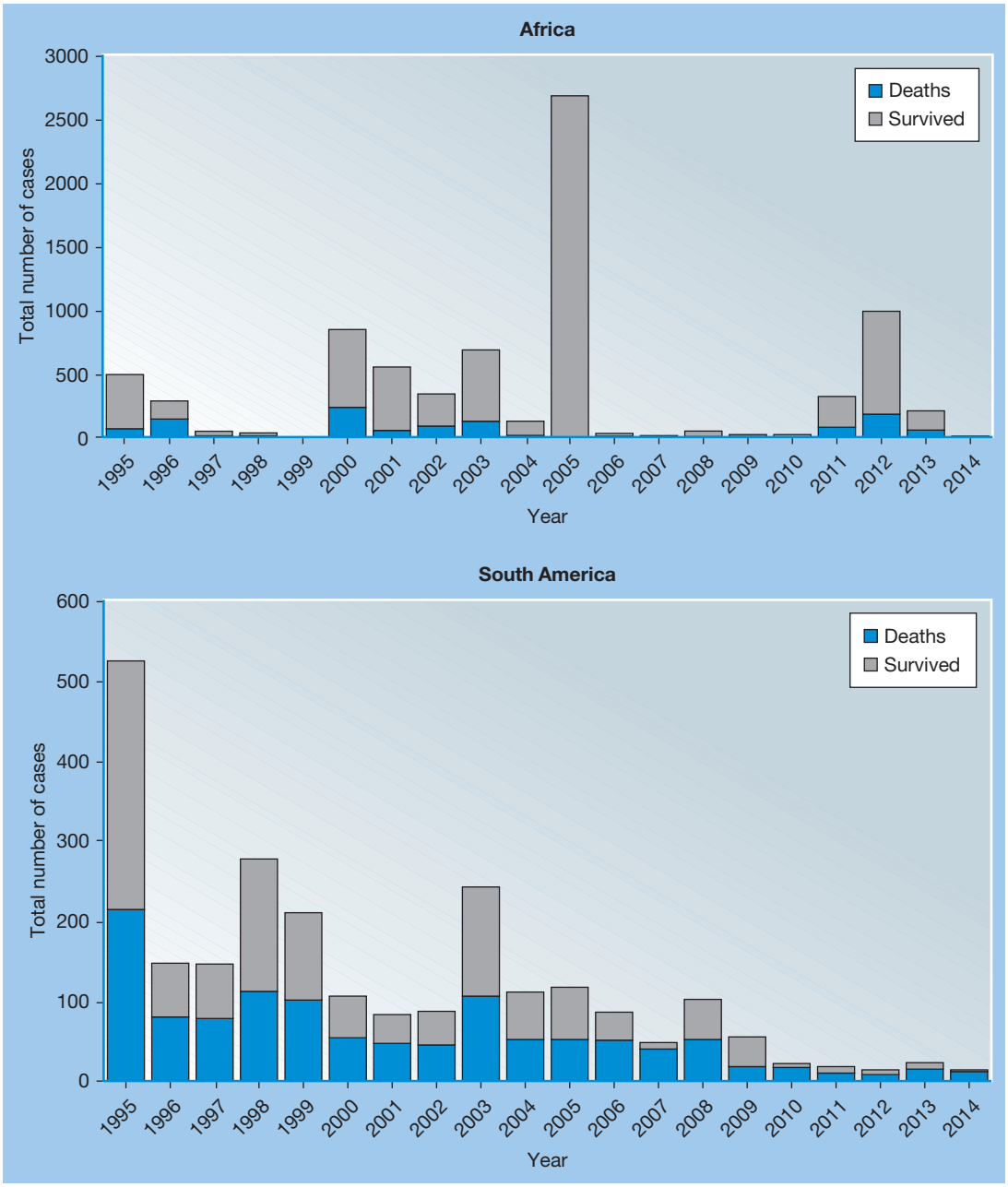


Figure 63.5. Annual number of yellow fever cases by outcome in Africa and South America reported to the World Health Organization, 1995–2014. Note the difference in scale. Overall, more cases were reported in Africa, but the proportion of cases who died following the diagnosis of yellow fever is much higher in South America.

Yellow Fever in Expatriate Residents, Travelers, and Military Personnel

In the prevaccine era, yellow fever virus was a major threat to expatriates living in and travelers to tropical America and Africa, including U.S. military personnel stationed overseas.[443] Fourteen cases occurred in Navy personnel in Honduras and Nicaragua in 1919,[444] and a suspected case was reported in Brazil in 1929.[445] A single case, acquired in Fernando Po in 1938, was reported among 2300 seamen of the Polish merchant fleet serving in the tropics.[446] In the United States, the last imported case in the prevaccine era occurred in a Mexican immigrant who died in Texas in 1924.

After yellow fever vaccination came into general use, yellow fever disease cases became increasingly uncommon. During World War II, a few cases among Europeans were reported during[212] and after[447] the 1941 outbreak in the Sudan and among British forces in West Africa in 1942.[448] In 1952, a fatal case of yellow fever occurred in a previously vaccinated European working in Uganda.[449] In 1979, two fatal cases occurred in unvaccinated French tourists who visited an area of Senegal bordering The Gambia.[450–452] These infections were acquired 1 year after a major outbreak in a region where the resident population had a high background of vaccine immunity. The French tourists returned to France during the early phase of illness and died in hospital in Paris. In 1979, a nonfatal case also occurred in a European resident of Dakar who visited the Gambian border,[450] and in 1981, an unvaccinated Lebanese resident in Senegal died of yellow fever after visiting a camp near The Gambia.[453] In 1985, a nonfatal case of yellow fever

occurred in The Netherlands in a female traveler to The Gambia, Senegal, and Guinea-Bissau.[454] In 1988, a vaccinated Spanish woman acquired yellow fever during travels through Mali, Burkina Faso, Niger, and Mauritania,[455] 1 year after a widespread increase in yellow fever activity in West Africa.

From 1996 to 2015, there have been six cases in unvaccinated tourists from the United States and Europe to South America and West Africa (Table 63.5). In all cases, the affected patients died after returning to their country of residence. Two fatal cases occurred in an unvaccinated American[369] and a Swiss[456] tourists who had visited jungle areas near Manaus, Brazil, in 1996, and returned home during the early phase of illness or during the incubation period, respectively. These cases occurred during a period of increased yellow fever viral activity in the Amazon basin. In August 1999, a 40-year-old unvaccinated man became ill in Germany after a visit to Côte d'Ivoire and died 4 days later in hospital.[362,457] In September 1999, a 48-year-old unvaccinated male U.S. citizen became ill on a trip to southern Venezuela; he returned to California on the fifth day of illness and died in hospital on the 11th day after onset.[457] In November 2001, a 47-year-old female unvaccinated Belgian tourist acquired yellow fever in The Gambia, returned home while ill, and died in hospital.[362,385,458,459] In March 2002, an unvaccinated U.S. citizen acquired yellow fever while on a fishing trip on the Rio Negro (Amazonas, Brazil) and died in a Texas hospital.[370,460]

During 2016–17, an unprecedented number of disease cases have been reported among travelers or persons from a nonendemic area who were working in an area undergoing an outbreak. At least 16 cases have been reported among residents from countries not contiguous with the outbreak areas in both African and South America (E. Staples, unpublished data).[463a,463b]

Risk of Acquiring Yellow Fever During Travel. The risk of a traveler acquiring yellow fever is determined by vaccination status, geographic location, season, duration of travel, activities that lead to exposure to mosquito bite, and the intensity of yellow fever virus transmission occurring at the time of travel. Exposure in an area undergoing an outbreak, even for a short period, may be associated with a high risk of infection. Although nearly all recent episodes of yellow fever among unvaccinated travelers have occurred in conjunction with increases in yellow fever activity, information about increased risk was often not readily available at the time. Increased yellow fever viral activity in a region often spans 2 or more years and affects contiguous areas in an expanding fashion, but such extensions in time and place may be invisible to passive surveillance systems. Moreover, in areas where vaccination is widely practiced, yellow fever virus can circulate silently between monkeys and mosquitoes, with few human cases in the indigenous population. A Belgian traveler who acquired yellow fever after spending 1 week in The Gambia in 2001 is a case in point.[385] There was no evidence of yellow fever virus transmission at the time, and there was a high prevalence of vaccine immunity in the local population. Season is also an important risk factor for yellow fever with most cases in travelers occurring during the period of virus amplification (late rainy season to early dry season).

During interepidemic periods in Africa, the incidence of overt disease is below the threshold of detection by existing surveillance. Such interepidemic conditions can last years or even decades in specific countries or regions. Epidemiologic silence can provide a sense of false security and lead to travel without the benefit of vaccination. Surveys in endemic areas of rural West Africa during silent periods indicate that the annual incidence of yellow fever illness approximates 1.1 to 2.4 per 1000 population and yellow fever death 0.2 to 0.5 per 1000 population, below detection by existing surveillance.[31] In unvaccinated travelers to these areas, the risk of yellow fever illness may be estimated at one per 1000 population per month and death at one per 5000 (1 : 2000 risk of illness and 1 : 10,000 risk of death from yellow fever for a typical 2-week journey),[461] but risk varies considerably with season and location (e.g., West Africa having a higher risk than East or Central Africa). These estimates, which are based on risk to local residents, might overestimate the risk to travelers who take precautions against mosquito bites and have less outdoor exposure than the indigenous population.

In South America, the incidence of disease is lower than in Africa. The risk of illness and death for the traveler is probably 10 times lower than in rural West Africa—approximately one per 20,000 population for illness and approximately one per 100,000 population for death per 2-week journey—but the risk varies greatly with specific location and season.[461] As in Africa, zoonotic virus transmission is often epidemiologically silent. The low reported incidence of yellow fever has diminished concern among travelers. In Brazil, for example, where the majority of the population lives in coastal regions outside the endemic zone, unimmunized recreational or vocational

TABLE 63.5 Reported Yellow Fever Cases in Travelers, 1970–2015

Month and Year	Age (y)	Sex	Vaccinated	Residence	Exposure	Outcome	Reference
Oct 1979	42	M	No	France	Senegal	Died	Bendersky et al, 1980[452]
Oct 1979	25	M	No	France	Senegal	Died	Bendersky et al, 1980[452]
Aug 1985	27	F	No	Netherlands	Guinea-Bissau, Gambia, Senegal	Survived	World Health Organization, 1986[454]
Oct 1988	37	F	Yes	Spain	Niger, Mali, Burkina Faso, Mauritania	Survived	Nolla-Salas et al, 1989[455]
Apr 1996	53	M	No	Switzerland	Brazil (Amazonas)	Died	Barros and Boecken, 1996[456]
Aug 1996	42	M	No	United States	Brazil (Amazonas)	Died	McFarland et al, 1997[369]
Aug 1999	40	M	No	Germany	Côte d'Ivoire	Died	Bae et al, 2005[362]; World Health Organization, 2000[457]
Sep 1999	48	M	No	United States	Venezuela	Died	World Health Organization, 2000[457]
Nov 2001	47	F	No	Belgium	Gambia	Died	Bae et al, 2005[362]; Parent et al, 2005[458]; Berneau, 2006[459]
Mar 2002	47	M	No	United States	Brazil (Amazonas)	Died	Hall et al, 2002[370]

travelers to the interior are the usual victims of yellow fever. Four of the six cases among travelers from the United States and Europe between 1996 and 2002 were exposed in South America (see Table 63.5).

Current yellow fever virus activity is found on the WHO website (http://www.who.int/csr/don/archive/disease/yellow_fever/en/) and is published in the WHO *Weekly Epidemiological Record*, and by the CDC's Division of Global Migration and Quarantine. Annual or biannual summaries of morbidity and epidemiologic trends are also published in the *Weekly Epidemiological Record*. Although these materials have significant limitations owing to underreporting, they provide a picture of current hot spots of yellow fever virus activity. The user should keep in mind that endemic (and even epidemic) yellow fever occurs in areas that are silent with respect to official reports.

Many nonendemic countries with *Ae. aegypti* require a valid yellow fever International Certificate of Vaccination or Prophylaxis (ICVP) for entry from a yellow fever–endemic region. A current listing of receptive countries that require a valid certificate is provided in the most recent WHO *International Travel and Health* (green book) and the CDC's *Health Information for International Travel* (yellow book).[413,414]

Risk of Spread to Nonendemic Areas

Areas at highest risk for the introduction and secondary spread are areas with *Ae. aegypti* and tend to be in the Americas and include coastal regions and interior towns infested by this vector in Argentina, Brazil, Peru, Bolivia, Ecuador, Venezuela, Colombia, the Guianas, Central America, the West Indies, Mexico, and the southern United States. All these areas have been affected by yellow fever in the past and have had recent circulation of dengue and Zika viruses, related flaviviruses with a similar urban transmission cycle. Within individual countries currently affected by yellow fever, the disease may be acquired by unvaccinated residents who travel from an uninfected area to a region of endemic activity.[462] This is a recurring theme in South America, where unvaccinated migrant workers move from a coastal area or the Andean highlands into the Amazon region. In Brazil during the first half of 2000, 11 (14%) of 77 reported yellow fever cases were in tourists from coastal areas to the endemic part of the country.[419,463] In Africa, similar episodes undoubtedly are common, but tend only to be recognized when larger cluster of disease cases are detected by the surveillance system or viral activity is recognized first in sylvatic areas before being introduced into an urban area with *Ae. aegypti*.[418,464]

The risk that urban yellow fever will reemerge continues to exist owing to the expansion of urban centers, increased air travel, and the continued spread of *Ae. aegypti*.[170,418,465,466] These factors have also likely led to the rising global incidence of other *Ae. aegypti*–transmitted viruses, such as dengue, chikungunya, and Zika.[465,467,468] *Ae. aegypti*–infested regions of southern Europe, the Middle East, Asia, Australia, and Oceania are also at risk for introduction of yellow fever virus. The virus has never been recorded in India or other parts of Asia. The possible reasons for its absence include both demographic and biological factors.[336] The most likely mode of introduction of yellow fever from endemic areas to Asia is by air travel of viremic humans, and all receptive areas can be reached by air from an endemic region within less than the incubation period of yellow fever.[465] However, yellow fever occurs in remote areas and affects persons engaged in subsistence farming, who are infrequent international travelers. Even if the disease occurs in more urban areas, like it did in Luanda, Angola, in 2016, there will need to be some degree of unchecked expansion of the virus (e.g., no vaccination coverage in the population) to increase the risk of introducing the virus to a nonendemic area in Asia, and the virus will need to be introduced during a time when *Ae. aegypti* are active.[469] Biological factors that could limit the risk of introduction include cross-protection from dengue or Japanese encephalitis viruses, as the majority of persons residing in Asia are immune to dengue or frequently are immunized against Japanese encephalitis. A third hypothesis is that *Ae. aegypti* strains in Asia could have low vector competence for yellow fever virus.[470,471] It is likely that all of these mechanisms combine to reduce the likelihood of introduction and spread of yellow fever virus in Asia.

Spread of yellow fever virus outside its traditional boundaries and reemergence of the urban disease in Africa or the Americas would greatly increase the demand for yellow fever vaccine, as was seen in 2016 when demand outstripped supply during the response in Angola. During periods of increased epizootic transmission, a high risk of introduction of yellow fever into large unvaccinated coastal populations juxtaposed to endemic areas has been postulated.[472] Ecological changes resulting from deforestation are also changing the landscape of sylvatic yellow fever.[473] Destruction of habitats of nonhuman primates and certain vectors might reduce transmission in some areas, but in general such effects are counterbalanced by increased human encroachment into enzootic areas and opportunities for interhuman transmission of the virus.[469]

PASSIVE IMMUNIZATION AND PASSIVE-ACTIVE IMMUNIZATION

Antiserum to yellow fever virus produced in horses, monkeys, or chimpanzees protects rhesus monkeys against lethal yellow fever when given 1 to 3 days after challenge.[381,474,475]

As noted under "Treatment" above, IFN-α when given to monkeys shortly before and shortly after their exposure to wild-type yellow fever virus, resulted in lower viremias and increased survival compared to untreated controls.[391] The requirement to administer IFNs very shortly before infection or during the incubation period likely precludes their clinical use in prophylaxis of wild-type yellow fever. However, in a patient with known exposure (e.g., in the case of an unimmunized laboratory worker with accidental infection), early postexposure prophylaxis with IFN-α, or preferably with immune plasma, would be indicated. As noted previously, a defective adenovirus type 5 vector carrying the gene for the consensus sequence of IFN-α protected hamsters in the 2-day postchallenge period.[394] It is possible to envisage use of such a strategy in a population affected by a yellow fever outbreak during the time period before an effective vaccination campaign could be mounted.

Before vaccines were developed, passive immunization was widely used for preexposure prophylaxis. The concept was established in the 19th century,[476] and convalescent serum was first used in the early 20th century.[477] Experimental validation occurred after isolation of yellow fever virus. In 1928, Stokes and colleagues reported that pretreatment with a small volume of convalescent serum protected monkeys against lethal challenge.[11] Because of an increasing number of laboratory infections, standard practice was to administer convalescent serum to at-risk laboratory workers and to inject large amounts after accidental exposures. The potency of passive antibody and the schedule of immunization were not carefully controlled, and laboratory-acquired disease occurred in persons who had been previously immunized[478]; such cases were the result of inadequate dosage of antibody and the long interval between transfer of serum and exposure.[280] In monkey studies, transfer of immune serum prior to challenge was protective, immunization 24 hours after experimental challenge protected 55% of the animals, and transfer at 48 or 72 hours protected only

15% to 20%.[479] Administration of antibody after clinical onset was ineffective.

Because of the difficulty in obtaining sufficient amounts of potent human serum, hyperimmune antibodies were prepared in nonhuman primates, horses, and goats.[480,481] Antibodies raised in monkeys and horses were shown to protect susceptible rhesus macaques. Heterologous antisera had limited usefulness for repeated administration to humans but were applied together with partially attenuated virus to achieve active immunization. Passive-active immunization using an excess of human or animal serum mixed with partially attenuated virus (French neurotropic strain) elicited active immunity without untoward effects, and this became standard practice.[17,18] However, it was a cumbersome method, requiring that the amount of immune serum be sufficient to protect against disease but not completely neutralize the virus and prevent active infection.[482] Heterologous serum was problematic, because allergic reactions occurred in 85% of recipients of goat serum, and late reactions similar to abortive yellow fever infection were noted in some cases, possibly the result of rapid clearance of the heterologous serum, allowing replication of the underattenuated vaccines used in these studies (e.g., the 17E strain). Monkey serum was safer (with respect to hypersensitivity reactions), but frequent failure to seroconvert was attributed to vaccine neutralization by the antiserum. Passive-active immunization was discontinued in 1936 with the advent of vaccines that could be administered safely without serum.[22]

Recent Application of Passive Immunization

Commercial lots of serum Ig contain high titers of neutralizing antibodies to yellow fever (Monath TP, unpublished observations, 2004),[483,484] presumably because 5% to 10% of plasma donors have been immunized during military service in the United States. There are no published reports of off-label use of IVIg for passive immunoprophylaxis of persons with contraindications to active vaccination. A patient with chronic lymphocytic leukemia, in whom active vaccination was contraindicated, was planning a short trip to the Amazon region and was passively immunized with commercial IVIg having a $\log_{10}$ neutralization index (LNI) of 3.0, to achieve a high initial passive titer and a protective level of antibody throughout several weeks of travel (McMullen R, et al, unpublished data, 2000). The level of antibody considered to be protective was an LNI of 0.7 based on studies in nonhuman primates.[485] In a second case report, a kidney transplant recipient on maintenance immunosuppression was given three doses of IVIg from days 20 to 22 post vaccination in response to an elevation in his liver enzymes.[484] The patient did not develop any serious adverse events and was found to develop a neutralizing antibody and T-cell response following vaccination, albeit lower than normal controls. Although these approaches might have merit for certain patients who cannot avoid travel to a high-risk area, they are expensive, require monitoring of antibody levels in IVIg and the patient, and are not supported by clinical data on efficacy.

The development of humanized mouse MAbs that target epitopes present in both wild-type and vaccine yellow fever viruses holds some promise as a potential passive immunization strategy. These antibodies provide several potential advantages such as being easily produced, being more capable of quantitating the amount delivered, and being relatively safe compared IVIg, which is a blood product. Studies of a humanized MAb (2C9-cIgG) that targets the E protein domain II epitope in both immunocompromised mice and immunocompetent hamsters conferred increased survival in the animals when challenged.[386,387] Human/mouse chimeric IgG and IgM monoclonals were derived from this antibody and the IgG construct (but not IgM) was shown to protect IFN knockout mice when given before or up to 48 hours after lethal challenge with 17D virus.[386] However, more work is needed to determine if these antibodies will provide adequate protection if given to humans who cannot safely receive vaccine.

Maternal Antibody

Transplacental transfer of yellow fever virus–specific neutralizing antibodies has been documented in monkeys[486] and humans,[167] and antibody has also been found in breast milk of immune mothers.[487] Because yellow fever 17D vaccine is not administered to infants younger than 6 months of age for safety reasons, maternal immunity typically does not pose an obstacle to effective immunization but should, in endemic areas, offer infants some initial protection against wild-type disease.

ACTIVE IMMUNIZATION

Inactivated Vaccines

After isolation of the virus in 1927, attempts to develop an inactivated vaccine were made at the Wellcome Bureau of Scientific Research in London,[16,488] at the Oswaldo Cruz Institute in Brazil,[489] and at the Pasteur Institute, Paris.[474] The vaccines were prepared by phenol or formaldehyde treatment of infected monkey liver and/or spleen. Hindle inoculated rhesus monkeys with a single dose of inactivated liver tissue emulsion containing more than 10,000 monkey lethal doses per gram and found that 80% of the immunized monkeys were protected against challenge.[15] However, his and others' studies were not controlled for residual live virus, nor were serologic studies performed. Subsequent studies in monkeys yielded erratic results,[490,491] and human trials in Brazil involving 25,000 persons were inconclusive.[474] Burke and Davis[478] reported yellow fever in a person who had previously received inactivated liver-tissue vaccine.

As virologic techniques improved, antigens were tested that were more suitable for vaccine production than infected monkey viscera. Gordon and Hughes[492] prepared vaccines from heat- or UV-inactivated virus tissue cultures (viscerotropic virus) or from mouse brain (neurotropic virus) containing known amounts of virus. Preparations containing residual live virus caused illness in monkeys, and survivors were immune to challenge. However, monkeys given various doses of fully inactivated vaccines did not develop antibodies and were not protected. It appeared that the preparations lacked sufficient antigenic potency or the methods that inactivated virus also destroyed its antigenic properties. However, all studies employed a single dose of vaccine, a procedure that would not be expected to be very effective in the case of an inactivated vaccine. Sellards inoculated mice with multiple sequential doses of phenol-inactivated mouse brain and demonstrated protection against challenge, and rabbits immunized sequentially with inactivated virus developed neutralizing antibodies.[493] However, prime-boost vaccination strategies were not used in monkeys or humans.

Methodological problems in antigen production, virus inactivation and potency assays, ignorance about immunologic principles, and the lack of adjuvants were obstacles to the early development of inactivated vaccines. Given the growth characteristics of yellow fever virus, with titers of 10^7 to 10^8 achievable in cell culture and the success of inactivated vaccines against other flaviviruses (Japanese encephalitis, tick-borne encephalitis), there is no doubt that a satisfactory

product could be developed today. The failure of the early efforts to produce inactivated vaccines led, in part, to development of live vaccines.

With the recognition of serious adverse events following the 17D vaccine, there is renewed interest in inactivated whole virus and recombinant subunit vaccines.[494] Three companies (Xcellerex, Bio-Manguinhos, and Najit Pharma) have reported development of whole-virus inactivated vaccines prepared from the 17D or 17DD strains propagated in Vero cells, purified, and inactivated by different methods.[495–498] Analysis of the inactivated vaccine identified mutations in E protein and NS4B protein that suggested viral adaptation to Vero cells.[499] At least two vaccines inactivated with β-propiolactone and adjuvanted with aluminum hydroxide have been developed.[496,498] Both of these vaccines induced neutralizing antibodies in rodents and protected them against lethal challenge. In addition, the vaccine developed by Xcellerex was also found to protect hamsters against lethal challenge and induce neutralizing antibodies in monkeys and humans.[496,500] In a Phase I clinical trial, two doses of 4.8 μg (a dose similar to that in a commercial vaccine against Japanese encephalitis) spaced 21 days apart induced neutralizing antibodies within 10 days of the second vaccination in all subjects.[500] Neutralizing antibody titers were similar to those induced by commercial vaccines against Japanese encephalitis and tickborne encephalitis.

Live Vaccines

French Neurotropic Vaccine (Discontinued)

In 1930, Theiler showed that mice were susceptible to intracerebral inoculation of yellow fever virus.[501] Following the lead of Pasteur, who had attenuated rabies virus by passage in rabbit brains, Theiler made a series of mouse brain passages of the French strain and showed that the adapted virus lost its viscerotropism for monkeys and protected them against challenge. However, in subsequent studies the neuroadapted virus caused fever and viremia in a high proportion of monkeys, and it caused lethal encephalitis after intracerebral or intranasal inoculation and, in some animals, after subcutaneous inoculation.[16,225,271,493,502] The virus was considered too dangerous without the coadministration of immune serum. Consequently, the first human volunteers were given immune human serum and neuroadapted virus (see "Passive Immunization and Passive-Active Immunization" earlier).[16] This regimen resulted in the development of active immunity.

In 1932, Sellards and Laigret reported the first immunizations with the French strain in the absence of immune serum.[17,503] Systemic adverse reactions were attributed to underattenuation. To address this problem, the mouse brain virus was attenuated by aging at room temperature. Subjects received three inoculations at intervals of 20 days with virus that had been aged for decreasing lengths of time.[504] Preliminary studies indicated that the vaccine regimen was well tolerated and that 90% of inoculated subjects seroconverted after the second dose. By 1934, 3196 persons in French West Africa had received immunizations with this regimen, and 70% of those studied developed antibodies after the first dose.[18] One-third of the recipients experienced a febrile reaction after the first dose, and there were two serious adverse events (myelitis, meningitis). In 1935, Nicolle and Laigret[505] simplified the method to a single inoculation of mouse-brain virus aged for 24 hours and treated with olive oil or egg yolk to retard diffusion from the inoculation site. By 1939, more than 20,000 persons in West Africa had received either the single- or three-dose schedule. Concerns about reactogenicity decreased with increased vaccine use, despite occasional reports of postvaccine encephalitis.[25,506] Careful follow-up studies were not routinely conducted, and the threat of natural infection superseded concerns about vaccine safety.

In 1939, Peltier and colleagues[507] simplified yellow fever immunization by conversion from subcutaneous inoculation to the scarification technique used to deliver smallpox vaccine. In 1939, nearly 100,000 persons were given French neurotropic vaccine and smallpox vaccines simultaneously, without recognized adverse events. Of 1387 subjects followed, 96% developed neutralizing antibodies 1 month after immunization. By 1941, French neurotropic vaccine administered by scarification had been given to 1.9 million people in francophone Africa, and immunization was then made compulsory. By 1947, 14 million persons had been immunized.[24] In 1946, the UNRRA Standing Technical Committee on Health approved the French neurotropic vaccine, and the WHO granted similar approval in 1948.

French neurotropic vaccine was prepared at the Pasteur Institute in Dakar by the intracerebral inoculation of 2.5- to 3-month-old mice with approximately 20,000 LD_{50} (median lethal dose) of the French virus.[508] A seed lot system was not used, although passage level was restricted. Mice showing illness were killed and the brains were aseptically removed and lyophilized. After sterility testing, brains were pooled, ground to powdered form, and again tested for sterility. The vaccine powder was filled into ampoules containing one-tenth of a mouse brain (0.4 g), equivalent to 100 doses, and tested for sterility and potency. After reconstitution in 2 mL of diluent, the recommended minimum potency was 5000 mouse LD_{50}/dose. The vaccine was quite stable and was stored at 4°C but shipped at ambient temperature. After reconstitution in a solution of gum arabic, a drop of the solution was placed on the skin, and scarification was performed with a vaccinostyle or similar instrument typically used to perform smallpox vaccinations.

By 1953, 56 million doses had been delivered in francophone Africa (twice the population of the region).[26] As a result the incidence of yellow fever declined in francophone countries (Fig. 63.6), but not in neighboring Nigeria and Ghana, where immunization was not practiced. Seroconversion rates were shown to exceed 95%,[509] and population surveys in French West Africa showed that the prevalence of immunity rose from approximately 20% before vaccination to 86% in 1952 to 1953.[510]

The safety of French neurotropic vaccine was not carefully evaluated during the ramp-up to achieve full coverage. It was known that French neurotropic vaccine caused viremia in two-thirds of subjects, that 10% to 15% experienced a mild reaction with fever, headache, and backache on days 4 to 6, and that rare cases of meningoencephalitis occurred 10 to 15 days after vaccination.[25,511,512] The incidence of encephalitis was initially estimated at between one per 3000 and one per 10,000,[512] but its importance was minimized because full recovery was the rule and because no serious reactions were noted during campaigns in French West Africa involving more than 40 million persons.[24,513] However, contemporary reports described outbreaks of encephalitis and deaths following yellow fever immunization in French Equatorial Africa.[514]

English and American workers considered French neurotropic vaccine too dangerous for routine use, but when epidemics struck in Nigeria (1951–52) and Central America (1950–52), the danger of yellow fever exceeded those of vaccine-associated risks. In Nigeria, use of French neurotropic vaccine was followed by an outbreak of encephalitis principally in children, with an incidence of 3% to 4% and a case fatality of 40%.[515,516] Autopsies showed lesions of encephalitis consistent with direct viral injury, and yellow fever virus was isolated from brain tissue, confirming that French neurotropic vaccine, and not an adventitious agent, was responsible. In

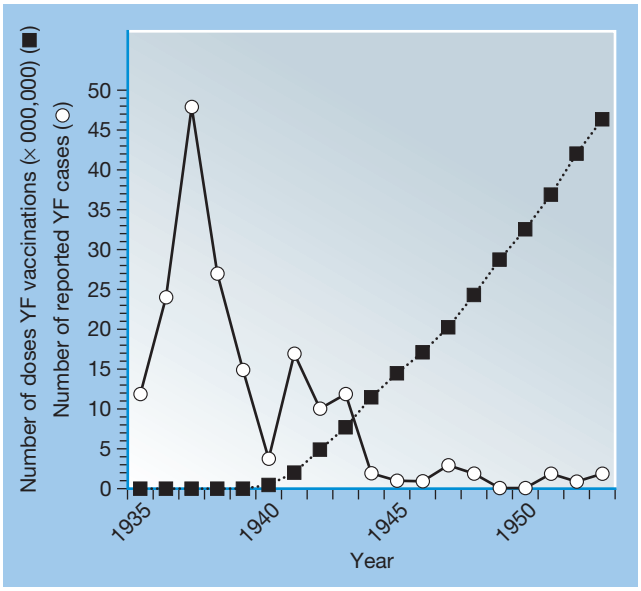


Figure 63.6. Cumulative number of doses of French neurotropic vaccine administered and incidence of yellow fever, French West Africa, 1935–53. Compulsory vaccination of the indigenous population resulted in virtual disappearance of the disease. Neighboring Anglophone countries, which did not practice immunization, continued to have epidemic yellow fever (YF). *(From Monath TP. Yellow fever vaccines: the success of empiricism, pitfalls of application, and transition to molecular vaccinology. In: Plotkin S, Fantini M, eds.* Vaccinia, Vaccination, and Vaccinology: Jenner, Pasteur and their Successors. *Paris, France: Elsevier; 1996:157–182. Data from Durieux C. Mass yellow fever vaccination in French Africa south of the Sahara. In: Smithburn KC, Durieux C, Koerber R, et al, eds.* Yellow Fever Vaccination. *Geneva, Switzerland: World Health Organization; 1956:115–121.)*

Costa Rica and Honduras, 10 definite and five possible cases of postvaccination encephalitis occurred in children.[517]

The increased recognition of severe reactions in children led to a change in the policy for use of French neurotropic vaccine. In 1959 to 1960, vaccination was restricted to persons older than 10 years of age.[518] The distribution of vaccine decreased from approximately 8 million doses to 4 million doses per year. Within 5 years of cessation of routine immunization of children, epidemic yellow fever reappeared in Senegal for the first time in 28 years.[247] The 1965 epidemic at Diourbel was the largest on record in West Africa, affecting up to 20,000 persons. Because of the high incidence of yellow fever in children and limited supplies of the safer 17D vaccine, the age limit for use of French neurotropic vaccine was reduced to the original 2 years. Among 498,887 persons vaccinated with French neurotropic vaccine, there were 231 cases of postvaccination encephalitis.[519] The majority of cases occurred in children 2 to 11 years of age, in whom the incidence of encephalitis was approximately 1.4 per 1000 and the case-fatality rate was 9%.[519,520] Collomb and colleagues have described the clinical syndromes associated with acute encephalitis and the neuropsychiatric residua.[521]

The high numbers of disease cases and vaccine adverse events in young children in West Africa confirmed the need to provide a high rate of coverage of the childhood population in Africa and the need to use a safer method of immunization. In 1966, the Pasteur Institute, with the assistance of the WHO, expanded production of 17D vaccine at Dakar, and by 1970 an official policy was established for use of 17D in persons younger than 5 years of age.[522] Small amounts of French neurotropic vaccine were used in regions with poor accessibility, where use of the more thermolabile 17D vaccine was problematic, but in 1982 production of the neurotropic vaccine was discontinued.

The potential contamination of the French neurotropic vaccine with murine viruses (e.g., lymphocytic choriomeningitis) has not been fully evaluated. The isolation of yellow fever virus from brain tissue of fatal cases[515] and absence of murine viruses in French neurotropic vaccine lots[523] supports the hypothesis that the vaccine and not an adventitious agent was responsible for encephalitis. In addition to its inherent neurotropism, French neurotropic vaccine appears to be genetically less stable than yellow fever 17D virus based on mutations and phenotypic changes occurring during laboratory passage.[524] It is possible that some adverse reactions to the vaccine reflected reversion of the virus during manufacture or during replication of the vaccine in human hosts.

Yellow Fever 17D Vaccine

The 17D vaccine is the only product currently approved for immunization against yellow fever. Several recombinant or subunit vaccines using 17D virus have been and are being explored (see "Future Yellow Fever Vaccines and Yellow Fever–Vectored Vaccines" later).

Development and Early Clinical Testing. The original development of this vaccine, described by Lloyd and colleagues[525] and Theiler and Smith,[19] was achieved by empirical methods of sequential passage of the prototype Asibi virus in a substrate that was restrictive for growth. This process enhanced the selection of variants with altered biological properties, without neuroadaptation by mouse brain passage as in the case of French neurotropic vaccine. The first successful in vitro passages of Asibi virus were achieved in cultures of minced mouse embryo tissues. After 18 subcultures, the virus was passed to cultures of minced whole-chick embryo. After 58 passages, the virus, now designated subculture series 17D, was propagated by subcultures in minced chick embryo cultures from which the brain and spinal cord had been removed. The final passage prior to human inoculation was in embryonated eggs.

A reduction in monkey neurovirulence and loss of viscerotropism occurred between the 89th and 114th passages, and a reduction in mouse neurovirulence occurred between the 114th and 176th passages. Monkeys inoculated by peripheral routes did not develop encephalitis; those inoculated intracerebrally developed histopathologic changes, but only 5% to 10% succumbed to encephalitis. The animals developed antibodies and resisted challenge with Asibi virus. Preclinical safety and efficacy were deemed sufficient to permit human studies. The initial trials using virus at the 227th and 229th passage levels were conducted in 1936, first in yellow fever immune and then in nonimmune volunteers.[21,22] The trials showed acceptable tolerability and development of neutralizing antibodies. In early 1937, 17D vaccine was taken to Brazil, where it was used in trials of increasing size, leading to the establishment of local manufacturing capacity and the initiation of a mass vaccination campaign in 1938.[22,23,526] Between 1938 and 1941, more than 2 million persons were immunized in Brazil.

Seed Lot System. During the initial phase of yellow fever vaccine production at the Rockefeller Foundation in New York and in Brazil between 1937 and 1941, a number of different substrains were used, representing independent parallel subculture lineages originating at about the 200th passage of the

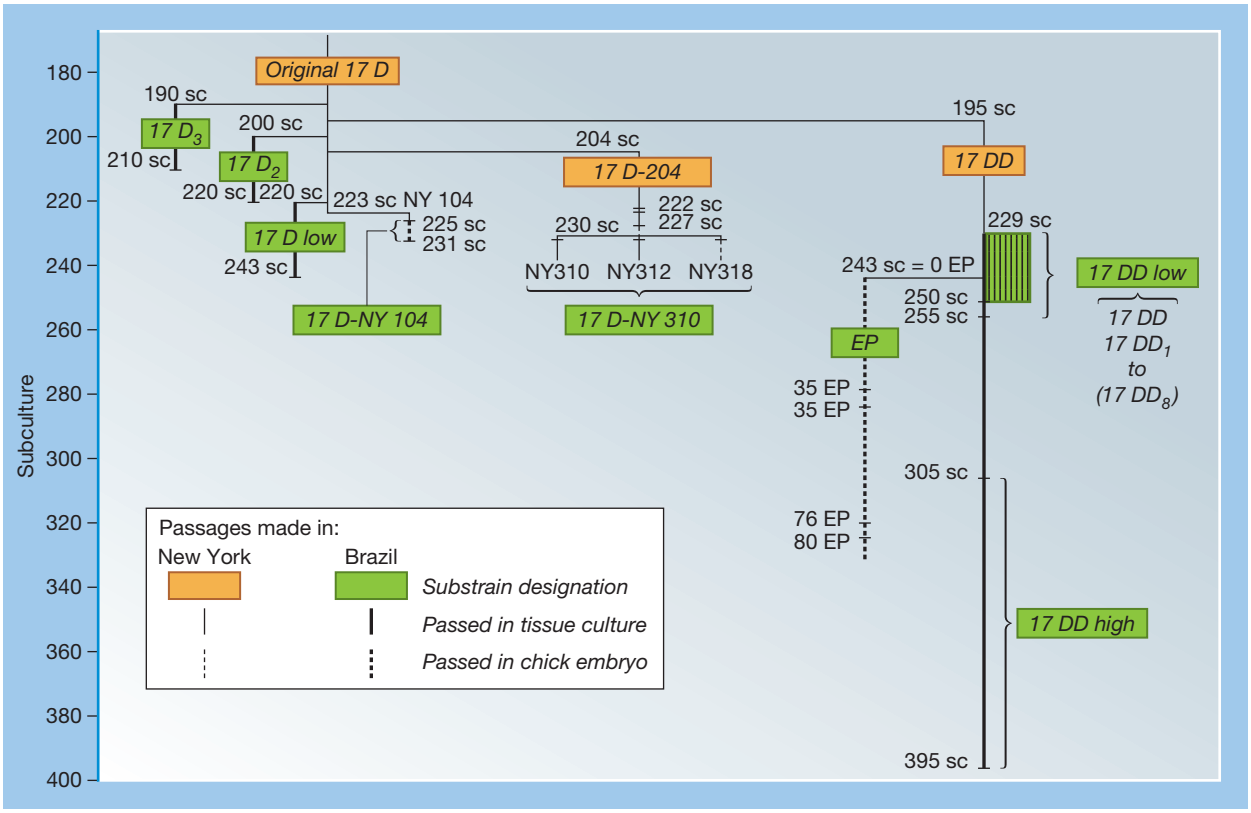


Figure 63.7. Derivation of early vaccine lots from the original 17D virus before the seed lot system was introduced (see text). *(From Fox JP, Penna HA. Behavior of 17D yellow fever virus in rhesus monkeys. Relation to substrain, dose and neural or extraneural inoculation.* Am J Hyg. *1943;38:152–172; and Brès P, Koch M.* Production and testing of the WHO yellow fever primary seed lot 213–77 and reference batch 168–73. WHO Expert Committee on Biological Standardization, 36th Report. *Geneva, Switzerland: World Health Organization; 1987.)*

original 17D line (Fig. 63.7). Two main lineages (17D-204 and 17DD) are used for vaccine production (see "Substrains" later).

Between 1938 and 1941, field trials and experimental studies revealed the importance of controlling passage level and virus substrain. Substrains 17DD high (305th to 395th passage levels) and $17D_2$ (passage 220) were found to be overattenuated, with poor seroconversion rates in humans and low levels of viremia and poor immunogenicity in monkeys,[269,527] indicating that loss of immunogenicity could occur in as few as 20 passages in minced chicken embryo tissue cultures. More important, some substrains were associated with the appearance of encephalitis. In 1941, following an outbreak of postvaccination encephalitis in Brazil, a survey of 55,073 persons who received different lots of the same substrain (NY17D-104) revealed the occurrence of 273 (0.5%) severe systemic reactions, including 199 (0.36%) with CNS signs and one fatal case of encephalitis.[528]

A controlled study was performed, in which more than 19,000 subjects received different vaccine lots, including EP, $17D_3$, 17D-NY 310, and 17D-NY 104 (see Fig. 63.7), that had been associated with the highest incidence of severe reactions or a control vaccine prepared from uninfected chick embryos. Children 5 to 14 years old sustained the highest incidence of encephalitis, with onset of CNS signs 9 to 12 days after immunization. The highest incidence of encephalitis (13/1000) was observed in recipients of the 17D-NY 104 vaccine. This substrain was shown also to produce the highest incidence of encephalitis in monkey neurovirulence tests. Other substrains (17D-NY 310 and $17D_3$) that were associated with encephalitis in humans, but at a lower incidence than 17D-NY 104, caused early-onset and prolonged fever in monkeys. Unfortunately, these substrains and vaccine lots have not been retained and are not available for sequencing studies.

Recognizing that continued serial passage could lead to unwanted alterations in the biological properties of 17D vaccine, the Rio de Janeiro laboratory instituted a "seed-lot" system in 1941, in which primary seed and secondary seed lots were prepared, and the latter used to prepare multiple vaccine batches. The primary and secondary seeds were extensively characterized, and all vaccine lots were restricted to a single passage from the secondary seed. This system was used from 1942 onwards by many manufacturers, and was formally established as a biological standard by UNRRA in 1945.[529] However, appropriate seed lots were not distributed to all manufacturers, and preparation of vaccines by serial passage and without adequate neurovirulence testing continued in some countries in the 1950s. Several cases of encephalitis were reported following use of such vaccines at the Pasteur Institute during that time.[530] In 1957, publication of the WHO Requirements for Yellow Fever Vaccine further standardized the seed lot and manufacturing procedures; this guidance has been retained with subsequent updates.[531]

Production

Vaccine Producers. Currently there are at least six manufacturers of yellow fever 17D vaccine (Table 63.6); Central Research Institute in Kasauli, India, stopped production in 2011, but plans to resume production in the future. Four manufacturers in Brazil, France, Russia, and Senegal are WHO prequalified to supply vaccine for the Expanded Programme on Immunization (EPI) and mass vaccination campaigns

TABLE 63.6 Manufacturers of Yellow Fever 17D Vaccine

Country	Manufacturer	Trade Name	Prequalified by the WHO	Comment
Brazil	Bio-Manguinhos, Rio de Janeiro	—	Yes	Only 17DD vaccine in use; national use, exported
China	China National Biotech Group, Beijing	—	No	National use
France	Sanofi Pasteur, Marcy l'Etoile	Stamaril	Yes	National use, exported
Russia	Chumakov Institute, Moscow	—	Yes	National use, exported
Senegal	Institut Pasteur, Dakar	—	Yes	National use, exported
United States	Sanofi Pasteur, Swiftwater, PA	YF-Vax	No	National use, limited export market

WHO, World Health Organization.

during routine and emergency operations. Prior to 2002, roughly 5 million doses of vaccine were produced by prequalified manufacturers (namely Sanofi Pasteur) and distributed by United Nations Children's Fund (UNICEF). Since 2002, there has been a concerted effort to increase the coverage of vaccine through large preventive campaigns being conducted in West Africa, as well as increasing demand through the EPI and establishment of a vaccine stockpile. In 2013, 31 million doses of vaccine were produced for use in 30 Global Alliance for Vaccines and Immunization (GAVI)-eligible countries.[532] However, it is projected that 62 million doses were needed per year for 2014–17 to supply the EPI, mass vaccination campaigns, and emergency response stockpiles for these countries. In addition, the Pan American Health Organization (PAHO) estimated that countries in its region will need at least 20 million doses of vaccine yearly. Vaccine supplies have been limited by a number of issues such as availability of pathogen-free eggs, manufacturing equipment, and maintenance of good manufacturing practices by some of the manufacturers of prequalified vaccine.[532] Vaccine demand is anticipated to continue to outstrip vaccine supply for the immediate future.

Sanofi Pasteur exports vaccine for the travel market while the other manufacturers offer vaccine to limited populations (see Table 63.6). The number of manufacturers has decreased since the 1990s, and facilities in the United Kingdom, The Netherlands, Germany, Colombia, Australia, Nigeria, and South Africa are no longer active. Berna Biotech acquired the 17D vaccine from Germany (Robert Koch Institute) and following acquisition of Berna by Crucell, Crucell brought the vaccine (BERNA-YF or Flavimun) through Phase III trials and preregistration in Switzerland; however, following Crucell was acquired by Johnson & Johnson in 2011, and the program was discontinued in 2014. Interestingly, this vaccine, derived from a seed stock prepared for the WHO (YF17D-213/77), was demonstrably less neurovirulent than two licensed 17D-204–derived viruses.[533]

Substrains. All yellow fever vaccines are live attenuated vaccines derived from the 17D strain and are produced in embryonated eggs. The WHO has established standards of safety and potency (see "Method of Manufacture" later). The biological performance of all 17D vaccines is presumed to be similar or identical with respect to seroconversion rate, quality of the immune response, durability of immunity, safety, and tolerability. Vaccines in current production differ, however, with respect to 17D substrain, passage level, formulation with stabilizers, thermostability, salt and other excipients, and the diluent used for reconstitution. 17D vaccines are not biologically cloned and are heterogeneous mixtures of multiple virion subpopulations (genetic swarms).[122] Unsurprisingly, differences have been found in plaque size[534,535] and in the nucleotide sequences of these vaccines in current use.[70–73,77]

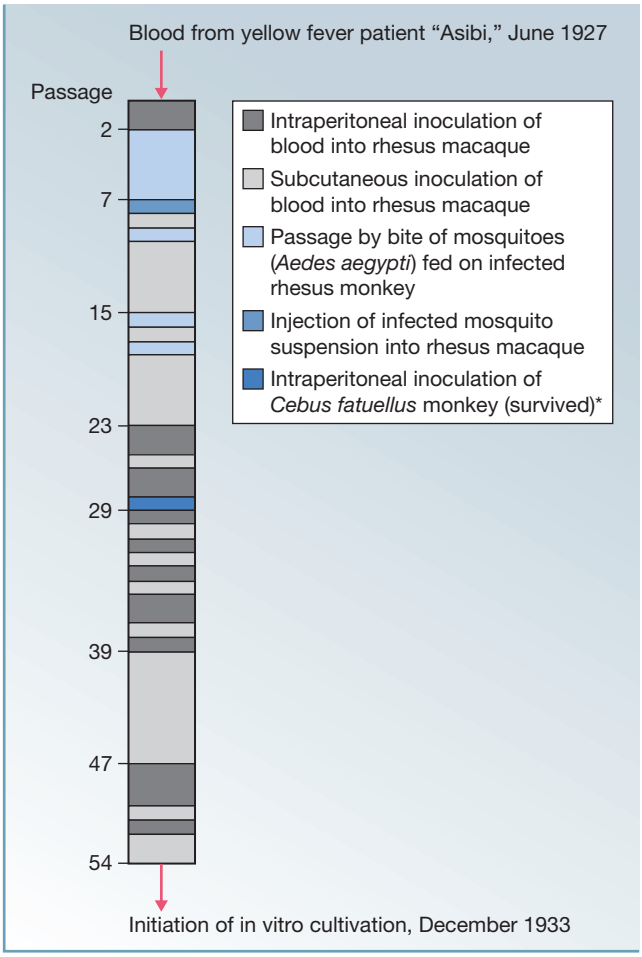


Figure 63.8. Passage history of Asibi virus from original isolation to the initiation of in vitro culture for development of the 17D vaccine. *This inoculation is not counted by the authors but represents a passage in the history of Asibi virus before cultivation in vitro.

There is no evidence to suggest that such variations affect safety or efficacy.

Before the attenuation process was initiated, Asibi virus was passaged sequentially 54 times in monkeys, either by direct injection of blood from the previous animal or with an intervening passage in *Ae. aegypti* mosquitoes (Fig. 63.8). Throughout this passage history the virus maintained its virulence for rhesus monkeys. Figs. 63.7 to 63.9 show the virus passage

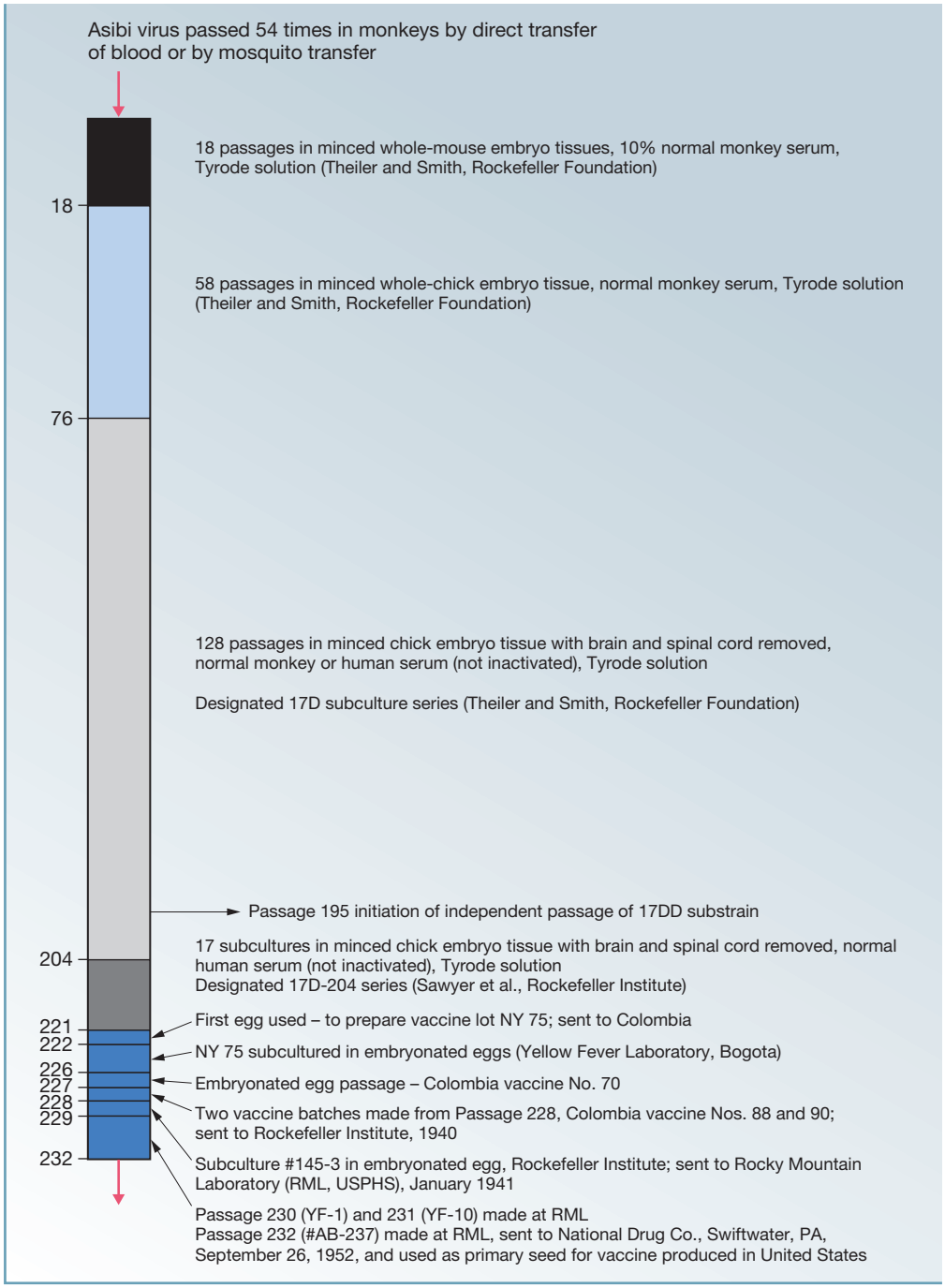


Figure 63.9. Passage history of the seed virus used to prepare yellow fever vaccines. USPHS, U.S. Public Health Service. *(Modified from Brès P, Koch M.* Production and testing of the WHO yellow fever primary seed lot 213–77 and reference batch 168–73.WHO Expert Committee on Biological Standardization, 36th Report. *Geneva, Switzerland: World Health Organization; 1987.)*

history during the original development of 17D vaccines. In vitro cultivation began in December 1933 with the passage of the virulent Asibi strain in mouse embryo tissue culture and subsequently in chick embryo tissue culture to produce attenuated 17D vaccines (Fig. 63.9).[525] Two 17D substrains—17DD and 17D-204—currently used for vaccine manufacture represent independent subcultures performed at the Rockefeller Foundation in New York (see Fig. 63.7). The 17DD and 17D-204 substrains were derived from passage levels 195 and 204, respectively, of the original 17D virus (see Figs. 63.7 and 63.9). 17DD virus was sent to Brazil at passage level 229, whereupon it was passed 14 times in minced chicken embryo tissue cultures and then, beginning at passage 243, in whole embryonated eggs (the EP lineage in Fig. 63.7). Primary and secondary seeds were prepared in Brazil at passages 284 and 285, respectively, and the current vaccine is at passage 286. One of two 17DD primary seeds was transferred to the Pasteur Institute in Dakar, Senegal, and was used for a time to prepare new seed stocks for vaccine manufacture; manufacture was subsequently switched to 17D.

The 17D-204 substrain (and a derivative, 17D-213/77) has been used by all other manufacturers. At passage level 222, the virus was propagated in embryonated eggs to prepare vaccine lot NY 75 (see Fig. 63.9). This vaccine was transferred to the Yellow Fever Laboratory in Bogota, Colombia, where additional egg passages were performed. Colombia No. 88 was returned to the Rockefeller Institute in 1940, whereupon a small number of passages in eggs were made at the Rockefeller Foundation or the Rocky Mountain Laboratory (National Institutes of Health) prior to the manufacture of primary seed stocks in France, the United States, Australia, The Netherlands, Germany, Colombia, South Africa, England, and India (Fig. 63.10). Deep sequencing of the parental Asibi strain and 17D-204 (YF-Vax) found both viruses to contain quasispecies though Asibi actually contained more than the vaccine virus.[122] Furthermore, the analysis indicated that none of the attenuating mutations in 17D-204 were present in Asibi virus quasispecies suggesting that the mutations in the vaccine were discrete and not due to selection of genomes in the wild-type population.

The Pasteur Institute in Dakar switched from 17DD to 17D-204 virus for preparation of secondary seed free from avian leukosis virus. In 1977, the Robert Koch Institute (Berlin) prepared for the WHO a primary seed free from avian leukosis virus, and it maintains this as a reference stock (designated 17D-213/77)[536] available to new manufacturers and as a source for emergency production. The Robert Koch vaccine was acquired by Berna Vaccines and produced in Switzerland. Crucell subsequently acquired Berna Vaccines and developed this vaccine (Flavimun) through late clinical stages but has yet to market the vaccine.[537] Russia is the only other manufacturing site using the 17D-213/77 virus for production. Current 17D-204 vaccines are produced at passage levels between 233 and 239. The 17DD vaccine is at higher passage, and the manufacturer in Brazil (Bio-Manguinhos) has on at least two occasions made new working virus seeds by making an additional passage in eggs rather than from the master virus seed, which was depleted. These additional passages were extensively characterized by sequencing and monkey neurovirulence testing.

The 17DD and 17D-204 substrains are distinguishable by MAb analysis, indicating variations in antigenic determinants on the E protein.[131,132,135,538] Antigenic differences have also been identified between vaccines produced from the 17D-204 substrain by different manufacturers.[132] Examination of yellow fever vaccines manufactured in 11 countries by T_1 oligonucleotide fingerprinting revealed a very high degree of genetic similarity, and sequencing showed all the mutations that distinguish Asibi parental strain from 17D are maintained.[536,539,540] The primary seed and vaccine produced in South Africa differed at one and two oligonucleotides, respectively. This difference was confirmed at the sequence level by Xie,[110] who found two changes in the 3′ noncoding region. A comparison of the nucleotide and amino acid sequences of the 17DD substrain vaccine produced in Brazil, the WHO reference vaccine (17D-213), and 17D-204 strains manufactured in the United States, France, and the United Kingdom, revealed that 17DD vaccine had accumulated fewer nucleotide and amino acid changes per passage during the process of development and attenuation than 17D-204 vaccine viruses.[71,72,539]

Depending on the specific substrain, 17DD and 17D vaccines differ in up to 17 amino acid residues in the coding region.[69,72,539] At 12 other amino acid residues, variability is observed between vaccines belonging to the 17D-204 substrain (including the WHO 17D-213/77 virus); six of these amino acid changes are unique to the China 17D-Tiantan substrain suggesting this is the most divergent.[72] These differences among vaccines do not appear to be related to attenuation, because all vaccine strains are attenuated and one or more vaccine strains has an identical residue at each position to that found in parental (virulent) Asibi virus (refer to Table 63.1 for shared differences of all vaccine strains and Asibi virus that might play a role in attenuation).

The neurovirulence phenotypes of 17DD and 17D-204 substrains have been examined in mice and monkeys.[268,534,541–543] Vaccines were compared with respect to average survival time and mortality after intranasal inoculation of young adult mice. 17DD substrain vaccines were neuroinvasive after intranasal inoculation, whereas those derived from 17D-204 were not. In monkeys subjected to the WHO standard intracerebral neurovirulence test, higher clinical and histopathologic lesion scores were found in the brains of animals inoculated with 17DD than in those inoculated with 17D-204 vaccines.[541,544] A newer study found further differences in the neurovirulence of 17D-204 and 17D-213, with 17D-213 vaccine having lower neurovirulence in monkeys and mice than 17D-204.[534] The difference in neurovirulence between the two substrain 17D vaccines was attributed to mutations in E envelope coding region and NS4B coding region. However, both 17DD and 17D vaccines passed WHO standards and were well within the limits of acceptable safety in the monkey test. Overall, the data suggest that 17DD vaccine may be somewhat more neurovirulent than 17D-204 substrain vaccines, as determined in animal models. In humans, however, this difference has not been appreciated as the rates of serious neurologic adverse events reported through passive surveillance have found slightly lower rates in Brazil (0.37 neurologic disease cases per 100,000 doses) where 17DD is used, compared to rates in the United States (0.8 per 100,000 doses) where 17D-204 is used.[255,259a,545] However, rates varied annually, particularly in Brazil, suggesting that the adverse event rates potentially were similar between the two vaccines. Given these data and the long history of use of both 17DD and 17D vaccines in many millions of persons, there is no reason to suspect that these vaccine substrains differ with respect to safety for humans.

The safety and immunogenicity of the 17DD and 17D-204 lineage (17D-213/77) vaccines have been compared in a clinical trial and had virtually identical safety profiles, seroconversion rates, geometric mean titers, and distribution of antibody titers.[546–548]

Method of Manufacture. Biological standards for yellow fever vaccines have been established by the WHO,[531] and all vaccines approved for international use must comply with these basic standards and be produced according to good manufacturing practices. National control authorities, such as the United States Food and Drug Administration (FDA), govern marketing approval, and thus biological standards differ somewhat from country to country. Manufacturers currently use seeds free from avian leukosis virus, but testing for other viral adventitious agents varies.

Primary (master) and secondary (manufacturer's working) virus seeds are tested for bacteria, fungi, mycoplasma, and adventitious viruses and are subjected to a standard safety and immunogenicity test in rhesus or cynomolgus monkeys.[531] A minimum of 10 monkeys are inoculated intracerebrally with 0.25 mL of virus containing 5000 to 50,000 MLD_{50}, the equivalent in international units to 3.7 to 4.7 $\log_{10}$ IU (see the discussion of potency measurements later in this section). Animals are monitored for clinical signs, viremia, antibody responses, and semiquantitative neuropathologic lesions at 30 days and are subjected to statistical analysis (Table 63.7).[531,549,550] For comparison, a reference control is inoculated into 10 other animals. Some authorities have advised using a reference vaccine with high neuropathologic scores that failed the safety test, such as 17D Lot 6766,[541,549] but generally, when making a new virus seed, manufacturers are advised to use an internal

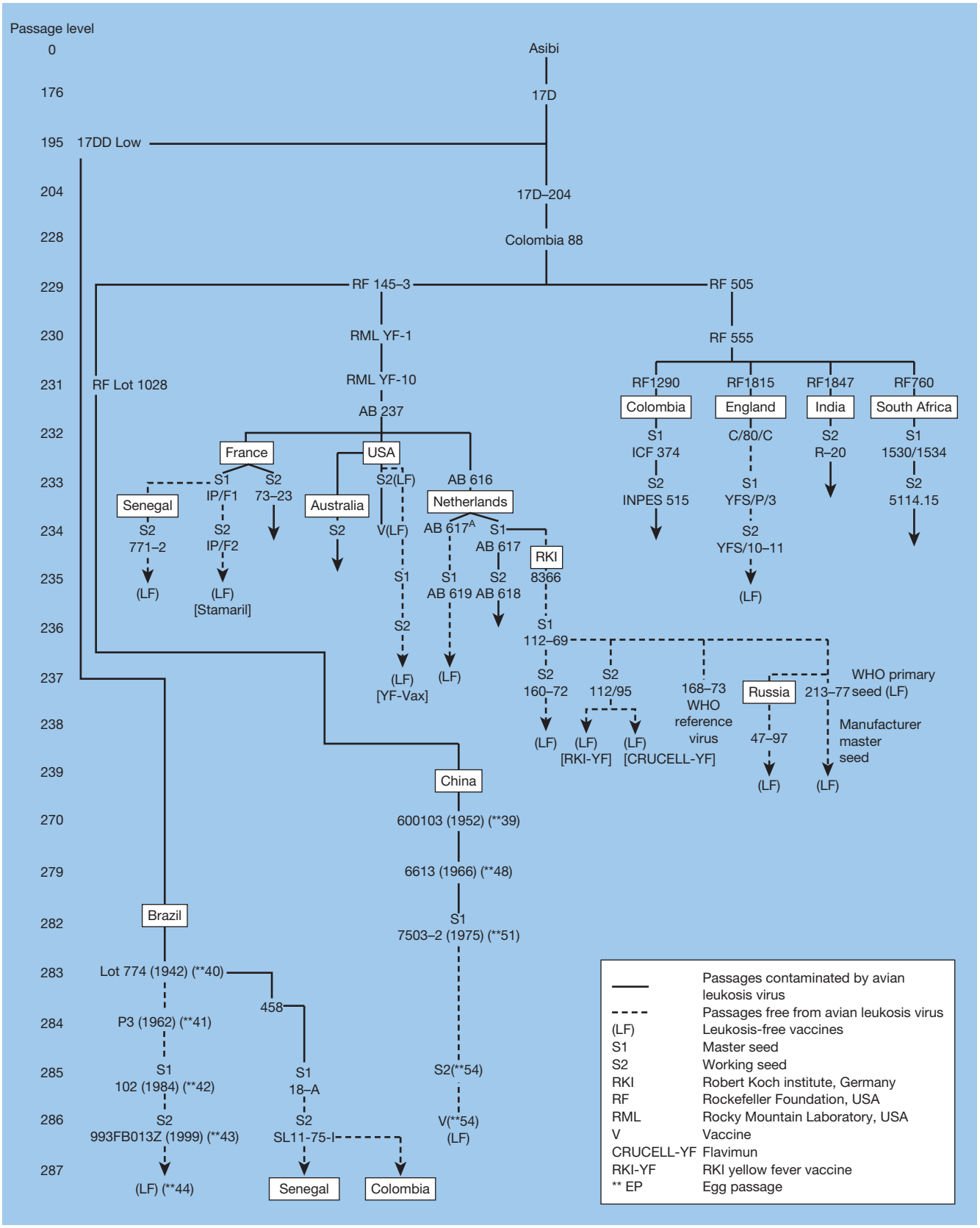


Figure 63.10. History and genealogy of 17D vaccines and reference viruses. *(From World Health Organization.* Recommendations to Assure the Quality, Safety, and Efficacy of Live-Attenuated Yellow Fever Viruses, WHO Document TRS 872, Annex 2. *Geneva, Switzerland: World Health Organization; 2010.)*

TABLE 63.7 Biological Standards for Monkey Safety Tests[a] on 17D Vaccine Seeds

Criterion	Test	Result
Viscerotropism	Viremia level on days 2, 4, and 6	Viremia <500 IU/0.03 mL in all samples, and not greater than 100 IU in more than one sample
Immunogenicity	Neutralizing antibody	>90% seroconversion on day 30
Neurotropism	Clinical observation daily for 30 days	Frequency of encephalitis and semiquantitative clinical score less than reference seed
	Histologic evaluation (day 30)	Mean lesion scores shall not be significantly greater than reference seed[b]

[a]Ten nonimmune rhesus or cynomolgus monkeys are inoculated in the frontal lobe with 5000–50,000 MLD_{50} and monitored for 30 days.

[b]Histologic grading performed on five levels of brain and six of cervical and lumbar spinal cord, using semiquantitative grading score of inflammation and neuronal degeneration. Areas of brain studied include target areas (areas that show more-severe lesions irrespective of degree of neurovirulence of virus tested), spared areas, and discriminator areas that distinguish between vaccines of low and high neurovirulence. Mean group scores for target and discriminator areas combined and for discriminator areas alone are calculated. The mean score for the test monkeys should not be significantly greater (at 5% significance level) than overall mean score for monkeys injected with reference virus.

MLD_{50}, mouse median lethal doses.

World Health Organization. Recommendations to Assure the Quality, Safety and Efficacy of Live Attenuated Yellow Fever Vaccines. *TRS 872, Annex 2. WHO Tech Rep. Ser. Geneva, Switzerland: WHO; 2010.*

reference control (e.g., a current working virus seed) that passed the test previously.[531,534,543]

The neurovirulence test has been extensively deployed for new chimeric vaccines that use 17D as a vector.[542] The sensitivity of this test is quite high, and single mutations in the virus were represented by changes in neuropathologic scores.[86] The value of the test to distinguish a 17D vaccine (seed) that has increased viscerotropism (by measuring viremia) has not been demonstrated and is uncertain.

In a study of rhesus monkeys inoculated by either the intracerebral or subcutaneous route with 17DD vaccine, viremia was measured daily by plaque assay and RT-PCR.[551] The proportion of animals developing viremia following intracerebral inoculation was higher than after subcutaneous inoculation, raising the question whether the neurovirulence test (intracerebral) is an appropriate way to measure viscerotropism. However, considering the combined groups (intracerebral and subcutaneous inoculation), the duration of viremia was only 2 to 6 days, and mean titers by plaque assay were 1.62 (standard deviation [SD], 0.76) $\log_{10}$ PFU/mL.

The genomes of many seed viruses used for production have been sequenced, and this will probably be a future requirement.[552] Minor differences and heterogeneities at individual amino acids occur across vaccines and passage levels used by different manufacturers (see "Substrains" earlier).

Vaccine is produced by aseptic inoculation of working virus seed into viable embryonated eggs.[553,554] Uninoculated control eggs (80 eggs per batch) are included and tested. All current manufacturers, as required, use eggs from closed, special pathogen-free flocks.[531] The dose of seed virus inoculated into 7- to 9-day-old embryonated eggs is 2000 to 5000 MLD_{50} or the equivalent in international units, in 0.1 mL (see the discussion of potency measurements later in this section). After incubation for 3 to 4 days, the peak titer of virus,[555] infected embryos are aseptically harvested. Embryos must be less than 12 days of age at the time of harvest. A single harvest is the pool of embryos inoculated together in a single production run.

Methods for recovery of virus from infected embryos vary, but all include addition of sterile water for injection (typically 1 to 2 mL/embryo), homogenization to pulp, and clarification by centrifugation to yield supernatant fluid harvest. Individual harvests containing homogenate from a single pool of embryos are held frozen pending sterility tests.

The vaccine must be prepared aseptically, because terminal sterile filtration cannot be performed owing to high loss of virus. Sterility tests are performed at one or more steps prior to pooling single harvests into the final bulk, which may contain one or a pool of single harvests. The amount of final bulk prepared is determined principally by the volume capacity of the lyophilizer. The final bulk is diluted and blended with stabilizers (e.g., hydrolyzed porcine gelatin and sorbitol, but stabilizers vary by manufacturer) based on potency (infectivity titer) to produce the drug product, filled into glass vials, and lyophilized. Sterile filtration is not performed because the consistency of the product renders filtration difficult and results in significant loss of titer. The manufacturing process is thus performed aseptically under Class 100 (Class A) conditions.

The target infectivity titer is adjusted for losses expected across lyophilization and losses expected between release and end of shelf life. Consequently, the release titer must exceed the compendial minimum (3 $\log_{10}$ IU). Data from current manufacturers indicate potencies range from 3.5 to 6.0 $\log_{10}$ IU/0.5 mL dose.[552] The excess at release is determined to provide for minimum potency at the end of shelf life (2 or 3 years, depending on the manufacturer). The yield per embryo is typically in the range of 100 to 300 doses.

The original WHO biological standard is tied to mouse MLD_{50} (determined by intracerebral inoculation of mice 4 to 6 weeks of age) or the equivalent in a plaque assay. Because of the variability of the mouse and plaque assays and difficulty in establishing the PFU-to-MLD_{50} ratio, and to assist manufacturers in the control of yellow fever vaccines, the National Institute for Biological Standards and Control (NIBSC, United Kingdom) conducted a study with the aim of establishing an international standard.[556] The study confirmed that the ratio of PFU to MLD_{50} was approximately 10:1. An international standard (NIBSC code No. 99/616) preparation of 17D vaccine was established and was approved by the WHO for use by manufacturers and national control laboratories.[552,556] The new standard for minimum potency is established at 3 $\log_{10}$ IU, which is approximately equivalent to 3 $\log_{10}$ MLD_{50} and 4 $\log_{10}$ PFU.[552]

There has never been an upper limit established for potency, and some vaccine batches have titers up to 6 $\log_{10}$ IU. The current view is that manufacturers should establish their own internal upper limit using trend analysis of multiple batches, and the upper limit should be approved by the national control authority. Interestingly, there are no clinical data comparing 17D vaccines at high versus standard dose but one dose-ranging study using a lower-than-normal standard dose of 17DD vaccine found that there were similar seroconversion rates and viremia levels seen with 10-fold lower doses than standard.[557,558] In animals it is possible to show prozone effects, with lower immunogenicity at high doses (see "Vaccine Substrain or Dose").

The final vaccine bulk is tested for absence of adventitious agents. Standards vary across national control authorities but include tests for bacteria (including *Mycobacterium avium*), fungi, and mycoplasma. Because yellow fever 17D vaccines were developed before the application of modern tests for adventitious viruses, the standards for testing may be less stringent than for new vaccines in some countries. Control (uninfected) eggs are routinely incubated along with eggs used for vaccine manufacture and are tested for viability, sterility, and hemagglutinating viruses. The in vitro virus test in cell cultures and the in vivo virus test in mice, guinea pigs, and eggs are required by some control authorities.

These tests, which require subculture or blind passage, are complicated by the requirement to neutralize the test article (yellow fever 17D virus) in order to observe cultures or in vivo hosts for inapparent viruses. Neutralization requires a potent antiserum that has been raised in a pathogen-free host against yellow fever virus antigen produced in a host other than eggs. The neutralization procedure may be difficult, particularly when high-titer preparations must be tested in sensitive indicator cells or in suckling mice. Protein content before addition of stabilizers may not exceed 0.25 mg/dose.

After filling, the final product is tested for identity by neutralization test, potency, thermostability, sterility, general safety in mice and guinea pigs, residual moisture, residual ovalbumin, and endotoxin. Some countries require that a monkey safety test be performed on each vaccine lot. As specified by the WHO standards,[531] potency must be at least 3 $\log_{10}$ IU/mL. The dose in the final container exceeds the minimum specification by 50-fold to account for potential losses during storage and losses during thermostability testing (see "Vaccine Thermostability" later).

Sanofi Pasteur has conducted extensive analyses of 17D vaccine batches produced over 12 years.[559] The vaccine was genetically stable and homogeneous with respect to plaque size, mouse LD_{50} and average survival time, and virus load measured by quantitative PCR.[560] An important aspect of this and related analyses was the definition of the ratio between genome equivalents and infectious units, which is approximately 1000 : 1,[559,561] which is similar to findings for other flaviviruses. Because the vaccine contains an excess of likely defective particles, this could explain prozone effects seen in animals at high doses. It is also uncertain whether the actual content of infectious virus could exceed that measured in mice or cell culture, and whether the defective virus in the vaccine modulates infectivity in vivo.

Constituents. There are no antibiotics or preservatives in yellow fever vaccines. Because of the risk of bacterial contamination and the thermal instability of the virus after reconstitution, single-dose and (especially) multidose vials must be used within a short time, as specified in the package label: typically 1 hour, or up to 8 hours if maintained on ice. Salt, buffer, and stabilizer excipients vary by manufacturer. The stabilized vaccine manufactured in France by Sanofi Pasteur (Stamaril) contains no animal-derived excipients and is reconstituted in 0.4% saline. Each dose contains 16.0 mg lactose, 8.0 mg sorbitol, 833 µg L-histidine hydrochloride, 362 µg L-alanine, 1.6 mg sodium chloride, 54 µg potassium chloride, 598 µg sodium phosphate-dibasic dodecahydrate, 63 µg potassium phosphate-monobasic, 39 µg calcium chloride, and 29 µg magnesium sulfate. In contrast, the same manufacturer's product made in the United States (YF-Vax) is stabilized with sorbitol and hydrolyzed porcine gelatin and is reconstituted with 0.9% saline. The amount of hydrolyzed porcine gelatin per dose in these stabilized vaccines is approximately 10 mg. Osmolality of 17D vaccines is slightly above normal serum levels.

Adventitious Viruses. In 1966, yellow fever 17D vaccine was discovered to be contaminated with avian leukosis virus.[562] All vaccines produced at that time contained the agent, owing to the high prevalence of infection of chicken embryos. New seeds free of avian leukosis virus were developed in the 1970s, and all manufacturers now employ leukosis-free seeds as stipulated by WHO standards.[531] Although the presence of leukosis virus in yellow fever vaccine is certainly undesirable because of the possibility of insertion of leukosis virus oncogenes, there is no evidence to implicate the virus in human disease. The question was addressed by a retrospective survey of World War II veterans for cancer deaths.[563] The incidence of all cancers, lymphoma, and leukemia was not significantly different (and in fact was lower) in persons vaccinated 5 to 22 years previously with 17D vaccine than in those not vaccinated. All manufacturers are required to use eggs from specific pathogen-free flocks.

Vaccines manufactured in eggs, including yellow fever 17D, test positive by the product-enhanced reverse-transcriptase assay, reflecting the presence of defective particles containing endogenous avian leukosis or retrovirus sequences. No evidence has been found for infectious or inducible replication of competent retroviruses or for infection with avian leukosis or endogenous avian retrovirus in humans.[564–567] However, one study found viral nucleic acid of avian leukosis virus that was attributed to endogenous retroviruses in the chicken germline.[564] Tests for retroviral contamination by amplification in avian cell culture and quantitative PCR may be required for future regulatory approval of yellow fever vaccines made in eggs.

During the original formulation and early use of 17D vaccines, pooled human serum was used as a stabilizer. Vaccines were thereby contaminated with hepatitis B virus, resulting in outbreaks of jaundice.[568–570] Human serum was eliminated from yellow fever vaccines by 1942. Current vaccines carry no inherent risk of contamination with human hepatitis viruses.

Preparations Available, Including Combinations. All yellow fever vaccines approved for distribution are live attenuated 17DD or 17D-204 substrain viruses, prepared in embryonated chicken eggs, and formulated as lyophilized powder. Vaccines vary with respect to stabilizer additives and salt content. Some contain sodium chloride and buffer salts and are reconstituted with sterile water, and others are reconstituted with saline solution.

Vaccines differ with respect to expiration date, but all require storage at the point of use at 2°C to 8°C and should be used within 1 hour after reconstitution. However, for use in mass immunization campaigns and the EPI, reconstituted vaccine may be used during an 8-hour workday if it is maintained on ice. Vaccines are supplied in single-dose and multidose containers with up to 20 doses.[574a]

Vaccines produced in France (Stamaril, Sanofi Pasteur); Brazil (Bio-Manguinhos), Russia (Chumakov Institute), and Senegal (Pasteur Institute) are exported for international use or supplied to the EPI through the UNICEF and PAHO bid markets (i.e., prequalified). The vaccines produced elsewhere are used almost exclusively in-country for travelers and military personnel.

Although certain combination vaccines have been clinically tested (including yellow fever–measles and yellow fever–typhoid), no products are currently commercialized.

Genetic Stability

Vaccine Thermostability. In 1987, the WHO published an addendum to the biological standards, with a guideline (not a formal requirement) that vaccines meet a stability standard.[571] The specification included two criteria: the lyophilized

vaccine held at 37°C for 14 days must maintain minimum potency (>1000 MLD_{50} per 0.5 mL dose) and show a mean loss of titer of less than 1 $\log_{10}$ LD_{50}. Of the vaccines produced by the 12 approved manufacturers at that time, only five met the stability specifications.[572] Current WHO requirements for yellow fever vaccine make the stability standard a requirement and the specification has been reconsidered and determined to be appropriate[531]; vaccines produced worldwide meet the specification.[552]

Without stabilizers, lyophilized yellow fever vaccine loses 1.5 to 2.5 $\log_{10}$/dose during 14 days at 37°C. Stabilized vaccines lose only 0.3 to 0.5 $\log_{10}$ per dose during this interval. In one study, a stabilized lyophilized vaccine produced in Senegal met WHO standards after storage at 2°C to 8°C for more than 3 years.[573,574] Storage at −20°C was superior to storage at 2°C to 8°C. Stabilized yellow fever vaccine has a similar or better stability profile than other thermolabile vaccines used in the EPI (including polio, measles, and pertussis vaccines). Thus, although the lyophilized vaccine requires proper storage and handling under cold-chain conditions in the field, yellow fever vaccine is not the weak link in the EPI system.

Stabilizers differ by manufacturer. The vaccine producers in the United States use sorbitol and gelatin. The YF-Vax product label provides stability data at 35°C to 37°C and 45°C to 47°C.[575] The half-life of the lyophilized vaccine at these temperatures is 14 and 4.5 days, respectively, determined by Vero cell plaque assay. The vaccine made in France is stabilized with sugars, amino acids, and divalent cations (see "Constituents" earlier). A disadvantage of yellow fever vaccine, particularly for multidose containers, is its instability after reconstitution. According to the product label, the vaccine must be held on ice after reconstitution and is discarded after 1 hour, although, for practical use in the field, the WHO recommends that a longer interval (6 hours) may be used if the vaccine is maintained on ice.[531]

During Replication in the Host. 17D vaccines are not plaque-purified and contain heterogeneous subpopulations with differing mouse neurovirulence.[85,110,534,535] One variant recovered from 17D vaccine by plaque purification had a wild-type epitope and amino acid change at position E-173.[84,85] The relative proportion of plaque size variants in 17D vaccines changes with passage.[534,535] Consensus sequencing and later deep sequencing of 17D vaccines revealed nucleotide heterogeneities within structural and nonstructural genes and the 3′ noncoding region, and studies on a 17DD secondary seed and vaccine lots derived from it revealed four nucleotide heterogeneities within structural and nonstructural genes, indicating the quasispecies nature of the vaccine.[71,122,543]

Given the safety record of 17D vaccines since stabilization of passage level, genotypic and phenotypic heterogeneity does not appear to pose a safety problem. No individual plaque variant of 17D virus has shown a mouse neurovirulence phenotype that exceeds the vaccine itself, and thus changes in the relative proportion of variants on replication in humans might not alter virulence. Xie and colleagues[576] sequenced 17D virus strains isolated from the sera of six subjects given the 17D-204 vaccine produced in the United States. No mutations were found in the structural genes, and no more than two nucleotide changes were found in NS5 regions. Similarly, virus strains recovered from sera of monkeys 12 days after intracerebral inoculation of 17D-204 vaccine contained no mutations or a single silent mutation in NS5. These results indicate a high degree of genetic stability of 17D virus during in vivo replication. Mutations tend to accumulate at a significantly lower rate than expected for an RNA virus.[577] In addition, studies employing a truly clonal yellow fever 17D virus as a vector for foreign genes have been very successful, indicating that the quasispecies nature of 17D vaccine is not critical to safety and efficacy and that it should be possible to develop a molecular (or biological) clone of 17D as a vaccine.

Rare mutational events in 17D vaccine during replication in the host have been shown to alter pathogenicity. The first of two published fatal cases of encephalitis occurred in 1965 in a 3-year-old child who received 17D-204 vaccine.[97] Jennings and colleagues[92] defined the characteristics of the virus from brain tissue and commercial 17D vaccines at similar laboratory passage level. Compared to commercial vaccine, the brain isolate had higher neurovirulence for mice, caused severe encephalitis in a cynomolgus monkey, and reacted with a wild-type–specific MAb. The brain isolate differed from 17D-204 at two residues in the E gene (E-155 and E-303) and one in NS4B. There is a potential role for these mutations in neurovirulence, particularly the change at position E-303. A single mutation occurring during in vitro passage in the heterologous E protein of a chimeric yellow fever vaccine increased the neurovirulence of the virus.[86]

The occurrence of serious adverse events, particularly viscerotropic adverse events, raises the question of whether such events are caused by mutations that change virulence or tropism of the virus or selection of virulent variants in vivo. The data obtained so far—consensus sequencing of multiple virus isolates from organs of patients—indicate that host susceptibility rather than a change in the virus is responsible for such events.

Biodistribution. In a study in cynomolgus macaques, the sites of virus replication of 17D vaccine were determined in vivo, and the relationship between neutralizing antibody response and viral clearance was described.[284] 17D vaccine virus was detected in sera of seven (47%) of 15 monkeys between 2 and 5 days after vaccination, with a mean peak titer (±SD) of 67.3 (±94.8) PFU/mL, and a mean number of viremic days of 1.4 (±1.6). Neutralizing antibodies appeared by day 7 in 12 of 15 (80%) monkeys and by day 14 in 100%. Viral RNA was detected on day 3 in the skin (inoculation site), lymph nodes, and bone marrow and on day 7 in the skin, lymph nodes, spleen, thymus, adrenal gland, liver, and bone marrow. By day 14, viral RNA was present only in lymphoid tissues, and by day 46, viral clearance was complete (no data were collected in between days 15 and 45). Virus loads were generally low, in the range of 50 to 500 PFU equivalents (Eq/g).

The observation that human vaccinees shed 17D virus (genomes detected by RT-PCR) in urine after clearance of virus from blood[300] was noted earlier in "Pathogenesis." This observation does not necessarily mean that 17D virus infects the kidney or collecting system, because virus produced elsewhere could be shed in the urine (no 17D virus was found in kidney of vaccinated monkeys).[284] The finding of genomes in urine in humans raises the possibility of persistent infection and a possible explanation for highly durable immunity and persisting IgM antibodies. There are no known clinical consequences; the patients of some concern might be those who sustained neurotropic adverse events, but neurologic abnormalities appearing late (years) after the acute event have not been described.

Dose and Route of Administration. Yellow fever 17D vaccine is given by the subcutaneous route in a volume of 0.5 mL, generally in the upper arm. Use of the subcutaneous route of inoculation in humans followed the exhaustive preclinical evaluations of 17D virus in nonhuman primates at the Rockefeller Institute in the 1930s, in which this route of inoculation was employed, and the subcutaneous route is nearly universally used in practice. The Summary of Product Characteristics approved by the European Union Committee for Medicinal Products for Human Use for the 17D vaccine

manufactured in France (Stamaril) specifies that the vaccine may be given by either the subcutaneous or intramuscular route. However, there are no clinical data comparing the two routes of immunization with 17D virus, and there is only one extant published report of intramuscular inoculation.[483]

The minimum dosage requirement is 3 $\log_{10}$ IU/dose. Because commercial vaccines contain an excess of virus to provide for losses during storage, the delivered dose is higher than the minimum standard. Most vaccines contain around 3.7 to 4.7 $\log_{10}$ IU/dose on release, but some have potencies as high as 6 $\log_{10}$ IU/dose.[552]

As discussed in detail under "Vaccine Substrain and Dose," there are safety and efficacy concerns related to dosage, both at the low and high ends of the dosage range. To stretch vaccine supplies, there is renewed interest in dosage-sparing approaches. The WHO recently released a white paper (information paper) that was endorsed by the Strategic Advisory Group of Experts (SAGE) for the use of fractional doses of the vaccine for outbreak responses.[574a] Clinical studies have shown some promising results with 10-fold reduction in vaccine dose producing similar viremia levels and seroconversion rates to a standard (full) dose.[557,558]

Attempts to immunize monkeys with 17D vaccine by the oral route or by the intestinal route using acid-stable capsules were not successful,[199] but application of virus by intranasal spray resulted in uniform seroconversion.[579] Intranasal immunization has not been attempted in humans. The residual neurotropism of 17D virus raises safety concerns because of the possible exposure of olfactory neurons in the nasal cavity and potential for direct access to the brain via the ethmoid route. The finding of neurotropic disease in three breastfed infants whose mothers were vaccinated[203–205] does suggest that either intranasal or gastric inoculation might result in effective inoculation with seroconversion but could also result in neurologic adverse events.

Skin Immunization. In 1950, a decision was made in Nigeria to prepare 17D vaccine for immunization by scarification, which would simplify and reduce the cost of delivery and would permit use of formulations with less-stringent requirements for sterility.[580] Beginning in 1951, trials were initiated using 17D vaccine alone, 17D combined with smallpox vaccine, or 17D and smallpox vaccine given together but at different sites.[580–584] The vaccine was prepared in eggs or by passage of the egg vaccine in mouse brain. Clinical trials revealed lack of seroconversion in up to 15% of those vaccinated with 17D alone and in 35% of those receiving combined yellow fever and smallpox immunization.[585] Revaccination with 17D at an interval of 14 days increased the seroconversion rate.[586]

Studies in West Africa and Malaya suggested that the cause of these vaccine failures might have been cross-protective heterologous flavivirus immunity.[587–589] A head-to-head comparison of 17D and French neurotropic vaccine delivered by scarification showed a higher seroconversion rate in subjects given the French vaccine.[580] The apparent lower seroconversion rate of 17D applied by scarification was probably not because of vaccine titer differences,[586] but rather a result of the inherently higher capacity of French neurotropic vaccine to replicate at the dose delivered by this method. A newer study that delivered 17D vaccine either subcutaneously or transcutaneously to persons with and without atopic dermatitis found that persons who received 17D vaccine transcutaneously had lower rates of seroconversion (82%) 30 days postvaccination than did those receiving the vaccine subcutaneously (91%) (P = .059).[590] When seroconversion rates for normal controls were compared, the difference was significant at 100% (21/21) for subcutaneous and 74% (14/19) for transcutaneous delivery (P = .02). Seroconversion rates for patients with atopic dermatitis were similar between subcutaneous (93%) and transcutaneous (90%) delivery but were lower overall; in this group an inverse correlation between the immune response and IgE levels were observed. The authors thought that the lower seroconversion rates with transcutaneous delivery were partly from inadequate viral penetration and infection of the skin. Previously, scarification was not adopted for routine delivery of 17D vaccine in anglophone Africa owing to similar uncertainties about efficacy, as well as lack of an efficient cold-chain, absence of a public health policy for yellow fever vaccine production and immunization after independence from colonial rule, and the development of improved methods for subcutaneous vaccine delivery (the jet injector).

The early workers who used the epidermal route of immunization (scarification) had no specific knowledge of the role of Langerhans cells as the initial target cell in human infection with flaviviruses. Needleless immunization of the skin, or the use of microneedles that deliver live vaccine to the epidermis in a more controlled fashion than the original scarification methods, offer considerable promise as an improved vaccination method for 17D and other live flavivirus vaccines.[591] Monkeys were efficiently immunized with a chimeric yellow fever 17D and Japanese encephalitis vaccine delivered by rubbing the skin with vaccine and a device that strips the stratum corneum.[201]

A clinical trial of 17D vaccine was conducted in 155 naïve and 20 previously vaccinated subjects randomized 1:1 to receive intradermal injection of 0.1 mL (one-fifth of a standard dose) or subcutaneous injection of a full dose.[592] One hundred percent of subjects developed neutralizing antibodies, and no differences in titer were observed between intradermal and subcutaneous delivery. The intradermal group had increased erythema and swelling at the site of inoculation, consistent with other vaccines given by this route. The intradermal route is attractive as a means of needleless delivery and dose sparing.

Cost of Manufacture and Vaccine Price. The cost of manufacture of yellow fever vaccine is modest, but it is highly dependent upon local conditions (personnel wages, overhead, etc.). Price and sales margins vary by market segment. In 2013, the cost of yellow fever vaccine for UNICEF, which procures the vaccine for use in Africa, and for prices on PAHO bid orders for vaccine supplied to South American countries in five- and 10-dose containers, varied between US$0.67 and US$1.40 per dose.[532] In contrast, the price charged to the patient in travel clinics in the United States is US$150 to US$350 per dose.[461,593]

RESULTS OF VACCINATION

Immune Responses to Vaccination

Innate Immune Responses to Vaccination

Innate immune responses to yellow fever vaccine serve two important functions: they provide relatively immediate protective expression of antiviral molecules that stimulates and modulates the adaptive immune response, including a mixed T-helper (Th)1/Th2 $CD4^+$ response and rapid activation of $CD8^+$ cytotoxic T cells.[594,595] The immunologic basis for susceptibility to serious (viscerotropic) adverse events following 17D vaccine is suspected to lie in deficits or dysfunctions in the innate immune response. It is generally believed that the strong activation of innate immunity is central to why yellow fever vaccine is such a strong immunogen and generates such long-lived immunity by virtue of early development of Th2 memory $CD4^+$ cells and persistent B-cell populations.

Within the first few days after vaccination, a robust innate immune response is generated, peaking on day 7 and

diminishing thereafter, that includes NK cell activation, Toll-like receptor (TLR) activation, non–TLR-dependent sensor activation, proinflammatory cytokines, IFNs, and various IFN-stimulated gene products with antiviral activity appearing during the early phase of virus replication, prior to the advent of virus-specific cytotoxic T cells and Ig.[596,597] In peripheral blood, increases in percentages of $CD86^+$ myeloid dendritic cells, plasmacytoid dendritic cells, monocytes and $CD14^+$ $CD16^+$ inflammatory monocytes are observed.[595]

In vaccinated humans, a pattern of early activation of NK cells and monocytes has been observed, including upregulated expression of TLR3 and TLR9 and activation-related markers CD69, CD38 and CD16, increased expression of major histocompatibility complex (MHC) class II antigens, and increased levels of circulating IFN-γ.[598] CD38 is known to trigger cytotoxic activity of NK cells, and CD16 is involved in antibody-dependent cell-mediated cytotoxicity. TLR signaling also controls the life span of dendritic cells by modulating apoptotic and antiapoptotic molecules, an effect that can dramatically affect immune responses. Following vaccination, 10 of 15 subjects developed circulating IFN-α.[599] IFN appeared and peaked 24 hours after viremia, in concert with the appearance of HI antibodies, and levels declined rapidly thereafter.[599] These results are consistent with a subsequent study showing that 2′,5′-OAS activity in peripheral blood mononuclear cells increased by day 4 and peaked on day 7 after 17D immunization.[600]

The role of IFN in clearance of 17D and inhibition of the immune response to coadministered vaccines has not been clarified. It is likely that common side effects of 17D vaccine (headache, myalgia, asthenia) and the mild leukopenia associated with vaccination are related to release of IFN-α and other cytokines.[596,601] Increases in serum β_2-microglobulin, neopterin,[358] and TNF-α have been found also during the first week after 17D vaccination, but not after revaccination. The increase in β_2-microglobulin reflects activation of T lymphocytes bearing MHC class I antigens, and neopterin is released by monocyte/macrophages in response to IFN-γ released by activated T lymphocytes. Two cytokines found to be consistently elevated after 17D vaccination were IFN-γ–induced protein 10 (IP-10) and IL-1α.[594] This finding is similar to results obtained with dengue, West Nile virus, and tickborne encephalitis.

Silva and colleagues[602] phenotyped proinflammatory and regulatory cytokines expressed on peripheral blood cells of patients following yellow fever vaccination. During the first week, innate immune cells increased including TNF^+ neutrophils and monocytes and $IFN\text{-}\gamma^+$ NK cells. Later (days 15 to 30), regulatory cytokine expression, such as $IL\text{-}10^+$ monocytes, was increased. Their studies showed that a proinflammatory cytokine pattern occurred early, followed later by appearance of a mixed proinflammatory–regulatory cytokine pattern.

Systems biology approaches have now been applied to the elucidation of genes activated and downregulated in healthy human subjects following 17D vaccination.[594–596,603–605] In several studies, as many as 125 different genes were upregulated after 17D vaccination. Genes that involved recognition of foreign (viral) RNA included membrane-associated sensors, activated TLR2, TLR7, TLR8, and TLR9 (TLR3 is likely activated also), non–TLR-dependent cytoplasmic sensors, retinoic acid–inducible gene I *(RIG-I)*, and melanoma differentiation–associated gene 5 *(MDA5)*. For example, TLR7 sensors transduce a signal, via the adaptor MyD88 (myeloid differentiation 88), to upregulate expression of inflammatory cytokines such as IL-6, IL-12, and TNF (via mitogen-activated protein kinase and nuclear factor κB activation) and type I IFNs (via IFN regulatory factor 7 activation). These pathways involve multiple intermediate molecules, such as TRIM (tripartite motif) and TNFR-associated factor (TRAF), and ubiquitination steps, and the transcriptional profiling demonstrated the activation of these gene pathways. TLR2 pathways are associated with cytokines promoting a Th2 phenotype, and TLR7, TLR8, and TLR9 are associated with Th1 pathways. The pattern of TLR activation is consistent with the finding of a mixed Th1/Th2 $CD4^+$ cell response to 17D vaccine.

With the resulting production of IFN-α and IFN-β, there was broad activation of multiple IFN-induced genes involved in antiviral responses including STAT1; OAS 1, OAS 2, OAS 3, and IFN-stimulating gene (ISG) 20, which degrade viral RNA double-stranded RNA-activated protein kinase (PKR); MX1, MX2, and EIF2AK2, which inhibit viral replication; and ISG15 involved in ISGylation and ubiquitination.[594,595,600,603] Other major pathways and transcriptional elements that were upregulated include integrated stress response genes, complement system genes, and monocyte/macrophage and NK cell–associated genes. Activation of the complement system included C1Qa, C1Qb, C3AR1, and SERPING1. The complement system has been shown in studies of West Nile virus to be critical to survival and T- and B-cell responses.[606] NK cell–associated genes are upregulated, and these cells show increased expression of TLR3 and TLR9 and various markers, including CD69, CD38, and CD16.[598]

Activation of inflammasome includes upregulation of caspase-1, caspase-5, and IL-1β production. Activation of the inflammasome may in turn depend on TLR triggers, IFN regulatory factor 3, and activation of nuclear factor κB. IL-1β may serve as an adjuvant to T- and B-cell responses. An important transcriptional element upregulates ETS2, which transduces multiple genes involved in activating NK cells, antigen-presenting cells, and T and B cells. TNF receptor superfamily (TNFRSF) genes are activated, including TNFRSF17, a receptor for B-cell activating factor (BAFF, also known as TNFSF13B) that is known to regulate B-cell responses.

Investigation of the genes activated following vaccination with 17D have provided a more-detailed view of the interplay of innate and adaptive responses and have elucidated specific gene signatures correlating with T- and B-cell responses.[594,595,603] A gene signature, including EIF2AK4 and C1qB, accurately correlated with $CD8^+$ T cell responses, whereas B-cell growth factor TNFRS17 predicted the neutralizing antibody response with up to 100% accuracy.[594] Activation of genes associated with B-cell activation tended to occur late (days 10 to 14), in concert with the appearance of neutralizing antibodies. *FOXO3A* and *E2F1* gene activation is also notable in these studies[595] because these genes are mediators of apoptosis, which is an early event in 17D infected cells, compared to wild-type yellow fever, and may be an important factor in the attenuation phenotype of 17D.[607]

The bridge between the innate and adaptive response depends on upregulation of MHC and costimulatory molecules (e.g., CD40, CD80, and CD86) on dendritic cells, recruitment of and antigen presentation to $CD4^+$ and $CD8^+$ T cells, control of dendritic cell life span, and IL-6–induced suppression of regulatory T cells. Owing to the activation of multiple TLRs, a mixed Th1/Th2 cytokine and T-helper response is evoked.[603,608] The very robust innate response to 17D virus probably underlies the rapid, strong, and durable adaptive responses. It is also likely that patients who have an unfavorable outcome to disease and vaccination have defects in innate and adaptive immune responses that result in virus outracing immunity during the early stage of infection or develop immunopathologic responses during the later stages, such as cytokine storm, through a dysregulated immune response characterized by an overexuberant proinflammatory cytokine response.[275]

Adaptive Immune Responses to Vaccination

The key protective elements of immunity to yellow fever are T cells and neutralizing antibodies. Yellow fever vaccine induces cytotoxic $CD4^+$ and $CD8^+$ cells that destroy cells infected with the vaccine virus (or wild-type virus, if exposed) and a mixed Th1/Th2 $CD4^+$ helper response for antibody production during the second week. Neutralizing antibodies elicited by the vaccine in advance of exposure to the bite of an infected mosquito are the key mediators of immunity and prevent virus replication and spread. Patients with defective innate or adaptive immunity can develop severe infections caused by 17D vaccine; fortunately these events are very rare.

Humoral Immune Responses to Vaccination. Although the immune response to 17D vaccine is broad and involves all arms of the immune system, humoral immunity mediated by viral-specific antibodies is the primary protective element of immunity. Using knockout mouse models, $CD4^+$ T cells, B cells, and antibodies were all found to be required to provide the mice full protection against a subsequent challenge.[609] The principal role of humoral antibodies in protection was also shown by transferring serum from immune hamsters and monkeys before or shortly after challenge with virulent yellow fever.[280,479,610]

Test Methods Affecting Interpretation of Antibody Responses. The neutralization test is the gold standard for measuring immunity following vaccination. Unfortunately, it is not widely available and is difficult and time consuming.

Various methods have been used to measure neutralizing antibodies, and these methods vary with respect to sensitivity (Table 63.8). Tests in mice were used through the 1950s and in some laboratories thereafter, but these tests were subject to considerable variability (reviewed by Smithburn[611]). The intraperitoneal neutralization test in newborn or 18- to 21-day-old mice was shown to be more sensitive for detecting neutralizing antibodies than the intracerebral test, owing to the higher and more rapid lethality of virus for mice inoculated by the latter route.[612,613]

Tissue culture neutralization tests have replaced tests in mice. These tests avoid the need for animals, are considerably more convenient and less costly, provide results in a shorter time (5 to 7 days vs 21 days in mice), and are more reliable and quantitative.[612,614,615] A standardized plaque-reduction test has been described,[612,616] but these reports are quite old.

A variety of continuous cell lines, including monkey kidney cells (MA-104, LLC-MK2, and Vero), hamster kidney (BHK-21), porcine kidney (PS), and primary chick or duck embryo cells, may be used for plaque assays. A comparative study showed that the intraperitoneal neutralization test performed in newborn mice was a somewhat more sensitive assay for neutralizing antibodies than the constant serum-varying virus plaque-reduction test in MA-104 cells.[612] The cell culture test was, however, more sensitive than the intracerebral suckling or adult mouse tests. Similarly, Poland and colleagues[614] showed that a constant virus-serum dilution plaque-reduction test in Vero cells was more sensitive than the intracerebral adult mouse neutralization test.

Differences in test sensitivity account for the variable geometric mean antibody responses noted in clinical trials (Table 63.9).[329,358,361,537,547,548,592,601,617–647] However, they do not appear to influence seroconversion rates, when seroconversion is measured relatively early (e.g., 1 month or several months) after vaccination. The determination of low antibody levels, for example many years after vaccination, may be problematic if an insensitive test is used. In a study of persons vaccinated 30 to 35 years earlier, Poland and colleagues[614] found that the intracerebral adult mouse neutralization test had a 52% false-negative rate compared to plaque reduction, with the false-negative sera having low antibody titers.

A standardized assay was not used across clinical trials (see Table 63.9). The serum-dilution plaque-neutralization test has been used in recent years as the method of choice in many clinical studies of yellow fever vaccines, including randomized trials.[496,547] The percentage of plaque reduction used to define the titer end point has varied between 50% and 90% (50% has been the most commonly used end point for vaccine

Text continued on p. 1226

TABLE 63.8 Methods Used to Measure Yellow Fever Antibody Responses in Animals and Humans

Host	Description	End Point	Comment
Monkey (rhesus)	Simultaneous SC or IP inoculation of test serum and virus	Death	Used in early studies before development of mouse model
Mouse (adult)	IC inoculation of preincubated mixture of serum with constant virus dose (100 LD_{50})	Survival ratio or survival time; quantitative test (log_{10} neutralization index) measures difference in virus titer between test and control sera	In screening test, survival time end point was more sensitive
Mouse (adult)	IC inoculation of starch followed by IP inoculation of serum–virus mixture	Survival ratio	Extensively employed in early serologic surveys
Mouse (18–21 days old)	IP inoculation of serum–virus mixture	Survival ratio	More sensitive (higher serum antibody titers) than adult mouse tests
Mouse (newborn)	SC, IP, or IC inoculation of serum–virus mixtures	Survival ratio	
Cell culture	Constant serum-varying virus dilution	Log_{10} neutralization index determined by plaque reduction or cytopathic effect	Measures quantity of virus neutralized by undiluted or low dilution of serum
Cell culture	Constant virus-varying serum dilution	Highest serum dilution reducing a defined proportion of plaques	Sensitivity varies with end point selected (50%, 70%, 90% plaque reduction)

IC, intracerebral; IP, intraperitoneal; LD_{50}, median lethal dose for 50% of test subjects; SC, subcutaneous.

TABLE 63.9 Seroconversion Rates and Neutralizing Antibody Responses to Yellow Fever 17D Vaccine in Clinical Trials

Vaccine Manufacturer	Vaccine Specification	Dose	Method	Interval[a]	Age	Study Site	N	Seroconversion (%)	Titer	Test Used	Reference
National Drug Co. (U.S.)		6.1–6.4 $\log_{10}$ $SMLD_{50}$	NSI	21 days	Adults	U.S., Japan	41[b]	100	>500	SDNT (IC, mice)[c]	Wisseman et al, 1962[633]
National Drug Co. (U.S.)		6.8 $\log_{10}$ MLD_{50}	JI	3 wk	5–54 mo	Burkina Faso	72	97	NT	LNI (IC, mice)	Meyer et al, 1964[626]
Wellcome (U.K.)	ALV contaminated	4.1–4.4 $\log_{10}$ MLD_{50}	NSI	1 mo	Adults	England	38	97.4	1.6	LNI (IC, mice)	Draper, 1967[646]
	ALV free	4.1–4.4 $\log_{10}$ MLD_{50}	NSI	1 mo	Adults	England	59	98.3	1.7	LNI (IC, mice)	
National Drug Co. (U.S.)	ALV contaminated	6.2–6.7 $\log_{10}$ MLD_{50}	NSI or JI	28 days	Adults	U.S.	187	>99	2.2–3.5	LNI (cell cult)	Tauraso et al, 1972[632]
	ALV free	5.6–6.0 $\log_{10}$ MLD_{50}	NSI	28 days	Adults	U.S.	187	>98	2.2–3.1	LNI (cell cult)	
National Drug Co. (U.S.)	Administered with or without smallpox vaccine at varying intervals	4.3–5.0 $\log_{10}$ MLD_{50}	NSI	28 days	Adults	U.S.	483	99.8	2.2–2.6	LNI (cell cult)	Tauraso et al, 1972[627]
Wellcome (U.K.)	Not stabilized	4.4 $\log_{10}$ MLD_{50}	NSI	28 days	Adults	England	10	100	2.7	LNI (cell cult)	Freestone et al, 1977[634]
	Stabilized	4.2 $\log_{10}$ MLD_{50}	NSI	28 days	Adults	England	20	100	2.9	LNI (cell cult)	
Institut Pasteur (Senegal)		ND	JI	25 days	Adults and children	Gambia	41	92.7	≥13.1	PRNT	Monath et al, 1980[329]
Wellcome (U.K.)	ALV free, stabilized	>3 $\log_{10}$ MLD_{50}	NSI	2–11 wk	Adults	England	600	96.0	2.19	LNI (cell cult)	Moss-Blundell et al, 1981[624]
Connaught (U.S.)	ALV free	3.7 $\log_{10}$ PFU ($LLC\text{-}MK_2$)	NSI	1 mo	Adults	U.S.	28[d]	100	415	PRNT	Bancroft et al, 1984[635]
Pasteur Mérieux (France)	Stabilized, ALV free	5.3–5.4 $\log_{10}$ PFU (PS)	NSI	1 mo	1–5 yr	Central African Republic	209	94	See note[e]	PRNT	Georges et al, 1985[628]
Pasteur Mérieux (France)	Not stabilized	ND	NSI	1 mo +	Adults	France	143	99.3	14	PRNT	Roche et al, 1986[636]
	Stabilized	ND	NSI	1 mo +	Adults	France	115	100	13	PRNT	
Institut Pasteur (Senegal)	Combined with DTP-polio, measles with or without HBV	ND	NSI	60 days	18–26 mo	Senegal	188	91.5–93.6	19.4–31.8	PRNT	Yvonnet et al, 1986[637]

FioCruz (Brazil)	17DD substrain; not stabilized	3.7 $\log_{10}$ MLD_{50}	NSI	28 days	Adults	Brazil	15	100	1656	PRNT	Lopes Ode et al, 1988[638]
	17DD substrain; stabilized	3.8 $\log_{10}$ MLD_{50}	NSI	28 days	Adults	Brazil	31	100	1790	PRNT	
Pasteur Mérieux (France)	Stabilized, ALV free	ND	NSI	45 days	6–7 mo	Côte d'Ivoire	50	91	>1:5	PRNT	Lhuillier et al, 1989[629]
Pasteur Mérieux (France)	Stabilized, ALV free	3.91 $\log_{10}$ MLD_{50}	NSI	30 days	6–12 mo	Cameroon	68	92.6	22.63	PRNT	Mouchon et al, 1990[639]
Pasteur Mérieux (France)	Stabilized, ALV free	ND	NSI	195–240 days	4–8 mo	Mali	52	96.2	19.8	PRNT	Soula et al, 1991[640]
	Stabilized, ALV free	ND	NSI	195– 240 days	12–24 mo	Mali	19	94.7	29.5		
Connaught (U.S.)		ND	BJS	4–6 wk	Adults	U.S.	32	81	49.5	PRNT	Jackson et al, 1993[641]
Pasteur Mérieux (France)	Stabilized, ALV free	ND	JI	2–4 wk	Adults	Nigeria	331[f]	88.5	NT	PRNT	Nasidi et al, 1993[625]
Pasteur Mérieux (France)	Stabilized, ALV free	ND	NSI	45 days	Adults	France	36	100	NT	PRNT	Receveur et al, 1993[630]
Pasteur Mérieux (France)	Stabilized, ALV free	3.9 $\log_{10}$ $TCID_{50}$	NSI	35 days	Adults	Europe	41	100	26.6	PRNT	Ambrosch et al, 1994[642]
Pasteur Mérieux (France)	Combined with measles or HBV + measles	ND	NSI	30 days	9 mo	Senegal	172	96	16.8	PRNT	Coursaget et al, 1995[643]
BioManguinhos (Brazil)	17DD substrain; stabilized	ND	JI	6 mo	>6 y	Brazil	161	86.8	NT	PRNT	Guerra et al, 1997[631]
Pasteur Mérieux (France)	Combined with HAV and typhoid	ND	NSI	28 days	Adults	Switzerland	56	100	752	PRNT	Dumas et al, 1997[644]
Connaught (U.S.)	Stabilized, ALV free	ND	NSI	28–30 days	Adults	U.S.	~35	Not specified	226.3	PRNT	Dukes et al, 1996[645]
Robert Koch (Germany)	Stabilized, ALV free	ND	NSI	26 days	Adults	Germany	12	100	88	PRNT	Reinhardt et al, 1998[358]
Institut Pasteur (Senegal)	Stabilized, ALV free	ND	NSI	3 mo	6 mo, 9 mo	Ghana	139, 150	98.6, 98.0	158.5, 129.8	SDNT (Vero)[g]	Osei-Kwasi et al, 2001[620]

Continued on following page

TABLE 63.9 Seroconversion Rates and Neutralizing Antibody Responses to Yellow Fever 17D Vaccine in Clinical Trials *(Continued)*

Vaccine Manufacturer	Vaccine Specification	Dose	Method	Interval[a]	Age	Study Site	N	Seroconversion (%)	Titer	Test Used	Reference
Pasteur Mérieux (France)	Stabilized, ALV free	ND	NSI	10–14 and 28 days	Adults	U.K.	93	D10–14: 86 D28: 100	124 (D28)	PRNT	Lang et al, 1999[622]
Evans Medical[h] (U.K.)	Stabilized, ALV free	ND	NSI	10–14 and 28 days	Adults	U.K.	92	D10–14: 88 D28: 99	91 (D28)	PRNT	
Aventis Pasteur[i] (U.S.)	Stabilized, ALV free	ND	NSI	30 days	Adults	U.S.	291	99	2.21	LNI	Monath et al, 2002[601]
Evans Medical (U.K.)	Stabilized, ALV free	ND	NSI	30 days	Adults		283	98	2.06	LNI	
Aventis Pasteur[i] (U.S.)	Stabilized, ALV free	ND	NSI	30 days	Children 9 mo-10 y	Peru	369	90.6	1.26	LNI	Belmusto-Worn et al, 2005[619]
Evans Medical (U.K.)	Stabilized, ALV free	ND	NSI	30 days	Children 9 mo-10 y	Peru	738	94.9	1.32	LNI	
Berna Biotech[j] (Switzerland)	Stabilized, ALV free	$\geq 6.3 \times 10^3$ PFU/0.5 mL	NSI	29 days	Adults	Switzerland	143	100	1,184	PRNT	Barban et al, 2007[559]
Robert Koch (Germany)	Stabilized, ALV free	$\geq 6.3 \times 10^3$ PFU/0.5 mL dose	NSI	29 days			73	100	1,050	PRNT	
Aventis Pasteur (France)	Stabilized, ALV free	$\geq 6.3 \times 10^3$ PFU/0.5 mL dose	NSI	29 days			72	100	612	PRNT	
Aventis Pasteur[i] (U.S.)	Stabilized, ALV free	ND	NSI	30 days	Adults	U.S.	11	91	2.29	LNI	Monath et al, 2003[617]
Bio-Manguinhos (Brazil) 17DD 013Z[k]	Stabilized, ALV free	≥ 1000 MLD_{50}/0.5 mL	NSI	≥30 days	14.8–67.7 y	Brazil	270	98	14,536	PRNT	Camacho et al, 2004[547]
17DD 102–84	Stabilized, ALV free	≥ 1000 MLD_{50}/0.5 mL	NSI	≥30 days			270	99.5	15,691	PRNT	
17D-213/77	Stabilized, ALV free	≥ 1000 MLD_{50}/0.5 mL	NSI	≥30 days			270	99.5	18,649	PRNT	
Placebo	N/A	N/A	NSI	≥30 days			271	6.4	32	PRNT	
Bio-Manguinhos (Brazil) 17DD	Stabilized, ALV free	ND ND	NSI NSI	30 days 30 days	Adult primary vaccinees Adult revaccinees	Brazil Brazil	8 9	100 88	30,321 mIU/mL 5840 mIU/mL→28,526 mIU/mL	PRNT PRNT	Santos et al, 2005[618]

Aventis Pasteur (Sanofi Pasteur) (U.S.)	Stabilized, ALV free	ND	NSI	35–365 days	Adult revaccinees Prevaccination PRNT 1:10	U.S.	829	78	GMT 10→101	PRNT	Hepburn et al, 2006[621]
		ND	NSI	35–365 days	Prevaccination PRNT 1:20–40	U.S.	121	65	GMT 26→109	PRNT	
		ND	NSI	35–365 days	Prevaccination PRNT >1:40	U.S.	79	10	GMT 152→177	PRNT	
Sanofi Pasteur (France)	Stabilized, ALV free	3.5×10^4 PFU	NSI	2,4,8, 52 wk	Adult, primary vaccinees	Netherlands	78	100	~ 128 (peak, 4 wk)	PRNT	Roukens et al, 2008[592]
		7.0×10^3 PFU	Intradermal	2, 4, 8, 52 wk			77	100	~ 128 (peak, 4 wk)	PRNT	
Bio-Manguinhos (Brazil) 17DD	Stabilized, ALV free	ND	NSI	Multiple times to 1 y	Adult, primary vaccinees (82% seropositive to dengue)	Brazil	238	100	GMT (1–2 mo) 912	PRNT	de Melo et al, 2011[623]
Bio-Manguinhos (Brazil) 17DD	Stabilized, ALV free	4.7 log10 PFU	NSI	30 days	12–23 mo	Brazil	457	89	3,664 mIU/mL	PRNT	Nascimento Silva et al, 2011[548]
17D-213/77	Stabilized, ALV free	4.91 log10 PFU	NSI				428	87	3106 mIU/mL	PRNT	
Sanofi Pasteur (U.S.)	Stabilized, ALV free	ND	NSI	30 days	18–40 y	U.S.	80	100	Log_2 8.3–8.90	PRNT	Edupuganti et al, 2013[361]
Sanofi Pasteur (France)	Stabilized, ALV free	ND	NSI	29 days	18–60 y	Germany, Czech Republic	201	97–100	GMTs 9→5244	PRNT	Alberer et al, 2015[647]

[a]Interval between vaccination and serologic testing.
[b]Volunteers in the study with no preexisting yellow fever immunity.
[c]Serum dilution–constant virus neutralization test performed in suckling mice inoculated IC.
[d]Seventeen of 28 subjects had previously received an experimental vaccine against dengue type 2.
[e]Seventy-eight percent of vaccinees had high N antibody titers (≥320).
[f]Includes all subjects in the study except pregnant women.
[g]Serum dilution–constant virus neutralization test in Vero cell cultures using cytopathic effect as the end point.
[h]Vaccine bulk manufactured by Wellcome and formulated for sale by Evans Medical; Powderject Corp, and then Chiron Vaccines acquired this company.
[i]Formerly Connaught, then Pasteur Mérieux Connaught, then Aventis Pasteur, currently Sanofi Pasteur.
[j]Flavimun, derived from the 17D-213–77 secondary seed stock also used to produce the Robert Koch Institute vaccine, manufacture of which was discontinued.
[k]Lot produced from a new secondary seed lot; 17DD 102–84 is vaccine prepared from the previous secondary seed lot.
ALV, avian leukosis virus; BJS, BioJect system; cult, culture; DTP, diphtheria, tetanus, pertussis vaccine; GMT, geometric mean titer; HAV, hepatitis A virus; HBV, hepatitis B virus; IC, intracerebral [injection]; JI, jet injector; LD_{50}, median lethal dose; LLC-MK_2, monkey kidney cells; LNI, log neutralization index; MLD_{50}, mouse LD_{50} performed per WHO requirements in 4- to 6-week-old animals; M, mouse neutralization test; N/A, not available; ND, not determined, but vaccine meets WHO standards (>1000 MLD_{50}); NSI, needle and syringe injection; NT, not tested; PFU, plaque-forming unit (titration in cell culture; cell type specified); PRNT, plaque-reduction neutralization test with geometric mean serum dilution; PS, porcine kidney cells; SDNT, serum dilution neutralization test; $SMLD_{50}$, suckling mouse LD_{50}; $TCID_{50}$, median tissue culture infective dose; WHO, World Health Organization.

studies but 90% is preferred for diagnostic evaluation). Some investigators have expressed the titer end point as a reciprocal dilution of the highest serum dilution reducing plaques by the specified percentage; others have attempted to standardize the test with an international reference serum and expressed the titer in IU/mL.[547] Hence the sensitivity (reflected by geometric mean antibody titer) has varied considerably, presumably owing to the methodological differences in virus strains and cell lines used, as well as the use of accessory factor (complement), agar overlay composition, and plaque-detection staining methods.[556] These variables make it impossible to compare immunogenicity in terms of antibody titers across different clinical trials.

The constant serum–varying virus neutralization test has been considered by some to be a preferable method for determining the antibody response to yellow fever vaccine.[193,601] This method has been compared to the mouse neutralization test[612] and was formally validated prior to a Phase III trial.[601] More importantly, the test serves as a surrogate for protection, because the cutoff for a positive antibody test (LNI of 0.7) has been established by studies in monkeys in which animals were challenged with lethal wild-type virus.[485] Moreover, the level of neutralization (quantity of virus neutralized) in undiluted or minimally diluted serum may be biologically more meaningful than a serum dilution end point titer. In contrast, the level of seroprotection in the serum dilution–constant virus neutralization test has not yet been agreed on by any regulatory agency. The LNI test may, however, be slightly less sensitive than the serum dilution–constant virus plaque-reduction method and appears to be influenced by antibody affinity, which can change over time. For measurement of the immune response in an individual, the difference in titer between prevaccination and postvaccination sera expressed as the LNI represents the neutralizing capacity of the serum.

The test is performed by mixing a volume of normal or test serum (undiluted or at a very low dilution) with an equal volume of a series of 10-fold dilutions of virus over a range that includes the virus titer end point (e.g., 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}). After incubation for a suitable period (e.g., 1 hour at 37°C) the serum–virus mixtures are titrated in cell culture by plaque assay. The titer of virus (expressed as $\log_{10}$ PFU/mL) of the test series is subtracted from the titer in the control series to determine the LNI. For example, if the virus titer with normal serum is 5 $\log_{10}$ PFU/mL and with the test serum it is 2 $\log_{10}$ PFU/mL, the LNI is 3.0, reflecting neutralization of 3 logs of virus. If a prevaccination serum is not available, the LNI is calculated using a normal serum control. Because complement increases the sensitivity of the yellow fever neutralization test, and because complement levels may be unstable in stored sera, it is preferable to heat-inactivate test samples; some laboratories add complement (or a standard source of fresh-frozen serum) to the virus-serum mixture.

The IFA, ELISA, hemagglutination-inhibition, and complement-fixation methods have been used to measure responses to yellow fever vaccine.[376,536,612,632,648,649] When compared to the plaque-reduction neutralization test, a commercial indirect fluorescent antibody assay (Euroimmun AG, Lübeck, Germany) showed greater than 95% specificity and sensitivity for detecting IgG antibodies following primary yellow fever vaccination, and it appears to be suitable for rapid determination of serologic response.[376] There was a high (98%) correlation in categorical response but no correlation between indirect fluorescent antibody and neutralizing antibody titer.[364] Where critical decisions on protective immunity are required, however, confirmation by neutralization test may be warranted until sufficient data on sensitivity of indirect fluorescent antibody tests accumulate. The hemagglutination-inhibition test is less sensitive than neutralization and is complicated by low specificity in persons with prior flavivirus exposure.[650] The choice of yellow fever antigen might affect results; use of 17D viral antigen provided a more sensitive assay for detecting vaccine-induced immunity than antigens prepared from the French neurotropic virus or a wild-type (JSS) strain.[649]

Persons without prior flavivirus exposure generally do not develop CF antibodies after administration of 17D vaccine.[329,330] Consequently, it was thought that the complement-fixation test distinguishes recent infection with wild-type virus from vaccine-induced immunity. However, in one study, subjects with prior heterologous flavivirus immunity developed broadly cross-reactive CF antibodies to yellow fever following 17D vaccination.[329] The complement-fixation test might correlate with protein-specific antibodies to NS1. When measured by ELISA or Western blot, anti-NS1 antibodies are absent after primary 17D vaccination, but they can be found in vaccinated persons who have preexisting flavivirus immunity. IgG ELISA is insensitive and unsatisfactory for detecting seroconversion to 17D vaccine.[364,600,651]

IgM antibody responses measured by IFA and ELISA are further discussed in "Antibody Subclass Response" later.

Neutralizing Antibody Response in Clinical Trials. The neutralizing antibody response to 17D vaccine has been evaluated in numerous studies since the vaccine was developed in the late 1930s. Theiler and Smith[21] and Smith and colleagues[22] demonstrated appearance of neutralizing antibodies within 1 to 2 weeks after immunization. In a field study in Brazil (1937–38), 94% of 882 vaccinees seroconverted after vaccination.[22] Subsequent clinical trials have confirmed the high immunogenicity of yellow fever 17D vaccine, with development of neutralizing antibodies exceeding 90% in nearly all studies (see Table 63.9). Since 2000, there have been at least five large randomized immunogenicity trials in adults or children, the purpose of which was to compare vaccines for registration by local national control authorities[537,548,601,619] or to compare lots made from new secondary seeds and 17D and 17DD substrain vaccines.[547] Response rates have been similar for vaccines produced from the 17DD and 17D-204 (or 17D-213) substrains, for vaccines formulated with or without stabilizers, and for vaccines with and without avian leukosis virus.

Neutralizing antibody levels following 17D vaccine show a degree of individual variability (Fig. 63.11).[317,601] However, when a validated LNI assay was applied in a randomized clinical trial setting, the 95% confidence limits were quite narrow: Among seronegative subjects treated with YF-Vax (N = 291), geometric mean LNI was 2.20, 95% CI was 2.12, 2.28; for ARILVAX-treated subjects (N = 283), geometric mean LNI was 2.08 and 95% CI was 1.97, 2.13.[601] These data are similar to those reported in other trials using the plaque-reduction neutralization method.[547]

Kinetics of the Antibody Immune Response. Studies in rhesus monkeys established that neutralizing antibodies were detectable in serum on day 6 or 7 following inoculation of 17D virus, at which time the animals were completely refractory to challenge. Significant protection was present 1 to 2 days before the appearance of detectable neutralizing antibodies,[611,613] suggesting that very low levels of antibody were protective. Occasional animals survived challenge at even earlier times after 17D immunization, suggesting that IFN or other antiviral mechanisms may also play a role in protection.

Human immunization with 17D virus is also followed by the rapid appearance of neutralizing antibodies, detection of antibodies being dependent upon the sensitivity of the neutralization assay. Early studies using the mouse protection test failed to detect antibodies 7 days after vaccination, but the

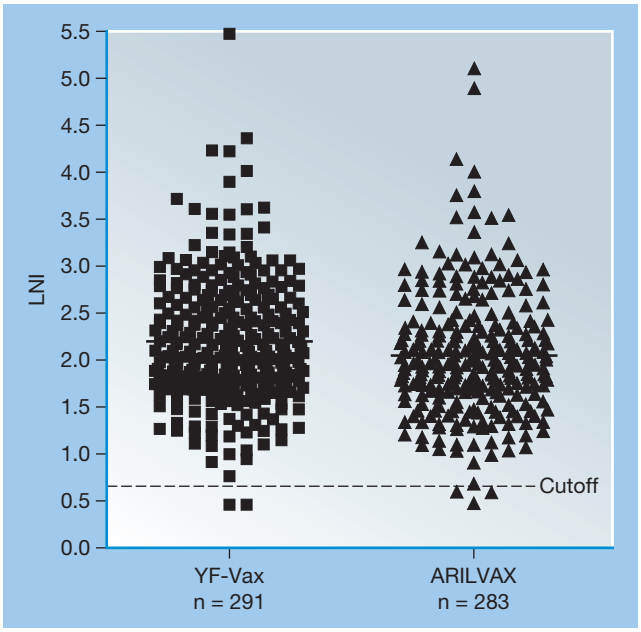


Figure 63.11. Variability of the neutralizing antibody responses in seronegative healthy adults in a Phase III clinical trial of YF-Vax and ARILVAX. LNI, $\log_{10}$ neutralization index. *(From Acambis Inc. Clinical study report, Protocol H-070-005, 1999; see Monath TP, Nichols R, Archambault WT, et al. Comparative safety and immunogenicity of two yellow fever 17D vaccines [ARILVAX and YF-VAX] in a phase III multicenter, double-blind clinical trial.* Am J Trop Med Hyg. *2002;66[5]:533–541.)*

majority of subjects had seroconverted by day 14.[21,22] Smithburn and Mahaffy[613] found neutralizing antibodies in 10% of a small group of subjects on day 7 and in 90% on day 10 after immunization. Wisseman and colleagues[633] found no evidence of immunity on day 6, but all subjects had seroconverted by day 14 after vaccination. In a newer study, Lang and colleagues[622] found that 86% to 88% of adult subjects seroconverted by day 14, and 99% to 100% had seroconverted by day 28 after vaccination. Similarly, Reinhardt and colleagues[358] showed that all subjects seroconverted between days 6 and 13 and that mean antibody titers increased slightly from 1:71 on day 13 to 1:88 on day 26 after vaccination. Monath and colleagues[193] found that 80% of subjects seroconverted by day 11 and 100% by day 31, with a marked increase in antibody level measured by LNI from 1.26 to 3.98. Silva and colleagues[602] found that 30% of subjects developed neutralizing antibodies by day 7 and 100% by day 15 after vaccination. Based on human and monkey studies, Courtois[652] concluded that "man seems unable to form antibodies so early as the rhesus but by the 10th day his serum has a very high degree of protective activity. Judging from the studies carried out in monkeys, it seems probable that a protective mechanism begins to operate in man by the 8th or 9th day." This conclusion was incorporated into the International Health Regulations,[653] which stipulate that the vaccination certificate for yellow fever is valid 10 days after administration of 17D vaccine.

Studies employing more-sensitive neutralization tests have demonstrated antibodies at earlier times following administration of 17D vaccine. Monath[654] found the earliest evidence for neutralizing antibodies by plaque-reduction assay on day 4 in one of four volunteers, and all subjects had seroconverted by day 8. Time to appearance of antibodies was not shortened by use of a kinetic neutralization test. Bonnevie-Nielsen and colleagues[600] found no antibodies by plaque-reduction neutralization test on day 4 after vaccination; 25% were immune on day 7 and 88% on day 12. Thus, it appears that immunity to yellow fever can occur in a minority subset of vaccinees during the first week after immunization. Neutralizing antibody levels continue to increase during the first month after immunization, with peak titers found at 3 to 4 weeks.[633,654] In one study of 238 subjects in Brazil, who were tested at various time points up to 17 months after vaccination, neutralizing antibody titers began to decline at around 13 months.[623]

Antibody Subclass Response. IgM antibodies appear rapidly after 17D vaccination, but as discussed later they can persist for long periods of time and therefore do not indicate recent immunization. The ELISA and indirect fluorescent antibody methods are more-practical approaches for measuring IgM antibodies than fractionating serum for neutralization testing.

The primary immune response is characterized by the appearance of neutralizing antibodies of the IgM class between days 4 and 7, several days before detection of IgG antibodies.[654] Titers of IgM neutralizing antibodies were 16- to 256-fold higher than IgG antibodies during the first 4 to 6 weeks after immunization. During a field study in Nigeria, 141 (37%) of 385 persons vaccinated with 17D seroconverted by IgM antibody-capture ELISA.[163] However, prior flavivirus exposure in this population was high, and this might have reduced IgM responses. In clinical studies in Europe, IgM antibodies measured by capture ELISA appeared in 83% of volunteers within 2 weeks after primary immunization.[358,601] De Melo and colleagues[623] studied 238 subjects in Brazil who received 17DD vaccine, of whom 82% had had prior dengue infections. Prior dengue immunity did not significantly reduce the IgM antibody response to yellow fever vaccine. In this study, 71% of the subjects developed IgM antibodies as determined by ELISA.

Nogueira and colleagues[655] found persistence of IgM antibodies for at least 2 months after vaccination with 17DD vaccine and de Melo and colleagues[623] found 43% of subjects remained IgM positive when tested between 100 and 349 days after vaccination. More recently, Gibney and colleagues[656] found that 73% of persons vaccinated 3 to 4 years previously were IgM-positive. In contrast, IgM antibodies were not detected in another cohort of subjects tested 2 years after primary immunization.[601] (See "Duration of Immunity" later for more discussion on reasons for persistence.)

The memory B-cell response after yellow fever revaccination was not associated with an IgM response in one study where the interval between primary and revaccination was relatively short (2 years),[601] whereas in another study four of five subjects revaccinated with an interval longer than 10 years developed anti–yellow fever IgM.[358] Two individuals who had received yellow fever vaccine 23 and 45 years previously and then received yellow fever vaccine through transfused blood products developed IgM antibodies against yellow fever virus.[657] IgM responses measured by indirect fluorescent antibody test were less common in subjects who had preexisting heterologous flavivirus immunity, but all responded with IgG antibodies.[376]

The IgM antibody capture ELISA was evaluated in 21 Cuban subjects who received 17D vaccine.[377] The test was similar in sensitivity to the plaque-reduction neutralization. Of 17 naïve subjects, 16 (94%) seroconverted by IgM ELISA. The test was highly specific and showed no cross reactions with dengue antigen. However, 11% of dengue immune sera cross-reacted by IgM ELISA using yellow fever antigen. Similar results were reported in subjects with and without preexisting Japanese encephalitis antibody who received 17D vaccine.[658] On day 10 after 17D vaccination, yellow fever virus–specific neutralizing antibodies were detected in six of 19 subjects and IgM

antibodies were detected in seven of 19 subjects. On day 14, all subjects had IgM and neutralizing antibody responses. From these limited studies, it appears that the IgM capture ELISA may be similar in sensitivity to the neutralization test in detecting serologic response to 17D vaccine when sera are obtained early after vaccination.

The indirect fluorescent antibody test was compared with plaque reduction in 17D primary vaccinees.[376] An overall correlation of 99% with neutralization was observed in primary vaccinees. Sensitivity and specificity of both the IgG and IgM responses were greater than 94%.

Despite the early work showing functional (neutralization) activity of IgM, the role of IgM antibody in protection or recovery from infection has not been clearly shown. In one study of 2C9 MAb with the same antigen recombining site but different isotypes (IgG or IgM), only the IgG molecule protected AG129 mice from peripheral yellow fever virus challenge.[386] This observation suggests that the ability of 2C9 IgG to protect was owing to the Fc portion of the IgG molecule. On the other hand, protection against another flavivirus (West Nile virus) in the mouse model was found to be highly dependent on the early IgM response.[659]

Serologic Specificity. The primary neutralizing antibody response to 17D vaccine is highly specific, characterized by no or very-low-titer antibodies to other flaviviruses.[342,633] In addition, neutralization titers in persons receiving 17D vaccine are higher against the homologous (vaccine virus) than against wild strains but antigenic conservation of neutralization epitopes is sufficient to ensure vaccine efficacy. Neutralizing antibodies in the IgG and IgM fractions of serum display similar specificity in primary vaccinees, whereas IgG (but not IgM) from a person who had been vaccinated three times and was tested 18 months after the last immunization showed some flavivirus cross-reactivity.[654] IgM responses determined by indirect fluorescent antibody assay or ELISA tend to be yellow fever–specific even in subjects with preexisting heterologous flavivirus experience, although fewer such subjects develop IgM responses than in primary vaccination.[376] In contrast, one report described a high rate of IgM seroconversion to dengue in yellow fever vaccinees, although prior dengue exposure could not be excluded in all subjects.[339] The HI antibody response to primary immunization is also yellow fever specific.[650] In contrast, persons with heterologous immunity respond to 17D vaccination with the development of a broadened response and appearance of both homologous (yellow fever) and heterologous N, HI, IgG ELISA, and IFA and CF antibodies.[329,633,654] Similarly, persons with prior 17D immunity respond to natural infection with heterologous flaviviruses with the development of a broadened flavivirus group response.[342,660] Yellow fever–immune subjects given a monotypic ChimeriVax dengue-2 vaccine developed high titers of heterotypic neutralizing antibodies to dengue types 1, 3, and 4.[661]

The phenomenon of "original antigenic sin" has been noted in persons previously vaccinated with 17D virus and subsequently infected with another flavivirus. In such cases, an anamnestic response leads to a rapid rise in yellow fever antibodies, whereas the response to the heterologous antigen is delayed. This phenomenon was observed in yellow fever–immune volunteers who were vaccinated with experimental dengue-2 vaccines.[348,662] Original antigenic sin might lead to diagnostic confusion in patients with clinical syndromes resembling naturally acquired yellow fever.[378]

Antibody-Dependent Enhancement. Clinical experience indicates that there is no evidence that yellow fever vaccination increases the risk of severe dengue disease. This is a question of immense importance, because dengue viruses are encroaching on yellow fever endemic areas in South America, where routine 17D vaccination is performed. Serologic studies should be performed in individual cases of dengue hemorrhagic fever to define the role of prior yellow fever immunity. In the case of Japanese encephalitis, preexisting immunity did not increase viremia following yellow fever 17D vaccination.[183]

Cellular Immune Response to Vaccination. Cytotoxic T lymphocytes mediate killing of flavivirus-infected cells and are an important effector mechanism of viral clearance during primary infections. T-cell responses following 17D vaccination are both broad (representing a diverse oligoclonal repertoire of cells) and long lasting (many years).[145,146,604,663,664] Beginning during the first week after vaccination, effector $CD8^+$ cytotoxic T cells with antiviral activity and $CD4^+$ cells with a mixed Th1/Th2 pattern appear very rapidly.[594,595,665,666] At 2 to 4 weeks after vaccination, effector cell populations peak before contracting into a memory $CD45RA^+$ T-cell population.[667]

The first study of antigen-specific T-cell responses was by Co and colleagues,[145] who studied the T-cell responses in four human subjects vaccinated with 17D. All four subjects responded by lymphoproliferation, cytotoxic T-cell, and IFN-γ ELISPOT (enzyme-linked immunospot) assays. Antigen-specific responses by IFN-γ on an ELISPOT assay were observed at the earliest time point studied (14 days) and were detectable at 19 months after vaccination. $CD8^+$ T-cell lines were established, the majority of which were HLA-B35 restricted. $CD8^+$ T-cell epitopes were recognized in viral proteins E, NS1, NS2B, and NS3. T-cell frequencies specific for these epitopes were in the range of 10^{-4}. Subsequently, Co and colleagues studied the dynamics of $CD8^+$ T-cell responses to defined HLA-B35–restricted 17D epitopes in human subjects. Positive cells were detected as early at 7 days and peaked 2 weeks after vaccination, with approximately 1% of peptide-specific cells at that time point.[663]

Miller and colleagues[665] and Akondy and colleagues[146] studied $CD8^+$ T-cell responses following inoculation of 17D vaccine in healthy adults. Two weeks after vaccination, there was a maximum expansion of a pool of effector $CD8^+$ T cells (Fig. 63.12). These cells were characterized by expression of

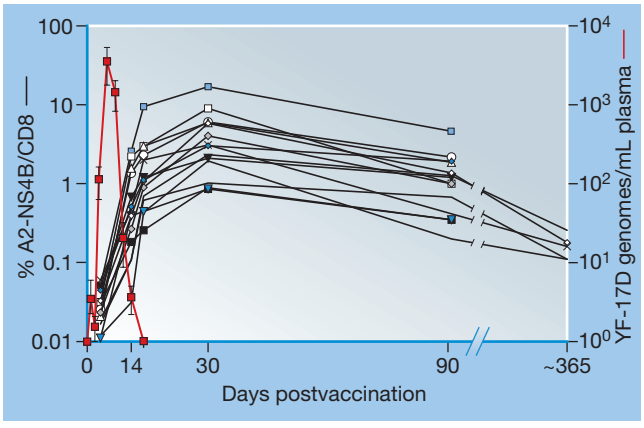


Figure 63.12. Kinetics of the yellow fever 17D–specific $CD8^+$ T-cell response by major histocompatibility complex (MHC) class I tetramer staining in human subjects. The red line indicates the level of 17D RNA in blood (mean ± standard deviation). The black lines are responses of individual subjects, showing the percentage of positive cells against an immunodominant epitope on NS4B. *(From Akondy RS, Monson ND, Miller JD, et al. The yellow fever virus vaccine induces a broad and polyfunctional human memory CD8+ T cell response.* J Immunol. *2009;183:7919–7930.)*

T-cell activation markers, CD38, CD127, CCR7, and HLA-DR. In addition, at the peak of the response on day 15, up to 15% of $CD8^+$ T cells were specifically activated; they also expressed Ki-67, perforin, and granzyme B. Interestingly, these cells downregulated expression of antiapoptotic Bcl-2 molecules, suggesting that the responding T cells proliferate, acquire cytolytic potential, and have increased susceptibility to apoptosis. At 2 to 4 weeks after vaccination, virus-specific effector populations contracted 90%, downregulated expression of the activation markers, and gradually differentiated into long-lived memory cells that reexpressed CD127, Bcl-2, and CD45RA. These differentiated $CCR7^-$ $CD45RA^+$ memory $CD8^+$ cells were polyfunctional; produced multiple cytokines, including IFN-γ, TNF-α, IL-2, and macrophage inflammatory protein (MIP)-1β; demonstrated robust proliferation upon restimulation; and are likely important in long-term protection against exposure to yellow fever virus.[146] Additional experiments monitoring the activation status of cytomegalovirus, Epstein-Barr, and influenza-specific $CD8^+$ T cells in subjects inoculated with 17D vaccine showed that bystander activation does not contribute to the peak of the anti-17D $CD8^+$ T-cell response and indicated that the observed T-cell responses were primarily 17D specific. A separate study was able to show a positive correlation between the initial viral load in persons receiving 17D vaccine and the magnitude of the $CD8^+$ T-cell response.[668] However, above a certain viral level the magnitude of the $CD8^+$ T-cell response is saturated or plateaus. These results in combination with that of dose-sparing studies suggest that sufficient levels of viral antigen need to be given with certain viral vaccines, like yellow fever vaccine, to ensure a complete immunologic response.[557,558,668]

In other studies, lymphocyte subsets were defined in 17D first-time vaccinees and revaccinees.[618] The results confirmed the observations just described, in that activated $CD8^+$ cells expressing CD38 proliferated after primary and revaccination. In addition $CD3^+$ and $CD4^+$ cell subsets increased in both groups; the increase in $CD4^+$ and $CD8^+$ memory cells was greater in revaccinated subjects. In persons with prior flavivirus experience, memory T-helper and B cells reactive to specific and cross-reactive antigenic determinants contribute to the rapid anamnestic antibody response.

Using different knockout mice with various deficiencies in T and B cells, Bassi and colleagues found that $CD8^+$ cells were in fact not needed to develop protective immune response following vaccination when the mice were challenged intracerebrally with 17D virus.[609] However, $CD8^+$ cells facilitated virus clearance and were necessary in B-cell deficient mice to ensure their survival when challenged. Furthermore, $CD8^+$ cells also appeared to facilitate viral clearance in mice that were vaccinated and then challenged 28 days later; without $CD8^+$ cells, virus was found to persist in the brains of the inoculated animals. These results suggest that $CD8^+$ cells help control viral proliferation and might be necessary for complete viral clearance.

A detailed analysis of cell surface markers in peripheral blood of subjects following yellow fever 17D vaccination showed a complex sequential activation and regulation of B and T cells.[604] The authors speculated that this finely tuned activation of innate and adaptive immune responses determined the outcome of infection (recovery-immunity vs runaway infection and vaccine-related adverse events). The most striking changes occurred between days 7 and 15, the period in which the adaptive immune response occurs. Changes in B cells reflected both activation and modulation, upregulation of IL-1OR, $CD69^+$, CD32 (FcγR), and decreased $CD23^+$ expression. $CD4^+$ T-cell activation occurred early and $CD8^+$ activation occurred later. Again, both T-cell activation markers and simultaneous increases in regulatory T cells were found. This study provides a basis for future analyses of B- and T-cell dysregulation in patients with viscerotropic adverse events.

Effects on Immune Response

Age. Some reports suggested that young children did not respond as well as older persons to 17D vaccine or that they lost immunity more rapidly,[669,670] but this was not confirmed in other studies.[648,671] Newer studies have also had conflicting results.

In a study of seronegative Ghanaian infants 6 and 9 months of age immunized with 17D vaccine manufactured by the Institut Pasteur in Senegal, seroconversion rates 3 months after vaccination were 99% and 98%, with a mean 50% plaque-reduction neutralization titers of 159 and 130, respectively.[620] In comparison, two pediatric studies performed in South America showed lower seroconversions rates for children.[548,619] One trial had lower neutralizing antibody responses in children to YF-Vax and ARILVAX (90.6% and 94.9% seroconversion and geometric mean LNIs of 1.26 and 1.32, respectively)[619] compared to a study of the same vaccines in adults (99.3% and 98.6% seroconversion and geometric mean LNIs of 2.21 and 2.06, respectively) (see Table 63.9).[601] The two trials used the same serologic assay and testing laboratory, but they were conducted in different populations (in children in Peru and in adults in the United States). Although antibodies to dengue were present at baseline in approximately 15% of the pediatric subjects, there was no effect of this covariate on seroconversion or on geometric mean antibody titers.[619] In a large double-blind clinical study performed in Brazil that compared 17DD and 17D-213/77 vaccines in children found seroconversion rates of 89% (95%CI, 85% to 91%) and 87% (95%CI, 83% to 90%), respectively.[548] These seroconversion rates are lower than the 99% seroconversion seen in a previous trial of similar design in adults.[547] Given these data, the WHO has called for additional study on this topic.[672] However, when a review of all pediatric data was recently conducted, Staples and colleagues[673] found an estimated seroconversion rate, when study size differences and variability between studies were accounted for using a random effects model, of 93% (95% CI, 88% to 96%) among 4675 children at 1 to 2 months of age following their primary yellow fever vaccination. Furthermore, when the random effects model was used to compare seroconversion rates between children younger than 9 months of age and those 9 months of age or older, there was no difference. For children younger than 9 months of age, data from four studies provided an estimated seroconversion rate of 95% (95% CI, 91% to 98%). In children 9 months of age or older, data from 11 studies provided an estimated seroconversion rate of 92% (95% CI, 86% to 96%).

In a large clinical trial, there was no difference between young adult and elderly (60 years of age and older) subjects in the rate of seroconversion to 17D vaccines.[674] Seroconversion rates were 97% to 100% in all ages, and the rare primary vaccine failures were observed only in the younger age groups. Geometric mean LNIs were similar across age groups (Fig. 63.13). In a smaller trial comparing persons 18 to 30 years of age to those 65 years of age and older, similar geometric mean titers were found at 28 days after vaccination.[256] However, at 10 days postvaccination, the older adults had lower seroconversion rates (50% vs 77%) and significantly lower geometric mean titers when compared to young adults. Furthermore, the study also found older adults to have higher viremia levels detectable for longer periods. These results in combination with others suggest that T-cell immune response might be delayed but the magnitude of the B-cell response, in terms of antibody production, appears to be intact.

Sex. There does not appear to be a strong gender bias. In one large trial, the magnitude of the neutralizing antibody response was statistically significantly higher in male than in female subjects,[601] although the seroconversion rates did not differ and the difference in geometric mean LNI was not biologically relevant (<0.3 $\log_{10}$) (Table 63.10). In a study in Brazil, the seroconversion rate in male subjects (but not the antibody titer) was higher than in female subjects.[547] Similarly, Niedrig and colleagues also reported higher neutralizing and IgM antibody titers in male vaccinees.[376] Naturally acquired yellow fever disease is more common in men and could to some degree, though not completely, be attributed to epidemiologic factors. Postvaccinal encephalitis caused by French neurotropic vaccine was also higher in male than in female patients, suggesting that male patients undergo a more active infection. However, it has been noted that men have greater serologic responses to some nonreplicating vaccines (e.g., pneumococcal and meningococcal polysaccharide),[675] so that the gender difference seen with yellow fever might represent an immunologic mechanism unrelated to replication of the virus.

Race. European Americans were noted to have higher mean neutralizing antibody response than African Americans in two studies (see Table 63.10).[601,621] The response was also statistically higher in European Americans than in Latin Americans and Africans.[601,676] Anecdotal evidence suggests that the lethality of wild-type yellow fever appears to be higher in whites than blacks. The trial data showing higher antibody responses in whites might reflect a higher level of susceptibility (e.g., lack of previous flavivirus immunity), virus replication, and antigen expression in this racial group.

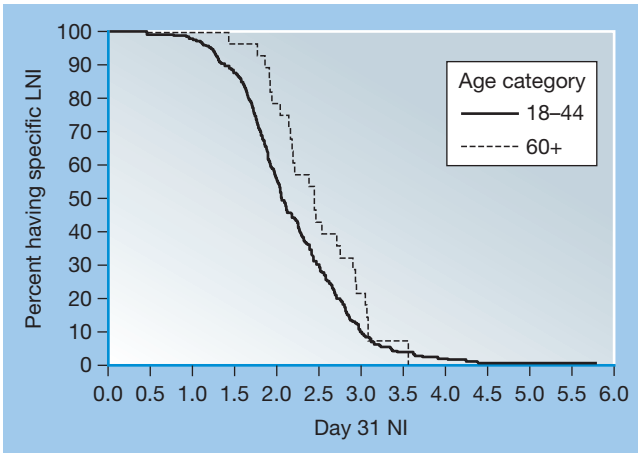


Figure 63.13. Reverse cumulative distribution of neutralizing antibody titers ($\log_{10}$ neutralization index [LNI]) for persons 18 to 44 years of age and 60 years of age and older. *(From Monath TP, Cetron MS, McCarthy K, et al. Yellow fever 17D vaccine safety and immunogenicity in the elderly.* Hum Vaccin. *2005;1:207–214.)*

Genetic Restriction. No studies have been reported on genetic restriction of immune responses to yellow fever vaccine. This is an area deserving study, because studies in mice indicate that haplotype influences replication of flaviviruses[261,262] and the immune response to live viruses.[267,677]

Vaccine Substrain or Dose. All yellow fever 17D vaccines contain a minimum of 3 $\log_{10}$ IU/dose.[531] Vaccine potency of manufactured lots exceeds this minimal limit by approximately five- to 50-fold to account for losses upon storage. The infectivity assay underestimates the number of genome equivalents (measured by RT-PCR) by approximately 1000-fold,[559] and the difference can be composed of both noninfectious (defective) and infectious virions that were not detected by the assay used.

Although there have been several studies documenting similar neutralizing antibody responses and titers following vaccination with different substrains of yellow fever vaccine, few studies have explored the innate and cellular immune responses to different substrains. In one comparative study, both 17DD and 17D-213/77 elicited a balanced cytokine profile in children 9 to 12 months of age.[678] However, there were subtle differences in the cytokine profiles between the substrains with 17DD eliciting a slight predominance of neutrophil-derived TNF-α and neutrophils and monocyte-producing IL-12, whereas 17D-213/77 had more of a predominance of IL-12$^+$CD8$^+$ T cells and IL-10 production. Whether these differences are substantial enough to impact longer-term immunity is unclear.

A dose–response relationship was observed in rhesus monkeys inoculated intramuscularly with 17D vaccine.[485,679] The dose at which 90% of the animals developed a rise in LNI greater than 0.7 was approximately 1000 MLD_{50} (approximately 4 $\log_{10}$ PFU) and the 90% protective dose against lethal challenge was approximately 200 MLD_{50} (approximately 400 PFU). The 50% immunizing dose was approximately 2 MLD_{50} (approximately 20 PFU). The relationship between PFU and MLD_{50} is approximately 10 : 1.[263]

Dose–response in humans was first determined by Fox and colleagues[680] in 1943. The minimal dose resulting in seroconversion was between 14 and 140 MLD_{50} (presumed equivalent doses in IU and estimated to represent 140 and 1400 PFU,

TABLE 63.10 Host Factor Effects on the Log_{10} Neutralization Index in a Clinical Trial of Two Yellow Fever 17D Vaccines

Host Factor	Group	ARILVAX (n = 283)		YF-Vax (n = 291)		*P* Value (ANOVA) Host Effect
		No. Tested	Mean LNI	No. Tested	Mean LNI	
Sex[a]	Male	108	2.16	111	2.35	.001
	Female	175	1.99	180	2.12	
Race	African American	37	1.93	42	2.06	.017
	Latin American	15	1.95	15	1.85	
	Other (white, Asian)	231	2.09	234	2.26	
Smoking	Yes	54	2.26	73	2.27	.017
	No	229	2.01	218	2.19	

[a]Niedrig et al[376] also reported higher neutralizing (and IgM ELISA antibody) titers in male than in female vaccinees.
ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; IgM, immunoglobulin M; LNI, log neutralization index.
Data from Monath TP, Nichols R, Archambault WT, et al. Comparative safety and immunogenicity of two yellow fever 17D vaccines (ARILVAX and YELLOW FEVER-VAX) in a Phase III multicenter, double-blind clinical trial. Am J Trop Med Hyg. *2002;66(5):533–541.*

respectively). At a dose of 14 MLD_{50}, 70% of the volunteers seroconverted. Large scale-field trials of various vaccine lots, some of which were of suboptimal potency, indicated that administration of doses in the range of 10 to 50 MLD_{50} immunized more than 85% of vaccinees.

More recent dose–response studies (Table 63.11) indicate that doses of 100 to 300 PFU result in the development of neutralizing antibodies in approximately 90% of yellow fever naïve persons vaccinated.[557,558,638,681,682] In addition, an unpublished study of ARILVAX (17D-204 vaccine previously manufactured in the United Kingdom) found that 13 of 15 (93%) subjects given 200 PFU seroconverted and that all subjects seroconverted at a dose of 2000 PFU or more. Roukens and colleagues[592] gave 17D vaccine by the intradermal route at one-fifth dose and found no differences in antibody response compared to the standard dose given subcutaneously, but the trial design did not elucidate whether this was the result of a more effective route of inoculation or simply because (as expected from the foregoing) 17D vaccine efficacy drops off only at very low doses.

These data are promising that the potential amount of antigen in a dose of the vaccine could be lowered and thus have a dramatic effect on vaccine supply. Due to the large outbreaks in 2016 in Angola and later DRC, WHO reviewed available data and with the approval of SAGE endorsed the use of fractional doses in response to an emergency situation where extension of the outbreak is imminent and in which current vaccine supply is insufficient.[575] In fact, one-fifth doses of 17DD vaccine were used in Kinshasha, DRC, to preempt the spread of disease. Fractional doses were delivered to all persons aged 2 years and older; children younger than 2 years and pregnant women were given a full dose of the vaccine.[574a]

To reset the potency specifications for release of vaccine at a lower dose would require amending both national licenses and WHO requirements, with data on long-term stability, statistical noninferiority end points in clinical studies, effects of heterologous flavivirus immunity, and follow-up studies on duration of immunity.

Interestingly, an inverse relationship between vaccine dose and antibody titer has been consistently observed.[269,634,638,681] Smith and colleagues[681] found significantly higher antibody responses in subjects given doses of 5 to 50 MLD_{50} than in those given doses of 500 to 5000 MLD_{50}. In monkeys, inoculation of large doses of 17D virus resulted in earlier appearance of viremia, but viremia was inconsistent, lower in magnitude, and briefer in duration than after inoculation of diluted virus.[479] Limited replication of the virus after the inoculation of very large doses might explain the lower magnitude of the immune response. This dose prozone effect is also clearly evident in mice (Fig. 63.14) and may be explained by the presence of IFN (produced in the infected egg), defective interfering particles, noninfectious antigen competing for cell receptors, or induction of a strong innate immune response by virus inoculated at the high dose range. Similar effects have been also noted with chimeric viruses using yellow fever as a vector for the envelope genes of heterologous flaviviruses.[193,284]

Panthier[530] suggested that a low dose of 17D vaccine might cause a delay in antibody response and an increased risk of neurologic accidents (encephalitis). This hypothesis was based on a single case of encephalitis in a 4-week-old infant after pricking the skin with a needle dipped in 17D vaccine and on the work of Fox and Penna[269] in monkeys. Low-dose vaccine administration attributable to loss of vaccine potency was suggested as a factor in encephalitis caused by French neurotropic vaccine in some epidemics.[682] It is now known, however, that vaccination of infants younger than 4 months of age carries a higher risk of encephalitis owing to age susceptibility, not vaccine dose. Moreover, application of low doses of 17D

TABLE 63.11 Relationship Between Dose of Yellow Fever 17D and Neutralizing Antibody Response[a]

Vaccine Manufacturer	Dose	No. Subjects	Seroconversion Rate (%)	Mean Antibody Titer (LNI)	50% Immunizing Dose[b]	Reference
South African Institute for Medical Research	5000 MLD_{50}	4	100%	1.55 LNI	~5 MLD_{50}	Smith et al, 1962[681]
	500	7	100%	1.80 LNI		
	50	8	100%	3.04 LNI		
	5	8	62.5%	2.80 LNI		
Wellcome (United Kingdom)	200,000 PFU	20	100%	2.93–2.96 LNI	42 PFU (~7 MLD_{50})	Stuart, 1956[682]
	200	13	85%	2.97 LNI		
	50	12	58%	3.10 LNI		
	10	13	0%	—		
Bio-Manguinhos (Brazil)	2000 PFU[c]	12	100%	—	~20 PFU (~60 MLD_{50})	Lopes Ode et al, 1988[638]
	1000–2000	34	100%	—		
	500–1000	10	100%	—		
	200–500	34	100%	—		
	100–200	32	93.7%	—		
	50–100	59	81.3%	—		
	20–50	25	84.0%	—		
	<20	53	41.5%	—		
Bio-Manguinhos (Brazil)	27,476 IU	131	97.7%	13,479 mIU/mL	Not established	Martins et al, 2013[557]
	10,477 IU	115	99.1%	12,190 mIU/mL		
	3013 IU	132	97.7%	11,608 mIU/mL		
	587 IU	131	96.9%	12,145 mIU/mL		
	158 IU	122	88.5%	6,837 mIU/mL		
	31 IU	118	66.9%	1970 mIU/mL		

[a]Seroconversion is defined by development of neutralizing antibodies in subjects who were seronegative at baseline or fourfold increase in titers.
[b]The LD_{50}-to-PFU ratio varies for different vaccines, based on differences between the sensitivity of the assays employed or (possibly) mouse virulence of the vaccines. The Wellcome vaccine had an MLD_{50}-to-PFU ratio of 0.17,[634] whereas the Bio-Manguinhos vaccine had an MLD_{50}-to-PFU ratio of 2.9.[638]
[c]Range of doses for volunteers receiving different vaccine lots tested in the study.
LNI, log neutralization index; MLD_{50}, mouse median lethal dose; PFU, plaque-forming units.

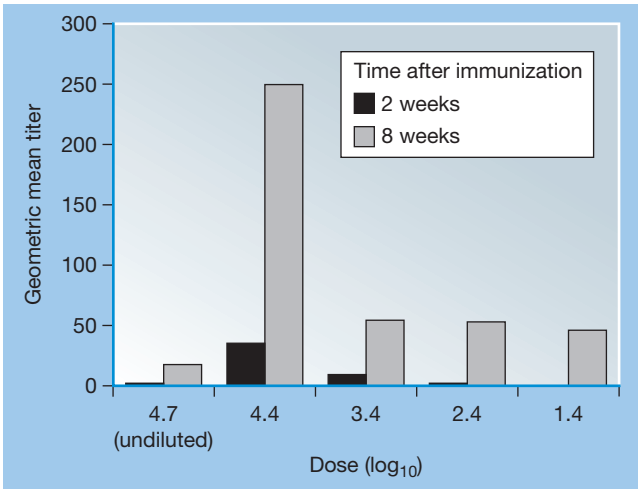


Figure 63.14. Neutralizing antibody response to yellow fever 17D vaccine in mice. Geometric mean titer, groups of five Swiss Webster mice inoculated subcutaneously with 0.1 mL of graded doses of commercial yellow fever 17D vaccine and tested 2 and 8 weeks after immunization. Antibody titers are geometric mean 50% plaque-reduction end points. A prozone was observed, with lower responses in mice given the highest virus dose. *(Courtesy F. Guirakhoo and TP Monath, unpublished data, 1997.)*

vaccine by scarification was not associated with adverse reactions and a large cluster of postvaccinal encephalitis have also occurred when the French neurotropic vaccine was used at a standard dose.[68,519,580]

Heterologous Flavivirus Immunity

Immunization in Persons With Heterologous Immunity. Using neutralization tests, yellow fever is distinct from other flaviviruses,[38] but it shares antigenic determinants detected by binding assays, hemagglutination inhibition, and complement-fixation.[305] Owing to these cross-reactive determinants, the immune response to 17D vaccine is qualitatively different in naïve persons and in those with prior heterologous immunity.

Interference With 17D Vaccine Caused by Heterologous Immunity. The evidence for interference with 17D vaccine in humans is conflicting, and discrepancies across studies may be because of the number of prior flavivirus infections, the breadth of the heterotypic response, or the identity of the viruses responsible for prior infection. Prior infection with members of the Japanese encephalitis complex does not appear to alter the immune response to 17D vaccine. Pond and colleagues[650] observed that natural immunity to St. Louis encephalitis virus had no effect on seroconversion rates to 17D vaccine. Persons with monotypic neutralizing antibodies to Japanese encephalitis virus or with broad flavivirus cross reactions had neutralizing antibody responses to 17D equivalent to those of immunologic-naïve subjects.[633] Moreover, viremic responses to 17D were not significantly different in persons with and without prior Japanese encephalitis immunity, indicating that replication of 17D virus was not reduced.[183] The results of these studies and those of Pond and colleagues[650] are consistent because St. Louis and Japanese encephalitis viruses are close antigenic relatives.

Pond and colleagues[650] also observed that persons with natural immunity to dengue had lower seroconversion rates to 17D vaccine than nonimmune subjects, an observation that appears to be consistent with the experimental data in dengue-immune monkeys challenged with wild-type yellow fever virus[337] and with epidemiologic evidence suggesting cross protection in human populations.[334–336] However, in a large clinical study of dengue-immune versus nonimmune children, there was no statistical influence of preexisting dengue neutralizing antibodies on seroconversion rates and geometric mean neutralizing antibody titers following 17D immunization.[619] In a study of primary vaccinees in Brazil, de Melo and colleagues[623] found that subjects with prior dengue immunity had lower geometric mean neutralizing antibody titers (geometric mean titers 860) than those without dengue immunity (geometric mean titers 1120), although the difference was not statistically significant.

Yellow fever antibodies acquired by natural infection with heterologous flaviviruses significantly blunted the magnitude of the neutralizing antibody response to 17D vaccine.[329,681] Interference was particularly evident when 17D vaccine was given by scarification, possibly owing to the lower dose of 17D administered by this route. Low response rates to 17D vaccine administered by scarification were associated with preexisting heterologous flavivirus immunity in multiple studies in Africa.[586,588,589] In contrast, volunteers who had previously received a live attenuated dengue vaccine and were subsequently given 17D vaccine had similar immune responses to yellow fever as dengue nonimmune controls, suggesting no interference with yellow fever vaccine replication.[348] It is possible that the lack of interference was related to the attenuated properties of the dengue infection. In a study conducted in Nigeria, Omilabu and colleagues[684] found no restriction of neutralizing antibody responses to 17D vaccine in persons with various patterns of preexisting flavivirus immunity. In general, however, immune activation at the time of vaccination will likely impede the response, as was shown when the immunologic response was compared between vaccine recipients in Uganda and recipients in Switzerland.[676] Uganda had a lower proportion of individuals with detectable viremia and lower levels of viremia; individuals also developed lower neutralizing antibody titers when compared to vaccinated persons in Switzerland. The authors found that at baseline, persons in Uganda had higher levels of $CD8^+$ T cells and B cells, as well as proinflammatory monocytes, and this negatively correlated with subsequent antibody titers. These findings suggest that immune activation prior to vaccination decreased the response to the vaccine and could explain some of the findings seen related to dengue immunity.

A number of new vaccine candidates have been developed using 17D virus as a vector for foreign genes, including chimeric viruses in which the prM-E genes of 17D virus have been exchanged for the corresponding genes of a heterologous flavivirus.[685–687] Because the chimeras contain nonstructural genes that could induce antivector immunity, studies were performed to investigate interactions between 17D and the chimeric vaccines. In one published study, there was a suggestion (not statistically significant) that prior immunization with a chimeric yellow fever–Japanese encephalitis vaccine (ChimeriVax-JE) reduced the seroconversion rate and geometric mean neutralizing antibody titer to 17D vaccine given 30 days later.[617] However, in a larger clinical trial, there was no interference with respect to the response to 17D vaccine in subjects who had been given ChimeriVax-JE vaccine 30 days previously.[688] The interactions between dengue and yellow fever appear to differ (see the next section). There is no clear evidence that prior flavivirus immunity enhances replication of yellow fever 17D virus or the immune response to 17D virus (see "Antibody-Dependent Enhancement" earlier).

Heterologous Immunization in Persons Immune to Yellow Fever 17D. Several live attenuated flavivirus vaccines have been evaluated in persons with prior immunity to 17D vaccine, including empirically derived dengue type 2 vaccines.[349,662] In yellow fever–immune persons, the response to dengue vaccine was independent of dose and was of greater magnitude and

duration than in nonimmune persons. The results suggested that cross-reactive nonneutralizing antibodies might have enhanced dengue virus replication in vivo. Similarly, a clinical study of a chimeric vaccine in which 17D vectored the prM-E genes of dengue type 2 virus showed significantly higher, durable neutralizing antibody responses to dengue in yellow fever–immune subjects. In addition, a notable broadening of the response occurred across all four dengue serotypes, indicating that the initial 17D vaccine had primed for an anamnestic response to cross-reactive shared epitopes.[661,689] This was also true when a tetravalent mixture of chimeric 17D viruses with envelope genes of dengue types 1 to 4 was given to yellow fever immune and nonimmune subjects.[690,691] There were also slightly higher geometric mean titers seen when the chimeric tetravalent dengue vaccine was given within 3 years of their yellow fever vaccination rather than 3 or more years after.[692] Finally, tetravalent dengue vaccination induced a slight increase in the geometric mean titers of yellow fever in children previously vaccinated against yellow fever.[692]

Data on interactions between yellow fever 17D and chimeric vaccines are inconsistent. In two published studies, prior yellow fever vaccination did not influence the immune response to ChimeriVax-JE vaccine.[193,617] However, in a third study, yellow fever 17D vaccine administered 30 days prior to ChimeriVax-JE vaccine did not affect seroconversion to the Japanese encephalitis virus, but it blunted the neutralizing antibody titer.[688] The data suggested that when a more-immunogenic vaccine (17D) preceded a more-attenuated live vaccine (ChimeriVax-JE), cross-reactive antibody and/or T-cell responses to the yellow fever backbone could blunt the infection and neutralizing antibody response to the second vaccine.

Interestingly, in studies in which viremia was evaluated in yellow fever immune and nonimmune subjects who received ChimeriVax–dengue-2[661] and ChimeriVax-JE vaccines,[193] a higher proportion of yellow fever–immune subjects developed viremia, and viremia levels were slightly higher than in nonimmune subjects, suggesting in vivo immune enhancement. However, the differences in viremia were subtle and were not statistically significant. In a study of tetravalent dengue vaccine, the early viremia profiles were quite similar in the yellow fever–immune and –naïve groups,[689] suggesting that, in this case, prior yellow fever immunity modulated the antibody response (through immunologic memory) rather than abrogating the infection. Now that dengue vaccines are undergoing clinical evaluation, it is very important to determine whether prior dengue vaccination diminishes the viremia following 17D vaccination, which might be expected based on epidemiologic and experimental studies suggesting unidirectional cross-protection between dengue and yellow fever.[337,341]

Prior 17D immunization modulates the response to inactivated flavivirus vaccines through memory responses of IgG-producing B cells and restimulation of memory T cells. In yellow fever–immune subjects receiving inactivated tickborne encephalitis vaccine, anti–tickborne encephalitis antibodies appeared earlier and in higher titers than in nonimmune subjects.[693] The anamnestic response was also characterized by the appearance of cross-reactive heterologous antibodies.

Simultaneous and Combined Vaccination. Yellow fever vaccine has been given simultaneously at different sites or as a mixture, combined with a variety of other vaccines with at least 18 unique antigens contained in 11 inactivated and 10 live-attenuated vaccines (Table 63.12).* These studies are relevant to the use of 17D vaccine in routine childhood immunization programs and to the immunization of travelers but there are limitations to many of the studies including: (a) studies were conducted several decades ago, using different vaccine preparation than currently available; (b) low numbers of subjects; (c) exclusion of targeted populations (e.g., children or adults); and (d) exclusion of special populations (e.g., those with altered immune system).

Based on the available data for inactivated vaccines, there are no safety concerns with the coadministration of yellow fever vaccine and inactivated vaccines. Immunogenicity for most vaccines appears not to be compromised when yellow fever vaccine is coadministered with inactivated vaccines.

For the coadministration of yellow fever vaccine with other live-attenuated vaccines, there are no safety concerns with coadministration with most vaccines. However, data from two studies on the coadministration of yellow fever vaccine and a dengue chimeric vaccine based on the yellow fever vaccine backbone found increased rates of both systemic and local adverse events.[661,691] Although there was no increase in serious adverse events noted, there was limited power to detect serious events. Immunogenicity for most vaccines is also not compromised when yellow fever vaccine is coadministered with other live-attenuated vaccines. Possible interference with yellow fever immune responses were noted when live vaccinia or measles vaccine[626] or inactivated hepatitis B vaccine[637] were concurrently administered with 17D, but these results were not confirmed in other trials for these specific antigens. However, a trial of the concurrent administration of yellow fever vaccine and the combined measles, mumps, and rubella vaccine to children 12 to 23 months of age found a significant decrease in the seroconversion rates and geometric mean titers obtained against yellow fever, mumps, and rubella when the vaccines were coadministered versus administered 28 days apart; no decreases were noted in the immune response to measles.[548] A second study in children receiving polio, yellow fever, and measles and rubella vaccines in Africa found similar rates of seroconversion when the vaccines were administered alone versus together, although the GMTs for yellow fever were significantly reduced when the vaccine was given at the same time as measles and rubella compared to alone.[720a] Immunologic interference was also noted in persons who received yellow fever vaccine 1 year before the chimeric tetravalent dengue vaccine based on the yellow fever vaccine backbone.[690] The study found an initial delay in the antibody response to dengue-1 among yellow fever vaccine-primed persons. In a separate study, the immunologic response to the chimeric dengue vaccine was not different between two groups of children who had either received yellow fever vaccine 2 to 5 years or 6 to 11 years previously.[692]

An interesting observation, not relevant to current vaccination practices, was the mutual interference resulting from simultaneous or sequential administration of 17D and inactivated parenteral whole-cell cholera (vi) vaccines.[696,698] The interference induced by 17D vaccine with vibriocidal responses was not confirmed in another study[697] and might not be real. The basis for interference is obscure, because the interval between cholera and yellow fever immunization was as long as 4 weeks. Trials of combined yellow fever and live oral cholera vaccine (alone or with Ty21a live typhoid vaccine) showed no one-way interference with anticholera immunity or yellow fever antibody responses.[701,707]

Lipopolysaccharide is known to have adjuvant activity, and coadministration of the typhoid vi vaccine appeared to enhance antibody titers to yellow fever, especially when the typhoid vaccine was concurrently administered with yellow fever.[642] In other studies, however, no enhancement was observed when typhoid vi and meningococcus conjugate vaccines were coadministered with 17D.[645,647] An enhanced neutralizing antibody response to yellow fever was observed in a

*References 483, 548, 617, 626, 627, 629, 630, 637, 639, 640, 642–645, 647, 661, 688, 690, 691, 694–711.

TABLE 63.12 Immunogenicity and Reactogenicity of Yellow Fever Vaccine and Other Vaccines When Coadministered

	Age Group	Immune Response Yellow Fever Vaccine	Reactogenicity	Immune Response Other Vaccine	References
INACTIVATED VACCINES[a]					
Cholera	Adults and children	Potentially decreased[c]	Not altered	Potentially decreased[c]	Wolga et al, 1986[694]; Felsenfeld et al, 1973[696]; Gateff et al, 1975[697]
Diphtheria	Adults and children	Not altered	Not altered	Not altered	Wolga et al, 1986[694]; Yvonnet et al, 1986[637]; Ruben et al, 1973[699]
Hepatitis A	Adults	Not altered	Not altered	Potentially decreased[c]	Receveur et al, 1993[630]; Jong et al, 2002[705]; Gil et al, 1996[706]; Dumas et al, 1997[644]; Bovier et al, 1999[700]; Bock et al, 2000[704]; Bienzle et al, 1996[703]
Hepatitis B	Children	Potentially decreased[c]	Not altered	Not altered	Yvonnet et al, 1986[637]; Coursaget et al, 1995[643]
Influenza	Adults	Not altered	Not assessed	Not altered	Goullin et al, 1993[709]
Meningococcal	Adults	Not altered	Primarily not altered[e]	Enhanced	Dukes et al, 1996[645]; Alberer et al, 2015[647]
Pertussis	Children	Not altered	Not altered	Not altered	Ruben et al, 1973[699]
Polio	Adults and children	Not altered	Not altered	Not assessed	Wolga et al, 1986[694]; Yvonnet et al, 1986[637]
Tetanus	Adults and children	Not altered	Not altered	Potentially decreased[c]	Wolga et al, 1986[694]; Yvonnet et al, 1986[637]; Ruben et al, 1973[699]; Gateff et al, 1973[710]
Typhoid	Adults	Not altered	Not altered	Not altered	Jong et al, 2002[705]; Dumas et al, 1997[644]; Dukes et al, 1996[645]; Ambrosch et al, 1994[642]; Alberer et al, 2015[647]
LIVE, ATTENUATED VACCINES[a]					
Tuberculosis (BCG)	Children	Not altered	Not altered	Not altered[d]	Gateff et al, 1973[710]
Cholera	Adults	Not altered	Not altered	Not altered	Kollartsch et al, 1997[701]; Tsai et al, 1999[711]; Foster et al, 1999[707]; Wolga et al, 1986[694]
Dengue[b]	Adults and children	Not altered[d]	Worse[d]	Not altered	Qiao et al, 2011[690]; Poo et al, 2011[691]; Guirakhoo et al, 2006[661]
Japanese encephalitis[b]	Adults	Not altered[d]	Not altered	Potentially decreased[c]	Nasveld et al, 2010[688]; Monath et al, 2003[617]
Measles (only)	Children	Not altered	Not altered	Not altered	Stefano et al, 1999[695]; Adu et al, 1996[702]; Soula et al, 1991[640]; Mouchon et al, 1990[639]; Lhuillier et al, 1989[629]; Gateff et al, 1973[710]; Yvonnet et al, 1986[637]; Ruben et al, 1973[699]
Measles, mumps, rubella combined	Children	Decreased	Not altered	Decreased	Nascimento Silva et al, 2011[548]
Polio	Adults	Not altered	Not altered	Not assessed	Wolga et al, 1986[694]; Kaplan et al, 1984[483]
Vaccinia	Adults and children	Not altered	Not altered	Not altered[d]	Gateff et al, 1973[710]; Ruben et al, 1973[699]; Tauraso et al, 1972[627]; Meyer et al, 1964[626]
Typhoid	Adults	Not altered	Not altered	Not altered	Kollaritsch et al, 1997[701]; Tsai et al, 1999[711]

[a]Listed by antigen component rather than specific vaccines; the specific vaccine and manufacturer often varied between studies.
[b]Specifically chimeric vaccines that use 17D vaccine backbone and insert the prM-Env genes from the respective viruses.
[c]Significant decrease in geometric mean titers or seroconversion rates in at least one study but was not reported for all studies.
[d]Specific numerical data not provided for all studies.
[e]More adverse events reported in one study when meningococcal vaccine was administered with yellow fever and typhoid vaccines when compared to meningococcal vaccine alone.

study of combined 17D-measles vaccine, perhaps owing to cytokine induction by the unrelated virus.[639] Interactions with an experimental Japanese encephalitis vaccine (ChimeriVax-JE) using 17D as a live vector are discussed in "Interference with 17D Vaccine Caused by Heterologous Immunity" earlier.

There are no published studies on the coadministration of yellow fever vaccine and anthrax, *Haemophilus influenzae* b, human papillomavirus, live-attenuated influenza, inactivated Japanese encephalitis, pneumococcal, rabies, rotavirus, tickborne encephalitis, and varicella zoster vaccines. One individual received zoster vaccine 21 days prior to receiving yellow fever vaccine[712] and there were no safety concerns; they developed protective immunity against yellow fever virus.

Coadministration of Immune Serum Globulin and Yellow Fever Vaccine. Seroconversion rates and neutralizing antibody titers were not affected by the intramuscular administration of 5 mL of commercial pooled immune serum globulin containing high titers of yellow fever–neutralizing antibodies 0 to 7 days before or at intervals after yellow fever 17D immunization[483]; this was a finding of practical importance before the advent of vaccines against hepatitis A. The results are not unexpected given the success of passive–active immunization and the observation that revaccination of persons with neutralizing antibodies often results in a booster response.[621,713] In 2007, available units of IVIg manufactured from plasma donations sourced in the United States contained yellow fever–neutralizing antibodies ranging in titer from 80 to 320.[484]

Administration of immunoglobulin for hepatitis A prophylaxis concurrently with 17D vaccine for travel, practiced until the mid-1990s before hepatitis A vaccines were available, has been proposed to explain the absence of cases of YEL-AVD. A clinical study performed in 2006 to investigate the effect of immune serum globulin on viremia and immune responses following 17D vaccine, as a possible means of managing subjects who have contraindications to 17D vaccine or who have risk factors for adverse events such as advanced age, did not show any significant alteration in immune response to the vaccine when coadministered with immune serum globulin.[361]

EVIDENCE THAT YELLOW FEVER VACCINE PROTECTS AGAINST DISEASE

Although a large body of preclinical data in nonhuman primates has demonstrated the protective activity of yellow fever 17D vaccination against lethal challenge,[485,611] there have been no controlled clinical trials performed showing the effectiveness of 17D vaccine in humans. Several observational studies, however, attest to the effectiveness of the vaccine in humans.

Laboratory infections with yellow fever were commonplace before routine immunization, but disappeared thereafter. Observations over 50 years showed that jungle yellow fever in Brazil and other South American countries occurred only in persons who had not been immunized with 17D and that immunization during outbreaks resulted in rapid disappearance of cases.[167] Yellow fever in francophone Africa virtually disappeared after the institution in 1941 of mandatory immunization with French neurotropic vaccine (see Fig. 63.6).[714] A high rate of coverage of the population was followed by a marked reduction in the incidence of yellow fever, despite continued human exposure to the enzootic cycle. Yellow fever epidemics were reported during the 1940s and 1950s from neighboring anglophone countries, particularly Nigeria, in which routine vaccination was not practiced.

An assessment of the effectiveness of 17D vaccine was made during an epidemic in Nigeria in 1986 (De Cock KM and Monath TP, unpublished observation, 1986). By determining vaccine coverage and the number of serologically confirmed yellow fever cases among vaccinated and unvaccinated subjects, vaccine efficacy was estimated to be 85%. The assessment was complicated by the simultaneous occurrence of natural infection and immunization in the population, with the potential for natural infection prior to onset of vaccine immunity, and by the reliance on historical data about disease and vaccination.

Because yellow fever vaccination is the standard of care, placebo-controlled efficacy trials cannot be performed today. New yellow fever vaccines will be licensed based on noninferiority of immunologic surrogates. Further studies on the effectiveness of yellow fever vaccines, which could be done using case-control methodology, are nevertheless warranted, particularly in populations affected by HIV or malnutrition, where vaccine efficacy may be impaired.[715]

DURATION OF IMMUNITY

Neutralizing antibody titers peak approximately 30 days after vaccination and wane thereafter. Persons with a detectable neutralizing antibody titer (LNI = 0.7; plaque-reduction titer using a 50% cutoff = 10) are believed to be protected against clinical infection with wild-type virus, although some studies in animal models suggest a higher level of antibody (e.g., titer = 40) may be required for protection.[485,617,679,716] The level of neutralizing antibodies determines the degree of protection, as observed in the case of persons who are revaccinated with 17D virus and in whom a high antibody level might result in sterilizing immunity, whereas lower levels allow sufficient replication for a boost in immunity (Fig. 63.15). As might be expected, the level of neutralizing antibody after primary or revaccination with 17D determined the level of antibodies present over time.[621]

Immunity following 17D vaccination is remarkably durable, and it has been concluded by many advisory committees that vaccination confers lifelong immunity.[672,673] In the last two years both SAGE and the Advisory Committee on Immunization Practices (ACIP) have revisited the data on long-term

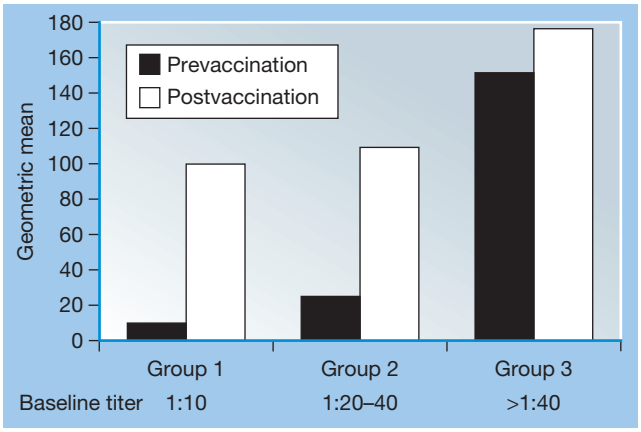


Figure 63.15. Relationship between preexisting neutralizing antibody level and the response (increase in antibody titer) to revaccination with yellow fever 17D. The higher the baseline titer, the lower the increase after revaccination. *(Data from Hepburn MJ, Kortepeter MG, Pittman PR, et al. Neutralizing antibody response to booster vaccination with the 17D yellow fever vaccine.* Vaccine. *2006;24[15]: 2843–2849.)*

immunity following yellow fever vaccination. In a comprehensive review, Gotuzzo and associates found that 75% to 100% of vaccinated persons had protective levels of antibodies more than 10 years postvaccination.[717] In addition, they also found very few vaccine failures (n = 10) reported following yellow fever vaccination. When a similar review was later conducted by the yellow fever vaccine work group of ACIP, one additional study was identified yielding immunogenicity data on 1137 persons vaccinated 10 or more years previously (Table 63.13).[340,358,614,623,649,651,660,718–723] Using a random effects model to account for the number of individuals enrolled in the different studies, the estimated seropositivity rate for persons vaccinated 10 or more years previously was 92% (95% CI, 85–96%).[673] Of the 164 persons vaccinated 20 or more years previously, the estimated seropositivity rate was 80% (95% CI, 74–86%). The ACIP working group, also identified an additional 13 vaccine failures for a total of 24 vaccine failures in more than 540 million doses delivered.[448,449,455,724–726] Of the 24 vaccine failures, five occurred within 10 days of vaccination and likely never seroconverted before developing disease; 16 occurred in individuals who were vaccinated in the last 10 years; and only three occurred in persons who were vaccinated more than 10 years previously, namely at 11, 20, and 27 years postvaccination.[673,726] Given these data, SAGE and ACIP no longer recommend booster doses for most healthy individuals.

Following the recommendation that booster doses of yellow fever vaccine are no longer needed, the World Health Assembly reviewed and approved a proposal to remove the booster dose requirement from International Health Regulations.[727] This change became effective in June 2016. The previous recommendation for requiring proof of vaccination every 10 years was established based on published studies from the 1960s showing that neutralizing antibodies were present in 92% to 97% of persons 16 to 19 years after vaccination.[649,660]

Despite the WHO recommendation to remove booster doses for yellow fever vaccine, certain regulatory authorities have not accepted this recommendation. Based on studies of neutralizing antibody titers and subsets of memory T and B cells at various times postvaccination,[720,728] Brazilian authorities have recommended one booster dose 10 years after primary vaccination in adults and one booster dose at 5 years of age in children who received their initial dose at 9 months of age. Data from a cohort of 651 adults in Brazil found that 93% (95% CI, 88–96%) of vaccine recipients were seropositive at 30 days postvaccination compared to 85% (95% CI, 80–90%) at 12 or more years postvaccination.[720] Interestingly, lower rates of seropositivity (76%; 95% CI, 68–83%) were seen in persons who were 5 to 9 years postvaccination. The authors noted the difference in their results compared to other studies and suggested these differences might be the result of variation in the immune response to specific vaccine strains (i.e., 17DD vs 17D-204) or to preexisting immunity or immune stimulation that might exist in endemic areas compared to nonendemic areas as was seen in a separate study comparing the immune response to yellow fever vaccination

TABLE 63.13 Number and Proportion of Persons With Detected Antibody Levels at ≥10 Years Following Yellow Fever Vaccination

Location	Assay and Cutoffs Used to Detect Antibodies[a]	Years Postvaccination	Detectable Antibody Levels		Reference
			No.	%	
NONENDEMIC					
Germany	$PRNT_{90}$ ≥10	≥10	5/5	100	Reinhardt et al, 1998[358]
United States	Mouse protection	10–15	24/24[b]	100	Rosenzweig et al, 1963[660]
Brazil[c]	Mouse protection	17	105/108	97	Groot et al, 1962[649]
Germany	$PRNT_{90}$ >10	11–38	38/51	75	Niedrig et al, 1999[651]
France	$PRNT_{80}$ ≥10	10–60	80/84	95[d]	Coulange Bodilis et al, 2011[721]
United States	$PRNT_{90}$ ≥10	10–69	68/81	84[e]	CDC, 2014[723]
United States	$PRNT_{90}$ ≥2	30–35	91/116	78	Poland et al, 1981[614]
ENDEMIC					
Uganda	Mouse protection	10	156/202	77	Dick et al, 1952[718]
Brazil	$PRNT_{50}$ ≥20	10	20/20	100	de Melo et al, 2011[623]
Brazil	$PRNT_{80}$ ≥10	≥10	19/19	100	Machado et al, 2013[722]
Brazil	$PRNT_{50}$ ≥10	10–18	307/329	93	Collaborative Group for Studies on Yellow Fever Vaccines, 2014[720]
Democratic Republic of Congo[f]	Mouse protection	12	76/79	96	Courtois, 1954[719]
Colombia	$PRNT_{75}$ ≥10	10–24	13/19[b]	68	Gomez et al, 2008[340]

[a]For mouse protection assays, vaccine recipient serum for which ≥3 of 6 mice survived are included as seropositive. PRNTx% is the reciprocal of the highest serum dilution at which x% of yellow fever virus is inhibited.
[b]Numbers are estimated as some cases included in the data received their vaccination <10 years previously.
[c]In Pouso Alegre in Minas Gerais State of Brazil that at the time of the survey had never reported a human disease case of yellow fever.
[d]Of subjects who received yellow fever vaccine ≥20 years previously, 88% (15/17) were seropositive.[721]
[e]Of subjects who received yellow fever vaccine ≥20 years previously, 84% (26/31)were seropositive.[723]
[f]Uélé in Belgian Congo; corresponds to areas currently found in Democratic Republic of Congo.
CDC, Centers for Disease Control and Prevention; PRNT, plaque-reduction neutralization test.

among Swiss and African cohorts.[676] The Brazilian Collaborative Group for Studies on Yellow Fever Vaccines implemented a more comprehensive, prospective longitudinal study to further explore the duration of immunity following yellow fever vaccination in Brazil.

Although booster doses of yellow fever vaccine are no longer being recommended for most travelers, certain groups, such as pregnant women, hematopoietic stem cell recipients, and HIV-infected persons, might need additional doses of yellow fever vaccine either because of a lack of initial seroconversion or of more rapidly declining antibody titers caused by an underlying condition (see "Primary Vaccine Failure" later for more information on these groups).[673,729] Although initial seroconversion data on children suggest their immune responses to yellow fever vaccine are similar to that of adults,[673] additional work assessing antibody duration should be performed to ensure that persons who receive the vaccine as children will not develop disease decades later as a consequence of declining antibody titers over time.[672]

The basis of durable yellow fever immunity is a matter of speculation. Persistent infection of human cells in culture by yellow fever 17D virus has been described.[730] A human macrophage cell line and human peripheral blood mononuclear cells became persistently infected, with continued production of infectious virus without the appearance of cytopathic effects, in a fluctuating pattern suggesting a role for defective interfering particles.[344] Yellow fever virus was recovered from the brains of rhesus monkeys up to 159 days[181] and from sera 30 days after intracerebral inoculation.[180] A variant virus recovered from a 17D vaccine pool caused persistent infection of mouse brain without overt disease.[84] Although the brain may be a privileged site with respect to immune clearance and is not infected in the course of the vast majority of persons who receive 17D vaccine, these observations, as well as the prolonged synthesis of IgM antibodies in persons immunized with 17D virus,[654,656] suggest that chronic persistent infection or storage of antigen in vivo, possibly in follicular dendritic cells, might explain the durability of the human immune response. In one study of 44 travelers, yellow fever vaccine viral RNA was detected in the urine of two subjects at 21 days and 6 months postvaccination.[731] However, yellow fever vaccine virus RNA was not detected in urine of the remaining 42 persons tested from 0 to 11 months postvaccination. Another hypothesis for the persistence of IgM antibodies following vaccination is incomplete class switching either caused by alterations in the vaccine virus because of the attenuation process or potential interference of the vaccine virus in the regulation or recognition of molecules needed for class switching.[656]

Yellow fever 17D elicits a robust innate immune response in mice and humans, activating TLRs 2, 7, 8, and 9.[608] This drives a strong adaptive immune response, which apparently is associated with a long-lived population of plasma cells secreting antibody. Whether or not restimulation is required (as suggested earlier) or whether the long-lived antibody production occurs simply as a result of the early strong innate response is unclear.

REVACCINATION

Yellow fever vaccination establishes a large pool of memory B and T cells that respond to subsequent exposure to wild-type virus or revaccination. 17D vaccine induces prolonged and continuous antibody production by long-lived plasma cells as a first line of defense, and memory B cells respond with a boost in antibody production on reexposure to the antigen. In addition, a pool of highly functional $CD45RA^+$ memory $CD8^+$ T cells is established after 17D vaccination, in which cells respond rapidly after restimulation, proliferate, and produce multiple effector cytokines and cytotoxic enzymes.[146,665]

According to recent changes to International Health Regulations,[732] the yellow fever immunization certificate for international travel is valid for the lifetime of the vaccinee. Most countries have adopted this change, which is noted under the yellow fever vaccine requirements on both the WHO and CDC's travelers' health websites.[742a] However, not all countries with yellow fever vaccination requirements have adopted this change. In practice, at travel clinics, the vast majority of people receiving 17D vaccine are primary vaccinees. In one study of 3207 travelers who received yellow fever vaccine at a travel clinic visit in the United States, only 149 (5%) had reportedly received a previous dose of yellow fever vaccine 10 or more years previously.[733] These data are similar to unpublished data from three clinics in France (2003–05) indicating that 6% (6211 of 102,112) of persons to whom 17D vaccine was given were revaccinees (Goujon C and Godineau J-P, personal communication, 2006). In contrast, in countries such as Brazil that have conducted repeated mass immunization campaigns in the endemic areas, (multiple) revaccination has been commonplace.

In some studies, prior immunity inhibited the response to revaccination, an expected finding for a live vaccine. A booster response to revaccination was found to be more likely in persons with a low neutralizing antibody titer.[621,734] In others, revaccination or vaccination of persons with naturally acquired immunity was followed by a booster response in the majority of subjects, indicating that sufficient virus replication had occurred or that the antigenic mass of neutralized virus in the vaccine dose was sufficient to elicit a memory response.

Smith and colleagues[681] revaccinated eight subjects who had received 17D vaccine at least once (and up to five times) between 1 and 14 years previously and who had LNIs of 2.1 to 3.6. Only one subject developed a significant rise in neutralizing antibodies. Wisseman and Sweet[713] revaccinated 11 adults 14 months after primary immunization. None had detectable viremia after revaccination, but all responded with a rise in neutralizing antibodies, and seven (64%) had greater than fourfold increases in titer. The geometric mean titers rose from 121 to 576 after revaccination, but in most subjects the response was lower than the response to primary immunization. The dose of virus administered by Wisseman and Sweet was 10- to 30-fold higher than that administered by Smith and colleagues[681]; this plus the lower number of prior immunizations might explain the difference between the studies. Fox and Cabral[670] also concluded that a higher dose of 17D was required to boost immunity in persons with preexisting immunity. In another study, two subjects who were revaccinated 3 to 18 months after their last immunization had a rise in hemagglutination-inhibition but not neutralizing antibody titers.[654] Reinhardt and colleagues[358] noted modest increases in neutralizing antibodies in five of five subjects revaccinated after an interval of more than 10 years, whereas Monath and colleagues[193] found that only three of six subjects revaccinated after a short interval (9 months) developed increases in neutralizing antibodies.

Hepburn and colleagues[621] compared the responses of healthy adult subjects according to the level of neutralizing antibodies present before revaccination. The intervals between previous vaccination and revaccination were not established in this study. As expected, the level of preexisting neutralizing antibodies had a profound effect on the magnitude of the neutralizing antibody response to revaccination (see Fig. 63.15). Subjects with baseline antibody titers of 10 had a 10.1-fold rise (95% CI, 9.26–10.95) in antibody titer versus 2.46 (95% CI, 3.63–5.01) in subjects who had a baseline titer of 20 to 40 (geometric mean, 25.6). Subjects with high baseline

titers (>40; geometric mean, 152) had little or no response to revaccination (sterile immunity). However, the loss of neutralizing antibodies after revaccination was more rapid in subjects with low antibody titers before revaccination. Subjects with low pre-revaccination titers (1:10) dropped to low titers (<1:40) faster (median, 768 days) than those with titers of 20 to 40 (3340 days) or greater than 40 (7709 days).

CORRELATES OF PROTECTION

In the majority of vaccinees, antibody titers are lower and their appearance is delayed compared to natural infection with yellow fever virus,[14,22,308] reflecting less virus replication and antigen expression of the attenuated strain. The minimal protective level of neutralizing antibodies induced by 17D vaccine has been estimated by dose–response studies in rhesus monkeys that were challenged after immunization with virulent yellow fever.[485,539,679] An LNI, measured by plaque reduction, of greater than 0.7 of that measured before challenge (20 weeks after immunization) was strongly associated with protection (Table 63.14). Of 11 vaccinated monkeys that succumbed to challenge, 10 had a prechallenge LNI lower than 0.5 and one had an LNI of 0.9. Clinical trials of 17D vaccine have shown geometric mean LNI values greater than 2.2 measured within a relatively brief interval (usually 1 month) after vaccination (see Table 63.9). Antibody titers measured by serum-dilution plaque-reduction tests have been more variable. WHO currently considers a titer of 10 or greater in the serum-dilution plaque-reduction test with a 50% cutoff as protective for nonhuman primates in new seed lot production guidelines for manufacturers.[531]

Other experimental evidence supports the concept that minimal immunity is required for protection. Neutralizing antibodies appear in the sera of rhesus monkeys on days 6 to 7 after inoculation of 17D virus. However, some monkeys survive challenge performed after a shorter interval (1, 3, or 5 days) after vaccination, despite the absence of detectable antibodies.[20,613] Early protection may be mediated by a low-level specific antibody response (see "Kinetics of the Antibody Immune Response" earlier) or by innate immune responses.

For pivotal trials of a yellow fever 17D vaccine between 1999 and 2001, the FDA recognized neutralizing antibodies as a surrogate for protective immunity against yellow fever virus and accepted the LNI 0.7 as the minimum level for protection based on published animal studies[485,601,619] and data showing that passive immunization with antibodies is sufficient for protection (see "Passive Immunization and Passive-Active Immunization" earlier). Because there was an accepted immune surrogate, it was possible to use the seroconversion rate (using the LNI 0.7 cutoff) as the primary end point in pivotal trials.

Passive immunization is an established method for defining the level of antibody required to protect against virus challenge. Hamsters were injected with graded doses of homologous yellow fever immune serum or control serum and challenged 24 hours after transfer. Before challenge, hamsters were bled for measurement of passively acquired neutralizing antibody (i.e., plaque-reduction neutralization test with 50% cutoff [$PRNT_{50}$]). After challenge, hamsters were followed for illness, death, and weight change. Full protection was observed in animals with $PRNT_{50}$ titers 40 or greater, and partial protection was observed in animals with passive titers of 10 to 20.[716] These are somewhat higher titers than established as the immune correlate for encephalitis viruses (e.g., Japanese encephalitis), where a titer of 10 is considered protective in both mice and humans.[735] It is possible that higher neutralizing antibody titers are required to protect against viscerotropic infections than are required to protect the brain against neuroinvasion.

TABLE 63.14 Neutralizing Antibody Response to Yellow Fever 17D Vaccine Correlates With Protection Against Challenge With 5.0 Log_{10} LD_{50} of Virulent Asibi Virus

Log Neutralization Index (Plaque Reduction)	Protected	
	Yes	No
>0.7	51/54 (94%)	1/11 (9%)
<0.7	3/54 (6%)	10/11 (91%)

Chi-square: $P < .0001$.
LD_{50}, median lethal dose for 50% of test subjects.
From Mason RA, Tauraso NM, Spertzel RO, et al. Yellow fever vaccine: direct challenge of monkeys given graded doses of 17D vaccine. Appl Microbiol. *1973;25(4):539–544.*

PRIMARY VACCINE FAILURE

A small minority of healthy persons inoculated with 17D vaccine do not develop detectable neutralizing antibodies (see Table 63.9). Although there are few reports on this question, it appears that failure to mount a response on primary immunization does not represent an absolute refractoriness, because revaccination may be followed by development of neutralizing antibodies.[600]

Development of clinical yellow fever in persons with a history of 17D vaccination has been reported rarely, with approximately 24 cases reported among at least 540 million persons vaccinated (see "Kinetics of the Antibody Immune Response" earlier for more details).[448,449,455,672,673,724–726]

Malnutrition and Vitamin Deficiency

Protein-calorie malnutrition has been associated with failure to respond immunologically to 17D vaccine. In a study of eight children (mean age, 2 years) with kwashiorkor, only one seroconverted after 17D vaccination, compared to five of six control children.[736] Further studies are required to assess the relevance of this finding to use of 17D vaccine in the EPI in Africa, particularly where covariates, such as HIV infection, can diminish immunoresponsiveness. Although there were no obvious adverse effects of immunization in the small series of kwashiorkor patients, the safety of yellow fever immunization in infants with malnutrition has not been fully assessed. Gandra and Scrimshaw[737] showed that 17D vaccine administered to children 4 to 11 years old recovering from malnutrition resulted in a significant catabolic effect lasting up to 12 days, in the absence of fever or other signs of increased metabolic rate. This suggests that 17D vaccine could result in a net loss of body nitrogen and aggravate the clinical effects of protein malnutrition. Further studies in fasted adults showed that metabolic changes and mobilization of protein associated with yellow fever immunization were related to dietary factors; a catabolic effect was not observed in subjects consuming a high-protein diet.[738,739]

Vitamin A deficiency is associated with impaired T-helper function, antibody responses, and susceptibility to infectious diseases. Adults with low and high vitamin A levels were vaccinated against yellow fever in a controlled double-blind trial. T-cell responses measured by proliferation, some cytokines, and neutralizing antibodies tended to be lower in the subjects with low vitamin A.[740]

Pregnancy

In a field study conducted in Nigeria, the IgM ELISA and neutralizing antibody responses to 17D vaccine were statistically

significantly impaired in pregnant women compared to nonpregnant girls and women of childbearing age, male students, and the general population.[625] The majority (88%) of the subjects were immunized in the third trimester of pregnancy. Only 39% of the pregnant women developed neutralizing antibodies, compared to 82% to 94% of the other groups. The difference was attributed to the immunosuppression associated with pregnancy, emphasizing the need to reimmunize at-risk women who have been inadvertently vaccinated during pregnancy.

In contrast, a study in Brazil of 433 women inadvertently immunized with the 17DD vaccine during pregnancy showed a 98% seroconversion rate by neutralization test.[741] The majority of the subjects were in the early stage of pregnancy (mean gestational age at immunization, 5.7 weeks; SD, 4.9 weeks). The difference between this study and that of Nasidi and colleagues might be explained based on gestational age, nutritional factors, or preexisting heterologous flavivirus immunity (not defined). It is unlikely that difference in the vaccine substrain played a role.

Hematopoietic Stem Cell Transplant Recipients

Data are limited on safety and immunogenicity for yellow fever vaccine in hematopoietic stem cell transplant recipients. However, data suggest most recipients become seronegative to live viral vaccine antigens after transplantation.[742] Infectious Diseases Society of America guidelines recommend readministering live viral vaccines, such as measles, mumps, and rubella vaccine and varicella vaccine, to posttransplantation patients if the recipient is seronegative and is no longer immunosuppressed.[743]

HIV Infection

There is limited evidence to suggest that asymptomatic HIV infection reduces immune responses to yellow fever vaccine.[715,744] HIV infection reduces immunologic responsiveness to a number of nonreplicating and live childhood vaccines. In the case of inactivated flaviviral vaccines, HIV infection reduced the seroconversion rate or resulted in lower geometric titers to Japanese encephalitis vaccine[745,746] and reduced seroconversion rates to tickborne encephalitis vaccine when compared to uninfected controls.[747] Some case reports indicate that vaccination of HIV-infected subjects who are not immunosuppressed ($CD4^+$ T lymphocytes >500/mm^3) was followed by seroconversion.[748] Tattevin and colleagues[749] vaccinated 12 subjects with $CD4^+$ counts as low as 240/mm^3 without adverse effects; all developed yellow fever–neutralizing antibodies, and there were no significant changes in $CD4^+$ cell counts or viral loads. On the other hand, 17D vaccine was administered to 33 adult travelers with HIV infection and $CD4^+$ cell counts greater than 200 per mm[750]; only 70% responded by 1 month after vaccination, and one person seroreverted between 1 and 3 months after vaccination. A separate study found 93% (13/14) of HIV-infected persons with no baseline immunity seroconverted following vaccination, but the time needed to achieve seroconversion was prolonged.[751]

A retrospective cohort study found that significantly fewer HIV-infected persons had yellow fever–neutralizing antibodies at 1 year after vaccination compared with vaccinated uninfected persons (83% and 97%, respectively; $P = .01$).[715] Among HIV-infected infants in one developing nation, only 17% developed neutralizing antibodies within 10 months of yellow fever vaccine compared with 74% of HIV-uninfected controls matched for age and nutritional status.[752] In addition to a suboptimal immune response in primary vaccine recipients, a small study also found that there was a booster effect following repeat vaccination in only three of nine HIV-infected persons.[751]

Antiretroviral therapy with resulting improvements in $CD4^+$ counts is associated with an improved response to vaccines. If feasible, patients starting on antiretroviral therapy should be deferred for 17D vaccination until $CD4^+$ counts exceed 350 to 400 per mm^3 for several months and HIV RNA levels become undetectable.

The results of these various studies indicate that HIV infection can impair the immune response to 17D vaccine. Because both HIV and 17D viruses exhibit tropism for human lymphoid cells,[753] it is possible that HIV infection interferes with replication of 17D vaccine. This is supported by data that found HIV viral load was more likely to determine immune response to the vaccine than the CD4 count was.[744,754] In vitro studies and clinical trials comparing 17D viremia in HIV-infected and -uninfected subjects would help address how HIV infection affects the immune response to the vaccine.

Yellow Fever Vaccine, Antimalarial Drugs, and Tuberculin Skin Tests

Chloroquine is known to inhibit the immune response to inactivated rabies vaccine and live oral cholera vaccine, and it interferes with flavivirus replication in vitro. In humans, there was no inhibitory effect of chloroquine at doses used for malaria prophylaxis on the response to yellow fever 17D.[755]

Despite an earlier study to the contrary, yellow fever vaccine does not appear to depress tuberculin skin test sensitivity[756] and is unlikely to adversely affect the course of active tuberculosis. However, there are no field studies of this question.

Other Host-Specific Effects

Evaluation of neutralizing antibody responses in a large clinical trial revealed some heretofore unknown and subtle host effects on the immune response (see Table 63.10).[601] The mean LNI was higher in smokers than in nonsmokers. There are few studies on the interaction of smoking and the immune system, but most report a suppressive effect, for example after hepatitis B vaccine.[757] In the case of 17D vaccine, it was postulated that the adaptive immune response might be enhanced by the suppression of innate immune responses (e.g., NK cell function) attributable to smoking.

ADVERSE EVENTS

17D vaccines are widely acknowledged as one of the safest vaccines in use. More than 540 million persons have been immunized, and the vaccine has a long record of tolerability and safety.[672,682,758,759] However, the vaccine has been under close scrutiny as clinical, histopathologic, and virologic evidence linked 17D vaccine in 2001 to a previously unrecognized serious adverse events, namely viscerotropic disease.

Viremia Following 17D Vaccination

Whereas wild-type yellow fever virus causes high levels of viremia in monkeys and humans, 17D vaccine induces minimal virus titers in circulating blood. A control test for the reduced viscerotropism of 17D vaccines is the measurement of viremia in monkeys on days 2, 4, and 6 following intracerebral inoculation, which must not exceed 500 IU per 0.03 mL on any day and must not exceed 100 IU per 0.03 mL on more than one day.[531]

Viremia following 17D vaccination has been measured in adult humans. Smith and colleagues[22] found virus in serum of 13 (46%) of 28 subjects given 3.0 to 4.0 $\log_{10}$ MLD_{50} of

17D vaccine. The earliest onset of viremia occurred on day 4 and the latest on day 10. Of the 13 viremic subjects, four were viremic for 1 day, five for 2 days, and four for 3 days. The quantity of virus in blood was extremely small in all cases (<2 MLD_{50}/0.03 mL). Sweet and Kitaoka[183] measured viremias in subjects with and without prevaccination neutralizing antibodies to Japanese encephalitis virus. The 17D vaccine (National Drug Co., Philadelphia, PA) contained about 6.4 log_{10} suckling mouse intracerebral LD_{50}/dose (equivalent to approximately 5.3 to 5.8 log_{10} MLD_{50},[679] and thus substantially higher than the dose used by Smith and colleagues).[22] Viremia was measured by intracerebral inoculation of infant mice with undiluted serum. Of 25 subjects without preexisting flavivirus immunity, 15 (60%) had detectable viremia on 1 or more days during the 6-day sampling period (days 3 to 8 after vaccination). The highest incidence of viremia (48%) was on day 5, and the mean duration was 1.9 days. Titers were very low, because none of the sera caused 100% mortality in infant mice. There was no difference in the incidence or duration of viremia in subjects with preexisting immunity to Japanese encephalitis virus.[679]

In a study of 15 young adults, Wheelock and Sibley[599] determined 17D viremia by a more-sensitive method: inoculation of 0.1 mL of plasma into tube cultures of BHK-21 cells. Fourteen subjects (93.3%) had detectable viremia. The average time to onset of viremia was 4.4 days (range, 3 to 6 days), and the mean duration was 2.5 days (range, 1 to 5 days). Viremic days were sequential and continuous in most cases, but one subject had detectable viremia on days 4, 8, and 9. A rough estimation of the level of viremia was possible based on the detection in cell cultures inoculated with varying volumes of plasma. Virus titers were exceedingly low; in only three of 14 subjects was virus detected in cultures inoculated with 0.001 mL of plasma.

Actis and Sa Fleitas[760] studied 12 adults who received an unspecified dose of 17DD-EP vaccine produced in Brazil. The duration and level of viremia, detected by intracerebral inoculation of weanling mice, was similar to those in previous studies, but a somewhat higher proportion of subjects were viremic than in other studies using mice for detection of viremia. In two clinical trials of a chimeric yellow fever–Japanese encephalitis vaccine, yellow fever 17D (YF-Vax) was used as a control for safety in healthy adults 18 to 59 years of age and viremia was measured by plaque assay in Vero cells.[193,617] Nearly all subjects were viremic on one or more days. Approximately 30% to 60% of the subjects were viremic between days 4 and 6 after vaccination, with lower percentages at earlier and later time points (Fig. 63.16). The mean duration of viremia was approximately 2 days. Viremia levels were low (mean peak titers <20 PFU) and in no case exceeded 2 log_{10} PFU/mL. Six previously vaccinated subjects experienced no viremia after revaccination.

Similarly, Reinhardt and colleagues[358] inoculated 12 naïve and five previously vaccinated healthy adults 18 to 50 years of age with the yellow fever 17D vaccine manufactured by the Robert Koch Institute. Viremia levels were measured by plaque assay in PS cells and by RT-PCR. The pattern and level of viremia were similar to those described earlier. There was no correlation between the level of viremia and the height of the neutralizing antibody response. The highest incidence of viremia by plaque assay was on days 5 (42%) and 6 (33%), the duration of viremia was 1 to 3 days, and the peak titer was 97 PFU/mL, with most values less than 20 PFU/mL. All 12 naïve subjects, including five who had no viremia by plaque assay, tested positive by RT-PCR, and sera contained detectable viral RNA at least 1 day longer than by infectivity assay. None of the revaccinated subjects tested positive by RT-PCR or plaque assay.

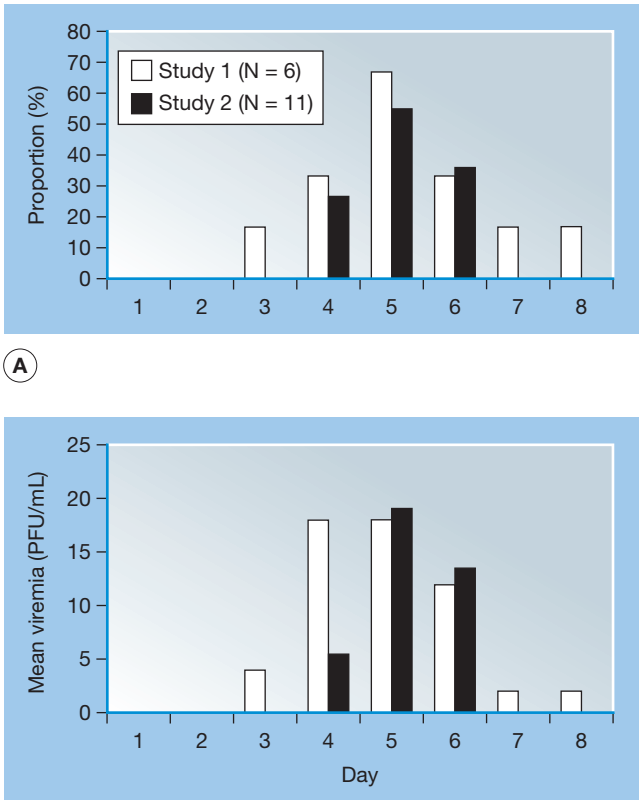


Figure 63.16. A, Percentages of healthy adults in two studies with viremia detectable by plaque assay following inoculation of yellow fever 17D vaccine (YF-Vax). **B,** Mean viremia levels in the same studies. Viremia occurred principally between days 4 and 6 and did not exceed 2 log_{10} PFU/mL in any subject. PFU, plaque-forming units. *(Data from Monath TP, McCarthy K, Bedford P, et al. Clinical proof of principle for ChimeriVax: recombinant live, attenuated vaccines against flavivirus infections.* Vaccine*. 2002;20[7–8]:1004–1018; and an unpublished study, 2004.)*

In two newer studies using the same cohort of 80 flavivirus-naïve adult subjects, infectivity assays found 54 (68%) were positive and all had detectable viral genomes by TaqMan real-time PCR.[361,668] Viral genomes were detected as early as 2 days after vaccination and were no longer detected in most recipients 11 days after vaccination. For all individuals, the peak viremia occurred between 5 and 7 days postvaccination, which is similar to previous studies that have used less-sensitive techniques.

Surprisingly, in a study of eight seronegative healthy adults who were 18 to 55 years old, had no history of prior yellow fever immunization, and who received the 17DD Brazilian vaccine, no viremia could be detected by plaque assay or RT-PCR on days 2, 4, and 7 after inoculation.[618] There are no previous data to suggest that the 17DD strain would be less viremogenic than the 17D-204 used in all the studies of viremia cited earlier, and the viremia profile in monkeys is similar.[551] In fact, a newer study using quantitative RT-PCR following 17DD vaccination documented viremia in up to 15% of vaccine recipients with peak viremia occurring 5 days postvaccination.[557] In a much larger study of 1087 adults in Brazil, viremia was detected in 2.2% and 3.7% of subjects who received 17D-DD and 17D vaccines, respectively, suggesting no difference between vaccines.[547] However, the publication

did not provide exact denominators or covariates (baseline immunity), and the overall incidence of viremia is lower than expected from previous studies.

The objectives of dose sparing and needleless immunization have spurred renewed interest in epidermal and intradermal immunization, as well as administration of lower amounts of vaccine virus. Monkeys given a chimeric yellow fever–Japanese encephalitis vaccine developed higher viremias after epidermal than subcutaneous inoculation.[201] However, a clinical trial of intradermal 17D at one-fifth dose versus subcutaneous inoculation at full dose did not show a difference in the proportion viremic or titers estimated by RT-PCR.[592] In a study exploring the viremia and immune response to different doses of 17DD vaccine, a 10-fold lower dose than what is currently used was able to solicit equivalent viremia levels and kinetics as full dose vaccine.[557,558] Doses 50- to 1000-fold lower than reference were also able to induce low levels of detectable viremia but the kinetics were different and doses containing 200-fold lower than reference amounts of 17DD virus produced lower rates of seroconversions.

Taken together, these studies confirm that very low levels of virus are present in circulating blood for a brief period following 17D vaccination. Viremia occurs during the latter half of the first week after vaccination, and thus the incubation period and duration of viremia are not dissimilar to natural infection with yellow fever virus. Cessation of viremia corresponds to the time of appearance of neutralizing antibodies 8 to 9 days after immunization (see "Kinetics of the Antibody Immune Response" earlier). Titers of virus in blood are far below the infection threshold for mosquito vectors. The low viremia following 17D vaccine might also explain the apparent low risk of transmission of the virus to the fetus in women who have been immunized during pregnancy (see "Pregnancy and Adverse Events" later) and the low incidence of neuroinvasion and yellow fever vaccine–associated neurologic disease (YEL-AND), formerly called postvaccinal encephalitis. Detection of viremia is influenced by test methodology. PCR is more sensitive than infectivity assays[358,559] and might detect viral RNA in the absence of infectivity; for example, after the appearance of antibody.

There are no data on viremia levels in infants or children given 17D vaccine. The higher risk of YEL-AND in young infants suggests the possibility that viremia levels may be higher or more prolonged; alternatively, susceptibility could reflect immaturity of the blood-brain barrier. There are limited data on the viremia levels seen for older adults. One study found that travelers 60 years of age and older were more likely to have detectable viremia with higher levels that were present for longer when compared to younger adults.[256] This finding, in addition to the delayed antibody response in older adults compared to younger adults, has been hypothesized as a reason more serious adverse events have been identified among older vaccine recipients. No data are available on the height and duration of viremia in persons who are immunosuppressed.

Viremia is an accepted marker of viscerotropism of wild-type yellow fever virus and is the basis for the 17D vaccine safety test in monkeys that specifies limits on the level of viremia for release of virus seeds. To differentiate the normal, low-level transient viremia observed in the course of normal vaccination from the viremia that may be anticipated in a viscerotropic vaccine accident, prolonged or abnormally high viremia has been incorporated into the yellow fever vaccine algorithm that is provided with the Brighton case definition for viscerotropic disease.[761] In one subject with YEL-AVD, in whom the pattern of viremia was adequately measured, viremia was prolonged and reached a level approximately 100-fold higher than seen following normal vaccination.[266]

Historical Problems

There have been two notable adverse events in the history of 17D vaccine that have led to changes in manufacturing and delivery of the vaccine. The first (described in "Seed Lot System" under "Yellow Fever 17D Vaccine" earlier) was the occurrence of YEL-AND associated with uncontrolled passage of 17D substrains during the early years of vaccine manufacture.[269,528,682] The problem was resolved when stabilization of passage level during vaccine manufacture was instituted in 1941. The second event was the development of acute hepatitis in persons who received 17D vaccine, which was recognized as early as 1937 by Findlay and MacCallum.[762] Cases were reported in Brazil during the vaccination campaigns between 1938 and 1940 and, after careful study, were attributed to an adventitious agent in the vaccine rather than to viscerotropism of 17D virus.[763] In 1942, a massive outbreak of hepatitis appeared in United States military personnel immunized with 17D vaccine, resulting in approximately 28,000 cases and 62 deaths.[570] These reactions were the result of using pooled human serum (contaminated with hepatitis B virus) as a vaccine stabilizer.[568] This practice was discontinued in Brazil in 1940 and in the United States in 1943,[764] and the problem resolved.

Common Adverse Events and Clinical Studies

Historical Events and Studies

Fever, headache, and backache, described as mild, were noted since the earliest studies of 17D vaccine.[21] During large-scale field studies in Brazil in 1937 to 1938, Soper and colleagues noted mild systemic reactions in 5% to 8% of vaccinees.[527] Among 2457 persons in Brazil from whom "reasonably accurate" clinical follow-up was obtained by Smith and colleagues, 15% complained of headache for 1 to 2 days, 10% developed pains in the body (usually accompanied by headache), 1.4% missed time from work (usually only 1 day), and 0.2% spent 1 or more days in bed.[22] These reactions, which were generally considered mild, occurred on the fifth sixth, or seventh day after immunization. Local reactions at the site of inoculation were not observed, and no systemic allergic reactions were noted.

Recent Clinical Studies

Reactogenicity of 17D vaccine was monitored in twenty-four clinical studies conducted between 1953 and 2014, including one placebo-controlled study (Table 63.15).* Self-limited and mild local reactions (erythema and pain at the inoculation site) and systemic reactions (headache, headache and fever, and fever without symptoms) occurred in a minority of subjects 3 to 7 days after immunization. Reactogenicity in children typically less than 1 year of age is no greater (or perhaps less) than in adults; this conclusion was also made during the early studies in Brazil in 1937 to 1938.[22]

When subjects were under daily surveillance using a diary card to record local and systemic adverse events, a higher incidence of adverse events was detected (Tables 63.16 and 63.17).[624,634]

In one study, 1440 subjects were monitored; half of subjects received YF-Vax (U.S.-produced vaccine) and half received ARILVAX (UK-produced vaccine).[601] Safety was assessed through a diary card and by clinic visits, recording all signs

*References 537, 546, 548, 557, 601, 619, 620, 622, 624, 627, 629, 632, 634, 636, 639, 640, 642, 644, 647, 694, 765–768.

TABLE 63.15 Adverse Events Noted in Clinical Trials of 17D Vaccines Manufactured Using Standardized Methods, in Which Adverse Events Were Specifically Monitored

Reference	Vaccine	Placebo Controlled?	Subjects	Country	No. Tested	Result
Kouwenaar W, 1953[765]	Rocky Mountain Laboratory, U.S.	No	Adults	Netherlands	1130[a]	4.5% with low-grade fever, 7.6% with mild headache, muscle ache, malaise lasting hours to 4 days
Tauraso, et al, 1972[632]	National Drug Co., U.S.	No	Adult prisoners	United States	1676	Low reactogenicity, minimal subjective and objective reactions
Tauraso, et al., 1972[627]	National Drug Co., U.S.	No	Merchant Marine cadets	United States	181[b]	No local or generalized adverse reactions
Freestone, et al, 1977[634]	Wellcome, U.K.	No	Adults	United Kingdom	30[c]	See Table 63.16
Moss-Blundell, et al, 1981[624]	Wellcome, U.K.	No	Adult male military	United Kingdom	514[d] seronegative 64[d] seropositive	3.9% reported any reaction; 0.2% headache only; 3.3% local reactions; 0.4% other 1.6% reported local reaction
Fabiyi, et al, 1983[766]	Pasteur-Mérieux, France	No	Adults	Nigeria	762	No adverse events
Wolga, et al, 1986[694]	Pasteur-Mérieux, France	No	Adults	France	195	No adverse events
Pivetaud, et al, 1986[767]	Pasteur-Mérieux, France	No	Adults	France	370	See Table 63.16
Roche, et al, 1986[636]	Pasteur-Mérieux, France	No	Adult male military	France	297	No adverse events attributable to vaccine
Lhuillier, et al, 1989[629]	Pasteur-Mérieux, France	No	6–9-month-old infants	Côte d'Ivoire	74[e]	No adverse reactions
Mouchon, et al, 1990[639]	Pasteur-Mérieux, France	No	6–12-month-old infants	Cameroon	75[e]	No adverse reactions
Soula, et al, 1991[640]	Pasteur-Mérieux, France	No	4–24-month-old infants	Mali	115[e]	21% with fever >38°C; 2.6% with local induration; 1.7% with rash; 7% with conjunctivitis
Ambrosch, et al, 1994[642]	Pasteur-Mérieux, France	No	Young adults	Austria	41	2.4% fever >38°C; 4.8% malaise; short duration
Dumas, et al, 1997[644]	Pasteur-Mérieux, France	No	Adults	Switzerland	59	Asthenia, GI disorders, headache, myalgia, malaise, or fever in 42%
Lang, et al, 1999[622]	Pasteur-Mérieux, France, and Evans Medical, United Kingdom	No	Adults	United Kingdom	211	See Table 63.16
Osei-Kwasi, et al, 2001[620]	Institute Pasteur, Senegal	No	6–9-month-old infants	Ghana	420	23.18% fever in 6 mo and 21.5% fever in 9 mo; cough, diarrhea, mild injection reactions also common in both age groups
Monath, et al, 2002[601]	Aventis-Pasteur, U.S., and Evans Medical, U.K.	No	Adults	United States	1440	See Table 63.17
Camacho, et al, 2005[546]	Bio-Manguinhos, Brazil	Yes	Adults	Brazil	1081	See Table 63.17
Pfister, et al, 2005[537]	Berna-YF, Switzerland; Robert Koch Institute, Germany; Aventis Pasteur, France	No	Adults	Switzerland	340	See Table 63.16

TABLE 63.15 Adverse Events Noted in Clinical Trials of 17D Vaccines Manufactured Using Standardized Methods, in Which Adverse Events Were Specifically Monitored *(Continued)*

Reference	Vaccine	Placebo Controlled?	Subjects	Country	No. Tested	Result
Belmusto-Worn, et al, 2005[619]	Aventis-Pasteur, U.S., and Evans Medical, U.K.	No	Children	Peru	1107	See Table 63.17
Ripoll, et al, 2008[768]	Sanofi Pasteur (Stamaril) and Bio-Manguinhos (17DD)	No	Adults	Argentina		See Table 63.16
Nascimento Silva, et al, 2011[548]	Bio-Manguinhos (17DD) and 17D-213/77	No	Children (12–23 mo)	Brazil	1828	14.2% fever; 1.9% vomiting; 3.3% irritability; 2.8% injection site pain; 1.5% injection site erthyema
Martins, et al, 2013[557]	Bio-Manguinhos (17DD)	No (dose-ranging study)	Adults	Brazil	900	15.2% fever; ~20% headache; ~20% fatigue; 21.3% local pain (for normal dose)
Alberer, et al, 2015[647]	Sanofi Pasteur (Stamaril)	No	Adults	Germany, Czech Republic	299	2–12% injection-site pain; headache 4–8%; 2–3% fatigue; 1–8% influenza-like illness

[a]Including 465 with a history of atopy or other allergic reactions.
[b]This was a study of sequential or simultaneous inoculation of smallpox and yellow fever 17D vaccines; in the group of 181 subjects included here, the vaccines were separated by 28 days.
[c]Subjects receiving standard dose of 17D vaccine.
[d]Yellow fever neutralizing antibody status before 17D vaccination; numbers differ from Table 63.16 because not all subjects were interviewed for adverse events.
[e]This was a study of combined or sequential vaccination; only subjects receiving 17D alone are included in the table.
GI, gastrointestinal.

and symptoms. There were no serious adverse events attributable to either vaccine. Significantly more subjects in the YF-Vax group (72%) experienced one or more nonserious vaccine-related adverse events than in the ARILVAX group (65%, $P = .008$) (see Table 63.17). The difference was because of a higher rate of local reactions of mild to moderate severity caused by YF-Vax.

The most common systemic side effects were headache, asthenia, myalgia, malaise, fever, and chills. These systemic adverse events are presumably caused by activation of T lymphocytes and the release of cytokines, including IFNs and TNF-α during the viremic period.[358] These were generally mild or moderate, but 7% to 8% of all treatment-emergent events were severe and were reported to interfere with normal activities. Rash was noted in approximately 3% of the subjects and urticaria in 0.3%. There were no cases of anaphylaxis, serum sickness, or other severe allergic reactions. The incidence of nonserious adverse events was lower in elderly persons than in younger subjects, and the difference was statistically significant for headache, malaise, injection site edema, and pain. Lower reactogenicity of 17D vaccine in elderly versus young adult travelers was also noted in a report by Philipps and colleagues.[769] Injection-site reactions occurred between days 1 and 5. Systemic adverse events also occurred at highest incidence during days 1 to 5, but they continued between days 6 and 11. The mean white blood cell count decreased slightly between baseline and day 11, with a mild neutropenia. No subjects developed clinically significant thrombocytopenia ($<0.5 \times 10^5/\mu L$).

Elevations in aspartate aminotransferase were seen in 3.5% to 3.9% of subjects; 3.9% to 4.6% of subjects had an increase in alanine aminotransferase from normal to abnormal range between baseline and day 11. In most cases, levels were minimally elevated and levels returned to normal or had decreased by day 31. Subjects with elevated serum enzymes had no associated syndrome or constellation of other clinical laboratory abnormalities. There was no relationship between elevated serum enzymes and use of concomitant medications.

In a second randomized, double-blind Phase III 17D vaccine study, a total of 1107 healthy children 9 months to 10 years of age in Peru were recruited (see Table 63.17).[619] Safety was assessed through a diary card and by clinic visits, recording all signs and symptoms. No treatment-related serious adverse events were reported. A similar proportion of subjects in each group reported at least one adverse event: 441 of 738 (59.8%) for ARILVAX versus 211 of 369 (59.9%) for YF-Vax. Most (591; 96.7%) of the adverse events were mild and resolved without treatment. These trials were comparative studies of two vaccines, intended to show noninferiority against a comparator vaccine. Without a placebo group, it is difficult to ascribe systemic adverse events and minor laboratory abnormalities to the vaccines under study.

One double-blind trial included a placebo control.[546] This study, conducted in Brazil, enrolled 1087 subjects 15 to 68 years of age; 272 received a 17D-213 yellow fever vaccine, 543 received two lots of the 17DD vaccine, and 272 received placebo (see Table 63.17). The study participants recorded on a diary card all signs and symptoms for a 10-day period. There were no severe or immediate adverse events, and four hospitalizations within 30 days of vaccination were for reasons unrelated to vaccination.[546] Compared to placebo, there was slightly more local reactions reported among vaccinees compared to the placebo group (5% vs 2.6%; $P = .23$) and more systemic adverse events reported among vaccinees compared to placebo group (20% vs 14%, $P = .11$). The proportion of vaccinees seeking medical care or missing work related to their

TABLE 63.16 Incidence of Nonserious Adverse Events After Yellow Fever 17D Vaccination[a]

Reference	Subjects	Adverse Event		
		Type	No.	%
Freestone, et al, 1977[634]	30 adults	Any reaction	10	33
		Headache only	1	3
		Headache and fever	2	7
		Local reaction only[b]	2	7
		Local reaction and headache	4	13
		Lymphadenopathy	1	3
Moss-Blundell, et al, 1981[624]	86 adults	Any reaction	36	42
		Headache only	9	10
		Fever only	7	8
		Headache and fever	4	5
		Local reaction only	1	1
		Local reaction and headache	6	7
		Other[c]	9	10
Pivetaud, et al, 1986[767]	370 ages 1–84 y; majority ages 20–39 y	Any reaction	94	25
		Flu-like systemic reaction	42	11
		Fatigue, weakness	28	8
		Gastrointestinal complaints, with or without vomiting	9	2
		Headache only	5	1
		Headache, fever, vomiting	1	0.3
		Headache, dizziness	1	0.3
		Local reaction[b]	17	5.0
Lang, et al, 1999[622]	211 adults Stamaril and ARILVAX	Percentage with ≥1 events		
		Systemic events	36	17.0
		Number of events:		
		Fever only	0	0
		Asthenia	19	9.0
		Headache	25	11.8
		Myalgia	15	7.1
		Arthralgia	2	0.9
		Gastrointestinal events	7	3.3
		Local reactions[d]	33	16.0
Pfister, et al, 2005[537]	304 adults Berna-YF, Robert Koch Institut, and Stamaril	Systemic events	155	51.0
		Arthralgia	38	12.5
		Asthenia	72	23.7
		Fever	26	8.6
		Gastrointestinal disorders	40	13.2
		Headache	96	31.6
		Lymphadenopathy	20	6.6
		Myalgia	55	18.1
		Skin rash	13	4.3
		Local events	127	41.8
		Erythema	84	27.8
		Induration	53	17.4
		Pain	69	22.7
Ripoll, et al, 2008[768]	2496 1252 subjects received 17D-204 vaccine (Stamaril) 1244 subjects received 17DD (Bio-Manguinhos) There were no differences, and data are combined for analysis	Any solicited systemic event	774	31.0
		Pyrexia	222	8.9
		Asthenia	406	16.3
		Arthralgia	188	7.5
		Headache	433	17.3
		Myalgia	322	12.9
		Nausea	125	5.0
		Vomiting	40	1.6
		Rash	27	1.1
		Any severe systemic	39	1.6
		Any local solicited	410	16.4
		Pain	378	15.1
		Erythema	35	1.4
		Induration	38	1.5
		Any severe local	20	0.8

[a]In these studies, subjects without preexisting yellow fever immunity completed a daily diary or were actively followed up after yellow fever 17D vaccination. The trials were not blinded and placebo-controlled.
[b]Pain and/or redness at site of inoculation.
[c]Abdominal pain (1), nausea and vomiting (1), upper respiratory infection (6), rash (1).
[d]Pain, erythema, induration/swelling, or hematoma.

TABLE 63.17 Three Controlled Trials in Which Adverse Events[a] Were Assessed and Actively Monitored With Diaries and Frequent Clinic Visits

	Incidence of Related AEs Occurring in ≥2% of Adult Subjects (Monath et al[601])					Incidence of Related AEs Occurring in Adult Subjects (Camacho et al[546])								Incidence of Related AEs Occurring in ≥5% of Children (Belmusto-Worn et al[619])			
	ARILVAX		YF-Vax			17DD Lot 1		17DD Lot 2		17D-213		Placebo		ARILVAX		YF-Vax	
Body Class System	**N**	**%**	**N**	**%**	***P***[b]	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Subjects	715	100	725	100		269		269		269		271		738		369	
Subjects with no AE	248	34.7	204	28.1													
Subjects with 1 AE	467	65.3	521	71.9	.008	48	17.8	57	20.9	59	21.7	39	14.3	441	59.8	221	59.9
Body as a whole	435	60.8	496	68.4	.003												
Chills	76	10.6	73	10.1	.730												
Fever	102	14.3	109	15.0	.710	15	5.6	8	3.0	17	6.3	12	4.4	197	26.7	98	26.6
Fever (>38.5°C axillary)						12	4.5	7	2.6	11	4.1	6	2.2				
Headache	225	31.5	228	31.4	1.000	28	10.4	35	12.8	25	9.2	19	7.0	44	6.0	26	7.0
Myalgia	171	23.9	182	25.1	.624	14	5.2	18	6.7	20	7.4	7	2.6				
Flu-like symptoms[c]						14	5.2	19	7.1	20	7.4	7	2.6				
Infection	16	2.2	9	2.6													
Malaise	134	18.7	129	17.8	.682									57	7.7	26	7.0
Pain	15	2.1	11	1.5													
Other symptoms						23	8.6	26	9.7	31	11.5	17	6.3				
LOCAL REACTIONS (INJECTION SITE)																	
Edema	61	8.5	144	19.9	<.001												
Inflammation	113	15.8	213	29.4	<.001												
Erythema						9	3.3	11	4.0	14	5.1	7	2.6				
Pain	171	23.9	286	39.4	<.001	9	3.3	9	3.3	13	14.8	7	2.6				
Reaction	26	3.6	41	5.7	.080	9	3.3	11	4.0	14	5.1	7	2.6				
DIGESTIVE SYSTEM																	
General	48	6.7	37	5.1	.219	11	4.1	13	4.8	10	4.3	12	3.7				
Diarrhea														91	12.3	40	10.8
Nausea	23	3.2	22	3.0		7	2.6	8	3.0	6	2.2	8	3.0				
Appetite decreased														62	8.4	34	9.2
Vomiting														43	5.8	11	3.0
MUSCULOSKELETAL SYSTEM																	
All	174	24.3	185	25.5	.626												
NERVOUS SYSTEM																	
All	34	4.8	35	4.8	1.000												
RESPIRATORY SYSTEM																	
General	44	6.2	29	4.0	.071												
Nasopharyngitis														64	8.7	32	8.7
Pharyngitis	16	2.2	10	1.4										129	17.5	54	14.6
Pharyngotonsillitis														45	6.1	21	5.7
Cough														55	7.5	20	5.4
Rhinitis	18	2.5	12	1.7													

Continued on following page

TABLE 63.17 Three Controlled Trials in Which Adverse Events[a] Were Assessed and Actively Monitored With Diaries and Frequent Clinic Visits *(Continued)*

	Incidence of Related AEs Occurring in ≥2% of Adult Subjects (Monath et al[601])					Incidence of Related AEs Occurring in Adult Subjects (Camacho et al[546])								Incidence of Related AEs Occurring in ≥5% of Children (Belmusto-Worn et al[619])			
	ARILVAX		YF-Vax			17DD Lot 1		17DD Lot 2		17D-213		Placebo		ARILVAX		YF-Vax	
Body Class System	N	%	N	%	P[b]	N	%	N	%	N	%	N	%	N	%	N	%
SKIN AND APPENDAGES																	
All	25	3.5	31	4.3	.496												
Rash	21	2.9	23	3.2													

[a]Adverse events considered remotely, possibly, probably, or definitely related to clinical trial materials.
[b]Based on Fisher's exact test; *P* value is provided for each adverse event wherever the percentage for either treatment group is not less than 5%.
[c]Defined as myalgia plus headache or fever.
AE, adverse event.
Data from Belmusto-Worn VE, Sanchez JL, McCarthy K, et al. Randomized, double-blind, phase III, pivotal field trial of the comparative immunogenicity, safety, and tolerability of two yellow fever 17D vaccines (ARILVAX and Yellow Fever-VAX) in healthy infants and children in Peru. Am J Trop Med Hyg. *2005;72(2):189–197; Camacho LA, de Aguiar SG, Freire S, et al. Reactogenicity of yellow fever vaccines in a randomized, placebo-controlled trial.* Rev Saude Publica. *2005;39(3):413–420; and Monath TP, Nichols R, Archambault WT, et al. Comparative safety and immunogenicity of two yellow fever 17D vaccines (ARILVAX and Yellow Fever-VAX) in a phase III multicenter, double-blind clinical trial.* Am J Trop Med Hyg. *2002;66(5):533–541.*

systemic signs and symptoms were again slightly higher than placebo (7.9% vs 5.1%, $P = .26$).

Fever, myalgia, and headache were the most common symptoms, and the incidence of flu-like symptoms (myalgia and/or headache with fever) was more prominent in vaccinees when compared to placebo controls (6.5% vs 2.6%, $P = .06$). In contrast, nausea, vomiting, and gastrointestinal events occurred as often in vaccinees as in placebo controls (4.2% vs 3.7%, $P = .93$). It may be concluded that 50% of local events and 34% of systemic events among subjects inoculated with 17D-213/17DD yellow fever vaccine could have been explained by the vaccine virus. Among subjects who were seropositive before vaccination the difference between vaccinees pooled together and the placebo group was almost nil for the rate of systemic and local adverse events. Viremia was detected in 3% to 6% of vaccinees up to 10 days after vaccination, and variations in liver enzyme levels after vaccination were similar in placebo and vaccine recipients.

The safety of the 17D vaccine was also studied during the 2001 vaccination campaign against yellow fever in Abidjan, Côte d'Ivoire.[770] Active surveillance system for adverse events following immunization (AEFI) during a 4-week period was established. More than 2.6 million doses were administered and 87 adverse events were reported, including one male subject with a fatal outcome. He developed a severe abscess, resulting in myositis, and died from complications of diabetes, which had been previously unrecognized. Of those with AEFI, hospitalization was required for 13 (15%) patients, 54 (62%) were treated in ambulatory settings, and 20 (23%) recovered without further intervention. No sequelae were observed among the 86 survivors. The male-to-female ratio was 1 : 1. The mean age of the persons reporting an adverse event was 25 years (median, 23 years; range, 1 to 61 years). No case of YEL-AVD was reported.

Serious Adverse Events

Immediate-Onset Adverse Events

Hypersensitivity reactions to egg proteins in yellow fever vaccine have been extremely uncommon. No severe reactions were observed during initial use of the vaccine in more than 2 million persons. Guinea pig sensitization tests with 17D vaccine showed that anaphylactic reactions were reduced when the test vaccine was prepared from embryos less than 13 days old.[771] For this reason, the age of embryos at time of harvest for production of 17D vaccine is 12 days or less.[531,554] Yellow fever vaccine contains multiple proteins derived from egg white and yolk.[772] The WHO requirements for 17D vaccine specify that each dose contain a level of residual ovalbumin acceptable to the national regulatory agency.[531] One major commercial 17D vaccine specifies an upper limit of ovalbumin of 5 µg per 0.5 mL dose. In one study, 17D vaccine produced in the United States contained 7.8 µg ovalbumin per 0.5 mL dose.[773]

The first report of allergic phenomena associated with use of 17D vaccine was in 1942, during large-scale immunization of military personnel. Sulzberger and Asher described three patients with a serum sickness syndrome (urticaria or erythema multiforme-like rash accompanied by malaise, fever, arthralgia, pruritus, nausea, and vomiting) with onset between 3 and 7 days after receipt of different lots of 17D vaccine.[774] In 1943, Swartz described a patient who had a strong history of egg and other food allergies and who developed anaphylaxis 5 minutes after receiving simultaneous injections of 17D and cholera vaccines.[775] Skin testing revealed marked reactions to egg white and chicken meat and a moderate reaction to 17D (but not cholera) vaccine. Sprague and Barnard reported a case of severe anaphylaxis occurring within 15 minutes after 17D vaccine in a man with known egg allergy.[776]

The consensus based on observations during the first 20 years of use of 17D vaccine was that allergic reactions were extremely rare, occurring at an incidence of less than 1 per million, with reactions occurring principally in persons with known egg sensitivity. Reactions are typically mild and thus might be underreported.

Kouwenaar found the incidence of allergic reactions to yellow fever vaccine to be higher than expected, particularly in persons with a history of various allergies.[765] He vaccinated 242 persons having allergic histories with 0.1 mL of 17D vaccine by the intradermal route; if no reaction occurred within 45 minutes, the subjects received the remaining 0.4 mL subcutaneously. Nine (3.7%) of the subjects experienced allergic reactions; these were characterized clinically as exacerbations of

known but dormant allergy (eczema, asthma, rhinitis) in four patients, urticaria occurring less than 3 days after vaccination (two patients), and "serum sickness–like disease" urticaria or rash occurring 6 to 14 days after vaccination (three patients). Of the nine subjects reacting to yellow fever vaccination, two had a known history of egg allergy and the others had various other food allergies, asthma, or hay fever. In a control group of 465 persons without a history of allergy, only three patients (0.6%) had a late reaction to yellow fever vaccine: facial erythema in two and urticaria in one. In an additional group of 185 nonallergic persons who were reimmunized with 17D vaccine, one (0.5%) experienced generalized urticaria. The skin test has a very low positive predictive value for the development of allergic response to yellow fever vaccine and there are insufficient data on the more important question of the negative predictive value of the skin test.

More recent and definitive data on the incidence of allergic reactions are few, principally because a prior history of intolerance or allergy to eggs, egg products, chicken proteins, or egg-based vaccines or gelatin stabilizer in vaccines is considered a contraindication to the use of 17D vaccine, and few immunizations are given to such persons. Guidelines for use of yellow fever and other egg-based vaccines recommend that egg-sensitive patients undergo scratch, prick, or needle puncture testing with 1 : 10 diluted vaccine and a negative and positive (histamine) control. If the test is negative, an intradermal test is performed with 0.02 mL of 1 : 100 vaccine.[575,777] Application of this skin test procedure in clinical practice was described by Mosimann and colleagues.[778] In the case of a positive intradermal test (a wheal 5 mm or larger), and an established need for yellow fever vaccine, the patient may undergo a desensitization procedure consisting of increasing subcutaneous doses at 15- to 20-minute intervals under the supervision of an experienced physician in the management of allergic complications. In a newer study of six persons with reported egg allergies, the skin prick test was used to determine the level of reactivity.[779] Persons with skin prick tests of less than 3 mm were administered 1 : 10 dilution intradermal and then the rest of the vaccine subcutaneously and those with a positive skin prick test had a seven-step desensitization protocol. All six patients were successfully vaccinated with one skin-prick-positive patient developing generalized urticaria.

In one study, 30,000 Navy and Marine Corps personnel were screened for a history of egg sensitivity and 42 allergic patients underwent scratch and intradermal testing with crude egg white and a variety of egg-based vaccines including yellow fever 17D; most patients also underwent oral egg challenge.[780] The study demonstrated that a history of egg allergy was not a strong contraindication to administration of egg-based vaccines, because only 16% of the egg-sensitive subjects experienced any reaction, and these were mild in all cases. Skin tests were positive in 31% of the egg-sensitive subjects. Vaccination was performed in 39 subjects (excluding three subjects with strongly positive intradermal tests) using the egg-based vaccines. Intradermal skin testing with vaccine had reasonably high negative predictive value (0.80) but a lower positive predictive value (0.57) for an allergic reaction. However, the strength of positivity of the intradermal test appeared to correlate with severity of symptoms after vaccination.

Sensitization to egg-white protein by prior immunization with rabies vaccine produced in chick embryo cell culture was reported to cause a positive intradermal or scratch skin test to yellow fever vaccine.[781] No systemic allergic reactions have been noted in such cases. Similarly, prior yellow fever vaccination was reported to sensitize against a rabies vaccine manufactured in duck embryo cells.[782] There are no current precautions regarding sequential immunization with vaccines produced in chick tissue or cells, but few studies of egg- or chick cell–based vaccine interactions have been performed. Influenza vaccine, which is manufactured in eggs but contains little residual egg protein, can generally be safely given to egg-allergic persons.[783] As noted later, gelatin (used as a vaccine stabilizer) must be considered in the evaluation of cross-sensitization to vaccines.

A case report of a woman who had no history of egg allergy and suffered an anaphylactic reaction following yellow fever vaccine is of interest.[784] The patient had a history of intolerance to foods containing raw, but not cooked, eggs and had positive skin tests to raw egg antigen and 17D vaccine prepared from egg tissues without exposure to heat. Because few foods contain raw eggs and these are mixed with other ingredients, persons sensitized to raw eggs might not give an allergic history when queried before vaccination.

Since 1990, the CDC and FDA have enhanced procedures for reporting AEFI through the Vaccine Adverse Events Reporting System (VAERS).[785] Data collected between 1990 and 1997 revealed 40 cases of nonfatal hypersensitivity-type reactions (urticaria, angioedema, bronchospasm, anaphylaxis) temporally associated with yellow fever vaccine.[786] The incidence of anaphylactic reactions cannot be established with accuracy. However, based on the number of vaccine doses distributed in the United States during the interval under study (5.2 million) and the assumption that all reported events are caused by 17D vaccine, the incidence of anaphylactic reactions was estimated to be 0.8 per 100,000 doses. A second analysis identified 28 cases of anaphylaxis following 17D vaccination reported to VAERS between 2000 and 2006. Based on the number of vaccine doses distributed in the United States during this study period (1.5 million), the incidence of anaphylactic reactions was estimated to be 1.8 per 100,000 doses.[255] In the most recent analysis, 30 anaphylactic reactions were reported to VAERS from 2007–13 for an incidence of 1.3 per 100,000 doses.[259a] Although egg protein has been implicated in hypersensitivity reactions to yellow fever vaccine, other components might also play a role; for example, hydrolyzed porcine gelatin incorporated as a stabilizer by some manufacturers. Gelatin has been implicated in allergic reactions to measles, varicella, and Japanese encephalitis vaccines.[787,788]

After large-scale vaccination campaigns in which 17D vaccine was administered to 38 million people in eight west African countries during 2007 to 2010, 11 hypersensitivity reactions were identified, one of which was fatal.[789] The incidence of hypersensitivity reactions was calculated to be 0.03 per 100,000 doses. A published review of 17 years of worldwide use of the 17D-204 vaccine Stamaril, involving 276 million doses distributed, estimated a reporting rate of anaphylaxis of 0.01 per 100,000 doses.[790] In yellow fever nonendemic countries (Europe, Australia, and New Zealand), where 16.2 million doses were distributed during the same period, there were 33 reports meeting any level of the Brighton Collaboration anaphylaxis case definition, yielding a reporting rate of 0.2 per 100,000 doses. It is unclear why these reporting rates are lower than those published from United States, but it might be partly because of low sensitivity of vaccine adverse event surveillance systems in endemic countries, where the vast majority of doses of 17D vaccine are administered.

Rare Adverse Events Temporally Related to 17D Vaccine

There are a number of individual case reports of adverse events possibly associated with yellow fever vaccination.[791–806] Although some are credible, such as the occurrence of ketoacidosis in an insulin-dependent diabetic patient 4 days after vaccination,[791] others, such as chronic lymphocytic leukemia[792] and malaria recrudescence,[804] likely represent chance

associations in time. The absence of similar observations over the long history of use of yellow fever vaccine supports this view.

A case of severe gastroparesis was reported in temporal association with 17D vaccine in a patient in the United States. Gastroparesis has been described as a rare adverse event following other vaccines.[807] A case of multiple evanescent white dot syndrome, an inflammatory chorioretinal disorder, was reported in a 50-year-old woman who developed rapidly progressive left eye visual loss starting 10 days after 17D and hepatitis A vaccination.[793] Her visual symptoms and funduscopic examination normalized spontaneously within 6 weeks. A 33-year-old man presented 3 weeks after receiving hepatitis A, yellow fever, and typhoid vaccinations with sudden onset of intermittent floaters and flashing lights in his right eye.[806] He was diagnosed with acute multifocal choroiditis that resolved after 8 weeks. A case of Kawasaki disease occurring in a 12-year-old boy with symptom onset 20 days after 17D vaccination was reported.[794] A case of autoimmune hepatitis was reported in a 31-year-old woman who received 17D and hepatitis A vaccines.[795] Her symptoms, including itching, started 11 days following vaccination. She later developed jaundice, significant elevation of liver function tests, and histopathologic findings from liver biopsy consistent with autoimmune hepatitis. Another case report described an immunocompetent 64-year-old woman who developed localized herpes zoster 3 days after yellow fever vaccination.[797] Similar associations have been made with live (smallpox and varicella) and inactivated (hepatitis A, influenza, rabies, and Japanese encephalitis) vaccines. Mention is made in the literature of a favorable effect of yellow fever vaccine on reducing the incidence of recurrent herpes labialis in 11 patients,[801] but this observation was not confirmed in a subsequent clinical trial.[808]

A case report described a patient who developed confusion and difficulty walking 1 week after yellow fever vaccination and in whom YEL-AND was suspected; however, her symptoms did not resolve. She was discovered to have an auditory neuroma and hydrocephalus; her physicians concluded that YEL-AND was responsible for the acute decompensation of her underlying disease.[809] Two case reports of dermatologic symptoms following 17D vaccination include a male with an uncommon presentation of pityriasis rosea[810] and a woman who developed a localized bullous fixed drug eruption.[811] One report describes the isolation of yellow fever vaccine virus in cell culture from a respiratory swab taken from a female college student with an influenza-like illness.[812]

Reactions Resulting From Improper Handling and Use of Yellow Fever Vaccine

Because yellow fever vaccine contains no preservative, improper handling of multiple-dose vials can lead to bacterial contamination, sometimes with serious consequences. Repeated use of the same needle and syringe, rubbing dirt or other materials into the inoculation site, and other practices can also contribute to infection. Four known outbreaks of serious illness have been associated with contamination of yellow fever vaccine vials or inoculation equipment in Africa (Table 63.18). The clinical picture in all four episodes was similar, with marked swelling and pain of the vaccinated arm beginning hours after immunization and progressing in the most severe cases to cardiovascular shock and death within hours to several days.[813–815] Some, but not all, cases had signs of necrotizing myositis (gangrene). In all episodes, known or potential problems with use of multidose vaccine containers contaminated after reconstitution, improperly sterilized jet injector equipment, or reuse of syringes and needles were involved. Although no etiologic agent was implicated, *Clostridia* and group A or anaerobic *Streptococcus* were suspected.

There have been at least three reports in the literature of an inadvertent administration of an overdose of the vaccine.[816–818] In one report, 14 adults were unintentionally given 25 times the recommended dose of the vaccine during an outbreak control immunization campaign in Minas Gerais (Brazil).[816] No adverse events were observed. Another case was reported in which 12.5 times the recommended disease of the vaccine was given[817]; again, no adverse events were observed. In the last report, 49 people were inadvertently administered 10 times the normal dose of yellow fever vaccine during a reactive campaign in Brazil.[818] All were followed for 45 days and only one, a 6-year-old girl, required emergency care 2 days after vaccination for fever, abdominal pain, and vomiting. She was found to have mild elevated bilirubin and the authors concluded she might have had mild viscerotropic disease.

Pregnancy and Adverse Events

Because spontaneous abortion, stillbirth, and congenital malformation have not been observed in the aftermath of yellow fever epidemics, there was little concern about the use of a live attenuated vaccine derived from a wild-type virus. In one report of (wild-type) yellow fever death of a pregnant woman,

TABLE 63.18 Episodes Involving Multiple Serious Adverse Events Characterized by Cellulitis or Necrotizing Myositis Associated With Probable Bacterial Contamination of 17D Vaccine After Reconstitution or Infection of Inoculation Site

			Deaths				
Year	Location	No. Cases	No.	%	Incidence[a]	Circumstances	Reference
1974	Côte d'Ivoire	39	8	20.5	5.3/100,000 immunizations	Cases occurred at 9 designated vaccinating centers; 5-dose vials pooled to prepare 50–100 doses for jet injection	World Health Organization, 1986[815]
1982	Ghana	6	2	33.3		Cases occurred at a single center; possible contaminated 50-dose vial or syringe	World Health Organization, 1986[815]
1984	Benin	31	11	35.5	4/10,000	Cases associated with one vaccinating team, six vaccinating sessions using multidose vials, jet injectors	World Health Organization, 1985[814]
1987	Nigeria	25	5	20		Illegal clinics, unauthorized inoculators, reuse of syringes, multidose vials	Oyelami et al, 1994[813]

[a]Estimated, because number of persons vaccinated with vaccine vial(s) implicated in the contamination is unknown.

there was no evidence that the fetus had developed hepatitis.[819] In another report, two women 2 and 5 months pregnant died of yellow fever; in neither case did the fetal livers show necrosis.[820] However, in a case in Brazil, a newborn infant acquired a fatal case of wild-type yellow fever 3 days after birth.[202] The mother had a mild case of yellow fever beginning 3 days before delivery. Transmission could have occurred via the placenta shortly before birth, during childbirth, or possibly via breast milk after birth.

Initially, the use of yellow fever vaccine in pregnancy was not constrained, and many women were vaccinated without any reported adverse effects. In Paris, Stefanopoulo and Duvolon immunized more than 200 pregnant women between 1936 and 1946,[821] and in Brazil, Smith and colleagues vaccinated "a considerable number of women in all stages of pregnancy with no untoward effects."[22]

A few studies have addressed the risk of congenital infection in pregnant women who were inadvertently immunized with 17D vaccine during emergency vaccination campaigns. Nasidi and colleagues studied 101 women who were immunized in Nigeria in 1986.[625] Four women (4%) were immunized in the first trimester, eight (8%) in the second trimester, and 89 (88%) in the third trimester. There were no adverse effects on fetuses or neonates attributable to vaccination among 40 infants who were carefully followed up. No evidence for transplacental infection was obtained in 40 babies whose cord blood was tested for IgM antibodies. However, the women immunized during pregnancy had a significantly lower seroconversion rate (39%) compared to control groups immunized during the campaign (see "Pregnancy" under "Primary Vaccine Failure" earlier for more details).

A second study was conducted following a vaccination campaign in Trinidad in 1989.[822] Approximately 400,000 persons were immunized and 100 to 200 were estimated to have inadvertently received 17D vaccine during pregnancy. Forty-one cord bloods were obtained from babies born to mothers who had received 17D vaccine during the first trimester. One infant (normal, full term) had IgM antibody in cord blood, suggesting that congenital infection with 17D virus might have occurred.

In a separate case-control study of 17D vaccination during early pregnancy, the relative risk of spontaneous abortion was estimated to be 2.3, but statistical power was low.[823]

In one additional study, pregnant women who were inadvertently immunized against yellow fever during a mass vaccination campaign conducted in Brazil in 2000 were followed to evaluate the possible effects of the 17D vaccine on conception and pregnancy and to assess congenital infection resulting from immunization, if any.[741] A total of 480 pregnant women were identified, having received the vaccine at a mean gestational age of 5.7 weeks (95% CI, 5.2–6.2). After a minimum 6-week interval, a total of 20% of women reported mild adverse events, such as headache, fever, or myalgia. At birth, no yellow fever IgM antibodies were detected in the infants and no placental or umbilical cord blood was PCR positive. The incidence of malformations (2%, or 7/304 babies), miscarriages (3%, or 11/441 pregnancies), stillbirths (0.7%), and premature delivery (8%) was similar to that found in the general population. Of 304 babies born to pregnant women vaccinated during the campaign, no increase in the rates of serious defects were detected when compared to a reference population of approximately 10,000 births before the vaccination campaign.[824] The only dysmorphism that was statistically more frequent in infants born to yellow fever vaccinees was nevus but the authors suggested that the finding could have resulted from assessment bias. One infant experienced a transient bilateral hypoacousia, three infants had an abnormal funduscopic examination, and four infants with neurologic abnormalities were IgG negative at 1 year of age. An accurate evaluation of a risk of miscarriage could not be performed owing to a delayed presentation of women following vaccination.

A manufacturer's review of 17 years of pharmacovigilance data for Stamaril, which is distributed worldwide, excluding North America, identified reports of 195 cases of inadvertent vaccination during pregnancy, with all but one being from Europe.[790] Spontaneous abortion was reported in 24 cases (12%), which was not higher than the background rate of 10% to 15% reported for the general population in Europe. There were 14 reports of congenital abnormalities or psychomotor retardation, with no consistent pattern. The observed reporting rate for congenital abnormalities was felt to be consistent with the known incidence of major congenital anomalies in Europe.

Rate of Occurrence

A serious adverse event is defined as any adverse experience that occurs after administration of a vaccine, including 17D vaccine, and results in death, life-threatening illness, congenital anomaly, inpatient hospitalization, prolongation of existing hospitalization, or persistent or significant disability. In some countries, significant medical events are also reported as serious adverse events.

From late 1930s to 2014, the total number of yellow fever immunizations administered worldwide approximates 540 million; the majority of which have been performed in developing countries. Surveillance for adverse events has been passive and insensitive to the discovery of rare events.

Khromava and associates used VAERS data to estimate the incidence of all serious adverse events in the civilian population in the United States, where approximately 300,000 doses are distributed annually.[253] The VAERS database contains reports of adverse events and does not assign causality, other than temporal association. The overall incidence of serious adverse events following yellow fever vaccination between 1990 and 2002 was 1.6 per 100,000 doses. Rates in the (younger) military population were about one half that in the civilian sector and were not different from those associated with typhoid vaccine. Lindsey and associates performed an analysis of VAERS data to estimate the incidence of serious adverse events in the United States civilian population for the years 2000–13, during which an average of 268,000 doses were distributed annually.[255,259a] The overall incidence for that time period was 4.2 per 100,000 doses. Cottin and associates used pharmacovigilance data from 16.2 million doses of Stamaril distributed to Europe, Australia, and New Zealand during 1993 to 2010 to estimate the incidence of serious adverse events to be 5.1 per 100,000 doses.[790] Bruegelmans and colleagues estimated the incidence of serious adverse events to be 0.43 per 100,000 doses in preventive vaccination campaigns in eight West African countries during 2007 to 2010, in which approximately 38 million doses of yellow fever vaccine were administered.[789] Biscayart and associates estimated an incidence of 1.8 serious adverse events per 100,000 doses administered in various provinces of Argentina in 2008 after a yellow fever outbreak in the northeastern province of Misiones resulted in approximately 2 million doses being administered.[825] However, it is not clear that the classification of adverse events as "serious" was the same in each of these recent studies as in the prior published studies using VAERS data from the United States.

Age is an important determinant of severity of adverse events to 17D vaccines. Several publications reported on the age and the occurrence of serious adverse events following the administration of 17D vaccines. Three publications

reported on age-specific incidence of serious adverse events with similar age stratification.[674,826,827] In U.S. and Australian residents, persons older than 65 years of age had a nine to 16 times higher risk of developing a serious adverse event following administration of 17D vaccines than did adults 25 to 44 years of age, which suggests that immune senescence might play a role (Table 63.19). When applying a decennial age stratification for the civilian population, the relative risk of developing serious adverse events for persons 60 years of age and older was six to 10 times higher compared to younger adults in one study[253] and 1.5 to 4.1 times higher in two other studies.[255,259a] Table 63.20 summarizes the findings for U.S. civilian and U.S. military observations. None of these retrospective studies adequately determined causal relationships between vaccine and adverse events, and elderly persons may be more likely to develop coincident illnesses than younger persons.

A similar conclusion with respect to age-related risk was reached after analysis of postmarketing surveillance data for ARILVAX in the United Kingdom.[674] This report also compared the neutralizing antibody responses in elderly and young adults. No differences were found, suggesting that if immune senescence plays a role, it might reflect an age-dependent impairment of cellular immunity.

A mathematical model was developed to evaluate the risk of developing a serious adverse event.[828] The authors used the U.S. data[253] and reached a conclusion that contraindicated yellow fever vaccination in patients 60 years of age and older, taking into account that the risk of serious adverse events (including deaths) is higher for that age group. A number of issues in this analysis, which applied U.S. data to the situation in a yellow fever–endemic country, were raised by Martins and associates.[545] There is a higher fatal outcome of wild-type yellow fever infection in older adults, a risk of a reintroduction of yellow fever by travelers into new countries, and lower risk of serious adverse events from vaccine in revaccinated or naturally immune people in endemic countries. In an analysis of the manufacturer's pharmacovigilance database for Stamaril, involving a 17-year time period during which 16.2 million doses were distributed to yellow fever nonendemic countries, Cottin and associates concluded that there was no evidence for a higher risk of serious adverse events after vaccination for travelers older than 60 years of age compared to that for younger travelers.[790] This age analysis was limited by the absence of age distribution data of vaccine recipients. However, to obtain a crude estimate of the age distribution of Stamaril recipients in yellow fever nonendemic countries, the authors used a computerized primary care database from the United Kingdom containing data for patients who received Stamaril.

TABLE 63.19 Retrospective Analysis of the Incidence of Serious Adverse Events in Persons Receiving Yellow Fever Vaccine in the United States and Australia

Age (y)	Doses	Serious Adverse Events[a]	Reporting Rate per 100,000	Relative Reporting Rate (95% CI)
UNITED STATES, 1990–98				
15–24	189.991	2	1.05	
25–44	702.783	2	0.29	Reference reporting rate
45–64	442.605	5	1.13	3.97 (0.77–20)
>65	108.307	5	4.52	16.2 (3.2–84)
Total	1,443,686	14	9.7	—
AUSTRALIA, 1993–2002				
15–24	32.423	0	—	—
25–44	120.552	3	2.49	Reference reporting rate
45–64	48.697	4	8.21	3.3 (0.62–9.9)
>65	8.984	2	22.26	8.95 (1.5–53.5)
Total	210.656	9	4.27	—

[a]Number of reports of serious systemic adverse events, including neurologic and multisystemic adverse events requiring hospitalization or resulting in death.

CI, confidence interval.

Australia data from Lawrence GL, Burgess MA, Kass RB. Age-related risk of adverse events following yellow fever vaccination in Australia. Commun Dis Intell. *2004;28(2):244–248. Erratum in* Commun Dis Intell. *2004;28(3):348.*

U.S. data adapted from Martin M, Weld LH, Tsai TF, et al. Advanced age a risk factor for illness temporally associated with yellow fever vaccination. Emerg Infect Dis. *2001;7(6):945–951.*

TABLE 63.20 Estimated Incidence of Serious Adverse Events in the United States

Sex and Age (y)	Doses	Reporting Rate per 100,000			
		SAE (N = 72)	Anaphylaxis (N = 28)	YEL-AND (N = 12)	YEL-AVD (N = 6)
Male	743,305	5.9	2.3	1.2	0.7
Female	790,865	3.5	1.3	0.4	0.1
1–18	178,454	4.6	3.4	1.1	0.0
19–29	389,018	4.1	3.1	0.3	0.5
30–39	210,545	3.8	4.3	0.5	0.0
40–49	223,233	4.5	0.0	0.9	0.0
50–59	254,719	2.7	0.4	0.4	0.0
60–69	191,025	6.3	0.0	1.6	1.0
>70	87,177	12.6	0.0	2.3	2.3
Total	1,534,170	4.7	1.8	0.8	0.4

SAE, serious adverse event; YEL-AND, yellow fever–associated neurologic disease; YEL-AVD, yellow fever–associated viscerotropic disease.

Data from a retrospective survey reported in Lindsey NP, Schroeder BA, Miller ER, et al. Adverse event reports following yellow fever vaccination. Vaccine. *2008;26:6077–6082.*

Yellow Fever Vaccine–Associated Neurologic Disease

Two types of YEL-AND have been recognized following yellow fever vaccination. In the first type, yellow fever vaccine virus invades the CNS to cause encephalitis or meningitis; this is often referred to as *neurotropic disease*. In the second, yellow fever vaccine virus elicits an autoimmune reaction in which antibodies or T cells produced in response to the vaccine causes either central or peripheral demyelination that can result in a number of clinical syndromes such as ADEM or Guillain-Barré syndrome.

History of Neurovirulence of Vaccine

The 17D vaccine strains retain a degree of neurovirulence as demonstrated by intracerebral inoculation of mice and monkeys[542] and by the occurrence of rare cases of neurologic accidents in humans. A bimodal age-related risk of these accidents has been illustrated, with young infants and the elderly being at highest risk.

Historically, the typical syndrome associated with 17D vaccine has been encephalitis, characterized by onset of signs and symptoms 2 to 24 days after immunization. These include fever and variable neurologic signs including meningismus, convulsions, obtundation, and paresis. The CSF contains 100 to 500 cells (mixed polymorphonuclear and lymphocytic) and increased protein concentration. The clinical course has typically been brief and recovery generally complete. However, two fatal cases have been reported, including a 3-year-old girl who died of encephalitis following vaccination with a strain of yellow fever vaccine virus that had mutated to be more neurovirulent, and a 53-year-old man who had asymptomatic HIV with a CD4+ count of 108 cells/mm^3 and died from meningoencephalitis following yellow fever vaccination.[587,829] There has also been another complication described, including a 29-year-old man who had residual mild ataxia 11 months after onset.[830] The overall case-fatality rate of neurologic adverse events is very low and appears to be less than 1% based on the limited number of deaths reported among persons with neurologic disease following yellow fever vaccination.

In neurotropic cases, with a clinical picture of acute viral meningoencephalitis or meningitis, the mechanism is thought to be neuroinvasion and direct viral injury by 17D virus. Virus was recovered from brain tissue of a patient with a fatal case of encephalitis,[92] and 17D virus genome has been detected by PCR in the CSF from other neurotropic cases (Centers for Disease Control and Prevention, unpublished data, 2015).[203] In addition, in a number of cases, CSF has been tested for IgM and found positive.[545,831] However, as pointed out below, a substantial number of subjects have other manifestations of an autoimmune nature, such as ADEM and Guillain-Barré syndrome; it is uncertain whether low-grade viral infection of the brain might occur in these cases or is necessary for this type of pathogenesis to occur.

After standardized manufacturing procedures were instituted in the early 1940s, no cases of encephalitis temporally associated with 17D vaccine were reported for approximately 10 years.[682,821] From 1945 to 1988, a total of 17 yellow fever–associated encephalitis cases were reported worldwide, with only one reported fatal outcome.[587,682,832–842] In 1952–53, five cases occurred among 1800 children vaccinated at earlier than 1 year of age at the Pasteur Institute in Paris for an incidence of 2.8 cases per 1000 vaccinated (Table 63.21).[682] The incidence was even higher at four per 1000 vaccinated among the infants younger than 6 months of age. These cases constitute the first evidence that 17D vaccine manufactured according to biological standards and with restricted passage may be encephalitogenic in very young infants. Fifteen cases occurred during the 1950s, when there was no age restriction on use of the vaccine in infants. Of the 15 cases, 13 (87%) occurred in infants younger than 4 months of age, and all were younger than 7 months of age. Recommendations for restriction of use of 17D vaccine to infants older than 6 months of age were followed by a reduction in incidence of encephalitis.[843] Between 1960 and 1988, only two cases were reported, one of which occurred in a 1-month-old infant in France, at the time the age limitation was not universally practiced (see Table 63.21).[832] The only fatal case reported during this period was reported in 1965 and was a 3-year-old girl who died of encephalitis.[587] The virus was found to have a mutation in the yellow fever virus isolated.[92]

There are no reports of encephalitis in infants vaccinated for international travel since the recommendation for restricted use of the vaccine in children younger than 6 months of age has been universally adopted. Current recommendations for the minimum age for vaccination differ, typically from 9 months to 12 months of age, and depend on the recommendations of national health authorities based on risk of exposure to natural infection with yellow fever. During ongoing or threatened epidemics, it may be advisable to immunize infants from the age of 6 months, but in no case should the vaccine be administered to infants younger than 6 months.

The basis for increased risk of encephalitis in young infants is unknown, but it parallels the increased susceptibility of neonatal mice to neuroinvasion and neurovirulence of yellow fever and other flaviviruses. Possibilities include immaturity of the blood-brain barrier, prolonged or higher viremia, and immaturity of the immune system and delayed clearance of the infection. There are no data on 17D viremia levels or on the kinetics of the immune response in infants or children.

The incidence of encephalitis following 17D vaccine has been more common in male than in female patients. A slight excess of male patients (56%) was noted also in the cases of encephalitis following use of the French neurotropic vaccine.[519]

Case Definitions

In 2002, the Yellow Fever Vaccine Safety working group was organized by the CDC to review case reports of encephalitis in the VAERS database. As a result, the Yellow Fever Vaccine Safety working group refined and broadened the identification of the syndrome from encephalitis to YEL-AND to encompass all the neurologic syndromes reported as adverse events following 17D vaccines. The working group developed case

TABLE 63.21 Incidence of Yellow Fever Vaccine–Associated Neurologic Disease in Infants Vaccinated With 17D Vaccines in France

Location	Years	Age	No. Vaccinations	No. Encephalitis Cases	Incidence per 1000	Case Fatality (%)	Reference
Paris	1952–53	<6 mo	1000	4	4	0	Stuart, 1956[682]
		7–12 mo	800	1	1.25	0	
Lyon	1958–78	<1 mo	1830	1	0.5	0	Louis et al, 1981[832]

definitions for cases with a neurotropic involvement (encephalitis), with an autoimmune involvement in the CNS (yellow fever vaccine–associated autoimmune disease in the CNS [YEL-AAD-CNS]) or with an autoimmune involvement in the peripheral nervous system (YEL-AAD-PNS).[729,831]

In addition to the case definitions, the Yellow Fever Vaccine Safety working group devised causality determinations for 17D vaccine.[729,831] For neurotropic disease, causality was principally based on testing CSF for virus, viral RNA, or IgM antibodies. There was no specific testing available for the autoimmune-mediated events of ADEM and Guillain-Barré syndrome, although their clinical diagnosis was made using appropriate studies such as neuroimaging, electroencephalography, electromyelography, nerve conduction studies, and tests for myelin antibody. Because no yellow fever vaccine-specific confirmatory laboratory tests exist for ADEM and Guillain-Barré syndrome, their causality determinations only specify "suspect" and "probable" levels of certainty. If there was no evidence of another diagnosis, a case was further classified depending on whether 17D vaccine was the only vaccine administered.[831]

The distinction between neurotropic disease (meningoencephalitis) and ADEM is not always clear, either clinically or through diagnostic testing. For neurotropic cases, the detection of the 17D virus in CSF by either culture or nucleic acid amplification is diagnostic. The detection of yellow fever virus IgM and neutralizing antibodies in the CSF also supports the diagnosis. Because IgM antibodies do not normally cross the blood-brain barrier, their production in the CSF is considered indicative of local CNS infection. There have been a number of ADEM cases following yellow fever vaccination in which IgM was detected in CSF, but neutralizing antibody tests were not routinely perform to determine if antibodies were in fact specific for yellow fever virus.[831,844] The pathogenesis of ADEM could result in the production of cross-reacting nonspecific autoantibodies, or it might involve low-level viral neuroinvasion insufficient to cause overt disease before viral clearance but sufficient to stimulate yellow fever virus-specific IgM antibody production and to trigger an autoimmune process. Detection of yellow fever virus neutralizing antibodies in CSF might differentiate specific IgM antibodies associated with neuroinvasion from nonspecific cross-reactive antibodies produced as a result of the autoimmune pathogenesis of ADEM.

Since 2007, the Brighton Collaboration, whose goal is to improve the comparability of vaccine safety data through the development of standardized cases definitions,[845] has published a series of case definitions for neurologic adverse events that have been reported postvaccination, including aseptic meningitis,[846] Guillain-Barré syndrome,[847] encephalitis, myelitis, and ADEM.[848] Having more standardized case definitions that can be used in variety of settings, regardless of resources, should help improve the capture and comparability of YEL-AND cases moving forward.

Summary of Cases Reported Between 1992 and 2014

Between 1992 and 2014, more than 200 YEL-AND cases have been reported worldwide. These predominantly included cases of meningoencephalitis, ADEM, and Guillain-Barré syndrome,[253,255,259a,545,790,825,829,831,844,849–854] but they also included cases of unusual neurologic syndromes such as ataxia, Bell palsy, transverse myelitis, longitudinal myelitis, bulbar palsy, mononeuritis, optic neuritis, and CNS vasculitis with stroke.[259a,796,799,800,803,805,855,856] Not all these unusual cases had a clear relationship between the event and vaccination, particularly as several of these events also occur as independent events in the population.

Although the neurotropic manifestation of YEL-AND has classically been regarded to be encephalitis or meningoencephalitis, aseptic meningitis also appears to be part of this disease spectrum. Several more recent published reports discuss patients with aseptic meningitis,[851,853] including 35 cases identified following a mass 17D vaccination campaign in the Brazilian state of Rio Grande do Sul in 2009. In practice, the clinical distinction between aseptic meningitis and encephalitis is not always clear.

Comparing more recent YEL-AND cases with earlier cases reported prior to 1992, there has been a notable increase in the number of cases reported and a change in the epidemiology of cases. Since 1992, YEL-AND cases have been predominantly adults, whereas the earlier timeframe largely involved cases among infants (see "History" above). Some of these differences are likely explained by establishment of a minimal age for vaccination, increased sensitivity of case detection, and a probable increase in vaccine use in elderly travelers.

Detailed descriptive statistics for all cases reported worldwide since 1992 are not possible because data are incomplete and reporting details are variable. However, data for all YEL-AND cases reported to United States VAERS (the inception of VAERS in the United States was 1990) from 1992 through 2013 are sufficiently complete to describe basic demographic features and explore differences between the syndromes (Table 63.22). Among these 52 cases, the distribution of YEL-AND syndromes is 23 neurotropic (44%), 10 ADEM (19%), and 20 Guillain-Barré (38%). One case was classified as encephalitis/ADEM owing to overlapping clinical and laboratory features. The median age of all cases is 45 years, with a range of 16 to 78 years; 83% (43/52) were male. The median onset of symptoms is 13 days (range, 2 to 56)

TABLE 63.22 Descriptive Statistics, U.S. Cases of Neurologic Disease Following Yellow Fever Vaccination, 1992–2013[a]

Category	No. of Cases	Male	Female	Mean Age (y)	Median Age (y [Range])	Mean Onset (Days)	Median Onset (Days [Range])
Encephalitis	16	14[b]	2	52.3	65 (16–78)	13.2	11.5 (2–34)
Meningitis	7	6	1	39	34 (19–67)	19.3	13 (10–56)
ADEM	10	9[b]	1	29.9	21.5 (17–61)	13.2	10.5 (2–44)
GBS	20	15	5	47	48 (17–76)	13.5	13.5 (2–29)
Total	52[b]	43[b]	9	44	45 (16–78)	14.3	12.5 (2–56)

[a]All yellow fever vaccine-associated neurologic disease (YEL-AND) cases meet either the CDC Yellow Fever Vaccine Safety Working Group YEL-AND case definition or the Brighton Collaboration case definitions for encephalitis, aseptic meningitis, ADEM, or GBS.
[b]One case in a male patient was classified as encephalitis/ADEM. This case was only counted once in the total count of cases, but was included in the category calculations for both encephalitis and ADEM.
ADEM, acute disseminated encephalomyelitis; GBS, Guillain-Barré Syndrome.

following vaccination. Among the four neurologic syndromes, males made up the majority of cases, but the median age ranged between the syndromes with persons with ADEM and meningitis having lower median ages than encephalitis. The differences in the age between syndromes, particularly for encephalitis compared to meningitis, is similar to what has been reported with West Nile virus disease cases.[857]

Although the higher proportion of YEL-AND cases among males might be partly because of differences in vaccination rates between sexes, the finding in VAERS cases is similar to the higher rate of YEL-AND reported among male infants before 1989 and an excess of encephalitis cases being identified among males who had received the French neurotropic vaccine.[519] The reason for the apparent predilection for YEL-AND among males who receive yellow fever vaccine is not known.

Risk of Occurrence

The incidence of YEL-AND in very young infants may be estimated at 0.5 to 4.0 per 1000 doses based on two reports that provided denominator data (see Table 63.21). In contrast, the risk of developing encephalitis in persons older than 9 months of age (the current minimum age recommended for routine immunization in the United States[729]) is much lower. Three studies of VAERS data have estimated the overall incidence of YEL-AND in the civilian U.S. population between 1990 and 2013 to be 0.4 to 0.8 per 100,000 doses (Table 63.23).[253,255,259a] This incidence is identical to that estimated from postmarketing surveillance data in the United Kingdom.[849] However, rates reported from other locations have varied substantially from 0.016 cases per 100,000 doses in several African countries[789] to a high of 9.9 cases per 100,000 doses in France (see Table 63.23).[850]

Reporting rates of YEL-AND often vary between nonendemic and endemic areas and based on the level of surveillance (see Table 63.23). In 2013, Cottin and associates[790] identified 24 cases of YEL-AND being reported following the administration of 276 million doses of Stamaril (17D vaccine manufactured in France) for an overall reporting rate of 0.008 per 100,000 doses distributed worldwide. In comparison, the rate among travelers from nonendemic areas (e.g., Europe, Australia, and New Zealand) was higher at 0.15 cases per 100,000 doses. Data from the Brazil's National System for Surveillance of Adverse Events Following Immunization for 2007–12 found an overall reporting rate of 0.2 YEL-AND cases per 100,000 doses, but it was as high as 1.1 per 100,000 doses in Rio Grande do Sul in 2009.[545] The discrepancy between YEL-AND reporting rates for Brazil as a whole versus Rio Grande do Sul state was attributed to the intensity of surveillance for AEFI that varies throughout this large country. Interestingly, the rates of YEL-AND among primary vaccine recipients in Brazil are not dissimilar to those reported from other countries that looked primarily at a traveler population (e.g., VAERS data).

As noted earlier, the incidence of YEL-AND is age dependent, particularly in travelers. In persons older than 60 years of age, the incidence of YEL-AND was estimated at 1.4 to 2.2 per 100,000 doses.[253,255,259a] The relative risk of YEL-AND in persons older than age 60 years is 2.4 to 3.5 times higher than in young adults. Roukens and associates found that following primary 17D vaccination, compared to younger subjects, elderly (60 to 81 years old) travelers had a delayed antibody response and higher PCR viremia levels.[256] If viremia is related to neuroinvasion, this might provide an explanation for increased risk of YEL-AND in the elderly. Further studies to confirm this theory are needed. In endemic areas of Brazil, the rates of YEL-AND were found in be highest in 5- to 9-year-olds

TABLE 63.23 Reporting Rates for Yellow Fever Vaccine–Associated Neurologic Disease[a]

Location	Reporting Period	No. of Cases	No. of Doses	Reporting Rate per 100,000 Doses	Reference
NONENDEMIC AREAS					
U.K.	1991–2003	4	3,046,007	0.13	Kitchener, 2004[849]
Europe, Australia, New Zealand	1993–2011	24	16,200,000	0.15	Cottin et al, 2013[b,790]
U.S.	1990–2002	8	2,230,760	0.4	Khromava et al, 2005[c,253]
U.S.	2000–06	12[d]	1,534,170	0.8	Lindsey et al, 2008[c,255]
France	2000–08	4	40,404	9.9	Guimard et al, 2009[850]
U.S.	2007–13	17	2,224,790	0.8	Linsey et al, 2016
ENDEMIC AREAS					
Brazil	2000–08	85[e]	101,564,083	0.084	Martins et al, 2010[f,851]
West African Countries	2007–10	6	38,009,411	0.016	Breugelmans et al, 2013[789]
Argentina	2008–09	12	1,943,000	0.6	Biscayart et al, 2014[825]
Brazil, all regions	2007–12	65	31,434,631	0.21	Martins et al, 2014[545]
Brazil, Rio Grande do Sul only	2007–12	46	4,895,123	0.94	Martins et al, 2014[545]

[a]YEL-AND includes meningitis, encephalitis, meningoencephalitis, ADEM, GBS, and other less-common neurologic syndromes.
[b]This study calculated a worldwide reporting rate for YEL-AND of 0.008/100,00 doses, based on 276 million doses of vaccine distributed globally. However, since all 24 cases of YEL-AND were reported from nonendemic countries, the low worldwide reporting rate likely reflects significant underreporting from endemic countries.
[c]Data reported are only for U.S. civilian cases.
[d]This published study includes an overlap period from years 2000 through 2002 with the Khromava 2005 study. The number of unique YEL-AND cases reported during 2003 through 2006 is six.
[e]This published study includes an overlap period from years 2007 through 2008 with the Martins 2014 study. The number of unique YEL-AND cases reported during 2000 through 2006 is 79.
[f]Data are reported for the entire country of Brazil.
ADEM, acute disseminated encephalomyelitis; GBS, Guillain-Barré syndrome; YEL-AND, yellow fever vaccine–associated neurologic disease.

even when looking specifically at first-time vaccine recipients.[545] It is unclear why the age-based risk groups are different based on the population but it could be hypothesized that it is related to base-line rates of exposures to flaviviruses, such as yellow fever and dengue viruses.

Yellow Fever Vaccine–Associated Viscerotropic Disease

YEL-AVD is a fulminant 17D virus infection of liver and visceral organs clinically and, to some degree, pathophysiologically resembling naturally acquired yellow fever. Fortunately, YEL-AVD is rare, but it can be lethal, with more than 60% of recognized cases dying.[729] The severity of YEL-AVD mandates a careful analysis of risk versus benefit of vaccination[494] and underlies the efforts to revise geographic risk of exposure to yellow fever during travel (see Fig. 63.4).

History

YEL-AVD was first recognized in 2001 when several cases of multiorgan system failure were described following 17D vaccination.[374,858–860] The original seven reported cases were associated with vaccines manufactured in Brazil (17DD substrain), France, and the United States (17D-204 substrain). The signs and symptoms of the syndrome resembled wild-type yellow fever virus infections and disease and included a rapid onset of high fever, malaise, and myalgia within 2 to 5 days of vaccination, followed by jaundice, oliguria, cardiovascular instability, and hemorrhage. Six of the seven cases died. Large amounts of yellow fever viral antigen were found in liver, heart, and other affected organs.

Shortly after the initial published reports of YEL-AVD in 2001, three additional brief reports describing milder nonfatal cases of visceral dysfunction following 17D vaccination were published.[861–863] Since 2001, additional cases of YEL-AVD have been published, both fatal and nonfatal.[265,266,360,849,854,864–874] A number of unpublished cases have also been reported by vaccine manufacturers, including 18 cases from Brazil,[851] most of which were fatal (Bio-Manguinhos, unpublished work, 2011).

In addition to the prospective identification of YEL-AVD cases, both confirmed and suspect cases of YEL-AVD have been identified retrospectively, dating back to at least the 1970s, if not before.[44,380,515,864] A retrospective analysis of 79 yellow fever samples in Brazil identified a patient who died in 1975 with "yellow fever" but whose partial genomic sequence provided evidence of the presence of a 17DD vaccine virus.[44,380] In 2010, a review of historical case reports in the United States described a 75-year-old man who became ill 6 days after receiving 17D-204 vaccine in 1973.[864] The man developed sepsis-like syndrome with multiorgan failure and died 11 days after vaccination. Histopathologic examination of the liver from this patient was not considered to be entirely characteristic for yellow fever, but the case met current criteria for viscerotropic disease and might have been confirmed if clinical suspicion for YEL-AVD existed at the time and there was more conclusive testing available. Even dating back to the 1950s, there was a description of potential YEL-AVD cases by Macnamara.[515] He reported a "number of cases of sickness which commenced several days after vaccination and exhibited jaundice, vomiting and albuminuria were seen by the doctor at a hospital serving an area in which a vaccination campaign was conducted. An epidemic of yellow fever, however, had been in progress so that naturally occurring yellow fever cannot be excluded, although it was felt by the doctor in charge that vaccination might well be the cause."

Case Definitions

The first case definition for YEL-AVD was developed by the Yellow Fever Vaccine Safety working group in 2002.[729] A suspect case was defined as a person with clinical symptoms and signs suggestive of organ failure following yellow fever vaccination. Probable and definitive cases were suspect cases with either abnormal histopathology or specific testing performed to document the abnormal presence and concentration of yellow fever vaccine virus or specific antigens in blood or tissues.[729] The case definition and causality classifications were also adopted by other yellow fever vaccine safety working groups in South America and Europe.[360,790]

In 2008, the Brighton Collaboration assembled a working group to devise a new case definition of viscerotropic disease as an adverse event following immunization. This case definition, with an unlinked yellow fever vaccine causality assessment, was published in 2012.[761]

Summary of Reported Cases

As of February 2017, at least 100 cases of viscerotropic disease have been reported worldwide following the administration of 17D vaccines from various manufacturers. As might be expected, with one exception, YEL-AVD has been observed only in persons with first-time vaccination and presumably without preexisting yellow fever immunity. The solitary reported case of YEL-AVD after a booster dose was never confirmed with yellow fever virus–specific testing. It was reported with minimal clinical detail in an aggregate report of YEL-AVD cases retrieved from a vaccine manufacturer's pharmacovigilance database.[790] From the little information provided, it is likely that this case would not meet either of the existing case definitions.

Detailed descriptive statistics for all reported cases worldwide are not possible because data are incomplete and reporting details and case classifications are variable, often case reports. However, data for all 24 YEL-AVD cases reported in the United States are sufficiently complete to describe basic demographic features and clinical outcomes of cases (Table 63.24). The median age of patients is 66 years (range, 21–79) and 71% (17/24) are male. The median time to symptom onset is 4 days (range, 1–18 days). Overall, 46% (11/24) of patients died with a median time to death from vaccination of 11 days (range, 8–30). There are a few notable demographic and clinical differences between males and females reported to with YEL-AVD. The median age for women is substantially lower at 31 years when compared to men who had a median age of 70 years but the age ranges are similar. Furthermore, the case-fatality rate is 71% in women but only 35% in men (Table 63.24). Although the numbers are small and might reflect a surveillance detection artifact, overall, the disease appears to be more severe in young girls and women.[875,876] This gender difference remains unexplained.

Review of Signs and Symptoms

Symptoms of YEL-AVD typically start 3 to 4 days after vaccination (range, 1–18 days) (Centers for Disease Control and Prevention, unpublished data, 2015)[729] and commonly include fever, myalgias, malaise, headache, and gastrointestinal symptoms (e.g., anorexia and diarrhea).[877] Laboratory abnormalities include elevations of liver transaminases, total bilirubin and creatinine in serum, decreased platelet counts in blood, and elevated proinflammatory cytokines. Less commonly reported abnormalities include elevated creatine phosphokinase, indicating rhabdomyolysis, and prolonged clotting times and/or elevated fibrin degradation products, which, when severe enough, indicate DIC.

TABLE 63.24 Descriptive Statistics, U.S. Cases of Viscerotropic Disease Following Yellow Fever Vaccination, 1973–2014[a,b]

		Age (y)			Onset (Days Postvaccination)			Death (Days Postvaccination)				
Patients	Number	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Number Fatal	Case-Fatality Rate (%)
Male	17	61.6	70	21–77	4.9	4	1–18	15.2	12	8–30	6	35
Female	7	44.6	31	22–79	2.9	2	2–5	11.8	10	8–21	5	71
Total	24	56.6	65.5	21–79	4.3	4	1–18	13.6	11	8–30	11	46

[a]Viscerotropic disease cases in the United States obtained from reports to VAERS or publication in medical literature.
[b]All viscerotropic disease cases meet either the CDC Yellow Fever Vaccine Safety Working Group YEL-AVD case definition or the Brighton Collaboration viscerotropic disease case definition.

TABLE 63.25 Reporting Rates for Yellow Fever Vaccine–Associated Viscerotropic Disease

Location	Reporting Period	Number of Cases	Number of Doses	Reporting Rate per 100,000 Doses	Reference
NONENDEMIC					
U.K.	1991–2003	4	3,046,007	0.13	Kitchener, 2004[849]
Europe, Australia, New Zealand	1993–2011	12	16,200,000	0.07	Cottin et al, 2013[a,790]
U.S.	1990–2002	7	2,230,760	0.3	Khromava et al, 2005[b,253]
U.S.	2000–06	6[c]	1,534,170	0.4	Lindsey et al, 2008[b,255]
U.S.	2007–13	6[b]	2,224,790	0.3	Linsey et al, 2016
ENDEMIC					
Peru	2007	5	63,174	7.9	Whittembury et al, 2009[360]
Brazil	1999–2009	20	107,649,393	0.019	Martins et al, 2010[851]
West African Countries	2007–10	5	38,009,411	0.013	Breugelmans et al, 2013[789]
Argentina	2008–09	12	1,943,000	0.6	Biscayart et al, 2014[825]

[a]This study calculated a worldwide reporting rate for YEL-AVD of 0.004/100,000 doses, based on 276 million doses of vaccine distributed globally. However, 11 of 12 cases of YEL-AVD were reported from nonendemic countries, and for one case the country of origin was unknown. This suggests that the low worldwide reporting rate reflects significant underreporting from endemic countries.
[b]Data reported are only for U.S. civilian cases.
[c]This published study includes an overlap from years 2000 through 2002 with the Khromava 2005 study. However, all six YEL-AVD cases were reported during the nonoverlapping period of 2003 through 2006.
YEL-AVD, yellow fever vaccine–associated viscerotropic disease.

Several patients with more mild disease thought to be YEL-AVD have been reported.[360,790] However, the majority of reported cases have had more severe disease with evidence of shock characterized by hypotension, organ hypoperfusion, and respiratory failure. These patients often require intravenous vasopressor medication, mechanical ventilation, and hemodialysis. An apparent difference between life-threatening wild-type yellow fever and YEL-AVD is the absence of severe hemorrhage (black vomit) in the latter. In several persons surviving long enough for an assessment of their immune response, antibody titers to yellow fever virus were unusually high compared to the typical response to 17D vaccination (≥1 : 10,240).[360,374] For details of individual case clinical features and laboratory abnormalities, see Martin M and associates,[374] Vasconcelos PF and associates,[858] Whittembury A and associates,[360] and Monath TP and associates.[878]

Reporting Rate

Worldwide, there have been a number of studies estimating reporting rates for YEL-AVD from endemic and nonendemic countries, including some after large national vaccination campaigns involving the administration of millions of doses of 17D vaccine (Table 63.25). The reporting rates vary from a low of 0.013 cases per 100,000 doses in several African countries[789] to a high of 7.9 cases per 100,000 doses in Peru.[360] The lowest rate is likely represents significant underreporting from the involved African countries; the high rate reported in Peru is an unexplained unique occurrence, and is discussed in more detail below.

Similar to YEL-AND, reporting rates for YEL-AVD tend to vary between nonendemic and endemic areas (see Table 63.25).[254] From VAERS data, the rate of YEL-AVD in United States civilians is estimated to be 0.25 to 0.4 per 100,000 doses distributed.[253,255,259a] This is similar to the rate reported from postmarketing surveillance data in the United Kingdom.[849] However, the rate of YEL-AVD following Stamaril in nonendemic areas (e.g., Europe, Australia, and New Zealand) was lower at 0.07 per 100,000 doses.[790] In comparison, the rates in endemic areas range from 0.004 to 0.6 per 100,000 doses[789,790,825,851] The highest rate of 0.6 per 100,000 doses was reported in Argentina[825] (other rates in endemic areas are <0.02 per 100,000 doses) and was similar to the rate reported in nonendemic countries. One potential explanation for this is that the national data in Argentina includes a fair number of travelers receiving the vaccine beyond those receiving the vaccine in the small endemic region in the north of the country (Misiones Province). Finally, reporting rates of YEL-AVD also vary by age with older age groups found to have increased rates (See "Advanced Age" under "Risk Factors for Yellow Fever

Vaccine–Associated Viscerotropic Disease" below for more details).[254]

Pathogenesis

The biodistribution of 17D virus in normal nonhuman primates[284] indicates that 17D virus replication is generally limited to lymphoid tissues. In contrast, the individual patients who have developed YEL-AVD have a different biodistribution of 17D virus that involves widespread replication in visceral organs.[276,360,858,859,868] Indeed, the pathogenesis of YEL-AVD closely resembles that of wild-type yellow fever. The disease is characterized by replication of 17D virus in liver, kidney, heart, and other vital organs, and by pathoanatomic changes similar to those observed in wild-type disease, including apoptotic Councilman bodies in the liver. Major hemorrhage appears to be less prominent in YEL-AVD than in wild-type disease. As in the wild-type disease, the terminal events with circulatory shock and multiorgan failure resemble systemic inflammatory response syndrome.

The immune responses in a few cases of YEL-AVD have been described. These studies have shown a cytokine activation profile similar to that seen in wild-type yellow fever, with elevations of both proinflammatory (IL-6, IL-8, IL-17, MCP-1, growth-related oncogene [GRO], and RANTES) and regulatory cytokines (IL-4, IL-5, IL-10), increased incidence of NK cells, cells expressing cytokines, and massive activation of T and B cells.[867,879] These exaggerated cytokine changes and immune responses, reflecting an overwhelming infection, are likely to play a pathologic role. The innate responses in such patients might be defective.[266,865,880] The role of the adaptive response in the pathogenesis of YEL-AVD (and wild-type yellow fever) is speculative, but it has been suggested that a vigorous cytotoxic T-cell and antibody response in the presence of widespread tissue antigen might have pathologic consequences.[381]

Risk Factors for Yellow Fever Vaccine–Associated Viscerotropic Disease

Advanced Age. Advanced age was initially associated with the occurrence of YEL-AVD cases in the United States.[826] Subsequent analyses showed that serious adverse events, in general, were more common in the elderly. An analysis of VAERS reports between 1990 and 2002 yielded an incidence of YEL-AVD in the civilian population ages 60 to 69 years and 70 years and older in the United States that was 4.4 and 13.4 times higher, respectively, than in young adults.[253] During this period, the overall incidence of YEL-AVD in persons older than age 60 years was 1.8 per 100,000 doses compared to 0.1 per 100,000 doses in persons younger than age 60 years, based on an experience of 2.2 million doses of 17D vaccine administered to civilians. In an analysis of VAERS reports from between 2000 and 2006, based on an experience of 1.5 million doses administered to civilians, the overall incidence of YEL-AVD was 0.4 per 100,000 doses. For persons 60 to 69 years of age and for persons 70 years of age and older, the incidence was estimated to be 1 per 100,000 doses and 2.3 per 100,000 doses, respectively.[255] An analysis of the reporting rate ratios for several studies found the ratios to be two to 37 times higher for persons 60 years of age or older (or 65 years of age or older in some studies) compared to young age cohorts.[254] However, whereas the reporting rate of YEL-AVD is higher in the elderly, severity (as reflected by the case-fatality rate) appears to be greater in young persons and possibly in young females.[875,876] The reason for the increased severity of YEL-AVD in younger persons is not known but could be related to a more robust immune response associated with systemic inflammatory response syndrome and cytokine storm similar to what is seen with dengue hemorrhagic fever.

Until recently, the majority of cases of YEL-AVD reported from Brazil occurred in children and young-to-middle-aged adults.[851] It was previously thought that a potential predilection for severe reactions in the elderly might not be apparent in Brazil, because many older persons were previously vaccinated and therefore immune to YEL-AVD. Three cases of YEL-AVD were reported in primary vaccine recipients older than age 60 years following the mass vaccination campaigns in the southern states of Rio Grande do Sul and São Paulo in 2008–09, and conducted in response to a large epizootic expansion (Bio-Manguinhos, unpublished work, 2011). This population was largely unvaccinated, because these areas had not experienced outbreaks in many years.[881] This experience suggests that in Brazil, advanced age might be a risk factor for YEL-AVD in primary vaccinees, just as in the United States. Indeed, calculations using age-stratified data for YEL-AVD cases and 17DD doses administered between 1999 and 2009 yield a crude estimate of 0.017 cases per 100,000 doses for persons younger than age 60 years versus 0.047 cases per 100,000 doses for persons 60 years of age and older.[851]

Thymic Disease. A history of thymus disease has been also identified as a risk factor for YEL-AVD.[869] Four (17%) of the initial 23 17D vaccine recipients with YEL-AVD worldwide had a history of diseases involving the thymus, all of which are extremely rare, suggesting that compromised thymic function may be another independent risk factor for developing YEL-AVD. One fatal case in the United States occurred in a 67-year-old woman who had a history of thymectomy for a malignant thymoma approximately 2 years before vaccination. A second case in the United States occurred in a 70-year-old man who had a history of hyperthyroidism, myasthenia gravis, and thymectomy for thymoma 20 years before vaccination. This patient survived. A third case was reported from Switzerland and occurred in a 50-year-old man who had a history of thymectomy for thymoma 8 years prior to vaccination. This patient also survived. The last case was reported from Colombia and was a 44-year-old man who developed fulminant hepatic failure and died following 17DD yellow fever vaccine. This patient had a thymectomy for benign thymoma 2 years before vaccination.

Following the initial description of the four cases, a history of thymic disease was added to the package insert and vaccine policy statements as a contraindication to vaccination. Since then only one case of YEL-AVD has been identified in a patient with a thymoma. The patient's thymoma was occult at the time of vaccination and was only discovered after she presented with symptoms of YEL-AVD.[873] Autopsy performed after her death confirmed the diagnosis of a thymoma and YEL-AVD.

Genetic Factors. Genetic factors determining susceptibility to 17D (and perhaps flaviviruses in general) are suspected to play a role in cases of YEL-AVD. Two familial clusters, each involving two cases, have been reported in Brazil. The first cluster was recognized after a 19-year-old woman died from a fatal case of YEL-AVD (confirmed by 17D RNA amplification from multiple organs) in 2001.[870] Upon interviewing the family, they reported that the woman's sister, who had received yellow fever vaccine in 1999 when she was 12 years old, had a very similar reaction after vaccination, with jaundice, hypotension, bleeding, thrombocytopenia, and renal dysfunction. No yellow fever virus-specific testing had been done on clinical specimens from the younger sister so the case was classified retrospectively as suspect. The second cluster occurred in 2009 when two brothers ages 30 and 34 years, both with Addison

disease treated with physiologic corticosteroid replacement therapy, died from illnesses clinically compatible with YEL-AVD after receiving 17DD vaccine.[851] An autopsy was not performed on either brother; however, immunohistochemistry was positive for yellow fever antigen in liver tissue (from postmortem liver biopsy) from one brother and 17D RNA was amplified from serum drawn 9 days after vaccination from the other brother (Bio-Manguinhos, unpublished work, 2011).

Flavivirus susceptibility in mice has been mapped to a mutation in an allele in one of eight genes *(Oas1b)* encoding 2′,5′-OAS, an enzyme important in type 1 IFN-mediated innate immunity.[262,264] Belsher and associates determined the OAS genotype in a 22-year-old woman with fatal YEL-AVD and found polymorphisms in *Oas1* and *Oas2* genes that could have impaired enzymatic functions important in innate immune responses to infection.[265] There were no abnormalities in CCR5 or RANTES genes, TLR3 (a susceptibility factor in West Nile), or DC-SIGN (dendritic cell–specific intercellular adhesion molecule-3-grabbing nonintegrin), a major Flavivirus receptor.

Pulendran and associates studied a 64-year-old patient with a severe but nonfatal case of YEL-AVD.[266] In this case, no mutations were found in OAS genes, TLR3, or DC-SIGN, but the patient had markedly elevated levels of RANTES and $CD14^{+}$ $CD16^{bright}$ monocytes in blood, which expressed CCR5 receptors for RANTES and could have reflected some impairment in trafficking of these cells. This patient was found to be heterozygous for the CCR5-Δ32 mutation (which is known to determine resistance to HIV) and to have a polymorphism in the promoter region of RANTES gene that regulates gene activity. The significance of the CCR5 and RANTES gene polymorphisms is not clear, but they could impair important effector mechanisms in innate immunity. Because of the CCR5-Δ32 mutation in this case and the role of CCR5 deficiency in susceptibility to West Nile virus,[882] it has been suggested that caution be exercised in yellow fever vaccination of HIV-infected subjects treated with CCR5 inhibitors, such as maraviroc.[883] Host genetics have been studied in a few other cases of YEL-AVD without finding a consistent defect.[360]

Because the viscerotropic response to 17D is likely related to host genetics, it is advisable to ask travelers whether severe reactions to 17D vaccine have occurred in a family member and to avoid vaccinating someone with a family history of a serious adverse event.

Other Considerations

Vaccine Virus. Almost all reported cases of YEL-AVD have occurred in persons given different lots of the vaccine. However, there has been one known cluster of five cases of YEL-AVD associated with the same lot of yellow fever vaccine.[360] This occurred in 2007 after a vaccination campaign in a nonendemic area of Peru. Four patients died of confirmed YEL-AVD. The overall incidence of YEL-AVD in this campaign was 7.9 per 100,000 doses, and the lot-specific incidence was 11.7 per 100,000 doses of the implicated lot. A thorough investigation detected no abnormalities of the implicated vaccine lot and no common risk factors among the five patients. The incidence of YEL-AVD associated with this campaign in Peru was substantially higher than previous risk estimates from other countries and remains unexplained, and no other clusters of YEL-AVD have been reported.

The genetic stability of the 17D-204 yellow fever vaccine strain, used in Stamaril, was studied by genetic sequence analysis of virus obtained from 12 bulk lots manufactured between 1990 and 2002, with identical sequences being confirmed.[559] In addition, comprehensive analyses of viral plaque size distributions, an additional marker for genetic stability, also was consistent with homogeneity of the viral population in those bulk lots. The total virus load, as measured by quantitative RT-PCR, was equally homogeneous and hence confirmed the consistency in the manufacturing process. Data obtained in animal models with yellow fever virus from these bulk lots were also consistent.

Analyses of the vaccine lots and seed viruses associated with cases revealed no evidence for mutations in the vaccine that could explain the adverse events. More important, analyses of 17D and 17DD viruses recovered from patients' tissues did not reveal mutations compared to the vaccine strains, indicating that even with in vivo amplification and selection as a filter to select for variants associated with pathogenicity, no evidence for a change in the virus was obtained.[360,374,858,868] Additionally, vaccine viral isolates from patients with YEL-AVD do not reproduce a similar syndrome when inoculated into healthy primates and golden hamsters.[380,868,884] Overall, these data suggest that mutations to the vaccine during manufacturing are unlikely to occur and be a risk factor for YEL-AVD. Although deep sequencing methods were not available at the time the isolates from patients were examined, the consensus sequence defined the majority virus population recovered from vital organs and should be reflective of the virus population causing the pathological process.

Autoimmune Disease. Twelve patients who developed YEL-AVD had a history of autoimmune disease. These include three patients with systemic lupus erythematosus, one with cutaneous lupus, two with Addison disease, one with Crohn disease in remission, one with a history of polymyalgia rheumatica and hypothyroidism, one with ulcerative colitis, two with myasthenia gravis, and one with pernicious anemia (Centers for Disease Control and Prevention, unpublished data, 2015).[360,374,851,864,867,869] Complete details are not available regarding medications these patients were taking, which might have included immunosuppressive medications. Six of the patients with underlying autoimmune disease were older than 60 years, and two of these six had a history of thymectomy for a thymoma; both factors are considered to be independently associated with increased risk of YEL-AVD.

More information is needed regarding the number of patients with underlying autoimmune disease who have been safely vaccinated to better address the potential risk of YEL-AVD in persons with autoimmune disease. Nevertheless, it is concerning that 12 (approximately 12%) of approximately 100 reported patients with YEL-AVD had a history of autoimmune disease, whereas the prevalence of autoimmune disease in the United States is 3%.[885] This suggests that autoimmune diseases, possibly in conjunction with other risk factors, including immunosuppressive medication, could increase the risk of YEL-AVD. Definitive risk assessment data are lacking to guide decision making about yellow fever vaccination in these patients. However, healthcare providers should consider the possibility of diminished immune function resulting from the autoimmune disease state itself and/or the treating medication in deciding whether or not to administer yellow fever vaccine to these patients.

Surveillance. The recognition of viscerotropic adverse events is especially difficult in endemic areas, where the syndrome could be confused with wild-type yellow fever.[380] During summer 2001 in Minas Gerais (Brazil), health authorities initiated a mass immunization campaign to control an outbreak involving 81 suspected cases and 32 confirmed cases, including 17 fatal outcomes (for a 53% case-fatality rate).Two cases of a fatal yellow fever disease in a 39-year-old man and a 69-year-old man who received the 17D vaccine 2 and 14 days, respectively, before the onset of signs and symptoms were of

special interest as suspect YEL-AVD. The molecular characterization of the virus isolated from fresh tissue and serum, however, revealed wild-type yellow fever virus.[886] Therefore, it is important to carefully analyze virus isolates by molecular techniques from case reports of yellow fever disease temporally associated with the administration of yellow fever vaccine in endemic areas.

Immunoglobulin Administration. The relatively recent recognition of YEL-AVD in developed countries may be attributable to improved surveillance for vaccine-associated adverse events. Other explanations have been suggested, in particular the possibility that immunoglobulin administered for hepatitis A prophylaxis in the era before marketing approval of hepatitis A vaccines (1995) might have provided protection against viscerotropic disease. However, this theory was not substantiated by a randomized, double-blind, controlled trial.[361] In the trial, 40 subjects were given both 17D vaccine and intramuscular human immunoglobulin and 40 subjects were given 17D vaccine and saline injection placebo. The coadministration of immunoglobulin with 17D vaccine did not reduce the incidence of vaccine-induced viremia nor were yellow fever antibody or T-cell responses to the vaccine altered. However, there were some limitations of the study design[887] and the results were unexpected given the documented role of passive antibody in protection against yellow fever.

Recommendations for Evaluation of Suspect Yellow Fever Vaccine–Associated Viscerotropic Disease Cases

Patients with an undiagnosed febrile illness occurring within 10 days after vaccination should be investigated and those with elevated liver enzyme levels and reduced platelet counts should be hospitalized for observation. To provide much-needed data on causality, serial samples for quantitative viremia, antibody, and cytokine studies should be undertaken and buffy coat cells or peripheral blood mononuclear cells frozen appropriately for future immunologic and genetic studies. If autopsy is performed on fatal cases, samples of multiple organs should be preserved for histopathology and immunocytochemistry and frozen (on dry ice or liquid nitrogen) for virologic studies. In the United States, such cases should be promptly reported to the VAERS (800–822–7967) and consultation sought with the Division of Vector-Borne Diseases at the CDC in Fort Collins, CO (970–221–6400) for diagnostic testing support. In other countries, such cases should be promptly reported to the country-specific health authorities and advice sought about obtaining diagnostic testing.

Impact of Serious Adverse Events

Impact on Travel Vaccination

The risk of acquiring yellow fever virus infection during travel to an endemic and epidemic area may exceed the risk of YEL-AVD for the vast majority of persons and travel destinations.[461] However, the epidemiologic risk of transmission is variable in time and place even in endemic areas. Hence, physicians should be aware of this serious complication of 17D vaccines and that it occurs at higher incidence in primary vaccination of elderly persons. Physicians should also stay up-to-date on yellow fever endemic and epidemic areas.[412] Information on occurrence of human cases may not be an adequate guide, as monkey-to-monkey transmission can occur in remote and sparsely inhabited areas, and vaccination rates can be high in endemic areas. Special attention is also drawn to the fact that in at least one case the intended travel destination was not within the yellow fever endemic zone, and therefore vaccination was not necessary.[374] This is likely also true (though not published) in two other cases, and a fourth was immunized in the military for deployment (a practice that did not generally take into account whether the actual destination was in a yellow fever–endemic area).[888]

Impact in the Endemic Areas

YEL-AVD and YEL-AND are recognized as significant factors to be considered in the implementation of yellow fever vaccination strategies in South America, in particular when mass vaccination is conducted in areas where routine vaccinations have not heretofore been performed. Only five young children (10 months to 5 years of age) have been reported with cases of YEL-AVD (all in Brazil), although millions of doses of yellow fever vaccine are given annually in the EPI in endemic countries of South America, generally at 9 to 12 months of age. Thus, vaccine safety does not appear to be a major concern for use of 17D vaccines in this age group, where it is most cost effective as a means of long-term protection of the population against epidemic and endemic disease.

On the other hand, when mass immunizations have been given in areas of periodic epizootic expansions outside the traditional endemic area, such as occurred in 1998 to 2000 and 2007 to 2008 in eastern and southern Brazil or during the campaign in Ica Province, Peru, in 2007, involving very large numbers of vaccinations in nonimmune adults, cases of YEL-AVD have appeared.[360,858,881,889] The authorities have argued that without vaccinations, many lives would have been lost to wild-type yellow fever, and that the penalty to pay for prevention was a relatively smaller number of lives lost to vaccine accidents. However, in Ica where there were five YEL-AVD cases, vaccination was undertaken in an area where yellow fever virus is not found and probably should not have been given, demonstrating the need to carefully evaluate risk factors when considering vaccination.

In Africa, routine immunization of infants at 9 months of age is established in many endemic countries, and catch-up campaigns are being implemented as part of the Yellow Fever Initiative described later in the discussion under "Public Health Considerations." To date, 11 potential YEL-AND or YEL-AVD cases have been documented with the enhanced surveillance efforts that were performed these mass campaigns.[789] However, this is in the context of over 38 million doses of the vaccine being delivered and offering protection to persons living in areas at high risk of yellow fever virus infection.

INDICATIONS, CONTRAINDICATIONS, AND PRECAUTIONS FOR VACCINATION

Indications

All inhabitants of countries or areas within countries endemic for yellow fever should be routinely immunized, preferably at 9 months of age. Fig. 63.4 shows the areas endemic for yellow fever. Within these countries, priorities for immunization may be established related to risk of exposure to yellow fever virus. For example, residents in rural areas and areas of historical yellow fever epidemic activity are at higher risk than residents in large cities or areas in which yellow fever has not occurred in many years. Persons migrating from nonendemic to endemic areas are at high risk and should be immunized. Because such movements are difficult to control, and the potential exists for spread of yellow fever virus from rural to

urban areas, a policy of universal immunization in many countries within the endemic zone is favored.

However, in many countries a distinction is drawn between endemic and nonendemic regions within national borders. For example, in many countries in South America, the coastal zone is traditionally outside the endemic region. Many of these areas contain very large human populations and major urbanizations, and these regions have been excluded from routine yellow fever immunization. New safety concerns for use of 17D vaccine have reinforced the policy to exclude these coastal zones. Mass vaccination of adults in a nonendemic region of Peru (Ica Province) was associated with a high incidence of YEL-AVD in 2007.[360] Adverse events also led to the cessation of a mass vaccination program in the nonendemic region of coastal Brazil in 2000.

During epidemics of yellow fever, mass immunization should be instituted at the earliest possible stage of the outbreak. Priorities for immunizing population subsets according to geography or age group are determined by local information on the progress of the outbreak and on the history of prior vaccination coverage.

Immigrants, travelers, and military personnel and dependents require immunization at least 10 days before arrival in endemic areas as even a short stay in an area of virus transmission is dangerous.[461,732] Because native inhabitants may be immune and the virus can circulate in a silent zoonotic cycle, the absence of recent notification of yellow fever in an area is not an indication that it is safe to enter without vaccination. On the other hand, the notification of human cases within the past 1 to 2 years is an indication of high risk. Short-stay travelers to large cities within endemic areas, such as the coastal metropolises of West Africa (e.g., Accra, Lagos, Dakar, Abidjan) are at lower risk in the absence of a reported outbreak. However, cases of urban yellow fever were reported within the city of Abidjan (Côte d'Ivoire) in 2001 and again in 2008.[428,890] Similarly, urban areas located within endemic regions, such as Iquitos, Peru, represent areas of risk. Clearly, visitors to urban regions reporting yellow fever or juxtaposed to areas of ongoing virus activity require vaccination. Coastal areas of East Africa and most of South America are outside the area of yellow fever transmission; however, there was expansion of yellow fever activity in coastal states in Brazil in late 2008 through early 2009 and again in late 2016 through early 2017. Current information of yellow fever endemic and epidemic activity may be obtained on the WHO website[891] and the CDC's traveler's health website.[892]

Some countries in the endemic zones and some countries outside the endemic zones but infested with *Ae. aegypti* and receptive to the introduction of yellow fever virus require a valid certificate of immunization for travelers from endemic countries. A full listing is given in the WHO document International Travel and Health[414,893] and in CDC's Health Information for International Travel.[413,894] Some countries require a valid certificate even if the traveler has been in transit through an endemic country and even if the disembarking traveler is in transit. Controls at airports and borders are highly variable, but travelers respecting the regulations avoid unnecessary delays. Persons with a contraindication to immunization (see the next section) should have their medical provider fill out the Medical Contraindication to Vaccination section of the ICVP and also obtain a letter from the provider stating why immunization could not be performed.

Contraindications and Precautions

Contraindications and precautions to the administration of yellow fever 17D vaccine specified by the ACIP[729] are shown in Box 63.1 and described in this section. The medical practitioner administering yellow fever vaccine has the responsibility to assess the risks associated with 17D vaccine, the risk of exposure to natural infection with yellow fever virus, and the statutory and practical requirements for a certificate of immunization. This should take into account the details of the patient's medical history, the itinerary of travel, and existing regulations. In two surveys of subjects seeking travel health advice, 2% to 6% had one or more potential precautions or contraindications for yellow fever vaccination.[733,895]

BOX 63.1 Contraindications to and Precautions for Yellow Fever Vaccine Administration Based on the Recommendations of the Advisory Committee on Immunization Practices[a,729]

CONTRAINDICATIONS

- Allergy to vaccine components[b]
- Age <6 months
- Symptomatic HIV infection or $CD4^+$ T-lymphocytes <200/mm^3 (or <15% of total in children <6 years)
- Thymus disorder associated with abnormal immune function; history of thymectomy
- Primary immunodeficiencies
- Malignant neoplasms
- Transplantation
- Immunosuppressive and immunomodulatory therapies

PRECAUTIONS

- Age 6–8 months
- Age ≥60 years
- Asymptomatic HIV infection and $CD4^+$ T-lymphocytes 200 to 499/mm^3 (or 15–24% of total in children <6 years)
- Pregnancy
- Breastfeeding

[a]Recommendations are made for travelers and laboratory workers in the United States and might differ from vaccine recommendations in other countries and in areas with endemic yellow fever disease.
[b]Includes egg allergy (oral intolerance to eggs).

Young Age

Infants are at higher risk for YEL-AND, and this risk is inversely proportional to age. The vaccine should never be administered to infants younger than 6 months.[672,729] Routine immunization may be performed at 9 months of age. Age 6 to 8 months is a precaution for yellow fever vaccination.[729] Such infants should be immunized only if there is a significant risk of natural infection, such as residence in or travel to a rural area in the yellow fever endemic zone or in the context of an ongoing epidemic. If possible, travel should be delayed until the infant is 9 months of age. Where the risk of exposure to yellow fever virus is very low (e.g., in large urban areas in endemic countries, especially in the context of brief visits by tourists), it is advisable to delay immunization until 12 months of age.

Advanced Age

Age 60 years or older is considered a precaution for yellow fever vaccination for primary vaccine recipients[729] owing to higher reporting rates of severe and serious adverse events, including YEL-AND and YEL-AVD in this population (see "Adverse Events" earlier).[253–255,259a,826,827,849] The relative risk of a serious adverse event increases with advancing age beyond 60 years, and the precaution for vaccination should be

considered in that light. The risk for YEL-AND and YEL-AVD also are increased in this age group, at 1.8 and 1.4 cases per 100,000 doses, respectively, compared with 0.8 and 0.4 cases per 100,000 doses, respectively, for all yellow fever vaccine recipients.[255] YEL-AND and YEL-AVD have occurred exclusively in the setting of primary vaccination, and there is far less risk to persons 60 years of age or older who are revaccinated, unless they have some underlying acquired immunosuppressive disorder. The reason for the higher risk of adverse events is not completely elucidated but one study did find that older adults have a delayed immunologic response to yellow fever vaccination and develop higher levels of viremia post vaccination than younger adults.[256]

Pregnancy

For a discussion of the risks of congenital infection, see "Pregnancy" under "Adverse Events" earlier.

The hypothetical risk of transplacental infection and the recognition that young infants (and, thus, potentially, the unborn fetus) were susceptible to neuroinvasion by 17D virus led to the general recommendation that the vaccine not be administered during pregnancy unless it is clearly required based on a high risk of natural infection.[672,729] Both the ACIP and the WHO recommend that a risk-to-benefit assessment be performed for all pregnant women for whom yellow fever vaccine is being considered. Inadvertent immunization of women (generally in the early stages of pregnancy) is definitely not an indication for therapeutic abortion. Women should be cautioned that there is a hypothetical risk, but they should be reassured that no harm to the fetus has ever been demonstrated. Fear of potential adverse effects might have led to an increase in therapeutic abortions during a vaccination campaign in Trinidad in 1977 to 1978.[896] If possible, women should wait until 4 weeks after vaccination before conceiving.[729]

Pregnant women who have received the vaccine should be reassured that there is no risk to them and very low risk to the fetus. These women should be followed to parturition, and if fetal abnormality is noted, an infant blood sample should be obtained for IgM test to determine whether congenital infection had occurred. Data on whether the immune response to yellow fever vaccination during pregnancy is impaired are conflicting (Goujon C and Godineau J-P, personal communication, 2006) and might be related to the timing of the vaccination during pregnancy.[625,741]

Breastfeeding

Very limited safety data are available on the use of 17D vaccine in breastfeeding women and their infants. Although a very large number of lactating women have been successfully immunized during emergency vaccination campaigns without a significant number of adverse events being reported, cases of serious adverse events occurring in breastfed infants whose mothers were vaccinated raises concern over the use of the vaccine in lactating women.[729,897] Two probable and one confirmed case of YEL-AND have been reported in infants whose mothers were vaccinated with yellow fever vaccine.[203–205] The infants were breastfed exclusively, were younger than 1 month at the time of the exposure, and had not received the vaccine themselves. The interval between vaccination and onset of symptoms in breastfeeding infants was 3 to 4 weeks. Yellow fever vaccine virus was recovered from the CSF of the confirmed case patient, and yellow fever–specific IgM antibodies were detected in the CSF of the probable case patients. Testing was not performed on the breast milk in any case to confirm the presence of vaccine virus.

West Nile virus RNA has been found in human breast milk[898] and some tickborne flaviviruses (tickborne encephalitis, Powassan encephalitis) are secreted in milk of domesticated livestock, which can be a source of oral infection of humans. Given these findings, the ACIP states that breastfeeding is a precaution to yellow fever vaccination.[729] The WHO recommends that a risk-to-benefit assessment be conducted for lactating women for whom yellow fever vaccination is being considered, but also states that lactating women should be advised that the benefits of breastfeeding far outweigh alternatives.[672] Further research is needed to document the risk for potential vaccine exposure through breastfeeding.

Altered Immune State

Data on the safety and efficacy of yellow fever vaccination in persons with altered immune function remain limited. However, yellow fever vaccine is contraindicated in patients who are immunosuppressed as a result of primary or acquired immune deficiency or medication because of the theoretical and occasionally documented risk of serious adverse events. Specifically, yellow fever vaccine is contraindicated in persons with primary immune deficiency disorders, malignant neoplasms, transplantation, or treatment with radiation or immunosuppressive drugs including high-dose corticosteroids, alkylating agents, antimetabolites, TNF-α inhibitors, and IL-1 blockers, and MAbs targeting immune cells (e.g., rituximab).[729] In addition, yellow fever vaccine is contraindicated in persons with symptomatic HIV infection or $CD4^+$ counts less than 200/mm^3 or less than 15% of total lymphocytes for children younger than age 6 years.[729] The theoretical concern about use of CCR5-receptor blocking agents was noted in the section on HIV, earlier. Low-dose corticosteroid treatment (20 mg prednisone or equivalent per day), short-term corticosteroids (course of <2 weeks), and topical, inhaled, or intraarticular or bursal injections of corticosteroids do not pose a contraindication to yellow fever vaccination.[729] If feasible, immunosuppressive medication should be stopped until immune function is restored or improved before yellow fever vaccination.

Four (17%) of the initial 23 reported YEL-AVD cases were noted to occur in persons who had had thymectomies performed for thymomas,[869] and in 2003, "thymus disorder" was added to the 17D vaccine package insert as a contraindication. Since this recommendations was made, there has been only one fatal case of YEL-AVD reported in a person with an occult thymoma that was only detected after vaccination and when she developed symptoms to YEL-AVD.[873] To date, no evidence has been identified of immune dysfunction or increased risk for yellow fever vaccine-associated serious adverse events in persons who have undergone incidental surgical removal of their thymus or have had indirect radiation therapy in the distant past.

Although there has been a recent increase in the number of persons who are HIV-infected and have been vaccinated against yellow fever, data on the safety and immunogenicity of the vaccine are limited to approximately 800 HIV-infected persons with less than 25 of these individuals having $CD4^+$ counts of less than 200 per mm^3 when they were vaccinated.[715,744,748–752,899–901] As noted above, the vaccine is contraindicated for persons with symptoms HIV or $CD4^+$ counts of less than 200 per mm^3. This recommendation is made partly because of the report of a healthy adult who had low $CD4^+$ cell counts as a result of HIV infection and who developed fatal YEL-AND following 17D vaccination.[829] The ACIP also considers asymptomatic HIV infection and $CD4^+$ T-lymphocytes counts of 200 to 499/mm^3 (or 15% to 24%

of total in children younger than age 6 years) to be a precaution for vaccination. This is partly because of the theoretical safety concerns but also because of the lower rates of seroconversion and more rapid antibody decay seen among persons infected with HIV who are vaccinated when compared to healthy persons (see "HIV Infection" under "Primary Vaccine Failure" earlier).[715,744] For HIV-infected persons who experience immune reconstitution in response to antiretroviral therapy, their current CD4 count and symptoms of HIV infection (if stable over a 3-month period) rather than a CD4 count nadir and a history of prior opportunistic infections/symptomatic HIV should be used to categorize their HIV status.[413] Finally, if an asymptomatic HIV-infected person has no evidence of immune suppression based on $CD4^+$ counts ($\geq$500 cells/mm^3), yellow fever vaccine can be administered, if recommended on the basis of their destination-specific yellow fever risk.

Policy decisions regarding use of 17D vaccine in endemic areas, including emergency vaccination to control outbreaks or in routine immunization programs, must also take into account benefits and potential risks related to the prevalence of HIV in the population and the sensitivity of surveillance for adverse events.[902] Where emergency and preventive vaccination has been deployed in areas of Africa with a relatively high prevalence of HIV infection and follow-up was conducted, no safety concerns have been noted.[770,901,902] Current policy supports use of live vaccines, including yellow fever 17D for children, regardless of possible HIV exposure, as a means of ensuring immunization coverage and controlling epidemic yellow fever.[903]

There is increasing evidence from case reports that autoimmune diseases constitute a risk factor for YEL-AVD (see "Autoimmune Disease" under "Other Considerations" for YEL-AVD above). In 2010, 11 (17%) of 65 YEL-AVD cases had a history of autoimmune disease, such as systemic lupus erythematosus, or diseases with the potential for autoimmune etiology. There may have been other confounding risk factors also (e.g., advanced age, thymectomy, medications). However, the association with autoimmune diseases emphasizes the need for caution in patients with any immune dysregulation and further analysis of the risk and pathogenesis of adverse events.

An individual risk-to-benefit assessment must be performed for every patient who has a history of immunosuppression and who is traveling to an endemic area. If travel is unavoidable and contraindications to vaccination exist, the physician should provide a medical waiver and should counsel the patient about measures to avoid mosquito exposure. For select travelers, passive immunization could be considered (see "Passive Immunization and Passive-Active Immunization" above for more information) but this practice is complex and expensive and likely is not a viable option for most immunosuppressed individuals.

Blood, Bone Marrow, and Organ Donation

Transfusion-related transmission of yellow fever vaccine virus has been documented among persons receiving blood products that were collected from recently vaccinated persons.[657] Blood donation centers should screen blood donors to determine if they received a yellow fever vaccine within the past 2 weeks and defer these donors. However, practices vary among blood collection centers, and questions about vaccination history might not routinely be asked. Vaccine recipients should be advised to delay blood donations until 30 days after vaccination.

No formal recommendations have been made on the suitability of persons who have received 17D vaccine at a remote point in time as organ or bone marrow donors. This issue has been raised because of the possibility that 17D virus causes a latent, persistent infection (reviewed in "Duration of Immunity" under "Results of Vaccination" earlier) that is contained and irrelevant in the immune host but that might cause systemic infection in an immunosuppressed transplant recipient. The risk to recipients is purely hypothetical. Unless the vaccine changed its tropism and virulence during latency, the risk to the recipient, even if infected, appears to be small. In a single report, bone marrow from a donor twin immunized 1 month previously with yellow fever 17D was grafted to an identical twin.[904] The recipient failed to develop yellow fever antibodies, indicating that no yellow fever B cell clone had been transferred or that immunosuppressive therapy might have masked an immune response by the recipient to transferred virus. There were no untoward events attributable to the potential transfer of 17D virus.

There are no data on whether yellow fever vaccine induces transient false-positive serologic tests for HIV, syphilis, or other bloodborne viruses, as has been reported for influenza and smallpox vaccines.[905]

Allergy to Vaccine Constituents

Documented allergy to a vaccine component, including eggs (cooked or raw), egg products, chicken meat, or gelatin or a severe allergic reaction to previous administration of the vaccine (or other vaccines prepared in eggs, such as influenza) constitute a contraindication to vaccine administration. The product label for YF-Vax also notes that the vaccine stopper contains latex rubber, which might cause allergic reactions.[575] Generally, persons who are able to eat eggs and egg products without allergic reactions can receive the vaccine. Egg allergy is more common in children than adults. If there is doubt about egg allergy, a test may be performed to determine egg-white IgE (by skin prick or serum test) or allergy to the yellow fever vaccine itself using a scratch or prick test with 1 : 10-diluted vaccine followed (if positive wheal and flare) by an intradermal test with 0.02 mL of 1 : 100-diluted vaccine, using appropriate controls. The methods are described in the YF-Vax package insert.[575] Desensitization may be considered in the event of demonstrated allergy if vaccination is required for travel and has been shown to be effective in a limited number of persons with egg allergies.[779] This should ideally be performed by an allergist.

It should be noted that the small dose of vaccine given in the allergy skin test may be sufficient for effective immunization. Roukens and associates[906] used an intradermal test dose of 0.1 mL (undiluted vaccine) in seven egg-allergic subjects and found that all seroconverted. However, the procedure (using much smaller doses than in the U.S. product [YF-Vax] label) was not followed in this case. Nevertheless, before performing desensitization in a known allergic patient who has received an intradermal test dose of vaccine, it is useful (if feasible) to wait 14 days and perform a neutralization test. If the patient is found to have neutralizing antibodies, the patient may be considered to have been satisfactorily vaccinated.

Waiver of Yellow Fever Vaccination

If a contraindication to yellow fever vaccination exists or a precaution exists for which the physician judges that the risk of adverse events outweighs the risk of exposure to wild-type virus, a waiver may be provided. The Medical Contraindications to Vaccination form is part of the ICVP and should be signed and validated with the stamp of a designated yellow fever vaccinating center.[413,732]

Patients Who Receive Yellow Fever Vaccine but Have Contraindications and Are at Risk for Adverse Events

For immunosuppressed patients who are inadvertently vaccinated, combinations of IVIg (derived from U.S. plasma donations only), IFN-α, or IFN-ribavirin[408] (all of which are commercially available) may be considered. Treatment should be initiated as early as possible, and certainly within 48 to 72 hours after vaccination. Although there are no clinical data to provide a guide, it appears preferable to use IFN-α rather than a pegylated IFN, because the pharmacokinetics of IFN-α are short (peak blood levels of 3 to 12 hours) and pegylated IFN has delayed action (15 to 44 hours); IFN-α treatment may be repeated daily for several days during the period of active vaccine virus replication.

Because these treatments are not labeled for this indication, and only preclinical data are available, no dosing regimens are provided here. Physicians who encounter such cases should seek advice from an infectious diseases specialist. Interference with the vaccine's efficacy could potentially result from treatment, and the patient's neutralizing antibody level should be confirmed 14 days or more after vaccination and before travel to an endemic area.

PUBLIC HEALTH CONSIDERATIONS

Recommended Use and Epidemiologic Results of Vaccination

South America

It is estimated that approximately 25% of the total population in the endemic countries of South America inhabit the yellow fever endemic zone, meaning that the majority of the population lives in more densely populated areas along the east and west coasts of the South American continent (see Fig. 63.4). Yellow fever immunization has been implemented for decades in all countries with endemic yellow fever in South America, but vaccination coverage and strategies vary by country. Vaccination policy has changed, encouraged by PAHO, toward implementing yellow fever vaccination in the EPI and away from intermittent mass campaigns. Currently, 10 of the 13 countries considered to be endemic for yellow fever vaccinate all children from 9 to 12 months of age against yellow fever as part of the EPI. Only Argentina, Brazil, and Suriname limit their coverage to children residing in at-risk areas. However, vaccination coverage rates have varied and are lower than wanted to protect the at-risk population and protect against outbreaks of the disease. From 2010 to 2014, the vaccination coverage for yellow fever in the region was approximately 75% but varied from 51% to 99% depending on the country. One reason cited for the lower vaccination rates was limited availability of the vaccine.[440]

Enhanced efforts to cover the population have occurred during periods of increased viral activity or geographic expansion. An example of geographic expansion is the period 1999 to 2000, when a large epizootic epidemic swept Brazil, causing 192 human cases and threatening densely populated areas on the fringe of the endemic zone. During that interval more than 35 million doses of vaccine were administered in mass campaigns, and consideration was given to introducing routine immunization in coastal areas outside the endemic zone. Following other major epizootic waves between 2007 and 2009 and starting in late 2016, which involved states in southern and coastal Brazil, additional mass campaigns were instituted. Between 1999 and 2009, the interval covering these major expansions of yellow fever in Brazil, 104 million doses were used.[907]

A continuing debate for policymakers is the need for vaccination of the large population in the nonendemic coastal region of Brazil at risk for urban yellow fever.[908] The risk of serious adverse events, particularly YEL-AVD, has been judged to outweigh the benefits,[828] although it is acknowledged that the introduction into the EPI would exclude higher-risk elderly persons and gradually provide an immune barrier to urban yellow fever. The dramatic clinical presentation of yellow fever and improved surveillance for infectious diseases are important factors in making healthcare policy decisions of this sort, because an urban outbreak of yellow fever would come to light quickly and could be contained rapidly.

The result of vaccination policies in South America is difficult to assess by historical comparisons with the prevaccine era, because confounding events coincided with the introduction of wide-scale immunization, including the introduction of active surveillance (using viscerotomy, initiated in 1930) and the expansion of human settlements in endemic areas. Nevertheless, in countries like Brazil, which instituted a program of immunization, the incidence of jungle yellow fever has declined as vaccination coverage increased.[909]

Africa

The increased incidence of epidemic yellow fever in Africa, beginning in the mid-1980s[28,170,910] and the recognition that the disease predominantly affects children,[28,163] led to a reassessment of vaccination policy for Africa. In 1988, the joint UNICEF and WHO Technical Group on Immunization for the African Region and the EPI Global Advisory Group recommended that countries endemic for yellow fever incorporate 17D vaccine into the routine EPI schedule, either at 6 months of age or at 9 months of age, together with measles vaccine.[911,912] In 1990, this recommendation was reemphasized, with the additional suggestion that catch-up immunization of older children is needed in countries at high risk. Surveys conducted between 1987 and 1990 indicated coverage of approximately 80% of infants by 1 year of age in The Gambia, Côte d'Ivoire, and Senegal, but rates of only approximately 40% in Burkina Faso, Chad, Mauritania, and the Central African Republic.

By 1991, 14 countries (Angola, Burkina Faso, Cameroon, Central African Republic, Chad, Côte d'Ivoire, Gambia, Ghana, Mali, Mauritania, Niger, Nigeria, Senegal, and Togo) at risk for yellow fever had officially incorporated 17D vaccine in the EPI, but uptake of the vaccine was poor in most countries, principally because of lack of donor funding for purchase of vaccine. In 1992 (13 countries reporting), the overall coverage was 19%; in 1993 (12 countries reporting) coverage was 14%; in 1993 (11 countries reporting), coverage was 29%.[913] By 1995, rates were less than 50% in all countries, except The Gambia, the Central African Republic, and Burkina Faso. The Gambia, which suffered a major outbreak in 1978 to 1979[252] and responded with mass immunization of children and adults, followed by sustained high rates of coverage of infants in the EPI, is one of the few African countries that has maintained a high level of vaccine coverage against yellow fever.

Another technical consensus meeting was held at the WHO in 1998, at which time 17 of the 34 countries endemic for yellow fever had incorporated vaccine into the EPI, although coverage remained low. Vaccination coverage has gradually improved over the last few years, in part because donor agencies, including the Bill & Melinda Gates Foundation and the Global Alliance for Vaccines and Immunization (GAVI), have provided funds for yellow fever vaccine purchases. Of interest was the forecast for vaccine doses required under assumptions of increased coverage rates. In 1997, only 4 million doses of vaccine were supplied by UNICEF to African countries, whereas 24 million doses were required to achieve 80% coverage of

children living in high- and moderate-risk areas in the 34 affected countries, and 240 million doses were required to undertake preventive mass campaigns. Demands for vaccine in response to outbreaks could severely stretch the capacity of manufacturers. These scenarios should be compared to the annual production capacity of approximately 30 million doses from manufacturers serving the African market. Shortages of vaccine for control of epidemics have been experienced, for example, during an outbreak in Guinea in 2000 and 2001,[911,914] leading to a recommendation for a strategic stockpile of vaccine to be held by UNICEF.

The Guinea case study led to an initiative whereby GAVI encouraged and supported inclusion of yellow fever vaccine in the EPI, encouraged catch-up campaigns, and provided a 6-million dose emergency stockpile of vaccine for rapid distribution in emergencies. In 2007, $58 million was pledged by GAVI to perform catch-up yellow fever immunization campaigns with the target of reaching 48 million people in West Africa.[915] By 2010, the number of African countries practicing routine infant immunization had risen to 24 compared with 15 in 1999.

In 2006, the WHO launched the Yellow Fever Initiative.[27,916] The purpose of the initiative is to ensure inclusion of yellow fever vaccine in the EPI at age 9 months and older, implement preventive mass immunization campaigns for older persons in high-risk areas, and ensure adequate supplies of the vaccine. The initiative has been very successful, providing more than 95 million persons protection against yellow fever disease in Africa and increasing the production capacity of the vaccine from 30 million doses in 2000 to more than 75 million doses in 2009.[426,916] From 2007 to 2014, preventive campaigns have been conducted in 13 countries, including 11 high-risk countries in West Africa as well as the Central African Republic and Sudan. Because of larger-than-anticipated vaccine needs in Nigeria, Nigeria has been partially vaccinated and all at-risk persons are expected to be vaccinated (assuming sufficient vaccine supplies) by 2017.[426] Reported vaccine coverage rates are greater than 80% for these preventive campaigns and no outbreaks have been reported in areas that have been vaccinated.

To guide the deployment of vaccine in catch-up campaigns in Africa, the WHO prepared a model to identify high-risk areas.[436] The risk assessment model employed indicators of exposure to yellow fever virus and susceptibility of the population and was used to prioritize areas to vaccine within West African countries. Following the use of this model, the WHO has led efforts with other partners to better estimate additional areas at risk in other countries in Africa.[149] The second risk assessment approach divides countries into zones in which the ecology is considered to be similar for the circulation of yellow fever virus. Areas are then randomly selected in each zone and teams travel to these locations to conduct human serosurveys and surveillance for mosquito vectors.[243] The rates of yellow fever virus–specific antibodies among humans and the number and types of vectors in ecologic zones are then used to recommend whether preventive vaccination or introduction of routine yellow fever vaccination is required.[426] These substantial efforts to better define risk areas and improve vaccination coverage have greatly reduced the risk of disease among those most at risk and have helped to decrease the potential of subsequent large outbreaks like those that were seen in the 1980s in Nigeria. However, until all risk assessments and campaigns are completed and high vaccination coverage sustained, there will continue to be a risk of outbreaks of disease in the region, as was seen in Angola and DRC in 2016. Finally, although these measures will help lower disease risk, these approaches will not guarantee that unvaccinated persons will not be infected if they live or travel to an endemic area.

Distribution of Yellow Fever Vaccine to At-Risk Travelers

Tourism and travel continue to increase at a rapid rate. The International Tourism Association estimates that 3.2 million persons traveled from the United States to South America and Africa in 2014.[917] Yellow fever is one of the most important vaccines for travel, because it is a lethal disease and because a certificate of vaccination is required for entry to some countries. Yellow fever is the one of the only vaccines for which such a certificate is required under the International Health Regulations. The International Health Regulations also specify that yellow fever vaccine be administered only by approved vaccinating centers. This restriction provides assurances that the vaccine will be properly stored and delivered using aseptic technique within the specified time after reconstitution. A vaccination certificate, specifying the date of immunization and the vaccine lot number and signed by personnel at the vaccinating center and validated with a uniform stamp, constitutes proof of valid immunization for the purposes of international travel.

In the United States, the CDC has the statutory authority to approve centers. This authority is delegated, in turn, to state health departments.[918] There are currently just over 6700 certified yellow fever vaccination centers in the United States, concentrated principally in large metropolitan areas and in gateway cities and represented by organized travel clinics.[919] Difficulty in accessing a yellow fever vaccinating center can contribute to undervaccination.[369,920]

A study of travelers to yellow fever–endemic African destinations from Johannesburg International Airport found that 76% had proof of yellow fever vaccination.[921] Among U.S. travelers attending a travel health clinic in Boston, 90% to 93% of the travelers going to a yellow fever endemic or partially endemic country were vaccinated prior to traveling.[733] However, these findings need to be tempered by the fact that many travelers never seek pretravel consultation. A study also performed in Boston at Logan Internal Airport found that only 46% of travelers to low- or low-middle-income countries sought pretravel advice.[922] Fear of adverse events can also contribute to declining vaccine uptake. The unfortunate result may be reports of fatal yellow fever among travelers.

Yellow fever vaccine should be administered 10 days or more before entering a country with endemic yellow fever. One survey of Israeli travelers showed that 11% of travelers to South America and 33% of travelers to Africa received their vaccinations fewer than 10 days before departure.[923] In practice, because travelers generally disembark some time before entering a rural area where exposure could occur, delayed immunization is of little consequence. However, delayed vaccination could represent a risk under certain circumstances; for example, in persons moving from uninfected to infected areas within a country or from an infected to a receptive zone.

Unnecessary use of yellow fever vaccine is also a significant problem. At least one case of fatal iatrogenic YEL-AVD occurred in a traveler bound from Australia for the Hajj to Saudi Arabia,[924] a country in which there was no risk of natural exposure and whose regulations did not require a certificate of yellow fever vaccination for travelers from a nonendemic area.[924] In another case, a Spanish traveler received 17D vaccine for travel to Mauritius, where yellow fever does not occur. Finally, a U.S. traveler recent died of YEL-AVD after he was given yellow fever vaccine for travel to Pakistan (Centers for Disease Control and Prevention, unpublished data, 2015). These unfortunate cases emphasize the importance of determining the need for vaccination on a case-by-case basis.

Cost-to-Benefit Analysis of Immunization

In an analysis of the benefit of immunization versus risk of yellow fever, Brès[758] concluded that a "fire-fighting" approach (emergency mass immunization in the face of outbreaks) was less costly than preventive immunization. Monath and Nasidi,[439] using a model in which routine yellow fever vaccination was hypothetically introduced into the EPI in Nigeria, explored this question. They concluded that 15 to 18 years would be required to achieve an effective immune barrier to epidemic spread, at which time routine immunization was seven- to eightfold more efficient than emergency control in the number of cases and deaths prevented. The cost of routine immunization was estimated at US$763 per case and US$3817 per death prevented during epidemics, with lower costs if prevention of endemic disease was taken into account. These cost-to-effectiveness ratios compared favorably to those for other infections preventable by EPI vaccines in Africa.[925] A more recent cost-to-benefit analysis performed when the WHO position paper on yellow fever vaccination was updated, found that the cost per dose of yellow fever vaccine is similar for various strategies.[926] Cost was estimated to be approximately US$0.67 per dose for each strategy (e.g., EPI, preventive and reactive vaccination campaigns) but the breakdown of cost (e.g., cold chain, vaccine price) varied based on strategy. These data suggest that vaccine utilization strategies should be driven by factors other than cost (e.g., vaccine availability).

Eradication or Elimination? Level of Herd Immunity Required

It was established as early as the 1930s that eradication by means of human vaccination is not feasible, because yellow fever virus circulates independently of humans in nonhuman primates and mosquitoes.[927] To eliminate the disease through vaccination nearly 100% coverage would be necessary to prevent cases of jungle yellow fever acquired by exposure to enzootic vectors. Prevention of epidemics involving interhuman transmission by *Ae. aegypti* or sylvatic vectors in Africa or South America would also require a high prevalence of immunity. This was shown in the case of the severe epidemic in Senegal in 1965, where the prevalence of immunity before the epidemic in children younger than 10 years (the age group affected during the outbreak) was estimated to have been 57%.[247]

Brès concluded that herd immunity must exceed 90% to preclude epidemic yellow fever.[758] Mathematical models have been applied to the calculation of the proportion of human hosts susceptible to infection required to sustain an epidemic (reproductive rate of infection >1), but they require an understanding of the biting rate, probability of feeding on a human host, and transmission efficiency of the vector.[272,421,928] The effect of herd immunity on yellow fever transmission under different assumptions of vectorial capacity was explored by Monath and Nasidi.[439] The prevalence of immunity in the human population required to preclude an epidemic was estimated to be between 60% and 90%. In a separate analysis, Massad and associates[928] reached a very similar conclusion, estimating that 58% to 88% yellow fever vaccination coverage rates would be required to prevent urban yellow fever in Sao Paulo State, Brazil. A more recent model estimated that 67% vaccination coverage in an area would result in 1 to 12 local cases in an area and no spread of activity to new areas only 7% of the time using a moderate transmissibility scenario with an R_0 of 4.1.[469]

FUTURE YELLOW FEVER VACCINES AND YELLOW FEVER–VECTORED VACCINES

At the Oswaldo Cruz Foundation in Rio de Janeiro, research has been sponsored by the WHO aimed at developing a novel method for manufacturing 17D vaccine. The approach used a full-length complementary DNA (cDNA) clone of 17D-204 virus. The cDNA was reverse transcribed to full-length positive strand RNA and transfected into primary chick-embryo cells. The progeny virus was investigated as a vaccine candidate but was found to have a marginally acceptable neurovirulence profile.[541] Unlike current yellow fever vaccines, which are heterogeneous mixtures of virion subpopulations, the new vaccine is genetically homogeneous. More recently, a group inserted full length 17D cDNA after a cytomegalovirus promoter and showed that they were able to get seroconversion and neutralizing antibodies formed in mice.[929] It is likely that with additional work, cDNA-based vaccines could be derived that would be safe and effective. The benefits of this approach are genetic homogeneity, reduced likelihood of selection of a subpopulation during replication in vitro or in vivo, manufacture of seed virus from bacterial plasmids (reducing the risk of adventitious viruses), production of vaccine in a cell culture system, and potentially longer-term immunity than might be achieved with an inactivated vaccine. However, it must be recognizes that DNA vaccines in general have been weakly immunogenic, unless delivered by electroporation, a method that would not be well suited to routine infant immunization.

An important aspect of the infectious clone work is that it set a precedent for novel chimeric vaccines in which heterologous flavivirus prM-E genes (e.g., of Japanese encephalitis virus, West Nile, or dengue) have been inserted into the yellow fever 17D virus infectious clone.[193,284,617,685–687,930–933] The Japanese encephalitis vaccine produced by this method (Imojev, Sanofi Pasteur) is licensed for use in some countries and the dengue vaccine is in licensing registration. Characterization of chimeric viruses has shown that the nonstructural genes of yellow fever 17D confer a highly attenuated phenotype on the vaccine candidates, including the restricted ability to replicate in mosquito vectors, while retaining the ability of the vaccines to efficiently immunize mice, monkeys, and humans. 17D virus has also been investigated as a vector for unrelated antigens (review in Bonaldo and associates 2014[934]) including the glycoprotein of Lassa virus,[935,936] a malaria cytotoxic T-lymphocyte epitope,[937] simian immunodeficiency virus and HIV *gag* sequences,[938–940] amastigote surface protein-2 of *Trypanosoma cruzi*,[941,942] influenza A viral epitopes,[943] and a tumor antigen.[944] The foreign gene carrying capacity is limited by the small size of the 17D genome. The foreign genes were inserted at viral protease cleavage sites, such as between NS2B and NS3, or between E and NS1,[945] leaving the E gene intact, so that antivector immunity would not be a problem in previously vaccinated subjects or for boosting immune responses. Foreign epitopes have also been inserted in the E protein fg loop,[946] but the carrying capacity of such constructs is a limitation.

Workers at Bio-Manguinhos in Brazil have produced a new seed stock and vaccines in primary chick embryo cell culture, rather than embryonated eggs. A cell-based method would have substantial advantages for manufacture and control. Good yields (>6 $\log_{10}$ PFU/mL) were obtained, and the virus obtained showed no changes in consensus sequence or immunogenicity; however, higher scores were obtained in the monkey neurovirulence test, and the vaccine was not pursued.[947] Yields are very robust (7 to 8 $\log_{10}$ PFU/mL), and effective dose requirements are low. The emerging data with chimeric yellow fever vaccines indicates that a similar approach could be used to manufacture yellow fever vaccines.

Several groups are engaged in developing nonreplicating vaccines, including whole-virion chemically inactivated vaccines produced in Vero cells[495,496,498,500] and a recombinant subunit vaccine produced in plants. The advantage of these vaccines is that they will not cause serious adverse events related to viral replication and can likely be given to persons with a contraindication to receiving 17D vaccine. Safety and immunogenicity have been demonstrated for one inactivated vaccine in a Phase I trial.[500] Fraunhofer USA used a tobacco plant expression system to produce yellow fever E protein. Fraunhofer entered into an agreement with iBio Corporation for commercial applications of the technology. Recently iBio entered into a licensing agreement with Bio-Manguinhos to further develop the subunit vaccine.[948] A disadvantage for use in endemic areas is the need to boost immunity at intervals, whereas the live vaccine induces long-lasting protection.

Combination vaccines are not being pursued actively by industry. Potential combinations of value might include yellow fever and measles vaccines for the EPI and the combinations of 17D vaccine with inactivated vaccines (e.g., hepatitis A, typhoid), as a means of simplifying immunization regimens for travelers. Again, it is likely that the development of chimeric yellow fever–vectored vaccines will lead to combination vaccines against yellow fever and dengue and against dengue and Japanese encephalitis; the combination of these vaccines with measles or meningococcal vaccines would be of interest for use in the EPI.

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64 Zika Virus

Stanley A. Plotkin and Barney S. Graham

Although development of vaccines against the Zika virus is still in early stages, the rapidity and extent of research efforts to create a vaccine justify a brief chapter on the subject. The virus is native to Africa and was first isolated from sentinel monkeys placed in the Zika Forest in Uganda in the 1940s as part of surveillance efforts for mosquito-borne viruses at what is now the Ugandan Virology Research Institute. The virus eventually spread to other parts of Africa, Southeast Asia, and, most notably, Micronesia, in particular the island of Yap, where it caused widespread disease, including, in retrospect, the manifestations described below.[1] No animal reservoir is known. Genetic mutations in the virus have been documented as Zika virus spread from Africa to other parts of the world, but a direct association between new RNA sequences and pathogenesis or transmission has not been demonstrated.[2,3]

The world began paying attention to Zika after the virus was transferred from Micronesia to Brazil sometime in 2013 or 2014, possibly through the agency of infected people participating in athletic events in northern Brazil.[1] The vectors of the virus are *Aedes aegyptii* and *Aedes albopictus* mosquitoes, which are ubiquitous. From Brazil, Zika virus has spread to Columbia, Central America, Mexico, the Caribbean islands, and Florida.[4] The fact that Zika virus can persist in blood, semen, urine, and perhaps also in female genital secretions for extended periods has allowed transmission through sexual exposure and the potential for infection from blood transfusions.[5,6]

The Zika virus is a typical flavivirus, with a positive strand RNA genome coding for the envelope (E), premembrane (prM), and capsid structural antigens as well as nonstructural proteins (Fig. 64.1). Infection by Zika elicits antibody responses, particularly to the E, prM, and the nonstructural NS1 protein. Both $CD4^+$ and $CD8^+$ T cells are elicited by infection, and although passive administration of antibodies is protective, cellular responses may also participate in controlling replication.[7] Antibodies directed against domain III of the E protein are known to be particularly important for protection against other flaviviruses.[7,8] The impact of preceding dengue virus infection on Zika and vice versa is the subject of active investigation, particularly with respect to antibody-dependent enhancement.[9,10] It has been shown that to date Zika virus consists of only one serotype and that neutralization of one ZIKV strain will predict neutralization of all others.[3]

Although 20% of people infected with Zika have fever and related symptoms, and Guillain-Barré syndrome occurs in approximately 1 in 4000 infections,[11–13] the effect on fetuses carried by women infected in pregnancy has called particular attention to the virus. Zika can invade and destroy neurological stem cells in the fetus, resulting in microcephaly and other adverse effects on the fetal brain.[14–17] The risk of abnormal development of the brain is highest after infections in the first trimester of pregnancy, but infections even later in pregnancy can apparently lead to fetal damage (Box 64.1). The full spectrum of the congenital Zika syndrome is currently unknown, but microcephaly occurs in a minimum of 0.88% of infants born to infected mothers and may be as high as 13% after first trimester infections.[14] Testicular damage has also been demonstrated in rodents.[18]

VACCINE DEVELOPMENT

Development of vaccines against other flaviviruses suggests that a vaccine against Zika is feasible. The objective of vaccination is the induction of neutralizing antibodies against the virus primarily directed against epitopes on the E protein. Aside from inactivated virus vaccines, most candidate vaccines contain the E protein together with the prM or M proteins for conformational purposes. Live, attenuated vaccines have the advantage of also stimulating $CD8^+$ T-cell responses that prevent replication but also carry more risk of persistence or shedding and unintentional infections.

Table 64.1 lists some of the Zika vaccine approaches now in preclinical and clinical development, although other approaches not currently known to us are probably also being studied.[3,7,19–21] The strategies being pursued are those classic

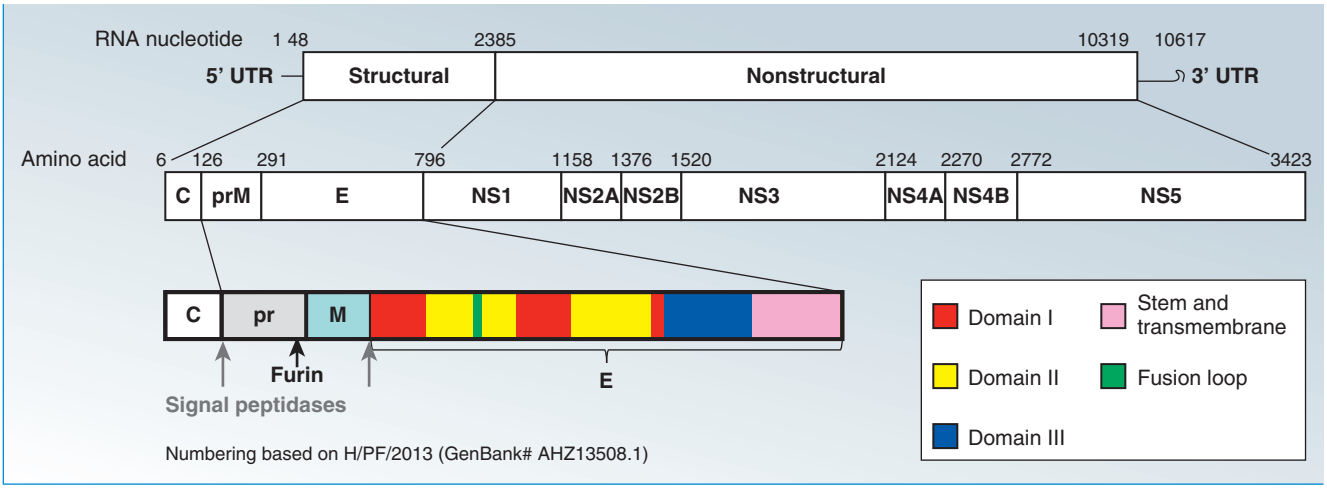


Figure 64.1. Organization of the Zika genome. Based on Zika virus H/PF/2013 (GenBank#AHZ13508.1).

BOX 64.1 Fetal Abnormalities Caused by Zika[17]

Microcephaly
Thin cerebral cortices with subcortical calcifications
Macular scarring
Contractures
Hypertonia

TABLE 64.1 Zika Vaccines in Development (Incomplete List)

Vaccine Type	Antigen	Developer
DNA plasmids	M-E	Beth Israel
DNA plasmids	prM-E	NIAID, Inovio
mRNA	prM-E	Moderna, University of Pennsylvania
Inactivated virus	All	Walter Reed, Sanofi Pasteur, Takeda, Emergent Biosolutions, Valneva
Chimeric dengue	prM-E	NIAID
Chimeric yellow fever	prM-E	Sanofi Pasteur
Self-amplifying RNA	prM-E	GlaxoSmithKline
Live attenuated	All	UTMB by mutation, Codagenix by codon-deoptimization
VSV vector	prM-E+/–C	Harvard
Adenovirus vector	M-E	Beth Israel (possibly J&J)
Measles vector	prM-E	Institut Pasteur (with Themis)

for vaccine development against flaviviruses and other viruses, including live-attenuated strains, chemically inactivated whole virus, DNA plasmids, or mRNA coding for critical proteins; self-amplifying viral RNA; subviral particles or viruslike particles; and chimeric Zika–yellow fever or dengue replicating viruses, subunit proteins, and peptides. Critically important to success is that passive antibody is protective in animal models and that neutralization titers correlate with protection.[3,19–21] Thus, the correlate of protection is likely to be conformation-dependent E protein–specific antibody. The envelope protein also contains a fusion loop region that induces neutralizing antibodies that cross-react with other flaviviruses.[22,23] In preclinical studies, vaccines based on DNA plasmids, mRNA, adenovirus vectors, or inactivated virus have been protective against Zika virus challenge in rodent and monkey models. Clinical data will be available by the time this book is published.

Evidence for efficacy in animals as well as immunogenicity in humans is promising, and a Zika vaccine usable in emergencies could be available within a few years. However, licensing authorities will require considerable safety testing relative to concerns about Guillain-Barré syndrome or fetal abnormalities after vaccination. Although Zika vaccines will probably have commercial interest, the formation of the global fund for development of vaccines against emerging diseases, called Coalition for Epidemic Preparedness and Innovations (CEPI), will aid in achieving the goal of protection against Zika. This international fund has been created to accelerate vaccine development against epidemic infection even if a large commercial market is not anticipated.

The use of a Zika vaccine will depend on its future epidemiology, but unless the virus extinguishes itself because of high immunity in the population, residents in endemic countries as well as travelers to those countries would be candidates for vaccination. Because the most devastating effects of Zika virus occur as a result of infection during pregnancy, vaccinating women of child-bearing age will have a high priority.

References for this chapter are available at ExpertConsult.com.

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65 Zoster Vaccines

Myron J. Levin

Herpes zoster (HZ), also called shingles, is a dermatomal-vesicular disease. (A dermatome is the area of skin innervated by one sensory nerve, and thus has bilateral symmetry and does not cross the midline.) Fig. 65.1 depicts the right C4-C5 cervical nerve dermatome. The cutaneous component of HZ is usually preceded by prodromal pain in the affected dermatome, followed 1 to 3 days later by the appearance of vesicular skin lesions that are accompanied by pain that often persists after the skin lesions heal.[1]

In 1892, Bokay made the seminal observation that hinted at the cause of HZ. He reported five instances in which varicella occurred in children exposed to adults with HZ, leading him to query: "I would like to bring up the question of whether or not the unknown infectious material of chickenpox could under certain circumstances manifest itself, instead of as a generalized eruption, as a zoster eruption."[2] In the ensuing half century, clinical and histologic evidence lent support to this prescient suggestion.[3–6]

In the 1950s, viruses isolated from varicella or HZ were recognized to have similar properties in tissue culture. Convalescent serum samples from varicella or HZ fixed complement using antigen from vesicle fluid from either varicella or HZ cases, and immunofluorescence techniques, demonstrated that a new antibody appeared in blood 3 days after varicella infection that equally stained cells infected with virus isolated from either varicella or HZ.[7] Antibody appearing after varicella was subsequently shown to neutralize viral isolates from either varicella or HZ. Weller and coworkers concluded that the same virus caused both diseases and named it "varicella-zoster virus" (VZV).[8]

The etiologic relationship between these two diseases was elegantly proved when two isolates were obtained from an immunocompromised child. The first isolate was obtained when the child developed varicella, and the second isolate was obtained several years later when HZ developed. DNA extracted from the two isolates were identical by restriction endonuclease mapping using multiple enzymes that focused on known variable regions of the VZV genome.[9]

PATHOGENESIS

Localization of Latent Varicella-Zoster Virus in Sensory Ganglia

The VZV and herpes simplex viruses are α-herpesviruses that cause human infections. The molecular virology of VZV, a member of the Herpesviridae family, is described in Chapter 62. An essential and unique feature of α-herpesviruses is their ability to become latent in neurons in sensory ganglia during their initial (primary) infection. The primary infection with VZV is manifest clinically as varicella (chickenpox), which is endemic worldwide. Varicella usually occurs in childhood in temperate climates, generally as winter–spring epidemics.[10,11] In countries with widespread immunization against varicella, this pattern is disappearing. In tropical climates, the prevalence of varicella remains high in late adolescence or young adulthood. Because varicella vaccine was not introduced into the United States until 1995, more than 97% of adults have had varicella.[12]

VZV is transmitted by an airborne route as droplet spread from the pharynx or from aerosols from skin lesions of an index case of varicella or HZ (see Chapter 62). It is postulated that the initial replication of VZV occurs in epithelial cells lining the nasopharynx and subsequently spreads to adjacent lymphoid tissue, thereby infecting memory $CD4^+$ T cells, such as those that are abundant in tonsillar lymphoid tissue.[13–15] Dendritic cells have a central role in this process.[16] Activated $CD4^+$ T cells expressing cutaneous homing markers are preferentially infected and are then able to deliver VZV to cutaneous epithelia within a few days of infection.[14,15,17] Infection of cells at the dermal–epidermal junction leads to the most visible consequence of VZV viremia, which is the characteristic vesicular lesion.[18] The incubation period prior to varicella symptoms after exposure may be explained by the time required for dissemination of VZV by circulating T cells, initial cell-to-cell transmission in tissues, innate immune responses that delay VZV replication, and the development of specific immune pathogenesis that results in skin lesions.[15]

The significance of the varicella lesions for the pathogenesis of HZ is that the termini of sensory axons are located at the dermal–epidermal junction at the base of vesicles. It is hypothesized that from this locus, VZV enters and ascends by retrograde axonal transport to become latent in the soma of neurons in sensory ganglia. This mechanism is suggested by the clinical observation that the dermatomes most frequently affected with HZ are those that have the highest density of skin lesions during varicella and vesicular fluid has very high titers of cell-free VZV.[1,10,13] Moreover, early studies with the varicella vaccine indicated that vaccine recipients were much more likely to develop subsequent HZ with the vaccine strain virus if they had a vaccine-related rash in the post-vaccine period.[19] In addition, vaccine-related HZ occurring in recipients of the varicella vaccine most often appears in the dermatome where the vaccine is administered.[20] However, this is not always the case, suggesting that viremia occurs after varicella vaccine administration. As viremia is characteristic of varicella, this could provide an alternative or additional mechanism for VZV to gain access to sensory ganglia.[13,15,17] The presence of VZV in autonomic ganglia and enteric neurons also indicates that the viremia of varicella can seed these ganglia.[21,22]

Nature of Latent Varicella-Zoster Virus DNA

Cranial and dorsal root sensory ganglia, autonomic ganglia, and enteric neurons contain VZV DNA detectable by polymerase chain reaction (PCR). This is true of more than 90% of adult trigeminal ganglia and 70% of thoracic ganglia, reflecting the prevaccine epidemiology of varicella; other cranial nerve and autonomic ganglia contain latent VZV DNA.[21–26]

Approximately 2% to 5% of neurons in sensory ganglia contain latent VZV DNA, which is absent in nonneuronal satellite cells.[27,28] The VZV DNA in neurons is latent in the sense that infectious virus cannot be isolated from ganglia. The latent VZV DNA is present in the nuclei of neurons in a circular form different from that present in intact virions.[24,29]

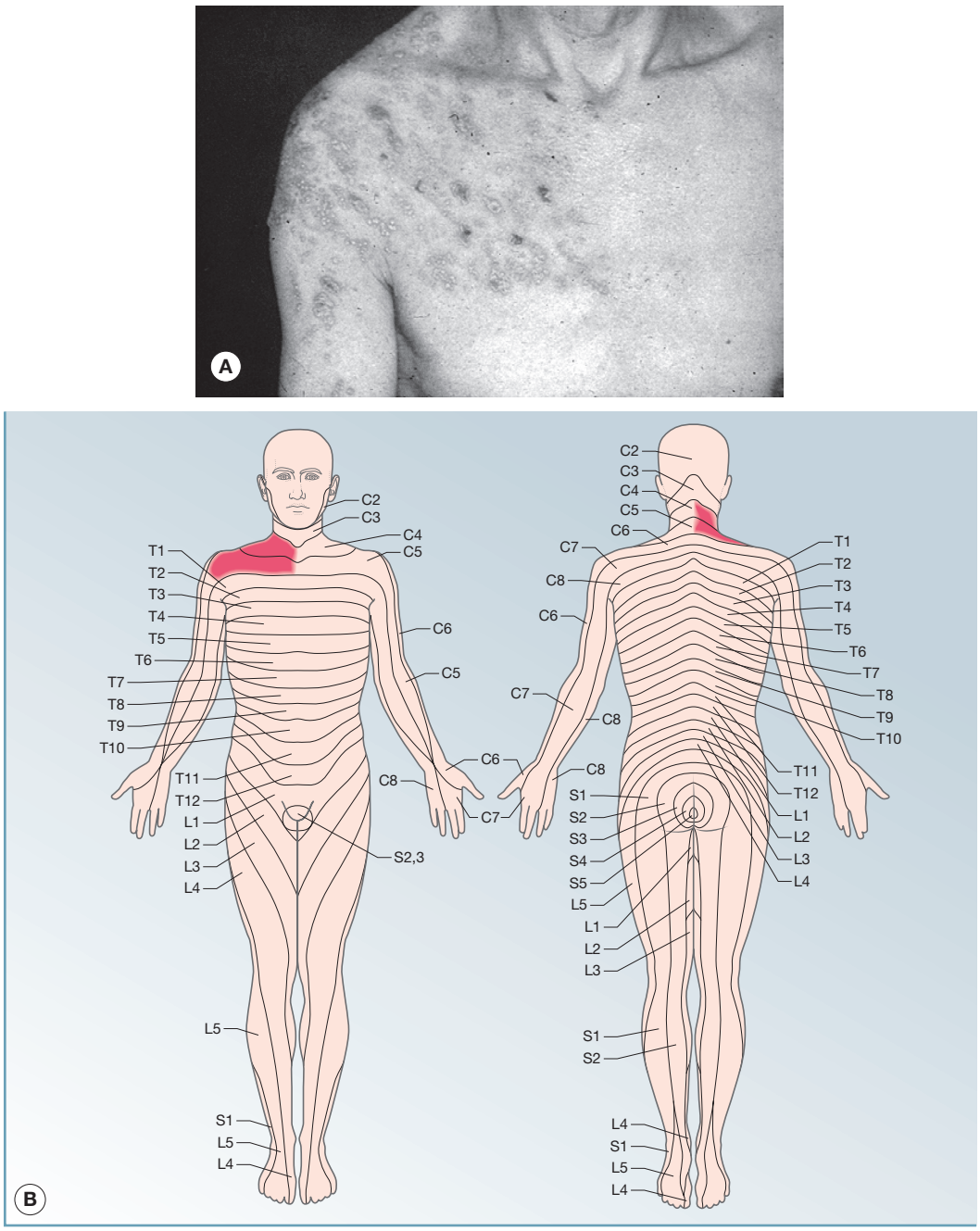


Figure 65.1. A, Herpes zoster in the right C4-C5 dermatome. **B,** Dermatome map indicates the area of skin innervated by individual sensory ganglia; gradation indicates dermatomes involved in **A**.

Episomal DNA also characterizes the physical state of latent herpes simplex virus in sensory ganglia. The number of copies of VZV DNA in a neuron is 1000-fold lower than the burst size in infected fibroblasts, indicating rapid shut off of VZV replication in infected neurons.[27,28,30] VZV DNA serves as an active template during latency from which five or six immediate-early or early genes of VZV are transcribed during latency and their gene products detected.[24,31] Some of the latency gene products, many of which are strong transactivators, are not transported to the nucleus during latency, whereas they are found in the nucleus during lytic infection. It is hypothesized that this failure to translocate one or more of the early gene products prevents the cascade of events in the nucleus that normally leads to viral replication.[32]

Maintenance of Latency by Varicella-Zoster Virus–Specific Immune Responses

Although the mechanism of latency is not understood, strong evidence exists that it is related to a decline in the VZV-specific immunity of the host that initially developed at the time of, or shortly after, the appearance of skin lesions with varicella.[33] These immune responses include polyclonal VZV-specific antibody and T-cell–mediated immune (VZV-CMI) responses, including $CD4^+$ and $CD8^+$ effector and memory T cells. These persist lifelong to protect the host against subsequent cases of varicella and to prevent HZ.[34,35]

The relationship between VZV-CMI and HZ is evident from numerous and varied clinical observations. It was recognized

early that the age-specific incidence and severity of HZ was greatly increased in patients with immune compromise resulting from underlying illness (e.g., HIV infection) or immunosuppressive therapies for malignancy, autoimmune disease, or organ transplantation.[36–40] Natural and iatrogenic experiments indicate that VZV-CMI is necessary *and* sufficient to maintain latency of VZV and prevent HZ.[41] For example, children born with isolated γ-globulin deficiencies do not develop severe varicella or experience an increased incidence or severity of HZ, whereas children with severe combined immune deficiency often have severe morbidity with VZV infections.[42,43] The likelihood of chemotherapy-treated patients with lymphoma developing HZ correlated with the preservation or recovery of VZV-CMI and was not influenced by the presence of anti-VZV antibody, and anti–varicella antibody before bone marrow transplantation was not predictive of the occurrence of HZ.[40,44] Moreover, recipients of allogeneic hematopoietic stem cell transplants, who have their immune responses totally ablated and then receive replacement therapy with intravenous γ-globulin (which has high levels of anti–VZV antibody), nevertheless have a high incidence of HZ, often with severe manifestations. The risk of HZ in this setting abates only with engraftment and return of the potential for pathogen-specific CMI.[45] The essential role of VZV-CMI in maintaining latency is verified by the success of an experimental, inactivated vaccine that boosted VZV-CMI (but not anti–VZV antibody) after stem cell transplantation and thereby reduced the frequency and severity of HZ.[46,47]

Clinical Manifestations

VZV, because of the global endemicity of varicella, is latent in many ganglia in most humans and has the potential to reactivate intermittently in a subclinical manner. This is suspected because unexplained increases in VZV-specific antibody and activated VZV-specific T cells occur intermittently, and VZV DNA is intermittently detected in the blood of asymptomatic immunocompromised and immunocompetent people.[38,48–52] Presumably these random events, *of unknown frequency*, are of no consequence because the VZV-CMI present when they occur is sufficient to prevent propagation of VZV infection in the ganglion. The subsequent boost in immunity that accompanies subclinical reactivation may be a factor in maintaining VZV-CMI throughout life (termed "endogenous boosting"). When an immune person is exposed to someone with varicella or HZ, VZV-specific immunity may also be boosted (termed "exogenous boosting").[53,54] The accumulation of activated VZV-specific $CD4^+$ T cells in older people may be a reflection of these phenomena.[55]

HZ occurs following reactivation caused by the waning of some (unknown) critical level of VZV-CMI. In this scenario, the appearance of infectious VZV in a ganglion, unchecked by the ambient VZV-CMI, leads to ganglionitis, during which many neurons and supporting cells are damaged by the widespread infection and/or the intense inflammatory response that follows.[56–61] An alternative potential role of VZV-CMI is to directly prevent (rather than limit) VZV reactivation. There is insufficient information to distinguish between these two possibilities. Although the inadequate immune response to a VZV reactivation that culminates in HZ may result from therapy-related or disease-related immune suppression, much more often it is the result of the decline in VZV-CMI that accompanies the normal aging process.[62–67] It is also likely that the level of VZV-specific immunity, at any age, can be temporarily blunted by changes in sense of well-being,[68] depression,[69–71] stress,[72] or intercurrent infection with viruses that can alter CMI responses, such as Epstein-Barr virus and cytomegalovirus.[73,74] Trauma to a dermatome may also lower the threshold for symptomatic reactivation in the ganglion innervating that dermatome.[75] These additional factors explain why children sometimes develop HZ.[76,77] Race and family history may also influence the age-specific incidence of HZ and its complications.[70,78–81]

The ganglionitis and related neuronal damage that accompany extensive VZV reactivation cause the neuropathic prodromal pain in the dermatome where skin lesions subsequently appear. Prodromal pain accompanies 70% to 80% of HZ cases in older adults.[1,82–84] The prodromal pain typically lasts 3 to 4 days, but may last a week or longer. Its character varies with the patient: shooting, boring, aching, and throbbing are some descriptors. The pain may be constant or intermittent. Intense itching is common.[85] The cause of this localized pain in the involved dermatome is initially unclear to the medical provider, often leading to a search for visceral disease suggested by the location of the pain, such as myocardial infarction when a left upper thoracic dermatome is involved; renal stone or intervertebral disk disease when a lumbar dermatome is involved; or intraabdominal disease (e.g., cholecystitis or appendicitis) when right-sided mid-lower thoracic dermatomes are involved. Approximately 12% of medical costs for HZ in the United States occur before the appearance of skin lesions.[86,87]

The duration of the prodrome represents the rate and extent of VZV replication in the ganglion and subsequent descent in the sensory nerve to the dermal-epidermal junction, where VZV replicates in the skin to induce the characteristic rash. The appearance of skin lesions reveals the origin of the prodromal pain and provides the diagnosis. Mild constitutional symptoms are infrequent (10% to 20%). The rash of HZ in immunocompetent patients characteristically involves a single dermatome; hence, the lesions do not cross the midline (see Fig. 65.1).[82,83] Several contiguous dermatomes may develop lesions, or individual variation in innervation may give this impression. Infection of epithelial cells causes a varicelliform rash that is limited to the dermatome involved. After brief macular and papular stages, the characteristic rash (i.e., vesicles on an erythematous base) appears. New vesicles appear for 3 to 4 days in groups that tend to cluster where there are branches of the involved cutaneous sensory nerve. Pustulation of vesicles begins within 1 week, followed 3 to 5 days later by lesion ulceration, crusting, or both. The lesions may evolve over a longer time or become hemorrhagic in patients with advanced age or immune suppression.[1] The rash is usually accompanied by the same pain experienced during the prodrome, but this acute phase pain can worsen, improve, or appear for the first time after the skin lesions appear.[88] The acute pain and the preceding prodrome can be very severe, disabling, and a barrier to employment and activities of daily living. The nociceptive pain, which accompanies extensive skin involvement, is additive to the neuropathic pain. Itching may also increase in prominence.[85,89]

VZV frequently gains access to the bloodstream, as demonstrated by VZV DNAemia during the early stages of HZ.[51,90,91] This is of little consequence to most patients, but in immunocompromised patients, extensive viremia in the absence of a vigorous immune response can result in a disseminated form of HZ that includes severe, multiorgan disease.[39,92–94] VZV is also present in the saliva shortly after the onset of HZ.[51]

Additional insight into the age-related decline in VZV-CMI is gained from the observation that elderly patients with HZ frequently have cutaneous VZV lesions at a distance from the involved dermatome.[1] This reflects their inability to muster adequate VZV-CMI in a timely manner after VZV reactivation, thereby permitting more extensive viremia, unimpeded VZV replication at distant sites, or both.

In addition to the acute pain, complications occur in approximately 8% of patients with HZ who are 50 to 59 years

old and in more than 12% of persons 70 years of age or older.[87] Complications include bacterial superinfection of the skin (2%); segmental motor nerve damage, including nerves of the face, limb weakness, and other neurologic complications (3–5%); and dysfunction of bowel or bladder when sacral nerves are involved.[83,87]

The presence of motor involvement may exceed 10% if this finding is carefully sought by a neurologist.[95] Motor deficits are rarely permanent, although recovery is less likely to be complete in older patients. The occurrence of motor deficits is consistent with data that VZV often extends through the ganglionic root to the central nervous system.

Lymphocytic pleocytosis and VZV DNA are commonly found in the cerebrospinal fluid of patients with HZ, and magnetic resonance imaging studies obtained during HZ often demonstrate inflammation in the spinal cord at the level of HZ involvement.[96] Spread to the spinal cord has been confirmed histologically in patients who had HZ when they died of unrelated diseases.[26,57,97] While these abnormalities are generally inconsequential, they probably explain why contiguous dermatomes are often involved and why the uncommon complications of transverse myelitis and bilateral dermatomal involvement are occasionally seen in both immunocompetent and immunocompromised patients.[98,99] VZV meningitis and meningoencephalitis can occur with HZ.[26,100,101] Because the ophthalmic branch of the trigeminal nerve is involved in 10% to 15% of cases, damage to ocular structures is a common occurrence (5% of HZ in older patients).[87,102,103]

The most common complication of HZ is the persistence of significant pain for months after onset of the rash.[88,89,104–106] The frequency of this postherpetic neuralgia (PHN) is strongly influenced by the age of the patient. PHN has been defined as pain persisting for an extended period after the onset of HZ (i.e., prodromal pain) or after the onset of HZ rash. Thus, the age-specific frequency of PHN varies with its definition. The postevent interval used to define PHN has most often been between 30 and 90 days. Because the onset of rash is easily recognized and remembered, most investigators now use this starting point and many now define PHN as pain being present at 90 days after the rash appears.[83,84,106,107]

Age is the strongest prognostic factor for the occurrence of PHN, which is uncommon for people younger than age 40 years, but becomes common when HZ occurs in people older than age 50 years. The various definitions have led to estimates of PHN ranging from 7% to 25% of HZ cases. HZ cases that occurred during a prospective zoster vaccine trial in subjects at least 60 years old resulted in significant pain lasting or beginning (sometimes pain occurs after the rash appears) at least 30 days after rash onset in 30% of placebo recipients; for 60 days in 17%; and for 90 days in 12%.[107] In some patients, PHN may last for a year or longer.

PHN may be intermittent or constant, stabbing or lancinating, deep burning or throbbing, or allodynia, which is a painful sensation resulting from an otherwise normal stimulation of the skin. It is the third most common cause of chronic neuropathic pain in the United States, with a point estimate of 500,000 cases annually.[108] Acute and chronic pain strongly influence the quality of life, with effects on physical, psychological, social, and functional domains. These effects are particularly profound in elderly patients.[109–112]

EPIDEMIOLOGY

HZ is of endogenous origin, resulting solely from reactivation of latent VZV in sensory ganglia. Thus, the at-risk population includes more than 95% of U.S.-born adults 20 to 29 years old and more than 99.6% of persons older than 40 years of age who have anti–VZV antibody.[12] The risk of HZ may change in future years as a result of the 1995 recommendation for universal immunization with the varicella vaccine. Although recipients of the varicella vaccine can develop HZ with the vaccine strain VZV,[20,76,113] the frequency and the severity of this occurrence are believed to be three- to fivefold less than after natural infection.[76,114,115]

In the seminal study by Hope-Simpson, who recorded 16 years of HZ in his medical practice, the frequency of HZ correlated closely with increasing age.[116] These findings have been replicated in many countries during an extended observation period. The age-specific incidence of HZ is similar worldwide (Fig. 65.2).[87,117–119] HZ is 5- to 10-fold more likely to occur after

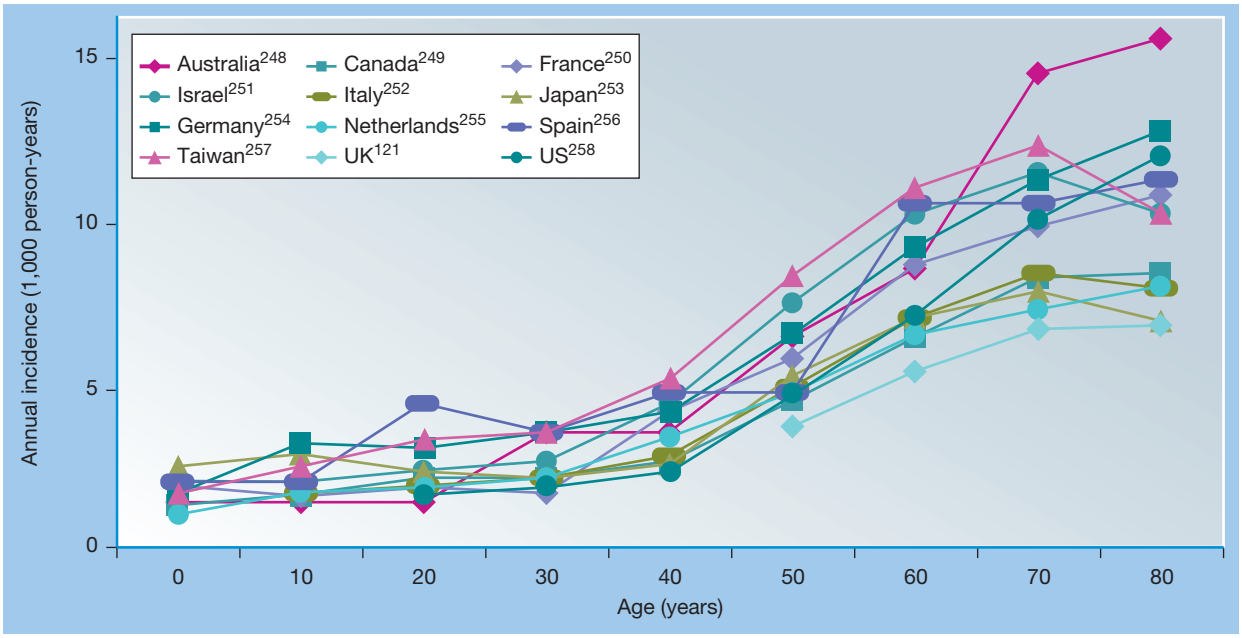


Figure 65.2. Age-specific incidence of herpes zoster as a function of age. *(Courtesy J. Pellissier and M. Brisson, Merck & Co, Inc.)*

60 years of age than in childhood. Thus, although the frequency of HZ in the general population has been reported to be 1.2 to 4.8 per 1000 person-years, the frequency increases to 7.2 to 11.8 per 1000 person-years for people at least 60 years old.[87,118,120] Using current census data and these age-specific rates, it is likely that more than 1 million cases of HZ occur annually in the United States. Of these, 45% to 50% occur in people 60 years of age or older; almost 20% occur in people 50 to 59 years old.[87] The lifetime risk of HZ approaches 50% for people who reach 80 years of age. The annual risk of HZ in people older than 60 years is greater than 1.1 per 100 persons.[107,121] Female gender and a family history of HZ increase the risk of HZ, whereas the risk is decreased in black individuals.[122–124] The severity of HZ also increases with age, as shown by the greater frequency and duration of PHN with age and the increase in complications with age.[87,106,118,120,125] In addition to age, another important risk factor for HZ is immune compromise from disease or immunosuppressive therapy, but this probably contributes less than 10% of the societal burden of HZ.[36,86,126] Other strong risk factors repeatedly identified for PHN and other complications of HZ include the intensity of the prodromal pain, the intensity of the acute pain, and the extent of the rash.[83,87,89,105,127] Each of these is probably a marker for the inability of the patient to rapidly mobilize VZV-CMI responses early after VZV reactivation occurs, and thereby fail to limit virus-induced damage within the involved ganglion.[128]

TREATMENT

The acute phase of HZ should be treated as soon as possible with nucleoside analogs (e.g., acyclovir, valacyclovir, famciclovir).[106,129] These are excellent antiviral drugs that inhibit VZV in vitro and limit virus replication in vivo (i.e., reduce duration of shedding). Their use is invariably suboptimal because of the delay in appreciating the diagnosis, during which time the ganglionitis proceeds. Nevertheless, six placebo-controlled clinical trials with these antivirals administered within 72 hours of rash onset significantly limited new lesion formation, shortened the time to healing, and decreased the severity and duration of acute pain.[83,130,131]

Valacyclovir and famciclovir have superior bioavailability to that of acyclovir and, therefore, can be dosed less frequently, but the clinical superiority of one of these drugs has not been established. The effect of antiviral drugs on PHN is controversial. Three metaanalyses and some (but not all) controlled trials failed to determine that antivirals reduced the incidence of PHN, but some suggested that the duration of prolonged pain was shortened for some patients.[83,106,130–132] Positive results on acute and chronic pain were obtained under ideal prospective trials conditions, which would be difficult to achieve in routine practice, especially because fewer than 60% of patients receive antiviral drugs within 72 hours after rash onset, as mandated by the successful trials. Moreover, the proportion of advanced elderly patients participating in the clinical trials was generally lower than found in most practices. There may be some value in beginning antiviral drugs even later than the 72-hour window after rash onset recommended for administration, especially in very old patients whose immune response may be delayed.[83]

Additional treatment in the acute phase of HZ often requires the use of strong analgesics for pain.[83] Opioids are effective in the early phase of HZ and are a mainstay for persistent pain.[133–135] Corticosteroids shorten the duration of healing and acute pain and hasten the return to a normal quality of life when used with concomitant antiviral therapy, but their use must be weighed against potential side effects.[136,137] Corticosteroids do not prevent PHN. Drugs that act on nerve transmission, such as amitriptyline and gabapentin or pregabalin, are under study for use in the acute phase of HZ.[133,138,139]

Other than analgesics, the clinical impact of drugs used to treat HZ in the acute period, although statistically significant, is modest when applied to PHN. Many patients get additional relief from gabapentin or pregabalin and another class of neural-active drugs, tricyclic antidepressants.[106,140,141] Significant PHN often requires multiple drugs.[83,106,142] Strong analgesics, usually opioids, are often required, although there is controversy about their efficacy.[83,133–135,140,143] Some patients benefit from topical application of lidocaine patches, which inhibit skin sensations that are perceived as allodynia.[144] Capsaicin is a topical therapy that desensitizes receptors on sensory nerve axons and inhibits initiation of pain transmission. Because capsaicin initially causes a local burning sensation, it is difficult to study in controlled trials, but there are credible reports that topical capsaicin ameliorates PHN in some patients.[145,146] More complex treatment approaches for unresponsive PHN can be provided by pain specialists.[82,83]

Despite these numerous therapies, the management of acute HZ pain in the acute phase, and especially PHN, is difficult.[106] In general, only approximately 50% of patients benefit from each of the therapies mentioned and they experience only partial relief. Moreover, many of these drugs frequently produce disabling side effects, especially in elderly patients who are most likely to get HZ and PHN. Some of the side effects, such as difficulty concentrating, difficulty with balance, and bowel or bladder dysfunction, are preexisting problems for many older patients. Furthermore, adverse drug effects are more common when combinations of drugs are required for intractable pain, when they are metabolized more slowly in older patients, and when they are used for patients already receiving many other drugs with potent side effects or with the potential for drug interactions. The therapy for severe PHN is time consuming for treating physicians and is expensive. *As with any infection, prevention is preferable to treatment.*

PASSIVE IMMUNIZATION

Given the epidemiology of HZ, passive immunization would not be feasible as a preventive measure. Furthermore, neither prevention nor treatment of HZ with VZV-specific antibody is successful, as indicated by the frequency of HZ in patients after allogeneic hematopoietic cell transplantation, even though they receive large amounts of VZV-specific antibody contained in the intravenous immunoglobulin typically administered for many months after transplantation. In addition, older people who frequently develop HZ have high levels of VZV-specific antibody.[62–64,147–150]

ACTIVE IMMUNIZATION

Rationale for a Zoster Vaccine

When Hope-Simpson showed in his seminal monograph that the frequency of HZ increased with age, he also suggested that this was the consequence of an age-related decline in VZV-specific immunity.[116] His conclusion was consistent with the clinical information available at that time, since corroborated, that immunocompromising diseases and therapies are associated with a large increase in the frequency and severity of HZ. We now know that the essential component in protection against HZ is VZV-CMI. Distinct humoral and T cell–mediated immunity had not been defined when Hope-Simpson discerned the relationship between age and HZ frequency. The hypothesized progressive decline in VZV-specific immunity has been identified repeatedly as VZV-CMI, and not as

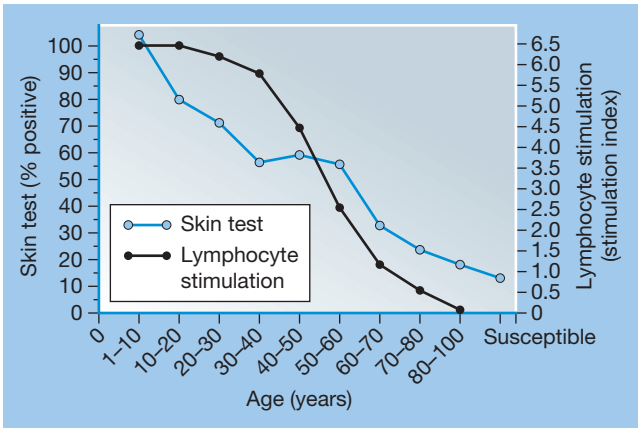


Figure 65.3. Herpes zoster and aging worldwide. *(Courtesy E. Bresnitz, Merck Vaccine Division.)*

antibody, as conjectured by Hope-Simpson. This *immunosenescence* is now considered to be one characteristic of the normal aging process (Fig. 65.3).[62–64,66,151,152] The close relationship between the decline in VZV-CMI and the increasing frequency of HZ with aging is assumed to represent cause and effect. This relationship and the continuing decline in VZV-CMI are maintained into the sixth, seventh, and eighth decades.[65,67] Anti–VZV antibody levels do not decline with age.[62–64,147,148] In addition, Hope-Simpson noted that second cases of HZ were rare in elderly patients, suggesting that the occurrence of HZ in most cases sufficiently stimulated VZV-specific immunity to prevent second attacks. Many investigators have documented that second attacks of HZ are uncommon (2% to 5%),[87,107,116,118,120,153] although a recent study questioned this conclusion.[154] Overall, the data available in the 1980s led many investigators to suggest that a vaccine capable of boosting VZV-CMI in elderly subjects might prevent or attenuate HZ in this population.[155] A similar hypothesis, suggested for transplant recipients at high risk for HZ, was confirmed by boosting VZV-CMI with an inactivated VZV vaccine.[46,47]

Preliminary Trials of Live Varicella-Zoster Virus Vaccine to Prevent Herpes Zoster in Older People

Relevant trials with a live VZV vaccine were undertaken from 1984 to 1999.[155] It was appreciated that the subjects had preexisting VZV-specific immunity and that an investigational vaccine would be unique *in preventing reactivation of an endogenous latent infection, rather than preventing a new infection*. Thus, there were considerations that preexisting immunity in vaccinees might limit replication of the administered live vaccine, while there was also concern that this live attenuated vaccine was being administered by a nonnatural subcutaneous route to a host population known to have waning immunity to VZV.

Two studies of vaccine immunogenicity were especially important. The first, by Trannoy and associates, involved vaccination of 200 subjects at least 55 years old with doses of 3200 to 41,000 plaque-forming units (PFU) of Oka strain VZV.[156] By comparison the amount of VZV in the current varicella vaccine is 1340 PFU/dose. A boost of approximately 2.5- to threefold in VZV-CMI was documented by two assay methods; no dose–response relationship was observed. The second study, by Levin and associates, involved 240 subjects at least 60 years old who received from 3000 to 12,000 PFU of Oka/Merck strain VZV.[157] The number of VZV-specific $CD4^+$ memory T cells in vaccinees was increased approximately twofold. A dose–response effect was not observed, but an important observation was that the duration of the response correlated with the titer of vaccine virus administered. For all age groups combined, the half-life for the booster effect was 56 months. At the highest dose, the vaccine effect declined little over 5 years.[147,157,158] Injection-site reactions were similar in frequency and severity to those occurring with the pneumococcal vaccine.[156] No age effect on the vaccine-induced boost was observed in these pilot studies.

By the late 1990s, a body of work had established that the vaccine was safe and well tolerated in elderly vaccinees. The Phase I/II studies demonstrated that an increase in VZV-specific $CD4^+$ T cells, measured by a variety of techniques, could be achieved and that the booster effect persisted for long periods, although it was not known whether the VZV-CMI responses elicited were biomarkers for protection against HZ.

Subsequent studies determined that cytotoxic $CD8^+$ T cells were also increased in number following vaccine.[159] An inactivated VZV vaccine induced a $CD4^+$ T-cell memory response similar in magnitude to that induced by the live vaccine, but the cytotoxic T-cell response was greater with the live attenuated vaccine. Prior to embarking on a definitive efficacy trial of a zoster vaccine, a questionnaire to record HZ pain and discomfort was validated and laboratory methods were chosen and validated for measuring VZV-CMI.[160–163] A study to inform the choice of dose for a definitive trial determined that approximately 25,000 PFU of the Oka/Merck strain (at expiry) was practical from the manufacturing perspective and would provide optimal boosting of VZV-CMI with minimal side effects.[163] Safety was demonstrated in older subjects with comorbidities, specifically persons with controlled diabetes mellitus or chronic obstructive pulmonary disease.[163,164]

Composition of the Zoster Vaccine

The zoster vaccine contains the Oka strain of VZV developed for the varicella vaccine (see Chapter 62).[165–167] It is produced by Merck & Co, and marketed as Zostavax. It was licensed in the United States in May 2006 and is licensed in the European Union, Canada, Australia, and much of Asia and the Middle East (>50 countries).

In the United States, the zoster vaccine is a lyophilized preparation designed to improve stability during prolonged storage, but elsewhere is provided as a refrigerator-stable vaccine.[168] This formulation is being considered for the United States. When reconstituted (0.5 mL of diluent), it contains at least 19,400 PFU (calculated for final expiration date) (14 times more potent than the licensed varicella vaccine) in 0.65 mL. Diluent is provided with the lyophilized vaccine. Incipient substances include porcine gelatin, L-glutamate and stabilizing salts, trace DNA and proteins from MRC-5 cells, and trace quantities of neomycin and bovine calf serum. There are no preservatives.

Immunologic Response to Zoster Vaccine

An immunologic substudy of 1200 subjects from the pivotal efficacy trial (1999–2004) was undertaken at two study sites to measure VZV-specific antibody titers and VZV-CMI before vaccination and at intervals thereafter for 3 years in vaccinees and placebo recipients.[65] At baseline the level of antibody was not influenced by age, whereas there was a strong correlation between increasing age and a decline in the magnitude of VZV-CMI responses. This finding confirmed that the age-related decline in VZV-CMI continues into the eighth decade of life and beyond and explains why the frequency of HZ continues to increase with increasing age. It also explains why

HZ becomes increasingly more severe with advancing age and explains the increased frequency and severity of PHN.

Both antibody and several measures of VZV-CMI responses in the immunologic substudy were significantly boosted after administration of the zoster vaccine.[65] The roughly twofold increase in VZV-CMI that resulted from vaccination was similar to that reported by Trannoy and colleagues[156] and Levin and colleagues[157] in the pilot studies. However, the magnitude of the vaccine-induced boost in antibody and VZV-CMI responses was also a function of age, and overall the boost in immune response was significantly less in persons 70 years of age or older than in younger vaccinees (Fig. 65.4). This finding is consistent with, and probably explains, the results of the vaccine trial (described in the next section) in that significant efficacy was observed in both age groups, but greater protection occurred in the younger vaccinees. The immunologic data also could explain why the efficacy in younger vaccinees was primarily achieved by preventing HZ, since it is likely that the boosted VZV-CMI in most younger vaccinees was sufficient to completely eliminate any clinical consequences of VZV reactivation in their ganglia (i.e., remained subclinical). In contrast, in older vaccinees, the lesser boost in VZV-CMI from zoster vaccine might not be sufficient to prevent early reactivation events in their ganglia, but would still be sufficient to support timely and adequate mobilization of immune defenses sufficient to limit ganglionitis and thereby attenuate the HZ. This relationship between severity of HZ and ambient VZV-CMI responses has been confirmed in subjects who developed HZ.[128]

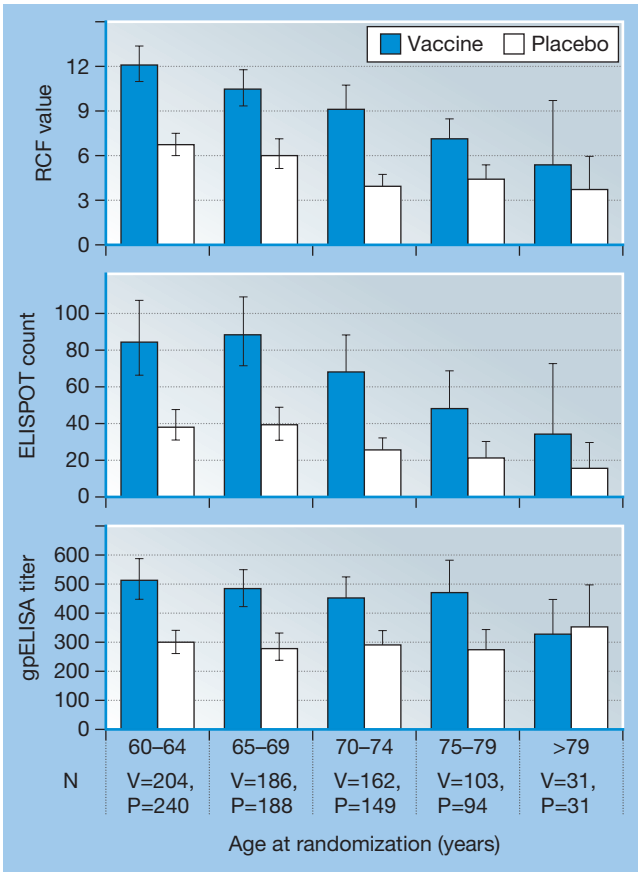


Figure 65.4. Varicella-zoster virus–specific immune responses at 6 weeks after vaccination in the pivotal zoster vaccine trial by age group. Among vaccinees there was a significant linear age effect (age slope) for the week 6 responses for RCF and ELISPOT ($P < .001$). There was a lesser association of age and gpELISA response (slope is 1% per year of age with $P = .034$). *Responder cell frequency (RCF) value*, number of responding cells per 10^5 peripheral blood mononuclear cells (PBMCs); *ELISPOT (enzyme-linked immunosorbent spot) counts*, number of spot-forming cells per 10^6 PBMCs; *glycoprotein-based enzyme-linked immunosorbent assay (gpELISA) titer*, gpELISA units/mL. *Error bars*, 95% confidence intervals for the geometric mean. *N*, number of subjects who had blood samples collected in the age group. *V*, number of subjects in the vaccine group. *P* values for differences between age groups are shown below the graphs. *(From Levin MJ, Oxman MN, Zhang JH, et al. Varicella-zoster virus–specific immune responses in elderly recipients of a herpes zoster vaccine.* J Infect Dis. *2008;197:825–835.)*

The peak boost in VZV-CMI occurs at 1 to 3 weeks after zoster vaccination.[169] This is consistent with unpublished information from the HZ vaccine trial that demonstrated prevention of HZ (approximately 50%) was observed within the initial month after vaccination. The immunology substudy of the clinical trial also indicated that the magnitude of the boost in VZV-CMI declined by 40% to 50% in the first year post-vaccination and then remained constant in the second and third years.[65] These observations are consistent with the trial results wherein vaccine efficacy was greatest in the 6 months after vaccination, and thereafter a lower efficacy was established and maintained for the remainder of the 3-year study (Fig. 65.5).

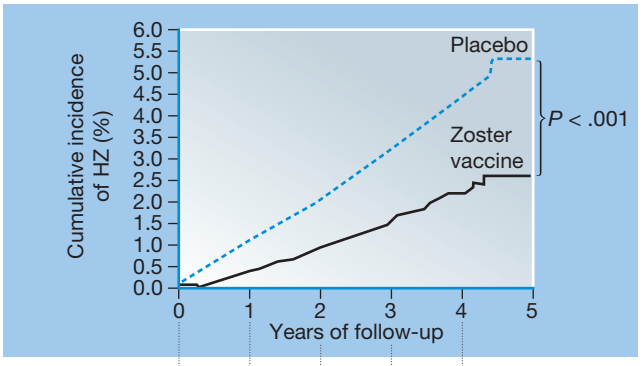
A subsequent immunologic study measured VZV-CMI in elderly versus young subjects before and after zoster vaccine. Before zoster vaccine, elderly subjects had significantly fewer VZV-specific T-helper types 1 and 2 $CD4^+$ T cells, fewer early effector $CD4^+$ T cells, and fewer $CD8^+$ early effector and effector memory T cells than a young comparator population. These differences in older subjects were largely abolished by administration of zoster vaccine.[55] Intradermal administration of zoster vaccine has also been evaluated. This was dose-sparing, in that a two-third reduction in dose administered intradermally induced VZV-specific antibody and VZV-CMI responses equivalent to a standard dose of vaccine.[170]

Efficacy of the Zoster Vaccine

Persons 60 Years of Age or Older

The efficacy trial of the zoster vaccine was a randomized, double-masked, placebo-controlled study involving 38,500 subjects 60 years of age or older; 46% of whom were at least 70 years old and 6.5% of whom were at least 80 years old.[107] Equal numbers received either a single subcutaneous injection containing a median potency of 24,600 PFU of the Oka/Merck strain of attenuated VZV or a placebo containing excipients without VZV or cell components; all had a history of varicella or resided in the United States for at least 30 years. They were taught by video at each study site to recognize HZ and instructed to immediately contact the study site if they suspected HZ. Each month, the subjects called an automated telephone response system that queried them about interval symptoms suggestive of HZ, reeducated them about the clinical signs and symptoms of HZ, and reminded them to contact the study site if they suspected HZ. The automated telephone response system notified the study site when suspected HZ was reported or if there was no contact.

Site investigators examined subjects as soon as possible after suspected HZ was reported and described each suspected episode in detail. Samples for PCR (also local culture at the site's discretion) and digital photographs were obtained. Pain was assessed with a questionnaire that measured typical pain, as well as allodynia and disturbing pruritus.[161] Pain assessment was repeated at defined intervals as long as the pain remained



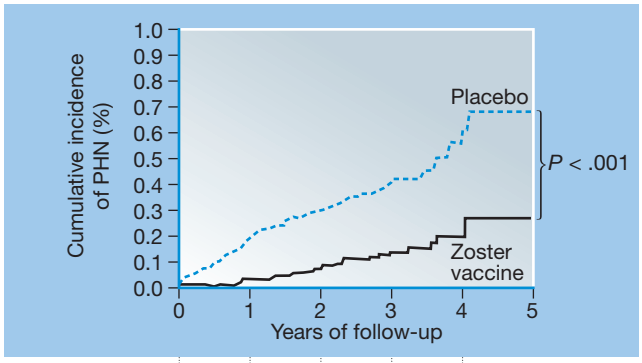


Figure 65.5. A, Vaccination reduces incidence of herpes zoster (HZ). **B,** Vaccination reduces incidence of postherpetic neuralgia (PHN). *(From Oxman MN, Levin MJ, Johnson GR, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults.* N Engl J Med. *2005;352:2271–2284.)*

TABLE 65.1 Herpes Zoster and Related Events in the Live Herpes Zoster Vaccine[a] Trial

	Vaccine				Placebo			
Age (y)	**HZ Cases**	**HZ Incidence[b]**	**BOI Score**	**PHN Cases**	**HZ Cases**	**HZ Incidence[b]**	**BOI Score**	**PHN Cases**
All	315	5.42	2.21	27	642	11.12	5.68	80
60–69	122	3.90	1.50	8	334	10.79	4.33	23
≥70	193	7.18	3.47	19	308	11.50	7.78	57

[a]Herpes zoster vaccine is the licensed vaccine Zostavax.
[b]HZ cases per 1000 person-years.
BOI, burden of illness; HZ, herpes zoster; PHN, postherpetic neuralgia.
Data from Oxman MN, Levin MJ, Johnson GR, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N Engl J Med. *2005;352:2271–2284.*

at 3 or greater (scale of 1 to 10) up to a maximum of 181 days. A prior validation of the pain instrument indicated that pain scores less than 3 were inconsequential with respect to quality of life. Complications of HZ were recorded. Subjects with HZ were offered a standard course of antiviral therapy and provided symptomatic care per local standard.

The prespecified primary end point for efficacy analysis was burden of illness (BOI). BOI was defined as the sum for each treatment group of the area-under-the-curve measurements from plots of pain severity over time for each individual case of HZ. Subjects without HZ contributed nothing to this sum. Thus, BOI was a metric that encompassed frequency, duration, and severity of HZ in a population, and in this trial represented the average severity of illness from HZ among all subjects in the vaccine and placebo groups. PHN, defined as the presence of pain with a score of 3 or greater at least 90 days after the onset of rash, was the secondary end point. The incidence of HZ, which was not initially chosen as an end point, was later added as an end point. The mean time in the study was 3.13 years; 95% of subjects completed the study; 1% were lost to follow-up or withdrew; and 4% died during the study.

A low threshold for suspecting HZ was adopted by the study sites. There were 1308 suspected cases of HZ: 481 were in the vaccine group and 827 in the placebo group. Of the suspected cases, 315 vaccinees and 642 placebo recipients were confirmed as HZ by prespecified criteria, which excluded HZ occurring during the 30-day postvaccination period (Table 65.1). Confirmation of the diagnosis was made by PCR in approximately 93.5% of cases,[171] by local culture in approximately 1% of cases, and (in the absence of a laboratory diagnosis) by an experienced central clinical evaluation committee in 5% to 6% of cases. Clinical adjudication was the basis of an HZ diagnosis only when definitive laboratory testing was not available.

The overall vaccine efficacy against HZ (VE_{HZ}) was 51.3% (5.42 cases/1000 person-years vs 11.12/1000 person-years; Table 65.2; $P < .001$). The efficacy was the same for either sex. The overall vaccine efficacy for BOI (VE_{BOI}) was 61.1% (2.21 vs 5.68 score; $P < .001$). The overall vaccine efficacy against PHN (VE_{PHN}) was 66.5% (27 vs 80 cases; $P < .001$). The VE_{PHN} was not altered when the definition of PHN was varied for intervals between 30 to 180 days after rash onset in a sensitivity analysis. In a time-to-event analysis, the cumulative incidence of HZ was significantly lower in the vaccine group (see Fig. 65.5A) for 5 years, as was the cumulative incidence of PHN (see Fig. 65.5B).

The effectiveness of zoster vaccine against HZ was confirmed in three retrospective studies. One study undertaken in a managed care setting, which included almost 76,000 vaccinated persons 60 years of age or older, showed a 55% reduction in HZ (hazard ratio, 0.45; 95% confidence interval [CI], 0.42–0.48) compared with more than 227,000 matched unvaccinated control subjects.[172] Zoster vaccine also reduced the number of complications, including hospitalization and

TABLE 65.2 Efficacy (%) With Live Herpes Zoster Vaccine[a] by Age Stratum

Measure	Overall	Age (y)		
		60–69	70–79	≥80
VE_{BOI} (CI 95%)	61.1 (51–69)[b]	65.5 (52–69)	55.4 (40–70)	NA
VE_{PHN} (CI 95%) if HZ develops	39 (7–59)	5 (–107–56)	55 (18–76)	26 (–69–68)
VE_{HZ} (CI 95%)	51.3 (44–58)	64 (56–71)	41 (28–52)	18 (–29 to 48)

[a]Herpes zoster vaccine is the licensed vaccine Zostavax.
[b]Data in parentheses are 95% confidence intervals.
CI, confidence interval; HZ, herpes zoster; NA, not available; VE_{BOI}, vaccine efficacy for burden of illness; VE_{HZ}, vaccine efficacy for herpes zoster; VE_{PHN}, vaccine efficacy for postherpetic neuralgia.
Data from Oxman MN, Levin MJ, Johnson GR, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N Engl J Med. *2005;352:2271–2284; Merck & Co. Zostavax (Zoster Vaccine Live) [package insert]. Available at: http://www.merck.com/product/usa/pi_circulars/z/zostavax/zostavax_pi.pdf.*

TABLE 65.3 Persistence of Live Zoster Vaccine[a] Efficacy

Years Since Immunization (Range in Years)	Person-Years	Efficacy Against HZ (95% CI)	Efficacy Against PHN (95% CI)
Pivotal trial (0–4.9)	58,200	51.3 (44.2–57.6)	66.5 (47.5–79.2)
Short-term persistence study (3.3–7.8)	9975	39.6 (18.2–55.5)	60.1 (–8.8 to 86.7)
Long-term persistence study (4.7–11.6)	25,250	21.1 (10.9–30.4)	35.4 (8.8–55.8)

[a]Herpes zoster vaccine is the licensed vaccine Zostavax.
CI, confidence interval; HZ, herpes zoster; PHN, postherpetic neuralgia.
Data from Morrison VA, Johnson GR, Schmader KE, et al. Long-term persistence of zoster vaccine efficacy. Clin Infect Dis. *2015;60:900–909.*

ophthalmic HZ, but unlike the controlled trial, no age effect was noted on the protection provided by the vaccine. Additional studies using a Medicare database and smaller private insurance database had similar findings, including an effectiveness against postzoster pain of 48% to 61%.[173,174] These studies specifically addressed the value of zoster vaccine in a community setting with routine clinical practices.

Persistence of the Efficacy of Zoster Vaccine

The persistence of vaccine-induced protection is a major issue for elderly vaccinees. The 51.3% efficacy against HZ determined in the pivotal zoster vaccine trial was based on approximately 58,000 subject-years (s-y), with subjects followed for a mean of 3.1 years (range, 0.0–4.1 years). A subsequent short-term persistence study included approximately 10,000 s-y with follow-up for 3.3 to 7.8 years, which determined an overall efficacy against HZ of 39.6%.[175] This was complicated by a commitment to provide zoster vaccine to placebo recipients, resulting in the placebo control group diminishing over the course of the study. An additional approximately 25,000 s-y, with subjects in follow-up for 7 to 11 years, determined an overall efficacy against HZ of 21.3% (Table 65.3).[176] This result was hampered by the absence of a placebo control group, thereby requiring that a control group be generated with a statistical model based on age-specific HZ rates observed throughout the pivotal trial; this was further adjusted for the calendar effect observed on those rates. In addition, the estimates for the latest years of follow-up have wide confidence intervals. Efficacy against BOI and PHN was somewhat higher, 35% to 37%.

Prevention Versus Attenuation

The effect of aging on vaccine efficacy in elderly people has been repeatedly demonstrated for other vaccines, such as influenza vaccine and pneumococcal vaccine, reflecting age-related immune senescence.[152,177] This effect for zoster vaccine is shown in Table 65.2. The VE_{HZ} decreased from 64% (CI, 56–71%) in the 60- to 69-year-old group to 41% (CI, 28–52%) in vaccinees 70 to 79 years old, and 18% (CI, –29% to 48%) in vaccinees 80 years of age or older.[165] However, the VE_{BOI} was similar in 60- to 69-year-old vaccinees and in vaccinees older than 70 years of age, indicating that VE_{BOI} was largely achieved by preventing HZ in the younger age group, while attenuation of incident HZ was a major contributor to reducing BOI in the older age group. Confirmation of this attenuation is provided by the VE_{PHN}, which was preserved at least through 79 years of age. Individual HZ pain scores of 600 or more (equivalent to the worst imaginable pain for 60 days, or less pain for longer periods) occurred with HZ in one vaccinee in the 60- to 69-year-old group compared with nine placebo recipients in this age group; HZ cases with this pain intensity in subjects 70 years of age or older occurred in 10 vaccinees compared with 31 placebo recipients.[178]

The importance of attenuation against HZ in elderly persons is also demonstrated by measuring the practical consequences of the acute and chronic effects of HZ on elderly people. This was done by recording the effects of HZ on the activities of daily living (e.g., work, mood, relationships, sleep, self-care, grooming) in subjects in the zoster vaccine efficacy trial.[111] These data demonstrated (Table 65.4) that zoster vaccination had a small effect on the HZ-related loss in activities of daily living in younger vaccinees who developed HZ despite vaccination, whereas the efficacy of zoster vaccine in preventing this loss was greater in the older age cohorts that developed HZ. Ultimately, the absolute benefit of zoster vaccine for attenuating HZ is greater in older subjects because the frequency and severity of HZ, including PHN, increase disproportionately with age.

Correlates of Immunity

The clinical and laboratory information previously described indicates that VZV-specific CMI is not only sufficient, but also necessary, to maintain latency of VZV. The extent of pain

TABLE 65.4 Live Herpes Zoster Vaccine[a] Preserves Activities of Daily Living in Subjects Developing Herpes Zoster

Age Group (y)	HZ-Induced Loss in ADL[b]		VE[e] (95% CI) Preventing Loss of ADL
	Zoster Vaccine[c]	Placebo[d]	
All ages	57.8	81.6	29.2 (7 to 46)
60–64	50.7	66.2	23.4 (–5 to 61)
65–69	53.8	62.0	13.3 (–49 to 49)
70–74	59.2	85.3	30.6 (–15 to 58)
75–79	63.4	106.6	40.5 (–11 to 68)
≥80	72.1	146.1	50.7 (–7 to 77)

[a]Herpes zoster vaccine is the licensed vaccine Zostavax.
[b]Higher numbers indicates more loss of ADL.
[c]Zoster vaccine, *n* = 19,254 (315 HZ cases).
[d]Placebo, *n* = 19,247 (642 HZ cases).
[e]Efficacy in preventing HZ-induced loss in ADL calculated from area-under-the-curve of plots of interference with ADL vs time after HZ onset. Measured as arbitrary units derived from the plots. See Schmader, Johnson, Ciarleglio, et al.[111]
ADL, activities of daily living; HZ, herpes zoster; VE, vaccine efficacy.

associated with HZ is also closely correlated with this specific CMI and not with antibody.[128] Nevertheless, in the large pivotal vaccine trial for older subjects, the magnitude of the antibody boost (measured by glycoprotein-based enzyme-linked immunosorbent assay) and the magnitude of the two measures of VZV-CMI (responder cell frequency and enzyme-linked immunosorbent spot assay) all correlated with protection against HZ.[65] The interpretation of these correlations is uncertain because these two types of immune responses were not correlated with each other. At best, antibody responses to zoster vaccine may be a nonmechanistic correlate of immunity, indicating responsiveness to the vaccine, although not the final path to protection. Antibody responses alone were measured in the large trial of zoster vaccine in subjects 50 to 59 years old. The boost in antibody in these younger vaccinees also correlated with protection against HZ.[179] A further analysis of their antibody responses to zoster vaccine revealed that the rise in antibody titers 6 weeks after vaccination inversely correlated strongly with the likelihood of developing HZ; more so than the magnitude of the response.[180]

Safety of the Zoster Vaccine

Deaths and all serious adverse events were similar in frequency in both treatment arms.[107,181] Reactions at the injection site were more frequent in vaccine recipients (48%) than in placebo recipients (16%), but the severity and frequency of these reactions were similar to those observed with other vaccines administered to older people. A single or small group of vesicle(s) occurred at the inoculation site in 0.11% of vaccinees compared with 0.04% of placebo recipients. Mild systemic events, such as headache, were also more common in vaccinees (6.3% vs 1.4%), but there was no difference in the occurrence of fever.

A prespecified substudy (6000 subjects) in the 42-day postvaccine period recorded more serious adverse events in vaccine recipients (1.9% vs 1.3%; $P < .03$), but there were no significant differences in the distribution of these events according to body system or type of event, and no clinically meaningful differences between the groups were observed in the pathophysiology, nature, timing, intensity, or outcome of these events. A time-to-event analysis did not suggest any temporal clustering of vaccine-associated serious adverse events. When all 38,500 subjects were considered in the 42-day postvaccine interval, the incidence of adverse events was similar (1.4%) in the two treatment groups.

In the 42-day postvaccine period, there were seven cases of HZ in vaccine recipients and 24 cases in placebo recipients, indicating that the vaccine did not cause or induce HZ and that zoster vaccine was effective shortly after administration. Moreover, no vaccine strain VZV (as typed by PCR) was recovered from 321 samples of DNA from HZ cases that occurred in vaccine recipients. Vaccine safety was confirmed in an additional placebo-controlled study and an analysis of eight managed-care organizations encompassing 193,000 older adults.[182,183] Five cases of optic neuritis, uveitis, or keratitis were reported as occurring within 2 weeks of zoster vaccination, usually in patients with prior quiescent disease.[184] A causal relationship has not been established. A single case of HZ caused by zoster vaccine has been confirmed (>20 million doses).[185]

Administration of Zoster Vaccine at 50 to 59 Years of Age

Zoster vaccine was licensed for this age group in the European Union, based on bridging experiments that showed a boost in VZV-specific antibody responses comparable to that observed in vaccinees 60 years of age or older. Subsequently, in a double-blind, placebo-controlled trial of almost 22,500 subjects 50 to 59 years old in the United States and Europe, the efficacy of zoster vaccine was demonstrated to be 69.8% and the safety profile was excellent.[186] The vaccine is now licensed for this age group in the United States. This is important because almost 20% of HZ cases (170,000 to 210,000) occur in this age group in the United States, compared with 47% of cases occurring in persons 60 years of age or older. More than 11% of all PHN occurs in the 50- to 59-year-old group.[87] The severity of acute disease, the complications, and the costs are similar to those experienced by older people; it is primarily the severity and duration of prolonged pain that distinguishes older patients with HZ. Moreover, 70% of people 50 to 59 years old are employed; approximately 3.4 days of employment are lost when HZ occurs at this age.[187,188] The prevention of work lost strongly influences the cost-to-benefit analyses of administration of zoster vaccine to persons 50 to 59 years old.

INDICATIONS

Zoster vaccine was licensed in May 2006 for people at least 60 years of age who had not had prior HZ for the prevention of HZ and related morbidity and subsequently recommended for these individuals by the Advisory Committee on Immunization Practices (ACIP). The target age was lowered to 50 years of age in 2011, but 50- to 59-year-old individuals were not added to the ACIP general recommendation. The vaccine is administered as a single subcutaneous dose.[165] The U.S.–licensed vaccine must be stored frozen (less than −15°C) in a lyophilized form and must be used within 30 minutes of reconstitution, except this formulation may be stored and/or transported at refrigerator temperature (2°C to 8°C) for up to 72 hours before reconstitution.

CONTRAINDICATIONS

Zoster vaccine should not be given to immunosuppressed persons; pregnant women; persons with active tuberculosis; or persons with a history of anaphylactic/anaphylactoid reactions to any of the vaccine components. The Oka/Merck strain

of VZV used in the zoster vaccine has caused severe or fatal disease in at least 23 people, but all but one were VZV-naive susceptibles receiving the varicella vaccine.[189] The vaccine should not be given to people who are receiving or have recently received (i.e., within 48 hours) antiviral drugs active against herpesviruses because these drugs may interfere with the protective effect of zoster vaccine. These drugs should be avoided for 10 days after zoster vaccine administration.[190] National blood bank policy is to delay blood donation until 30 days after zoster vaccine.

Prior Episodes of Herpes Zoster

The ACIP reaffirmed the indications stated at licensure, except for indicating that the vaccine is recommended for persons at least 60 years old (current age for licensure is now 50 years of age and older) regardless of a prior episode of HZ.[190] Considerations for this change include the following: (1) the incidence of second cases of HZ is uncertain, but it is 2% to 5% in some reports[87,116,118,120]; (2) by age 60 years, approximately 15% to 20% of the target population will have had HZ; (3) misdiagnosis and erroneous recall of past HZ are likely to be substantial and often not possible to confirm; (4) safety issues are unlikely[190]; and (5) the vaccine is likely to add protection, especially when the initial HZ was remote.[192]

Most likely, a prior attack of HZ will confer substantial protection against subsequent attacks for some years.[128] This boost in immune response is also likely to wane with aging, although the interval after an attack of HZ at which administration of zoster vaccine should occur is undefined. Some experts recommend that vaccination be given 3 to 5 years after prior HZ. The safety and immunogenicity of vaccinating in this setting was studied in 101 subjects.[192] Moreover, after the completion of the zoster vaccine efficacy trial, 420 placebo recipients who developed HZ received a dose of zoster vaccine without experiencing any vaccine-related serious adverse events.[193]

CONCOMITANT ADMINISTRATION

Although it might be convenient to administer zoster vaccine at the same visit as the annual influenza vaccine, administration of zoster vaccine should not be delayed for this purpose and should be given to qualifying adults as soon as an opportunity is available. In general, administration of widely used live attenuated and inactivated vaccines has not resulted in an increase in adverse events or a reduction in immunogenicity of the concomitantly administered vaccines.[194] In fact, simultaneous administration studies of HZ vaccine with trivalent inactivated influenza vaccine in more than 750 subjects showed that the immunogenicity of either vaccine was not reduced.[195] Concomitant administration with the quadrivalent inactivated influenza vaccine is under study.

One report of simultaneous administration with pneumococcal polysaccharide vaccine demonstrated a significant reduction in VZV-specific antibody responses.[196]Although there were some inconsistencies in the clinical trial, and effects on VZV-CMI were not evaluated, the U.S. Food and Drug Administration requested a revision to the zoster vaccine package insert stating that the HZ and pneumococcal vaccines should not be administered concomitantly and should be separated by 30 days.[165] However, in a retrospective cohort study involving almost 76,000 vaccinees in a managed care setting, the efficacy of zoster vaccine was not affected by concomitant pneumococcal polysaccharide vaccine administration.[172] These two vaccines are now frequently administered together. Concomitant use studies with the adult acellular pertussis-tetanus-diphtheria vaccine have not been done.

PUBLIC HEALTH CONSIDERATIONS

Burden of Herpes Zoster and Complications in Various Age Groups

The pain in the acute phase of HZ has a profound effect on the quality of life of many patients.[197–200] The effect of HZ on a typical older patient is quantitatively similar to that of congestive heart failure, severe depression, acute myocardial infarction, and uncontrolled diabetes.[201] In younger employed patients, the ability to work is often severely affected by the acute pain.[187] An average of 27 hours is lost from work after HZ and 34 hours of additional decreased effectiveness.[202,203] Chronic pain has even more substantial effects on quality of life, such that physical disability and emotional distress are common in patients with PHN. The effect of PHN is most marked in older patients, in part because they may have more intense pain and in part because of frailty and comorbidities. They experience physical consequences (e.g., fatigue, anorexia, weight loss, and insomnia), psychological consequences (e.g., depression and difficulty concentrating), social consequences (e.g., decreased social interaction and change in social role), and functional impairment (e.g., dressing, bathing, mobility, and shopping).[109,111,197,199,204] These consequences in older patients, which often are not readily appreciated, can lead to a downward spiral that ends with dependent care or even death. Table 65.4 indicates that zoster vaccine significantly reduces the loss of routine activities of daily living in people who develop HZ despite vaccination.[111] This attenuation effect is greater for older patients.

Societal Value of Zoster Vaccine

Administration of zoster vaccine to 1 million people in the target population will prevent approximately 70,000 cases of HZ, 20,000 cases of PHN, 250,000 visits to a medical office, 350,000 prescriptions, 8700 emergency department visits, and 8000 hospitalizations.[126] There are 54 million Americans in the 60- to 79-year-old age group (64 million in 2020); an additional 12 million are 80 to 100 years old (13.1 million in 2020). A case of HZ generates a cost (as of 2014) of about US$500 in a 50-year-old patient and more than US$1000 in a 70-year-old patient. Complications such as eye involvement (US$1500), neurologic involvement (US$2100), and skin superinfection (US$2115) add to the medical costs.[126,203] Hospitalization for HZ, especially cases occurring in an immunosuppressed patients, greatly increases the cost.[203,205] Total cost of HZ in the United States is almost US$2.2 billion.[203]

There have been more than 15 analyses of the cost effectiveness of zoster vaccine, including three from the United States, two from Canada, and eight from Europe, as well as Australia and Korea. Most of these rely, in large part, on published information from the HZ vaccine trial.[119,126,205–208] Most, but not all studies support the widespread administration of zoster vaccine. The strength of support in individual studies is very sensitive to assumptions about prevention of complications (especially PHN), age of vaccination, effect of age on vaccine efficacy, durability of protection, local healthcare costs, cost of vaccine, and utilities used to determine quality-adjusted life-years.

Until recently, the data on durability were limited, and most studies did not fully incorporate the vaccine effect on severity of illness. Some of these studies have influenced national policies on vaccine usage. For example, the United Kingdom recommends zoster vaccine for people who reach 70 years of age with a gradual catch-up program that includes people each year who reach 79 years of age.[209] Younger and older people are not covered by the vaccine program.

In the United States the cost per quality-adjusted life-year was calculated as US$86,000 when vaccination is undertaken at age 60 years and US$37,000 for vaccination at age 70 years.[210]

Barriers to Vaccine Uptake

The currently licensed zoster vaccine costs US$118 to the federal government and US$188 in the private market (the most expensive vaccine recommended for adults), not counting an administration fee. Thus, coverage by health maintenance organizations and governmental providers is essential for wide use. Although approximately 85% of the target population for the vaccine has some coverage from these sources, coverage is often partial and most people are left with a significant copayment. This and other barriers are responsible for an approximate zoster vaccination coverage of less than 25%, although in some managed care organizations coverage has exceeded 60%.

The largest barrier is financial, including the difficult Medicare Part D reimbursement procedures for physicians and patients. Other factors are the following: (1) lack of a strong recommendation by the caretaker (<50% for zoster vaccine vs >90% for vaccination against influenza and pneumococcus); (2) lack of patient awareness; (3) expense of stocking zoster vaccine and the requirement for storage in a freezer; and (4) the lack of a strong medical culture for vaccination of adults.[211]

Potential Effects of Universal Varicella Vaccination on the Epidemiology of Herpes Zoster and Use of the Zoster Vaccine

It has been suggested that administration of zoster vaccine at a younger age could become necessary if the age-specific incidence of HZ shifted toward younger ages as a consequence of universal vaccination against varicella. This concern was raised because environmental exposure of adults to varicella was known to boost VZV-CMI, and could be important in maintaining latency of VZV.[50,53,54] The absence of this environmental exposure was hypothesized to lead to a more rapid decline in VZV-CMI and occurrence of HZ at a younger age. This was the conclusion of two case control studies examining the effect of potential varicella exposure to the age of onset of HZ.[50,212] There are two studies in which mathematical modeling predicted the magnitude of this presumed negative effect of universal varicella vaccination.[213,214] These results have influenced public health vaccine policies concerning varicella immunization in some countries. The validity of this concern is difficult to assess by epidemiologic means because the data quality and surveillance methods vary greatly between prevaccine and postvaccine periods. In addition, there are potential biases from longitudinal changes that have occurred in the medical care system and study populations. Many studies have demonstrated an increase in incidence in HZ that began *before* the varicella vaccine era ("calendar effect") or failed to show an increase in incidence in the current era,[87,118,120,215,216] but most have found that HZ has been increasing over an extended period that *preceded the introduction of varicella vaccine*, and has increased in countries where the vaccine is not used.[217,218] A detailed analysis of a Medicare database of 3 million older adults found no significant change in the rate of HZ increase after the introduction of varicella vaccine (which was rising prior to its introduction), and also found that HZ incidence did not vary with individual state coverage for varicella vaccination.[219] This finding was replicated using a private medical insurance database of equal size.[220] Nuns and monks who were cloistered for decades without contact with children had the same incidence of HZ as age- and gender-matched villagers from the same locale.[221]

OTHER CONSIDERATIONS

Immunization Before Immunocompromise

There are additional settings in which zoster vaccine might be administered to patients younger than the currently approved age of 50 years. These include younger patients with latent VZV (i.e., prior varicella) who are likely to become immunosuppressed, such as patients scheduled for organ transplantation, therapy for autoimmune diseases, or chemotherapy for malignancy. The efficacy of this approach has not been demonstrated. However, zoster vaccination of immunocompetent older people who subsequently develop an unexpected immunocompromising illness are protected for at least several years after vaccination.[222]

While zoster vaccine is contraindicated for immune compromised patients, this definition of immunocompromise is not rigorously defined. For example, treated patients in the early stage of HIV infection (with ≥15% $CD4^+$ cell count) are likely candidates for zoster vaccine, including those younger than age 50 years.[223] Zoster vaccine was safe and immunogenic for patients receiving 5 to 20 mg of prednisone-equivalent on a chronic basis.[224] Moreover, there are likely to be levels of mild immunosuppression, or windows between cycles of immunosuppressive therapy, when patients might benefit from zoster vaccine despite the current general contraindication. This paradigm works for administration of the same Oka/Merck VZV strain as varicella vaccine in susceptible persons, such as in children scheduled for renal transplantation.[225] This type of off-label use should proceed with caution, since this strain of VZV can be very morbid in severely immunocompromised patients.[189] Mitigating the risk are the following factors: this proposed use of zoster vaccine is likely to occur in patients with preexisting immunity, which adds a large measure of safety, and the vaccine strain of VZV is sensitive to nucleoside analog antiviral therapy. Investigations are underway to establish the safety of administration to patients in a variety of clinical settings to determine if a significant boost in VZV-CMI is safely induced and maintained. It would be prudent to administer the zoster vaccine at least 2 weeks after the most recent immunosuppressive therapy and 2 weeks before anticipated additional immunosuppression.[190] This window should be broader when high-dose corticosteroids are a component of therapy for an extended period.

Unintended Administration of Zoster Vaccine to an Elderly Person Without Prior Varicella

This occurrence should be infrequent because most adults have preexisting immunity to VZV. In the two zoster vaccine efficacy trials (50–59 years of age; ≥60 years of age), subjects were recruited on the basis of a history of varicella or of residence in the United States for at least 30 years. In these two trials, five of 3754 serum samples lacked serologic evidence of past VZV infection. Even if a VZV-susceptible person were to receive zoster vaccine, it is unlikely to cause any serious adverse event given the attenuated nature of the vaccine virus, as demonstrated by approximately 150 VZV-susceptible subjects older than 13 years of age who received zoster vaccine in clinical trials; approximately 20 of them were older than 30 years. There were no significant systemic events, including the absence of signs or symptoms suggesting rash or dissemination of the administered virus.[226]

Elderly Persons Who Are Household Contacts of Varicella-Zoster Virus–Susceptible Immunocompromised Patients and Pregnant Women

It is especially important that elderly persons receive zoster vaccine if they are in regular contact with people who are VZV-naïve and at high risk to develop severe varicella.

Investigational Vaccines and Additional Studies to Improve Prevention of Herpes Zoster

The problem of immunosenescence complicates efforts to develop a vaccine with improved efficacy to prevent HZ. The administration of two doses of the current vaccine was not significantly more immunogenic than a single dose in boosting VZV-CMI responses.[169,227] However, the outcome measure, VZV antibody responses, was evaluated 4 weeks after the second dose, whereas some earlier experiments indicated that increasing the dose did not increase peak VZV-CMI responses, but greatly increased persistence of those responses.[158] Much higher doses—up to fivefold—can be safely given, but it is unclear that these will significantly improve VZV-CMI responses (Levin et al, unpublished data),[228] and this may not be commercially feasible. Given the significant waning of zoster vaccine protection after 7 to 8 years, a booster dose of zoster vaccine was safely administered to 200 subjects 10 years after a first dose. This trial indicated that VZV-CMI had declined substantially in the 10-year interval, but was still higher than levels measured in age-matched controls who had never received zoster vaccine. The booster dose restored the loss of VZV-CMI, and the immune response after 1-year in the boosted group was higher than that achieved in the control group.[229]

Heat-Inactivated Vaccine

Given that the live zoster vaccine cannot be used in patients with significant immunosuppression, an inactivated formulation (Merck Research Laboratories) was prepared by heating infectious Oka-Merck strain VZV. The initial controlled experiment used 2900 PFU of 50°C heat-inactivated VZV (4.5 U of antigen) to vaccinate patients undergoing autologous bone marrow or hematopoietic stem cell transplantation or allogeneic bone marrow transplantation.[46] A single-dose regimen was ineffective. Three monthly doses resulted in early restoration of VZV-CMI. Although this regimen did not prevent HZ, disease severity was markedly reduced. A subsequent controlled trial was performed in patients with lymphoma undergoing autologous transplantation.[47] Four monthly doses (6115 PFU, inactivated) were administered starting 1 month prior to transplantation. VZV-CMI recovered significantly sooner in the vaccinated patients, which correlated with significant efficacy against HZ. The four-dose regimen (now 4.8 U antigen) was evaluated in additional hematopoietic stem cell transplantations and immunosuppressed patients.[230] Significant increases in VZV-CMI were elicited in immunosuppressed patients with solid tumors or hematologic malignancies, autologous stem cell transplantation, and HIV with 200 or fewer $CD4^+$ cells/mm^3, but not in allogeneic bone marrow transplant recipients. Efficacy was not an end point. Additional studies are in progress (new proprietary inactivation procedure) in patients with a variety of immune compromising conditions, including biological response modifiers, with or without methotrexate, but not in immunocompetent older individuals who manifest age-related decline in immunity.

Recombinant Varicella-Zoster Virus Glycoprotein E Vaccine

Recombinant subunit vaccines provide another alternative for immunocompromised patients and, together with appropriate adjuvants,[231] may also provide an alternative for preventing HZ in elderly people. VZV glycoprotein E (gE) is a major component of the VZV capsid; the most abundant glycoprotein expressed on VZV-infected cells; the major target for neutralizing antibodies; required for replication and cell-to-cell spread; and a primary target of VZV-specific B- and $CD4^+$ T-cell responses.[52,232–237] The investigational recombinant subunit vaccine contains 50 μg of VZV gE and the AS01B adjuvant system. The vaccine combines gE antigen with monophosphoryl lipid A (a Toll-like receptor 4 agonist prepared from bacterial lipopolysaccharide) and QS-21 (a plant-derived triterpene glycoside) in a liposomal formulation. Phase I/II and Phase II clinical trials conducted in older adults showed that this vaccine administered in a two-dose schedule (60-day interval) elicited robust immune responses that were significantly elevated for at least 3 years.[238–240] The responses included stimulation of VZV- and gE-specific antibody in much greater quantities than a comparator live VZV vaccine. Importantly, multifunctional gE- and VZV-specific $CD4^+$ T cells were stimulated to high levels, including cells with a memory phenotype. These effects have been linked to a transient stimulation of the innate immune system and generation of a high number of efficient antigen-presenting dendritic cells.[241] Strikingly, in the Phase I and II trials, age had no significant effect on these responses. Strong immune responses did not occur when the adjuvant was omitted. Local and systemic side effects were reported more frequently than had been observed with the licensed live vaccine, but these were mostly transient, mild to moderate, and well tolerated.

A randomized, Phase III, placebo-controlled trial of this vaccine (Shingrix; GlaxoSmithKline) was undertaken in 15,400 subjects, stratified by 50 to 59 years, 60 to 69 years, and 70 years of age and older.[242] The vaccine was greater than 97% effective in preventing HZ, with very similar efficacy for all the three age strata (Table 65.5). Local and systemic reactions were significantly more frequent and severe than observed with some other commonly administered vaccines, including the licensed zoster vaccine. In general, these were well tolerated and 96% of subjects received the second dose of study vaccine. However, 9.4% had grade 3 systemic reactions (unable to perform usual daily activity) that lasted 1 to 2 days.

The efficacy of this vaccine alters our current paradigm about vaccination of older individuals, including results with the live zoster vaccine. Namely, that efficacy was strikingly better and did not decline with increasing age. These two outcomes mirror the immunologic profiles obtained in the Phase II studies. Persistence studies continue.

A confirmatory placebo controlled trial of 14,800 subjects 70 years of age and older (2850 subjects 80 years of age or older) was subsequently reported.[243] Data from subjects 70 years of age and older from the two trials were combined to determine the efficacy of the investigational vaccine in these older subjects (Table 65.6). Efficacy was 90% for subjects 70 to 79 years of age and was 89% for subjects 80 years of age and older. The safety profile was similar to that of the initial study. Efficacy declined very little during a 4-year follow-up. Efficacy against PHN was 88.8% in subjects 70 years of age and older. Information on durability of protection is not yet available. The efficacy from both studies may be too good to determine a correlate of protection against HZ or PHN. The vaccine protection against PHN was predominantly from prevention of HZ.

TABLE 65.5 Efficacy of an Adjuvanted Glycoprotein E Vaccine[a]—Initial Placebo-Controlled Trial (≥50 Years of Age)

	gE Vaccine			Placebo		
Age Group[b] (y)	**HZ Cases**	**Rate per 10^3 p-y**	**Age Group (y)**	**HZ Cases**	**Rate per 10^3 p-y**	**Vaccine Efficacy (95% CI)**
50–59 (n = 3492)	3	6.3	50–59 (n= 3525)	87	7.8	96.6 (89.6–99.31)
60–69 (n = 2141)	2	0.3	60–69 (n = 2166)	75	10.8	97.4 (90.1–99.7)
≥70 (n = 1711)	1	0.2	≥70 (n =1724)	48	9.4	97.9 (87.9–100.0)
All (n = 7344)	6	0.3	All (n = 7415)	210	9.1	97.2 (93.7–99.0)

[a]Herpes zoster vaccine is the recombinant gE vaccine (Shingrix; GlaxoSmithKline).
[b]According to protocol.
CI, confidence interval; gE, glycoprotein E; HZ, herpes zoster; p-y, person-years.
Data from Lal H, Cunningham AL, Godeaux O, et al. Efficacy of an adjuvanted herpes zoster subunit vaccine in older adults. N Engl J Med. *2015;372: 2087–2096.*

TABLE 65.6 Efficacy of an Adjuvanted Glycoprotein E Vaccine[a]—Confirmatory Placebo-Controlled Trial (≥70 Years of Age)

	gE Vaccine			Placebo		
Age Group[b] (y)	**HZ Cases**	**Rate per 10^3 p-y**	**Age Group (y)**	**HZ Cases**	**Rate per 10^3 p-y**	**Vaccine Efficacy (95% CI)**
All (n = 6541)	23	0.9	All (n = 6622)	223	9.2	89.8 (84.2–93.7)
70–79 (n = 5114)	17	0.9	70–79 (n= 5189)	169	8.8	90.0 (83.5–94.4)
≥80 (n = 1427)	6	1.2	≥80 (n = 1433)	54	11.0	89.1 (74.6–96.2)
Year			**Year**			
Year 1 (n = 6541)	2	0.3	Year 1 (n = 6622)	68	10.4	97.0 (88.8–99.7)
Year 2 (n = 6379)	6	1.0	Year 2 (n = 6372)	68	10.9	91.3 (79.9 -96.9)
Year 3 (n = 6137)	9	1.5	Year 3 (n = 6372)	48	8.1	81.6 (61.9–92.1)
Year 4 (n = 5896)	6	1.1	Year 4 (n = 5776)	39	7.1	85.1 (64.4–94.9)

[a]Herpes zoster vaccine is the recombinant gE vaccine (Shingrix; GlaxoSmithKline).
[b]According to protocol.
CI, confidence interval; gE, glycoprotein E; HZ, herpes zoster; "n" denotes sample size; p-y, person-years.
Data from Cunningham AL, Lal H, Chlibek R, Hwang S-J, Diez-Domingo J, et al. Efficacy of the herpes zoster subunit vaccine in adults ≥70 years of age. N Engl J Med. *2016;375:1019–1032.*

Additional information will be desirable on the persistence of this protection against HZ and on the long-term safety, given that a strong adjuvant is being administered in this older population. AS01B adjuvant is not present in any vaccines licensed in the United States, although it has been studied for many years as a component of a hepatitis B vaccine[244] and a malaria vaccine.[245] To date, the committee monitoring the safety of the two pivotal trials did not find any apparent safety signal.

The adjuvanted recombinant gE vaccine may be especially valuable for immunosuppressed patients who cannot receive the live attenuated vaccine. The immunogenicity and safety of the recombinant gE vaccine was demonstrated in HIV-infected patients and after autologous hematopoietic stem cell transplantation.[246,247] For these studies a third dose was administered 6 months after the first dose. Acceptable tolerability was observed. No alteration in the status of HIV infection or the hematopoietic transplant was attributed to vaccination, and gE-specific VZV-CMI and antibody responses were robust. At least eight additional studies are planned or ongoing to include special clinical situations (such as prior live zoster vaccine or prior HZ) and additional immune compromising conditions.

References for this chapter are available at ExpertConsult.com.

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SECTION 3 New Technologies

66 Technologies for Making New Vaccines

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The technological approaches for making new vaccines have been growing rapidly in recent decades owing to significant advances in a broad range of interrelated fields, including next-generation sequencing and antibody repertoire analysis, molecular and structural biology, genetics (reverse vaccinology), protein and polysaccharide chemistry, immunology, virology, bacteriology, fermentation, macromolecular purification, and formulation.

The majority of available vaccines have been for the prevention (prophylaxis) of infectious diseases. However, new technologies have extended this scope to vaccines for noninfectious diseases (e.g., autoimmune diseases, cancer, allergy, drug addiction) and therapeutic vaccines (e.g., certain infectious and noninfectious diseases). This broadening of scope is continuing to redefine the term *vaccine*.

There are two broad categories of vaccination, *active* and *passive*. Active vaccination stimulates the immune system to produce specific antibodies or cellular immune responses or both and prevents or protects against, ameliorates, or eliminates a disease. Passive vaccination uses a preparation of antibodies that neutralizes a pathogen or binds to a human cellular antigen and is administered before or around the time of known or potential exposure to a pathogen or to a subject with disease or infection. References to the term *vaccine* generally refer to vaccines administered by active immunization. Nevertheless, passive vaccination is desirable or essential in specific cases, particularly if no active vaccine is available or feasible, for immunocompromised persons, for certain types of cancer treatment, and for the immediate efficacy of immunotherapy in acute situations when the immune response to active vaccination will not be rapid enough to prevent a new disease. Passive vaccination is discussed under "Antibody Preparations" later.

Vaccines are stored in solution (liquid or frozen) or in freeze-dried (lyophilized) form depending on their stability and physical characteristics. Lyophilized vaccines are resuspended in diluent (resuspending fluid) at around the time of administration. The vaccine solution and diluent may contain the following additives: (a) preservatives or antibiotics to prevent bacterial growth in multidose containers; (b) stabilizers, including proteins or other organic compounds, to extend the dating period or shelf life for the product; (c) adjuvants for enhancing immune responses (see "Adjuvants" later); and (d) delivery systems for presenting the vaccine antigen(s) to appropriate cells of the immune system or for preserving or stabilizing the integrity or conformation of antigen(s) in vivo (see "Delivery Systems" later). The vaccine and the respective added components comprise the *vaccine formulation*.

This chapter summarizes the major technologies, key issues associated with their development, and immunological objectives underlying different kinds of vaccines. The status of development of key vaccines made by each approach is identified, whether licensed or in preclinical or clinical evaluations (Table 66.1). While most examples are for viruses and bacteria, there also are licensed vaccines and new developments for noninfectious diseases (Table 66.2). The technologies and examples should provide readers with an appreciation of the diversity of approaches being taken in the research and development of vaccines for new targets not previously approachable.

ACTIVE VACCINES

The protective immunity elicited by an active vaccine ideally would be lifelong and robust after one or a few doses with minimal side effects (reactogenicity). Available vaccines and vaccines under development generally fall short of this ideal, which continues to stimulate new research and development initiatives in this field. The term *immunogen* refers to the property of eliciting an immune response, whereas the term *antigen* denotes the property of in vitro immunological reactivity.

There are three general categories of active vaccines; Table 66.3 outlines the salient features. A *live* vaccine generally is a microorganism that replicates on its own in the host or infects cells and functions as an immunogen without causing disease. An *inactivated* vaccine contains an immunogen that cannot replicate in the host as the disease-causing microbe has been killed with chemicals, heat, or radiation.[1] A *nucleic acid–based* vaccine (usually DNA), also incapable of replicating in humans, is taken up by cells and directs the synthesis of the vaccine antigen(s).

The strategic choice for developing a live, inactivated, or nucleic acid–based vaccine should take into account the pathogenesis, epidemiology, and immunobiology of the target infection or disease and the technical feasibility of alternative vaccine designs. Epidemiology dictates the target population for the vaccine. The age and state of health of this population may favor certain designs as more appropriate for eliciting protective immunity. In the case of a vaccine intended for healthy subjects (especially infants), minimizing acute reactogenicity is critically important as is minimizing the risk of chronic autoimmune disease development in genetically susceptible subjects. Toxicology studies address the former while in regards to the latter, bioinformatics technologies have been developed to help identify microbial antigens that may lead to a cross-reactive immune response to normal human tissue (self) through molecular mimicry (discussed in greater detail in "Adjuvants" later and in "Bioinformatics Technologies and Vaccine Safety" later). On the other hand, the tolerance for reactogenicity and other potential adverse events is greater for a therapeutic cancer vaccine as the benefit far outweighs the risk. Finally, knowledge of immunobiology should enable the identification of the type(s) of immunity that should be elicited by the vaccine to be effective.

TABLE 66.1 Status of Development of Representative Human Vaccines Made by Different Technologies

Type of Vaccine[b]	Status of Development[a]: Preclinical Evaluation[c]	Clinical Evaluation[d]	Licensed Product[e]	Example[f]	Reference
ACTIVE					
Live					
Classic virus					
Attenuation in cell culture			xx	Poliovirus	2
			xx	Rotavirus	3
			xx	Measles virus	4
			xx	Mumps virus	5
			x	Varicella-zoster virus (VZV)	6, 299
Variants from other species			xx	Smallpox (Vaccinia virus)	11
		x		Rotavirus	13
Reassorted genomes			x[g]	Rotavirus	2, 16, 18
			x	Influenza virus	17
Temperature-selected mutants			xx	Rubella virus	19
			xx	Influenza virus	20
			x	Influenza virus	21
		x		Respiratory syncytial virus (RSV)	22
Recombinant virus					
Engineered genome		x		Herpes simplex virus (HSV)	23, 24
Deoptimized codons		x		Poliovirus	25
Recombinant viral vector		x		HIV using Vaccinia virus[h]	26–28
		x		Tuberculosis	34, 35
		x		HIV using adenovirus[i]	38
		xx		Ebola virus, allergy, and cytomegalovirus (CMV) using alphavirus[i]	20, 43–46
		x			
		x			
		xx		Dengue virus using yellow fever virus	48
Classic bacteria			xx	Tuberculosis (bacille Calmette-Guérin [BCG])	51
			x	Bladder cancer (BCG)	55
			x	Typhoid fever (*Salmonella typhi*)	57
Recombinant bacteria		x		Cholera (*Vibrio cholerae*)	58
		x		*Shigella*	59
Recombinant bacterial vector		x		*S. typhi*[k]	60
		x		*V. cholerae*[l]	61
		x		*Shigella flexneri*[m]	62
		x		*Streptococcus gordonii*	68
		x		*Listeria monocytogenes*	63
		x		BCG	64, 66
Dendritic cells			x	Prostate	70–72
		x		Glioblastoma	73
		x		Autoimmunity	75, 76
Inactivated					
Whole pathogen					
Inactivated bacteria			xx	Pertussis (*Bordetella pertussis*)	79
			x	Cholera	80
			x	Enterotoxigenic *Escherichia coli*	81
Inactivated virus			xx	Poliovirus	82
			xx	Influenza virus	83
			x	Rabies virus	84
			x	Japanese encephalitis virus	85
			xx	Hepatitis A virus	78, 86
Whole human cell			x	Melanoma	90–94
		x		Multiple myeloma	95

TABLE 66.1 Status of Development of Representative Human Vaccines Made by Different Technologies *(Continued)*

Type of Vaccine[b]	Status of Development[a]			Example[f]	Reference
	Preclinical Evaluation[c]	Clinical Evaluation[d]	Licensed Product[e]		
Protein-Based/Subunit					
Viral/bacterial			xx	Hepatitis B virus (HBV)	100
			xx	Pertussis	101–103
Tumor cells		x		Cancer	106
Chemically inactivated			xx	Tetanus (*Clostridium tetani*)	107
			xx	Diphtheria (*Corynebacterium diphtheriae)*	104
Genetically inactivated			x	Pertussis	108
Recombinant polypeptide			xx	HBV	110
			x[g]	Lyme disease (*Borrelia burgdorferi*)	115
			x	Cholera	262
			xx	Human papillomavirus	112, 141
		xx		HIV	116
		xx		HSV	119
			x	Meningococcal meningitis (*Neisseria meningitidis*, serogroup B)	120
		xx		Varicella zoster virus	122
Fusion proteins		x		Pneumococcal (*Streptococcus pneumoniae*)	124
		x		Group A strep (*Streptococcus pyogenes*)	125
			xx	Meningococcal meningitis (*N. meningitidis*, serogroup B)	126–128
		x		RSV	136
		x		Allergy	137
		x		Diabetes	138–140
Peptide based					
B-cell epitope					
Aggregate		x		Alzheimer disease	147
Fusion protein			x	Malaria[l]	149
		x		Lung cancer	154
Fusion peptide		x		Hypercholesterolemia	155
Conjugate		x		Malaria[m]	157
		x		Fertility[m]	158
		x		Pancreatic cancer[g]	159
		x		Non–small cell lung carcinoma[m]	160
Complex peptide	x			Malaria	162
Mimotopes	x			Cancer	164
		x		HIV	165
T-cell epitope					
CTL epitope		x		HBV	166
		x		HIV	167
T-cell activation		xx		Kidney cancer	168
T-cell receptor			x	Multiple sclerosis	170
Carbohydrate and polysaccharide based					
Polysaccharide			x	*Haemophilus influenza* type *b* (Hib)	171
			x	Meningococcal meningitis (*N. meningitidis, serogroup A,C,W,Y)*	172
			xx	Pneumococcal	173
Polysaccharide conjugate			xx	Hib[n]	174
			xx	Pneumococcal[o]	175,176
			x	Meningococcal meningitis (*N. meningitidis*, serogroup A,C,W,Y)	180, 181
		x		Group B strep (*Streptococcus agalactiae*)[l]	182
Other carbohydrate		x		Ovarian, breast cancer	183
		x		Melanoma	184

Continued on following page

TABLE 66.1 Status of Development of Representative Human Vaccines Made by Different Technologies *(Continued)*

Type of Vaccine[b]	Status of Development[a]			Example[f]	Reference
	Preclinical Evaluation[c]	Clinical Evaluation[d]	Licensed Product[e]		
Other		x		Breast cancer	188
		x		Schistosome	189
		x		Cocaine	190
Nucleic Acid Based					
Naked DNA		x		Influenza	192
Facilitated DNA		x		HBV	196
		x		HIV	199
		x		Metastatic renal cell carcinoma	200
	x			Influenza	197
Viral vector	x			Cancer	203
	x			Flavivirus	204
Viral delivery		x		HIV, malaria, melanoma, and prostate cancer using fowlpox virus[p]	205, 207, 208
		x		HIV using canarypox virus[p]	206, 209, 210, 266
Bacterial delivery	x			*S. flexneri*	211
Nonmicrobial delivery (RNA)	x			Melanoma	215
PASSIVE (ANTIBODY PREPARATIONS)					
Polyclonal					
Human immunoglobulin (Ig)		x		HBV (HBIG)	271
			x	VZV (VZIG)	272
			x	CMV (CMVIG)	273
Antibody fragment			x	RSV (RSVIG)	274
			x	Tetanus (TIG)	275
			x	Digoxin	276
Monoclonal					
Nonhuman		xx		Ovarian cancer	285
Natural human		x		CMV	286
Recombinant human			x	Melanoma	287, 291, 292
Human derived		x		HIV	288
Mouse derived		x		*Candida albicans*	290
Recombinant humanized		x		Colorectal and kidney cancer	294
			x	RSV	297
			x	Breast cancer	277
			x	Allergy	298
Recombinant chimeric			x	Non-Hodgkin lymphoma	278

[a]This denotes the single most advanced status achieved by each example.
[b]These categories are presented in the same order and outlined as in the text.
[c]Not yet evaluated in a human clinical trial.
[d]In clinical trial but not yet licensed, whereby "xx" denotes large-scale or Phase III trials on the path to licensure.
[e]Licensed in one or more major countries in the world, whereby "xx" denotes widespread use in multiple countries.
[f]These are representative examples for each vaccine strategy and not a comprehensive list of all examples, with key reference(s) illustrative of each example.
[g]Withdrawn postlicensure.
[h]Expressing more than 50 different foreign polypeptides, especially including HIV and colorectal cancer.
[i]Expressing foreign polypeptides, including HIV-1 *gag* and *env* genes.
[j]Expressing foreign polypeptides including Ebola and Marburg virus glycoproteins.
[k]Expressing foreign polypeptides include toxoids from *E. coli, V. cholerae,* and *C. tetani* and cancer-specific antigens.
[l]Fusion partner is hepatitis B surface antigen (HBsAg).
[m]Conjugate carrier is tetanus toxin (TT).
[n]Conjugate carriers are TT, diphtheria and tetanus toxoids–pediatric (DT), CRM_{197} (cross-reactive material 197), and an outer membrane protein complex.
[o]Conjugate carrier is CRM_{197}.
[p]Expressing foreign polypeptides, especially including HIV.

Live Vaccines

Some live vaccines are close to ideal in their capability to elicit lifelong protection in one or two doses with minimal reactogenicity. Such vaccines may be feasible in cases in which the natural infection or disease confers lifelong protection on the host. Live vaccines (with the exception of dendritic cell vaccines; see "Dendritic Cells as Autologous Vaccines" later) consist of viruses or bacteria that replicate to a limited degree in the host in a manner resembling that of the natural microorganism, thereby eliciting an immune response similar to that elicited by the natural infection. The live vaccine is attenuated; that is, its disease-causing capacity has been reduced by biological or technical manipulations. The live vaccine needs to be balanced by being neither overattenuated (no longer infectious enough to function as a vaccine) nor underattenuated (retaining pathogenicity even to a limited extent). Such vaccines usually elicit both humoral (antibodies) and cellular immunity (e.g., cytotoxic T lymphocytes [CTLs]).

Live vaccines are not technically feasible for most vaccines currently under development. The balance between incomplete attenuation (and consequent disease-causing capability) and complete attenuation (inadequately immunogenic) is delicate and, for some viruses or bacteria, technically unachievable at present. Because a live vaccine can replicate, it may be possible for it to revert to its more naturally pathogenic form unless there are multiple attenuating mutations. Moreover, some live vaccines can be transmitted from a vaccinee to a nonimmunized person, which can be quite serious if the recipient has an immune deficiency (e.g., AIDS or as a result of cancer chemotherapy). In cases in which the natural viral infection per se fails to induce a protective immune response, an attenuated virus would not be expected to be protective without further engineering. As is discussed in the following sections, live vaccines may be produced using either a classic strategy or one using recombinant technology.

TABLE 66.2 Targets for Vaccines Outside the Field of Infectious Diseases

Target Cancer	Condition
Bladder cell surface	Superficial bladder cancer
Prostatic acid phosphatase	Prostate cancer
GM_2 ganglioside	Melanoma
MUC-1 mucinous glycoprotein	Non–small cell lung carcinoma
HER2 glycoprotein	Breast cancer
CD20 glycoprotein	Non-Hodgkin lymphoma
Carcinoembryonic antigen	Carcinoma
ADDICTION	
Cocaine	Drug dependency
Nicotine	Cigarette smoking Cardiovascular
Angiotensin	Hypertension
Cholesterol ester transfer protein	Hypercholesterolemia
GASTROINTESTINAL	
Gastrin 17	Gastroesophageal reflux disease
AUTOIMMUNE	
Myelin basic protein	Multiple sclerosis
Tumor necrosis factor α	Crohn disease
Insulin, proinsulin, others	Diabetes
Ragweed, others	Allergy
TOXICITY	
Digoxin	Toxicity
REPRODUCTION	
Human chorionic gonadotropin	Fertility

Classic Strategy for Viral Targets

Attenuation in Cell Culture. The production of live viral vaccines depends on efficiently propagating virus in cell culture. The approach is empirical, in that wild-type virus isolated from a natural human infection is passaged in vitro through one or more cell types, with the goal of attenuating its pathogenicity. In contrast, a strategy to increase pathogenicity is to passage a viral or bacterial strain serially in a host species. The mechanism by which a mutation is introduced during the course of empirical attenuation is not well understood. In some cases (e.g., poliovirus),[2] it has been possible to demonstrate attenuation in a primate species, whereas in most cases, attenuation is proven only through extensive clinical trials. The success of this empirical approach, which has been applied to oral vaccines (oral poliovirus vaccine[2] and

TABLE 66.3 Comparative Properties of Active Vaccines

Characteristics	Advantages	Challenges
LIVE VACCINES		
• Able to replicate in the host • Attenuated in pathogenicity • Elicit antibodies and cell-mediated immunity	• May elicit broader immune responses • May require fewer doses • Generally longer lasting protection	• Uncertain window for attenuation • Uncertain safety before large-scale use • Stability • Ability to analyze the final product
INACTIVATED VACCINES		
• Unable to replicate in the host • Elicit mostly antibodies	• Cannot multiply or revert to pathogenicity • Nontransmissable to another person • Generally less reactogenic • Usually more feasible technically	• May require adjuvant • May require delivery system • Immunogenic potency • Variable efficacy
NUCLEIC ACID–BASED VACCINES		
• Stimulate synthesis of antigens only in cells • Elicit mostly cell-mediated immunity	• Standardized method of production and analysis • Potential sustained immunological stimulation	• Establishing proof-of-principle • Immunogenic potency

rotavirus[3]) and to injected (parenteral) vaccines (measles,[4] mumps,[5] varicella[6]), has been borne out by the significant number of available licensed vaccines. The reactogenicity of such vaccines has been low enough that some of them (polio, measles) are broadly accepted worldwide for routine pediatric use. Measles, mumps, and rubella (MMR) vaccines have been widely used for decades as the trivalent MMR vaccine. More recently, following many attempts to achieve a stable and immunogenic formulation, the MMR and varicella vaccines have been combined into the measles-mumps-rubella-varicella vaccine.[7] By means of intensive immunization programs with oral poliovirus vaccine, polio is approaching worldwide eradication. As a striking example of the challenge to proving attenuation, the Urabe strain of mumps virus was licensed after showing apparent safety in clinical trials. After several years of use in millions of children, vaccination with this strain was observed to cause aseptic meningitis at a rate of approximately 1 : 10,000.[8] Given the availability of an alternative mumps vaccine without this serious effect, the Urabe strain was withdrawn and is no longer available as a result of the manufacturer's exit from the vaccine market.[9] Another highly relevant example is vaccine-associated paralytic poliomyelitis which is a very rare adverse event (estimated at one case per 2.4 million doses administered) associated with the live-attenuated oral poliovirus vaccine. It is still the vaccine of choice for controlling poliomyelitis in many countries because of its ease of use, low cost, and superiority in conferring intestinal immunity in immunologically naïve individuals.[10]

Variants From Other Species. An animal virus that causes a veterinary disease similar to human disease can be isolated and cultivated, anticipating that the animal virus will be attenuated for humans yet be sufficiently related to elicit protection against the human virus. Smallpox, the first vaccine, is the prototype of this approach. More than 200 years ago, Jenner discerned that persons exposed to cows were resistant to smallpox, so he used the virus isolated from cows (vaccinia virus) for human vaccination against smallpox (caused by variola virus). The immunization program was applied worldwide using vaccinia virus grown in vivo in the skin of calves[11] and resulted in the complete eradication of smallpox worldwide by the mid-1970s, the only infectious disease ever eradicated. Unfortunately, the vaccine had to reenter production in a modern cell culture–based process[12] for potential use as a safeguard against the dissemination of variola virus as a weapon of bioterrorism.

Similarly, first-generation vaccines for rotavirus consisted of nonreassortant animal viruses isolated from rhesus monkeys[13] and cows. However, such rotavirus vaccines were not reproducibly efficacious as human vaccines and, thus, new vaccines were developed that incorporated the VP7 and VP4 proteins associated with disease protection and recovery.[14] RV1 is a live attenuated oral vaccine licensed in the United States since 2008.[14] RV5, a live vaccine containing reassortants, is discussed in the following section.

Reassorted Genomes. A reassortant virus, which contains genes from two parental viruses, is derived following coinfection of a cell with two viruses with segmented genomes. To improve the efficacy of animal rotaviruses, reassortant rotaviruses were isolated containing mostly animal rotavirus genes, which confer the attenuation phenotype for humans, and the gene(s) for human rotavirus surface protein(s) that elicit serotype-specific neutralizing antibodies for human rotavirus.[15,16] These reassortant rotaviruses have elicited higher efficacy rates as vaccines than their nonreassortant parental animal viruses. The same approach has been applied to influenza vaccines, in which a virulent human influenza virus provides the genes that encode the immunogenic surface glycoproteins (hemagglutinin and neuraminidase), and an attenuated virus provides all other genes with an attenuated phenotype.[17] The quadrivalent reassortant rhesus rotavirus vaccine[15] is another example of the challenge in establishing the attenuation profile of a live vaccine. After 1 year of commercial distribution in the United States, it was observed that the vaccine was associated with an increased incidence of intussusception of approximately 1 : 10,000 immediately postvaccination. Although the overall benefit-to-risk remained very high, the vaccine was withdrawn from distribution by the manufacturer. A pentavalent reassortant bovine rotavirus vaccine has been licensed since 2006.[3,18]

Temperature-Sensitive Mutants. Viral mutants can be selected according to their relative growth properties at different temperatures. Such viruses are called temperature-sensitive, being unable to grow at elevated temperatures, or cold-adapted, having been selected for growth in vitro at lower than physiological (37°C) temperatures, that is, down to 25°C. The strategy behind this approach is that cold-adapted or temperature-sensitive viruses will be less vigorous in their in vivo growth than their wild-type parental virus, hence less virulent and phenotypically attenuated. A cold-adapted rubella vaccine[19] has been widely used as part of MMR vaccine. A cold-adapted influenza vaccine based on reassortants[20] was the first live vaccine licensed for intranasal administration and one has been used widely in Russia.[21] A double temperature-sensitive respiratory syncytial virus (RSV) vaccine has been tested clinically,[22] and the use of a double mutant (one that contains two independent mutations) is an important additional refinement resulting in a much lower frequency of reversion to wild-type virulence than for a single mutant.

Recombinant Technology-Based Strategies for Viral Targets

Specific modifications or deletions can be made in viral genes so that the virus is more stably attenuated. The increased stability of the attenuation phenotype results from making the modification(s) extensive enough that reversion through back-mutation is impossible or unlikely. In contrast, attenuated viruses derived by classic strategies may have only point mutations and therefore retain the capability to revert. In attempts to generate a new vaccine, herpes simplex virus (HSV) was genetically engineered in three ways[23]: (a) to be attenuated, (b) to provide for antibody markers of vaccination that are differentiable from those of wild-type HSV infection, and (c) to protect against both HSV-1 and HSV-2. In another example, a particular HSV glycoprotein gene was deleted, resulting in an HSV strain that cannot replicate; this recombinant virus is produced in vitro in a cell line that supplies the deleted glycoprotein gene in *trans*, and the resultant virus can initiate infection in the host without further replication[24] (termed *replication-defective*).

A new molecular strategy for attenuation is to create deoptimized codons with the goal of generating a replication-defective strain. Polioviruses with deoptimized codons in the capsid region produce less infectious virus and are more highly attenuated, yet still efficiently elicit neutralizing antibodies that are protective in a mouse challenge model.[25]

Recombinant technology also has been applied to developing live vaccines in which foreign polypeptide antigens from pathogens have been engineered into carriers or vectors. The strategy in creating such vectors is to present the foreign antigen to the immune system in the context of a live virus infection to stimulate the immune system to respond to the foreign polypeptide antigen as a *live* immunogen, with the

expectation of developing a broader immunity (humoral and cellular) to the corresponding human pathogen than that attainable by a subunit polypeptide vaccine. The foreign polypeptide is expressed within the infected cell and is transported to the cell surface to stimulate antibody production or is broken down into peptides, which are transported to the cell surface where they may elicit CTL responses. This strategy also has the potential advantage of amplification of the immunogenic signal when the live vector initiates multiple rounds of replication (nonreplicating vectors are discussed in "Delivery Systems" later). If the vector virus is a commonly used vaccine, one could immunize simultaneously against the vector virus and another pathogen, ideally in a single dose. However, the preexisting or induced immune response to the live viral vector per se ordinarily limits the effectiveness of revaccination.[26]

DNA Viruses

The prototype viral vector is vaccinia virus in which dozens of different foreign polypeptides have been expressed.[27] Immunizations of animals in a wide range of infection and oncology models have protected against the pathogen encoded in the foreign polypeptide or against tumor development. Recombinant vaccinia virus expressing HIV-1 gp160 has been tested clinically[28] for prophylactic and therapeutic applications.

Given the known adverse events after vaccinia (which are more serious in immunocompromised persons) observed in the worldwide eradication program, vaccinia virus itself has been engineered to reduce its virulence without compromising its efficacy as a live viral vector.[29,30] By passaging vaccinia virus several hundred times in chicken embryo fibroblasts, the highly attenuated strain modified vaccinia Ankara was developed that, owing to its safety under conditions of immune suppression in animal studies, has also led to its use in clinical trials with recombinant HIV vaccines,[31] including modified vaccinia Ankara expressing monomeric gp120.[32,33] Modified vaccinia Ankara has also been used to express antigen 85A from *Mycobacterium tuberculosis* and was well tolerated and immunogenic, but not efficacious, in infants and adults.[34,35] Two other members of the poxvirus family, fowlpox and canarypox viruses, are being developed as naturally attenuated live virus vectors that can infect human cells but not produce infectious viral progeny. This inability to spread makes fowlpox and canarypox viral vectors classifiable as DNA-based vaccines (see "Nucleic Acid–Based Vaccines" later). Recombinant vaccinia and fowlpox viruses expressing carcinoembryonic antigen have shown clinical activity against tumors bearing this antigen.[36]

Other mammalian viruses have also been engineered into live vectors. Adenoviruses are attractive candidates for vaccine vectors as the genome is well characterized, easy to manipulate, causes mild disease in immunocompetent human adults, and can be rendered replication-defective to reduce unwanted side effects.[37] Fifty-one serotypes have been isolated from humans and 27 serotypes from simians (including seven from chimpanzees). The human serotypes are subdivided into six subgroups (serotypes A to F) of which group B contains adenoviruses 7 and 35 and group D contains adenoviruses 4 and 26. Vectors based on adenoviruses 4 and 7 are replication-competent and have been used for oral vaccination of military personnel to prevent acute respiratory disease, whereas vectors based on adenoviruses 26 and 35 are nonreplicating.[37] These vectors have been engineered to express foreign polypeptides, especially for HIV vaccine, and have elicited protective immunity in several viral challenge models in animals[38] and have been tested in clinical studies.[39] As previously described, HSV also has been engineered into a live vector[23] and so has CMV.[40] Optimizing foreign polypeptide expression for all these live viral vectors remains an important immunological objective.

RNA Viruses

RNA viruses also can be engineered into vectors for expressing foreign polypeptides. Lymphocytic choriomeningitis is one example of an RNA virus used as a vector.[41] Sindbis and other alphaviruses have received extensive attention owing to their broad host range, ability to infect nondividing cells, and potential high-level expression per cell.[42] Sindbis has been developed into a viral vector–DNA-based vaccine (see "Nucleic Acid–Based Vaccines" later). Vesicular stomatitis virus vectors expressing filovirus proteins have been shown to protect against lethal challenge by Ebola or Marburg viruses,[43] and alphavirus expressing cytomegalovirus (CMV) glycoprotein has been developed as a candidate CMV vaccine.[44] The versatility of alphavirus vectors as a vaccine strategy is further manifested for noninfectious-disease targets, in that a vector encoding a grass pollen allergen is able to prevent allergic sensitization in a mouse model.[45]

Given the Ebola outbreak that started in Africa in May 2014, global vaccine efforts were accelerated leading to the entry of two vector-based vaccine candidates into efficacy trials in humans. One is derived from a chimpanzee adenovirus engineered to express glycoproteins of ebolavirus, and the other is based on the vesicular stomatitis virus vector that has been engineered to express ebolavirus glycoproteins.[46] Recent results from a Phase III study using the vaccine based on the vesicular stomatitis virus vector demonstrated an efficacy of 100%.[47] Another growing threat that has made the development of an effective vaccine an international priority is dengue disease. Vaccine development programs in both academic and industry settings have investigated various technologies, and the only successful dengue vaccine to date (recently reporting positive Phase III efficacy data)[48] is a recombinant, live, attenuated, tetravalent dengue vaccine (CYD-TDV). This vaccine consists of four recombinant dengue vaccine viruses (CYD 1 to 4), each constructed by replacing the genes coding for the premembrane and envelope proteins of the yellow fever 17D vaccine virus with those from the wild-type dengue viruses, generating chimeric complementary DNAs that are individually transcribed to RNA, and then RNA transfection in Vero cells.[49] Results from a long-term follow-up interim analysis and an integrated efficacy analysis demonstrated lower risk for hospitalization in vaccinated children 9 to 16 years of age and a pooled rate of efficacy during the first 25 months of 60.3% for all participants.[50]

Classic Versus Recombinant Technology-Based Strategies for Bacterial Targets

It has been difficult to develop live attenuated bacterial vaccines by classic strategies because attenuation is difficult to achieve without reversion. Also, bacteria could stop expressing virulence factors in vitro, then turn on expression when in the host.

Serial Passage

The one widely available live bacterial vaccine is a live attenuated strain of *Mycobacterium bovis*, also known as bacille Calmette-Guérin (BCG).[51] Early in the 20th century, this bovine strain was attenuated by 231 successive in vitro subculturings over 13 years; there are many strains of BCG vaccine available worldwide that are derived from this original strain. These vaccines vary in terms of tolerability, immunogenicity, and rate of protective efficacy for tuberculosis in clinical trials

(range: 0% to 80% protection) for reasons that may relate to the actual vaccine strains used or to differences in clinical study designs or populations. BCG vaccines have been inoculated into more than 3 billion people worldwide, have a generally acceptable tolerability profiles, and have an important role in protection against childhood tuberculosis.[52] BCG vaccines also have been administered orally.[53,54] Following the observation that patients with tuberculosis have reduced rates of cancer, BCG vaccine was developed into the first cancer vaccine, being indicated for the treatment of superficial bladder cancer by intravesicular instillation.[55] BCG vaccine also has been used as an adjuvant for the priming doses of certain cancer vaccines.

Mutagenesis

Chemical mutagenesis followed by selection was used to derive the attenuated Ty21a strain of *Salmonella typhi*.[56] This vaccine was licensed for preventing typhoid fever based on its record of safety and efficacy (approximately 60% for several years) after a regimen of three or four doses.[57]

Recombinant DNA (rDNA) technology would be expected to be applied to attenuating any new bacterial strain that had promise as a live attenuated vaccine. Therefore, it seems unlikely that a new live bacterial vaccine attenuated by a classic strategy, alone, will be currently developed.

Recombinant Bacteria

The engineering of bacteria for attenuation is much more complex than for viruses because bacterial genomes are approximately 100-fold larger than those of viruses. The strategy for attenuation, like that for viruses, is to identify the gene(s) responsible for the virulence of bacteria or for their ability to colonize and survive in particular tissue(s) in the host and then eliminate the gene (preferred) or abolish or modulate its in vivo expression. As is the case for modification mutants of viruses, there must be a balance between the virulence of a bacterial strain and its activity as a vaccine, and it is possible to overattenuate a bacterial strain to the point that it no longer replicates sufficiently in vivo and is no longer immunogenic. *Vibrio cholerae* strains have been developed as live cholera vaccines; attenuation has been accomplished by rDNA-directed deletion of genes encoding virulence factors (e.g., cholera toxin [CT]).[58] To ensure attenuation by reducing the probability of reversion, deleting at least two independent genes or genetic loci that contribute to virulence is highly desirable. There have been attempts to develop *Shigella* strains into live vaccines through particular chromosomal or plasmid-based gene mutations to reduce pathogenicity.[59]

Recombinant Bacterial Vectors

Suitable bacterial strains have been engineered into live vectors for expressing foreign polypeptides. The most common development has been to engineer enteric pathogens for inducing mucosal immunity against the foreign polypeptide following oral delivery. *S. typhi* vectors have been the most commonly developed strains in terms of immunology, molecular design, and clinical testing.[60] Strains of *V. cholerae*,[61] *Shigella flexneri*,[62] and *Listeria monocytogenes*[63] have also been engineered. The challenges for these vectors are to retain sufficient virulence for replication in the gut while achieving sufficient attenuation and expressing appropriate levels of foreign polypeptides. The BCG vaccine strain also has been engineered as a live vector to express foreign polypeptides[64]; one aspect of the versatility of BCG is its efficacy in mice by different routes of administration (oral, intranasal, and intradermal).[65] The first human clinical trials of a recombinant BCG vaccine (rBCG30) were initiated with BCG30 (Phase I), which is a recombinant BCG Tice strain derived from the *Mycobacterium–Escherichia coli* shuttle vector pSMT3 and expresses approximately fivefold more of the *M. tuberculosis* 30-kDa protein than the parental BCG strain.[66] rBCG30 was found to be well tolerated and more immunogenic than BCG.[67] The ability of some bacterial species to replicate intracellularly may augment the ability of expressed foreign polypeptides to elicit broad and vigorous immune responses against their respective pathogens.

Streptococcus gordonii, a gram-positive commensal (nonpathogenic) bacterium, has been engineered to express foreign polypeptides on its surface by genetic fusion to attachment sequences of *S. gordonii* M protein.[68] One significant challenge is whether the foreign polypeptide will be sufficiently immunogenic given that this bacterial species does not naturally elicit an immune response that clears them.

Dendritic Cells as Autologous Vaccines

Dendritic cells (DCs) can be exploited as potent professional antigen-presenting cells for tumor-specific antigens by isolating these from the patient and mixing ex vivo with mixed antigens from a person's tumor or alternatively with a recombinant immunostimulatory protein. The DCs ingest and process the antigens and reexpress them on the DC surface. The cells then are reintroduced into the patient for antigen presentation and immune stimulation of T cells.[69] DCs loaded with prostate-specific antigen genetically linked to the granulocyte-macrophage colony-stimulating factor cytokine have shown evidence for clinical efficacy in immunotherapy of prostate cancer[70–72] and were licensed in 2010. The mixed-antigen approach has shown promise for glioblastoma.[73,74] Because autoimmune diseases are characterized by the loss of immunological tolerance to self-antigenic determinants, it may be possible to exploit DCs as antigen-presenting cells to achieve tolerance, as has been demonstrated in animal models of diabetes, multiple sclerosis, and autoimmune encephalitis[75] and for which clinical trials have commenced (e.g., diabetes and rheumatoid arthritis) since 2011.[76,77]

Inactivated Vaccines

Inactivated vaccines have safety advantages as they are unable to replicate (see Table 66.3). Such vaccines are generally well tolerated, especially those that are purified to remove other macromolecules (i.e., subunit vaccines). Subunit vaccines include primarily the antigens that best stimulate the immune system and in some cases, use very specific parts of the antigen (epitopes) that antibodies or T cells recognize and bind. Because subunit vaccines contain only the essential antigens and not all the other components of the microbe, the chances for adverse reactions are less.[1] Subunit vaccine technology is discussed in greater detail later in "Protein-Based/Subunit Vaccines." Given the broad range of available approaches, it is generally easier to produce an inactivated vaccine than a live vaccine. The immunogenicity of such vaccines generally is enhanced by administration with adjuvant or delivery system (see "Adjuvants" later). Nevertheless, this type of vaccine usually requires multiple doses, often followed by booster doses, for attaining long-term protective immunity. In one case, short-term protection was demonstrated following a single dose of a formalin-inactivated hepatitis A vaccine in children 2 to 16 years of age who remained free of infection at 10 months follow-up.[78] These vaccines usually function by stimulating humoral immune responses and by priming for immunological memory and may stimulate CTL immunity when administered with certain adjuvants and delivery

systems. Multiple approaches exist for developing inactivated vaccines; they are discussed below starting with the classic approaches, followed by recombinant strategies.

Whole Pathogen

The earliest technology in the 20th century was the only one technically feasible at that time when there was essentially no definition of specific antigens and their role in immunity. The inactivation of whole bacteria or whole viruses had the objective of eliciting the formation of antibodies to many antigens, from which some antibodies presumably would neutralize the pathogen or its virulence determinants.

Inactivated Bacteria. These vaccines are prepared by cultivating the bacteria (e.g., *Bordetella pertussis*) and inactivating the whole cells with heat or chemical agents (e.g., thimerosal or phenol[79]) without further purification. Owing to their highly crude nature, the reactogenicity of such vaccines when given parenterally was greater than that of other types of vaccines. This led to the development of a series of new acellular vaccines (subunit vaccines) which is described in Chapter 44. On the other hand, inactivated whole-cell *V. cholerae*[80] and enterotoxigenic *Escherichia coli* (ETEC)[81] vaccines are well tolerated by the oral route. Even though the ETEC vaccine is now under active development, it seems less likely that new bacterial vaccines will be made in this manner given the number of alternative technologies available for preparing purified vaccines and the more exacting regulatory standards that have developed over time.

Inactivated Virus. Inactivated viral vaccines, prepared by this old technology, have been available for many decades, and are usually very well tolerated. Viruses are shed into cell culture media, cell-free media from infected cultures is collected, and the large size of the virus particles relative to other macromolecules in the media enables the particles to be enriched readily by straightforward purification techniques. Successes include poliovirus,[82] influenza virus,[83] rabies virus,[84] and Japanese encephalitis virus[85] vaccines. Alternatively, in the case of killed hepatitis A virus vaccine, infected cells are lysed, and virus particles are purified.[86] Virus particles are inactivated chemically, typically by treatment with formalin, and then may be adjuvanted by adsorption onto an aluminum salt. The key epitopes on the surface of many nonenveloped small viruses that elicit a protective immune response (protective epitope) often are conformational, being formed by the highly ordered assembly of viral proteins into precise structures. For most of the viruses from which inactivated vaccines have been developed,[82–86] it is not possible to mimic the conformation of such epitopes by other technologies (e.g., recombinant polypeptides). Inactivated viral vaccines tend to be highly potent immunogens. Thus, this classic strategy, which has an excellent track record of producing well-tolerated and efficacious vaccines, remains the technology of choice for many viral vaccines. Similar to live vaccines, there have been inactivated vaccines associated with rare adverse events including atypical measles seen with measles vaccine[87] (characterized by febrile illness with headache, photophobia, cough, and unilateral pulmonary infiltrates) and enhanced pulmonary disease with formalin-inactivated RSV vaccine.[88] A newer inactivated adjuvanted influenza vaccine produced using a split-virus approach (where the virus has been disrupted by a detergent)[89] has demonstrated a significant association to a very rare adverse event, narcolepsy, which is not seen with another adjuvanted influenza vaccine produced using a subunit approach (a process that removed/reduced the amount of other influenza viral components besides hemagglutinin and neuraminidase) and is discussed in greater detail later in "Adjuvants."

Whole Human Cells

Some of the designs for cancer vaccines are based on the use of individual tumor-specific antigens, either purified or expressed in a vector. A concern regarding using individual antigens in a vaccine is that the induced immune response following vaccination or administration of antibodies may stimulate antigenic modulation and diminished expression of the target antigen or loss of major histocompatibility complex class I expression or loss of other T-cell receptor–associated signaling, thereby reducing efficacy. The advantage of inactivated allogeneic whole-tumor-cell vaccines is the opportunity to present a wide array of tumor-specific antigens to the immune system. Tumor cell lines are expanded in vitro, pooled, and inactivated. A melanoma vaccine derived from two tumor cell lines and given with a synthetic adjuvant is licensed[90] in Canada, but further development for the European and U.S. market was discontinued.[91] In addition, autologous tumor cells modified with the hapten dinitrophenol and adjuvanted with BCG have shown evidence of clinical benefit for melanoma and was initially licensed in Australia but subsequently withdrawn and eventually discontinued because of financial constraints and subsequent decision to discontinue manufacturing.[92,93]

Tumor cells also can be genetically modified to express immunomodulators that may enhance antigen-specific antitumor immune responses. Irradiation of cells transduced with the recombinant vector renders them unable to proliferate, yet still able to secrete the immunomodulator. Autologous melanoma cells are transduced with a recombinant adenovirus expressing granulocyte-macrophage colony-stimulating factor; cells then are irradiated and reinfused to patients with melanoma, resulting in an antitumor effect.[94] Likewise, autologous plasma cells transduced with recombinant adenovirus expressing interleukin-2 are irradiated and reinfused to patients with multiple myeloma.[95]

Protein-Based/Subunit Vaccines

Given the complexities discussed previously for inactivated vaccines, developing a (purified) protein-based vaccine is the favored strategy for many pathogens in which the isolated polypeptides contain the protective epitopes. Protein-based approaches have relied on genetic, biochemical, and immunological analyses to identify the specificity of protective antibodies to polypeptide antigens. Genomics also has enabled the identification of new vaccine antigens. Once the complete sequence (or portions thereof) of the genomic DNA or RNA becomes available, open reading frames are identified. Then, the derived amino acid sequences are inspected for features suggestive of surface localization (e.g., homologies with other proteins that are vaccine candidates or a hydrophobic N-terminal sequence). The genes are expressed in a recombinant host cell (typically *E. coli*), and the recombinant polypeptide is purified and used to immunize animals. Alternatively, the gene is injected into mice as a DNA vaccine (see "Nucleic Acid–Based Vaccines" later). The derived antisera can be used in biological assays (neutralization of viruses, opsonization of bacteria, or binding to the pathogen or human cancer cell surface) to see whether the protein may be an attractive vaccine candidate. The new protein is also used in animal challenge models. One of the earliest applications of genomics technology to viruses was to hepatitis C virus following the cloning of its viral genome.[96] The application of genomics to *Neisseria meningitidis* was accompanied by the analysis of approximately

600 putative proteins, resulting in the identification of several leading candidate vaccine antigens through a process now termed *reverse vaccinology*.[97] This meningococcal vaccine has now been licensed for use in Europe, Canada, the United States, and Australia.[98]

Numerous discovery technologies have been used to identify human proteins (or carbohydrates) that are candidate cancer vaccine antigens, including unique tumor-specific and tissue-specific antigens and shared disease-related antigens expressed in a range of tumors but not in normal adult tissues.[99] These antigens have been engineered and formulated into vaccines based on their polypeptides (or carbohydrates) by means of many of the technologies described subsequently.

Viral Targets for Protein-Based Approaches. The first protein-based vaccines relied on natural sources of antigens. In this regard, the first hepatitis B vaccine is unique in using a human source (plasma) for the vaccine antigen. Liver cells of persons chronically infected with hepatitis B virus (HBV) shed excess viral surface protein into the blood; that is, hepatitis B surface antigen (HBsAg). HBsAg was identified as a 22-nm lipoprotein particle antigen with protective epitopes. To develop a vaccine, plasma was harvested from long-term chronic hepatitis B carriers, HBsAg purified, and the final preparation subjected to one to three inactivation techniques (depending on the manufacturer) to kill HBV and any other adventitious human agents possibly present in the starting plasma.[100] This vaccine was well tolerated and highly efficacious, but is no longer used as the vaccine can now be made by recombinant technology.

Bacterial Targets for Protein-Based Approaches. Proteins purified from cultures of *B. pertussis* have been combined to formulate acellular pertussis vaccines, which have replaced whole-cell pertussis vaccine for routine pediatric vaccinations in most developed countries. Depending on the number of different protein antigens, these licensed acellular pertussis vaccines are referred to as one-, two-, three-, four-, or five-component vaccines.[101–103] These vaccines all contain pertussis toxoid,[104] which when combined with other pertussis antigens, comprises the acellular pertussis vaccines. One preparation of the pertussis toxoid component is described in "Genetic Inactivation" later.

Tumor Cells. Proteins isolated from individual tumors have been used as cancer vaccines. In one application, individual tumors were isolated, the autologous gp96 heat-shock protein was purified from each tumor, and the heat-shock protein preparation was injected into the respective subject, resulting in stimulation of CTL responses.[105]

Chemical Inactivation. Many bacteria produce protein toxins that are responsible for the pathogenesis of disease, including tetanus and diphtheria. The toxin molecules are purified from bacterial cultures (*Clostridium tetani* [T], and *Corynebacterium diphtheriae* [D]), and then detoxified by incubation with a chemical such as formalin or glutaraldehyde. These detoxified toxins, referred to as *toxoids*, are included in the diphtheria, tetanus, and pertussis (DTP) combination vaccine.[106,107]

Genetic Inactivation. The chemical "toxoiding" procedure has the disadvantages of potential alteration of protective epitopes (with ensuing reduced immunogenicity) and the potential for reversion to a biologically active toxin. However, rDNA technology can be used to produce a stable toxoid. For example, pertussis toxin was mutated (in two locations to ensure stability) to reduce its enzymatic activity. The altered gene was substituted for the native gene in *B. pertussis*, resulting in an immunogenic but stably inactivated pertussis toxoid.[108] This double-mutant pertussis toxoid (which also is treated with formalin under mild conditions to improve its immunogenicity or stability) is a component of one particular acellular pertussis vaccine,[101] which is not in use clinically at this time, while other acellular pertussis vaccines use nonrecombinant pertussis toxoid and are described in more detail in Chapter 44. A genetic approach to derive a D toxoid also was successful following the mutation of *C. diphtheriae* cultures and screening for enzymatically inactive yet antigenic toxin molecules. This genetic toxoid (CRM_{197})[109] is the protein carrier for a licensed *Haemophilus influenzae* type b (Hib) conjugate vaccine and pneumococcal conjugate vaccine (see later section). This technology also has been applied to *V. cholerae* toxin and ETEC toxin to produce candidate mucosal adjuvants (see later section).

Recombinant Polypeptides. The expression of recombinant polypeptides as subunit vaccines arguably has been the most extensively used and widely accepted application of rDNA technology to the development of new vaccines and these approaches are summarized.

Particle-Based Vaccines. The first application of rDNA technology to the production of a vaccine was for hepatitis B through the expression of the HBsAg gene in baker's yeast *Saccharomyces cerevisiae*,[110] giving rise to 22-nm HBsAg particles. In contrast, expression of the HBsAg gene in *E. coli* gave rise to HBsAg polypeptides but not HBsAg particles. These HBsAg virus-like particles (VLPs) were very similar in structure to HBV virions. Purified yeast-derived HBsAg is adjuvanted with aluminum salts and this vaccine has largely supplanted the equally efficacious and well-tolerated plasma-derived vaccine that was initially approved by the U.S. Food and Drug Administration in 1981 and subsequently discontinued in 1990 (no longer available in the United States). With the goal of an edible vaccine, HBsAg also has been expressed in transgenic potato tubers and has been shown to be safe and immunogenic in clinical trials.[111]

Particles are almost always more immunogenic than individual polypeptides (Fig. 66.1). Furthermore, particles (including VLPs) usually elicit antibodies to conformational epitopes on the particle (and on the respective virus), whereas isolated surface polypeptides of the particle might not. Examples of such particle immunogens are hepatitis A virus virions (immunogenic in humans at dosage levels as low as 50 ng) and VLPs for human papillomavirus (HPV). The HPV virion is a highly ordered structure whose major protein is L1 and when expressed in eukaryotic cells, results in the formation of VLPs that elicit HPV-neutralizing antibodies. Bivalent, quadrivalent, and nonavalent vaccines have been shown to be highly effective and have been licensed.[112–114]

Natural Protein Sequences. There are innumerable ongoing applications of rDNA technology to produce natural proteins as candidate vaccine antigens for viral, bacterial, and parasitic infections. The major *Borrelia burgdorferi* surface protein (OspA [outer surface protein A]), expressed in *E. coli* as a recombinant lipoprotein,[115] was licensed for the prevention of Lyme disease, but is no longer produced. Recombinant HIV-1 gp120 (rgp120) expressed in Chinese hamster ovary (CHO) cells[116] has been formulated into a vaccine that was in Phase III clinical studies and was reported to have no efficacy in the populations studies.[117,118] Similarly, CHO cell–derived recombinant HSV glycoproteins were evaluated in Phase III clinical trials[119] and demonstrated overall efficacy of 9%, with no effect on the duration of the first clinical episode of genital HSV-2 or the frequency of subsequent recurrences. A *N. meningitidis* serogroup B vaccine composed of two recombinant lipidated

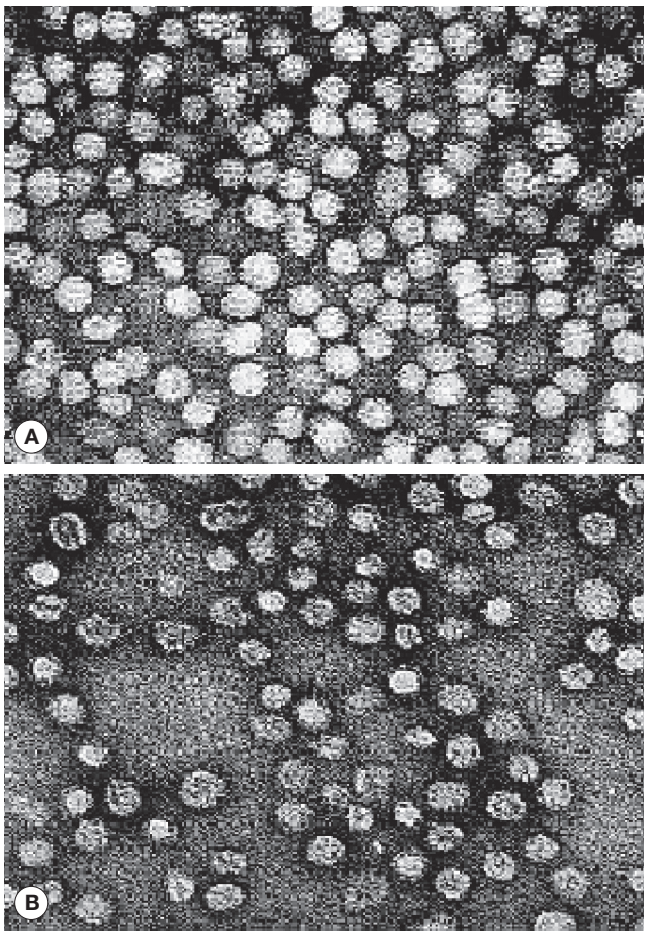


Figure 66.1. A, Recombinant hepatitis B virus–like particles. **B,** Recombinant human papillomavirus virus–like particles.

factor H binding protein variants individually produced in *E. coli* was licensed in the United States for individuals 10 to 25 years of age.[120] Finally, an AS01 adjuvanted[121] subunit vaccine containing recombinant varicella-zoster virus glycoprotein E has demonstrated 97% efficacy in a Phase III clinical trial for reducing the risk of shingles in adults older than 50 years of age[122] and provides a safer alternative for subjects with impaired cellular immunity (and at greatest risk for herpes zoster) for whom the previously approved live attenuated herpes zoster vaccines is contraindicated.[123]

Fusion Proteins. Recombinant fusion proteins containing multiple peptide sequences have been expressed as multiepitope vaccine antigens. A fusion protein containing the extracellular domains of two *Streptococcus pneumoniae* surface proteins has been evaluated in clinical studies.[124] A recombinant *Streptococcus pyogenes* vaccine consisting of 26 M protein type-specific peptide sequences integrated into four recombinant fusion proteins has been shown to stimulate responses to all 26 included *S. pyogenes* serotypes.[125] Similarly, the expression of four of five antigens, as fusion proteins, in the *N. meningitidis* serogroup B (MenB) vaccine increased immunogenicity,[126,127] and has been licensed for use in individuals from 2 months of age and older.[128]

Some of these vaccines are the products of the application of a new technology called *structural biology*. Purified proteins are grown into crystals whose X-ray diffraction pattern reveals the structure of these proteins. As of 2017, more than 126,000 biological molecular structures have been deposited into online protein data banks. Some of these structures for purified proteins help develop better vaccines by revealing their function and molecular interactions, while other structures, such as those for protective antibodies in subjects controlling their infections, help researchers understand why they are effective and what immunogens they are specifically recognizing so that recombinant immunogens can be designed that specifically reelicit these antibodies.[129] Two noteworthy efforts highlighting the power of structural biology are HIV-1 and RSV vaccine development. For example, structural biology has been used in the design of vaccines for HIV-1 by deciphering how neutralizing antibodies recognize the viral spike which targets the virus to specific receptors on host cells, an approach also being used for influenza and Ebola vaccines.[130–133] For RSV, structural biology revealed that RSV neutralization by the humanized monoclonal antibody motavizumab (which is 10-fold more potent than the licensed antibody palivizumab and differs only by 13 residues) was likely because of the recognition of a different quaternary configuration of the fusion (F) glycoprotein.[134] Motavizumab either binds to or induces a conformation of the trimeric F glycoprotein that is different from that observed in the PIV5 F prefusion structure. Subsequent structural biology studies on the RSV F glycoprotein identified prefusion-specific antibodies that were more potent than the licensed monoclonal antibody palivizumab and bound to a newly identified site of vulnerability named antigenic site ∅.[135] These effort led to the design of a fusion glycoprotein vaccine maintaining this newly identified site that elicited levels of RSV-specific neutralizing activity many times the protective threshold in immunized mice and macaques.[136]

Noninfectious Disease. Vaccine applications for allergy, oncology, and autoimmunity have also been evaluated. Because crude extracts from allergens such as ragweed can be administered to ameliorate allergic symptoms, allergen-encoding genes, expressed in a heterologous host cells, have also been designed. A recombinant dust-mite allergen polypeptide has been engineered to reduce its capacity to induce in vivo skin-test reactivity and in vitro histamine release from basophils of allergic patients, while retaining essential T-cell epitopes.[137]

Before the clinical symptoms of type 1 diabetes develop, autoantibodies become detectable to pancreatic β-cell autoantigens (e.g., insulin), following which β cells are destroyed by autoimmune attack. A range of recombinant autoantigens can prevent the development of type 1 diabetes in mouse models. Subsequent clinical trials have shown that subcutaneous injection of recombinant *E. coli*–derived insulin into prediabetic patients resulted in a significant delay in the development of clinical type 1 diabetes.[138] Studies of the intranasal instillation of recombinant insulin have also been promising.[139] An initial Phase I/II study using an engineered DNA plasmid encoding proinsulin has been demonstrated to preserve β-cell function in type 1 diabetes through the reduction of insulin-specific T cells.[140] While promising, the effects of this DNA-based vaccine need to be confirmed in larger clinical studies which are planned.

Custom Design. Recombinant fusion proteins can be custom designed for specific immunological purposes. Based on the observation that microbial heat-shock proteins can induce $CD8^+$ CTLs, immunization of mice with a fusion of *M. bovis* BCG heat-shock protein-65 with HPV-16 E7 protein was shown to induce $CD8^+$ therapeutic efficacy against HPV $E7^+$ tumor cells.[141] This fusion protein has advanced into clinical trials for treatment of HPV-associated malignancies.

DNA Shuffling. Rather than creating a specifically designed molecule, it is also possible to iteratively create new recombinant molecules and screen and select the ones with desired

BOX 66.1 Hosts for the Expression of Recombinant Proteins

BACTERIA

Escherichia coli
Bordetella pertussis
Vibrio cholerae

YEAST

Saccharomyces cerevisiae
Hansenula polymorpha

MAMMALIAN CELLS

Chinese hamster ovary
African green monkey kidney

LYMPHOID CELLS MAMMALS

Goat
Sheep
Cow

INSECT CELLS

Spodoptera frugiperda

PLANTS

Tomato
Potato
Tobacco

immunological properties as vaccine candidates. This *DNA shuffling* method has been applied to a range of vaccine candidates including *Plasmodium* falciparum, dengue virus, HIV-1, and hepatitis B but have not entered clinical testing.[142]

Host Cell Expression Systems. Many host cells have been used for the expression of heterologous recombinant genes (Box 66.1), including a range of bacterial and yeast cells and mammalian continuous cell lines (CCLs), such as CHO and African green monkey kidney (Vero). Smaller proteins not requiring posttranslational modifications generally can be expressed in immunogenic form in microbial expression systems. In 2013, an influenza vaccine was licensed that contains recombinant hemagglutinin protein produced in a continuous insect cell line using a baculovirus vector and is approved for use in people 18 to 49 years of age,[143] the first licensed pharmaceutical product made in insect cells.

Other nonvaccine proteins produced in microbial expression systems have been developed and licensed for human therapeutic applications; such proteins include insulin, growth hormone, interferons, and interleukin-2. In contrast, polypeptides that require appropriate folding and posttranslational modifications (e.g., glycosylation) for optimal immunogenicity or activity are expressed in mammalian CCLs. Licensed nonvaccine therapeutic proteins produced in mammalian CCLs include tissue plasminogen activator, erythropoietin, colony-stimulating factors, and monoclonal antibodies (MAbs). Whole animals (sheep, cattle, and goat) and plants (e.g., tobacco) also can be used as hosts for recombinant protein expression.[144,145]

Peptide Based

B-cell epitopes against which neutralizing antibodies are directed (neutralization epitopes) usually can be identified in a polypeptide. Some B-cell epitopes are conformational (as in VLPs), being formed by the juxtaposition in three-dimensional space of amino acid residues from different portions of the polypeptide, meaning that such epitopes require the full polypeptide for proper immunogenicity. In contrast, other peptide epitopes are linear, being fully antigenic as sequences of between six and 20 amino acid residues. Many linear epitopes are weakly immunogenic when presented in the context of the full polypeptide, such that the full or partial polypeptide containing a neutralization epitope might not be an effective immunogen for that epitope (a hypothetical mechanism for a pathogen to escape immunological surveillance). For example, a linear neutralization epitope has been defined for the *P. falciparum* (malaria) circumsporozoite protein (repetitive four-amino-acid sequence).[146] The circumsporozoite polypeptide contains a linear epitope that is recognized by neutralizing antibodies, yet the whole polypeptide elicits such antibodies only weakly. However, several approaches have addressed the immunogenicity of peptides containing B-cell epitopes and peptides with T-cell epitopes and are listed in Table 66.1, including fusion proteins, fusion peptides, and conjugation for example.

B-Cell Epitopes

Aggregate. Some peptides have the ability, under appropriate incubation conditions, to form small or large aggregates that are more immunogenic. The Aβ protein, associated with the development of Alzheimer disease, is processed into a 42-amino-acid peptide that forms large fibrils and soluble aggregates in vivo. A candidate Alzheimer disease vaccine was formulated by incubating Aβ peptide (1 to 42) into insoluble fibrils. This vaccine was shown to prevent the development of dementia in a transgenic mouse model of Alzheimer disease.[147] In initial clinical studies in humans, vaccination induced cellular immune responses that were associated with serious adverse events[148] (e.g., aseptic meningoencephalitis) such that the design of the vaccine is being refined.

Fusion Protein. The immunogenicity of peptide epitopes can be increased by genetic fusion to a carrier protein particle. Two such fusion partners are HBsAg[149] and hepatitis B core antigen,[150] a 28-nm HBV-encoded particle. The peptide fusion can be at the N- or C-terminus or at an internal sequence of the carrier protein, depending on which location provides the best immunogenic presentation while maintaining particle formation. A fusion of a circumsporozoite peptide to HBsAg has shown clinical promise as a malaria vaccine and results from an 18-month follow-up postvaccination demonstrated that three doses of this AS01-adjuvanted vaccine resulted in a vaccine efficacy of 46% in children and 27% in infants, and could be an important addition to current malaria control in Africa.[151] A positive opinion has been received from the European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP) on July 24, 2015, for use outside the European Union despite its limited efficacy because the benefits of the vaccine outweighed the risk in both age groups studied (especially to children in high transmission areas in which mortality is very high).[152] Melanoma-associated antigen 3 (MAGE-A3) is a tumor-specific protein identified on melanoma cells, non–small cell lung cancers, and hematologic malignancies.[153] A cancer vaccine composed of a fusion protein of MAGE-A3 and *H. influenzae* protein D and a proprietary immunoadjuvant was developed as a therapeutic vaccine against lung cancer, but development was recently halted after results from a Phase III study showed that it did not meet its primary end point in significantly extending disease-free survival.[154]

Fusion Peptide. A B-cell epitope from the target protein can be rendered immunogenic by fusion to a T-helper (Th) epitope. A 16-amino-acid peptide from cholesterol ester transfer protein fused to a 14-amino-acid Th epitope from tetanus toxoid (TT) increases plasma high-density lipoprotein and

decreases aortic lesions in rabbits fed a cholesterol-rich diet. It also has been shown to induce anti–cholesterol ester transfer protein antibodies in humans.[155] The Phase II study, however, did not meet the primary end point of increasing plasma high-density lipoprotein–cholesterol concentrations in the vaccine-treated groups as compared to the placebo groups, so further preclinical studies using adjuvanted vaccines are ongoing.[156]

Conjugate. The peptide is synthesized with a reactive amino acid residue, through which it can be covalently linked or conjugated to a carrier protein. The most commonly used carrier proteins in conjugates are bacterial proteins that humans commonly encounter such as TT, for which a conjugate with the malarial circumsporozoite epitope has been tested clinically.[157] Conjugation technology also has been applied to the formulation of a fertility vaccine based on human chorionic gonadotropin (hCG) or luteinizing hormone–releasing hormone. Antibodies to these peptide hormones have been shown to inhibit fertilization, although both luteinizing hormone–releasing hormone and hCG are poorly immunogenic. Conjugates of hCG or luteinizing hormone–releasing hormone to TT elicit antibodies that inhibit fertilization in laboratory animals and have been evaluated clinically as fertility vaccines.[158] Furthermore, because hCG is expressed on pancreatic and prostate tumor cells, an hCG conjugate vaccine has been evaluated clinically as a therapeutic cancer vaccine.[159] A peptide from the tumor-specific antigen MUC-1 conjugated to keyhole limpet hemocyanin has been evaluated for immunotherapy of non–small cell lung carcinoma.[160]

Complex Peptide. Multimers of the peptide sequence, such as multiple antigen peptides, can be synthesized for linkage in repeated arrays with resultant increase in peptide immunogenicity,[161] as shown with the malarial circumsporozoite peptide epitopes.[162] The application of the four aforementioned strategies to weakly immunogenic peptides has resulted in immunogenic presentations that elicit substantially increased titers of neutralizing antibody compared with those elicited by the epitope presented alone as a peptide or in the context of its natural full-length polypeptide. Nevertheless, the most effective strategy in terms of ultimate clinical usefulness is established on a case-by-case basis.

Mimotopes. By screening peptide libraries with antisera, peptides (mimotopes) can be identified that antigenically mimic the immunogen reactive with such antisera. This approach is a way to create an immunogen that cannot be produced readily by recombinant or synthetic methods. A peptide mimotope on the HBsAg S polypeptide, which reacts with antibodies to HBsAg, elicited antibodies to HBsAg when fused with a carrier protein.[163] The LeY antigen is a neolactoseries carbohydrate expressed on human tumor cells. Given the difficulty in isolating or synthesizing sufficient quantities of this carbohydrate for use as a vaccine immunogen, a peptide mimotope was isolated that mimics LeY; immunization of mice with a multiple antigen peptide mimotope elicited anti-LeY antibodies and reduced tumor growth in a mouse tumor model.[164] It also has been possible to use peptides that mimic carbohydrate epitopes on HIV-1 to elicit HIV-1–neutralizing antibodies.[165]

T-Cell Epitopes. Effective immune prophylaxis or therapy for some diseases requires CTL or other T-cell immunity rather than neutralizing antibodies. T-cell epitopes elicit various T-cell immune functions and have been applied mostly to therapeutic vaccines

Cytotoxic T-Lymphocyte Epitopes. Peptide epitopes recognized by CTLs may be useful immunogens for HIV and *M. tuberculosis* or immunotherapy for chronic diseases such as hepatitis B. CTL peptide epitopes generally are poor immunogens. Thus, for an immunotherapeutic hepatitis B vaccine, a CTL epitope from the HBV core protein was modified by covalent linkage to a Th epitope (from TT) and two palmitic acid molecules.[166] This vaccine was shown in clinical studies to be effective in eliciting HBV-specific CTLs and memory CTLs. T-cell epitope peptides conjugated to the cytosine phosphate guanine (CpG) oligonucleotides adjuvant show enhanced CTL activity against HIV.[167]

T-Cell Activation. Peptides that bind to human leukocyte antigen on the tumor surface can be formulated as mixtures of tumor-associated peptides. Injected tumor-associated peptides can activate T cells to attack and destroy tumor cells. The most advanced vaccine is in a Phase III clinical study for kidney cancer.[168]

T-Cell Receptor. Therapy of autoimmune disease can involve *T-cell vaccination*,[169] for which the target antigen is the T-cell receptor and the goal is to reduce T-cell–mediated autoimmune activity. This approach has been applied successfully for glatiramer acetate,[170] a peptide copolymer of alanine, lysine, glutamic acid, and tyrosine that is licensed for the immunotherapy of multiple sclerosis. It may function by inducing a shift from Th1 responses to glatiramer acetate–specific Th2 cells, which can mediate bystander suppression and, thus, ameliorate autoimmune antimyelin responses.

Carbohydrate and Polysaccharide Based. Many bacteria have an outer polysaccharide capsule. Antibodies directed against capsular polysaccharide are protective for most encapsulated bacteria, thus establishing capsular polysaccharide as leading vaccine antigens. Components of lipopolysaccharide, another abundant surface molecule on gram-negative bacteria, also may be vaccine antigens. Carbohydrates specific to the surface of certain types of cancer also have been used as vaccine antigens.

Polysaccharide. The natural capsular polysaccharide contains up to hundreds of defined repeat units distinct for each bacterial species and antigenic subtype; each monomer consists of monosaccharides, phosphate groups, and other small organic moieties. The polysaccharide is shed by the organism during its growth and is harvested and enriched from the culture medium. These polysaccharide preparations are usually immunogenic in adults and children older than age 2 years. The polysaccharide elicits antibodies that may mediate bacteriolysis or opsonization of the organism, thereby protecting against infection. Polysaccharide vaccines have been licensed for Hib[171] (monovalent), *N. meningitidis*[172] (quadrivalent), and *S. pneumoniae*[173] (tridecavalent). The shortcomings of these vaccines are that the polysaccharides, being T-cell–independent immunogens, are poorly immunogenic in children younger than age 2 years owing to the immature status of their immune systems, and polysaccharides do not elicit immunological memory or booster activity in older children and adults.

Conjugated Polysaccharide. Infants and children younger than age 2 years respond immunologically to T-cell–dependent immunogens such as proteins. Conjugation of a polysaccharide to a carrier protein converts the polysaccharide from a T-cell–independent to a T-cell–dependent immunogen. Consequently, polysaccharide conjugate vaccines can elicit protective immunoglobulin (Ig) G and immunological memory in infants and young children. Thus, conjugate vaccines using different carrier proteins display one or more differences in the following properties: response of 2-month-old infants to the first dose of vaccine, responses of 4- and 6-month-old infants to the second and third doses, response of children older than age 1 year to a booster dose, kinetics of decay of antibody levels, peak antibody titer, and age at which protection first can be shown. This strategy is important

particularly for encapsulated bacteria such as Hib, *S. pneumoniae* (pneumococcal), and *N. meningitidis* owing to the preponderance of invasive diseases caused by these bacteria in children younger than age 2 years.

The Hib bacteria are a single serotype (b). There are several licensed Hib conjugate vaccines[174] with different carrier proteins (TT, diphtheria toxoid, CRM_{197}, and an outer membrane protein complex from *N. meningitidis*) of different sizes, distinct polysaccharide chain lengths, and distinct conjugation chemistries that result in different immunological characteristics. For example, even though the Hib conjugate vaccine using outer membrane protein generates a rapid response, the Hib conjugate vaccine using CRM_{197} generates a much higher response.

Pneumococcal bacteria consist of approximately 90 serotypes, as reflected in distinct capsular polysaccharide structures. For designing a pediatric pneumococcal conjugate vaccine, seven serotypes have been established as responsible for approximately 60% to 75% of the major pediatric pneumococcal diseases (acute otitis media, pneumonia, meningitis) in developed countries, and a heptavalent pneumococcal conjugate vaccine was the first pneumococcal conjugate vaccine licensed for pediatric use.[175] A tridecavalent conjugate vaccine (PCV13) consisting of a mixture of 13 individual pneumococcal polysaccharide conjugates was licensed in 2010, and represents the vaccine with the most conjugate antigens included.[176–178] There was also licensed in 1983 a 23-valent pneumococcal polysaccharide vaccine (PPSV23) that contains more antigens, but these are not conjugated. Therefore, PPSV23 potentially affords broader protection against invasive pneumococcal disease because it covers a larger proportion of serotypes (85% to 90% compared to PCV13's greater than 60%) but does not demonstrate the higher immunogenicity seen with the conjugated vaccine.[179]

Invasive meningococcal disease is caused by *N. meningitidis* strains expressing one of five diverse capsular polysaccharides: A, B, C, W135, or Y. A quadrivalent meningococcal conjugate vaccine (A, C, W135, or Y) has been available for use in people in the United States and Canada based on the diphtheria toxoid carrier protein. A conjugate vaccine based on the mutant diphtheria toxin (CRM_{197}) also has been developed to provide immunity in children younger than age 2 years and is currently indicated for use in adolescents and adults.[180,181]

A conjugate of group B streptococcal polysaccharide has been used to immunize pregnant women[182] for the prevention of neonatal group B streptococcal meningitis. In this case, the polysaccharide conjugate vaccine would be advantageous over a polysaccharide vaccine in terms of eliciting high enough anti–polysaccharide antibody titers to enable sufficient protective IgG to cross the placenta to the fetus.

Other Carbohydrate. Tumor cells have specific carbohydrate structures lacking or deficient in other cell types. Such carbohydrates may have usefulness as cancer vaccines. Examples include the use of STn carbohydrate for ovarian and breast cancer[183] and GM_2 ganglioside for melanoma.[184] Such carbohydrates are rendered more immunogenic by conjugation to carrier proteins, as is done for capsular polysaccharide vaccines.

Other

Other technologies include those related to antiidiotypic antibodies and the conjugation of derivatives of drug substances to the B subunit of CT. Anti–idiotypic antibodies[185] represent a feasible vaccine strategy as demonstrated by the immunization of chimpanzees with anti–idiotypic antibodies mimicking HBsAg that protected from HBV infection.[186] Applications of this strategy to cancer therapy have involved the use of the anti–idiotypic antibodies as a mimic of a tumor antigen (but not identical and therefore not a self-antigen) to elicit an immune response against the tumor antigen.[187] Similar applications have been developed for metastatic breast cancer[188] and for parasitic infections caused by *Schistosoma mansoni*.[189] A highly novel approach has been to develop a vaccine to counter addiction to cocaine based on preclinical observations that antibodies can bind and clear cocaine. A derivative of cocaine conjugated to the B subunit of CT has been shown to induce anticocaine antibodies in cocaine abusers and in cocaine-abstinent subjects, with some indication of attenuation of cocaine's euphoric effects.[190]

Nucleic Acid–Based Vaccines

A technology that became fashionable during the 1990s was the use of DNA encoding vaccine antigens. The in vitro model for this technology lay in the transformation of cells in culture with a plasmid that directs the synthesis of a vaccine antigen. After cells in vivo take up DNA encoding vaccine antigens, the antigens can be secreted or associated with the cell surface in a way that triggers both humoral or cellular immune responses. DNA uptake was facilitated by chemical formulation, electroporation, or delivery by a nonreplicating virus or bacteria; the nonreplicating virus or bacteria approach fits the definition of a DNA-based vaccine (nonreplicating in vivo). Although this approach awaits clinical proof of principle, it is considered to have much long-term potential. Because the principles for both DNA and RNA approaches are similar in nature, the following examples focus on DNA but conclude with a summary of nonviral delivery approaches focusing on RNA.

Naked DNA

The initial strategy has been to inject intramuscularly a solution of uncoated or *naked* DNA encoding a vaccine antigen.[191] Cells take up the DNA, transcribe its expression cassette, and synthesize the antigen, which may be processed similarly to a live viral infection. Humoral or cellular immune responses to the encoded antigen are elicited (Fig. 66.2). The advantages of DNA vaccines are the relative technical ease of production and the ability to direct the synthesis of multiple copies of messenger RNA, hence amplification of antigen synthesis and

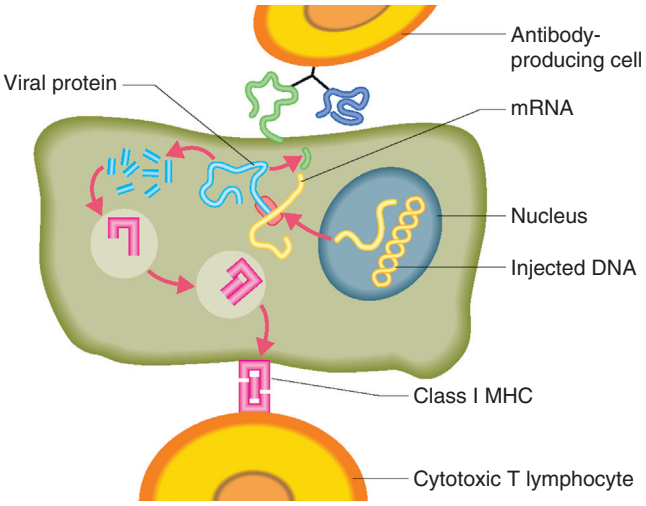


Figure 66.2. Injection of DNA encoding a foreign protein can elicit antibodies and cytotoxic T lymphocytes. MHC, major histocompatibility complex; mRNA, messenger RNA.

immune responses. Such vaccines have been shown to be effective in many animal models of infection, especially virus models.[192] Theoretical safety-related issues needed to be addressed for DNA vaccines as a novel technology. Preclinical studies showed that these vaccines did not induce detectable anti-DNA antibodies. Furthermore, the frequency of integration of plasmid DNA into chromosomal DNA was undetectable and was calculated to be much less than the rate of spontaneous integration events.[193]

Clinical studies with naked DNA vaccines for influenza, HIV, and malaria have shown these vaccines to be well tolerated.[194,195] This first generation of DNA vaccines was shown to elicit low and sporadic levels of specific antibodies and was ineffective. Subjects receiving approximately 1 mg of malaria DNA vaccine generated specific CTL activity. Beyond an excellent safety profile, these initial clinical data have shown that the immunogenicity of naked DNA vaccines needed to be augmented to be clinically effective, as discussed in the next section. These vaccines may have their best usefulness for inducing cell-mediated immune (CMI) responses or as priming doses in prime-boost regimens (see later section).

Facilitated DNA

Delivery. Facilitation can be at the level of cellular uptake of DNA, expression of messenger RNA, or immunological activation. DNA has been incorporated into microprojectiles that then are "shot" directly into cells, which then produce the encoded antigen that stimulates an immune response. DNA can be coated onto gold microparticles and delivered as dry powder into the epidermis with a needle-free device; such a hepatitis B vaccine was shown to be immunogenic.[196] For increasing uptake, DNA has been coated with cationic lipids, lipospermines, or other molecules that neutralize its charge and have lipid groups for facilitating transfer across membranes.[197] Such formulations also are being investigated for alternative routes of injection (besides parenteral) that may elicit mucosal immunity. The anesthetic bupivacaine given in conjunction with DNA enhances DNA uptake and expression.[198]

Composition. The base composition of the DNA may affect its potency. Unmethylated CpG dinucleotides, which are present in bacterial but greatly underrepresented in mammalian DNA, represent structures that stimulate innate immunity in response to bacterial infection. As part of DNA vaccines or as adjuvants per se (see later section), CpG dinucleotides have been shown to induce B-cell proliferation and Ig secretion, thereby modulating immunogenicity.[199]

While DNA vaccines elicit specific antibodies, they are particularly proficient at eliciting cellular immune responses, including CTLs. Therefore, naked (or facilitated) DNA vaccines may be useful in cancer immunotherapy as is being investigated with a plasmid DNA-lipid complex for metastatic renal cell carcinoma[200] or with interleukin-2 cytokine delivery as a plasmid formulation for treating melanoma which moved into Phase III studies.[195] A Phase I clinical trial for metastatic melanoma using plasmid pUMVC3-hIL-12-NGVL3 and electroporation to deliver antigens/cytokines directly into tumors demonstrated varying levels of tumors regression in patients.[201]

Viral DNA Vector

A variation on the design of the expression plasmid is to use a virus-based DNA expression system that can amplify the level of RNA and protein expression as occurs in a live virus infection. Such a system has been developed based on Sindbis virus DNA vectors.[202]

Viral RNA Vector

A self-replicating RNA encoding a tumor antigen can be used as a nucleic acid–based vaccine. Plasmids encoding RNA replicase of Semliki Forest virus and antigens of interest are subsequently digested to generate a DNA fragment which undergoes in vitro transcription into RNA. When injected, this RNA mediates the production of the replicase polyprotein and drives the replication of the antigen of interest. Using this approach, prophylactic and therapeutic RNA immunization elicited antibodies and CTL activity protecting mice from tumor challenge or prolonging survival, respectively.[203] Efficacy correlated with apoptotic death of transfected cells, followed by DC uptake of dead cells, which could provide a mechanism for enhanced immunogenicity.

Noninfectious RNA can be used to mimic immunization with a live flavivirus[204]; following transfection to host cells, noninfectious subviral particles are secreted. The delivery to mice of gold microparticles coated with RNA elicits neutralizing antibodies and protection against lethal challenge.

Viral Delivery

For DNA vaccine delivery by fowlpox or canarypox virus, the expression cassette for the recombinant protein is integrated into the viral genome. Although able to productively infect avian cells, these poxviruses can infect mammalian cells and produce protein products but not infectious virus.[205,206] This single round of self-limiting infection may suffice to elicit broad immunity to a pathogen whose recombinant polypeptide is expressed by these avian poxviruses in infected cells, while reactogenicity would be less than that associated with vaccinia virus (given the inability of the avian poxviruses to spread). Fowlpox virus has been tested as part of a DNA prime (pHIS-HIV-B) and a recombinant fowlpox virus (rFPV-HIV-B) boost regimen.[207,208] Canarypox virus (see "Viral RNA Vector" above) expressing HIV-1 rgp160 or rgp120 has been used as part of a prime-boost HIV-1 vaccination regimen (see later section).[209,210]

Bacterial Delivery

Bacteria that replicate intracellularly can be engineered to deliver plasmid DNA into host cells for expressing vaccine antigens.[211] *S. flexneri* has been attenuated by a deletion mutant in the *asd* gene, an essential gene. While such a strain can be propagated in vitro in the presence of diaminopimelic acid and can invade cells (as long as it maintains a plasmid encoding invasion- associated polypeptides), it cannot replicate in vivo, where diaminopimelic acid is unavailable. A plasmid harboring a eukaryotic promoter and recombinant gene was transformed into this strain. The resultant recombinant *S. flexneri* strain was able to invade mammalian cells in vitro and to express the plasmid-encoded protein as a vaccine antigen. Because *S. flexneri* replicates in the intestine and stimulates mucosal immunity, this vector may be delivered orally for delivering DNA to cells where mucosal immunity is stimulated. Other bacterial species that can invade mammalian cells but not divide also may be able to deliver recombinant plasmids to different cell types for expressing recombinant proteins as vaccine antigens.

Nonmicrobial RNA Delivery

Injection of naked messenger RNA or self-amplifying RNA generates immune responses but has limited potency because of RNA instability in vivo.[212] Therefore, messenger RNA vaccines have been formulated with delivery vehicles including

liposomes and cationic polymers for increasing potency,[213,214] and preclinical studies using this approach have demonstrated prevention of progression and metastasis of melanoma.[215] Based on innovations in systemic delivery of short interfering RNA using lipid nanoparticles, self-amplifying RNA vaccines have also been developed that contain an alphavirus genome encoding only the RNA replication machinery and with the structural protein genes being replaced with the protein antigens of interest. This approach has been found to elicit broad, potent, and protective immune responses comparable to viral delivery technology.[216]

Additional Considerations and Strategies for Active Vaccines

Formulation

The immunogenicity of inactivated, subunit, and DNA-based vaccines may be enhanced by their formulation, which refers to the final form of the vaccine to be administered. In addition to the vaccine *active substance* (antigen or DNA), the formulation also may contain an adjuvant and/or delivery system. The adjuvant is a substance that stimulates an increased humoral and/or CMI response to a coadministered antigen. The delivery system is a vehicle for ensuring the presentation of the vaccine in vivo to appropriate cells of the immune system or for stabilizing and releasing the antigen during an extended period. Many future vaccines are expected to contain new adjuvants and/or delivery systems. There are reviews[217,218] with extensive bibliographies for the wide variety of available experimental adjuvants and delivery systems. Some experimental adjuvants stimulate primarily antibody responses, whereas others stimulate primarily CMI responses, including CTLs. Table 66.4 outlines a range of benefits and challenges associated with the use of novel adjuvants and delivery systems.

TABLE 66.4 Adjuvants and Delivery Systems

Potential Benefits	Key Challenges
Dosage level ↓	Species-specific differences in cells, receptors, signaling pathways, and tissue distribution of common components of the immune system targeted by adjuvants may lead to erroneous conclusions regarding human safety and tolerability
Number of doses ↓	Long-term safety is difficult to ascertain given the indolent nature of chronic adverse events (e.g., autoimmune diseases), the lack of feasibility of long-term follow-up of subjects, and the contribution of differences in human leukocyte antigen genetics to certain safety signals
T-cell responses ↑	Demonstrated need for inclusion in prophylactic vaccines targeting healthy or young subjects has a different benefit-to-risk perception compared to patients with cancer or difficult-to-treat infectious diseases
Antibody level ↑	Analytical definition of adjuvants is complex and impacted by their classification based on source, mechanism of action, or physiochemical properties (e.g., active immunostimulants, carriers, vehicles, route of administration, salts, tensoactive agents, emulsions, particulates, lipopeptides)
Antibody quality ↑	Stability of adjuvants, long shelf life, and biodegradability are ideally expected but not always easily obtained
Responses ↑ for impaired persons	Pharmaceutical definition of what they really are, where the antigen is, and potential for long-term stability for all components
Mucosal immunity	Formulation needs to take into account route of administration and potentially adds complexity/costs to the manufacturing process

Adjuvants

Aluminum Salts. Aluminum salts (hydroxide or phosphate) are the only adjuvants broadly licensed for inclusion in human vaccines. They have been widely used for 70 years and injected into more than 1 billion people worldwide. The vaccine antigen binds ionically to the aluminum salt[219] and preferentially promotes a Th2-type immune response (i.e., antibody-based) and, thus, is not useful for inducing a CMI response. While aluminum salts improve immunogenicity for a number of licensed vaccines (e.g., hepatitis B, pertussis), they do not enhance immune responses to all vaccines.

Given the need to improve on the adjuvanting properties of aluminum salts, many chemicals, biochemicals from natural sources, and proteins with immunomodulatory activity (cytokines) have been studied as potential adjuvants. The adjuvanticity of most known formulations is associated with local or systemic side effects that may be mechanism-based or nonspecific.[220] The ideal adjuvant needs to achieve a balance between safety and immune enhancement. The immunobiology of the particular vaccine application dictates whether one would prefer an adjuvant that would enhance antibody responses or CMI responses.

Other Licensed Adjuvants: MF59, AS03, AS04, Liposomes, and AS01. MF59 (an oil-in-water emulsion) formulated with inactivated subunit influenza vaccine shows increased immunogenicity and acceptable clinical tolerability and has been licensed in Europe since 1997.[221] Another squalene-based oil-in-water emulsion called AS03, was widely used in vaccines for the 2009 H1N1 influenza pandemic.[222,223] Approximately 1 year following the influenza pandemic, reports from some European countries confirmed an association of an AS03-adjuvanted pandemic vaccine with the development of a neurologic sleep disorder, narcolepsy, in vaccinated individuals.[224,225] As of January 2015, more than 1300 cases of vaccine-associated narcolepsy have been spontaneously reported to the European Medicines Agency EudraVigilance database.[226] In contrast, no increased risk has been reported for the MF59-adjuvanted pandemic vaccine,[227] which could lead one to speculate about the safety of certain adjuvants used in vaccines. However, a study published in July 2015 may have established an immunologic link between influenza virus and narcolepsy through antigenic molecular mimicry, thus making the independent role of AS03 in triggering narcolepsy less likely.[228] Specifically, molecular mimicry was identified between a fragment of one of the influenza antigens (nucleoprotein) contained in the AS03-adjuvanted pandemic vaccine and a portion of the human brain receptor (hypocretin receptor 2) promoting wakefulness. The authors additionally demonstrated the presence of antibodies capable of cross-reacting with both influenza nucleoprotein and the hypocretin receptor which theoretically could disrupt wakefulness signaling triggered by binding of hypocretin protein to the hypocretin receptor 2. Using mass spectrometry, influenza nucleoprotein was demonstrated to be present in significant amounts in the AS03-adjuvanted inactivated split-virion pandemic vaccine compared to the MF59-adjuvanted inactivated subunit

pandemic vaccine (not associated with narcolepsy) that contained approximately 73% less influenza nucleoprotein as a result of an additional subunit purification step (see "Inactivated Vaccines" earlier). These findings and the association of narcolepsy also with influenza infection make the direct role of adjuvants alone in triggering autoimmune disease in genetically susceptible subjects less likely. This is attested to by both AS03- and MF59-adjuvanted pandemic vaccines not triggering or exacerbating disease activity in patients with established or active autoimmune disease, respectively.[225] Other adjuvants include AS04 (deacylated monophosphoryl lipid A [MPL] plus aluminum) formulated with recombinant yeast-derived hepatitis B vaccine that elicits significantly increased antibodies to HBsAg antibody responses[229] and is approved as formulations for HBV and HPV vaccines in Europe and, recently, in the United States.[222] Liposomes (virosomes) are another adjuvant technology that has gained approval and has been licensed as a component of a seasonal influenza vaccine and a hepatitis A vaccine.[230,231] AS01 is a liposome-based vaccine adjuvant containing 3-O-desacyl-4′-monophosporyl lipid A and the saponin QS-21 and is included in the RTS,S malaria vaccine and the subunit glycoprotein E varicella zoster vaccines.[121]

Adjuvants in Development. As noted previously, oligonucleotides containing unmethylated CpG structural motifs have been shown to enhance protein and peptide vaccine responses in diverse clinical experiences.[232]

Certain bacterial toxins with adenosine diphosphate (ADP)-ribosylating activity have been engineered as mucosal adjuvants. In particular, CT was shown to be active as a mucosal adjuvant for a coadministered antigen[233] when presented by the oral, nasal, vaginal, or rectal route, as was shown subsequently for the heat-labile toxin of ETEC. These toxins are composed of a catalytic A subunit and a pentameric B subunit that binds to GM_1 ganglioside on many cell types. CT and labile toxin are toxic in humans, especially by the oral route (through which they induce diarrhea); there also has been concern about the use of these adjuvants by the nasal route, given the development of Bell palsy after administration of an influenza virosome vaccine containing labile toxin in humans.[234] Consequently, point mutations have been made to create toxoids with reduced or eliminated ADP-ribosylating activity, and hence toxicity, while retaining adjuvanticity in mice.[235] An alternative approach has been to eliminate the B subunit of CT and substitute a synthetic dimeric peptide derived from *Staphylococcus aureus* protein A (DD) that binds to Ig. The fusion of the CTA subunit with the DD domain binds to Ig-positive cells, appears devoid of toxicity, retains ADP-ribosylating activity, and is active as an adjuvant in mice.[236] The tolerability and effectiveness of these engineered adjuvants needs to be evaluated in humans.

Novel adjuvantation has been applied to cancer vaccines. A 25-amino-acid peptide from the MUC-1 tumor-associated antigen is formulated with MPL and three lipids into a liposomal product. Vaccination of subjects with non–small cell lung and prostate cancer has shown positive clinical effects.[237]

Bioinformatic Technologies and Vaccine Safety

As mentioned in the previous section on "Adjuvants," the association of vaccine-associated narcolepsy in 2010 with one pandemic influenza vaccine containing a squalene-based adjuvant but not another pandemic influenza vaccine containing a squalene-based adjuvant suggested a primary role for the vaccine antigen as the trigger for developing this chronic sleep disorder.[225] This antigen-related hypothesis is also consistent with the association of narcolepsy with the 2009 pandemic influenza infection.[238] Adjuvants are not intrinsically linked to autoimmunity, but may instead uncover potential cross-reactivity mediated by B or T cells to similar epitopes contained in microbes and normal human tissue. Computational tools have now been developed to help uncover the potential cross-reactive epitopes and avoid vaccine components that could induce potentially deleterious immune responses to self-tissue. In addition to the bioinformatics tools for comparing microbial peptides for similarity to human proteins (i.e., Basic Local Alignment Search [BLAST] algorithms and sequence alignments), there are databases containing the structures of human leukocyte antigen molecules that are associated with certain autoimmune diseases and are important to consider as these molecules provide the context in which T cells can recognize cross-reactive peptides.[228] T-cell epitopes are now rapidly discoverable by cutting edge computer algorithms which can now find cross-reactive epitopes that induce autoimmunity or immune regulation by looking at the T-cell receptor "side" of the epitope.[239,240] While the approach for future vaccines will undoubtedly evolve with innovations in technologies, what is clear is that some difficult targets will require adjuvants to elicit the necessary immune response to provide protection. Such vaccines have the potential to uncover cross-reactive epitopes that may lead to immune-mediated diseases in genetically susceptible subjects. The computational tools are now approaching maturity that may enable vaccines to be refined to precise formulations containing the exact proteins or epitopes that contribute to protective immunity and from which cross-reactive epitopes have been removed.

For example, in the case of influenza vaccine (where a cross-reactive epitope has been identified from influenza nucleoprotein with high similarity to the human hypocretin receptor implicated in narcolepsy), one could apply some of the technologies discussed in this chapter to reduce or remove this cross-reactive epitope as follows: (a) applying subunit purification steps that are known to effectively minimize the total amount of influenza nucleoprotein transferring into the final vaccine product[228] or (b) genetically engineering influenza virus reassortant strains (similar to the approach used for some live-attenuated HSV vaccines[23,24]) such that the cross-reactive epitope is deleted or mutated enough so that its similarity to the human hypocretin receptor is lost (thus enabling vaccine manufacturers to continue using their preferred technology for producing their specific influenza vaccine, i.e., live-attenuated, inactivated split-virion, or inactivated subunit). Alternatively, the recombinant influenza vaccine produced in a continuous insect cell line and licensed in 2013 only contains influenza hemagglutinin[143] but is not approved for use in people younger than 18 years of age. When such hemagglutinin-only vaccines receive approval for younger ages, yet another possible solution to the problem of vaccine-associated narcolepsy (mediated by cross-reactivity to a fragment of influenza nucleoprotein) will be within arm's reach. However, any advantages (e.g., generation of cross-clade protection/development of a "universal" influenza vaccine) afforded by other epitopes contained in the highly conserved influenza nucleoprotein or other nonhemagglutinin influenza proteins would not be retained in hemagglutinin-only vaccines. The dilemma illustrated by this particular example is applicable to other vaccines and illustrates the continuous challenge facing vaccine developers trying to balance vaccine safety with vaccine efficacy.

Delivery Systems

Besides presenting an antigen, DNA, or RNA to their in vivo target cells, a delivery system may mediate other key activities. There may be a depot effect, whereby the antigen

is maintained in an appropriate site for continual immune stimulation. There may be an enhancement of in vivo stability of DNA or antigen. The delivery system may enable efficient presentation of mucosally delivered vaccines for uptake by M cells, followed by transcytosis into Peyer patches and presentation to lymphocytes for inducing mucosal immunity.[241–243] Certain formulations may maintain the vaccine inside a physical structure for a significant in vivo period, during which the vaccine is released slowly or in a pulsatile manner to function as a one-dose vaccine. No delivery systems have yet been widely licensed. Gaining clinical and pharmaceutical experience with new delivery systems and adjuvants remains a key goal in the field. A few specific viral and bacterial proteins have been shown to have adjuvanting and mucosal delivery properties in preclinical studies. A reovirus protein complexed with poly-L-lysine can adjuvant and present DNA for mucosal immune responses.[244] The *S. pyogenes* Sfp-1 protein has been reported to provide for mucosal adjuvantation.[245]

Comparing Specific Vector Technologies. The development of specific technologies for delivering vaccines by different routes of immunization (e.g., oral, nasal, transcutaneous) also has been a rapidly growing field. Once the vaccine has been delivered to the appropriate region of the human body, sufficient quantities of the antigen (and adjuvant) must gain access to appropriate cells to activate the immune system. The vehicles or vectors that may be used for this purpose include live attenuated viruses, live attenuated bacteria, commensal bacterial vectors, virosomes, VLPs, liposomes, lipopeptides, immune-stimulating complexes, microparticles, and nanoparticles.[246–250] Viruses are prototypical antigen delivery vehicles because they enter and commandeer cells to replicate themselves, thus multiplying the available antigen that they encode and inducing a natural adjuvant effect through activation of chemokines and cytokines. Recombinant viruses acting as vectors by incorporation of a gene expressing a heterologous antigen have similar advantages as conventional attenuated live virus vaccines. They deliver the foreign DNA or RNA into cells and get it replicated to activate the immune system. Viruses used as vaccine vectors ideally should have very low pathogenic potential, even in immunocompromised persons, and the capacity to hold the necessary foreign genes expressing the desired antigens and adjuvants. Bacteria have a major advantage over viruses as vaccine vectors because of their higher capacity for insertion of the heterologous genes expressing antigens, adjuvants, or plasmids for DNA vaccination. DNA vaccination involves the delivery of eponymous plasmids directly into host cells to express the desired antigens.

Nonreplicating vaccine delivery systems, including immune-stimulating complexes, liposomes, microparticles, nanoparticles, virosomes, and VLPs, mimic live viruses in the manner they deliver antigen and adjuvant. They are particles about the same size as viruses, allowing similar uptake by antigen-presenting cells. Many include a lipid component to increase cell membrane permeability and viral or bacterial proteins to activate the immune system. Liposomes are vesicles composed of a phospholipid bilayer membrane. Antigen can be packaged in its aqueous core, inside the lipid bilayer, or on the outside of the membrane.[251–253] Immune-stimulating complexes are cage-like structures roughly 40 nm in diameter composed of 12 subunits of saponin (such as Quil A) and cholesterol. Delivery can also be enhanced by packaging antigens and adjuvants into microparticles or nanoparticles composed of polymers of biodegradable materials such as polylactide and polylactide coglycolide or into biopolymers such as chitin or chitosan.[254–260] Microparticles can be designed to slowly release antigens to increase the duration of antigen presentation. Many recombinant bacterial vectors are administered orally. Antigen mixed with CT, heat-labile toxin, or their toxoid derivatives and applied to the skin can stimulate transcutaneous immunization.[261]

Adapting Strategies

Vaccine development historically has used the direct approach of a vaccine made by a single technology delivered in one or more doses. More recently with increased appreciation of the immunobiology of particular infections and diseases that are resistant to conventional single-technology vaccine approaches, regimens have been developed that use mixtures of vaccines made by different technologies (mixed vaccines) or different types of vaccines given during the overall dosing regimen (prime-boost regimens).

Mixed Vaccines

Oral inactivated whole-cell cholera vaccine, which lacks CT and its toxic effects, is well tolerated and efficacious.[80] To elicit CT-neutralizing antibodies, recombinant CT B subunit (rCTB) is independently expressed, purified, and added back to the whole-cell cholera vaccine. This combined whole-cell cholera + recombinant CT B subunit vaccine has been shown to have a higher rate of efficacy than whole-cell cholera vaccine alone.[262] Since CT B subunit is immunologically cross-reactive with the B subunit of labile toxin, the combined vaccine also shows some clinical efficacy against labile toxin–positive strains of ETEC.

Kinoids are a derivative of inactivated cytokines, of which the second-generation kinoids are also coupled to a molecule of keyhole limpet hemocyanin (immunomodulatory foreign carrier protein). This heterocomplex enables a T-cell response against the foreign carrier protein, thereby helping a B-cell response against the selected cytokine. This strategy induces elevated titers of neutralizing antibodies to counteract the pathological effect of local overproduced cytokines. Tumor necrosis factor-α–kinoid vaccines have been tested in early phase clinical trials in patients with Crohn disease,[263] Phase II/III studies in asymptomatic HIV-1–infected patients,[263] and in a Phase II study in tumor necrosis factor antagonist–resistant rheumatoid arthritis patients.[264] An interferon-α–kinoid vaccine has also been tested in Phase I/II studies in systemic lupus erythematosus patients.[265] These studies have demonstrated safety/tolerability and a trend toward clinical improvement warranting further studies.

Prime-Boost Regimens

Vaccination regimens with a sequence of different vaccines has shown that some vaccines are more efficient at priming and others at boosting immunological memory; these prime-boost regimens have been evaluated, particularly for HIV-1 vaccines. Canarypox virus expressing recombinant HIV-1 rgp160 or rgp120 has been administered in two priming doses followed by boosting with rgp160 protein[266]; this regimen induced higher levels of neutralizing antibodies than immunizing with rgp160 protein alone. A regimen consisting of a canarypox-vector prime plus a protein-subunit boost was responsible for the first demonstration to date of protection against HIV infection in Thailand[267] and the administration of the same vaccination protocol to 100 volunteers in South Africa generated similar results.[210] Likewise, clinical studies of two priming doses of rgp160 followed by boosting with a peptide from the major type-specific neutralization epitope of gp160 demonstrated higher levels of HIV-1–neutralizing antibodies than those elicited by rgp160 alone.[268] Priming with adenovirus expressing HIV-1 gp160 followed by boosting with HIV-1

rgp120 elicited persistent titers of virus-neutralizing antibodies and CTL activity and protected chimpanzees from HIV-1 challenge.[269]

The aforementioned studies have demonstrated that using a vaccine with superior priming characteristics followed by boosting with the appropriate related vaccine may be an improved regimen for eliciting higher antibody titers. Such a regimen is more complicated to develop technically and clinically and would not be generally useful in a case where a one-vaccine regimen is effective,. However, it may be useful for targets such as HIV-1 or malaria that have proven refractile to more conventional vaccine approaches.

Combination Vaccines

The increasing number of vaccines available and the desire for fewer needle sticks at the time of vaccination has fostered the use of *combination vaccines*, which are defined as two or more vaccines given in a physically mixed preparation. Combination vaccines are of two types: (a) *multidisease*—mixtures of different types of vaccines, and (b) *multivalent*—mixtures of different serotype vaccines for the same vaccine target. Some combination vaccines are multidisease *and* multivalent. There are numerous challenges in successfully developing novel combination vaccines, which include physical compatibility, stability, and potential immunological interference. For example, the success in developing measles-mumps-rubella-varicella vaccine[8] came only after numerous attempts spanning almost two decades. Combination vaccine technology is a complex discipline that is beyond the technical scope of this chapter. Other chapters in this volume and in the textbook *Combination Vaccines*[270] present a plethora of technical issues associated with developing such vaccines.

ANTIBODY PREPARATIONS

Antibody preparations can be monoclonal or polyclonal. Table 66.5 lists some examples of polyclonal antibody preparations and licensed monoclonal antibody therapies providing immediate immunological activity necessary to prevent or treat certain infections, cancer, or pathological conditions. Examples include (a) postexposure prophylaxis for known or suspected exposure to HBV[271] or varicella-zoster virus[272] in persons who are unvaccinated or in immunosuppressed individuals; (b) preexposure prophylaxis for at-risk persons with an underlying pathology (transplant recipients infected with CMV)[273] or infants with pulmonary pathologies to prevent RSV infection[274]; and (c) administration to pregnant mothers who are viremic for CMV[273] for potential modulation of perinatal viral infection in newborns. The protective effect

TABLE 66.5 Examples of Targets of Polyclonal Antibody Preparations (Immunoglobulins) and Licensed Monoclonal Therapies for Infectious Diseases (All Years) and Noninfectious Diseases (Last 10 Years Only)

Polyclonal Products[a]	Monoclonal Products[b]
INFECTIOUS DISEASES	
Cytomegalovirus	Anthrax infection (Raxibacumab)
Hepatitis B	Respiratory syncytial virus (Palivizumab)
Rabies	
Tetanus	
Varicella zoster	
Immunodeficiencies/autoimmunity (using pooled, polyvalent, immunoglobulin G antibodies from plasma of healthy donors)	
NONINFECTIOUS DISEASES	
Lymphocyte	Neuroblastoma (dinutuximab)
Rho(D)	Psoriasis (secukinumab, ustekinumab) Melanoma (nivolumab, pembrolizumab) Non–small cell lung cancer (nivolumab) Acute lymphoblastic leukemia (blinatumomab) Gastric cancer (ramucirumab) Ulcerative colitis/Crohn disease (vedolizumab, certolizumab pegol [Crohn only]) Castleman disease (Siltuximab) Chronic lymphocytic leukemia (obinutuzumab, ofatumumab) Breast cancer (ado-trastuzumab emtansine, pertuzumab) Hodgkin lymphoma, systemic anaplastic large cell lymphoma (brentuximab vedotin) Systemic lupus erythematosus (belimumab) Metastatic melanoma (ipilimumab) Bone loss (denosumab) Rheumatoid arthritis (tocilizumab, golimumab) Muckle-Wells syndrome (canakinumab) Ankylosing spondylitis, psoriatic arthritis (golimumab) Malignant ascites (catumaxomab) Paroxysmal nocturnal hemoglobinuria (eculizumab) Macular degeneration (ranibizumab) Colorectal cancer (panitumumab, bevacizumab) Multiple sclerosis (natalizumab)

[a]Information from a search on "globulin" at PDR.net at: http://www.pdr.net/search-results?q=globulin.
[b]The Antibody Society. www.antibodysociety.org/news/approved-antibodies/.

mediated by most of these antibody preparations is to (a) neutralize virus infectivity; (b) bind to bacteria, which then are destroyed by phagocytic cells; or (c) bind to and neutralize molecules such as toxins elaborated by the pathogen (e.g., tetanus[275]) or therapeutic agents like digoxin.[276]

Polyclonal Antibodies

Human Immunoglobulin and Antibody Fragment

The earliest preparations of antibody or Ig were made in species such as horses that had been injected with bacterial toxoids. Although such antisera were therapeutically effective as antimicrobials, the xenogeneic antibody elicited serious side effects, such as serum sickness, in recipients. A newer product, equine rabies globulin, is widely used as a lifesaving therapy in developing countries (giving approximately 2% allergic reactions). Nevertheless, such Ig preparations tend not to be used in humans, especially in developed countries, except in emergencies when no alternative therapy is available.

A range of human polyclonal Ig products has been available for more than 20 years (see Table 66.5). Depending on the Ig under consideration, these products are prepared by pooling plasma from healthy volunteers (with known titers of the specific target antibody) who would have acquired high titers by vaccination or natural infection with possible silent boosting by the pathogen. The pooled plasma is fractionated with alcohol to enrich for antibody. The preparations are heat treated under conditions that are known to destroy the infectivity of human pathogens such as HIV-1. The final product is released on the basis of standardized specific antibody content. These products are generally efficacious for preventing infectious diseases.[271–275] However, the large injection volumes (normalized for body weight) and the high protein (antibody) content (as high as 1 g per administration) can result in adverse reactions in recipients. Polyclonal antibodies can be processed for clinical use into F_{ab}, that is, antigen-binding fragments of the whole Igs (Fig. 66.3).[276]

Monoclonal Antibodies

MAbs have been evaluated for cancer immunotherapy.[277,278] The underlying immunological mechanisms for such MAbs would include antibody-dependent cytotoxicity, direct CTL activity, the binding to tumor cells to mark them for attack and destruction by phagocytic cells, and stimulating apoptotic destruction. Another area in which there is renewed activity is HIV where first-generation monoclonal antibody combinations were found to be largely ineffective but new-generation antibodies derived using single-cell-based antibody cloning methods have revealed more potent broadly neutralizing activity against HIV-1.[279] These include MAbs to host cellular receptors, including CD4 and CCR5.[279,280]

The use of MAbs offers the prospects of avoiding human sources of Igs; improving the tolerability of passive vaccines by substantially reducing protein content and injection volume; and providing an antibody source of unlimited supply with unique specificity, absolute standardization, and high reproducibility. In some cases, a mixture of MAbs may be required to provide multiple epitope recognition akin to that of polyclonal sera. The invention of hybridoma technology in the mid-1970s provided for a source of murine MAbs (mMAbs) that required only the availability of the specific immunogen for mice.[281] Many developments in rDNA technology in the 1980s and 1990s have enabled the expression of high levels of recombinant MAbs in defined CCLs, the chimerization or humanization of mMAbs (Fig. 66.4), the isolation of human MAbs (hMAbs) from transgenic mice, and the screening for specific hMAbs from a "library" of MAbs developed in lieu of having enough antigen for immunization.

Furthermore, the development of next-generation sequencing technologies has enabled high-throughput DNA sequencing and characterization of functional antibody repertoires including both protective and pathogenic immune responses. Blood samples obtained from patients successfully responding to an infection (either quickly clearing or chronically sequestering an involved site) can be compared to patients unsuccessfully responding to an infection (either rapid mortality or requiring a much longer time to clear an infection). Bioinformatic identification of clonal families of antibodies uniquely associated with successful response to infection can narrow down representative antibodies for recombinant expression of therapeutic antibodies "passive vaccines/antibody preparations" in addition to identifying the antigen targets of functional immune responses relevant to vaccine development ("active vaccines").[282] The many technologies for

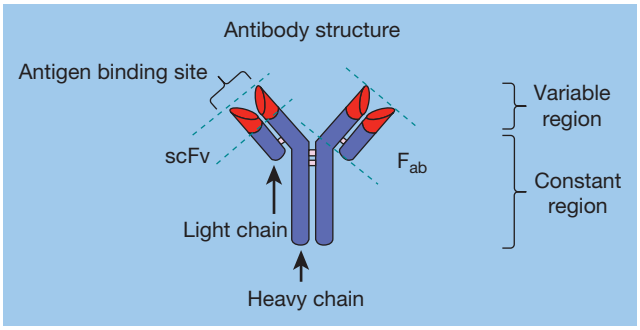


Figure 66.3. Structure of antibody molecule. F_{ab}, antigen-binding fragment; scFv, single-chain Fv molecule.

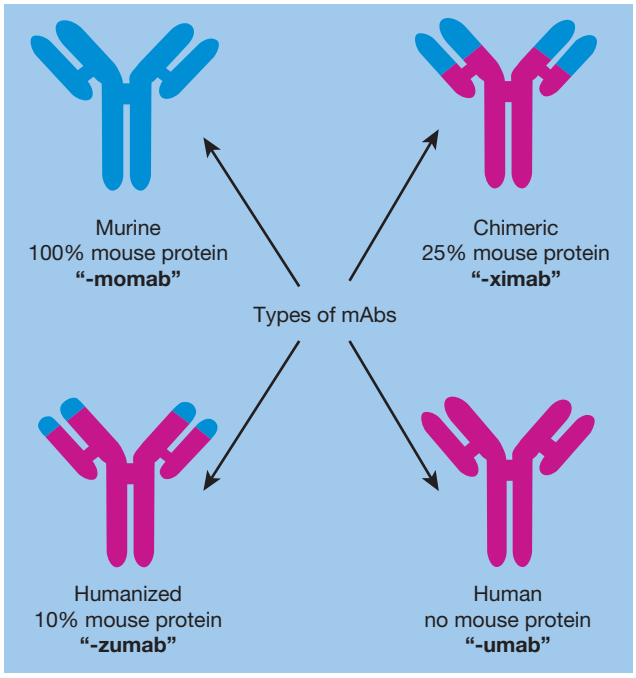


Figure 66.4. Comparison of the different types of monoclonal antibodies from murine to human and the nomenclature used to identify their composition.

developing whole MAbs or fragments or multimers thereof have been reviewed.[283]

Nonhuman

The first licensed therapeutic MAb of any type was a murine IgG_{2a} CD3-specific renal transplant rejection drug OKT-3 in 1986.[284] Given the technologies available to produce human, humanized, or chimeric MAbs and in light of issues regarding the tolerability of nonhuman antibodies, additional nonhuman mMAbs (see Fig. 66.4) are unlikely to become available for use as passive vaccines. A potential exception to this principle is a mMAb that stimulates a tumor-directed anti-Ig immune response by virtue of the presence of xenogeneic murine sequences.[285]

Natural Human

The first-generation technology for making hMAbs was adapted from murine hybridoma technology.[286,287] B lymphocytes are harvested from persons vaccinated for (or recently infected with) the desired pathogen; alternatively, B lymphocytes from healthy persons are cultured in vitro in the presence of the desired pathogen or antigen. Such B lymphocytes are immortalized by transformation in vitro with Epstein-Barr virus or by fusion with a human or murine myeloma cell. The resultant cell population is cloned and screened for the hMAb of the desired specificity. The positive cells are single-cell cloned, expanded, and preserved. The immortalized cells can be adapted to the desired growth medium and expanded to the scale necessary to manufacture a product. Alternatively, the hMAb genes can be cloned and expressed in a CCL to derive a recombinant hMAb. Given the technical challenges of maintaining the stability and large-scale consistent growth characteristics of the hybridoma cells in contrast with the reliable expression levels and stability of recombinant MAbs secreted from defined CCLs, the recombinant CCL-based route is generally preferred as a source of product.

Recombinant Human

Human Derived. The genes for the hMAb heavy (H) and light (L) chains from such hybridoma cells[288] are cloned and coexpressed in a CCL (e.g., CHO or NS0 cells) with known capability for high levels of recombinant MAb expression. Alternatively, human B lymphocytes are harvested, and the variable (V) regions of the IgG L and H chains are cloned en masse to produce an *E. coli* combinatorial V-region expression library, in which all combinations of different H and L chains are coexpressed in individual *E. coli* cells.[289] This combinatorial expression library is screened for specific antigen binding. The identified V genes of interest are recloned, reassembled with constant (C) regions into complete human H and L chain genes, and then coexpressed in a CCL (per natural hMAbs). Alternatively, the identified V regions may be used as less-than-complete antibody molecules (e.g., F_{ab} or single-chain F_v molecules) (see Fig. 66.3). Such a MAb fragment is being investigated for therapy of *Candida albicans* infections.[290] The efficiency of the combinatorial approach offers the advantages of flexibility of soliciting human B-lymphocyte donors without the need for antigen and the ability to find MAbs of higher affinity than found in a polyclonal antibody preparation. A recombinant hMAb binding to the CTLA-4 has been demonstrated to augment T-cell activation and proliferation of tumor infiltrating T-effector cells and reducing T-regulatory cell function and was licensed for the treatment of melanoma in 2011.[291] In 2014, two other hMAbs were licensed for the treatment of melanoma and block the inhibitory effect of either one or both of the programmed death (PD) receptor ligands (PD-L1 or PD-L2) on the PD-1 receptor expressed on the surface of activated T cells.[292]

Mouse Derived. A more recent and versatile technology that has attracted widespread use is the *XenoMouse*, which is a transgenic mouse into which transcriptionally active human Ig gene loci have replaced murine Ig loci.[293] MAbs derived from such mice through conventional hybridoma technology are fully hMAbs. Balancing the great versatility of deriving such hMAbs is the limitation that the human Ig gene repertoire in such XenoMice is incomplete. Such MAbs are advancing in clinical development for indications such as colorectal and kidney cancer.[294]

Recombinant Humanized

A mMAb can be changed into a *humanized* MAb (see Fig. 66.4) of the same antigenic specificity. The human immune system would not recognize the humanized MAb as foreign and, therefore, would not produce antibodies against it (other than antiidiotypic). The V regions of H and L chains contain three hypervariable regions or complementarity determining regions (CDRs), each five to 18 amino acid residues long. The six CDRs in an intact molecule come together in three-dimensional space to form the specific antigen-binding region of the antibody molecule (this region contains the idiotype and paratope, as discussed earlier). The three CDRs in each chain fall in linear sequence in the midst of four V-region less-variable framework regions. Humanization is accomplished by substituting on the DNA level the three CDRs from each murine H and L chain gene for the human CDRs in four human framework regions from individual H and L chain genes[295] (human framework region domains with sequence homology to the murine framework region domains are chosen for recombination). The substituted H and L chain V regions are reassembled with human H and L chain C regions, which are coexpressed in a CCL for scale-up and development as for any recombinant hMAb. The resultant humanized MAb contains only human-derived sequences except for the murine-derived CDRs and, consequently, has generally low immunogenicity, other than for an antiidiotypic response that may be elicited by any antibody.[296]

Humanized MAbs have been developed and licensed for applications in cancer, allergy, and infectious disease (see Table 66.5). Palivizumab, a humanized MAb that was developed based on its neutralizing activity, prevents RSV infections in at-risk newborns and is the first licensed MAb-based passive vaccine licensed for preventing an infectious disease.[297] Trastuzumab, which is specific for the HER2 (human epidermal growth receptor 2) protein, is licensed for immunotherapy of HER2-positive breast tumors.[277] Omalizumab, which binds to IgE, reduces symptoms of allergy by its ability to bind IgE and prevent IgE binding to mast cells and consequent histamine release.[298]

Recombinant Chimeric

There are potential technical limitations to being able to produce human or humanized MAbs; thus, chimeric MAbs (see Fig. 66.4) have been produced. The V region genes from mMAb H and L chains are combined with human C-region H and L chain genes, which then are expressed in CCLs as chimeric MAbs. Such MAbs have more murine-derived sequences than do humanized MAbs. Nevertheless, such MAbs have been shown to be well tolerated and effective in a range of applications. Rituximab, a chimerized MAb specific for CD20, is licensed for immunotherapy of non-Hodgkin lymphoma.[278]

CONCLUSION

The number of approaches for making new vaccines should continue to expand in the future such that almost all antigens or epitopes could be presented in a highly immunogenic form in the context of a live or inactivated vaccine or be expressed through a DNA-based vaccine. Further understanding of gene function in viral and bacterial pathogens should enable live vaccines to be more stably and predictably attenuated as vaccines and as live vectors for vaccinating against other pathogens. Adjuvant and delivery system technologies should provide formulations that are more potent than aluminum salts, yet are safe and well tolerated, and enable delivery by routes other than injection. Bioinformatics tools should enable the refinement of vaccine antigens to exclude those that are potentially cross-reactive with antigens found in normal human tissue thus limiting the generation of pathogenic autoimmune responses related to molecular mimicry. Similarly, formulations of DNA may improve potency and the ability of DNA to be delivered by routes that elicit mucosal immunity. MAb technologies will provide for finer epitope specificity and for increased diversity of antigen recognition. There also should be significant further developments in applications to noninfectious diseases, such as cancer and autoimmune diseases, building on successes to date in the fields of multiple sclerosis, whole-cell cancer vaccines, and MAbs for non-Hodgkin lymphoma and breast cancer (see Table 66.2).

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67 Development of Gene-Based Vectors for Immunization

David B. Weiner and Gary J. Nabel

Vaccines can confer immune protection against infectious agents through divergent arms of the adaptive immune response. The elaboration of antibodies through the humoral immune system has been highly effective in the neutralization of many bacteria, viruses, fungi, and parasites. The cell-mediated immune response also plays a major role in containment of infectious agents as well as in eliminating pathogenic cells. T lymphocytes comprise a diverse set of cells, and their functional activity depends on helper T cells, which elaborate a variety of cytokines and stimulate B cells to produce antibodies and modulate the induction and expansion of cytotoxic T lymphocytes (CTLs). CTLs recognize processed antigen in combination with major histocompatibility complex (MHC) molecules and release cytokines that can influence pathogen replication as well as lyse infected cells. In addition, T-regulatory cells are induced as part of an immune response and can play a negative role in the clearance of chronic infectious agents and in preventing clearance of pathogenic cells.

Both humoral and cellular immunity are elicited by vaccines, each with their own effector functions that can inactivate pathogens in different ways (Box 67.1). Although the humoral immune response is well known to confer protection, the role of CTL in protective immunity against viral infections has been recognized more recently. The function and specificity of CTLs have provided the foundation for understanding MHC restriction and its importance in protection against viral infection.[1,2] Cellular immune responses help control infectious diseases, in concert with antibody responses or when it is difficult to generate neutralizing antibodies, as in patients with AIDS, malaria, hepatitis C virus, or tuberculosis. Humoral immunity is readily induced by protein antigen preparations or inactivated viruses together with appropriate adjuvants. Gene-based vaccines, the engineering of nonviral RNA- or DNA-based systems, or viral recombinant systems to express foreign antigens when delivered to a host appear to be particularly effective at inducing T-cell responses, both CD4 and CD8. At the same time, some gene-based vaccines can induce humoral immune responses when used with specific vectors or in specific prime-boost combinations, or through enhanced delivery and formulation. A variety of vectors, nonviral and viral, have been developed for these purposes (Fig. 67.1), the most studied with a focus of being in or entering the clinic are reviewed here.

The majority of conventional adjuvants that have been previously used in vaccine development affect humoral immunity and enhance antibody responses without inducing cellular immunity. In contrast, gene-based vaccine vectors can stimulate both humoral and cellular immunity, thus providing greater selective pressure on infectious agents. In this chapter, the major gene-based vaccines progressing in clinical trials are discussed, together with the advantages and disadvantages of the individual vectors and their influence on the different effector arms of the immune system. Although there is considerable experience with inactivated viruses and protein-based vaccines, there is less experience with gene-based vaccine vectors as they are more recent developments. Their ability to induce both humoral and cellular immunity and their safety and mode of antigen presentation are attractive features that must be balanced with the limitations in knowledge about clinical efficacy, production methodology, and concerns about antivector immunity (Box 67.2). However, there is a growing clinical experience that is guiding the analysis of these approaches as well as recent compelling vaccine efficacy data. Based on their early successes and advantages in conceptual development, it is likely that these vectors will make important future contributions to vaccinology.

NONVIRAL VECTORS AND DNA VACCINES

Vaccination with plasmid DNA generated excitement in the early 1990s when four independent groups simultaneously reported that plasmid gene delivery resulted in in vivo antigen expression with resulting immune responses to the plasmid-encoded antigens. Stephan Johnson's laboratory used a device called the gene gun to "shoot" gold beads coated with a plasmid encoding human growth hormone into mice.[3] The animals generated antibody to growth hormone with this approach. Other groups also attempted to harness the power of plasmids for vaccination purposes and presented their work in a session at the Cold Spring Harbor Vaccines meeting in the fall of 1992. The laboratories of Margaret Liu (Merck) and Harriet Robinson (University of Massachusetts) vaccinated mice intramuscularly with plasmids expressing influenza antigens and demonstrated the generation of antigen-specific immunity.[4,5] In addition, David Weiner (University of Pennsylvania) reported that formulations of plasmids containing either HIV envelope or tumor antigens were capable of stimulating both cellular and humoral immune responses in mice or in the case of the tumor antigen formulations, impacting tumor growth.[6] These novel results, combined with the simplicity and ease of manufacturing, spawned research into DNA vaccines for a plethora of viral, bacterial, parasitic, and cancer targets.[7–12]

At this time the DNA vaccine platform was perceived by the scientific community to be an important new approach, as conceptually, DNA has multiple advantages over traditional live attenuated, killed, peptide-based, and viral vector vaccines.[13,14] For example, DNA is easy to manipulate, it combines the simplicity of synthetic chemistry or bacterial production with the power of genomics, and it allows the rapid design and construction of multiple potential vaccines by removing entirely the need to develop vaccines using pathogen-derived materials. In addition, DNA vectors are extremely stable, reducing the need for a cold chain and increasing product shelf life. Therefore, the ease of production, stability, and cost-effectiveness of this platform made it ideal for producing vaccines for the developing world. DNA vectors themselves are not immunogenic, which allows repeat administration without developing immune interference. Importantly, DNA vaccines combine the appeal of live replicating vaccines that induce broad cellular and humoral immune responses with the safety and ease of manufacturing of a nonlive, nonspreading platform.[15,16] As DNA vaccines are nonreplicating, they eliminate the risk of attenuation reversions in the host, dissemination in the recipient, the unintended consequences of secondary infections by transfer to unvaccinated populations, and they can be delivered to high-risk groups, including immunocompromised subjects. In the clinic, DNA vaccines

BOX 67.1 Mechanisms of Immune Protection by the Adaptive Immune Response

CELLULAR

- Lysis of infected cells
- Elimination of source of production of viruses and intracellular pathogens
- Elaboration of antimicrobial cytokines
- Recruitment of innate immune effector cells
- Induction of long-term immune memory
- Elaboration of chemokines to recruit inflammatory responses
- Secretion of proteins that block pathogen receptors

HUMORAL

- Reduction of initial microbial inoculum
- Direct neutralization of pathogen
- Complement-mediated lysis of bacteria and parasites
- Lysis of infected cells through antibody-dependent, cell-mediated cytotoxicity
- Recruitment of inflammatory cells via complement-dependent mechanisms
- Generation of secretory immunoglobulin A to facilitate mucosal elimination of pathogens

BOX 67.2 Advantages and Limitations of Gene-Based Vectors for Vaccines

ADVANTAGES

- Potent immunogenicity in animal and human infectious diseases
- Ability to induce cellular immunity with or without humoral immunity
- Relative ease of production for many viral and nonviral vectors
- Ease of analysis and screening in the laboratory
- Favorable safety profile and lack of persistence in vivo
- Efficient transduction of cells and reasonable production capability
- Many potential prime-boost combinations

LIMITATIONS

- High level of immunity to some vectors in humans
- Need for qualified packaging cell lines
- Induction of antivector immunity after initial injection of viral vaccines, limiting efficacy of homologous boost
- Potential complexity with multiple vectors in prime-boost
- Limited long-term safety data
- Need to develop large-scale manufacturing processes

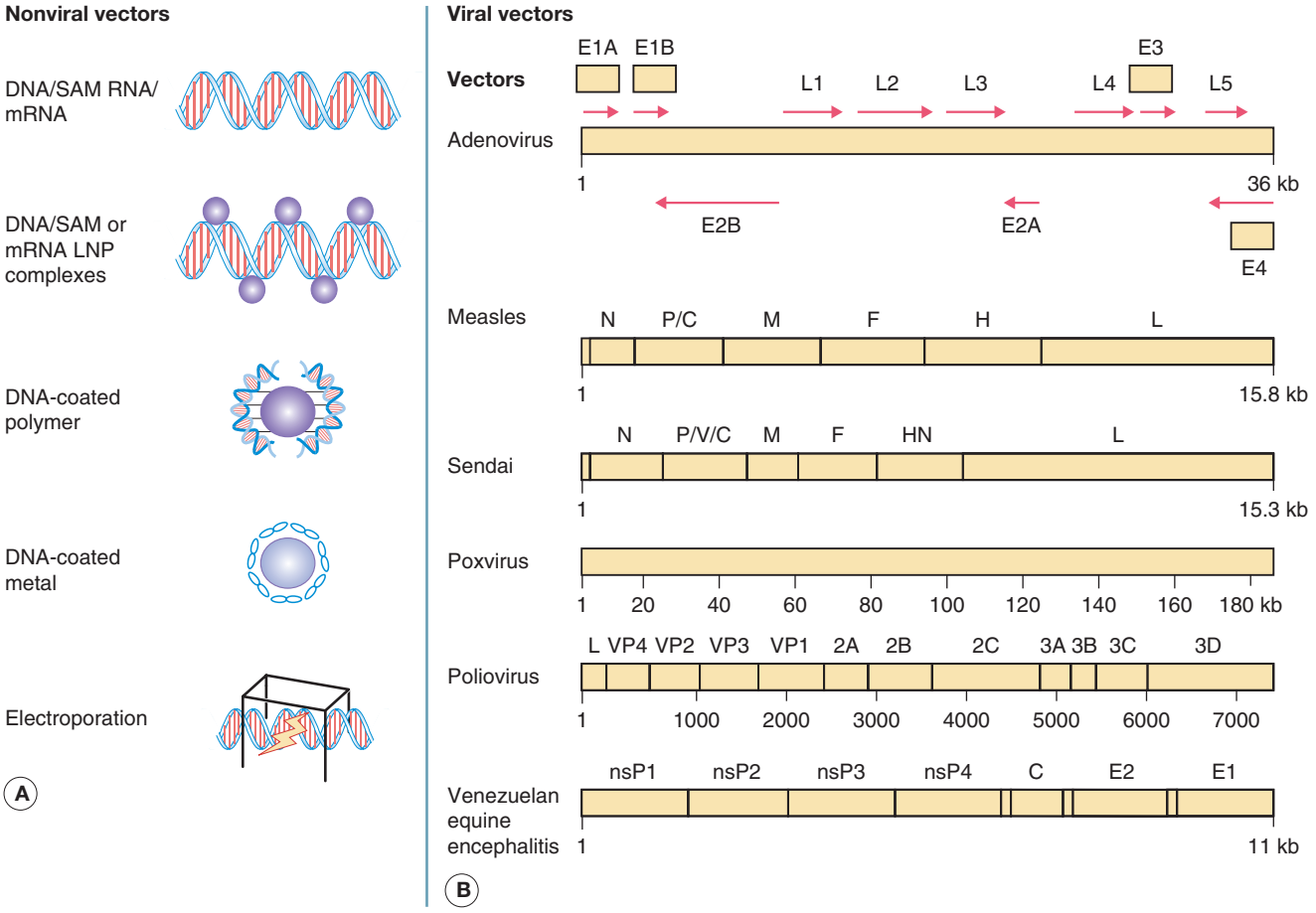


Figure 67.1. Representative vector platforms for gene-based vaccines that have advanced into clinical trials. Vaccination by gene delivery with nonviral **(A)** and replication-defective recombinant viral gene-based **(B)** vectors are shown. **B** shows the genetic organization and virus structure of the natural replication-competent virus.

have provided an unparalleled safety profile over the past decade and a half of study.[17] With some constraints, DNA vaccine products can be developed in repeat-use manufacturing facilities, thus providing enormous savings in product development and manufacturing.

Mechanism of Action

The mechanisms surrounding the generation of antigen-specific immunity with gene-based vaccines are important to understand. An antigen sequence of interest is designed to target a particular antigen or set of antigens of a pathogen or tumor antigen, potentially subjected to additional modifications (as described below), and inserted into a mammalian expression plasmid vector.[18–25] For use in humans, such vectors often share common features, including a high-copy-number origin of replication to enhance production, a human cytomegalovirus immediate-early promoter to drive in vivo expression, an RNA polyadenylation sequence that can be derived from bovine growth hormone or created synthetically to facilitate ribosome function, and a plasmid growth selection sequence such as a limited kanamycin bacterial gene or a nonantibiotic selection sequence to monitor the expression of the plasmid of interest during production.[15,17] The plasmid vaccine is then delivered to the skin by intradermal injection or intramuscularly by one of several delivery methods. Once inside the cytoplasm of the cell, the plasmid enters the nucleus of transfected cells (e.g., myocytes, keratinocytes, or local resident antigen-presenting cells [APCs]),[26–28] where the plasmid-encoded sequences drive host cell transcription, producing the foreign antigen in vivo. The host-synthesized antigens then become the subject of immune surveillance in the context of both MHC class I and class II molecules of the vaccinated host.

There are very specific differences between DNA vaccination and infection with the pathogen that the vaccine is intended to prevent. With DNA vaccines, antigen delivery remains local after plasmid transfection into cells, with little spreading of antigen expression to other regions of the body. The plasmids themselves represent a focused antigen, epitope, or multiple antigens of the pathogen and not the entire pathogen capable of dissemination.

The exact details of DNA vaccine-induced immunity remain a subject of debate. IM injection of the DNA vaccine has been shown to induce $CD8^+$ T-cell responses with more limited antibody production.[7] After IM injection, myocytes are likely transfected and dendritic cells present in the transfected muscle efficiently cross-present antigens to activate MHC class I–restricted T cells.[27] Alternatively, APCs in the muscle could be directly transfected and express antigen via the MHC class I pathway to activate CTL.[29] In contrast, intradermal (ID) administration has been reported to result in a more robust humoral response consisting of immunoglobulin (Ig) G_1 antibody production.[4,30] Because the dermis is rich in APCs, such as Langerhans and dendritic cells, ID vaccination, could result in APC transfection and antigen secretion, with either MHC class I or MHC class II presentation. APCs are also constantly sampling the environment through endocytosis, resulting in the uptake of secreted antigen predominant expression on MHC class II cells. It is thought that plasmid-encoded DNA triggers immune activation through stimulation of innate immune sensors, which include PAMPS (pathogen-associated molecular patterns) or the STING (stimulator of interferon genes)–TBK1 (TANK-binding kinase 1) pathways.[31,32] Such activated APCs express chemokines and cytokines that enhance immune cell trafficking and inflammation.

Animal Studies

Several DNA vaccines have been licensed for use in animals,[33–36] including dog melanoma immunotherapy, porcine recombinant growth hormone, vaccine for the prevention of rhabdovirus disease in fish, and West Nile virus vaccine for horses. These promising outcomes have yet to be translated to humans.

Prime-Boost Emergence

The success in preclinical models by DNA vaccines led to clinical studies in humans, initiated in the early 1990s. The goals of these human studies were to evaluate the safety, tolerability, and immune potency of the platform. Diverse DNA vaccines for a variety of prophylactic and therapeutic applications were studied, including HIV-1,[37] influenza, cancer antigens, hepatitis B, malaria,[38–42] and others.[43] Although the initial DNA vaccine studies in humans demonstrated excellent tolerability and safety,[15,43] the immune responses they elicited were much weaker than expected based on the preclinical data. Concerns about the ability of the technology to stimulate robust immune responses led to the development of the prime-boost strategy which sought to take advantage of properties of both DNA as well as recombinant viral vectors when combined resulting in focused immunity to the transgene (see below).[44]

Highly attenuated live recombinant poxviruses, including NYVAC, the modified vaccinia Ankara (MVA), the ALVAC canarypox-based vector,[45–49] and recombinant adenoviral platforms[50–52] were of great interest. Although these viral platforms induced strong antigen-specific cellular responses in preclinical models, preexisting poxvirus or adenovirus immunity diminished the immune responses induced by immunization with these recombinant vaccine vectors,[53–55] limiting the potency of the vectors in multidose regimens. Furthermore, other issues regarding preexisting immunity are also possible and problematic; these are further discussed below.[56]

Early DNA approaches induced small but focused immune responses that could be expanded with subsequent boosting with a recombinant vector encoding the same antigen as the DNA vaccine, with the prime boost strategy providing a more robust immune response.

This strategy was first suggested by studies in a murine model of malaria, in which DNA priming followed by MVA or NYVAC boost consisting of specific preerythrocytic antigens of *Plasmodium berghei*, induced higher CD8 T-cell responses than either platform delivered alone.[57,58] Schneider and coworkers, using a malaria vaccine, reported that priming with DNA and boosting with MVA vectors encoding the same antigen, led to enhanced immunity and greater protective efficacy than that achieved with either vaccine preparation alone.[57] These findings were quickly extended to many other DNA and recombinant vector combinations.

Important SHIV (simian-human immunodeficiency virus)/SIV (simian immunodeficiency virus) model vaccine studies using DNA priming followed by recombinant MVA boosts were reported to induce cell-mediated immune responses of improved magnitude in nonhuman primates and result in decreased viremia after viral challenge.[59–63] Accordingly, these heterologous-DNA-prime-followed-by-viral-vector-boosting for immunization became popular, as they presented a simple and available option to improve the immune response of two different vaccine platforms, resulting in a more robust level of vaccine-specific cellular and humoral immunity.

An early clinical evaluation of heterologous prime-boost vaccination by McConkey and colleagues is illustrative of these benefits.[64] This study of malaria antigen vaccination tested vaccination with a plasmid antigen cassette encoding

a preerythrocytic malaria antigen, thrombospondin-related adhesion protein (TRAP), followed by ID delivery of recombinant modified MVA containing the TRAP antigen as well. The DNA–MVA combination was safe, and induced cellular immune responses that provided partial protection against an irradiated-sporozoite malaria challenge in humans.

Studies by GeoVax (a biotechnology company) using HIV antigen cassettes showed that DNA priming followed by MVA boosting can be attractive as a combined vaccine modality.[65] The authors studied DNA and recombinant MVA HIV antigen vaccines which encode Gag, protease, reverse transcriptase, and the native, membrane-bound trimeric forms of envelope (Env) as vaccine antigens. They reported that DNA priming followed by recombinant poxviral boosting resulted in superior CD4 and CD8 T-cell immunity than poxviral vaccination alone. A follow-up study by these same investigators compared the data from an HIV gp120 env protein vaccination protocol where the protein vaccine was administered three times (group 1) versus a second group (group 2) which received four administrations of the immunizations with the HIV poxviral vector followed by two administrations of the HIV protein vaccination, versus a group (group 3) which received three administrations of the immunizations with an HIV env-encoding plasmid vaccine followed by boosting with gp120 protein twice.[66] The studies reported significant antibody response differences in the three arms. Group 2 exhibited the lowest neutralizing antibody titers, but a high level of binding antibody responses. Group 1 exhibited high neutralizing titer antibodies as did group 3. Interesting, the DNA prime–protein boost group (group 3) exhibited the highest level of broadly neutralizing titers observed suggesting a unique benefit of the DNA prime in the context of protein boosting.

The National Institutes of Health (NIH) Vaccine Research Center reported on several Phase I trials with the goal of developing a DNA prime followed by a recombinant adenovirus serotype 5 (rAd5) vector boost protocol for HIV.[67] For these studies both vector systems, the plasmid and the adenovirus serotype 5 (Ad5) contained similar inserts. The VRC-HIVDNA009-00-VP vaccine consisted of a four-construct mixture of plasmids encoding subtype B Gag-Pol-Nef fusion protein and three modified Env constructs from subtypes A, B, and C. Plasmid doses of 4 or 8 mg were studied but these appeared similar. The adenoviral vectors were delivered at 10^{10} particle units (PU) in a 1-mL volume, in the deltoid and as a single immunization in the prime-boost study. They observed that while each platform was capable of inducing immune responses as a standalone platform,[55,67,68] when the platforms were combined (DNA prime administered three times followed by Ad5 boost) improved immunogenicity was observed. The sequential DNA/rAd5 administration resulted in 7-fold higher magnitude Env-biased HIV-1-specific $CD8^+$ T-cell responses and 100-fold greater antibody binding titers measured by enzyme-linked immunosorbent assay.

An additional possible advantage of a heterologous prime-boost regimen is induction of responses that differed from those induced by repeated dosing of either vaccine modality. Specifically, a report by Cox and colleagues[69] showed that the cellular responses to an HIV vaccine expressing Gag, Pol, and Nef in a heterologous DNA/rAd5 regimen induced a greater Gag-specific CD4 T-cell response than that induced by the homologous rAd5/rAd5 regimen in humans.[70] Furthermore, studies by Schneider and coworkers[71] and Robinson and colleagues[72] of heterologous DNA/poxvirus prime-boost immunization strategies found that T-cell responses generated with a heterologous DNA/poxvirus strategy produce immune responses 10 times higher than either platform given separately.[64,73,74] Together these studies established that heterologous DNA prime-boost immunizations elicited immune responses of greater breadth than could be achieved by priming and boosting with the same vector (Fig. 67.2).

Specific studies, however, have provided unexpected and less-positive results. A focused Phase II study evaluated the regimen of an HIV-1 DNA prime followed by a rAd5 boost, to prevent infection or to reduce viral loads in study participants who became naturally infected after vaccination.[75] This study was designed after an earlier study entitled STEP, which tested an rAd5 vector that encoded gag pol and nef antigens of HIV. The STEP trial was ended early because of futility and a possible concern that persons in the vaccine arm acquired HIV infection more often than those in the control arm. The new trial was specifically designed to avoid potential risks observed in the STEP study, such as inclusion of env antigens in the 505 study as part of the vaccine. However, this trial also ended early because of futility,[76] supporting the conclusion that this prime-boost approach with these vaccine platforms was not effective. It has been speculated that rAd5 may not be the best genetic vaccine platform for HIV as its strong immune cell activation may work against the vaccine during HIV field challenge. An outcome of this study is a shift to other nonhuman adenoviral vectors, such as chimpanzee adenoviral vectors.[77] Overall, the prime boost strategy continues to have a central role in many vaccine efforts targeting diverse difficult pathogens, but as illustrated above, many challenges remain, and there is a major interest in further improving the immune potency of these approaches.

Improved Immune Potency of the DNA Platform

Many approaches have been taken to improve the immune responses induced by the DNA vaccine platform (Table 67.1). These start from reengineering the plasmid vector to better deliver and express antigens in vivo and include optimization of the promoter region and transcriptional elements to enhance antigen expression levels,[78–88] improved leader sequences,[89–95] and optimization of the plasmid backbone itself; development of improved gene sequences[26,28,96]; inclusion of molecular adjuvants in the formulation or as immune modulators; and development of a variety of next-generation delivery approaches.[97–108] Many of these areas have been reviewed,[15,109] and some are highlighted below in more detail.

DNA technology is highly malleable and a much-improved DNA vaccine immune profile has been described. Major areas of improvement are plasmid construction and design, including the optimization of promoters and enhancer elements; polyadenylation[78–88]; incorporation of novel leader sequences[89–95]; vector design; antibiotic resistance and selection sequences; origin of replication choices for production; and efficient and slimmer backbone designs deleted of extraneous DNA sequences, all of which may contribute to improved platform performance. An important consideration for increasing plasmid-driven immune potency involves sequence optimization.[18–26,28,96] Bacterial RNA is rich in AU sequences, whereas mammalian DNA is rich in GC. Therefore, the pool of transfer RNAs needed for translation in human cells is favored for sequences enriched in GC. Because of the redundancy in codon usage, unique transcriptional differences exist between bacteria, diverse viruses, parasites, and even host tumor antigens, which may benefit from attention to genetic design. DNA sequences can be codon-optimized to favor the transfer RNA pools available in human cells, allowing the encoded messenger RNA (mRNA) to be more efficiently translated.

An important modification for increased immune potency is RNA optimization, where changes are made to the RNA sequence that do not affect the amino acid sequence of the

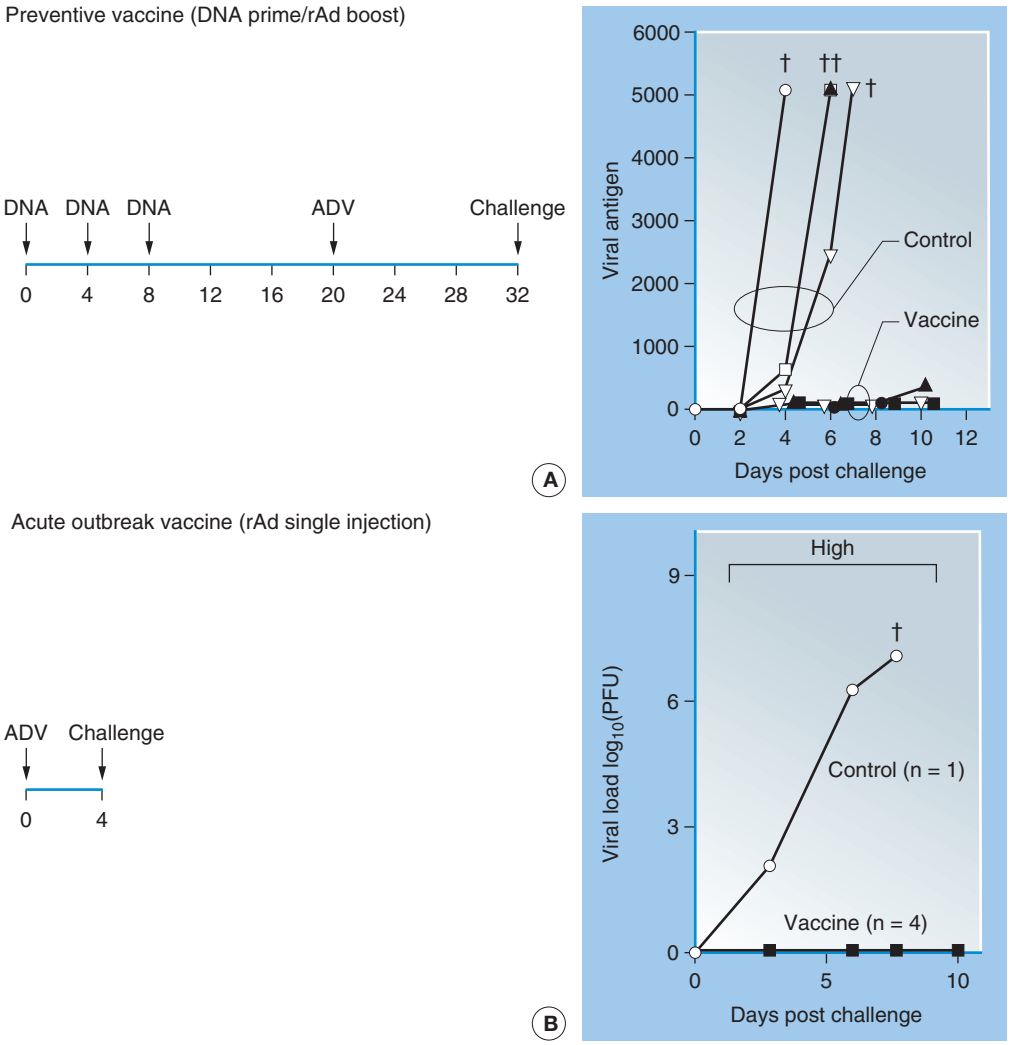


Figure 67.2. Prime-boost versus single shot in models of an Ebolavirus vaccine. Alternative approaches for vaccination can be used depending on the intended use of the vaccine. **A,** In the nonhuman primate challenge model, a DNA prime with recombinant adenoviral vector (rAd) boost of *GP* and *NP* genes confers protection in a lethal challenge model 8 months and longer after the initial immunization. **B,** In contrast, a single shot of an rAd vector encoding these genes stimulates a less potent immune response, but this rapid vaccination produces sufficient immunity to be useful during an acute outbreak. Symbols indicate individual subjects from experimental vaccine and control groups as labeled. The time frame shows the week of vaccination with DNA or ADV vector and subsequent challenge *(left)*. *(Modified from Sullivan NJ, Sanchez A, Rollin PE, et al. Development of a preventive vaccine for Ebolavirus infection in primates.* Nature*. 2000;408:605–609; and Sullivan NJ, Geisbert TW, Geisbert JB, et al. Accelerated vaccination for Ebolavirus haemorrhagic fever in non-human primates.* Nature*. 2003;424:681–684.)*

final vaccine antigen. For example, sequences rich in GC are more likely to form secondary structures and slow translation, lowering in vivo protein production. RNA optimization also involves removing internal *cis*-acting motifs such as TATA boxes, repeat sequences that can cause instability, cryptic splice sites, and unwanted ribosomal binding sites. A combination of these and other gene optimization strategies can have a dramatic positive effect on protein expression and vaccine immunogenicity.[110–112] A recent study of a West Nile virus vaccine DNA using an improved promoter induced relevant antibody responses in most of the 30 trial participants.[113] This study extended the findings reported in trials of severe acute respiratory syndrome (SARS) and Ebola plasmid vaccines, which resulted in low but positive serology induced by the vaccine, with however low T-cell responses suggesting more improvement is still important for this platform to be stand alone.[114,115]

A major advantage of the DNA platform is the ability to rapidly customize the vaccine antigens with high precision to address specific vaccine design limitations. For example, the diversity in HIV's Env sequence can reach greater than 15% within a subtype and greater than 30% between clades. Similar diversity issues plague influenza, hepatitis C virus, and malaria vaccine development, among others. Therefore, vaccination with a single viral sequence is unlikely to drive the diversity of responses necessary for cross-protection from the variety of sequences circulating in the population. Approaches to overcome these limitations combine computer predictions for immunogen design with synthetic chemistry to generate vaccine antigens that improve on nature. Important approaches include consensus antigen, ancestor gene, and center-of-tree designs[116–119]; mosaic antigens[120–122]; and epitope string approaches.[123–128] All these approaches seek to focus the immune response induced by a synthetic gene cassette to

TABLE 67.1 Some Major Approaches[a] in Plasmid Vaccine Optimization

Plasmid Modification	Sequence Modifications	Gene Adjuvants and Formulations	Delivery Enhancement
• Promoter choice • Backbone size • Enhancer elements • Transactivation sequences • Internal termination sequence • Poly AAA tract • Optimized ORI for production • Antibiotic selection sequence for stable production	• Modification of GC/AT content • Species codon optimization • RNA optimizations • Strong Kozack start sequence • Leader sequence • Termination sequence • Localization sequences • Glycosylation sequences • Immunogen sequence optimization • Epitope strings • Consensus • Mosaic • Center-of-tree • Matrix immunogens • Polyvalency or particle formation • Localization sequences • Designer immunogens	• Molecular adjuvants • Cytokines • Chemokines • Toll-like receptors • HSP • Costimulatory genes • Transcription factors • Adhesion molecules • Formulations • Alum • Saponin • Nanoparticles • Liposomes • Polymers	• Electroporation • Jet injector • Gene gun • Skin abrasion • Microneedle • Topical patch • Needle-free systems • Hydrodynamic delivery

[a]Alternative approaches to modification of plasmids, coding and noncoding sequence changes, formulation or adjuvanting, and delivery methods that can improve insert expression or immunogenicity are indicated.
HSP, heat shock proteins; ORI, origin of replication (site where DNA replication is initiated).

specific epitopes or regions of a native antigen or an antigen predicted by computer analysis to be more conserved or invariant in the population of divergent viruses. Such targeting would, in theory, preferentially expand the most desired T- and B-cell responses that nature would not drive. These strategies seek to maximize cross-reactivity of the T-cell responses induced against divergent strains of the pathogen. Consensus antigens, mosaic antigens, and epitope strings are currently being studied in the clinic for hepatitis C, hepatitis B, and HIV, among others. Data from HIV as well as human papillomavirus (HPV) are discussed further below.

Molecular Adjuvants

DNA vaccine approaches are particularly suited to deliver gene-encoded adjuvants to modulate the resulting immune response. As these adjuvants are derived from host genes with known biology, they allow an unprecedented level of insight into adjuvant choice. Unlike traditional adjuvants, molecular adjuvants are delivered as plasmid-encoded vectors as part of, or along with, the antigen-encoded vector.[93,129–139] On vaccination, the molecular adjuvant vector transduces cells at the site of vaccination that can then secrete the adjuvant molecule locally, thus coordinately and temporally targeting the same regional APCs[28,29] and draining lymph nodes as the vaccine antigen.

Granulocyte-macrophage colony-stimulating factor (GM-CSF), a white blood cell growth factor with considerable adjuvant properties, was one of the first gene adjuvants to clearly demonstrate that a cytokine plasmid could modulate DNA vaccine-induced immunity.[129] In mice vaccinated with a rabies virus antigen DNA, the addition of GM-CSF delivered as DNA increased antibody production, $CD4^+$ T-cell responses, and protection after lethal challenge. Accordingly, GM-CSF became a widely studied DNA molecular adjuvant that has been examined in macaques and in human clinical studies of a malaria vaccine.[140] However, in human studies, the adjuvant effect was not as clear as in the initial animal studies. It remains under investigation as part of a new delivery format in a prime-boost protocol.[139]

Another cytokine gene, interleukin (IL)-12, has also received a great deal of attention as a DNA vaccine adjuvant.[131,135] IL-12 is a strong T-helper cell type 1 adjuvant that expands T-cell immunity, including $CD8^+$ T-cell function. In mouse models, IL-12 increased $CD8^+$ T-cell lysis of target cells 4.5-fold.[131] Using an HIV-1 DNA vaccination of nonhuman primates (NHPs) along with plasmid encoded IL-12 demonstrated increased cellular responses that corresponded with control of viremia and improved clinical outcomes from a chimeric SHIV virus. The NHP challenge virus consists of SIV core antigens as well as an HIV-1 Env antigen and is designated SHIV98.6P. The ability of plasmid IL-12 vector to increase HIV-1–specific responses against an HIV-1 DNA vaccine is currently being studied in humans, where the adjuvant effect of the plasmid codelivered with IL-12 appears to significantly enhance the immune response, expanding both the CD4 and CD8 T-cell responses induced by the vaccine.[136] Additionally, IL-2–Ig, a T-cell growth expansion factor, administered with an HIV antigen DNA vaccine showed positive results in mouse and macaque model systems and was moved to human testing.[134] In the study of 70 persons which compared an HIV DNA vaccine alone to the HIV DNA vaccine given with IL-2–Ig either with the vaccine or 2 days later, the group receiving the IL-2–Ig adjuvant 2 days later exhibited improved enzyme-linked immunosorbent spot (ELISPOT) results compared to either of the other groups.[141] In a study of a DNA vaccine in the SIV model using a checkpoint inhibitor, anti–CTLA-4 or 41BB Ig adjuvants were reported.[142] This study showed that the 41BB adjuvanted groups showed superior CD8 T-cell responses to DNA alone or DNA + CTLA-4 adjuvanted groups. Furthermore, when the macaques were challenged with a highly pathogenic SIVmac251 monkey virus in the 41BB arm, seven of 14 animals showed several logs lower peak viremia and significant control of infection. The ease and specificity of such combination adjuvant approaches in the DNA vaccine arena has generated an enormous amount of data regarding vaccine effects of important cytokine genes, costimulatory molecules, chemokine genes, heat shock antigens, and other immune modulating molecules. It is likely that this area will continue to receive a great deal of attention,

particularly on the basis of the early positive effects that are being reported in the clinic.

Increased Vaccine Dose

As DNA vaccines are delivered without the benefits of a viral vector coat to facilitate host cell attachment and entry, they suffer intrinsically with a limited transfection efficiency, which compounds their lack of replication and spreading potential. Therefore, a major area of research has focused on improving DNA entry into target cells in vivo. To some extent, the efficacy of DNA vaccination in humans can be improved by increasing the dosage of plasmid delivered. Early studies in humans used doses of 1 mg or less but newer studies use doses of 8 mg, which increase the consistency and frequency of antibody production.[114] Formulations with doses as high as 12 mg, together with cytokine expression vectors and electroporation, have stimulated increased T-cell responses[143] and are promising approaches.

Novel Transfection Reagents

Formulations including novel transfection reagents were a major focus in early studies. In addition, physical delivery has become an important area of research. An important area for DNA delivery is the formulation of DNA into or on biodegradable polymeric microparticles (see a review[105]) as well as contained in liposomes.[97,101,108] Microparticle- and liposome-based DNA vaccine delivery systems are being studied for their utility of delivery and enhanced immunogenicity in several different host and antigenic vaccine platforms in small animals,[99,100,103,106] NHPs[97,104] and in humans in cytomegalovirus (CMV) and influenza DNA vaccines. These compounds can have dual roles, facilitating plasmid entry into the cell as well as providing an adjuvant effect. Polyethyleneimine, amine-functionalized polymethacrylates, cationic poly (β-amino) esters, poloxamers, and polyvinylpyrrolidone polymers are additional examples of molecules that can enhance DNA vaccine immune potency in specific systems.[103,106] The poloxamer CRL1005 has demonstrated improved immune potency in preclinical models for simian HIV vaccines.[97,108] In addition, ongoing studies show that liposome vehicles can improve DNA vaccine-induced immune responses. Studies with liposomes, in general, support an improved, but still modest, impact on antibody responses and lesser impact on T-cell responses. As liposomes have structural versatility with regard to the resulting vesicle surface charge (both cationic and anionic liposomes can be made), size, lipid content, and codelivery with other adjuvants, they offer the ability to be customized for specific DNA applications.[99,100,103–106]

The formulation of DNA vaccines in polyamine gels or nanoparticles[106,144,145] has also been reported to increase the uptake of plasmid vectors and increase antigen expression in vivo. Although there is increasing research in this area, they are not as well studied as the liposome and polymer approaches in their clinical development trajectories. However, alone none of these formulations appear capable of inducing immune responses from the DNA platform that rival the responses induced by viral vectors.

Vaccine Delivery Methods

Another major area of productive research has been in combining DNA vaccines with physical delivery methods. By physically forcing more plasmid DNA into cells, as well as increasing the number of transfected cells, expression levels should improve. Improvements in immune responses have been reported with physical delivery devices such as the gene gun or biolistic jet injection.[146–155] Jet injection involves using high pressure to deliver a liquid formulation of DNA just centimeters below the skin surface. The major limitations to clinical use of jet injection are the requirement for large amounts of DNA and the DNA degradation from the high-pressure delivery. However, it is a relatively simple technique that has shown improved immune responses when compared with IM injection in experimental model systems. The gene gun uses high pressure to deliver DNA-coated gold nanoparticles to the dermis and has demonstrated high transfection efficiency and enhanced antibody responses in several species including humans.[152,153] In contrast, it has had relatively little effect on improving cellular immunity.

Electroporation (EP) has been used to transfect cells in vitro for 3 decades and has more recently been used in vivo to increase the transfection efficiency of DNA vaccines.[17] EP involves applying a small electric field across the site of injection to cause temporary membrane instability and create an electric gradient, which increases plasmid uptake by cells in the field.[18,24] This technique has been studied for 2 decades as a method to improve delivery of chemotherapy drugs to kill specific tumor cells[147–149] and in several animal species (e.g., dogs, pigs, cattle, NHPs) to deliver genes that encode a variety of hormones, cytokines, enzymes, or antigens.[146,150–152,156] However, the conditions required for EP were initially considered too harsh for adoption into vaccination strategies. Yet, over the past 10 years, EP technology has developed more benign delivery devices that are computer controlled and capable of enhancing delivery through IM, ID, and microneedle transfection.[20,21] These approaches use lower voltage and are more tolerable. An exciting development is that in large-animal models, delivery of DNA vaccine by EP has led to both increased cellular and humoral immune responses,[22,23] rivaling those seen with viral vectors.[157] When these newer EP approaches are combined with other DNA optimization approaches, the magnitude of the immune responses generated by the combined DNA approach has increased more than a log.[110,156,158–164] EP technology can also be tailored to a particular DNA vaccine, using devices that control current, voltage, and timing settings. Combinations with molecular adjuvants also look highly promising,[136,165,166] and delivery to the skin, muscle, and the mucosal has been reported. Data from multiple primate challenge models have demonstrated much improved immunogenicity and efficacy against challenge by such delivery of plasmid vaccines.[167,168]

Clinical Studies

The initial studies of DNA vaccines in the clinic demonstrated safety and ease of production, but immune responses were not adequate. The initial fear regarding the use of DNA vaccines was the risk of integration into the host chromosome with subsequent activation of oncogenes or inactivation of tumor suppressor genes. However, studies have demonstrated that the rate of DNA integration in vivo is actually three times lower than the rate of spontaneous mutagenesis. In addition, no negative effects, such as induction of autoimmunity or transfer of antibiotic resistance markers, have been observed. With the renewed clinical interest in this approach, thousands of volunteers have received DNA vaccines without significant vaccine-related adverse events being reported. In fact, the number of DNA vaccines being tested in clinical studies compared with all other recombinant platforms has increased from just 4% in 2000 to comprise 18% of all such trials as of December 2016. Table 67.2 summarizes several pivotal trials of DNA vaccines.

One area that can serve as a barometer of the interest in an experimental platform is HIV vaccine trials. Seven of the

TABLE 67.2 Current DNA Vaccine Clinical Trials as of August 2015

Phase	Approach	No. of Trials	Vaccine Targets
I	DNA alone	17	Cancer, infectious disease
	Prime/boost[a]	3	Cancer, infectious disease
II	DNA alone	3	Cancer, infectious disease
	Prime/boost[a]	3	Cancer, infectious disease

[a]Boost studies include poxviral boosts, recombinant protein boosts, adenoviral boosts; seven DNA-only studies using electroporation. Genetic adjuvants, including granulocyte-macrophage colony-stimulating factor and interleukin-12, are found in multiple DNA studies. Both ID and cancer studies are recognized.

18 clinical trials sponsored by the HIV Vaccine Trials Network (HVTN) are evaluating DNA vaccines to elicit immune responses, either alone or in combination with multiple viral vectors as boosts. Additionally, new studies of EP-delivered DNA alone build on the data observed in HVTN 080 and EP DNA prime-boost strategies are being developed. An alternative approach uses a DNA prime to stimulate both T-cell and B-cell responses, followed by a recombinant protein-boost to generate more-potent antibody responses. DNA vaccines are also being studied in non–prime-boost settings using enhanced physical delivery. For example, in HVTN protocol 080, multiple-codon and genetically optimized DNA plus plasmid IL-12 as an adjuvant is delivered by EP to healthy volunteers. This study reported induction of T-cell immunity in 90% of study volunteers. In fact, this three-immunization protocol induced overall T-cell responses as high as responses previously reported to a DNA prime followed by poxviral or adenoviral boosts consisting of five immunizations. Furthermore, induced CD4 and CD8 T cells were still detectable at 6 months after the final immunization.[169] This was the first study to show that the combination of both EP and IL-12 can dramatically improve the T-cell responses in humans.

A Phase I study of a multioptimized (synthetic) consensus DNA-VGX3100 delivered by EP for treatment of early HPV disease was recently conducted in 18 women with precancerous cervical disease (Cin2 or Cin3). Participants were vaccinated with synthetic DNA encoding two different HPV oncogenes (E6 and E7) and two different oncogenic HPV strains (types 16 and 18) at week 1, week 4, and week 12. Three dose groups (0.6 mg, 3.0 mg, and 6.0 mg) of the plasmid vaccine were studied. Subjects were evaluated for induction of antibodies as well as CTL. In contrast to prior DNA vaccine studies, 100% of women in this study seroconverted, irrespective of dose group, greater than 90% after the second dose of vaccine. Antibodies persisted for at least 6 months after the final immunization. Cellular responses were demonstrated in 78% of women by ELISPOT and in more than 80% of women by cytotoxic assays. In the high-dose group six of six women exhibited killing capacity against targets.[167] Based on these encouraging data, a Phase IIB study was conducted in 147 women with cervical disease (CIN2 or CIN3) testing the same schedule as the Phase I study using the highest dose of vaccine. The primary end point was regression of disease, and the secondary end point was regression of disease as well as viral clearance. The study reported that 50% of the study participants had regression of their cervical disease, with 40% completely clearing the HPV infection ($P = .001$). Similar to the Phase I study, close to 100% of women seroconverted to the vaccine antigens, and greater than 90% exhibited strong T-cell responses. Furthermore, T cells were elevated in cervical samples examined from women who cleared their disease.[168]

It will be important to study optimized DNA vaccines delivered by EP in additional settings to extend these findings to additional targets, expanding the applications of the DNA platform. As with the report of the first efficacy data, it is clear from the preceding discussion that improvements in technologies are driving the field (see Table 67.2). The progress of clinical trials will have to be closely watched, as they will present an exciting picture over the next few years as various DNA approaches come to fruition.

Animal Studies

Several DNA vaccines have been licensed for use in animals. Products licensed for veterinary use[33–36] include a dog melanoma immunotherapy, porcine therapy, a porcine recombinant growth hormone, vaccine for the prevention of rhabdovirus disease in fish, and West Nile virus vaccine for horses based on successful field trials of DNA vaccines. The important safety record of this technology in animal health and in humans, the growing database regarding consistency of immune responses in the clinic, the first efficacy data reported in humans now reported, and the successful licensure of veterinary products suggest that DNA vaccination is well positioned to become an important platform for continued vaccine and immune therapeutic development.

RNA VACCINES

The application of nucleic acid encoded genes (nucleic acid vaccines) delivered in vivo adds another novel approach to the vaccine/immunotherapy pipeline. The use of plasmid DNA for in vivo antigen production and mRNA to deliver encoded antigens was first reported more than 2 decades ago.[170,171] Initially, DNA vaccines took center stage because of their ease of production and stability but problems with immunogenicity were soon appreciated. However, RNA-based approaches have reemerged as an important genetic vaccine platform. RNA-based vaccine approaches allow for infinite boosting because they are not subject to neutralization by the host immune response, even in previously seropositive individuals. RNA vaccines are produced, customized in vivo, and processed by the host. They are naturally folded and can be further modified by the endogenous host cell systems. Similar to DNA vaccine approaches the antigens produced in vivo can be presented on endogenous APC and stimulate both CD4 and CD8 T-cell responses, mimicking the immune responses induced by live infection. Mixtures of RNAs were also tested.[172] However, similar to the poor immunogenicity seen with DNA vaccines, the early studies using RNA vaccines were also plagued by low immunogenicity in small animals, as well as manufacturing issues resulting in low production yields and product instability.

Over the last decade, RNA vaccines have improved through ex vivo transfection studies.[173,174] RNA immunization by transfection of patient-derived dendritic cells showed induction of immunity in vivo. Based on these data, there was interest in cancer immune therapy using patient-derived cells, which are modified by transfecting them with either mRNA derived from their own cancer cells or antigen-specific synthesized mRNA to focus the immune response. These approaches were tested in pancreatic cancer, neuroblastoma, melanoma, colorectal cancer, lung cancer, and prostate cancer, among others. The early studies were well tolerated. Additionally, RNA-based approaches are being investigated for delivery of chimeric T-cell receptors for functionally targeting tumor antigens

directly.[175,176] This ex vivo approach is preferred because of difficulties in RNA production and storage, among other difficulties.

As a result of technological advances, the direct injection of RNA for vaccination purposes is growing in importance.[177] This reemergence is the result of separate advances in the field of RNA vaccines. One advance is the ability to stabilize mRNA during production. The second is the existence of more potent self-amplifying RNA vectors based on the alphavirus platform. In a recent study using a synthetic mRNA encoding influenza antigens, optimized for RNA GC content and complexed with protamine, mice or ferrets vaccinated with this formulation seroconverted to influenza antigens and were protected against influenza challenge[178] after a single immunization. The formulation was also immunogenic in animals as large as pigs, and while not fully protective after influenza challenge in pigs, the disease was attenuated. mRNA vaccines are now being tested in humans, with a focus on therapy of cancer, particularly prostatic cancer.[179–182] A prostate cancer mRNA vaccine containing "self-adjuvanted" mRNA encoding antigens of relevance to prostate disease, including prostate specific antigen, prostate stem cell antigen, prostate specific membrane antigen, and six transmembrane epithelial antigens of the prostate, was evaluated for immunogenicity and safety in 44 patients with advanced castration-resistant prostate cancer. These mRNA-based vaccines contained free and protamine-complexed mRNA to support the stability and delivery of the mRNA. In mice, the vaccine was immunogenic and suggested that these vaccines drove innate immune stimulation that was mediated in part via Toll-like receptor 7 activation. In clinical studies, the mRNA vaccines were administered with five ID injections at a recommended dose of 1280 µg. Results showed that immune responses could be detected against at least one vaccine antigen in 26 of 33 evaluable patients, with 15 of 33 patients responding to more than one of the vaccine antigens. Treatment-related adverse events were experienced by 39 (89%) patients, including injection-site erythema and injection-site reactions, fatigue (18%), pyrexia (16%), chills (11%), and influenza-like illness (11%). Although most of these reactions were considered mild in nature, additional safety studies are needed. The outcome results were interesting as the immune responders showed a trend toward increased survival compared to the non-immune responders. There was also a trend to better clinical outcome based on induction of more antigenic responses; patients who responded to three antigens had better outcomes than those who responded to only one or two antigens. Antibody responses induced by the vaccine were not as robust as the T-cell responses as only four of the patients showed increased titers to tested vaccine antigens. Additional studies in the mRNA vaccine area will be important to follow.

An additional area of importance in the RNA vaccine field is the reemergence of the alphavirus system as a nonviral RNA vaccine delivery platform.[183,184] For example, studies reported from Novartis have described this self-amplifying RNA technology.[185] In this approach the alphavirus genes encoding the RNA replication machinery along with the recombinant viral target antigens are synthesized in the laboratory. Users of this technology to vaccinate mice against respiratory syncytial virus F protein have reported rapid induction of potent antibody responses. This group also reported responses in an NHP study of HIV immunogenicity.[186] The self-amplifying HIV vaccine induced both T cells, measured by ELISPOT, and antibody responses, and were further boosted by MF59 adjuvanted HIV env antigen. Overall, the data clearly illustrate that this platform can induce immune responses. As with mRNA vaccine it will be important to monitor self-amplifying vaccines as they enter clinical evaluation.

RNA approaches have conceptual advantages as they are simple focused immunogens and do not require nuclear localization to generate expression. The replicating vectors such as self-amplifying vectors expand mRNA copies by providing the replication machinery from alphaviruses to maximize expression. The newer stabilized mRNA approaches exhibit a longer half-life compared to earlier-generation mRNA vaccines. These advances improve delivery and expression. Furthermore, RNA approaches strongly stimulate the host innate defense system in part through activation of the Toll-like receptor 3 and Toll-like receptor 7/8 pathways.[187] Innate activation may be an advantage for immune priming that needs further investigation. Overall, even though the RNA field has to catch up to other genetic vaccine platforms, this next generation of RNA vaccines presents interesting opportunities as a standalone platform or as part of prime-boost protocols.

VIRAL VECTORS

Advances in molecular virology have facilitated an understanding of the regulation of viral replication, gene expression, and molecular pathogenesis. At the same time, this understanding has enabled the development of novel viral vectors useful for vaccination. A variety of such vectors have now advanced to preclinical and clinical studies (see Fig. 67.1). Depending on their ability to target APCs, the ease of developing packaging lines, the inherent immunogenicity of both the vector and insert, and other factors (see Box 67.2), these viral vectors are helping to improve vaccine efficacy in a variety of infectious disease models. The properties of promising vectors and current progress in their development are summarized in the following sections.

Replication-Defective Adenoviral Vectors

Among the viral vectors that have been studied, recombinant adenoviral vectors have demonstrated immunogenicity and protective immunity in a variety of animal models. These vectors have been genetically modified so that they can deliver and express specific recombinant gene products but are unable to grow on their own and thus are replication-defective. Like DNA vaccines, these vectors transduce cells that can synthesize native gene products, and they appear to be quite potent in their ability to induce not only helper but specifically CTL immunity.[188] The majority of clinical vectors have been derived from Ad5, although more than 51 human serotypes in six subfamilies (A to F) are known. Ad5 is derived from the C subfamily and is the most common and best-studied serotype; however, the relatively high prevalence of immunity to Ad5 in human populations may pose limitations to the use of these vectors.[189]

Preexisting anti-Ad5 immunity may inhibit the response to rAd5 vaccine immunization, so alternative serotypes and chimeric vectors have been developed to circumvent this. The attraction to rAd5 for immunization has followed from its success with a variety of preclinical animal models and with human trials in Phase I or Phase II. With respect to animal models, the replication-defective adenovirus elicits potent immune responses and protection against Ebolavirus, administered either alone as a single injection, or in prime-boost combinations (see Fig. 67.2).[190,191] The prime-boost approach induces a more potent and durable immunity, desirable for a preventive vaccine in routine use, whereas a single rAd5 vaccination induces a more rapid response that is sufficient for immediate protection (see Fig. 67.2). This rAd5 approach may be useful in containing acute outbreaks of Ebola infection and could be applicable to other pathogens.[39] In addition, both rAd5 vaccines and DNA prime/rAd5 boost combinations

confer partial protection in rhesus macaques against multiple HIV isolates, including SHIV-89.6P,[37,192] SIVmac239,[193] and SIVmac251.[194–196] Replication-defective adenovirus has also been used in a variety of additional animal models of infectious disease, including plague, anthrax, influenza, and malaria.[51]

Phase I and Phase II clinical studies with replication-defective adenoviral vectors for HIV-1 have been conducted by several groups. The STEP trial, a clinical efficacy study of an rAd5 vector encoding *Gag*, *Pol*, and *Nef* genes of HIV-1, evaluated the effect of vaccine-induced T-cell responses on controlling viral load. Although the vaccine was immunogenic, there was no reduction in HIV acquisition or long-term control of postinfection viremia.[197] Further analyses revealed that persons with specific human lymphocyte antigen types, as well as those who developed a $CD8^+$ T-cell response to certain *Gag* and *Nef* HIV epitopes, selected against viruses that contained the vaccine epitope in vivo.[198] There was also an unexpected association between infection in vaccine recipients and those who were both uncircumcised and immune to Ad5 before vaccination. The rate of HIV infection during the first 18 months after the immunization regimen appeared higher in this subgroup, although this result remains controversial.

The clinical utility of the 505 DNA prime/rAd5 boost vaccines is as discussed above in the prime-boost section. The 505 trial was similar to the Merck STEP study, which also was ended early because of futility. The results support that improvement to Ad5 vector approaches must be considered for improving the outcome in the clinic. This could be a vector issue as the inclusion of env antigens did not impact positively on efficacy in the 505 trial. The serology issues associated with Ad5 vectors, as well as the controversy regarding whether there is an increased risk of HIV infection,[199] have driven interest in other non-Ad5 adenoviral vectors.

Effect of Preexisting Antivector Immunity and Alternative Adenovirus Serotypes

Despite the ability of rAd5 to induce potent and sustained immune responses against a variety of infectious pathogens, concerns remain that preexisting immunity against rAd5 may compromise its efficacy. In certain regions of Africa, the Ad5 seroprevalence is greater than 90% with a high degree of neutralizing antibody, limiting the use of this vector. Although both cellular and humoral immune responses contribute to anti-Ad5 immunity, it is likely that the Ad5 neutralizing antibodies play a major role in suppressing rAd5-induced immunogenicity, as seen in humans. This preexisting immunity has been shown to reduce the immunogenicity of Ad vaccines in mice,[200,201] rhesus monkeys,[202] and potentially in humans,[203,204] but it is not clear that preexisting immunity in humans will completely block vaccine immunogenicity.

Several strategies have been developed to overcome the potential problem of rAd immunity. Novel methods to deliver existing recombinant Ad vectors are being explored. For example, it is possible that the administration of higher doses of recombinant Ad5 vectors may overcome anti-Ad5 immunity, although this strategy may be limited by increased toxicity with dose escalation.[204–206] Ad boosting after DNA priming may potentially reduce its immunosuppressive effects, too, although this was not seen in the described HVTN 505 study.[200,201] Finally, the administration of Ad5 vectors through mucosal routes may help circumvent this problem.[207] However, the safety of this approach, particularly for intranasal delivery, has yet to be determined.[208] In addition, several investigators have explored the possibility of coating rAd5 particles with chemicals such as polyethylene glycol, which may block access of antibodies to the viral surface.

Alternative approaches to evasion of Ad5 immunity include engineering of the vectors to evade dominant Ad5 immune responses. A variety of chimeric fiber or hexon proteins have been described that maintain immunogenicity and can evade neutralizing antibodies, both against the fiber,[209–212] or through the use of hexon chimeras, which appear to be the targets of the major neutralizing antibody response.[213,214] Another approach to antivector immunity involves the development of novel vectors from alternative serotypes. To develop such vectors, investigators have evaluated rAd vectors from low-seroprevalence human adenoviruses, as well as from NHPs. Recombinant Ad vectors from human serotypes have been well described.[215–217] Seroprevalence of the 51 Ad serotypes suggests that the Ad11 and Ad35 subfamilies, as well as adenoviruses from subfamily D, including Ad26, are uncommon in humans,[218] and thus may offer advantages over Ad5 as vectors. Novel vectors based on rAd35 and rAd11 have been developed, and preclinical studies suggest that they are resistant to anti-Ad5 immunity in mice.[54,219] Some of the alternative vectors show less-potent antibody responses than seen with rAd5. There also appear to be regional differences in seropositivity to diverse "rare" serotype rAd vectors. For example, although the rAd26 and rAd28 B serotypes have shown promise in early clinical trials and their seroprevalence is low in North America, seropositivity to these viruses approaches 80% in parts of Africa,[220,221] complicating development and regulatory issues for such vectors.

In addition to these replication-incompetent Ad vectors, attenuated replication-competent vectors from Ad4 and Ad7 have been used as vaccine vectors to prevent adenoviral mediated disease in the military where there is a high incidence of this disease among recruits. These live vaccines appear well tolerated and highly effective against Ad4 and Ad7.[222,223] These serotypes have also been developed as recombinant vectors platforms—for example, against HIV.[222,224] These vaccines not only offer the potential of alternative serotypes but when used orally can deliver immune stimulus to the gut mucosa, which may have potentially desirable effects in protection against some viral challenges. Finally, recombinant Ad vectors have been developed from alternative species, including sheep, pigs, cows, macaques, and chimpanzees.[190,225–233] In particular such chimp adenoviral vectors appear to be gaining traction for clinical evaluation. Specifically, the Ad5 Ebola vaccine was superseded by a new chimp adenoviral vaccine.[234] A chimpanzee-derived replication-defective adenovirus (chAd) vaccine induced uniform protection against acute lethal Ebolavirus challenge in macaques. However, the protection was short lived. When chAd3 was boosted with a MVA Ebola vaccine, much more durable protection against lethal Ebolavirus challenge was generated. In human studies, the vaccine induced antibody responses were detected in 68% of vaccine recipients in the highest dose group studied (5×10^{-10}vp) with 100% of vaccinated persons responding in ELISPOT assays.[235] Overall, the data were encouraging. Surprisingly, preexisting neutralizing antibodies were observed against chimp adenoviral vectors in sub-Saharan Africa suggesting that at some level chimp adenoviruses or related viruses have crossed into the human population with some frequency. This finding has consequences beyond HIV vaccines as the chAd3-EBO-Z viral vaccine developed by the NIH is among the important new vaccine candidates being studied in the context of the recent Ebola outbreak in West Africa. The impact of preexisting immunity on this vaccine or others being developed will require additional investigation.[235,236]

In conclusion, the immunogenicity of rAd vectors has prompted their development as candidate vaccines for a variety of infectious diseases. These vectors are well tolerated and highly immunogenic at moderate doses. The concerns

over preexisting Ad5 immunity has generated a number of new approaches for Ad vectors. Novel delivery vectors, molecularly engineered rAd5, and alternative Ad serotypes from other species provide a number of options for clinical study in a variety of infectious disease as well as cancer immune therapy settings.

Poxvirus Vectors for Immunization

The efficacy of vaccinia virus against smallpox represents one of the best examples of the impact of vaccination on infectious disease. However, safety issues using vaccinia strains against smallpox were substantial,[237–240] and a number of alternative vaccinia virus strains have been developed as immunization vehicles (summarized in Box 67.3). These attenuated vaccinia viruses have also been used as delivery vectors for gene products against specific pathogens other than smallpox.

One of the two major attenuated strains of poxvirus is MVA, developed by repeated passaging of the Ankara vaccinia strain on primary chicken embryo fibroblasts (CEFs), as a safer alternative as a vaccine against smallpox. This resulted in multiple genetic changes that enabled the virus to replicate efficiently on a variety of nonavian cell types. A second alternative attenuated strain, the New York vaccinia strain (NYVAC), was developed by genetic modification including the deletion of 18 open reading frames associated with virulence and host range in the Copenhagen strain.[241–244] NYVAC, like MVA, is attenuated in animal models and shows favorable safety and immunogenicity in animals and humans.[242,245,246] NYVAC has a block at an early stage of replication, although it is able to replicate productively in African green monkey kidney cells and primary CEFs.

ALVAC, derived from a plaque-purified virus isolated from an existing canarypox strain, canapox,[247] is able to express inserted transgenes and is immunogenic in both animal and early clinical trials.[245,246,248–251] Additional avipox vectors include fowlpox and canarypox. These vectors have been evaluated both alone and in prime-boost combinations in a variety of infectious disease and cancer models (see review[245]).

In general, poxviruses are notable for their large genome size and their ability to express recombinant genes without an effect on their replication capacity. Polyvalent recombinants have been used to immunize experimental animals and have proved useful in a variety of infectious disease models, including rabies, measles, SIV, canine distemper, respiratory syncytial virus, malaria,[57,252] and influenza.[253] In addition, these vectors have been studied in a variety of HIV challenge models in animals. Human studies have also been conducted [45,254–258] with vaccinia,[259–267] NYVAC,[268–271] and ALVAC,[268,269,271–276] and have advanced into efficacy studies in humans. The ALVAC-EnvGag/Pol (clade B and AE) was evaluated in combination with gp120 protein boosting in a Phase III study in Thailand and demonstrated a 31% reduction in the frequency of acquisition of HIV infection among vaccinated heterosexual men and women when compared with placebo recipients.[277] This study represented a landmark trial, providing a proof of concept that a vaccine could prevent HIV-1 infection, although the degree of efficacy was modest. Although attenuated poxvectors have been evaluated in a variety of human studies, it is clear that developing these vaccines for use in humans has been challenging. In part, because the recombinant transgenes represent a small minority of gene products expressed in this otherwise large vector. Thus, there is no certainty that the immune response will be focused to the foreign transgene rather than to gene products synthesized endogenously by the poxvirus. In addition, as seen with rAd, antivector immunity remains problematic, although to a lesser degree with canarypox vectors.

Poxvirus vectors show thermostability, an ability to incorporate a large foreign transgene, a lack of persistence or genomic integration, and a demonstrable success in smallpox eradication. However, the difficulties in manufacturing virus in high yields from primary CEFs, as well as their antigenic complexity, reactogenicity and poor immunogenicity, possibly impacts their effect in multiple immunization regimens. A poxviral vaccine was tested in a human malaria challenge model.[278] The vaccine expressed a polyprotein insert which consisted of a string of six preerythrocytic antigens designed from *Plasmodium falciparum*. Following safety assessment of single doses, 15 volunteers received a heterologous prime-boost vaccination regime involving two difference poxvirus malaria subunit vaccines containing the insert, FP9-PP and MVA-PP. Following immunization the subjects underwent malaria sporozoite challenge. The vaccines were safe; however, T-cell interferon-γ ELISPOT responses were low and there was no vaccine efficacy observed. A novel MVA vaccine expressing the antigen 85A of *Mycobacterium tuberculosis* was evaluated in a placebo-controlled Phase IIb trial in South Africa in infants.[279] Infants who had previously received bacille Calmette-Guérin (BCG) vaccination were randomized to receive either the MVA85A or placebo intradermally and then followed for immune responses and protection against tuberculosis. Although the vaccine was safe and the immunogenicity was modest, there was no efficacy against tuberculosis infection or disease. Both of these studies with malaria and tuberculosis suggest that much more potent immune responses will be needed to impact these two infections. Thus, prime-boost strategies are being pursued.

In addition to studies in the infectious disease arena, poxviral vectors have been important tools in multiple cancer immune therapy protocols[280] targeting diverse tumor types. In these studies, T cells have been induced; however, the poxviral vectors appear to induce modest antibody responses. The first positive efficacy outcome has been reported in a Phase IIb study using a recombinant poxviral vector. Prostvac-V/F is a candidate, anticancer immunotherapy for therapy of prostate cancer which was originally championed by the National Cancer Institute. It was developed for men with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer. Therapy consists of priming with a vaccinia recombinant poxviral vector followed by up to five monthly immunizations with a fowlpox vector. The vectors contain the identical gene inserts. The inserts include a gene sequence encoding a modified immune stimulatory prostate-specific antigen cassette along with additional gene cassettes for three human immunostimulatory molecules (known as TRICOM), leukocyte function–associated antigen-3, intercellular adhesion molecule-1, and B7.1. In clinical trials, the treatment generated clear anti–prostate-specific antigen CTL responses in up to 57% of vaccinees, but almost no antibody responses.[281]

BOX 67.3 Poxvirus Strains Used as Immunization Vehicles

VACCINIA VIRUS

- New York vaccinia strain (NYVAC) (18 ORFs deleted)
- Modified vaccinia Ankara (MVA) (adapted to CEF)

AVIPOXVIRUS

- Fowlpox—FPV/TROVAC
- Canarypox—CPV/ALVAC (adapted to CEF)
- Canarypox—ALVAC (2) (+ *E3L* and *K3L* genes)

CEF, chicken embryonic fibroblasts; ORF, open reading frame.

These CTLs were shown to have the ability to lyse tumor targets.[282] In a randomized, placebo-controlled, double-blind, Phase II clinical trial (NCT00078585), Prostvac-V/F increased overall survival in patients with metastatic castration-resistant prostate cancer by 8.5 months which translated to a 44% reduction in risk of death.[283] Bavarian Nordic in conjunction with the National Cancer Institute is currently testing Prostvac with or without a GM-CSF adjuvant in a global, double-blind, randomized, Phase III efficacy trial dubbed PROSPECT (NCT01322490). It will be interesting to clinically evaluate this immune therapeutic vaccine in the context of checkpoint inhibitor therapy as well. Further improvement in poxviral immunogenicity remains important.

Adeno-Associated Viruses

The adeno-associated viruses (AAVs) were defined initially as "helper" viruses that facilitated the propagation of wild-type adenovirus in cell culture. In contrast to the large genome sizes of rAd and vaccinia vectors, AAV's genome sizes are much more limited in size, with an insert size of approximately 5 kb. Like other replication-defective viruses, these particles can be produced in packaging lines that provide complementary structural proteins made constitutively by the cell rather than the virus. A variety of serotypes have been defined,[284] and an HIV vaccine expressed in AAV2 has been evaluated in Phase I human studies, with poor immunogenicity results. Alternative serotypes, including AAV1, are currently under development and may be assessed both alone and in prime-boost combinations for efficacy in humans. An entirely unique use of these vectors has also been described recently. Recombinant adeno-associated virus vectors have been studied as a platform to deliver recombinant antibody genes for direct in vivo production of antibodies. This strategy, vector immune prophylaxis, allows previously identified rare neutralizing antibodies to be engineered into an Recombinant adeno-associated virus vector, which, upon infection, results in in vivo antibody production of protective antibodies. Vector immune prophylaxis also allows for production of protective antibodies in vivo that have not been achieved with vaccination.[285] Several studies have demonstrated the effectiveness of this delivery strategy in protecting NHPs against SIV,[285,286] humanized mice against HIV,[287,288] and mice and ferrets against influenza.[289,290] The first vector immune prophylaxis approach has reached the clinic to inhibit HIV infection. A similar approach has been described using DNA vectors delivered by EP. This DNA monoclonal antibody delivery approach showed in vivo production of broadly neutralizing HIV antibodies,[291] as well as antibodies that can protect against Dengue challenge.[292] Collectively the vector immune prophylaxis and related platforms are clearly exciting, even though they are early and have many hurdles to overcome. They illustrate how gene vectors are revolutionizing the way we think about traditional vaccination for protection against infectious disease.

VECTORS IN DEVELOPMENT

Alphaviruses are negative-stranded RNA viruses that can be modified to express foreign recombinant genes without producing pathogenic infections. Prototypes include Venezuelan equine encephalitis virus,[293,294] Sindbis virus,[295,296] and Semliki Forest virus. Replication-defective herpes simplex virus (HSV) can be produced using packaging cell lines similar to those described for replication-defective rAd5, AAV, or alphavirus vectors. These vaccines have been developed not only to deliver foreign genes as potential immunogens, but also to be vectors against HSV itself, including both HSV1 and HSV2.[297] Vesicular stomatitis virus, dengue virus type 4, yellow fever virus, and alphavirus have been modified to express heterologous viral genes for vaccines for infectious disease targets including HIV, West Nile virus, filoviruses, CMV, and other pathogens.[298–305]

Although not yet in the clinic, interesting data have been generated in NHP challenge models by a novel recombinant replicating simian CMV vector (RhCMV/SIV). This is engineered to contain SIV antigens env, gag, pol, vpr/vpx in four separate RhCMV vectors. The concept is that CMV infection is a potent T-cell memory driving infection, therefore recombinant antigens expressed from this vector may represent a novel CTL-inducing vaccine platform.[306] In an illustrative example, animals were vaccinated twice at 98-day intervals with the RhCMV/SIV vectors, then rested for 545 days and subjected to repeat low-dose pathogenic SIVmac[293] challenge. Over time, 50% of the vaccinated challenged animals exhibited close to complete control over the SIV challenge.[307] Surprisingly, CD8 T-cell responses were not responsible for this impressive control, rather it appears that CD8 T-cell killing is redirected in the context of MHC II molecules.[308] Important issues, such as development of a more field-ready vaccine protocol, understanding why protection is observed in specifically 50% of the animals, understanding the role of the observed CD8 class II killing and its relevance to humans, development of similar acting human appropriate vectors for study, as well as the unique differences between simian CMV and human CMV vectors will require more investigation. However, the interesting impact in the difficult SIV challenge model and the associated control and immune-based clearance is an important avenue for research for control of HIV and other chronic infections.

An unique vector that has moved quickly into the clinic is based on the vesicular stomatitis platform (VSV). The VSV Ebolavirus vaccine (rVSV-ZEBOV) was developed by the Public Health Agency of Canada and consists of a recombinant, replication-competent VSV engineered to express the surface glycoprotein from the Zaire strain of Ebolavirus (Fig. 67.3). Preclinical testing in mice and NHPs demonstrated that IM injections of rVSV-ZEBOV induced neutralizing antibodies that were capable of protecting the animals from a challenge with a lethal dose of Ebolavirus.[309] Phase I clinical trials studying the safety of this vaccine reported that vaccination with rVSV-ZEBOV at doses ranging from 3×10^5 to 50×10^6 plaque forming units (PFU) resulted in no serious adverse events with the only reported side effects being mild fever, joint pains, and vesicular dermatitis in some vaccines.[310] Importantly, vaccination resulted in a transient systemic infection that generated antibodies against Ebolavirus that were capable of neutralizing the virus in vitro (described more fully in the Chapter 20). This vaccine was deployed during the current outbreak. A Phase III study was designed using a ring vaccination strategy and a single immunization protocol. Excitingly, the preliminary results of this Phase III clinical trial of rVSV-ZEBOV in Guinea revealed that the vaccine was 100% effective in protecting close contacts of Ebolavirus patients from becoming infected when the vaccine was administered immediately as opposed to when it was delivered 3 weeks following the patient's diagnosis.[311] Based on these encouraging preliminary data, the World Health Organization approved the continuation of the trial with the elimination of the 3-week postexposure vaccination arm of the study. Further testing is needed to qualify and quantify the anti-Ebolavirus immune response(s) generated in vaccinees, to determine the longevity of protection, and to evaluate the protection generated against different strains of Ebolavirus. Nonetheless, these data are impressive and important for in the context of Ebola and other pandemic outbreaks.

The VSV Ebola is an example of pressing into service a recombinant platform resulting from the urgency of a serious

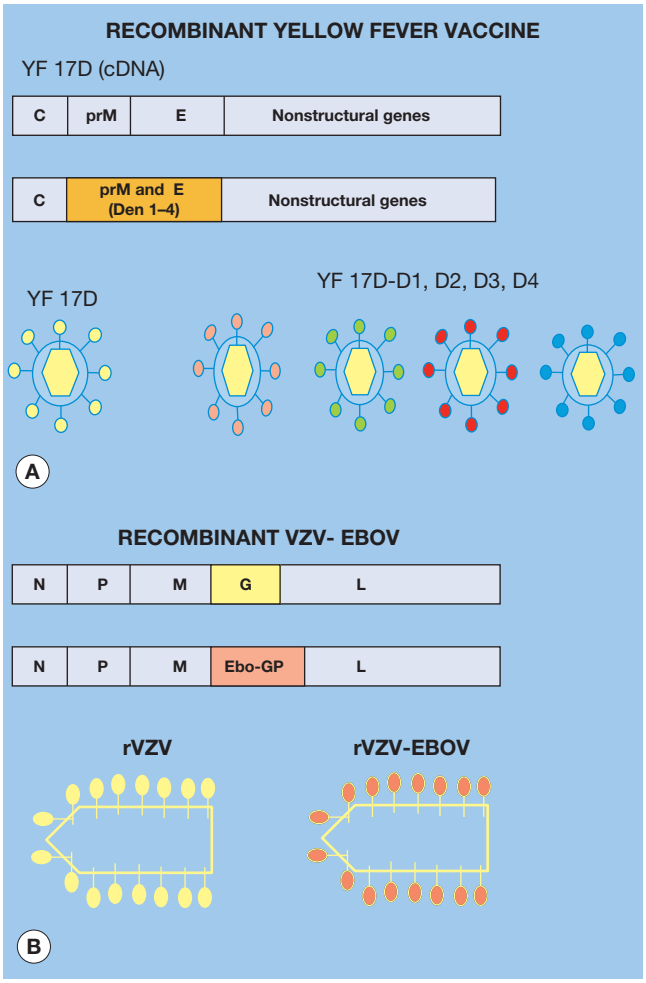


Figure 67.3. A, Recombinant chimeric dengue vaccine. The figure depicts the genome of the attenuated yellow fever vaccine *(top)* versus the chimeric vaccines genome, which has the *prM* and *E* genes *(in orange)* inserted in their place, one each in four different backbones. The *prM* and *E* genes associate to form a complex for env transport and correct surface orientation allowing attachment of the E antigen allowing infection and replication of the attenuated vaccine. The E antigen contains the important immune targets for protection. As there are four different Dengue viral serotypes *(Den 1-4)*, a different chimeric virus was constructed to contain each serotype. This is depicted in the schematic on the bottom of the figure to the left of the attenuated YF17D viral particle cartoon. **B,** Varicella zoster virus–Ebolavirus (VZV-EBOV). The figure depicts the genome of attenuated VZV *(top)* versus the genome of the attenuated VZV with the Ebola glycoprotein inserted upstream of the L antigen. *Yellow*, glycoprotein from VZV; *orange*, glycoprotein from Ebola strain. The glycoprotein switch between the attenuated vesicular stomatitis backbone and the constructed VZV-Ebolavirus vaccine as a viral particle is shown in the schematic below. To construct the vaccine the gene for the native envelope of VZV was deleted. The glycoprotein from the Ebolavirus Zaire-Kikwit 1995 strain is inserted into the recombinant viral genome.

life-threating emerging disease. In contrast, the development of chimeric attenuated yellow fever virus vector expressing dengue viral antigens is a story reminiscent of the development of recombinant poxviral vaccine vectors. The live attenuated 17D strain of yellow fever vaccine is considered one of the world's most effective and safest vaccines. It has been deployed since the 1950s with millions of doses administered. Originally developed by Max Theiler in 1937 through passage, a single dose of the vaccine can confer lifelong immunity. This passed virus has been used as the basis for construction of the new chimeric tetravalent dengue vaccine (CYD-TDV or ChimeriVax) which consists of four recombinant, live, attenuated yellow fever virus 17D vaccine backbones in which the surface Env and premembrane genes were swapped out for those of one of the four serotypes of dengue virus (see Fig. 67.3).[312] The administered vaccine dose consists of 1×10^5 CCID50 (median end point of the cell culture's infectious dose) of each chimeric vector, and each vaccinee receives three doses of vaccine, 6 months apart. Phase IIb clinical trials reported that the vaccine was 30% effective at preventing any dengue infection, and that the vaccine primarily provided protection from dengue serotypes 1, 3, and 4, and offered no protection from serotype 2.[313,314] In two Phase III trials in dengue endemic regions ChimeriVax was found to be 56% to 60% effective in preventing dengue virus infections including infections with dengue serotype 2. Additional testing and research are needed to determine the immune correlates of protection for the vaccine, and a Phase IV clinical trial will likely be needed after licensure to monitor the continued safety, efficacy, and feasibility of large scale vaccination with ChimeriVax. An important fact is that development of this vaccine cost more than $1.5 billion and more than 20 years of research to get to this milestone. Importantly, ChimeriVax is poised to be one of the first examples of successful licensure of a recombinant gene-based vaccine.

Cell Substrates

The progress of more recent viral vectors has depended on the development of appropriate packaging cell lines and cell substrates for viral production. Changes in regulatory requirements that allowed the advancement of transformed cell lines for virus production have proved invaluable in facilitating this effort. For recombinant adenoviral production, the PERC6 and GV11 cell lines have supported production of clinical-grade Ad5, and these have progressed into trials for HIV and are under study for other infectious agents, such as Ebolavirus, Marburg virus, tuberculosis, and malaria. Once approved, these cell lines can be used for diverse vectors. The PERC6 cell line has been used to develop a number of vaccines, including those for West Nile and influenza viruses. In these latter cases, the propagated virus is subsequently inactivated before administration to humans.

For the generation of replication-defective viral vectors, these cell lines allow the production of vectors that can be used in human vaccine studies. Of the viruses developed for such vaccines, representative members (summarized in Fig. 67.1B) include recombinant Ad, poxviruses, measles, Venezuelan equine encephalitis virus, and AAV, all of which have progressed into human trials. The development of transformed cell lines that are capable of propagation, in contrast to the previous standard, avian leukosis–free primary CEFs, represents a major advance in vaccine production technology. These cell lines facilitate the production of replication-defective viral vectors in stably transfected cell lines and offer potentially improved yields and stable production capacity. The development of these lines has taken years to implement because of regulatory concerns regarding adventitious agents, tumorigenicity, and other safety and consistency considerations. Oversight and evaluation of the strengths and limitations of these cell substrates continues,[315] based on guidelines created several years ago,[316,317] with an increasing number of such lines becoming better characterized and available.

Bacterial Vaccine Vectors

Because many infectious agents replicate at mucosal membranes and transit through the gastrointestinal tract for primary infection, the ability to elicit effective immune responses at these sites is desirable. A variety of bacteria are able to replicate at mucosal sites of natural infection, and it has been proposed that attenuation of these microorganisms and modification to facilitate the delivery of antigen might allow the development of improved vaccines to protect against pathogens that enter through the mucosa. Development of live bacterial vectors has therefore focused both on their ability to induce mucosal IgA responses and on cytolytic T-cell responses at mucosal sites. The synthesis of proteins in mammalian cells delivered by bacterial vectors has the potential to induce the cellular immunity that is the goal of many gene-based viral and nonviral vaccines. These approaches have been reviewed in detail elsewhere[318–320] and are summarized briefly here.

Among the live bacterial vectors used for antigen delivery, there are mucosal pathogens that have been attenuated, including strains of *Listeria monocytogenes*, *Salmonella*, *Vibrio* cholera, *Shigella*, *Mycobacteria bovis*, *Yersinia enterocolitica*, and *Bacillus anthracis*. In addition, there are commensal strains such as *Streptococcus gordonii*, lactobacilli, and staphylococci that have been used for the induction of humoral and cellular responses. For gene-based vaccination, *L. monocytogenes* has been a particular focus of research. This gram-positive intracellular pathogen has been studied as a model for understanding class I MHC-restricted immune responses. These responses are normally seen against the bacterial proteins or coexpressed antigens. This microorganism uses a specialized system to introduce proteins into cells and facilitate processing and presentation through MHC class I, and different mutations have been used to develop attenuated strains that retain the ability to deliver antigens. Similarly, *Salmonella* bacterial strains are intracellular pathogens that become restricted to the endosomal compartment of eukaryotic cells, where they are resistant to lysis.[321] A variety of mutations have been introduced into *Salmonella* to generate several different live vaccine carriers, and these vaccine prototypes have undergone further development for vaccine delivery. Among the other bacterial carriers, *M. bovis* Calmette-Guérin (bacille Calmette-Guérin) has been a widely used bacterial vaccine; for example, this organism has been used to express HIV antigens.[322,323] In some instances, expression of mammalian genes has required modification of codons more consistent with the host cell type, which has improved immunogenicity.[18,324] At present, however, the ability of such microorganisms to induce cellular immunity at levels similar to adenoviral or poxviral vectors in humans is still under investigation.

An area of intense interest has been the use of live bacterial vectors for the delivery of DNA vaccines. In this instance, the aim is for the bacteria to deliver plasmid DNA into the cytoplasm of infected cells; organisms such as *Shigella* and *Listeria* have been used for this purpose.[325,326] In addition, attenuated *Salmonella* has been evaluated for these purposes and has shown some promise in both infectious disease and tumor models in experimental animals.[327–329]

Although the use of such bacterial vectors is attractive in theory, it is more difficult to reduce this method to practice. Among the concerns is the possibility of reversion or reactogenicity of these potentially pathogenic bacteria to wild-type forms, the stability of the recombinant bacteria, and the possibility that preexisting immunity from exposure to natural pathogens may limit their infectivity. A variety of host genetic factors can modulate the immune response induced by the bacterial carrier, and variability in the innate immune responses to such pathogens may limit their consistency in vivo. Finally, perhaps the most challenging problem has been the ability to effect a gene transfer from bacteria into mammalian cells. It is likely that very specialized transport pathways are required for the successful implementation of this technology, and if additional improvements will be necessary to improve the efficacy of this approach remains to be established in the clinic.

CLINICAL APPLICATIONS OF GENE-BASED VECTOR TECHNOLOGY

There has been substantial work in animal models of genetic vaccine efficacy. Until very recently there were no reported successes in human studies for guidance; rather there were several failures that brought into question the potential of the technology. However, this status is rapidly changing. Some of the oldest of the recombinant vector platforms have been in clinical evaluation for close to 2 decades. Several trials using the poxvirus technology have been studied in efficacy trials. These include canarypox, MVA, and NYVAC, which have been evaluated in various Phase I to III human studies. As the production technology for poxviruses is well researched, and good-manufacturing-practice procedures for amplification of these viruses followed protocols similar to those developed for vaccinia virus, the path toward clinical studies appeared relatively straightforward. Over the past 3 decades efficacy data were elusive, but recently there has been some positive news. The "Thai" trail of a canarypox vector engineered to carry HIV antigens in which patients were boosted with gp120 antigen provided a possible glimpse of efficacy in a prime-boost platform study, with approximately 31% protection from infection reported.[277] Subsequently, the ProstaVac immune therapy efficacy trial using two different poxviral backbones in prime-boost fashion demonstrated overall increased survival in patients with metastatic castration-resistant prostate cancer of 8.5 months. These important results are supportive of potential impact of these vectors in other targets in the future. It is also important to remember that studies of recombinant MVA technology have given rise to a potentially safer next-generation vaccine for smallpox.[31]

Similarly, DNA vaccines have undergone Phase I testing targeting a diverse collection of infectious diseases, including Ebolavirus, West Nile virus, the SARS coronavirus, MERS, Zika, and influenza virus as well as in cancer studies. Proof-of-concept studies against these viruses have been performed first in animal models with either DNA or in prime-boost combinations or using newer synthetic EP systems. In such studies, impressive protection has been demonstrated in animals.[75,330] Based on these findings, several Phase I trials were completed for Ebola, SARS, and West Nile virus disease targets.[113–115]

In the case of influenza, both naked DNA and DNA adjuvanted with gold microparticles (by biolistics) have advanced into clinical testing. Of particular interest is the development of prime-boost strategies to stimulate the production of broadly neutralizing antibodies to influenza viruses, demonstrated initially in mice, ferrets, and monkeys.[331] Phase I studies testing this concept in humans revealed that even a single injection of a DNA vaccine can prime for an effective traditional vaccine boost against the H5N1 virus. This regimen also showed that more broadly neutralizing anti-stem antibodies can be elicited by vaccination in humans.[332]

A Phase IIb efficacy trial testing a synthetic DNA delivered by EP (VGX-3100) in cervical disease reported that 50% of the women in the vaccine arm regressed disease, and 40% of them cured their underlying infection, providing the first efficacy outcome for the DNA vaccine field.[333] It is likely that additional studies building off these more potent approaches will

be important to study. As previously discussed, DNA vaccines have been approved for veterinary use, including a DNA vaccine for West Nile virus in horses[334] and a DNA vaccine for infectious hematopoietic necrosis virus for protection of farm-raised fish. An additional vaccine is being developed against viral hemorrhagic septicemia virus in farmed salmon. In these studies, a single injection of microgram amounts of DNA induces rapid and long-lasting immune protection.[335]

Among many research and early clinical accomplishments, the efficacy data reported in multiple gene-based studies is a refreshing change. The protection reported for rVSV-ZEBOV vaccine studies which prevented Ebola transmission in West Africa, the efficacy of tetravalent ChimeriVax lowering hospitalizations and improving outcome in dengue endemic regions, the impact of ProstaVac for extending life of cancer patients, and the ability of VGX 3100 to regress and clear some women with neoplastic cervical disease serve as major milestones for gene based vaccine technologies. These efficacy outcomes have come to fruition only in the last few years suggesting that specific gene based platforms appear to be coming of age. The precedent set by these studies provide hope that additional gene-based vaccines will become available for human use and may contribute to the development of protective immunity for a variety of challenging infectious diseases as well as providing new therapies for cancer.

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68 Technologies to Improve Immunization

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NEW TECHNOLOGIES NEEDED TO REDUCE IMMUNIZATION LOGISTICS HURDLES

Immunization can be described as the process of delivering carefully packaged antigen to the appropriate destination in a vaccine recipient to produce a desired immune response. In this sense, immunization programs are package delivery systems: they manage the flow of antigens, formulated in vaccines and packaged in different presentations, between the point of origin at the vaccine manufacturer and the point of consumption, inside antigen-presenting cells (APCs) of the vaccinee. This simple concept of delivering antigen packages from point to point can help elucidate the complex logistical challenges inherent in the preservation, packaging, storage, transportation, and administration of vaccines.

Immunization is one of the most powerful tools for health, but many current vaccines are not affordable, accessible, and acceptable to everyone who needs them. Continual sharpening of this public health tool is needed to achieve the full potential of immunization for improving health. Some advances will come in the form of better vaccine antigens; however, significant potential also lies in improving the way vaccines are packaged and delivered. Reviewing immunization as a package delivery process and recognizing critical hurdles, bottlenecks, and barriers to vaccine flow is a first step toward making immunization programs more efficient and effective. Managing vaccine flow around those obstacles is the day-to-day work of immunization programs, which often requires heroic effort. This chapter briefly describes key restrictions to vaccine flow logistics in terms of complexity, cost, human resources requirements, distributability, and sources of errors in the immunization process. It reviews new technologies in various stages of development that have the potential to eliminate or reduce restrictions to vaccine flow. These new technologies have the potential to increase the capacity and efficiency of immunization programs and make immunization safer and more effective, affordable, accessible, and acceptable for everyone.

POINT-TO-POINT ANTIGEN PACKAGE DELIVERY

The four key points in the antigen package delivery system are the point of origin, storage and distribution point, the point of care (POC), and the final destination point in the recipient. The points of origin are the dozens of vaccine manufacturing plants where antigens are produced and, along with other components, formulated into vaccines, and where the vaccines are further packaged in multiple layers of containers for storage and distribution. The distribution and storage points form the network of thousands of sites from vaccine plants through national, regional, and local centers tasked with safely storing and transporting vaccines to the POCs. The POCs are the millions of hospitals, clinics, health posts, and homes where a competent vaccinator, an informed and willing vaccinee, and a safe and effective vaccine can be brought together for vaccine administration. The final destination points for antigen package delivery are the APCs inside the IM, subcutaneous (SC), cutaneous, or mucosal tissues of the billions of people who will benefit from immunization. Each of the four points along the package delivery system has distinct logistical challenges.

Points of Origin: Vaccine Manufacturing Plants

Purity and Stability

The antigen package delivery system originates in the vaccine manufacturing plant. The formulation, manufacture, and packaging of vaccines are well described in Chapter 5; this chapter highlights key factors relevant to immunization logistics. Vaccine manufacturing plants are highly capitalized, multimillion-dollar facilities that use sophisticated technology to mass-produce the billions of doses of vaccines used globally each year. Two critical vaccine flow logistics requirements start in the plant and span the entire immunization process up to vaccination of the individual: (a) maintenance of vaccine purity and (b) maintenance of vaccine potency by keeping it within the prescribed temperature range. These impose significant restrictions on vaccine flow logistics.

To maintain purity, the vaccine plant environment is highly regulated and monitored: people, equipment, and materials are introduced into the facilities in a precisely controlled manner. Expensive, high-speed filling equipment enables sterile packaging of bulk vaccine into specific-dose packages at low costs. Each step is carefully orchestrated, and even minor modifications to procedures or material may require approval from national regulatory authorities. Any change to the process implies regulatory consequences with significant associated economic costs (see Chapter 5).

For almost all currently licensed vaccines, the required storage conditions include constant maintenance at 2 °C to 8 °C (or −20 °C for several vaccines). This requirement, which is met by the system known as the cold chain, constricts the flow of vaccine to facilities and delivery mechanisms that have refrigeration or cold pack storage capabilities. Some vaccines, especially those that include aluminum adjuvants, are susceptible to damage from freezing temperatures. Others, especially live attenuated vaccines, are more susceptible to loss of potency at elevated temperatures. Through the addition of stabilizers and, in some cases, the additional step of lyophilization (freeze drying), vaccines maintain minimum potency under the required storage conditions, throughout the listed shelf life. When breaks in the cold chain are detected, valuable vaccines are discarded. When undetected, cold chain failures can result in administration of ineffective vaccine.

Presentation

Vaccine presentation affects flow logistics. In contrast to many pharmaceuticals, all currently licensed vaccines are administered as liquids—by needle and syringe injection, oral delivery, or nasal spray. Most vaccines are formulated, packaged, and shipped as liquids in single- or multidose vials or prefilled syringes. All others are first formulated as liquids, packaged in vials, and then lyophilized to enhance their stability. Although they are shipped in a dry format, lyophilized vaccines must be accompanied by a liquid diluent and reconstituted before on-site filling of the administration device and vaccination.

Liquid diluents increase the volume and weight of the vaccine, which may increase shipping costs and require more cold chain storage capacity. There is also the risk of spilling and leakage. Thus, the lyophilized format of vaccine with liquid diluent can slow down efficient vaccine flow.

There are three basic vaccine presentation schemes: prefilled delivery devices, liquid vaccine in vials or ampules, and lyophilized vaccine in vials. Prefilled delivery devices simplify the logistics at the POCs because they minimize on-site preparation. They also reduce the number of components to be shipped; eliminate the need for a separate supply chain for vaccine, diluent, and administration devices; and reduce filling overage required for vials and ampules. However, packaging vaccines in prefilled delivery devices is expensive, and the larger volumes of some devices increase the space needed in cold chain storage.

Liquid vaccines packaged and shipped in vials or ampules typically cost less per dose and occupy less cold chain space than prefilled vaccine presentations, but they require filling of the administration device at the POCs. This increases delivery complexity and creates an opportunity for errors, such as withdrawing the wrong dose amount from the vial or contamination of the vaccine. Vaccines in vials can be in single- or multidose format. Multidose vials typically cost less per dose and occupy less cold chain space than single-dose vials; however, vaccine wastage may be increased with multidose vials if a vial is opened when there are fewer people needing vaccine than the doses in the vial. Vaccinators also may be reluctant to open a 10-dose vial if only one or two people need vaccine, leading to missed opportunities to vaccinate those people. Repeated entry into a multidose vial and time lapse between vaccine withdrawals increase the risk of bacterial or fungal overgrowth, with subsequent injection of a contaminated vaccine. Single-dose vials avoid some of these problems but cost more per dose, occupy more cold chain space, and still require on-site filling of the delivery device.

Lyophilized vaccines can be packaged in single- or multidose vials, and they share the same problems as liquid vaccines in vials. The reconstitution step needed for lyophilized vaccines adds further complexity to delivery, creating the opportunity for errors such as use of a wrong amount of diluent or the wrong diluent. Other challenges with reconstitution include the potential for mismatched amounts of dry vaccine and diluent—which are often shipped and stored separately, with vaccine in the cold chain and diluent at ambient temperature—and a temperature difference between the diluent and the dried vaccine, which may render some vaccines ineffective upon reconstitution.

Inability to overcome the challenges presented by multidose liquid vial presentation and by lyophilized presentations can result in failure to immunize a vaccinee; cross-contamination of infectious pathogens among persons receiving vaccinations; and adverse reactions, including local abscesses, toxic shock syndrome, or even death.[1–9] Although rare, these errors can have grave consequences and may significantly undermine public confidence in vaccines. A recent example of a reconstitution error occurred in Syria in 2014, when an anesthetic agent was mistakenly used for a vaccine diluent, resulting in 15 infant deaths and many hospitalizations.[10]

Distribution and Storage Points

Complex networks of storage and shipping facilities manage the distribution of vaccines from dozens of manufacturers to millions of POCs. The challenges associated with timely ordering, purchase, inventory, and monitoring are staggering and result in ongoing attempts to improve immunization programs and develop software to assist with the logistics.

Vaccine distributability, a key logistics concept, is how easily a vaccine can be transported to POCs and administered to vaccine recipients. Cold chain requirements at every level of storage and distribution can significantly reduce vaccine distributability. In general, the complexity and difficulty associated with cold chain management increase with distance from the manufacturer. It is relatively easy to keep large quantities of vaccine refrigerated and monitored in cold rooms at vaccine plants or large national or regional storage facilities, and economies of scale reduce the per dose cost of storage. However, maintaining the refrigerated storage with around-the-clock monitoring and backup energy sources for the smaller quantities of vaccine present at each district and at the local level can be a daunting task.[11]

Package shipping must avoid delays that could allow vaccine temperatures to rise. Transporting vaccines at cold temperatures at the end of the cold chain, where transport is often by unrefrigerated vehicles, requires insulated boxes with cold packs, which significantly increase weight, expense, and difficulty.

Vaccination by needle and syringe injection requires highly skilled staff, which combined with limited staff supply can restrict distributability. Self-administered vaccines could significantly increase distributability, but only oral typhoid vaccine has been approved for self-administration.[12] Thermostable[13] self-administered vaccines distributed through regular mail systems and self-administered are a conceptual example of ideal distributability.

Points of Care: Vaccine Administration Settings

The POCs are the settings where three essential components meet: vaccine, vaccinator, and vaccinee. Ideally, a caring and competent vaccinator administers a safe and effective vaccine to an informed and willing vaccinee. While this chapter focuses on the logistics of getting vaccines safely to the POCs and technologies for vaccine administration, getting vaccinators and vaccinees to the POCs is equally important. As the complexity of the vaccination process increases, the skill level required of the vaccinator increases as well: safe injection requires highly trained staff, while oral vaccine delivery can be performed by minimally trained volunteers. Many pharmaceuticals and other treatments can be self-administered, but oral typhoid is currently the only vaccine approved for self-administration. Self-vaccination—enabling the vaccinee to be the vaccinator—could overcome the substantial logistical bottleneck resulting from skilled vaccinator shortages.

Getting potential vaccinees to the POCs can also be difficult, so these places need to be as close and convenient to everyone who needs vaccines as feasible. The vaccinee's home may be the most convenient POC, and many mass immunization campaigns in low-resource settings are conducted house-to-house to achieve maximum coverage. However, logistical challenges increase with rising numbers of POCs and with distance from distribution points. Vaccine hesitancy resulting from lack of confidence in vaccines or the health system, complacency about the risk of vaccine-preventable disease, or inconvenience of immunization can be another limiting factor in getting potential vaccinees to the POC. This is a multifaceted problem that must be addressed at many levels.[14] The pain associated with injection and needle phobia is one issue that may cause people not to seek vaccination. Many adults and children suffer from needle phobia.[15–17] Needle-free vaccine delivery technologies may increase the acceptability of vaccination.[12]

Vaccination, vaccine delivery, and vaccine administration are all terms that may refer to the act of transporting a vaccine across the skin of the vaccinee into the cutaneous, SC, or IM

tissue, or into an orifice to contact mucosal tissue. The vaccine delivery systems used for administration, such as injection by needle and syringe, jet injectors, or microarrays, may be considered *macrodelivery* systems. In contrast, vaccine *microdelivery* systems are the molecular antigen packaging technologies, such as viral vectors, microparticles, or virus-like particles (VLPs), that help transport the antigen to APCs once it has been administered to the vaccinee. Currently, most vaccines are administered by needle and syringe injection into the IM, SC, or intradermal (ID) tissues. Other vaccines are delivered mucosally by the oral or intranasal (IN) routes. Vaccine administration methods and devices are critical aspects of immunization logistics and many of the new immunization technologies described in this chapter focus on vaccine macrodelivery systems.

Current Vaccination Methods: Description and Logistical Hurdles

Vaccination by Injection. Hypodermic injection with a needle and syringe dates back to the mid-19th century and is such a predominant vaccine administration method that "shots" or "jabs" are often considered synonymous with vaccinations. Mass production of needles and syringes results in extremely low costs for these devices. Injection breaches the skin's stratum corneum, the protective layers of dead keratinized epithelial cells, to deposit vaccine in direct contact with the underlying dermal, SC, or muscle tissues. IM and SC delivery of vaccines by needle and syringe provides highly consistent dosing. For ID injection, because the dermal layer is so thin, precise targeted deposition is more difficult, so ID needle injection may provide less consistent delivery to the targeted tissues. Overall, needle and syringe vaccination produces excellent immune responses and is extremely safe when proper procedures are followed by trained vaccinators.

However, significant logistical limitations are inherent in needle and syringe injection. The high level of skill required for safe injections limits the availability of vaccinators. Reuse of contaminated syringes and needles is common in some developing countries and can lead to transmission of bloodborne diseases such as hepatitis and HIV.[18–22] Inexpensive autodisabling syringes and needles can prevent reuse and mitigate this problem. However, while autodisabling devices are used in many developing countries, risk of needlestick injuries to healthcare workers remains a concern and increases healthcare costs.[23,24] Safety syringes which include needlestick protection features, described later in this chapter, can reduce the risk of needlestick injuries. Finally, the cost and complexity of safe disposal of sharps in the medical waste stream represent major logistical challenges.

To overcome many of the difficulties of needle and syringe injection, multiuse nozzle jet injectors (MUNJIs) were commonly used in the past for IM and SC injection. However, repeated use of the jet injection nozzles without cleaning between patients was shown to have a risk of transmission of bloodborne pathogens, and use of these devices was discontinued.[25] Disposable syringe jet injectors (DSJIs) eliminated this contamination problem and are discussed in detail later in this chapter.

Mucosal Vaccination. In addition to vaccination by injection, current macrodelivery methods include mucosal vaccination via oral ingestion or nasal spray delivery. Both methods share the advantages of being needle free and present the possibility for simple administration methods. Mucosal vaccination mimics the route of entry, through portals to mucosal tissues, of many infectious agents and typically provides higher levels of mucosal immunity. The major challenge of mucosal vaccination is that delivery of antigen to the target tissues can be much less consistent than IM or SC vaccination by injection. Mucosal vaccination deposits vaccine on internal surfaces of the body. Although the vaccine is inside the body, it still must evade a variety of defense mechanisms to penetrate the mucosal surface and contact target tissues. Mucus flow, gastric acid, mucosal antibodies, and other antimicrobial substances continually destroy or remove substances on mucosal surfaces. As a result, mucosal vaccination generally requires higher doses of antigen, macrodelivery technologies to assure tissue contact, and more specialized antigen microdelivery packaging to reach APCs consistently.

Destination Points: Inside the Vaccinee's Antigen-Presenting Cells

Vaccination delivers the antigen package into the person receiving the vaccine, but delivery is not complete until APCs internalize the antigen. Immunization involves mimicry: vaccines must never be pathogenic, but must cause the APCs to respond to antigens as pathogens. One of the first principles of this mimicry is that the antigen package should be "pathogen-sized." Free antigen in solution is typically ignored by immune cells; however, APCs readily take up microparticle packages that are in the size range of pathogens, such as viruses and bacteria, by phagocytosis and endocytosis. These microparticle antigen packages are likely to be assessed as threatening and to initiate an immune response. Molecular antigen packages may include adjuvants, which are nonantigen components designed to trigger or modify an immune response. Some adjuvants act as package "warning labels" to alert activation of the innate immune system. Other adjuvants create a depot effect, which causes the antigen package to be opened gradually, prolonging the presence of antigen at the delivery site. Another molecular antigen packaging strategy is inclusion of specific cellular address labels. Typically, vaccine macrodelivery systems place the molecular antigen package in or near the tissues where APCs reside or traverse. For some vaccines, using molecules that match receptors on APCs such as dendritic cells or M cells increases the likelihood of delivery specifically to these cells. Many molecular packaging strategies for antigen delivery that are in use or in development are described in detail in Chapters 64 and Chapter 65, and examples are described briefly later in this chapter. Once the APCs have received the antigen package, the next step is processing of antigen and presentation to lymphocytes to initiate the immune response, which is well described in Chapter 2.

Logistical Hurdles in Special Settings

Emergency Settings

Preparation for emergencies should be included in every national immunization program plan. National programs in developed and developing countries have had varying degrees of success achieving high routine vaccine coverage rates, and strengthening routine systems is a key step for emergency immunization preparedness. Special situations and high-risk populations increase immunization logistical challenges and create the need for rapid delivery of vaccines to vastly increased numbers of people. War and other armed conflicts interrupt routine vaccination services and displace large populations away from available services, often concentrating people in refugee settings that significantly increase the risk of disease and the need for vaccines. Natural disasters such as earthquakes, tsunamis, or hurricanes disrupt local infrastructure, including transportation, electricity needed to maintain the

cold chain, and vaccine supply. Emergency situations expand the demand for skilled vaccinators and often the supply is diminished as vaccinators are personally affected by the emergency or pulled to other emergency duties. Reducing logistical barriers to routine vaccine delivery is critical to assuring the barriers are not insurmountable in an emergency situation.

Low- and Middle-Income Countries

All low- and middle-income countries (LMICs) have national immunization programs, and most provide vaccines recommended by the World Health Organization (WHO) for the following diseases: diphtheria, hepatitis B, *Haemophilus influenzae* type b (Hib), measles, pertussis, poliovirus, tetanus, and tuberculosis.[26] For routine immunization, vaccines can be administered at health posts or clinics (fixed post), or in communities through mobile outreach on a daily, weekly, or monthly schedule. Many countries also provide vaccines against pneumococcal disease, rotavirus, and other pathogens.[26] Supplemental immunization activities include mass campaigns that can be fixed post (often including extra posts), mobile outreach, or house-to-house campaigns. Three critical immunization delivery hurdles in LMICs are the cold chain requirement, the need for skilled vaccinators, and sharps safety.

The 2°C to 8°C cold chain storage requirement is especially challenging in countries with high ambient temperatures or with unreliable access to electricity.[27,28] Keeping vaccines tethered to refrigeration limits the capacity to distribute them to everyone in need because transporting them to remote locations requires cold packaging equipment many times larger and heavier than the vaccine itself. In a cold chain study in Vietnam, 46% of community health centers assessed did not have refrigeration.[11] This limits vaccination to days when vaccine can be transported from district facilities in cold boxes to the health centers for immediate use.

In many countries, cold chain equipment is antiquated and storage capacity is stretched to the limit, restricting the implementation of new vaccinations and eliminating surge capacity for mass-vaccination storage in case of an epidemic. For many immunization programs, purchase and maintenance of cold chain equipment consumes a significant portion of the immunization budget. One study estimated that cold chain equipment and overhead costs would account for 23% of the $25.4 billion needed for non-vaccine immunization program costs for low- and lower-middle-income countries from 2011 to 2020.[29]

Some vaccines may have thermostability that allows them to be managed outside the cold chain for limited periods of time. For example, a major reason for the successful MenAfriVac campaigns in Africa was the relaxation of the cold chain requirements in a number of countries for the last miles of delivery.[30,31] In these countries, a controlled temperature chain was implemented, allowing the vaccine to be shipped and stored at ambient temperatures for up to 4 days. In addition to increasing access to difficult-to-reach areas, a modeling study estimated that use of the controlled temperature chain could potentially reduce cold chain and associated logistics costs.[29] Additional studies based on field implementation are required. Research on the thermostability of other vaccines may enable their use under a controlled temperature chain to simplify logistics for the last difficult miles of delivery, improve vaccine access in remote areas, and potentially reduce costs.

The second hurdle for immunization programs in many LMICs—and often the rate-limiting factor—is the shortage of skilled vaccinators. There is a global shortage of all healthcare workers,[32] and safe and effective vaccination requires highly skilled workers for many vaccines, particularly for needle and syringe injection. Needle-free vaccines that could be administered by minimally trained staff or volunteers, or that could be self-administered, would significantly increase vaccine delivery capacity.

The third important logistics factor for immunizations in LMICs is sharps safety. Needlestick injuries are common; however, unlike in high-income countries, needlestick protection devices and prophylaxis to prevent bloodborne disease transmission following these injuries are often not available in low-resource settings because of cost. Safe disposal of used needles requires an expensive biowaste disposal infrastructure that is not always present. In some countries, used needles are harvested from the common waste stream and repackaged to the unsuspecting for reuse, presenting a high risk for transmission of bloodborne pathogens.

In the long run, thermostable needle-free vaccines could significantly reduce these logistical hurdles to vaccine delivery in developing countries and extend the benefits of immunization to all people. In the short term, intermediate technologies that are in development can improve cold chain shipping and storage and provide needlestick injury protection.

Bioterror and Pandemic Settings

The United States—used here as an example of a developed country—has a relatively strong immunization program infrastructure, which is reflected in high vaccination coverage rates (except for certain communities where vaccine hesitancy by parents has led to historically low coverage rates). In the United States, POCs include doctors' offices, health clinics, and, within the last several years, pharmacies. However, in an emergency, new vaccination POCs may be established—such as sports arenas, schools, convention centers, and other nontraditional locations—to allow mass vaccination to reach large populations rapidly.[33–35] At-risk individuals are a key consideration and include children, pregnant women, people with disabilities, and the elderly, among other groups.

In the event of a pandemic or bioterror event, local, regional, and even national healthcare resources and infrastructure may be overwhelmed. From the immunization logistics perspective, key factors will include ensuring vaccine supply and distribution, establishing accessible POCs for large numbers of people, and providing sufficiently skilled vaccinators to meet the vaccine demand. The U.S. Strategic National Stockpile maintains supplies of key vaccines. Cold chain logistics may become an issue in emergency settings if there is collateral damage to the power infrastructure, as in a natural disaster such as a hurricane, or if massive increases in vaccine volumes overwhelm local cold chain capacity.

In contrast to many developing countries, the United States has little recent experience with mass vaccination campaigns. In a pandemic setting or a bioterror event involving an infectious agent, gathering masses of people in central locations for vaccination may represent an increased risk for transmission of the disease; thus, more discrete methods of vaccine distribution may be preferable.

Summary: Desirable Features of Practically Ideal Vaccine Delivery Systems

The major immunization logistical challenges to vaccine flow include the following: distribution of multicomponent products, purity and sterility requirements, cold chain requirements, availability of highly skilled vaccinators, on-site filling and reconstitution, needle safety issues, and transfer of antigen into APCs.

Technologies described in this chapter address some or many of these challenges. Overall, a "practically ideal vaccine

delivery system" would be a thermostable vaccine in a prefilled unit dose for needle-free delivery with minimal waste. The vaccine would be optimized for shipping, even by mail in some cases, and would be self-administered or administered by minimally trained personnel. The molecular antigen packaging would ensure safe delivery of the antigen into the APC and induction of the immune response with minimal adverse reactions.

NEW TECHNOLOGIES FOR VACCINE STABILIZATION, PACKAGING, STORAGE, SHIPPING, AND ON-SITE PREPARATION: POINTS OF ORIGIN AND DISTRIBUTION POINTS

Maintaining the integrity, potency, and safety of vaccines from their point of origin at the vaccine manufacturer, through the point of preparation and use, requires attention to the vaccine's formulation, packaging (primary, secondary, and tertiary containers), and reconstitution.

Formulation

The development of vaccine formulations includes the chemical and physical characterization of the antigen, potency assays for lot release and to demonstrate stability, preclinical and clinical evaluation of the optimal administration route to include the potential use of adjuvants, and formulation stability development.[36] Current live-attenuated vaccines are not formulated with an adjuvant. Nonreplicating vaccines, including inactivated viruses and bacteria, VLPs, carbohydrate antigens, and purified or recombinant subunit protein antigens, are typically presented as liquid solutions or suspensions and usually contain adjuvants to induce the desired immune response.

A vaccine's formulation consists of the antigen as well as the other supporting ingredients, called excipients. Excipients include preservatives to prevent contamination and adjuvants to enhance potency. A carefully developed formulation can also increase the thermostability of the vaccine and avoid damage to the antigen due to freezing or high temperatures. Live-attenuated vaccines are often lyophilized and freeze stable but tend to be more heat sensitive for long-term storage (months to years) in the cold chain and immediately after reconstitution.[37] Nonreplicating vaccines can be more stable in high temperatures compared with live-attenuated vaccines, but can also be damaged by freezing, particularly if they include aluminum adjuvants.[37]

Preservatives

Thimerosal, an organic mercury preservative (approximately 50% by weight in the form of ethyl mercury), is used in some inactivated vaccines for multidose vial formats to prevent microbial growth in opened and partially used vials. It is also used during some vaccine manufacturing processes as an inactivation agent. Thimerosal is intended to kill or prevent the growth of a broad spectrum of pathogens (bacteria, fungi). The safety of thimerosal has been evaluated by the Global Advisory Committee on Vaccine Safety as well as other national-level expert groups and regulatory bodies, such as the European Agency for the Evaluation of Medicinal Products, the American Academy of Pediatrics, and the U.S. Food and Drug Administration (FDA). These evaluations concluded that, given the short biological half-life of ethyl mercury—which is excreted via the gut and does not accumulate in the body—evidence of long-term toxic effects has not been demonstrated.[38] In 1999, however, the U.S. Public Health Service urged vaccine manufacturers to reduce or eliminate thimerosal from vaccines as a precautionary measure. Currently in the United States, routine vaccines are thimerosal-free or have levels less than or equal to 1 µg of mercury per dose.[39] Two other preservatives in WHO prequalified vaccines are 2-phenoxyethanol, used with inactivated poliovirus vaccine (IPV), and phenol, used for inactivated typhoid vaccine.

Many industrialized countries, such as the United States, have switched to single-dose vials for use in routine immunization, and thimerosal is not used because the vial is accessed only once. However, in multidose vial formats for seasonal influenza vaccines and vaccines for epidemic or pandemic response, preservatives such as thimerosal continue to be used. Furthermore, many vaccines used in LMICs still contain thimerosal, including diphtheria, tetanus, and pertussis, hepatitis B, Hib, influenza, and meningococcal vaccines. LMIC use of single-dose vial presentations is limited by vaccine manufacturer production capacity, the increased cold chain volume, and cost, which many countries cannot absorb.

Thermostability

Extended exposure to elevated and freezing temperatures—those temperatures outside of the recommended range (normally 2°C to 8°C)—can damage vaccines. Heat can denature or otherwise alter the protein tertiary structure; this may reduce viability of live-attenuated vaccines or, in the case of polysaccharide conjugate vaccines, result in increased rates of hydrolysis of the polysaccharide from the protein in the vaccine formulation. Formation of ice crystals can result in freeze damage to the antigen, and freezing of vaccines containing aluminum adjuvants can reduce potency from agglomeration of the adjuvant.[40] Thermostability (resistance to high ambient temperatures or freezing) could reduce potency loss and have positive impacts on vaccine efficacy.[27,41] It could also allow removal of vaccines from the cold chain, reduce costs, and lead to increased coverage by allowing flexibility in time to reach remote populations. Some thermostable products could be dry cakes similar to lyophilized vaccine and would require reconstitution prior to injection. Other dry formulations could be incorporated into unit-dose dry-format delivery systems such as microarray patches, or dry powder aerosols for respiratory administration (see "Cutaneous Vaccination" and "Mucosal Vaccination" later). These formats would have the combined benefits of being needle free and thus simple to administer or self-administer. They would not require refrigeration or reconstitution.

Protection From High Ambient Temperatures. Enhanced thermostability of liquid formulation vaccines can be achieved through selection of buffering agents and by the use of excipients (vaccine formulation ingredients other than the antigen) that can further stabilize the formulation. Examples include nonreducing sugars, nonionic surfactants, and polymers or protein stabilizers. Excipient stabilization can enhance protection from shifts in pH, decrease antigen loss as a result of surface adsorption and aggregation, and prevent or reduce protein-to-protein interactions.[41]

Freeze Protection. Propylene glycol, polyethylene glycol, and glycerol have been used to protect aluminum-adjuvanted vaccines from freezing. Various concentrations of propylene glycol have prevented vaccine freezing or loss of potency and have prevented destructive particle aggregation if physical freezing occurred.[41]

Dry-Format Delivery. Alternative processes to lyophilization for vaccine drying (removing water molecules from the vaccine

suspension) include spray drying, spray-freeze drying (SFD), vacuum-foam drying, and supercritical fluid drying. These processes have been evaluated for increasing vaccine thermostability.[42] Research with aluminum-hydroxide–adjuvanted hepatitis B surface antigen (HBsAg) or human papillomavirus (HPV) vaccines and aluminum-phosphate–adjuvanted diphtheria and tetanus toxoids has been conducted using alternative freeze-drying processes. These studies have typically found aluminum coagulation and difficulty in reconstitution for lyophilized adjuvanted vaccines, although additional excipients and a thin film freeze-drying process may provide freeze protection.[43,44] The SFD method produced a homogenous suspension, indicating the feasibility of this approach for aluminum-adjuvanted vaccines.[45] Evaluation of SFD of HBsAg without aluminum in combination with inulin or dextran/trehalose stabilizers demonstrated enhanced thermostability (up to 60°C). However, preclinical immunogenicity studies of this formulation demonstrated immunoglobulin (Ig) G immune responses that were lower than responses to aluminum-adjuvanted HBsAg.[46] SFD has also been assessed for meningitis A and measles vaccines.[47,48]

In addition, alternative delivery or packaging methods, such as dry powder inhalation, microarray patches, biodegradable implants, or integrated reconstitution technologies (discussed later in this chapter), are currently being developed or used for biologics or pharmaceuticals and could be adapted for vaccines. Primary excipients include nonreducing sugars such as trehalose or sucrose because of the high glass-transition temperatures they exhibit. Glass (amorphous solid) is formed instead of crystals when these excipients are dried, which contributes to vaccine stability.[41]

Packaging

Vaccine packaging is the collection of components that surround the vaccine and protect its integrity (potency/stability/shelf life), from production through the supply chain to the POCs. Vaccine packaging is typically divided into three categories: primary, secondary, and tertiary.[49,50] Primary packaging protects against light, oxygen, and moisture vapor ingress, and it must not allow pH shifts that could affect vaccine stability or antigen binding to the material of the container that could reduce the available dose. The packaging also provides information and identification of its contents.[51] Labeling to identify the product must be integral to the packaging, or affixed to it. Primary containers are nested together in secondary packaging, and larger containers such as boxes or cartons provide tertiary packaging.

Primary Packaging

Vial and Ampoule (Vaccine and Diluent). Primary packaging, such as ampoules, vials, prefilled syringes, and prefilled oral dispensers, comes into direct contact with the vaccine product or diluent and may affect the vaccine formulation itself. The WHO has issued guidelines covering vaccine formulation, presentation, labeling, and packaging to ensure that vaccines submitted for prequalification have been optimized to address LMIC needs. These guidelines include mandatory, critical, unique and innovative, and preferred characteristics. Mandatory and critical characteristics must be met to achieve WHO prequalification. In the case of critical characteristics, some deviation from defined values may be allowed when taking into account public health needs. Unique and innovative characteristics may be vaccine specific and are reviewed as such. Preferred characteristics are not required, but they represent the buyer preference (procurement agencies and national immunization programs).[52]

Vaccines must be WHO prequalified for purchasing by UN agencies. The WHO requirements on vaccine quality, safety, and efficacy are included in the prequalification process, as well as compliance requirements for manufacturing and specifications for packaging and presentation. Prequalification provides assurance that vaccines used by national immunization programs are safe, effective, and meet quality standards.[53]

The Vaccine Presentation and Packaging Advisory Group (VPPAG) is a WHO and United Nations Children's Fund (UNICEF)-led forum for both the public sector and industry to discuss and provide advice on vaccine presentation and packaging. VPPAG has developed a generic preferred product profile for vaccines for LMIC use, which recommends "ready-to-use" presentations that do not require mixing (i.e., reconstitution) and formats that reduce the number of user steps (and potential errors). Vaccine formulations with improved heat and freeze stability also are recommended, to provide for higher temperature storage (target threshold 40°C) and potential use beyond the cold chain. Prefilled syringes or injection systems should reduce the volume required in the cold chain and should incorporate an autodisable feature that prevents reuse. These designs are designated as compact prefilled autodisable injection devices (cPADs). The generic preferred product profile also includes vaccine vial dimensional recommendations that conform to ISO 8362 and are the most efficient size for the cold chain. Vial labeling should include a vaccine vial monitor (per UNICEF and WHO recommendations), which consists of a temperature-sensitive material and serves as a visual indicator of cumulative heat exposure. Labeling should also include standard product, date, and lot information, among other requirements.

Unit Dose Versus Multidose Formats. Multidose presentations are common in LMICs, a result of both lower cost and reduced per-dose volume compared with single-dose presentations. Industrialized or high-income countries are less vaccine-price sensitive and have been switching to single-dose presentations for adult and childhood vaccines. The shift to single dose was accelerated by public concerns regarding thimerosal,[54–58] healthcare provider preference for single-dose presentations including prefilled syringes, and increased awareness of injection safety.[58] In the United States, the awareness of safety issues came in response to the Needlestick Safety and Prevention Act and subsequent revision of the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens standard. This led to requirements for workplace reporting and maintenance of a log of needlestick injuries as well as broader implementation of engineered safety features to reduce or prevent the risk of needlestick injury.[59]

The use of single-dose and small multidose presentations also has increased in LMICs, in part because of the higher costs of vaccine wastage for newer vaccines.[60,61] The use of preservatives in multidose vials is critically important for vaccines that are used in more than one immunization session because repeated access of the vial through the septum, as well as storage between sessions, presents potential contamination risk from pathogen ingress. The WHO's multidose vial policy permits open vials of vaccine with preservative that have been handled under specific conditions to be used for up to 28 days after the first dose is withdrawn.[5] These guidelines are consistent with U.S. recommendations, which also require that an open vial be discarded in 28 days except if there are different manufacturer requirements in the product labeling, which may specify an alternative number of days.[63]

These policies apply only to liquid vaccines that contain preservative; lyophilized live attenuated vaccines—such as measles-containing vaccines, bacillus Calmette-Guérin (BCG), and yellow fever vaccines—generally do not contain preservatives and must be discarded at a maximum of 6 hours after

reconstitution, per the multidose vial policy. Because of vaccine wastage concerns, some healthcare workers are hesitant to reconstitute a multidose vial if there are insufficient numbers of people available to be vaccinated.

Preservative-Free Multidose Primary Packaging. MEDInstill, Inc., a U.S.-based firm, has developed a primary packaging pharmaceutical filling and dispensing technology called Intact that may reduce or eliminate the need for preservatives in multidose vaccine vials. The technology is designed to reduce the risk of contamination during both filling and dispensing from a primary container. To maintain sterility during the filling process, a closed-system valve is used for the filling needle. The prevention of contamination allows for sterile filling in a nonaseptic facility.[64]

In addition, the Intact design has been incorporated into the dispensing port for MEDInstill primary containers. The valve allows multiple withdrawals from the container, maintaining sterility of the contents even in the presence of external contaminants. Multidose vials (Fig. 68.1A) and pouch designs are undergoing evaluation for pharmaceuticals and vaccines.[65]

Prefilled Syringes. Prefilled syringes represent a fully integrated vaccine presentation. Multiple studies comparing prefilled syringes with standard vial presentations have demonstrated increased efficiency and improved vaccination throughput with this packaging format. For example, a study of U.S. nurses preparing and delivering influenza vaccine reported that the time necessary for providing an injection was 12.4 seconds for a prefilled syringe compared with 49.7 seconds for a multidose vial.[66] The increased efficiencies possible with prefilled syringes for both parenteral and nonparenteral administration (oral/nasal) could greatly enhance pandemic/epidemic outbreak response capacity.

Glass Prefilled Syringes. Glass prefilled syringes are manufactured from type 1 borosilicate glass, which has high chemical resistance, low alkali content, and barrier properties appropriate for long-term storage of vaccines and other pharmaceuticals.[67] A leading example is the Becton, Dickinson and Company (BD) Hypak SCF (sterile, clean, ready to fill) glass prefilled syringe used widely in the United States and Europe (see Fig. 68.1B). This design comes in different models to include fixed-needle, Luer slip, and Luer-Lok varieties. Other manufacturers include Gerresheimer, Schott, Nuova Ompi, Nipro, and Catalent. Glass has been used for decades for a variety of primary containers (prefilled syringes, vials, cartridges). Problems associated with its use include the possibility of cracks or breakage.

Plastic Prefilled Syringes. Various plastic materials have been used as alternatives to glass, for both prefilled syringes and other primary container technologies. Plastic syringes are injection molded, allowing for tighter dimensional tolerances and ability to generate alternative geometries. Plastic prefilled syringes are lighter in weight and more resistant to breakage than glass during production, fill/finish, shipping, and programmatic use. Polypropylene (PP) is a polymer used for standard- and large-volume prefilled syringes (up to 50 mL) and vials.[68] Cyclic olefin copolymer and cyclic olefin polymer (COP) are highly transparent polymers that have been used increasingly for prefilled syringes.[69,70] Compared with PP, cyclic olefin copolymer and cyclic olefin polymer have lower water vapor and oxygen permeability, allowing long-term storage of vaccines[71] and have been demonstrated to be biocompatible, resistant to heat, and compatible with various terminal sterilization processes.[68]

The selection of glass or plastic is determined by the formulation or stability requirements of the pharmaceutical, needs of the patient/user, and other requirements. Plastic prefilled syringes are typically more expensive than glass.[72] Examples include the BD Sterifill SCF, SCHOTT TopPac, Gerresheimer ClearJect, Baxter Clearshot, and West Daikyo Crystal Zenith Ready-to-Use silicone-free syringes. Cyclic olefin copolymer and cyclic olefin polymer have also been used for vials; for example, Aseptic Technologies, Inc.'s, AT-Closed Vial composed of cyclic olefin copolymer (see Fig. 68.1C) has been validated for use with GlaxoSmithKline's Synflorix pneumococcal vaccine and was approved by the European Medicines Agency.[73]

Compact Prefilled Autodisable Devices. A cPAD is a prefilled single-dose injection system comparable to a prefilled syringe, but with an autodisable feature that prevents reuse. Like prefilled syringes, cPADs provide accurate doses, fast injection preparation, and quick delivery time (ready to use). In addition, because they are typically smaller than standard syringes, they bring the logistical benefits of decreased volume and weight.[74]

The BD Uniject injection system (see Fig. 68.1D) is a cPAD technology with a small reservoir prefilled with vaccine or other pharmaceutical. It has four main components: reservoir, port, needle assembly, and needle shield. The reservoir is a three-layer laminate film with linear low-density polyethylene in contact with the contained fluid. The port and valve are in contact with the fluid in the reservoir and are also constructed of low-density polyethylene. The needle assembly connected to the port consists of a polystyrene hub and a steel needle. The PP needle shield is removed from the needle for administration. Typically, a foil-laminate secondary pouch maintains stability of the material in the Uniject units. Crucell has developed a novel hybrid secondary packaging for the Uniject system that can store up to 20 filled devices.[75] A needleless version of the Uniject platform, designated "Uniject DP," also has been developed by BD for oral delivery and is commercially available.

To deliver the prefilled dose, after removal of the needle shield and insertion of the needle, the plastic reservoir of the Uniject system is squeezed between the thumb and fingers. The Uniject system is available in four dose-volume sizes: 0.25, 0.5, 1.0, and 2.0 mL. It is available with various needle gauges and lengths, ranging from 18 to 26 gauge and needle lengths of ⅜ to 1.5 inches (for SC or IM injection). The container is provided sterile in "ready-to-fill" reels of 1500. The sterile reel is loaded onto a custom filling machine where the containers are filled and then heat sealed. They are kept on the reel throughout the process and either accumulated in reel form or transferred out of the sterile area for final packaging.[76]

Hepatitis B and tetanus toxoid vaccines made by PT Bio Farma (Indonesia) have been WHO-prequalified in Uniject formats, and Crucell developed a new presentation of Quinvaxem fully liquid pentavalent vaccine in Uniject, which was prequalified but is not being commercialized.[77] A time-and-motion study in Kenya comparing the average time for health workers to prepare and inject 20 doses of pentavalent vaccine in five different presentations found that the prefilled Uniject system was faster to deliver than fill-onsite presentations (single-dose or multidose vials, liquid or lyophilized).[78]

Novel Primary Container Technologies. Blow-fill-seal (BFS) ampoules are plastic primary containers manufactured from polyethylene or PP and used for a variety of pharmaceuticals (see Fig. 68.1E). The containers are extruded, blown, filled, and sealed in an automated, continuous process. Although these ampoules have not yet been approved for vaccines, they have been evaluated with MedImmune's live attenuated influenza vaccine (LAIV)[79] and are being evaluated by rotavirus vaccine manufacturers.[80] For parenteral delivery, a Luer interface can be incorporated into the neck of the ampoule, allowing for connection of a Luer-tip needle. A needle and syringe can also simply withdraw a dose from a standard BFS ampoule

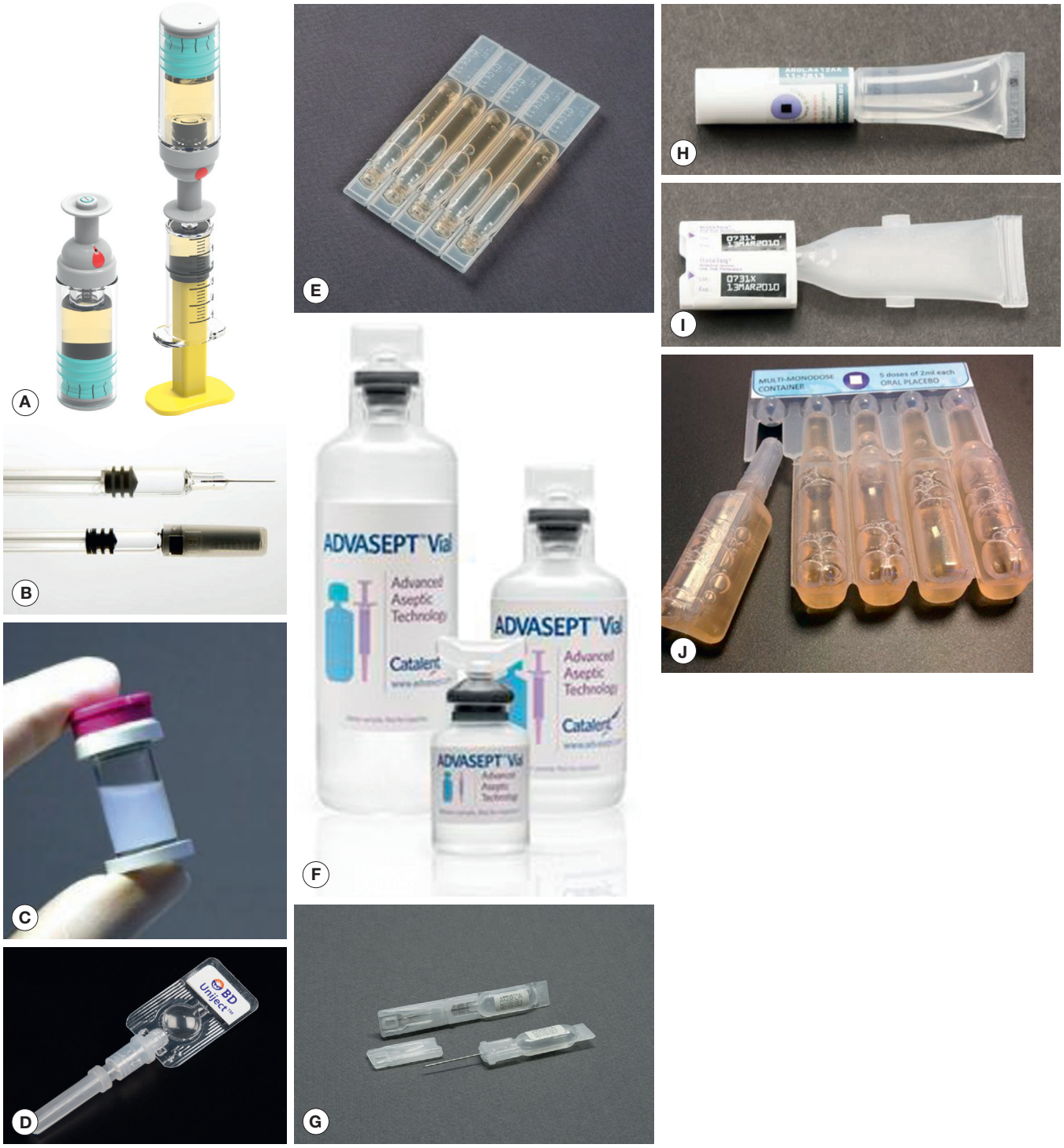


Figure 68.1. Vaccine primary containers. A, MEDInstill vial represents a preservative-free technology that incorporates a novel valve to prevent contamination. **B,** BD Hypack SCF is a widely used prefill glass syringe used for a variety of pharmaceutical applications. **C,** The Aseptic Technologies AT-Closed Vial uses cyclic olefin copolymer, an alternative material to glass. **D,** Uniject is the only WHO-prequalified compact prefilled autodisable (cPAD) technology. **E,** Rommelag produces blow-fill-seal (BFS) technology for small- and large-volume pharmaceuticals. **F,** Catalent ADVASEPT Vial technology uses the advanced aseptic manufacturing process of BFS to incorporate a septum through an insertion to form fill and seal the primary container. **G,** Brevetti Angela has also developed an integrated needle design for use in parenteral delivery, the SECURE-JECT- SQUEEZABLE. **H,** GlaxoSmithKline uses a polymer tube manufactured by Rexam for use with its Rotarix vaccine. **I,** Merck RotaTeq comes in a plastic injection molded tube composed of polyethylene. **J,** PATH in cooperation with Rommelag has developed a multi-mono-dose design with a single vaccine vial monitor to maximize cold chain volume. *(**A,** Courtesy of MEDInstill [Debashis Sahoo]. **B, D, E,** and **H–J,** Courtesy PATH. **C,** Courtesy Aseptic Technologies, Inc. **F,** Courtesy Catalent [Bill Hartzell]. **G,** Courtesy Brevetti Angela [Rajeev Kabbur, Daniel Martinez].)*

or vial with integrated septum such as Catalent's ADVASEPT Vial Technology (see Fig. 68.1F). Rommelag, Weiler Engineering, and Brevetti Angela are leading examples of BFS machine manufacturers. Brevetti Angela and Rommelag have BFS systems with integrated needles incorporated during the forming process (see Fig. 68.1G).

Plastic tubes that are injection molded or extruded have been developed primarily for oral administration. These containers are manufactured, terminally sterilized, and shipped to the pharmaceutical manufacturer for filling and heat sealing. Examples are the Rexam dispenser tube, used for GlaxoSmithKline's Rotarix vaccine, and the Lameplast tube, used for Merck's RotaTeq vaccine (see Figs. 68.1H and I). Under funding from the Bill & Melinda Gates Foundation, PATH, in collaboration with Rommelag, has developed a multi-mono-dose BFS container design targeted for rotavirus vaccine delivery. The design has individual containers conjoined by a shared tab. When one container is separated from the tab, it is rendered open and must be used to deliver the vaccine. This attribute provides key advantages compared with single-dose presentations, including cold chain volume reduction and potential cost savings (see Fig. 68.1J).

Safety Syringes. The Needlestick Safety and Prevention Act, signed into law in 2000, led to the standard use of syringes with engineered safety features for vaccine and drug delivery in the United States. One study found that beginning in 2001, this resulted in a drop of more than one-third in needlestick injuries—with approximate annual reductions of 100,000—and a cost savings of $69 million to $415 million.[81] However, a Cochrane review concluded there was no clear evidence of benefit with use of these devices, despite their increased costs.[82]

To achieve WHO prequalification, vaccines in prefilled syringes are required to have an autodisable or reuse-prevention feature.[52] Autodisable syringes have attached needles and a method to prevent reuse of needle and syringe after delivery of a fixed dose. A reuse-prevention feature syringe allows variable dosing, necessary for volume diluent transfer, for vaccine reconstitution. The WHO recommends autodisable syringes or other syringes with reuse-prevention features for vaccine administration.[84]

WHO also recommends syringes with a sharps injury protection (SIP) feature for immunizations.[85] A sharps injury protection feature covers, shields, or retracts the needle into the syringe barrel to prevent needlestick after use or during disposal. Multiple self-shielding needle technologies have been introduced to reduce the risk of needlestick injuries.[86,87] Currently, the costs of such syringes limit their use in LMICs.

The Unifill safety syringe (Unilife Medical Solutions) has a reuse-prevention feature that retracts the needle after delivery. Other developers, including Tip-Top, Safety Syringes, and Credence MedSystems, have prefilled syringe-compatible sharps injury protection designs that can be incorporated into the syringe after filling.[86,88] Merck's Gardasil HPV vaccine is delivered in a single-dose prefilled syringe with UltraSafe needle guards.[89] Clip-on prefilled syringe safety mechanisms also include the BD Preventis, the West Clip'n'Ject, and the Specialized Health Products International LuproLoc.

Secondary and Tertiary Packaging

Secondary packaging, such as cartons, trays, or foil pouches, hold one or more primary containers, such as vials or prefilled syringes of vaccine. These packages generally represent the volume for calculation of cold chain storage requirements.[52] Secondary packaging is important for vaccine stability; for example, a foil pouch protects a polymer-based primary container such as the Uniject system from water vapor loss, oxygen ingress, and light.

Tertiary packaging includes cartons or cases that enclose multiple secondary packaging units. Insulated shipping boxes with internal walls of polystyrene, isocyanurate panels, or polyurethane foam also maintain cold chain temperatures and prevent shifting during shipment. Pallets are used for international shipment of these containers. In the GS1 General Specifications, these are designated as export packaging or shipping containers, whereas the WHO puts them into the category of tertiary packaging.[90,91]

VPPAG secondary package recommendations include minimizing weight, volume, and the need for in-country repackaging for distribution in the cold chain. Specific vial arrays (rectangular) by number of vials for packing are also recommended. Tertiary packaging recommendations also include minimizing dimensions as well as limiting the need for repackaging, with vials in multiples of 100 for easier inventory control. Weight and width dimension recommendations are also included, with reference to ISO pallet sizes. Labeling recommendations also include the use of bar codes that conform to GS1 standards. Materials used for packaging (primary, secondary, tertiary) should minimize environmental impact.[92]

Environmentally Friendly Secondary Cartons and Tertiary Shipping Containers. Numerous companies have developed insulation products to replace polystyrene. Green Cell Foam technology is a biodegradable, starch-based polymer that can break down within four weeks, dissolves in water, and can be incinerated or burned cleanly (Fig. 68.2A). It maintains temperatures for 24 to 48 hours.[93,94] Ecovative is developing compostable plastics derived from agricultural byproducts and mushroom mycelium.[95] Wool packaging uses this renewable and compostable material for thermal insulation.[96] The Coldpack AirLiner is an inflatable insulation liner that can be shipped flat when empty for space saving and deflated for landfill disposal.[97] Placon plastics (polyethylene terephthalate) for secondary packaging are composed of at least 35% postconsumer content.[98] Softbox has developed a new thermal insulation foam with 100% recyclable materials that has increased thermal efficiency compared with Styrofoam. Softbox maintains the cold chain for more than half the world's top 50 pharmaceutical companies.[99]

The Credo container from Pelican Biothermal has been used for influenza vaccines and other temperature-sensitive drugs (see Fig. 68.2B).[100] Repeat flu vaccine shipments and alternative logistics/distribution models of use have been successfully demonstrated with this technology.[103] Container reuse could provide reduced environmental impact and disposal burden at the program level.[103] The Sonoco ThermoSafe Greenbox is another reusable container technology, composed of 100% biodegradable, bio-based materials.[105] A plant-based phase-change material allows cold chain temperature maintenance of up to 6 days.[96] The phase-change material can be reconditioned and used repeatedly, replacing the need for other refrigerants. Approximately 20,000 repeat uses have demonstrated the thermal management properties of this material.

On-Site Reconstitution and Filling

While shipping vaccines in lyophilized form has advantages such as improved thermostability, reconstitution can introduce errors noted earlier, such as using the wrong diluent. For reconstitution, diluent is withdrawn from its container, typically with a needle and syringe, and injected into the primary vaccine container for mixing. Then the reconstituted vaccine is withdrawn from the vaccine container for administration. Vaccines currently available in lyophilized form include yellow fever,

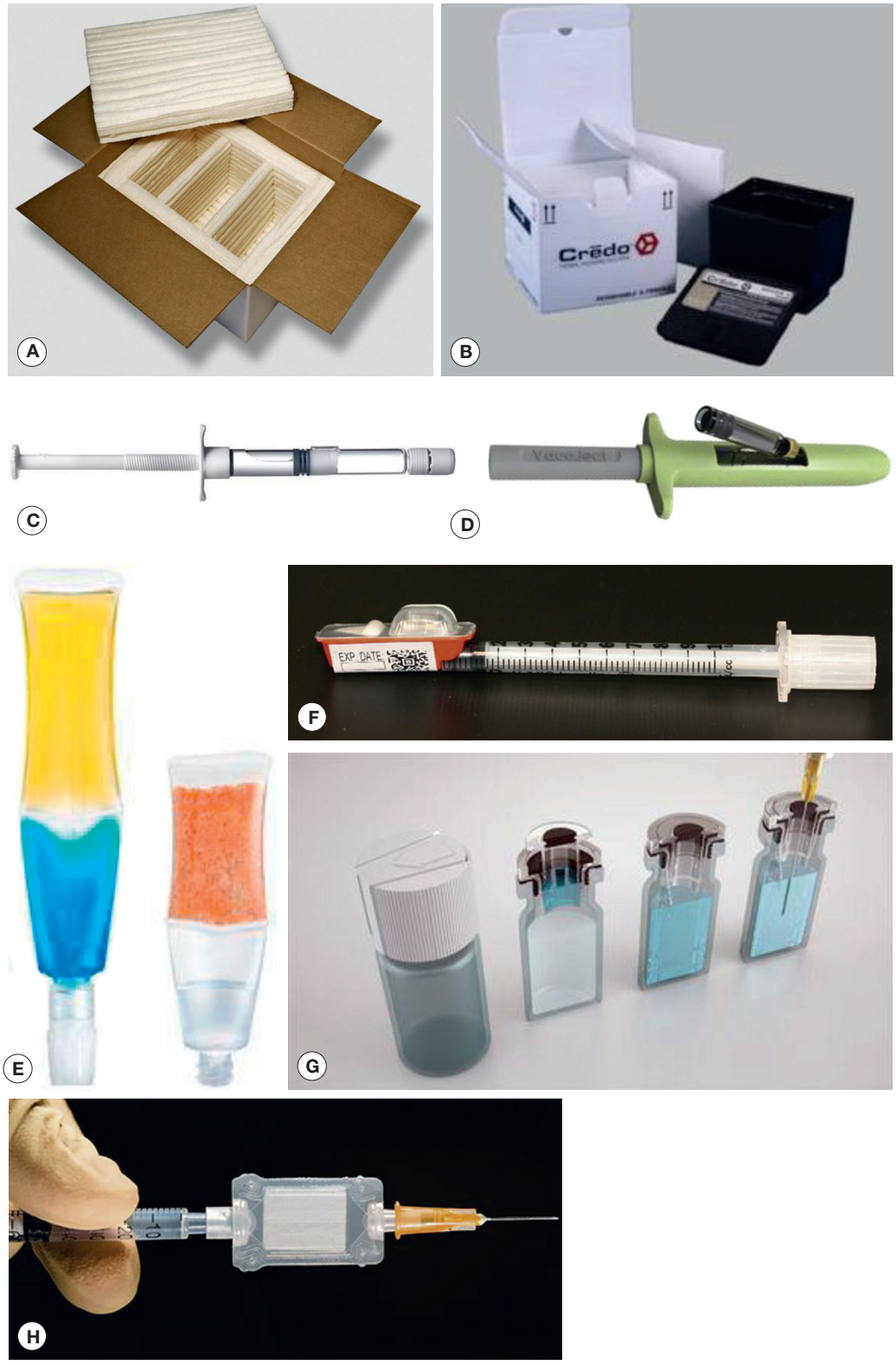


Figure 68.2. Vaccine packaging and reconstitution technologies. A, Landaal Green Cell Foam is a biodegradable polymer that can be easily disposed of by dissolving or incinerating. **B,** Credo Container from Pelican BioThermal can be repeatedly reused and has been used for influenza vaccines and other temperature-sensitive drugs. **C,** The Vetter Lyo-Ject (syringe) is a dual-chamber technology for simplified reconstitution. **D,** Duoject VaccJet is a cartridge-based technology that uses dual-sided needles for parenteral injection. **E,** Neopac's Fleximed Easymix uses a frangible seal separating the wet and dry compartments. Pressurizing (squeezing) one end breaks the seal allowing for reconstitution. **F,** AktiVax's ARCH design uses blisters with frangible seals to separate the dry vaccine from diluent prior to mixing. **G,** Eulysis' SVS is a vial-based design with a compartment that separates the dry powder/lyophilized pharmaceutical from the diluent, which is pierced by a piston cap allowing for mixing. **H,** HydRIS from NOVA Laboratories, is distal hub/attachment for prefilled diluent syringes that is filled with the lyophilized pharmaceutical in a sugar glass based membrane. Reconstitution occurs upon expelling the diluent from the syringe into the compartment during the process of delivery. (***A,*** *Courtesy Landaal Packaging Systems.* ***B,*** *Courtesy Pelican BioThermal.* ***C,*** *Courtesy Vetter.* ***D,*** *Courtesy Duoject.* ***E,*** *Courtesy Neopac [Ralf Künzi].* ***F,*** *Courtesy PATH.* ***G,*** *Courtesy Eulysis.* ***H,*** *Courtesy Nova Technologies.)*

measles, rubella, varicella, measles-containing combination vaccines (measles-rubella, measles-mumps-rubella [MMR], and measles-mumps-rubella-varicella), BCG, Hib (standalone and in some combination vaccines), rabies, rotavirus, Japanese encephalitis, meningococcus, and LAIVs. Vaccines in development, including those for cholera, dengue, enterotoxigenic *Escherichia coli*, influenza, tuberculosis, and rotavirus, will likely be available in a format requiring reconstitution.[37]

Preparation and delivery of reconstituted vaccines requires more vaccinator time than preparation and delivery of liquid vaccines. A time-motion study conducted in Kenya, comparing the amount of time required for vaccinators to prepare and deliver mock injections with various single-dose and 10-dose vaccine presentations, found that more time was required to administer lyophilized vaccines than liquid vaccines. The difference was greatest for single-dose, lyophilized presentations, since reconstitution is done once for each vial.[78]

Novel reconstitution technologies might be useful for current or future lyophilized vaccines. A variety of reconstitution technologies have been developed to reduce the chance of user error and simplify delivery. These technologies can be grouped into several categories: (1) fully integrated reconstitution technologies, which include primary packaging, diluent transfer, reconstitution, and delivery features; (2) partially integrated reconstitution technologies that include everything but the delivery device; (3) diluent transfer devices that facilitate reconstitution of existing lyophilized vaccine vials; and (4) hybrid reconstitution technologies that incorporate one or more features. Some of these technologies are currently in use for high-cost pharmaceuticals, particularly those intended for self-administration by patients at home. For vaccines, except diluent transfer devices such as vial adapters, reconstitution technologies are in varying stages of development, and no vaccines are available currently in these formats.

Fully Integrated Reconstitution Technologies

This type of technology incorporates packaging, reconstitution, and delivery and represents the most complex of the reconstitution technologies. Some technologies incorporate valved stoppers that are actuated with plungers in a glass syringe or a cartridge. Others use polymer tubes, pouches, or blister packs with frangible seals that rupture when pressure is applied.

Syringe- or Cartridge-Based Technologies. In this reconstitution technology, a prefilled syringe or cartridge contains both the dry and liquid components separated by an elastomeric internal stopper. Reconstitution occurs when mechanical pressure is applied to either overcome a valve in the plunger or push diluent through a bypass channel in the syringe. Parenteral delivery is possible with these technologies. These technologies may be more suitable for vaccine-drying methods other than lyophilization, which may represent a barrier to consideration because alternative drying methods are not currently in use for vaccines.

Examples of syringes include the Vetter Lyo-Ject Syringe (see Fig. 68.2C) and the LyoGo Dual Chamber Pre-Filled Syringe technologies. The commercially available Vetter Lyo-Ject Syringe incorporates a standard stopper with a bypass channel that is accessed upon advancement of the plunger, allowing diluent to flow through the channel into the dry powder chamber.[106] Vetter has also developed and marketed the Vetter V-LK Reconstitution Cartridge, which has a similar mechanism of action.

The LyoGo Dual Chamber Pre-Filled Syringe, currently in development,[107] has a chamber-separating stopper with a novel valve. Advancement of the plunger creates a pressure differential that opens the valve, allowing the diluent to enter the dry powder chamber. LyoGo also is developing a dual-chamber cartridge version of their novel valve technology. Another manufacturer, Unilife, has developed the dual-chamber syringe EZMix Genesis, which incorporates its retractable needle feature.[108]

Cartridge-based reconstitution technologies require incorporation into a separate novel delivery device. Duoject's VacJect technology is designed for single-use delivery using a retractable needle that allows cartridge access (see Fig. 68.2D). A reconstitution-capable version of the VacJect has been developed with a feature to allow for diluent transfer from one cartridge to a cartridge containing the lyophilized vaccine or drug.

Frangible Seal–Based Technologies (Tubes, Pouches, Blister Packs). An alternative to syringes and cartridges for reconstitution is the use of tubes, pouches, or blister packs with a frangible seal separating the diluent from the lyophilized or dry powder vaccine. Frangibility refers to property of breaking up into fragments when deformed or otherwise placed under sufficient stress. The frangible seal is ruptured upon application of pressure, allowing the diluent to mix with the pharmaceutical. Materials used for the seal must be compatible with heat sealing and can include PP, polyesters, or coextruded laminates.[98] Water vapor and oxygen transfer rates related to these different materials must be considered because they will affect product stability. An aluminum dual-chamber foil pouch was used for oral cholera vaccine (Orochol from Crucell [formerly Berna Biotech]); however, this product is no longer available. Frangible seal pouches have been used in non-pharmaceutical point-of-use component mixing.[109]

An example of a commercially available tube with a frangible seal is the Neopac Fleximed Easymix, a two-compartment, coextruded, polymer-based tube with the seal separating two compartments containing the dry and liquid or two liquid components (see Fig. 68.2E). Reconstitution occurs when the user applies pressure to rupture the seal between compartments. Nozzles for oral delivery are available, or a Luer interface can allow filling into a syringe or attachment of a Luer-fit needle. The pharmaceutical must be in dry format and dispensed into the tube and sealed. Use of this technology for vaccines may require shifting to a drying technique other than lyophilization (e.g., SFD).

The AktiVax ARCH (aseptic reconstitution cartridge hybrid) is a polymer prefilled syringe incorporating a flexible package on a solid backing (see Fig. 68.2F).[109a] The ARCH package can accommodate one or more constituents of the pharmaceutical, stored in adjacent high-barrier compartments separated by a frangible seal, which can be broken by depressing with a thumb for reconstitution just prior to injection. The mixed pharmaceutical is drawn into the attached syringe for injection. The ARCH syringe can incorporate a staked needle or Luer interface. As for the other technologies discussed above, use of flexible packaging may require updated vaccine lyophilization or other drying techniques. An integrated needle-based design using ARCH features—the Immunoject—also has been developed by AktiVax. In this design, the two-compartment blister is attached to a needle and a foldable plastic backing. The plastic backing shields the needle before use and allows compression of the blister compartments to rupture the frangible seal for reconstitution. Once reconstituted, the integrated needle allows parenteral administration, and refolding the plastic backing disables and covers the needle.[109b]

Partially Integrated Reconstitution Technologies

These technologies are characterized by an integrated primary container with a reconstitution feature such as a dual chamber, but they are without an integrated delivery feature for parenteral

delivery. The Eulysis Single Vial System (SVS) is composed of two compartments: the lyophilized or dry powder pharmaceutical is in a thin plastic cup resting on a glass vial that contains the diluent (see Fig. 68.2G). Actuation of a piston-like cap punctures the base of the plastic cup, causing the contents to fall into the diluent and permitting reconstitution; a needle and syringe must be used to withdraw a dose from the vial. A tamper-evident cap protects the piston cap from activation during shipment. This technology is in early-stage development and may potentially have wide-ranging applications for all freeze-dried injectable products. The Pfizer Injectable Act-O-Vial System demonstrates a similar concept. A rubber septum separates diluent and dry compartments of a nonstandard glass vial. Pushing down on the plastic cap dislodges the septum, forcing it into the lower dry compartment where reconstitution occurs. The Act-O-Vial is used with Pfizer's glucocorticoids Solu-Cortef and Solu-Medrol.[110,111]

Diluent Transfer Technologies

Diluent transfer technologies facilitate mixing of dry and liquid components of a pharmaceutical from two separate primary containers. These technologies eliminate the reconstitution needle to improve safety and reduce user error. An example, the Duoject E-Z-Link, incorporates a Luer lock fitting with a socket-style plastic spike vial adapter (socket sizes 13 mm and 20 mm) that can be used only once. A prefilled diluent syringe can be used to dispense into a standard-sized vial with lyophilized or dry powder pharmaceutical and draw from the contents once reconstituted. The E-Z-Link contains a protective disk to prevent contamination and shield the plastic spike.[112]

The Mix2Vial from West Pharmaceutical Services, Inc., allows connection of two vials with diluent and lyophilized pharmaceutical via two vial-adapter sockets with plastic spikes. The Mix2Vial requires the dry powder vial to be manufactured under vacuum to facilitate the transfer from the diluent vial. After reconstitution, a needle and syringe are used for dosing and delivery.[113] The West Needle-free Transfer Device has a Luer fitting to allow the attachment of a Luer lock syringe, which can be used to transfer the diluent from its vial into the lyophilized pharmaceutical vial and then back into the syringe. The syringe is then detached and a needle attached for parenteral delivery.[114] The West MixJect consists of a capped needle, a 13-mm vial adapter, and a Luer lock fitting for a prefilled syringe attachment. Diluent from the syringe is transferred into a vial of lyophilized pharmaceutical through the vial adapter, reconstituted, and drawn back into the syringe. The vial is then detached and the device is ready for drug administration.[115]

Hybrid Reconstitution Technologies

Some technologies present a fully or partially integrated hybrid approach to primary packaging, diluent transfer, reconstitution, and delivery. The Integrity Bio LyoTip distal hub incorporates a spiral-shaped channel to facilitate mixing of diluent and lyophilized pharmaceutical. The hub requires a Luer lock needle and syringe for delivery. The NOVA Laboratories Hydration Rehydration Injection System (HydRIS) is similar to the LyoTip: it is an attachment filled with lyophilized pharmaceutical for a prefilled diluent syringe (see Fig. 68.2H). A unique feature of the HydRIS is use of a stabilization technology based on sugar glass (an amorphous solid, clear crystallized form of the sugar): the pharmaceutical is dried onto a membrane, which is then integrated into the distal hub. The sugar-based membrane dissolves when it contacts the diluent, allowing the instantaneous mixing of diluent and lyophilized pharmaceutical.

Duoject has designed several reconstitution formats. One example is the PEN-PREP EVO device used to transfer reconstituted pharmaceuticals from a vial into multidose cartridges for use with traditional pen injectors. An integrated vial adapter incorporates a protective disk to shield the user from sharps injuries and to prevent contamination prior to use.[116] The Duoject Inter-Vial and the Duoject Smart-Rod XR combine a prefilled diluent syringe and a vial adapter. The vial adapter on the Inter-Vial detaches from the device once the drug is reconstituted and drawn back into the syringe. Detaching the vial adapter exposes a standard Luer lock for needle attachment. The vial adapter on the Smart-Rod is located on the plunger and cannot be removed for reuse. The Duoject Inter-Vial Plus and Duoject Smart-Rod Plus are nearly identical to the Inter-Vial and Smart-Rod XR except for the location of the diluent and lyophilized contents. The syringe components are prefilled with a lyophilized drug and attached to a diluent-filled vial.

Reconstitution Aids

Bundling or copackaging lyophilized vaccine and diluent in the same secondary packaging is common for many pharmaceutical applications as well as for select vaccine applications such as rabies vaccine. For the Rabipur rabies vaccine that is produced by CSL (formerly Chiron), the secondary package contains the vaccine vial, the diluent ampoule, and a syringe.[117]

Both vaccines and diluents can be supplied in vials or ampoule primary container presentations, and diluent can be provided in a prefilled syringe or other container such as a BFS ampoule. GlaxoSmithKline's Tritanrix HB+Hib vaccine presentation included a one-dose vial containing diphtheria, tetanus, pertussis (DTP)-hepatitis B (HepB) (liquid) and a one-dose vial of lyophilized Hib. A plastic clip bound both vials together to aid in easier reconstitution in that the "diluent" vial (the DTP-HepB vial) was immediately adjacent to the lyophilized vaccine vial.[118] The plastic clip binding either vial–vial or vial–ampoule combinations could reduce adverse events associated with incorrect diluent use.

Summary

The traditional glass vial and needle and syringe continue to be used with standard vaccine formulations and pharmaceutical filling techniques; however, new technologies are available that represent paradigm shifts in manufacturing and healthcare worker use. The use of polymer materials has enabled design and development of new primary packaging technologies, and alternative materials are informing new research in secondary and tertiary containers. Given the challenges of vaccine shipment and storage in the cold chain, new technologies that can reduce volume or the need for the cold chain would be of great benefit to high-income countries as well as LMICs.

NEW TECHNOLOGIES FOR VACCINE ADMINISTRATION: VACCINE MACRODELIVERY SYSTEMS AT THE POINTS OF CARE

New vaccine administration technologies are classified here into three major categories: SC and IM injection, cutaneous vaccine delivery, and mucosal vaccine delivery.

Subcutaneous and Intramuscular Injection

Most vaccines are delivered via SC or IM injection with needles and syringes. As described previously, in high-income–country

markets, immunizations are increasingly provided in prefilled syringes or single-dose presentations, while in lower-income countries serviced by UNICEF, vaccines are commonly delivered from multidose vials using autodisable needles and syringes, although use of single-dose vials and prefilled devices is increasing. An alternative to needle and syringe administration for SC and IM delivery is the jet injector, which can deliver vaccines in their current formulations. Another method in an early stage of development is solid dissolving needles, for which vaccines are reformulated as biodegradable implants.

Anatomy

The SC tissue (also known as the hypodermis) lies below the dermis of the skin and consists mainly of lobules of adipocytes as well as connective tissue (see Fig. 68.3A). Blood and lymphatic vessels transiting this layer serve the dermal layer of the skin. The thickness of the SC tissue layer varies widely between individuals of different age and body mass and at different body sites. For SC injection of vaccines, a $\frac{5}{8}$-inch needle (23 to 25 gauge) is recommended. The skin should be pinched up to avoid injecting intramuscularly and the needle inserted at a 45-degree angle. In infants younger than 12 months of age, SC injections are typically given in the thigh, while for adults and children older than 12 months, SC injections are given in the back of the upper arm, over the triceps.

Beneath the SC layer are skeletal muscles. Individual muscle cells are arranged into bundles called fascicles. These fascicles in turn are grouped in bundles within a layer of connective tissue to form muscles. For infants and toddlers up to 2 years of age, the vastus lateralis muscle in the anterolateral thigh is the preferred IM injection site, and the deltoid muscle in the upper arm is used for older children and adults. Recommended needle lengths vary by age, injection site, and body mass, because of the variation in thickness of the SC layer.

Immunological Rationale

The SC and IM routes of administration are typically used for vaccines because they are relatively convenient to access using a needle and syringe, enable consistent delivery, and provide adequately reproducible immunogenicity among individuals. However, compared with internal and external body surfaces that are more frequently in contact with the environment, SC and IM tissues have relatively fewer immune cells. Following injection of a vaccine, the antigen or adjuvant generates a local innate immune response, attracting APCs which ingest the vaccine package. These infiltrating dendritic cells (see Fig. 68.3B) and monocytes migrate to local draining lymph nodes and induce B- and T-cell immune responses. In general, SC and IM injection of vaccines tends to be less effective at producing mucosal immunity than mucosal vaccination.[119]

Jet Injection

Jet injectors force liquid under high pressure through a tiny orifice, producing a focused stream that penetrates the skin to deliver medication into targeted tissues without needles. Most modern jet injectors are powered by the release of energy stored in a compressed metal spring, and a few use compressed gas such as CO_2 or N_2. The velocity of the jet stream exceeds 100 meters per second. The depth achieved depends primarily on the power imparted to the liquid and then on variables such as orifice diameter, distance from the nozzle to the skin, and angle of injection.[120–125] This section focuses on jet injectors for SC and IM delivery of vaccines; devices capable of ID delivery are discussed "Cutaneous Vaccination" later.

Over the past half-century, jet injectors have been used to administer hundreds of millions, if not billions, of vaccine doses for mass campaigns in humans against smallpox,[126–132] measles,[133–135] polio,[136,137] meningitis,[138–140] influenza,[141,142] yellow fever,[143,144] cholera, and other diseases.[128,129,145–149] During the swine influenza mass campaign of 1976–77 in the United States, a substantial proportion of the approximately 43 million doses administered were by jet injection (Centers for Disease Control and Prevention [CDC], unpublished data).[150,151] Jet injectors have also been used for a wide variety of therapeutic drugs.[152–171] In recent years, the devices have been used to administer antigens to humans and animal models for a variety of investigational vaccines, including dengue,[172–175] herpes simplex type 2,[176] HIV/AIDS,[177–179] Japanese encephalitis,[180] malaria,[181] and melanoma.[182] A wide variety of investigational recombinant nucleic acid vaccines are being delivered in preclinical and clinical trials using various jet injectors.[183–198]

Because of safety issues with the earlier generation of MUNJIs, a new generation of DSJIs has been developed, many with features designed to be suitable for global immunization programs.

Multiuse Nozzle Jet Injectors. Jet injector technology was invented in France in the 1860s[199,200] and reintroduced in the 1940s as the Hypospray[157] for patient self-injection with insulin. In the 1950s, the U.S. military developed a high-speed system called the Ped-O-Jet[201] (Fig. 68.4A), and the units once referred to as jet guns were widely used for mass-vaccination programs.[127,202–206]

A large body of clinical literature shows the immunogenicity of vaccine delivered by jet injectors to be usually equal to, and sometimes better than, that induced by conventional needle and syringe for a wide variety of vaccines.[207,208] The pain associated with jet injection depends on the medication or vaccine involved. Insulin, other nonirritating drugs, and vaccines without adjuvants are usually reported to result in reduced or equivalent pain compared with needles,[121,124,133,141,154,171,209,210] but not always.[211] Vaccines with aluminum adjuvants or other irritating components tend to result in higher frequencies of mild, transient, local reactions (e.g., soreness, edema, erythema) when jet injected, probably because small amounts remain in the track left through the skin.[212–224] Bleeding and, less often, ecchymosis are reported to occur at the jet injection site more frequently than with needle injections.* Rarely, the jet stream causes a laceration if the injector moves during injection.[132,195,200,204] Safety features on some modern DSJIs reduce this risk.

Beginning in the 1960s, concerns arose for potential iatrogenic transmission of bloodborne pathogens by MUNJIs, which use the same nozzle to inject consecutive patients without intervening sterilization.[229,230] Bench and animal studies indicated cross-contamination could occur because blood or virus remained in nozzle orifices despite recommended alcohol swabbing between injections.[231–233] Fact superseded theory when a Med-E-Jet caused an outbreak of several dozen cases of hepatitis B among patients in a California clinic.[234] Subsequent clinical,[235] field,[236,237] bench,[238] animal,[239,240] and epidemiologic[241,242] studies added more evidence that MUNJIs could transmit pathogens between patients. This led to warnings and discontinuation of their use by public health authorities[24] and to market withdrawal of the Ped-O-Jet and discontinuation of its U.S. military use in 1997.[243,244]

*References 121, 124, 136, 137, 141, 154, 158, 167, 170, 202, 204, 209, 213, 225–228.

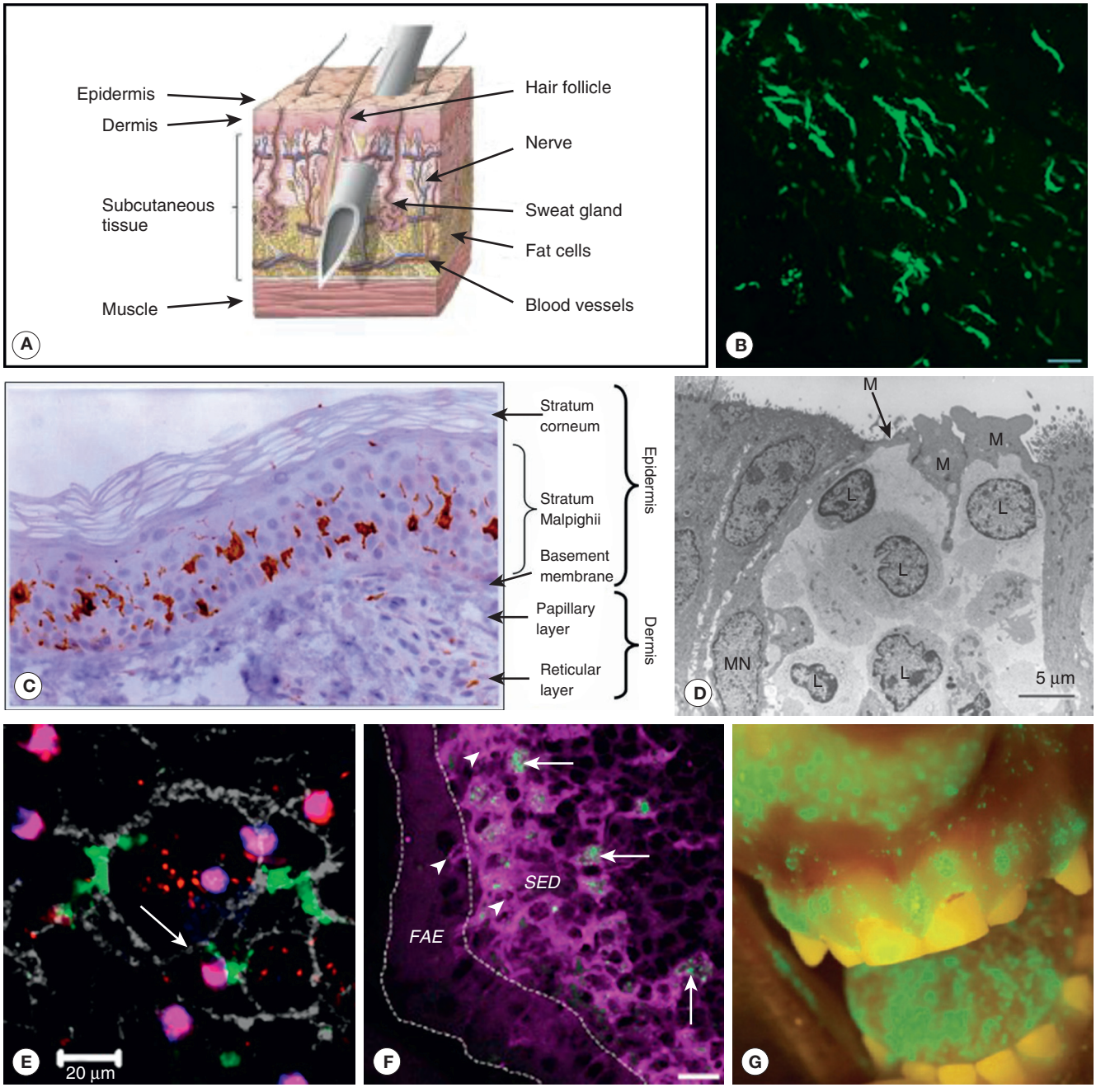


Figure 68.3. Examples of vaccine antigen delivery target cells and tissues. A, Diagram of subcutaneous injection showing epidermal, dermal, subcutaneous, and muscle tissue layers. **B,** Confocal microscopy of macaque muscle tissue 5 days following IM injection of live attenuated measles vaccine strain encoding enhanced green fluorescent protein (EGFP) demonstrates virus concentration in in dendritic cells by green fluorescence. Scale bar, 50 µm.[714] **C,** Activated Langerhans cells (dark stain) in the epidermal Malpighian layer 48 hours after immunization by application of cutaneous patch containing heat-labile enterotoxin of *Escherichia coli.* Full depth of dermis not shown. **D,** Transmission electron micrograph of nasal-associated lymphoid tissue from excised human adenoids, showing lack of apical cilia at the endothelial lumen *(top)* of an M cell *(M)*, the M cell nucleus *(MN)*, and the lymphocytes *(L)* enfolded in the cell's invaginated pocket, which remains contiguous with the extracellular space. M cells sample particulates from the lumen, presenting them to lymphocytes, macrophages, and dendritic cells, which congregate in the pockets. **E,** Two-photon intravital imaging of mice lungs: alveolar macrophages stained blue with rhodamine B-dextran appear purple when laden with *Bacillus anthracis* spores stained red with Alexa Fluor 633. Alveolar outline shown in gray. The white arrow on the lower image highlights the contact between infected macrophage and a green fluorescent protein (GFP) expressing dendritic cell (DC). Alveolar macrophages capture spores within minutes, DCs transport spores to the lymph nodes. Bar, 20 µm. **F,** Confocal laser scanning microscopy of murine Peyer patch. After administration by gavage, glucan particles *(green; arrows)* traverse follicle-associated epithelium (FAE) M cells and accumulate within $CD11c^+$ DCs *(magenta; arrowheads)* located within the subepithelial dome (SED). Scale bar, 50 mm. **G,** Macroscopic EGFP fluorescence in macaque mouth 9 days following infection with wild-type measles virus encoding EGFP shows extensive measles virus infection in tongue, buccal mucosa, gingiva, and tonsils (not shown). (***A,*** *From Clayton BD, Stock YN.* Basic Pharmacology for Nurses, *ed 13. St. Louis: Mosby; 2004.* ***B,*** *From Rennick LJ, de Vries RD, Carsillo TJ, et al. Live-attenuated measles virus vaccine targets dendritic cells and macrophages in muscle of nonhuman primates.* J Virol. *2015;89[4]:2192–2200.* ***C,*** *From Glenn GM, Taylor DN, Li X, et al. Transcutaneous immunization: a human vaccine delivery strategy using a patch.* Nat Med. *2000;6:1403–1406.* ***D,*** *From Fujimura Y. Evidence of M cells as portals of entry for antigens in the nasopharyngeal lymphoid tissue of humans.* Virchows Arch. *2000;436:560–566, 2000.* ***E,*** *From Fiole D, Deman P, Trescos Y, et al. Two-photon intravital imaging of lungs during anthrax infection reveals long-lasting macrophage-dendritic cell contacts.* Infect Immun. *2014;82[2]:864–872.* ***F,*** *From De Jesus M, Ostroff GR, Levitz SM, et al. A population of Langerin-positive dendritic cells in murine Peyer's patches involved in sampling beta-glucan microparticles.* PLoS One. *2014;9[3]:e91002.* ***G,*** *From Ludlow M, de Vries RD, Lemon K, et al. Infection of lymphoid tissues in the macaque upper respiratory tract contributes to the emergence of transmissible measles virus.* J Gen Virol. *2013;94[Pt 9]:1933–1944.)*



Figure 68.4. Selected devices for subcutaneous and intramuscular vaccine delivery. A, Ped-O-Jet (Keystone Industries), the most widely used multiuse nozzle jet injector (MUNJI) worldwide, before withdrawal from public health use by the 1990s for cross-contamination risk. Its metal spring is compressed by hydraulic fluid pumped by a foot pedal in its carrying case (**A,** *inset*) or by electric pump (not shown). Depth of delivery determined by removable nozzle used, either a subcutaneous (SC)/IM nozzle (shown here) or an intradermal (ID) nozzle (see Fig. 68.3E). **B,** Bioject Biojector 2000 DSJI, powered by gas. It has Food and Drug Administration (FDA) clearance for SC, IM, and ID delivery. **C,** PharmaJet Stratis DSJI, for 0.5-mL dose delivery. Delivery IM or SC set by vaccinator technique (fat layer pinched up for SC). Spring is reset using separate carrying case. Syringe is filled by pulling back and breaking off its blue shaft and thumb tab from conventional single-dose and multidose vials using needle-free vial adaptor (not shown). On insertion into device, any excess liquid is returned to vial to minimize wastage of overfill. Cleared for U.S. marketing by the FDA in 2011 and received World Health Organization (WHO) prequalification in 2013.[715,716] **D,** Biojet ZetaJet metal-spring-powered DSJI, features built-in crank for manual recocking of metal spring (Bioject Medical Technologies). Uses different autodisabling cartridges for SC, IM, and ID injections (SC and IM delivery was licensed by U.S. FDA in 2009; ID use is investigational). **E,** Investigational LectraJet HS (high-speed) motorized DSJI (D'Antonio Consultants International) features built-in motor and rechargeable battery for rapidly compressing metal spring between injections at rates exceeding 600 per hour, with battery capacity of >3000 injections per charge. Capable of rapid loading and unloading of single-use syringes from (**E,** *inset*) a sterile-packaged, 30-unit magazine for mass vaccination. **F,** Med-Jet H4 DSJI (Medical International Technology), gas-powered with entirely disposable single use fluid path. Licensed in Canada in 2014. A manually powered DSJI, the Dart (not shown), is in development and uses the same disposable cartridges. **G,** Investigational Solid Dose Injector (SDI) from Glide Pharma is powered by a compressed metal spring, which is released as the disposable drug cassette (white component extending beyond blue hub) is pressed fully against the skin. It inserts a (**G,** *inset*) pointed, hardened, approximately 1-mm-diameter drug formulation (shown compared with conventional matchstick tip) into subcutaneous tissues, where it dissolves. (***A*** *and* ***inset,*** *Courtesy James Gathany, Greg Knobloch [CDC Photographic Services].* ***B*** *and* ***D,*** *Courtesy Bioject Medical Technologies.* ***C,*** *Courtesy PharmaJet, Inc.* ***E,*** *Courtesy D'Antonio Consultants International, Inc.* ***F,*** *Courtesy Medical International Technologies [MIT Canada] Inc. [Karim Menassa].* ***G,*** *Courtesy Glide Pharma.)*

In the mid-2000s, a MUNJI was reengineered with disposable caps to try to prevent contaminating blood or tissue fluid from splashing back onto the reusable nozzle. However, after injections with saline of volunteers who carried hepatitis B virus, 8% of subsequent injections into vials—representing the next vaccinees in a clinic or mass campaign—were found to contain hepatitis B antigen.[245] High-speed microcinematography also revealed extensive splash back from the skin during injection with MUNJIs.

This body of evidence supports the conclusion that the design of MUNJIs is inherently unsafe for use in immunization settings, and any reuse of fluid pathways or unsterile components that are in direct or indirect contact with consecutive patients should be abandoned.[25] Even if contamination could be shown to be extremely rare, it is unlikely that policy makers could be convinced to set any level of acceptable risk.[246] Although public health authorities recommend against MUNJI use for vaccination,[25,247] MUNJIs continue to be used in the United States and other countries in clinical specialties such as dentistry, urology, and podiatry where cleaning and sterilization (such as autoclaving) of the entire device fluid path between patients is possible. Also, the Med-Jet line of MUNJIs has received licensure in multiple countries including Canada, China, and Russia for human applications including physiatrics, dermatology, and mesotherapy indications.[248] MUNJIs allowed a single health worker to vaccinate 600 or more patients per hour.[132] The withdrawal of the device posed challenges for conducting mass-immunization campaigns for disease control programs and in response to pandemic or bioterrorism threat.

Disposable-Syringe Jet Injectors. To overcome concerns about MUNJIs and their withdrawal, a new generation of safer DSJIs has been developed since the early 1990s.[249–252] Each sterile syringe (or cartridge) has its own orifice and nozzle and is discarded between patients. Although some are used for self-administration of insulin, other hormones, and drugs such as sumatriptan for migraines,[253] others are targeted for vaccine administration.

One early system, developed by predecessor companies of Sanofi Pasteur, was the manufacturer-prefilled Imule syringe for use in the Mini-Imojet DSJI.[254,255] Although demonstrated in the clinic and field to be immunogenic and safe for several vaccines,[256] the system was eventually abandoned upon corporate merger. The pioneering DSJI for the vaccine market was the Biojector 2000 (see Fig. 68.4B), introduced in the United States in the 1990s. During the first decade of the 2000s, it delivered approximately 1 million IM and SC vaccine doses per year at private, public, and U.S. Navy and Coast Guard immunization clinics in the United States, and it has been used in many vaccine studies, including a number of investigational recombinant nucleic acid vaccines.*

To meet developing-world needs, several DSJIs have been developed that are economical, autodisabling to prevent reuse, and suitable for mass campaigns and routine immunization. PharmaJet entered the market in 2009 with licensure of its eponymous device for IM and SC injections. A recent study with this now-discontinued DSJI found that delivery of MMR vaccine did not meet noninferiority criteria in comparison with needle and syringe delivery.[267] PharmaJet subsequently received market clearance for the Stratis device (see Fig. 68.4C), which was the first DSJI to receive WHO prequalification, in 2013. Various PharmaJet models have been studied for veterinary[186–188,268] and human[164,165,255,257,258,269–271] applications. In a bench study testing injection of MMR vaccine through pig skin into a vial with three PharmaJet injectors, there was no loss of measles or mumps vaccine virus infectivity but some loss of rubella infectivity with two of the devices.[271a] Other examples of DSJIs developed for vaccine delivery include the Bioject ZetaJet (see Fig. 68.4D),[272] the DCI Lectrajet (see Fig. 68.4E),[273,274] and the MIT Med-Jet H-4 (see Fig. 68.4F) and Dart.[248]

Use of DSJIs in the United States was curtailed by a 2011 FDA communication advising that vaccines should be delivered with a needle and syringe unless specifically labeled for jet injector delivery. Following consultation with the FDA, PharmaJet and bioCSL conducted a clinical trial demonstrating noninferiority of delivering bioCSL's Afluria influenza vaccine with a DSJI compared with a needle and syringe.[271] Based on these data, in 2014 Afluria was licensed by the FDA for delivery with the PharmaJet Stratis device. This "vaccine relabeling" has enabled DSJIs to reenter the U.S. market; however, the FDA-approved language limits delivery to a specific manufacturer's DSJI device.

Advantages of DSJIs are the elimination of needlestick injuries associated with possible transmission of bloodborne pathogens to health workers as well as reduction in the volume of sharps waste disposal, which is not required for used DSJI syringes. They also have the potential to increase acceptability and immunization coverage rates. Reduced crying has been observed when vaccines are delivered to infants with jet injection compared with needle and syringe injections.[258,267] However, like syringes with needles, DSJI syringes require onsite filling following vaccine reconstitution (if required). Prefilled DSJI syringes in development by PharmaJet and other manufacturers may enable greater efficiency and speed of administration, similar to that of the old generation of MUNJIs. Use of DSJIs likely will entail higher costs because of the higher cost of manufacturing a DSJI syringe and the cost of the durable injector, but modeling indicates the potential for overall cost savings compared with needles and syringes when the indirect costs of iatrogenic disease resulting from the latter are included.[257,275] As with all vaccination systems that require a durable administration device, distributability of the vaccine is restricted by the need to have a device available at every POC when vaccine is delivered. The cost of a jet injector can be negligible if amortized over many doses in high-vaccination–volume settings. However, in POCs with low-vaccination volumes, the cost of these devices may be prohibitive.

Solid Dissolving Needles

Dissolving needles are a novel biodegradable implant technology consisting of solid doses of vaccine that are administered subcutaneously. A disposable cartridge containing the implant is loaded into a reusable handheld applicator that uses gas or spring pressure to insert the implant into the skin. Once the implant is in the tissue, it begins to dissolve and the vaccine is released.

The Bioneedle Technologies Group (The Netherlands) has developed Bioneedles, small implants of biodegradable polymer (extruded starch) filled with concentrated liquid vaccine, which are freeze-dried. After insertion into the SC tissue, the implants have been shown to break down within a few hours and dissipate from the delivery site within 3 days. Administration of a placebo Bioneedle implant in a clinical trial found adequate safety and acceptability of the technology and no histological findings at the implantation site. Administration of tetanus toxoid, hepatitis B, influenza, and IPVs via Bioneedles has been studied in animal models.[276–279] In Wistar rats, the same dose of IPV given by SC or IM liquid injection or by Bioneedle implantation was found to produce similar antibody titers after two immunizations.

*References 154, 160, 161, 175–179, 182–185, 188–191, 198, 211, 214, 217, 254, 256a, 258–266.

Glide Pharma (United Kingdom) has also developed a solid-dose injector. It uses a spring-loaded device to quickly push into SC tissue a sharp, biodegradable implant the size of a grain of rice (see Fig. 68.4G). The solid-dose injector has been tested with influenza, diphtheria, and *H. influenzae* vaccines and is in development with an anthrax vaccine.[282]

Potential benefits of this technology class include dry-format unit-dose administration; increased thermostability of the solid vaccine formulation in comparison to a liquid formulation; reduced cold chain footprint because of the small dose and package size; avoidance of needles and syringes with reduced risk of needlestick injury and reuse; and potential for reduced number of booster doses if slow release is successfully demonstrated to provide improved immunogenicity. Challenges to development include demonstrating the feasibility of the formulation and manufacture of solid vaccine implants, acceptability, and the complexity and costliness of the administration device.

Cutaneous Vaccination

Cutaneous administration of vaccines includes delivery to either the dermis or epidermal layers of the skin. The skin was one of the first tissues into which variola (smallpox) virus and, later, cross-protecting cowpox virus were introduced to prevent smallpox. The cutaneous route for variolation involved breaking the skin with a sharp instrument, and it was used in India and China at least as early as the 16th century. Variolation was supplanted by safer cutaneous application of material from cowpox lesions (vaccinia virus), the 18th-century method first given the term *vaccination* and first published by Edward Jenner. The cutaneous route remains the standard for smallpox vaccine (see Chapter 33) as well as for administering BCG to prevent tuberculosis (see Chapter 54). Vaccines for poliovirus[284] and yellow fever[285] were also delivered by this route in the past. The cutaneous route has demonstrated and hypothetical advantages over other delivery methods, including the potential to enable dose-sparing.[286–307] Cutaneous delivery is often associated with an increase in mild local reactions, because of the visibility of the administration site, but is less likely to result in unanticipated serious adverse reactions than other routes. Mixed results for the immunogenicity of the ID route have been reported for a variety of live attenuated, inactivated, and subunit vaccines, including those from dose-sparing studies for currently available vaccine formulations as well as for investigational vaccines, particularly DNA vaccines.

In a classical ID injection, a bolus of liquid is deposited into the dermis to raise a visible bleb, as in the traditional Mantoux method. A variety of novel delivery methods and technologies have been developed to deliver vaccines into the skin with the goals of (a) improving the ease, acceptability, and safety of vaccination, and (b) taking advantage of the potential for skin tissue to enhance immunogenicity or enable dose-sparing.

Anatomy

The outermost layer of the skin is the epidermis (see Fig. 68.3A), a stratified squamous epithelium that is usually about 0.1-mm thick but can be from 0.8 to 1.4 mm on the palms and soles. The stratum Malpighi layer is the primary component of the epidermis, and its dividing and growing keratinocytes serve both a structural function—limiting the passage of water and other molecules—and an immunologic role. Keratinocytes germinate just above the basement membrane, which demarcates the boundary between epidermis and deeper dermis. These cells then grow, flatten, mature, and senesce in increasingly superficial strata until they reach the surface and are sloughed. The main product of this cell is keratohyalin, a dense lipid that helps form a waterproof barrier. The lateral edges of adjacent keratinocytes are tightly linked by desmosomes, which maintain the strength of the epidermis and also contribute to its resistance to the passage of foreign matter or molecules.[308,309]

The topmost horny layer of the epidermis is the stratum corneum, composed of staggered courses of dead keratinocytes—also known as corneocytes—in a lipid bilayer matrix. This stack of 10 to 20 cells, 10 to 20 µm thick, is the principal obstacle to the introduction of vaccine antigen for cutaneous vaccination. Below the epidermis and basement membrane lies the dermis, approximately 1.5 to 3.0 mm thick, in which fibroblasts, fine collagen, elastic fibers, and most skin organelles—including small blood vessels, lymphatic vessels, nerves, hair follicles, and sweat and sebaceous glands—are found.

Skin thicknesses have been mapped in children to identify the histologic suitability of sites for cutaneous vaccination. Equally important is selecting skin sites that are easily accessed so as to minimize disrobing and loss of privacy. In smallpox eradication, the volar surface of the forearm was commonly used because it was quickly accessible.[126]

Immunological Rationale

The specific mechanisms that produce an immune response when vaccine antigen is introduced into the skin are not entirely clear. With stimulation, keratinocytes can produce proinflammatory cytokines (e.g., interleukin-1) and can function as APCs by displaying major histocompatibility complex class II antigens (human leukocyte antigen–DR) as well as intercellular adhesion molecule-1. Epidermal Langerhans cells—a type of APC—are believed to play a key role in cutaneous immunization (see Fig. 68.3C), although dermal dendritic cells and other well-known immune system components such as $CD8^+$ and $CD4^+$ T lymphocytes, mast cells, and macrophages also circulate or reside in the epidermis or dermis.[298,299,302,310–314]

The immature Langerhans cells reside like sentinels among the keratinocytes in the epidermis, composing about a quarter of the skin surface area,[315] where they efficiently capture foreign antigens by phagocytosis or endocytosis. Like dendritic cells in other tissues (see Chapter 2), on activation (see Fig. 68.3C) these professional APCs process the antigen as they migrate to draining lymph nodes. There, now mature, they express high levels of class II major histocompatibility complex molecules and present the antigen brought from the skin to T-helper lymphocytes, a critical step for the subsequent immune responses orchestrated by the latter cells.

Macrodelivery of vaccines precisely into the extremely thin epidermal tissue or the dermis layer creates a technological challenge that has resulted in a wide array of cutaneous delivery methods and devices. Researchers investigating cutaneous delivery of existing vaccines and those in development have used the Mantoux method of injection by needle and syringe (see "Mantoux Method" later) as well as a variety of novel devices such as ID jet injectors, mini-needles, microneedles, electroporation devices, and microarray patches.

Traditional Methods

During the more than 200 years of cutaneous vaccination against smallpox (see Chapter 54), a variety of sharp

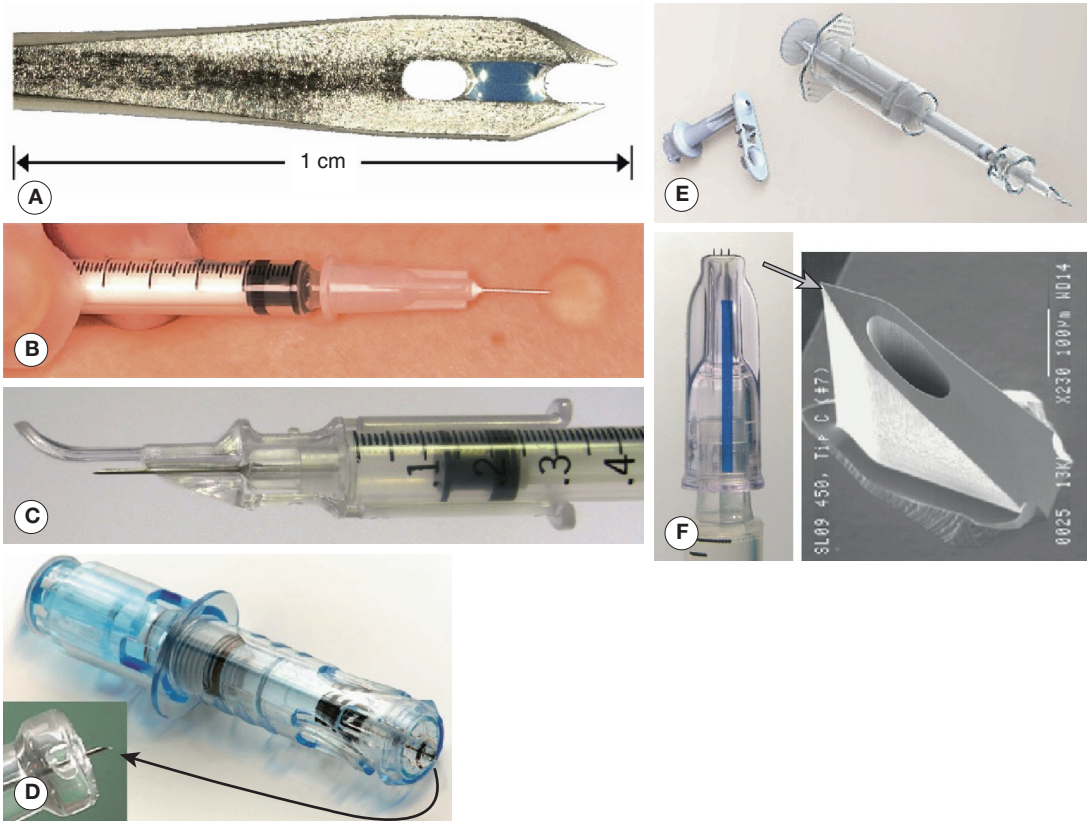


Figure 68.5. Selected needle-based technologies for cutaneous vaccine delivery. A, Pronged end of bifurcated needle (full length is 5 to 7 cm), which is the current preferred device for smallpox vaccination, holds between its tines approximately 2.5 μL of fluid by capillary action. **B,** Traditional Mantoux method for creating intradermal (ID) wheal using 26-gauge hypodermic needle and conventional 1-mL syringe. **C,** ID adapter for conventional needle and syringe for quick and consistent Mantoux ID injection (West Pharmaceutica Services), the length and depth of the protruding needle underneath the "Ski-tip" guide manipulates the skin to optimize needle placement regardless of bevel orientation.[338,339] **D,** Prefilled version of Soluvia mini-needle ID syringe (BD Micro-Delivery System; Becton, Dickinson and Co.). Used for ID delivery of Sanofi Pasteur brands of inactivated influenza vaccine (Intanza, IDflu,[717] Fluzone Intradermal[718]).[719] **D** *inset,* The 30-gauge staked mini-needle projects 1.5 mm beyond its hub to limit the depth of injection upon perpendicular insertion into the skin. Marketing of vaccine-device combination product approved in European Union in 2009 and in United States in 2011. **E,** ID syringe in development (Star Syringe) with an autodisable feature and an integrated plastic spike for filling from a vial, and a mini-needle adapter for providing an ID injection. **F,** MicronJet hollow microneedle device with Luer fitting onto conventional syringe for ID delivery (NanoPass Technologies Ltd). Cleared for marketing in the European Union and the United States. The blue line on the hub indicates to the user that the bevel and lumen of the microneedle needle are on the opposite side. **F** *inset*, Microphotograph shows the pyramidal shape and lumen of an individual microneedle. *(**A,** Courtesy James Gathany [CDC Photographic Services]. **B,** Courtesy of James Gathany and Greg Knobloch [CDC Photographic Services]; **C** and **F,** courtesy of Bruce G. Weniger; **D,** courtesy of Sanofi Pasteur; **E,** courtesy of Star Syringe Ltd [Paul Mallins]; **F,** from Van Damme P, Oosterhuis-Kafeja F, Van der Wielen M, et al. Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults.* Vaccine. *2009;27[3]:454–459.)*

instruments have been used to penetrate the epidermis (and avoid penetrating unnecessarily deeper into the dermis) for inoculation of cowpox or vaccinia virus. These included scarification with a needle, scalpel, lancet, or knife and rubbing vaccine into the resulting lesion; twirling a rotary lancet in a drop of vaccine on the skin; and the multiple-pressure method of pressing a straight surgical needle sideways into the skin through a drop of vaccine. Multitine devices consisting of multiple short needles in a circular or square array dipped in solution and pressed into the skin have also been used.[316,317]

The BCG vaccine for the prevention of disease from *Mycobacterium tuberculosis* was originally administered orally in the 1920s (see Chapter 60). Safety concerns prompted a shift to cutaneous administration by ID needle injection using the Mantoux technique in 1927. BCG has also been delivered cutaneously by multiple puncture devices,[318–321] scarification, jet injectors,[322] bifurcated needles (Fig. 68.5A),[323] and multitine devices,[316,324] but the Mantoux method remains the standard delivery method (see Fig. 68.5B).

Bifurcated Needle. In the 1960s, Benjamin Rubin invented the bifurcated needle (see Fig. 68.5A), for which Wyeth waived the royalties so that the WHO could produce it for smallpox eradication. The device holds approximately 2.5 μL of fluid by capillary action between its tines and is applied perpendicularly into the skin. This uses one-fifth of the typical dose volume needed by earlier multiple-pressure methods, but it

requires a higher virus concentration. Its simplicity, portability, and economy greatly facilitated the latter half of smallpox eradication, particularly in Asia and East Africa.

Mantoux Method. The needle technique for classical ID injection, as used for BCG, was developed in the early 20th century by Felix Mendel and separately by Charles Mantoux for the administration of tuberculin (now replaced by purified protein derivative) used for diagnosis of tuberculosis infection. Now referred to as the Mantoux method (see Fig. 68.5B), this procedure has become the common route for ID injection of both BCG and rabies vaccines. Although the latter was typically delivered intramuscularly, research found that reduced doses of rabies vaccines delivered via ID are similarly effective for both postexposure and preexposure prophylaxis regimens.[325–333] Because of the high cost of cell-culture rabies vaccines, the WHO has recommended ID delivery for resource-constrained settings since 1991, and this route is widely used in the developing world, particularly in Asia.[333a,334–336]

In the Mantoux technique, a short-bevel, fine-gauge needle, usually 27 gauge, is inserted, bevel up, at a 5- to 15-degree angle into slightly stretched skin, often the volar surface of the forearm. The tip is advanced approximately 3 mm until the entire bevel is covered. Upon injection of fluid, proper location of the bevel in the dermis creates a bleb, or a wheal, as the basement membrane and epidermis above are stretched by the fluid (see Fig. 68.5B). Leakage onto the skin indicates insufficient penetration to cover the bevel. Failure to produce a bleb indicates an improperly deep location of the fluid in the SC tissue. Drawbacks to the Mantoux method are the training, skill, and extra time needed to accomplish it correctly.

Needle-Based Technologies

Intradermal Adapters. A novel ID adapter (see Fig. 68.5C), designed to improve ease and consistency of the traditional Mantoux technique (see Fig. 68.5B), fits over a conventional syringe with a fixed needle and guides the needle to its appropriate position in the skin to produce the desired bleb. The device is marketed by West Pharmaceutical Services[337] and has FDA clearance. In clinical studies, the ID adapter has been found to reliably produce blebs, regardless of the orientation of the needle bevel during injection.[338,339] Similar to a fill-on-site mini-needle device, the ID adapter has the potential to be a relatively low-cost option to increase the ease and reliability of ID injections. Because the angle of injection mimics the Mantoux technique, it may also serve as a training tool for users inexperienced with Mantoux. One challenge is that the device design is syringe specific and must be paired with a particular brand of syringe.

Mini-Needles. As for cutaneous vaccination in general, a diverse terminology is applied to microscopic projections for perforating the superficial skin to deliver the drug or vaccine.[340–343] This chapter uses the term *mini-needles* for hollow projections of 1 mm or longer for liquid delivery. The term *microneedles* refers to hollow projections shorter than 1000 µm. Microarray patches are arrays of solid projections (without lumens) shorter than 1000 µm. These patches deliver vaccine in a dry format and are not classified as needles.

To circumvent the amount of skill and time needed for successful Mantoux injection, BD developed a prefilled glass syringe with a staked 30-gauge mini-needle that projects only 1.5 mm beyond its depth-limiting hub for intuitive perpendicular insertion into the skin (see Fig. 68.5D). Termed the Soluvia Micro-Delivery System, it was licensed exclusively by Sanofi Pasteur for certain vaccine applications. Sanofi Pasteur undertook a series of clinical trials with trivalent inactivated influenza vaccine,[345–349] which led to marketing approval in Europe in 2009 for ID delivery of its Intanza and IDflu products. These contained either 9 µg of viral hemagglutinin per strain per 0.1 mL for adults through age 59 years,[350] or a full (non–dosage-sparing) 15 µg for those age 60 years and older.[345–347] Sanofi Pasteur's U.S. brand of ID influenza vaccine (Fluzone Intradermal), similarly containing 9 µg per strain, was found to induce geometric mean titers of hemagglutination-inhibiting antibody that were noninferior to those of control patients receiving conventional Fluzone by the IM route with 15 µg per strain. In 2011, the FDA licensed the vaccine and its unique prefilled delivery system with an indication that it be used only for patients 18 to 64 years of age. Several other countries (e.g., Canada, Australia, and New Zealand) have also licensed a Sanofi influenza vaccine in the Soluvia mini-needle delivery system.

A fill-on-site version of the Soluvia mini-needle syringe was used in a rabies vaccine clinical trial and demonstrated protective seroconversion comparable to a full dose by the IM route. In preclinical animal experiments, delivery with this device produced good immune responses to anthrax recombinant protective antigen,[351–353] conventional hemagglutinin and plasmid DNA antigens for influenza, and live recombinant yellow fever vector for Japanese encephalitis vaccines.[354]

Novosanis has developed another prefilled mini-needle device, the VAX-ID, which was tested for delivery of saline in humans.[355] Star Syringe has developed a fill-on-site mini-needle ID syringe (see Fig. 68.5E). The Star ID device is designed to be low cost and suitable for use with existing vial formats, is filled through an integrated plastic spike, and has an autodisable feature. A clinical study assessing the injection performance with this device is planned.

Mini-needle devices can facilitate ID delivery and enable the dose-sparing potential of some vaccines while retaining the familiar interface and use method of common IM needle and syringe injections. ID injections with the Soluvia device frequently do not produce the characteristic wheals in the skin that are indicative of correct Mantoux injection technique,[356,357] so some education for healthcare workers may be needed. Prefilled presentations such as the Soluvia and VAX-ID offer additional benefits in ease of use and dose accuracy, but they are more expensive and require much more cold chain storage space than vial presentations. The recent development of low-cost fill-on-site devices such as the Star ID syringe, if demonstrated to perform clinically, could be more appropriate for the requirements of LMIC immunization programs.

Hollow Microneedles. Hollow microneedles, which deliver liquid vaccines intradermally, share attributes with the needle-based technologies for ID delivery described previously (see "Mini-Needles" and "Intradermal Adapters" earlier). Hollow microneedles are designed to inject therapeutic liquids through their tiny lumens and are used with either a syringe or a small wearable pump.[292,303,305,342,358] Flow rates of microneedles have been measured up to a remarkable 1 mL/min per cannula. Common lengths of 200 to 1000 µm are short enough, in theory, to be painless because they would not reach nerve endings in the dermis.[294,358–360]

The MicronJet 600 device developed by NanoPass is unique in its availability as a licensed, sterile, disposable microneedle device. It consists of three hollow microneedles 600 µm in height in a beveled pyramidal shape. These are mounted on an adapter with a Luer interface for fitting onto a conventional syringe for liquid vaccine or drug (see Fig. 68.5F). In 2010, it was cleared by the FDA for injection of any drug approved for ID delivery. It also holds a CE mark for marketing in Europe.

Adult volunteers vaccinated intradermally with a similar MicronJet version (four microneedles 450 µm in height)

received reduced single doses of 3 or 6 µg per strain, or full 15-µg doses by IM route, of licensed alpha-Rix (Fluarix, GlaxoSmithKline) 2007–08 seasonal influenza vaccine. By day 21, all three study arms developed comparable increases in antibody titers.[361] Local reactions were more frequent with MicronJet than by the IM route, but they were mild and transient. Similar dosage-sparing trials for 2009–10 monovalent H1N1 influenza vaccine and 2010–11 trivalent vaccine confirmed comparable or superior immune responses for the ID route versus IM. The MicronJet has also been studied for reduced-dose delivery of IPV[362,363] and varicella-zoster vaccine.[364]

Another hollow microneedle system is 3M's hollow microstructured transdermal system (hMTS).[289,369] Its patient-contact surface contains 18 microneedles of 500 to 900 µm in height, whose lumina of 10 to 40 µm in diameter delivers liquid volumes ranging from 0.3 to 1.5 mL. A spring-powered device contains liquid drug prefilled into a glass dose chamber. Upon triggering, a spike pierces the stopper of the chamber through which the dose passes and is forced slowly, over a period of 5 to 40 minutes, through the microneedles into the skin of the upper arm or thigh. Adhesive keeps the system in place until delivery is complete. Delivery of equine tetanus antitoxin to swine as a model for delivery of monoclonal antibodies resulted in pharmacokinetic profiles of tetanus antitoxin similar to dosages via SC injection.[369] Other industry and academic groups have pursued development of hollow microneedles, including Debiotech and the Georgia Institute of Technology.[249,370]

Hollow microneedles have advantages similar to those of other needle-based ID devices for delivery of liquid vaccines: they can increase reliability and ease of ID injection, potentially enabling vaccine dose-sparing. A clinical study found that delivery of influenza vaccine with the NanoPass MicronJet hollow microneedle device enhanced immunogenicity compared with the same dose of vaccine delivered ID by the Mantoux method, which suggests that the reliability and shallower depth of delivery of some hollow microneedle devices may enable superior immune responses.[371] However, separate needles or vial adapters are required to fill the syringes for hollow microneedle devices from a conventional vial, adding to the steps required for injection. Prefilled hollow microneedle technologies in development would simplify usage but are likely to increase costs and cold chain requirements.

Electroporation. Electroporation uses very short electrical pulses to produce temporary pores of nanometer-range diameters in the intercellular lipid matrix of the stratum corneum, which remain open and permeable for hours.[294,372–376] In vitro and in vivo preclinical studies of this technique demonstrated skin entry of larger molecules, such as heparin (12 kDa), peptides, proteins (such as luteinizing hormone–releasing hormone), and nucleic acids,[260,377–380] with potentially extensive use for investigational DNA vaccines in animals and humans.[194,381,382]

Inovio's CELLECTRA series of electroporation devices[383] and Ichor's TriGrid delivery system[385] are capable of ID and IM delivery. A hollow needle injects the drug conventionally, while parallel solid needles surrounding the injected dose create the current to generate pores in the target muscle tissue.[376,386,387] These devices have been tested preclinically and clinically for delivery of several DNA vaccines, including those for HPV,[388] HIV,[389,390] malaria,[391] and tuberculosis, with results demonstrating improved immunogenicity of the DNA antigen when electroporation is applied. Noninvasive surface electroporation devices have also been developed by Inovio and may have better acceptability.[392]

The Easy Vax and related Derma Vax epidermal electroporation systems combine the application of antigen- or drug-coated mini-needles 2 mm long, followed by electroporation. Smallpox antigen in plasmid DNA was dried onto the tips of arrays and inserted into the skin of mice, and when followed by six electric pulses, it induced protection from smallpox challenge.[393] A prostate cancer DNA vaccine was similarly administered.[394]

Electroporation has promise as an enabling technology for DNA vaccines, which have lower efficacy by traditional delivery methods. Acceptability is a challenge, particularly for IM electroporation devices, although ID devices have been found to be more tolerable.[395] The cost and complexity of the devices could preclude use in most low-resource settings.

Needle-Free Technologies

Jet Injection. Since the 1960s, multiuse nozzle jet injectors (see "Subcutaneous and Intramuscular Injection: Jet Injection" earlier) have allowed ID delivery of smallpox, BCG, and other vaccines via specialized nozzles or standoffs to achieve the shallower penetration required compared with IM or SC injection.[396,397–399] With discontinuation of the use of MUNJIs, DSJIs have been used for some ID injections.[128,129,249,400–402] For some of these devices, such as the Biojector 2000 and the Bioject ZetaJet, spacers can be added to a nozzle intended for SC delivery, creating a gap between orifice and skin, which weakens the jet and provides space for a bleb that leaves the dose in the skin. Other designs, such as the MIT Med-Jet line of gas-powered jet injectors and the MIT Dart spring-powered DSJI, can achieve ID delivery with variable pressure settings that allow the user to reduce the force of the injection stream. The PharmaJet Tropis (Fig. 68.6A) and the Bioject ID Pen (see Fig. 68.6B) are two new spring-powered DSJIs designed solely for ID delivery of 0.05 and 0.1-mL volumes.

The Ped-O-Jet (and, to a much lesser extent, other MUNJIs) administered tens of millions of smallpox vaccine doses for the first half of the WHO Smallpox Eradication Program in South America and West Africa from the late 1960s to the early 1970s, until invention of the simpler and swifter bifurcated needle.[126,132,399] MUNJIs also delivered yellow fever[128,143,404,405] and BCG vaccines[405a,406–410] by the ID route, as well as various tuberculosis skin testing antigens.[401,411–417] However, variations in subsequent tuberculosis skin testing reaction sizes when delivered by MUNJIs[322,418] led the WHO to discourage jet injector use for BCG and tuberculosis skin testing.[419,420]

To achieve polio eradication goals, injectable IPV is being introduced worldwide to supplement use of oral poliovirus vaccine (OPV). However, IPV is more expensive to produce and more difficult to deliver in campaign settings than OPV, promoting a search for cost-saving strategies that also avoid the introduction of needles into the polio eradication program. Recent clinical research has compared full doses of IPV given intramuscularly (0.5 mL) with ID delivery of 0.1 mL by either needle-free jet injectors or the Mantoux technique. In studies evaluating a primary series of immunizations in infants, some have found equivalent seroconversion rates, while others have shown reduced responses with ID jet injection.[258,259,421] In all the trials conducted in infants, ID delivery of reduced doses of IPV has resulted in lower titers of neutralizing antibodies, whether reported as geometric mean titers or median titers, and whether or not the study was performed in poliovirus-naïve infants or in infants receiving supplemental doses of IPV.[258,259,420,422] It has been suggested, however, that any detectable neutralizing antibody titer should be sufficient to prevent paralytic disease.[258] Clinical trials using a single dose of IPV for boosting immunity in previously immunized children and adults generally have found that ID delivery of a 20% (0.1 mL)



Figure 68.6. Selected needle-free technologies for cutaneous vaccine delivery. A, Investigational Tropis needle-free intradermal (ID) jet injector (PharmaJet). **B,** Investigational Bioject ID Pen needle-free jet injector for ID delivery of 0.1 mL (0.05-mL–dose model not shown) (Bioject Medical Technologies). Powered by metal spring reset with built-in lever. Disposable polypropylene spacer on disposable syringe creates the desired air gap to weaken the jet stream for ID delivery. **C,** *Left* and *middle*, Investigational Nanopatch microneedle array of silicon, after application to mouse skin. Microprojections are 30 μm wide at base and from 65 to 110 μm in height, and sputter-coated with 100 nm of gold. The red coating of antigen/adjuvant elutes to reveal the original gold coating.[721] **D,** Investigational dissolving microarray patch (Georgia Institute of Technology).[722] **E,** Corium dissolving microarray patch. Adhesive applicator provides correct pressure to apply patch and remains on the skin for several minutes while the patch projections dissolve. **F,** Investigational Particle-Mediated Epidermal Delivery (PMED) device (PowderMed) propels (usually gold) microparticles coated with (usually DNA) antigen into skin using a stream of supersonic helium gas. **G,** Investigational Skin Preparation System (SPS) for *transcutaneous immunization* (Intercell AG, originally developed by Iomai Corporation). Blue push-button requires the correct amount of abrasion pressure on the stratum corneum by a sandpaper-like strip pulled with the blue tab. **H,** Investigational PassPort thermoporation device and patch applied to patient arm. Heat induced by the device in metallic filaments embedded in the patch creates micropores in the stratum corneum for subsequent entry of drug within the patch (Altea Therapeutics). *(**A,** Courtesy of PharmaJet [Chris Cappello]. **B,** Courtesy Bioject Medical Technologies. **C,** Courtesy University of Queensland/Vaxxas [Mark Kendall]. **D,** Courtesy Jeong Woo Lee, Georgia Institute of Technology. **E,** Courtesy Corium International, Inc. [Bobby Singh]. **F,** Courtesy PowderMed. **G,** Courtesy Intercell AG [Andi Bruckner]. **H,** Courtesy Altea Therapeutics.)*

dose is immunogenic but often does not meet noninferiority criteria compared with full-dose IM delivery.[266,269,270]

A recent clinical trial of BCG vaccine given via ID found that DSJI delivery and Mantoux method delivery were similar in safety and immunogenicity.[423] Other vaccines that have been studied for ID delivery by DSJIs include those for HIV,[179,198] influenza, dengue,[424] and HPV.[425] Jet injectors for ID delivery offer the advantage of speed, simplicity, and patient acceptability as well as the ability to use off-the-shelf vaccines without reformulation. Challenges include developing devices that reliably deliver small ID doses to the correct depth,[266,269] obtaining clinical data to license vaccines for ID delivery by DSJIs, and the higher cost of DSJI devices compared with an ID needles and syringes.

Microarray Patches. Microarray patches (also referred to as microneedle patches) deliver solid formulations of vaccines into the skin and have a different set of potential benefits and development challenges from the liquid-delivery technologies for cutaneous vaccination that have been described. Microarray patches meet many of the criteria for a practically ideal vaccine delivery system because they are needle free in a prefilled unit-dose dry format, with potential for increased thermostability. They also can be shipped by common shipping methods such as mail and can potentially be self-administered or administered by minimally trained personnel. Microarray patches can be further subdivided into solid-coated and dissolving types.

Solid-Coated Microarray Patches. A common strategy pursued by a number of commercial and academic teams to carry antigens across the stratum corneum is to coat the vaccine onto solid microscopic projections, which are held for variable periods of time in the dermis or epidermal layer (depending on height) while the antigen (or other drug) elutes and diffuses.[290,293–295,297,301–305,342,343,426] Some microarray patch technologies have been designed for application by finger pressure, while other developers have found that a mechanical applicator is needed for consistency of skin penetration. To date, therapeutic drug applications have reached a more advanced stage of development than those for vaccines; nevertheless, a substantial amount of preclinical research has been conducted with vaccines, and several early-stage vaccine clinical trials are in progress.

One example of drug-coated microarray patches is the investigational Zosano Pharma ZP Patch platform (formerly known as Macroflux). Its titanium projections vary from 225 to 600 µm in height and are packed into an area of 1 to 2 cm^2 at densities from 140 to 650 tines/cm^2. They are inserted by a spring-mounted applicator and held in place by an adhesive patch. The most advanced applications for these microarray patches are delivery of parathyroid hormone to treat osteoporosis,[427] already studied clinically, and erythropoietin to treat anemia. Regarding vaccine applications,[428] Zosano Pharma's ZP-Flu influenza vaccine patch, applied for 5 or 10 minutes onto the skin, trended toward increased titers and seroprotection compared with an IM control injection.[430] Other preclinical studies of the system demonstrated delivery of ovalbumin,[428,431] oligonucleotides,[432] and the peptide hormone desmopressin.[433] The company reports animal work with tetanus, diphtheria, Lyme disease, and hepatitis B (DNA) vaccine antigens.

Another coated microarray patch platform is the solid microstructured transdermal system from 3M.[289,434] Its drug-coated pyramidal projections vary from 250 to 750 µm in height, in arrays of 300 to 1500 microarray patches mounted on an adhesive patch at a density of 1300 per square centimeter.[435–438] Application to the skin is by a manual finger-thumb Press&Patch device[439–441,480] or by a spring-powered applicator, shown elsewhere.[249] Coatings of the microarray patches are said to hold up to 0.5 mg of active pharmaceutical ingredient. In a rabbit model, coatings of tetanus toxoid and aluminum adjuvant in various ratios induced antibody using just a fraction of the standard IM dosage.[443] In swine, a model virus-like protein (HBsAg) demonstrated dosage sparing via solid microstructured transdermal system compared with antigen delivered by IM control route.[442] A more recent public registration described a safety trial without antigen.[444]

The Georgia Institute of Technology, a pioneering center for microarray technology, has worked with Emory University to conduct numerous studies of coated microarray patches[342,444a] in animal models for cutaneous vaccine delivery. In a series of murine studies using solid metal microarray patches coated with inactivated influenza viruses, cutaneous vaccination induced robust immune responses—often better than equivalent dosages in controls injected by the SC route—as well as protection against lethal viral challenge.[445–454]

When coated with BCG, the same microarray platform was highly immunogenic in guinea pigs, with robust cell-mediated responses in lungs and spleen, comparable to those with Mantoux injection.[455] Similarly, plasmid DNA antigen for hepatitis C, coated on needles 500 µm in length, primed specific cytotoxic T lymphocytes in vaccinated mice more readily than conventional needle.[456] Inactivated rotavirus vaccine—developed to avoid the inhibitory effect of maternal antibodies on live oral vaccines[457]—was coated onto this microarray platform and found immunogenic in an animal model.[458]

Another center for microarray research, the University of Queensland and the nearby company Vaxxas in Australia,[459,460] developed a novel nitrogen gas jet-drying method for coating antigen onto silicon that overcomes the challenges of dip-coating closely spaced projections,[444a,459,461] but still allows antigen to elute within 2 to 3 minutes upon skin entry. This Nanopatch technology has among the smallest projections (less than 300 µm in length) with more than 20,000 projections per square centimeter on silicon chips (see Fig. 68.6C). It has achieved $\frac{1}{30}$ to $\frac{1}{100}$ dosage-sparing compared with the IM route in a mouse model for influenza.[462,463] Other antigens studied with good results in murine models with this platform include HPV, chikungunya, DNA plasmid vaccines (herpes simplex virus 2, West Nile virus), and viral vector DNA vaccine (malaria).[463–470] Research on and development of coated microarray patches for vaccination are also underway by many other groups.[340,381,471]

Experimental placement of the solid microstructured transdermal system microarray patches device on human volunteers found it to be "well tolerated" and "nonintimidating and not painful." The Georgia Institute of Technology microarray patch (without coating) has been used in clinical studies to assess usability and completeness of skin penetration when applied by self-administration, and study participants indicated they would be more willing to be vaccinated with influenza vaccine if microarray patches were available.[12] In focus group discussions, both public and private healthcare providers were also positive toward microarray patches as a change from conventional needle and syringe delivery.[472] Microarray patches can also allow use of dry formulations, which improve vaccine thermostability and reduce the need for cold chain storage. For example, in formulations prepared at Georgia Institute of Technology, a key ingredient of the carboxycellulose matrix of the dried coating was trehalose, one of several sugars, including sucrose, that have been found useful in protecting protein antigens from damage by drying and freezing, thereby improving vaccine thermostability.[473,474] Microarray patches have the potential to enable dose sparing of some vaccines, and some patches may also provide an adjuvant-like effect themselves by increasing local cell death.[475]

Although they have the potential to be a transformative technology for vaccine delivery, microarray patches are in an early stage of development for use with vaccines, and challenges include generating clinical evidence and scaling up manufacturing processes. The required wear times for microarray patches are not yet fixed and differ among designs. In preclinical studies, wear times for some microarray patches for vaccines have been greater than 5 minutes but can also be around 2 minutes. Ideally, delivery times would be sufficiently rapid to allow for quick application, removal, and proper disposal by a healthcare worker. If longer wear times are required for successful delivery, early removal is a risk, leading to possible insufficient antigen delivery as well as disposal of vaccine antigen outside the location of the supervised immunization session. If the vaccine recipient remains in the clinic

or otherwise-supervised environment of delivery, increased vaccination time and thus reduced throughput might have a negative impact on overall program efficiencies. Coated microarray patches might be considered both sharps and infectious waste material after they have been used and in contact with bodily fluids, so the risk of needlestick injury remains a consideration. The need for an applicator (whether separate or integrated with the patch) to reliably apply the microarray patches is another key question, as are cost and logistical implications for immunization programs. A visual or auditory indicator may also be important to reassure users and recipients that the vaccine has been delivered correctly.[472,476]

Dissolving Microarray Patches. An elegant strategy to decrease risk from intentional reuse of, or inadvertent contact with, used microarray patches is for the sharps to dissolve in the skin with hydration, thus releasing the antigen.[249,305,477–481] The most common matrix for dissolvable microarray patches hard enough to penetrate skin is carboxymethylcellulose, "generally recognized as safe" for parenteral delivery by the FDA, among other compounds.[477,478] Georgia Institute of Technology and Emory University have encapsulated inactivated influenza vaccine virus into biocompatible polymer, which dissolves within minutes after its application to skin (see Fig. 68.6D). Robust antibody and cellular immune responses provided complete protection from lethal challenge in mice, and a clinical trial is in progress.[481a,482] Several sugars, such as trehalose, sucrose, and maltose, have been found to be key ingredients in stabilizing and maintaining the potency of antigen during the process of forming dissolvable microarray patches, and studies have shown up to 4 months of stability at 40°C for measles vaccine in a microarray patch.[473,483–485] Formal thermostability studies will be needed to assess whether such formulations would resist heat degradation and allow transport and storage outside the cold chain.

Corium International has developed the MicroCor dissolvable microarray patch, with an integrated applicator that adheres to the skin (see Fig. 68.6E). TheraJect has developed the DrugMAT and VaxMAT dissolvable microarray patches, which have been investigated preclinically for delivery of several pharmaceuticals, including influenza vaccine. TheraJect's patches are designed for manual application without an applicator, but an applicator is also in development if needed for reliable delivery. In Japan, CosMED markets a cosmetic MicroHyala microarray patch containing hyaluronate, which dissolves in 60 to 90 minutes, and a Phase I clinical trial using this technology to deliver influenza vaccine has been conducted.[486] Many others also pursue dissolvable microarray patches.[486a,487,488]

Advantages and challenges for dissolving microarray patches are similar to those for solid-coated patches (see "Solid-Coated Microarray Patches" earlier). An important distinction is that dissolving patches leave no sharps waste.

Powder Injection. The transfection of cells by kinetic methods to deposit DNA-coated gold particles into them was pioneered in the 1980s. The Helios or PDS 1000/He gene guns170 and the Accell injector[518] have become standard bench tools for biolistic delivery of nucleic acid plasmids into a wide variety of plants and animals to transfect them to express the coded genes.[304,489,490] Delivery of DNA into the skin overcomes the usual polarized T-helper cell type 1 response when DNA is delivered into muscle.[293,491,492] These devices are unavailable for human vaccination (patent rights are held by PowderMed). The proprietary terms "epidermal powder immunization" and "particle-mediated epidermal delivery" (PMED) refer to the use of helium gas to blow powdered proteins, polysaccharides, or inactivated pathogens (epidermal powder immunization) or DNA-coated particles (PMED) into the epidermis at supersonic speeds. This unique method of vaccination was developed in the early 1990s by Oxford BioSciences, which over the years was renamed PowderJect, acquired by Chiron,[493] spun off as PowderMed, and finally acquired by Pfizer[494] in 2006. Delivery is by either reusable (XR series) or single-use disposable (ND series) devices (see Fig. 68.6F), with the ND series targeted for commercialization.

Conventional protein antigens for delivery by epidermal powder immunization are spray-dried into powders of suitable density and size (20 to 70 μm),[495,496] but the economics of manufacturing such formulations may be an obstacle.[293] For DNA vaccines delivered by PMED, plasmids coding for desired antigens are coated onto gold beads (1 to 3 μm in diameter) and, when deposited into epidermal APCs, they are eluted and transcribed.[497] A number of preclinical studies in various animal models have been conducted.[495,498–500]

Human trials of DNA vaccines containing up to one order of magnitude less antigen than the amount used for IM routes have induced humoral and cellular immune responses for hepatitis B in subjects both naïve and previously vaccinated with conventional vaccine.[501,502] PMED vaccination has also been studied for DNA priming in trials of malaria vaccine,[503,504] has produced seroprotective immune responses by DNA vaccine for seasonal strains of influenza,[505] and has reduced influenza symptoms and viral shedding after human challenge.[506]

In the hepatitis B and influenza trials cited earlier, there were no severe local reactions, but erythema, swelling, and flaking or crust formation occurred in nearly all subjects, albeit resolving by day 28. Skin discoloration, however, persisted through day 56 in 29 (97%) of 30 subjects,[510] through day 180 in 21 (25%) of 84 injection sites,[511] and beyond 12 months in 5 (25%) of 20 patients with long-term follow-up.[510] No anti–double-stranded DNA antibodies were detected. The deposition of the gold particles was studied in pigs, in which most were deposited in the stratum corneum and epidermis and were eventually sloughed by exfoliation by 28 days.[512] At days 56 and 141 after administration, a few particles remained in the basal epidermal layer and in macrophages in the dermis and regional lymph nodes.

Preclinical studies of epidermal powder immunization or PMED in murine, porcine, and primate models have shown immunogenicity or protection for either powdered or DNA plasmid antigens for various other pathogens, including Eurasian encephalitic viruses,[514] hantaviruses,[515] HIV,[516,517] influenza H5N1,[499] malaria,[518] severe acute respiratory syndrome (SARS) coronavirus,[519] smallpox,[393] and Venezuelan equine encephalitis.[520]

Abrasion/Transcutaneous Delivery. Various commercial patch delivery systems developed since 1981 have demonstrated the ability of certain therapeutic agents (e.g., scopolamine, nitroglycerin, clonidine, estradiol, fentanyl, nicotine, testosterone) to diffuse passively into bare, untreated skin without the use of active technologies or enhancers. However, such passive diffusion usually works only for small molecules with certain physical characteristics. Thus, there are but a few animal models of immunization onto bare, untreated skin.[521–523]

Newer methods to facilitate antigen delivery to the epidermis involve painlessly stripping or abrading the skin to increase the permeability barrier of the stratum corneum. A variety of simple tools have been used to remove the stratum corneum. Common cellophane adhesive tape may be applied to the skin and pulled away, carrying away dead keratinocytes with each repetition. Such tape-stripping has been shown to enhance cytotoxic T-cell and cytokine immune responses on subsequent application of various antigens and adjuvants

to the skin in mice.[524–530] Similarly, rubbing gauze, emery paper, or pumice on the skin removes cells by their abrasive effects, and this has been found to enhance immune responses in humans. Application of cyanoacrylate glue followed by stripping the skin to apply antigen to the exposed hair follicles has been described,[531] but its practicality has been questioned.[299]

Among methods that strip the skin, perhaps the most advanced is one that combines this step with the use of a remarkably potent adjuvant, the heat-labile enterotoxin (LT) of *Escherichia coli*. This effort was originally championed by Gregory M. Glenn, first at the Walter Reed Army Institute of Research, then at Iomai Corporation, and later at Intercell. The vaccinator or the patient holds against the skin a device, the Skin Preparation System (see Fig. 68.6G). With the push of a button and the pull of a tab, a controlled pressure is applied to a sandpaper strip, which gently abrades and removes approximately 25% of the stratum corneum.[532] Then, a patch containing LT as antigen alone, or LT as an adjuvant for another antigen, is applied to the skin; the process is called transcutaneous immunization.[534–537] LT alone is intended to induce immunity against enterotoxigenic *E. coli* (ETEC), the cause of traveler's diarrhea, or against *Vibrio cholerae*, with[538] or without[539,540] ETEC colonization factor.

An initial randomized, blinded field trial among travelers to Guatemala and Mexico found 75% efficacy for the patch with LT alone in protecting from moderate to severe diarrhea.[541] In 2010, Intercell reported mixed results from two follow-up field studies. In a pivotal Phase III trial for traveler's diarrhea (N = 2036), again in Guatemala and Mexico, the trial's primary target end point of greater than 60% efficacy against moderate to severe ETEC diarrhea was not met, finding only approximately 35% protection. Nor was there an effect on the frequency of all causes of diarrhea. However, there was a 60% reduction in the incidence of LT-positive diarrhea of all degrees of severity, along with a significant reduction in duration and severity of all diarrhea causes. The patch also induced measurable immune responses and was well tolerated.[542]

In a smaller Phase II trial in India (N = 723), the LT patch also did not reach its targeted end point, perhaps because of a low attack rate (approximately 1%) for LT-positive ETEC.[543] As a result of these two trials, Intercell discontinued work on the LT patch for traveler's diarrhea but still pursues its use with the Skin Preparation System device for other applications.

Applying the Intercell LT patch near the site of injection of parenteral influenza vaccine (an application referred to as a vaccine enhancement patch) was found to improve hemagglutination inhibition titers in the serum and mucosa of both young and aged mice[544,545] and increase the hemagglutination inhibition titer or show an improving trend for adult human volunteers older than 60 years of age.[546] In preclinical studies of other applications, use of LT or a structurally similar cholera toxin as cutaneous adjuvants resulted in improved immune responses or challenge protection in animal models for tetanus,[547] anthrax,[548,549] malaria, *Helicobacter pylori*, and Shiga toxin–producing strains of enterohemorrhagic *E. coli*.[550,551]

Other methods take advantage of low-cost fabrication techniques adapted from the microelectronics industry to convert silicon, metal, or other material into arrays of micrometer- to millimeter-sized microrasps designed to abrade the stratum corneum.[294,297,301–303,325,552] One example is the microenhancer array (also known as Onvax), an investigational technology that scrapes the skin before or after topical application of the antigen or therapeutic agent.[553] The microenhancer array consists of a square or round chip containing about a 1-cm^2 area of silicon or plastic microprojections mounted on a finger-held applicator.[249,342,353] Preclinical studies of the microenhancer array device have been conducted using hepatitis B, anthrax, and Japanese encephalitis vaccines.[288,354] A human trial, however, in which rabies vaccine was applied before or after four "rubs" of the device over four separate deltoid skin sites did not detect any immune response after three dosings.[325]

Technologies for abrading the skin to enhance transdermal delivery have the potential to be low cost, acceptable to users, compatible with vaccines, and suitable for use in a variety of vaccination settings, although device design must ensure ease of correct use. Reproducibility of dosing and effectiveness of vaccination has been a challenge for this technology, particularly the disappointing results of the Intercell ETEC vaccine. As a result, the field of cutaneous vaccination has focused mainly on technologies that inject or deliver the vaccine into the skin, rather than relying on passive diffusion.

Microporation. Microporation, also termed thermoporation, uses heat to vaporize tiny openings in the stratum corneum.[294,295,377,554,555] In the PassPort system, developed by Altea and now owned by Nitto Denko,[289,556] this heat is generated by a disposable array of metallic filaments held momentarily against the skin by a device the size of a computer mouse (see Fig. 68.6H). At activation, electric pulses are induced to heat the filaments. An adhesive patch containing vaccine or therapeutic agent is then applied over the micropores just created. In a hairless mouse model, this technique elicited 10- to 100-fold greater cellular and humoral responses to an adenovirus vaccine than intact skin, as well as 100% protection to surrogate tumor challenge (27% for intact skin).[377] In the same model, adenovirus-vectored melanoma antigen applied to the micropores roughly doubled the average onset time of tumors after challenge, and it protected one of six mice compared with none of eight vaccinated controls with intact skin. Microporated recombinant influenza H5 hemagglutinin protected BALB/c mice from challenge with a lethal H5N1 strain.[557] Skin micropores also permitted the passage of insulin in pharmacokinetic human trials with historical controls, and in the other direction allowed interstitial fluid to be extracted for potential glucose monitoring.[558]

Another device similarly generates micropores with heat induced by radiofrequency waves (ViaDerm). A different technique uses short, 100-microsecond pulses of superheated steam in microliter amounts to remove the stratum corneum.[559] Pantec Biosolutions has developed a laser microporation platform that could also be used for cutaneous vaccine delivery.

Microporation systems provide a reliable means of forming pores in the stratum corneum to enhance transdermal delivery, although the dose and reproducibility of vaccine delivered across the skin following this procedure remain to be verified. Devices can be designed for rapid use and acceptable levels of local reactivity, but for any microporation system, the cost and distribution of the reusable device could be a challenge in low-resource settings, particularly due to electrical power requirements.

Mucosal Vaccination

Most human pathogens initiate infection through mucosal portals of entry—the respiratory, gastrointestinal, and genitourinary tracts. Mucosal immunity can prevent these infections. In contrast, systemic immunity clears infection only after successful invasion, by limiting replication and destroying the pathogens. Ideally, both mucosal and systemic immunity should be raised against targeted pathogens. Strong mucosal immunity may enhance the benefits of immunization for some diseases. For example, by preventing the initial infection, mucosal immunity can reduce the risk of transmission to

others in addition to preventing clinical disease. Prevention of infection at the mucosal surface may be especially important for diseases for which effective systemic immunity has been difficult to achieve, such as tuberculosis and AIDS.

Anatomy

As described above, the external surfaces of the human body are protected by the keratinized stratified squamous epithelium, which composes the outermost layer of the skin. In contrast, the internal surfaces of the mucosal tracts are protected by a variety of epithelial types commensurate with the different functions of the gastrointestinal, respiratory, and genitourinary tracts. Nonkeratinized stratified squamous epithelium predominates in the oral cavity, oropharynx, vagina, and anal canal. Ciliated and nonciliated pseudostratified epithelia line the nasal airway, nasopharynx, and bronchi. Various specialized simple (single layer) epithelia are found at other mucosal sites. For example, the enterocytes of the gastrointestinal tract have microvilli at their apical surfaces to facilitate nutrient absorption, and the pulmonary squamous alveolar cells are extremely thin to permit diffusion of oxygen and other gases between the alveoli and the vascular systems.

Lacking the skin's protective layer of keratin, mucosal epithelial surfaces are protected from pathogen invasion by several alternative mechanisms. First, a complex dynamic mucus coat restricts access to most mucosal epithelial surfaces. Next, proteolytic enzymes are abundant on many mucosal surfaces and the highly acidic gastric environment degrades most pathogens before entry into the small intestine. Mucosal systems also dynamically move potential threats to limit the duration of exposure. For example, the ciliated epithelia of the upper and lower respiratory tract continually drive the mucus layer and its contents to the oropharynx to be swallowed and degraded by gastric acid. Peristalsis and other gastrointestinal mechanisms result in the eventual expulsion of gastrointestinal contents. Finally, the importance of mucosal microbiota to the prevention of infection and promotion of healthy immune responses is becoming increasingly realized.[560–562]

In addition to the physical defenses described above, mucosal immunity provides additional defensive layers to prevent infection. Innate mucosal immune factors include endogenous antimicrobial peptides such as defensins and cathelicidins. Proteins such as lysozyme and lactoferrin also reside in many mucosal surfaces and inhibit pathogen incursions. Each of these pathogen invasion defenses is a barrier that mucosal vaccine delivery systems must overcome to deliver the antigen package to host cells and elicit a consistent protective immune response.

Anatomical features of the upper respiratory tract affect IN delivery of medications. Particles are first filtered by the hairs in the nostrils, whose surface is covered with keratinized stratified squamous epithelia. Next, the particles must traverse the external nasal valves, slit-like passages that limit airflow from the nostrils into the internal nasal airways. Djupesland and colleagues estimated that only 25% of large, high-speed droplets (average: 43 μm) of a traditional nasal spray reach beyond the external nasal valve.[563] Almost all large, high-speed particles that transit the nasal valve deposit in the internal nasal airway, which has an average of approximately 150 cm^2 (0.015 m^2) of surface area covered with columnar epithelia, most of which is ciliated. In the internal nasal airway, particles deposit on the nasal mucosa and then join the flow of mucus that is swept by ciliated epithelia toward the pharynx, where it is swallowed. Immune surveillance of antigens in the flow of mucus begins as they are taken up into epithelial cells, intraepithelial dendritic cells, surface macrophages, and M cells (see Fig. 68.3D).

For delivery into the lower respiratory tract, small particles inhaled via nose or mouth share a common pathway through the pharynx, larynx, and trachea. The bifurcation of the trachea into the right and left bronchi starts a series of bifurcations, providing further surfaces to trap airborne particles. Only very small, slow-moving particles succeed in navigating the tortuous passages to deposit in the lower airways. The smallest particles (≤3 μm) may reach the alveoli, where they can be rapidly absorbed into systemic circulation. The complex branching of the lung passages also results in an astonishing alveolar surface area, exceeding 100 m^2 in a human adult male. The lower airways in humans do not typically have organized lymphoid tissues, but they do have abundant intraepithelial dendritic cells and alveolar macrophages that process antigens (see Fig. 68.3E).[564]

Immunological Rationale

While IM, SC, and ID vaccine delivery can induce both mucosal and systemic immunity, administration of vaccines to mucosal tissues tends to induce the strongest mucosal response. Several cell types are active in the mucosal antigen surveillance system. Although epithelial cells may play a role in the immune response, critical targets include the professional APCs, such as dendritic cells and macrophages, which are present in all mucosal tissues (see Figs. 68.3E and F), and in the case of alveolar macrophages, on the mucosal surface (see Fig. 68.3E). Phagocytosis of foreign microparticles is an important component of innate mucosal immunity and of mucosal antigen surveillance. Because mucosal surfaces are exposed to a myriad of nonpathogenic macromolecules, there are mechanisms for downregulating the immune response to antigenic exposure. Thus the potential for inducing immunologic tolerance must be considered when developing mucosal immunization strategies.

Dendritic cells are active throughout mucosal tissues and are present in high concentrations in the mucosal-associated lymphoid tissue, which include the tonsils, adenoids, and Peyer patches (see Fig. 68.3F). Mucosal-associated lymphoid tissue are also rich in microfold (M) cells, specialized epithelial cells with apical surfaces that take up macromolecules, viruses, and bacteria by endocytosis. On their basal surfaces, M cells have invaginated pockets that communicate with the extracellular space and host collections of lymphocytes and dendritic cells (see Fig. 68.3D). M cells transfer the endocytosed materials to these APCs for processing.[565–568]

APCs from the mucosa track to regional lymph nodes, where B-cell activation occurs. These B cells preferentially switch to IgA plasmablasts that "home" back to the exposed mucosal tissue to provide antigen-specific IgA protection. T cells also home back to mucosal sites and play a major role in mucosal immunologic memory responses. Lymphocytes exposed to antigen from one mucosal site, such as the respiratory tract, will migrate to provide protection at remote mucosal sites, such as the vagina. This integrated network of immune cells and tissues is known as the common mucosal immune system.[119,569] In some studies, IN vaccination has resulted in higher vaginal IgA titers than has vaginal vaccination.[570]

Almost every mucosal surface available for administering vaccines has been studied with a variety of antigens in animal models, including oral, respiratory, rectal, vaginal, and ocular tissues. In general, the respiratory and gastrointestinal tracts have more organized lymphoid tissue sites and more capacity for induction of immune responses.[571]

Several human vaccines are licensed and have been used successfully for delivery to the enteric tract by oral ingestion. Preclinical studies have investigated the potential utility of administering oral vaccines that target the oral mucosa rather

than the enteric tract. IN LAIVs are the only respiratory-delivered vaccines in use. However, multiple clinical trials have assessed vaccine delivery to the respiratory tract.

Challenges for Mucosal Delivery of Vaccines

Many of the technologies in development for mucosal vaccination target the oral or respiratory route and these will be the major focus of this section. Although vaginal and rectal vaccines may be effective, they could have limited acceptability for social, cultural, and practical reasons.

The first challenge in mucosal immunization is determining the appropriate target tissue. The optimal target tissues are not fully understood and may vary for different vaccines. Oral and respiratory vaccination strategies each have two discrete target areas. Vaccination via the oral route may target the oral mucosa or the intestines. Respiratory vaccination may target the upper respiratory tract (nasal passages, pharynx and larynx) or the lower tract (trachea, bronchi, bronchioles and lungs). For both nasal and oral mucosal delivery, the pharyngeal tonsils are likely target tissues. Some respiratory vaccines may require deposition in the lower airways for uptake by alveolar macrophages and dendritic cells. Lower airway deposition can be achieved by oral or nasal inhalation. Scientific methods for evaluating and comparing different target tissues are not yet well developed.

A second challenge is selecting animal models and extrapolating results to human mucosal vaccine delivery. Interspecies differences in immunologic tissue limit interpretation of animal target-tissue research results for humans. Moreover, the size and anatomy of common research animals differ greatly from those in humans. For example, in small animals such as rodents, nose drops may deposit to the entire respiratory tract, which would not be the case in humans. Balmelli and colleagues estimated that 30% of 20 µL of vaccine given to mice as IN drops deposited into the lungs.[572] Many viruses and bacteria that infect humans do not grow well in animal models. For example, species-specific differences in the distribution of sialic acid receptors on cell surfaces is a crucial factor in tissue and host specificity of influenza A viruses, which limits the number of animal models suitable for influenza research. Such species-specific differences can make it difficult to use animals to study live attenuated vaccines or vaccine vectors, and difficult to challenge animals to assess protection. This impedes the development of safe and effective mucosal vaccines for humans.

A third hurdle for mucosal immunization is assuring delivery of an accurate dose to the target tissue. The mass or volume of the antigen delivered depends on many factors. For intestinal delivery, gastric acids may destroy much of the antigen dose. For respiratory delivery, variability in performance by delivery device, the technique of the vaccinator, and differences in the anatomy and physiology of persons being vaccinated affect the dose delivered.[573] Fortunately, for many vaccines there is a wide margin between the dosage necessary to induce protection and the dosage at which the risk of adverse events increases.

A fourth challenge is the lack of accepted correlates of protection for mucosal immunity. For many diseases, laboratory assays have well-established criteria for systemic immunity—such as antibody titers above certain cutoffs—that have served for many years to predict protection from disease. In the absence of accepted serologic or cellular correlates of protection induced by mucosal vaccines, clinical trials must use specific disease-prevention end points, which can make the studies required for licensure much larger and more expensive.

Several immunization safety concerns represent further challenges for mucosal vaccines. Live virus or bacterial vaccines might pose an increased risk to immunocompromised persons if administered mucosally. Live-attenuated vaccines that revert to more virulent genotypes can adversely affect the vaccinee and be spread to contacts in the community. For nasal vaccines, another risk is that vaccine antigen (live or inactivated), adjuvant, or excipients might affect nearby cranial nerves[574] or travel along the olfactory nerve through the cribriform plate into the brain, with resulting adverse nervous system effects. Vaccines targeting the lower airways may induce or exacerbate bronchospasm or pulmonary inflammation, which can be life threatening. Another risk is cross-contamination: respiratory pathogens from one patient may contaminate the respiratory immunization device and be spread to subsequent patients.[575] Also, vaccine aerosols may spread beyond the intended recipient and affect other persons in the vicinity.

With so many challenges, new delivery technologies to achieve mucosal immunization are required if this route is to become practical and acceptable. In this young field, published research on delivery systems in animals or humans is limited. In most reported animal studies, the delivery system is not mentioned at all. For most systems designed for human respiratory systems, testing is very difficult or impossible in an animal model.

Finally, perhaps the most significant challenges to implementation of novel vaccine delivery systems in routine immunization practice are the regulatory requirements needed to ensure that the systems are safe and effective. The required studies and clinical trials can be extremely expensive. Vaccine manufacturers typically are reluctant to assume such cost and risk to relicense an existing product already delivering profits unless the potential benefits and market advantages will be significant. The best opportunity to bring alternative delivery into routine practice may be to use new delivery systems—from the start—for new vaccines early in development and licensure processes.

Oral Vaccination

Oral Ingestion (Intestinal Delivery). Several human vaccines are licensed and have been used successfully for delivery by ingestion, including those for polio, cholera, rotavirus, typhoid, and adenovirus (see Chapters 10, 14, 48, 49, 52, and 61). OPV, a prototypical mucosal vaccine, demonstrates the key advantages and challenges of mucosal vaccination. The ease of delivery of OPV drops enables minimally trained volunteers to distribute it in house-to-house campaigns that have been essential for the current successes of the global polio eradication effort. OPV is a live attenuated vaccine, as are most successful mucosal vaccines to date. Vaccination results in increased mucosal immunity, particularly in the intestine, as evidenced by increased secretory IgA and decreased shedding of vaccine poliovirus on subsequent challenge compared with IPV given by injection (see Chapter 48). However, even minor shedding of vaccine virus can result in transmission of the vaccine virus to others. This is especially concerning because the vaccine virus has the potential for reversion to virulence, which can result in vaccine-associated paralytic poliomyelitis in vaccinees and their contacts, and even in sustained outbreaks of circulating vaccine-derived polioviruses. Another concern regarding mucosal vaccine delivery demonstrated by OPV is the inconsistency of the immune response across different populations. OPV produces immunogenic responses less consistently in children in developing countries than in children in industrialized countries. This may be caused by differences in intestinal microbiota (see Chapter 48).

Because intestinal delivery by oral ingestion is simple, especially for liquids such as OPV drops, there is little

technology development required for macrodelivery of oral vaccines. Most of the new technologies for intestinal vaccination focus on microdelivery systems, which are reviewed in a separate section. One of the biggest hurdles to consistent delivery of vaccine antigen to APCs in the intestinal tract is ensuring that the antigen survives degradation from gastric acids. Like wild poliovirus, OPV is stable in acid environments for several hours. Other enteric vaccines, such as cholera and rotavirus vaccine, are delivered with a buffer solution to neutralize gastric acid. Typhoid fever vaccine is delivered as a lyophilized powder protected in an enteric coated capsule. However, capsules are not appropriate for infant vaccination.

Oral Mucosal Vaccination (Sublingual or Buccal Delivery). Preclinical studies have been conducted to explore the potential of administering vaccines via the sublingual (SL) or buccal mucosa. These routes have long been used for low-molecular-weight drugs such as nitroglycerin SL tablets. Several SL immunotherapy vaccines are marketed in Europe to suppress allergic hypersensitivity.[576] The major advantages of oral mucosal immunization are the ease of administration, including the potential for self-administration, and the probability of inducing robust mucosal immunity. In contrast to intestinal delivery, orally absorbed antigens are not subjected to degradation by gastric acid.

The oral cavity is lined with stratified epithelium. Like the skin, the oral epithelium is replete with Langerhans cells, the principal target APCs for oral vaccination. Some oral surfaces, such as the hard palate, have a keratinized upper layer, while the epithelia of the buccal and SL mucosa are nonkeratinized.[576] The lack of keratin increases permeability and potential for transportation of antigen to the mucosal APCs without the disruption or penetration of the stratum corneum required for cutaneous vaccination. As an example, Fig. 68.3G demonstrates wild-type measles virus replication in the oral mucosa. However, salivary and mucus flow rapidly moves substances on oral surfaces toward the pharynx to be swallowed and digested, and many salivary components initiate the processes of digestion and degradation. The oral cavity does not have organized lymphoid tissue such as the intestinal Peyer patches; however, movement of antigen across the pharyngeal tonsils may play a role in induction of an immune response.[577] Unlike nasal delivery, oral delivery does not have the potential of exposing the cribriform plate to vaccine. Shim and colleagues reported that SL vaccination with a recombinant replication-deficient adenovirus expressing SARS-associated coronavirus antigen induced protective immunity against SARS-associated coronavirus comparable to that seen with IN vaccination. Adenoviral DNA was detected in the olfactory bulbs of the mice vaccinated intranasally, but not in those mice vaccinated sublingually.[578]

In addition to immunotherapy vaccines, two novel SL vaccines are commercially available in Spain for the prevention of recurrent infections. One of these, Uromune, is a mixture of inactivated whole bacteria of selected strains that are common causes of urinary tract infections (UTIs). The vaccine is self-administered as a daily SL spray, maintained under the tongue for a period of 1 to 2 minutes, and then swallowed. In a clinical study of 319 patients with recurrent UTIs, subjects who received the vaccination daily for 3 months had significantly reduced occurrence of UTIs compared with subjects who received the standard therapy of sulfamethoxazole/trimethoprim for a period of 6 months. The reduced incidence of UTIs persisted for 12 months after completion of vaccination.[579]

Immunotherapy for allergy patients and use of polyvalent inactivated bacterial preparations to suppress recurrent infections are the major focus of published clinical trials for oral mucosal vaccination.[580–583] There are few clinical data on traditional prophylactic vaccines delivered via the SL or buccal route, although studies are registered on ClinicalTrials.gov for influenza, cholera, and HPV.[584,585] Preclinical studies of SL vaccines have demonstrated immunogenicity in mice using viral vectors such as adenovirus-based vaccines[586–588] and bacterial vectors such as *Bacillus subtilis*.[589,590] For example, SL immunization of mice with recombinant adenovirus-based vaccine expressing influenza virus hemagglutinin induced significant levels of sustained hemagglutinin-specific mucosal IgA and IgG, and provided complete protection from challenge with a lethal dose of homologous virus.[590a]

Despite the potential benefits of oral mucosal immunization, significant challenges have delayed progress toward commercially available prophylactic vaccines. First, antigens presented to the oral mucosa may result in immune tolerance, as evidenced by the success of the SL immunotherapy products designed to induce tolerance. Producing the desired protective immune response may require manipulation of the antigen formulation and dose, as well as addition of adjuvants. Another problem is that the rapid flow of saliva limits the residence time of antigen in the mouth and the opportunity for contact with APCs. Several formats have been designed or adapted from oral drug delivery systems to increase oral residence time.[576] For preclinical testing of vaccines, oral delivery is complicated by the extremely small oral cavities in small animal models: delivery of liquid vaccines to the mouth in mice is limited to 5 to 15 μL and alternate delivery formats are extremely difficult to use. Also, unlike humans, most rodents have keratinized epithelia in the buccal mucosa, which limits comparability of antigen uptake.[576]

To overcome the inherent mucosal tolerance for antigens, a variety of mucosal adjuvants have been studied.[576,591–594] For example, mice sublingually immunized with influenza vaccine (hemagglutinin split vaccine) with a lipopolysaccharide adjuvant demonstrated both hemagglutinin-specific IgG (systemic) and IgA (mucosal) antibody responses, which led to a significant increase in survival rate against lethal influenza virus challenge compared with SC vaccination.[591] Two mucosal adjuvants—α-galactosylceramide, a potent stimulator of natural killer T cells, and cytosine phosphate guanine–oligodeoxynucleotide a Toll-like receptor-9 agonist—effectively increased gp140-specific serum IgG and vaginal IgA levels following SL vaccination in mice. Combining both significantly improved these responses, and serum and vaginal washes collected 60 days after immunization had significant neutralization activity against simian/human immunodeficiency virus.[595]

To enhance residence time on the oral mucosa, a thermoresponsive gel—which changes from aqueous solution to viscous gel upon contact with the mucosa at body temperature—was combined with a double mutant of a bacterial heat-labile toxin. SL immunization of mice with a trivalent IPV with the gel delivery system produced both mucosal and serum antibodies, including IgA.[596]

Interest in SL vaccination has accelerated significantly: a 2015 PubMed search of "sublingual vaccine" returned more than 175 published articles since 2010 compared with 115 prior to 2010.

Progress of candidate oral mucosal vaccines through clinical trials to commercialization will require significant additional research to increase our understanding of the pharmacokinetics and pharmacodynamics, as well as immunological mechanisms, of this route of delivery. Because of the inherent mucosal tolerance of antigens and limited oral residence time,[576] development of optimized delivery formulations and predictive assays for mucosal immunity are particularly challenging.

Respiratory Vaccination

Background. The earliest known route of vaccination was IN insufflation of powdered scab material from smallpox patients, reportedly practiced in China as early as the 10th century AD. However, IN LAIVs are the only respiratory vaccines in modern use (see Chapter 54). In contrast, the respiratory route is used to deliver a wide and expanding variety of pharmaceutical products.[597,598] Respiratory drug administration typically targets one of two major areas: the upper airway or the lower airway. Most common products, such as nasal decongestants and inhaled asthma medications, are intended for local therapy in the target area. Newer products, such as inhaled insulin, target deposition in alveoli to achieve systemic delivery. The first inhalable insulin (Exubera) was a commercial failure, partly because of a cumbersome delivery device.[599,600] However, another product for insulin inhalation, Afrezza, was approved by the FDA in 2014.[600a] Afrezza uses a small, simple dry powder inhaler, the Mannkind Dreamboat.[600b] Efficient and effective delivery to respiratory target tissues is determined largely by the aerodynamic size and speed of the vaccine particles, which are functions of the device engineering and the product formulation.

Devices for upper respiratory tract drug delivery include droppers, nasal sprayers, nasal nebulizers, and dry powder inhalers. Very few are designed for or have been tested with vaccines. Most drug devices deliver repetitive doses to a single patient. In contrast, vaccination delivers single doses to multiple patients, which raises the issue of device cross-contamination. Although unit-dose disposable devices or disposable components could solve this problem, they must be inexpensive to be cost-effective. Many drug-delivery devices are designed for self-administration; however, this requires a level of patient cooperation difficult to achieve in young children and impossible in infants.

With mucosal vaccination in general, the optimal target tissues are not yet understood for most potential respiratory vaccines, and they vary for different antigens. Liquid droppers for IN delivery are perhaps the simplest devices, and inexpensive prefilled single-use droppers are commonly used for administering drugs to the upper respiratory tract. However, drops administered intranasally tend to drip out of the nose or roll back to the pharynx where they are swallowed, resulting in less residence time in the nasal passages and nasopharynx and less contact with mucosal surfaces. Recumbent patient positioning may increase nasal residence time but may not be practical at all POCs. Prefilled and fill-on-site nasal spray devices generate smaller particles, typically in the 50- to 100-μm range, and nasal nebulizers generate even smaller (20 to 30 μm) particles. While these devices may increase mucosal surface contact and residence time, there are no definitive studies to their effect on immunogenicity.

Deposition of liquid vaccines in the lower airways requires a nebulizer device that generates the very small droplets required to pass the vocal cords and reach the trachea and bronchi (<10 μm) or the alveoli (<5 μm). Nebulizers are durable devices that require an energy source to generate aerosols. The device transits aerosol to the patient through a mask, oral prong, or nasal prongs. Single-use disposable components, including patient interfaces, are needed to prevent cross-contamination.

Dry powder aerosols can also be used to deposit vaccine in the upper or lower respiratory tract. Liquid delivery has the advantage of being the standard format: all currently licensed aerosol vaccines are in liquid presentation. Generating sprays and aerosols from existing liquid vaccine formulations could accelerate respiratory vaccination research. In contrast, a number of obstacles must be overcome to produce vaccines as dry particles of sizes suitable for upper or lower respiratory tract deposition.[601–603] First, formulating powders requires significant and extensive changes in manufacturing methods. Second, many potential dry-formulation ingredients are extremely hygroscopic and become sticky when exposed to humidity. Third, once the powders are deposited in the respiratory tract, they must be sufficiently hygroscopic to dissolve and release the vaccine for uptake. However, if these challenges can be met, dry aerosols have several advantages over liquid aerosols. Doses can be filled into inexpensive single-use presentations and delivered without on-site aqueous reconstitution. Secondary packaging that seals the dose container in an impermeable overwrap, such as metal foil, could maintain low humidity to prolong potency and increase shelf life. Minimal energy is required for powder dispersion compared with liquid droplet generation, so electromechanical devices are not necessary.

Upper Airway Vaccine Deposition

Nasal Sprays. The only vaccine currently licensed and in use in the United States for respiratory delivery is the cold-adapted LAIV FluMist, delivered with the AccuSpray IN syringe. LAIV development, testing, and licensure are reviewed in detail in Chapter 32. LAIV demonstrates several potential benefits of respiratory delivery. It produces both mucosal and systemic immunity, and it generally has been found to provide higher protective efficacy than injected inactivated vaccine in young children.[604–612] It also provides heterotypic immunity against nonvaccine strains.[604] IN delivery of LAIV may reduce the risk of influenza transmission because it reduces respiratory shedding among immunized children challenged later with a vaccine virus.[604] Finally, modest coverage with LAIV among schoolchildren reduced influenza-related illness rates in unvaccinated adults in a community.[612] However, despite the previous positive results for LAIV, vaccine effectiveness data for the 2015–16 influenza season found that FluMist did not provide protective benefit for children, and the Centers for Disease Control and Prevention (CDC)'s Advisory Committee on Immunization Practices has recommended that FluMist not be used in the United States.[613]

The AccuSpray device is a sterile, single-patient–use, disposable, prefilled glass syringe fixed with a nonremovable plastic nozzle (Figs. 68.7A and B). Its total dose is 0.2 mL of which 0.1 mL is sprayed consecutively into each nostril. An attachment on the plunger stops the first dose to allow the user to switch nostrils. FluMist vaccination delivered by AccuSpray is highly effective in most populations (see Chapter 54). Key advantages of AccuSpray delivery are that it is simple to use, low cost, capable of disposed without needing to use a sharps container, and has a low possibility of refill and reuse. The large particle sizes generated by the sprayer minimize deposition to the lower airways, reducing the risk of adverse pulmonary events. A limitation of the system is that the particle size emitted depends on the speed at which the vaccinator depresses the plunger. The median diameters of the particles can range from 200 μm or greater at plunger speeds of up to 33 mm per second to 50 μm or less at speeds of 80 mm per second and greater.[614] Although this wide variability might in theory affect the efficiency of vaccine deposition, LAIV by AccuSpray typically produces a high rate of protective immunity at the current dosage.

An IN LAIV developed in Russia has been in use in that country for more than 50 years (see Chapter 54). The vaccine is lyophilized and must be reconstituted and filled into the syringe at the POC. Each dose comes with a Lindal Group atomizer tip that fits the syringe, after removal of the reconstitution needle, for delivery into the nostril. No dose divider is provided and the 0.25-mL dose for each nostril must be drawn

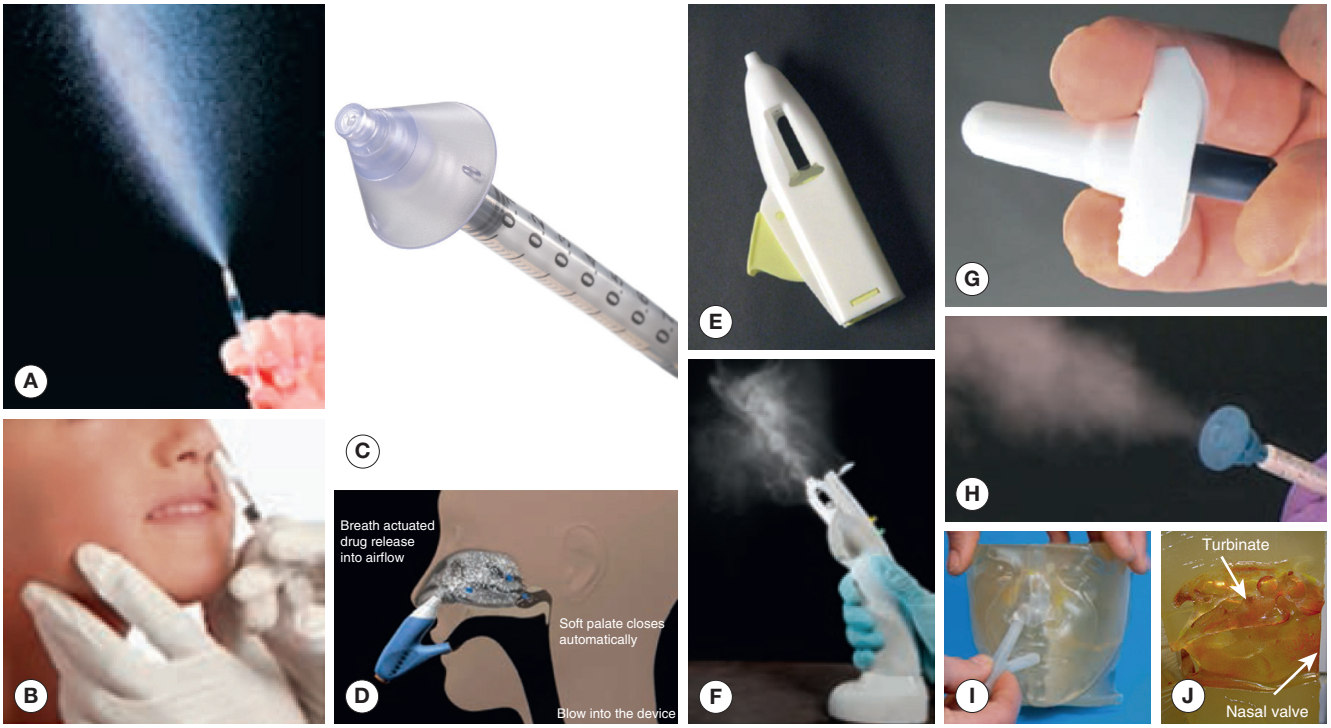


Figure 68.7. Selected devices for upper respiratory tract vaccine delivery. A and **B,** AccuSpray nasal spray syringe (Becton, Dickinson and Co.) **A,** AccuSpray produces an aerosol plume of particles reported from 50 to 200 μm in diameter, depending on plunger speed. **B,** AccuSpray used for intranasal delivery of FluMist influenza vaccine (MedImmune, Inc.). Prefilled liquid vaccine is stored refrigerated for single patient use. The total volume is 0.2 mL. A dose separator interrupts delivery at 0.1 mL and, when removed, allows the remaining 0.1 mL to be administered into the opposite nostril. **C,** Teleflex VaxINinator nasal spray device currently used to deliver Serum Institute of India Ltd's Nasovac-S influenza vaccine. The conical spray tip is attached to the syringe by a Luer fitting. **D,** Sagittal computer-assisted image illustrating intranasal delivery by the OptiNose delivery device (OptiNose, Inc.). **E,** Dolphin side-activated nasal spray disposable unit-spray device designed to deliver 200 μL of product in two 100 μL sprays. **F,** Investigational AeroVax prototype (Creare, Inc.) uses battery-powered piezoelectric energy to drive an aerosol from a disposable drug cartridge via a microperforated mesh plate through a disposable patient interface, such as nasal prong in patient nostril, oral prong, or mask (not shown). Droplet diameters can be tailored from <5 μm to 10 to 25 μm for upper or lower airway delivery, respectively. **G,** BesPak, UniDose DP a dry powder prefilled unit-dose device which can deliver up to 10 μg of medication. Requires a predefined amount of pressure to be applied to the plunger before the device will operate, thus ensuring proper dispersion. **H,** Investigational Solovent (Becton, Dickinson and Co.) dry powder inhaler prototype, adaptable for intranasal or pulmonary formats. The Solovent is a small cylindrical plastic capsule open on both ends. One end has a Luer fitting for attachment to any standard syringe to provide air flow, while the other end is the patient interface, either a nasal prong or a snap-fit adapter to mate with a mask or reservoir. The capsule contains the powder dose between two burstable plastic membranes, which rupture when airflow is provided by the syringe. Plume of powder upon release from Solovent in open air for visualization purposes. **I,** Investigational nasal dry powder inhaler developed by the CDC and Creare, Inc. A prefilled cup (shown between right thumb and forefinger) containing the powdered vaccine is opened by its attachment to the device. The breath of the patient blowing into the device tube carries the dry powder into the nose. Dispersion during patient exhalation limits pulmonary deposition from the posterior nasal space. The plastic face is a phantom model of the airway of a 5-year-old child (CFDRC, Inc.). **J,** Medial section of the right internal nasal airway of a plastic, phantom model shown and described in **I**. The external nares and face (not shown) are to the right, proximal to the nasal valve. The pharynx (not shown) is to the left, distal to the nasopharyngeal opening. The red-pigmented powder indicates the deposition pattern from the investigational nasal dry powder inhaler shown in **I**. *(**A**–**B,** Courtesy Nuphar Rozen-Alder [Becton, Dickinson and Co.]. **C,** Courtesy Teleflex Medical Europe Limited. Unauthorized use prohibited. **D,** Courtesy Gisle Djupesland. **E,** Courtesy Aptar Pharma and from Riddle MS, Kaminski RW, Williams C, et al. Safety and immunogenicity of an intranasal Shigella flexneri 2a Invaplex 50 vaccine.* Vaccine. *2011;29[40]:7009–7019. **F,** Courtesy James Gathany [CDC Photographic Services]. **G,** Courtesy BesPak [Ian Anderson] and from Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk Virus illness.* N Engl J Med. *2011;365[23]:2178–2187. **H,** Courtesy Becton, Dickinson and Co. [Vincent Sullivan]. **I,** Courtesy Creare, Inc. [Darin Knaus]. **J,** Courtesy Creare, Inc. The research was supported by the U.S. Department of Health and Human Services/CDD through SBIR Contract 200-2009-32519. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. We gratefully acknowledge Dr. Vincent Harrand of CFD Research Corporation for supplying the nasal phantom models used for this work.)*

up separately by reattaching the fill needle. Potential reuse of the device with cross-contamination is a risk.

BioDiem has commercialization rights for the Russian LAIV outside of Russia and has licensed the technology to the Serum Institute of India Ltd. The Serum Institute of India Nasovac-S vaccine is currently delivered with the Teleflex VaxINator, a single-use device that consists of an atomization spray tip compatible with any standard Luer lock fill-on-site syringe (see Fig. 68.7C). A dose divider attached to the syringe plunger separates the 0.5-mL dose for delivery of 0.25 mL per nostril. One advantage of the VaxINator is that the conical tip fits any nostril size and limits the depth of

insertion, providing the vaccinator with physical guidance to device placement. The device is potentially reusable. Teleflex offers an optional autodisable syringe, which automatically disables both the syringe and the VaxINator after the drug is delivered.

An inactivated virosomal-subunit influenza vaccine (Nasalflu, Berna Biotech) was licensed in Switzerland in 2000 for administration as a nasal spray. Nasalflu contained *E. coli* heat-labile toxin as a mucosal adjuvant. The vaccine was withdrawn from the market after an increased incidence of temporary paralysis of the seventh cranial nerve (Bell palsy), which was considered to be related to the heat-labile toxin adjuvant.[574,615]

Multiple clinical trials have been conducted with other LAIVs in addition to the licensed vaccines.[616,617] Clinical trials of other types of influenza vaccines—including inactivated, virosomal, live replication-deficient, and proteasome vaccines—also have demonstrated promising immune responses.[618–621] Most studies used standard nasal spray or did not describe the IN delivery method. One study used a novel breath-actuated nasal spray device, OptiMist, which delivers liquid or dry powder aerosols only during oral exhalation. Because this method raises the soft palate to close the connection between nose and throat, pulmonary deposition is avoided and delivery to the posterior nasal segments is increased (see Fig. 68.7D).[563] Inactivated influenza vaccine self-administered using the OptiMist resulted in significant increases in virus-specific IgA in nasal secretions, as well as protective levels of virus-specific serum antibodies, after two doses in more than 80% of subjects.[622]

Other IN vaccines for which clinical trials demonstrate encouraging immune responses include live attenuated respiratory syncytial virus, parainfluenza, *Bordetella pertussis* vaccines,[623–626] a Norwalk virus VLP vaccine, and hepatitis B vaccine. For example, live attenuated respiratory syncytial virus vaccines administered as IN drops to seronegative infants 5 to 24 months of age produced a functional immune response in 59% of subjects.[626] Riddle and colleagues reported that a *Shigella flexneri* subunit vaccine was safe and well tolerated, producing robust levels of antigen-specific intestinal IgA when administered IN with a Dolphin spray device (see Fig. 68.7E).[627]

Nasal Drops. Multiple clinical trials and almost all preclinical studies of IN vaccines have been conducted with nasal drops. Unfortunately, the dropper device and delivery method is often not described. Several studies compared LAIV delivery by nasal spray with liquid dropper delivery.[628] In a multicenter study of children 18 to 71 months of age, King and colleagues detected no statistically significant differences in reactogenicity or immunogenicity between subjects who received LAIV by drops or spray at four different doses.[628] In another study, four weekly doses of inactivated influenza vaccine were given as drops, 250 µL per nostril, with the vaccinee in a supine position on a tilted couch with the head lowered for 1 minute in order to retain the vaccine within the nasopharyngeal area. IN vaccine drops produced hemagglutination-inhibiting antibody titers ≥40, considered protective in more than 80% of subjects.[618]

Nasal Nebulizer. Several nebulizers have the capacity to deliver IN aerosols. Live attenuated measles vaccine administered via nasal prong with the AeroVax nebulizer (see Fig. 68.7F; Creare, Inc.) was shown to be safe and immunogenic in macaques.[629] AeroVax delivery of LAIV to ferrets elicited high levels of serum-neutralizing antibodies and protected them from homologous virus challenge at conventional (median tissue culture infective dose [$TCID_{50}$]: 10^7) and significantly reduced ($TCID_{50}$: 10^3) dosages, and it provided a significant level of subtype-specific cross-protection.[630]

Intranasal Dry Powders. Multiple clinical trials of an IN powder vaccine including chitosan as a mucoadhesive have demonstrated robust immune responses.[631,632] Two doses of a combination dry powder *Neisseria meningitidis*–diphtheria IN vaccine administered by a 5-mL Combitips Plus syringe produced protective antibody titers for both pathogens comparable to those generated by conventional injection. The Combitips is a sterile PP syringe with a solid displacement plunger that expels powder without residual airspace and has a very narrow opening that generates a fine aerosol. The tip of the syringe was placed as far into the nostril as possible without causing discomfort, with the subject in a semireclined position. One-third of IN vaccinees reported mild side effects compared with two-thirds of injection vaccinees who reported mild injection pain.[631] In another clinical study, IN powder diphtheria vaccine formulated with chitosan significantly augmented systemic T-cell responses compared with the same vaccine without chitosan.[632] An IN norovirus VLP vaccine that included chitosan and monophosphoryl lipid A as adjuvants significantly reduced the frequency of Norwalk virus gastroenteritis in human subjects following viral challenge with a homologous strain. The vaccine was delivered intranasally using the Bespak Unidose DP (dry powder) device[633,634] (see Fig. 68.7G), a single-use unit-dose design that allows for IN delivery of up to 100 µg of medication.

IN dry powder vaccines have been formulated and tested in preclinical studies for influenza, anthrax, diphtheria, meningococcus, measles, hepatitis B, plague, and Norwalk virus. The BD Solovent device has been used in several preclinical studies (see Fig. 68.7H). Live attenuated measles vaccine delivered intranasally to macaques with Solovent provided an immune response equivalent to SC injection, including protection from virus challenge.[635] Anthrax vaccine by the IN route with Solovent provided complete protection against inhalational challenge with roughly 90 times the median lethal dose in rabbits, while providing better stability than liquid formulations.[353,635a,636] Solovent IN delivery of a whole inactivated influenza virus product in rats elicited high titers of nasal antiinfluenza IgA, as well as serum antibody titers equivalent to those obtained with injected vaccine.[637] No loss of potency was found when it was stored at 25°C and 25% relative humidity for up to 12 weeks and at 40°C and 75% relative humidity for 2 weeks.[637]

An investigational nasal powder delivery device developed by Creare, Inc., operates by patient exhalation through the mouth, blowing the powder into the nose while simultaneously generating air flow that limits entry to the lower respiratory tract. In three-dimensional plastic models developed from computerized tomography airway anatomy of children ages 2, 5, 7, and 12 years of age, the device consistently delivered a significant fraction (>66%) of a fluorescein powder dose to the target IN airways (see Figs. 68.7I and J).

To increase nasal residence time, a dry powder formulation, GelVac, was developed with an inert in situ gelling polysaccharide. IN vaccination with GelVac Norwalk-virus VLPs induced systemic and mucosal antibody titers in rats and guinea pigs equal to or greater than those achieved by liquid VLPs plus adjuvant.[638] The GelVac powder vaccine was administered using a laboratory-constructed delivery device using a 5-mL syringe to provide airflow through a modified pipette tip, which served as a nasal prong. Although many dry powder and liquid-spray nasal devices are in use for drug delivery in humans, testing them in vaccine studies in animals is difficult or impossible because of the nostril size.

Lower Airway Vaccine Deposition. The vast alveolar surface area and abundant APCs make the lungs an attractive target for vaccine delivery. However, inflammatory adverse reactions in this vital organ could have grave consequences, so a high degree of caution is required. Significant development in

pulmonary drug delivery has been achieved, which might be adapted for vaccine delivery.

Pulmonary Liquid Aerosols. Measles vaccine is the most widely studied vaccine targeting pulmonary delivery. Early clinical studies of drops or sprays delivered to the conjunctiva, or the oral or nasal mucosa, produced inconsistent immune responses,[639–648] prompting researchers to consider aerosol pulmonary delivery. Albert Sabin was an early pioneer and advocate of this delivery method.[649–652] A significant body of clinical research in aerosol measles has been published and summarized in review articles and metaanalyses.[653–656] The primary finding is that delivery of nebulizer-generated small-particle liquid aerosols via pulmonary inhalation to children 10 months of age or older typically produced immune responses in very high proportions of subjects, usually equivalent to or greater than responses to injected vaccines.[630–632,640–642,656–671] For example, Dilraj and colleagues found that 96%, 94%, and 86% of schoolchildren who received the aerosol measles vaccine had antibody titers of greater than 300 IU/mL at 1, 2, and 6 years after vaccination, respectively, compared with 91%, 87%, and 73% among those receiving the injected vaccine.[659,669,672] In contrast, generally lower immune responses were found for the aerosol route compared with parenteral injection, among children younger than 10 months.[649–652,673–675] For example, Wong-Chew and coworkers found vaccination of 12- and 9-month-old infants by injection induced immunity in 100%, but by aerosol route in only 86% and 23%, respectively.[673,674] One hypothesis was that the very low respiratory volume of infants results in an inhaled dose of aerosol vaccine that is too small in that period. A follow-up study by Wong-Chew and colleagues demonstrated that increasing exposure time to aerosol measles vaccine elicits immune responses that are comparable to those seen by the SC route in 9-month-old infants.[676]

With regard to vaccine safety, the same reviews and metaanalyses[653–655] noted that no severe adverse events were reported after aerosol measles vaccination in any of the studies. Rates of minor adverse events, when reported, were typically less than or the same as vaccination by injection.[659,666,668,673,674] Experience in mass campaigns was similar, with de Castro and colleagues reporting no serious adverse events among more than 3.7 million children in Mexico vaccinated by aerosol.

The jet nebulizer system known as the Classic Mexican Device (see Figs. 68.8A and B) was used in many measles aerosol clinical studies and during mass campaigns in Mexico.[659,669,672,674,677] The system consists of a general-use (non–medical-grade) compressor that delivers air to a jet nebulizer, which holds the vaccine in crushed ice to maintain potency during the vaccination session. The vaccine aerosol is delivered through reusable plastic tubing to a single-use disposable paper cone (modified from a drinking cup), which is held close to the patient's face for 30 seconds. Typically, the aerosolized dose volume is roughly 0.15 mL, and the mass median aerosol diameter of droplets is 4.3 μm.[678] In a study that assessed the distribution of viable vaccine virus across the range of droplet sizes emitted from a Classic Mexican Device, Coates and coworkers estimated that 30% of infective viral particles were contained in droplets with diameters of 5 μm or less, and 23% were in droplets of greater than 10 μm. As with all vaccine delivery to the lower respiratory tract, it has not been possible to identify which target tissues are essential for production of an immune response. Although the Classic Mexican Device has demonstrated a level of safety and immunogenicity, it is heavy, cumbersome, and noisy, and it requires outlet (mains) electricity and crushed ice. It is thus not practical for routine vaccination.

Because of the encouraging results of early measles aerosol vaccine trials, in 2002 the WHO, in partnership with the CDC and the American Red Cross, initiated the Measles Aerosol Project. Its goal was licensure of at least one live attenuated measles vaccine and its associated aerosol delivery system in the developing world. The project documented immunogenicity and safety (i.e., the lack of local or systemic toxicity) in animal studies.[629] Three existing therapeutic nebulizers were used for Phase I clinical trials: the AeroEclipse, the CompAIR, and the Aeroneb.[679,680] Measles vaccine delivery by the three devices, delivered to 145 subjects in India, was reported to be safe, well tolerated, and immunogenic.[681] A modified version of the battery-operated Aeroneb vibrating mesh nebulizer (Aerogen) device (see Fig. 68.8C) was selected for use in the Phase II/III pivotal trial initiated in 2009 by the Measles Aerosol Project. The study was a randomized, open-label, active-control, noninferiority trial of the measles vaccine in unvaccinated healthy infants from 9 to 11.9 months of age. For aerosol delivery, vaccine was reconstituted in 2-mL diluent and administered as a single 0.2-mL dose, nebulized for 30 seconds through a single-use nonvented face mask. The nebulizer generated aerosol with a median diameter of 5.1 μm. In the full-analysis set, 673 (85.4%) of 788 children in the aerosol group and 754 (94.7%) of 796 children in the SC group were seropositive at day 91, a difference of 9.3 percentage points (95% confidence interval, 12.3 to 6.4), which failed to meet the predetermined 5% noninferiority margin. No serious adverse events were attributable to measles vaccination and adverse-event profiles were similar in the two groups.[682]

In other recent clinical studies, rubella and mumps vaccines have been used in combination with measles vaccine for aerosol delivery. Bennett and colleagues found that aerosol vaccination of preschool children with a combination MMR vaccine produced antibody responses to all antigens equivalent to those produced by injection.[658] Sepúlveda and coworkers found that aerosolized measles-rubella combination vaccine in school-age children produced levels of measles and rubella antibodies equivalent or higher than levels seen after SC injection.[666] Fewer adverse events were reported in the aerosol group. Diaz-Ortega and colleagues found that MMR vaccination by aerosol in college students produced immune responses similar to those produced by injection, with seropositivity retained in all vaccinees 1 year after vaccination.[670,671]

Few clinical trials of pulmonary aerosol delivery of other vaccines have been reported. In a small Phase I double-blind trial, aerosol delivery was compared with ID delivery for a modified vaccinia Ankara–vectored vaccine delivering the *Mycobacterium tuberculosis* Ag85A antigen. The aerosol was administered as a 1-mL liquid dose using an Omron MicroAIR U22 vibrating mesh nebulizer. Both administration routes were well tolerated with few adverse events and were immunogenic with Ag85A-specific systemic responses similar across groups. Ag85A-specific CD4 T cells were detected in bronchoalveolar lavage cells from both groups, and responses were higher in the aerosol group than in the ID group.[683]

Pulmonary Dry Powders. Measles vaccine has been a pathfinding application for respiratory dry powder vaccination. Early formulations were finely milled and retained adequate potency, but immune responses were poor when delivered to the respiratory tract of macaques.[601,603] Aktiv-Dry was awarded a Grand Challenges in Global Health grant in 2005 for a measles vaccine dry powder (MVDP) project. The project goals were to refine a dry powder measles vaccine formulation, establish production capacity at the Serum Institute of India, and complete preclinical and Phase I clinical testing[684] A novel spray-drying system was used to manufacture inhalable MVDP with minimal potency losses in the drying process.



Figure 68.8. Selected devices for lower respiratory tract vaccine delivery. A–B, Investigational Classic Mexican Device for aerosol vaccine delivery, illustrated by component diagram **(A)** and use in clinical trials **(B)**. A nonmedical electric compressor (not shown) delivers roughly 9 L of air per minute at a pressure of 30 to 40 psi (207 to 276 kPa) to a jet nebulizer that is kept in crushed ice to maintain vaccine potency. The vaccine aerosol (roughly 0.15 cm^3 of particles averaging 4.3 μm in diameter) is delivered through a disposable paper cone held close to the patient's face for 30 seconds. **C,** Aerogen Aeroneb GO battery powered vibrating mesh nebulizer used as the basis for the Measles Aerosol Project clinical trials device. Cap (between thumb and finger) is removed to fill upper chamber. Microperforated vibrating mesh disk at the base of the chamber aerosolizes dose into lower chamber. Patient inhales aerosol through the mouthpiece or attached mask (not shown). Cord connects to battery control pack. **D,** Investigational Solovent dry powder inhaler prototype pulmonary delivery format (Becton, Dickinson and Co.). Air from the empty syringe ruptures the membranes of the vaccine container, releasing into the cardstock spacer a plume of dry powder. Patient inhales from the cardstock spacer directly or via a mask. The vaccine container (capsule), spacer, and mask are single-use disposables. **E** and **F,** Investigational PuffHaler dry powder inhaler (Aktivdry, LLC). **E,** Air from the squeeze bulb lofts vaccine powder from the disperser into the reservoir once the pressure threshold of the burst valve is exceeded. **F,** Patient inhales from the single-use-only reservoir of PuffHaler after detachment from the device, either directly (as shown) or via a disposable mask (not shown). **G,** Investigational Twincer single-use, disposable, dry powder inhaler for pulmonary delivery (University of Groningen). The drug formulation is stored in the snap-together plates of the device in an aluminum blister for maximal moisture protection. The powder becomes available for inhalation upon pulling a foil cover that protrudes from the rear of the inhaler (not shown). *(**A–B,** Courtesy José Luis Valdespino [Instituto Nacional de Salud Pública, Mexico] and from Valdespino-Gómez JL, de Lourdes Garcia-Garcia M, Fernandez-de-Castro J, et al. Measles aerosol vaccination.* Curr Top Microbiol Immunol. *2006;304:165–193; **C,** Courtesy Philips Respironics and from Laube BL. The expanding role of aerosols in systemic drug delivery, gene therapy, and vaccination.* Respir Care. *2005;50[9]:1161–1176. **D–F,** Courtesy James Gathany [CDC Photographic Services] and [**D** and **F**] from MVDP author group, Cape S, Chaudhari A, et al. Safety and immunogenicity of dry powder measles vaccine administered by inhalation: a randomized controlled Phase I clinical trial.* Vaccine. *2014;32[50]:6791–6797. **G,** Courtesy University of Groningen [A.H. de Boer] and from de Boer AH, Hagedoorn P, Westerman EM, et al. Design and in vitro performance testing of multiple air classifier technology in a new disposable inhaler concept [Twincer] for high powder doses.* Eur J Pharm Sci. *2006;28[3]:171–178.)*

Pulmonary delivery of the vaccine powder with the Solovent device (see Fig. 68.8D) and the Aktiv-Dry developed PuffHaler device (see Figs. 68.8E and F) demonstrated robust immune responses in macaques, including serum antibody, T-cell responses, and protection from viral challenge. Both devices delivered aerosol into reservoirs for mask delivery.[635] Toxicology studies found no test-article–related effects or delayed onset of toxicity after inhalation by Sprague Dawley rats and no effects in mortality, clinical observations, respiratory function, clinical pathology, or histopathology in rhesus macaques.[685]

A Phase I MVDP safety trial of the vaccine was conducted in adults in India, with subjects randomly assigned in 1 : 1:1 ratio to receive MVDP via PuffHaler device, Solovent device, or the licensed SC measles vaccine. No adverse events were reported. MVDP produced serologic responses similar to SC vaccination.[686]

Other vaccines in development for pulmonary powder delivery include influenza, tuberculosis, and hepatitis B.[687] For example, a pulmonary powder formulation produced by SFD, with influenza subunit viral antigen and inulin stabilizer, induced humoral (IgG), cell-mediated (interleukin-4, interferon-γ), and mucosal (IgA, IgG) immune responses in BALB/c mice.[688] When tested separately with a novel dry powder inhaler, the single-use disposable Twincer (see Fig. 68.8G), inulin-based dry powder subunit influenza vaccine was dispersed with an aerodynamic particle size distribution suitable for pulmonary administration.[689]

VACCINE MICRODELIVERY SYSTEMS: ANTIGEN DELIVERY TO DESTINATION-POINT ANTIGEN-PRESENTING CELLS

Once vaccine has been delivered to the appropriate target tissue, sufficient quantities of the antigen must gain access to APCs to activate the immune system. The microdelivery vehicles or vectors that can be used for this purpose include live attenuated viruses (including those acting as vectors for exogenous antigen), live attenuated bacteria (including vectors), commensal bacterial vectors, virosomes, VLPs, liposomes, lipopeptides, immune stimulating complexes, microparticles, nanoparticles, and dendritic cells.[690–696] These vaccine microdelivery systems are described in detail in Chapter 64. Here we include an abbreviated description of these systems to complete the discussion of immunization as an antigen package delivery system.

Replicating Systems

Live Viruses and Bacteria

Viruses and bacteria are prototypical antigen delivery vehicles for several reasons: (a) the package size is optimal for cellular uptake; (b) viral surfaces are coated with molecules that attach to cellular receptors, a process that "rings the package delivery bell" (i.e., cells recognize them as potential pathogens, activating innate immunity and increasing cellular uptake); (c) once inside, viruses commandeer cellular systems for replication, multiplying exponentially the vaccine antigen that they encode; and (d) bacteria self-replicate, in tissues or inside cells, multiplying the vaccine antigen available to APCs. Live attenuated strains of pathogenic viruses and bacteria are among the most common type of vaccines and have been used for every delivery route. The major risks of attenuated pathogens as vaccines are potential pathogenic effects, which may occur in immunocompromised persons, following viral reversion to a virulent form, or with exposure of an unintended tissue, such as neurotoxicity following exposure via the olfactory route.[697]

Recombinant viruses and bacteria act as vaccine vectors by incorporation of genes that express a heterologous antigen. Some recombinant vectors also express adjuvants. The replicating systems have advantages similar to those of conventional live attenuated virus vaccines. They deliver the genetic code for the vaccine antigens and adjuvants to host cells for replication, and the cells manufacture the vaccine components to activate the immune system. Viruses and bacteria used as vaccine vectors should, ideally, have very low pathogenic potential, even in immunocompromised people, and they should have the capacity to incorporate the necessary foreign genes for desired antigens, promoters, and adjuvants. As vectors, bacteria have an advantage over viruses because of their higher capacity for insertion of heterologous genes expressing antigens, adjuvants, or plasmids (for DNA vaccination). One caveat for the use of vectored vaccines is that preexisting immunity to the vector may reduce vaccine effectiveness.[698]

DNA Vaccines

DNA vaccination involves the delivery of eponymous plasmids directly into host cells to express the desired antigens and adjuvants. Naked DNA lacks cellular entry mechanisms, so the critical hurdle is getting the DNA into the cells without degradation. Live attenuated bacteria, especially *Salmonella* and *Shigella*, have been vectored to produce DNA for IN vaccination.[699–704] Virosomes, liposomes, and microparticles have also been used to deliver DNA vaccines.[705–708]

Nonreplicating Vaccine Delivery Systems

Synthetic constructs, including liposomes, VLPs, virosomes, immune stimulating complexes, microparticles, and nanoparticles, are nonreplicating delivery systems that mimic live viruses in the way they appear to the immune system to enhance antigen delivery. They may also carry adjuvant. The particles are about the size of viruses, allowing similar uptake by APCs. Many include a lipid component to increase cell membrane permeability and may contain unrelated viral or bacterial proteins to activate the immune system.

One of the most novel methods of delivering antigen into a recipient's APCs is to extract the APCs, by peripheral blood draw for example, culture them with the antigen, and then administer the autologous antigen-laden APCs via intravenous, IM, or SC injection. Autologous dendritic cell-based vaccines are under development as curative cancer vaccines.[695,696]

Adjuvants

Nonreplicating antigens are typically poorly immunogenic and may require adjuvants to stimulate an appropriate immune response. One mechanism of adjuvant action is to provide "warning labels," implying the antigen package is a potential pathogen, to alert cells and induce innate immunity. Adjuvants studied for this purpose include pathogen components such as bacterial toxins and their derivatives, other bacterial components, and bacterial DNA motifs. Other adjuvants include the cytokines and chemokines that cells produce to induce innate immunity. Another adjuvant mechanism is to increase the residence time of the antigen in the target tissue, prolonging the opportunity for the antigen package to be taken up by APCs. Aluminum adjuvants provide this depot effect for many inactivated vaccines delivered intramuscularly or subcutaneously. For mucosal delivery, nanoemulsions and natural polymers such as chitosan have mucoadhesive properties that delay mucosal antigen clearance.[621,708a,708b,709–712]

CONCLUSION

New technologies for delivering antigen packages have the potential to overcome logistical hurdles that impede delivery of vaccines to all populations in need. Practically ideal vaccines would be safe, effective, and inexpensive. They would have these characteristics: (1) packaged as single-unit doses to minimize wastage and missed opportunities to vaccinate; (2) thermostable at ambient temperatures to make vaccines easy to store and ship, even via routine delivery systems such as postal services; (3) available in prefilled dose format to obviate on-site reconstitution or filling; (4) simple to administer or self-administer to reduce dependence on highly skilled healthcare workers; and (5) needle-free to reduce the risks of sharps injuries, needle reuse, and the burden of sharps waste disposal.

Combining new technologies for stabilizing and packaging vaccines with new vaccine administration technologies, new molecular antigen delivery systems, and adjuvants could yield practically ideal vaccines in the next few decades. However, the promising technologies described in this chapter face daunting obstacles to (1) bridge the gap between successful proof of principle and the expensive and complicated series of clinical trials, related studies, and regulatory steps to ensure that the novel systems are safe and effective, and (2) achieve licensure and general availability.

The initial costs of developing, licensing, and supplying practically ideal vaccines are very high. To encourage financing, a clear demand—a willingness to pay—must be expressed in advance by key stakeholders, including public health and immunization program policy makers, vaccine purchasers, and independent philanthropic entities. To create this demand, stakeholders must be aware of the potential of new technologies and convinced that the cost of developing practically ideal vaccines is outweighed by the benefit of reducing the long-term costs of current inefficiencies in the immunization system. In February 2014, the WHO held a meeting on Next-Generation Vaccine Delivery Technologies to conduct a rapid review of existing and future technologies, provide a vision for the future of vaccine technologies, and determine the strategic next steps to guide and enable the development, introduction, and uptake of new technologies with potential positive public health impact. Integrated products that would eliminate the need for reconstitution, provide improved thermostability, and offer needle-free delivery were all highlighted as priority technology development areas.[713]

DISCLOSURE

Mark Papania is a coinventor with corresponding financial interests in the AeroVax (CDC, Creare, Inc.) and dry powder inhaler (CDC, Creare, Inc.) devices illustrated in Figs. 68.7F and I.

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SECTION 4

Vaccination of Special Groups

69 Vaccination of Immunocompromised Hosts

Per Ljungman

Over the past decades, the number of immunocompromised patients has increased rapidly. These patients are vulnerable to a number of vaccine-preventable diseases and new vaccines are in development such as cytomegalovirus (CMV) vaccines—the "troll of transplantation"—and inactivated varicella vaccines.[1–3] The 2009 H1N1 influenza A pandemic also showed the need for rapid development of vaccination strategies in immunocompromised populations.[4–7] Thus, increased knowledge regarding responses to vaccinations in the immunocompromised host is needed. Yet, the issue is complex because the characteristics of the immunosuppressed states and their management differ greatly between the various patient groups and many immunosuppressive therapies are being developed and used in expanding numbers of patients.

PATIENTS WITH CANCER

Cancer chemo- and radiotherapy has changed substantially over the past several decades with the introduction of monoclonal antibodies and targeted anticancer drugs such as tyrosine kinase inhibitors. However, the number of clinical trials assessing vaccination responses in patients receiving these modern therapies is limited. Most vaccine studies have been performed in patients with hematological malignancies, and studies assessing immune responses in adult and pediatric solid-tumor patients are few. Many patients with non-Hodgkin lymphoma or chronic lymphocytic leukemia (CLL) will have received the monoclonal antibodies, rituximab or alemtuzumab, as part of their therapy and both of these agents persist in the circulation for prolonged periods impairing vaccine responses.[8–10] These complexities must be considered in interpreting the recommendations for immunizations, which are summarized in Table 69.1.

Inactivated Vaccines (Nonlive Vaccines)

These agents pose less risk to the patient because they are incapable of replicating in the immunocompromised subject. Several of the inactivated vaccines used in immunocompromised patients are summarized below.

Pneumococcal Vaccines

Pneumococci are important causes of infection in patients with hematological malignancies, particularly B-cell malignancies such as CLL, Hodgkin lymphoma, and multiple myeloma. Early studies were performed with the polysaccharide-based vaccine (23-valent pneumococcal polysaccharide vaccine [PPSV23]) in multiple myeloma patients and less than 40% had a serological response to vaccination.[11] The response to PPSV23 in patients with Hodgkin lymphoma varies with the time when therapy is delivered. Patients immunized after chemo- and/or radiotherapy have a severe impairment of the antibody response.[12,13] In contrast, improved responses to PPSV23 are obtained if immunizations are performed before therapy is initiated.[13,14] In children with Hodgkin disease, immunogenicity is improved if immunizations are administered prior to splenectomy.[15] Repeated vaccinations with PPSV23 given before and after splenectomy in patients with Hodgkin lymphoma induced antibody responses and were not associated with serious side effects.[16]

Pneumococcal conjugate vaccines (PCVs) have also been studied in patients with hematological malignancies. Molrine and colleagues showed that a single dose of the seven-valent PCV (PCV7) gave suboptimal responses in patients being treated for Hodgkin disease.[17] CLL patients responded to PCV7 but with lower responses than seen in healthy controls and with decreased responses later in the disease course.[18] Administering a single dose of the 13-valent PCV (PCV13) early after the diagnosis in patients with CLL resulted in an immune response in only 58% of CLL patients compared to 100% of healthy controls.[19] Whether repeated doses would enhance the immune response has not been studied.

In a noncontrolled follow-up study to the study by Molrine and colleagues, Chan and colleagues showed that priming with PCV7 improved the subsequent booster response to the PPSV23 in patients with previously treated Hodgkin lymphoma.[20] This prime-boost strategy has been successfully used in patients with sickle cell anemia[21] and HIV infection. The booster dose of PPSV23 broadens the coverage of additional pneumococcal serotypes and is recommended by the Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (ACIP).[22,23] It is unclear how effective this strategy is in patients with other hematological malignancies. Immunization against pneumococcal infections is recommended as early as possible after diagnosis and preferably before therapy is initiated in patients with hematological malignancies.[24,25]

Haemophilus influenzae Type B Vaccine

Severe infections with *Haemophilus influenzae* type b (Hib) in cancer patients are quite rare. Immunization with a conjugated Hib vaccine resulted in lower antibody responses than in normal children, and a booster dose was ineffective. Longer duration and intensity of antileukemic chemotherapy were associated with a poorer response.[26,27] Children undergoing therapy for solid tumors also have lower-than-normal responses to vaccination with an Hib vaccine.[28]

Very limited data are available regarding the risk for severe Hib infections and the response to vaccination in adult patients with cancer. Patients with multiple myeloma have antibody responses to Hib conjugate vaccines comparable to those of healthy adults.[11] Molrine and colleagues showed that

TABLE 69.1 Recommendations for Immunizations in Cancer Patients

Vaccine	Recommendation	Comments
Pneumococcal conjugate vaccine	Yes	Lymphoma and CLL patients. Responses <6 months after rituximab unlikely to be effective.
Pneumococcal polysaccharide vaccine	Yes	Lymphoma and CLL patients. Preferably before initiation of chemotherapy. Could be given at least after priming with conjugate vaccine. Responses <6 months after rituximab unlikely to be effective.
Conjugated Hib	Yes	Children with cancer. Patients with Hodgkin disease. Preferably before initiation of chemotherapy.
Inactivated influenza vaccine	Yes	Seasonal to all cancer patients.
Varicella	Yes	Seronegative children and young adults in remission from malignant disease. Not during active chemo- or radiotherapy.
Zoster	No Possible	During active therapy. At least 3 months after active therapy but at least 12 months after rituximab.
MMR	Yes Individual consideration	Children with cancer not previously vaccinated. Not during active chemo- or radiotherapy. Seronegative adults depending on the local epidemiological situation. Not during active chemotherapy or radiotherapy.
Tetanus toxoid, diphtheria toxoid acellular pertussis, poliovirus	Yes	Children to complete primary schedule. Booster dose after finishing intensive chemotherapy can be considered to retain long-term immunity.

CLL, chronic lymphocytic leukemia; Hib, *Haemophilus influenzae* type b; MMR, measles, mumps, and rubella.

99% of patients with Hodgkin lymphoma in whom therapy had been discontinued responded to vaccination.[17] In contrast, patients with CLL respond rather poorly to Hib conjugate vaccine, with response rates around 50%.[29,30] It is preferable for vaccination to be performed before the start of therapy.

Influenza Vaccine

Patients with cancer were more likely to die during hospitalization for influenza, especially if elderly and with comorbid conditions. The morbidity in adult patients is highest in patients with hematological malignancies, especially those with acute leukemia. Patients with lung cancer had the highest influenza-related case-fatality rates among patients with solid tumors.[31] Influenza disease can also interrupt scheduled chemotherapy with a negative impact on the ultimate disease outcome. Earle showed that in patients with colorectal cancer, patients who were immunized had fewer chemotherapy interruptions and were more likely to survive 1 year.[32] Efficacy data in adult patients with solid tumors are limited. In a study of lung cancer patients, the vaccination response was similar to that of healthy controls.[33] Patients with breast cancer vaccinated during ongoing chemotherapy had significantly lower responses than healthy controls, but these patients responded better if vaccinated early rather than late after chemotherapy.[34]

Most studies show that adult patients with hematological malignancies respond poorly to influenza vaccination. In a study of patients with multiple myeloma, the response rate to one dose of vaccine was 19%.[11] Similar results have been seen in patients with lymphoma.[35] In a study of patients with non-Hodgkin lymphoma, 32 patients were vaccinated, half with ongoing therapy (type not specified) and half without therapy. The immune responses were similar between the groups, although the responses tended to be more robust in patients without ongoing therapy but lower when compared to healthy controls.[36] A larger study from the same group gave similar results after influenza immunization comparing previously treated (within 2 months of vaccination) with untreated patients and controls.[37]

It may be possible to improve responses by giving repeated doses. Adults with lymphoma receiving a two-dose influenza vaccine schedule showed responses of approximately 30% after one dose and approximately 45% after two doses.[38] In a study of patients with multiple myeloma, two doses gave good seroconversion and seroprotection rates against H1N1 and H3N2 serotypes (27% and 34% seroconversion after first dose; 63% and 54% after the second dose, respectively) while the responses to influenza B were less.[39] However, the two studies failed to show an enhanced response after the second dose in patients with various hematological malignancies[9] or CLL.[40] De Lavallade and colleagues reported results of two doses of an adjuvanted pandemic H1N1 vaccine in patients with chronic myeloid leukemia and B-cell malignancies compared to one dose given to healthy controls.[8] The study showed improved seroconversion rates after the second dose compared to the first dose (39–68% in B-lymphoid malignancies; 85–95% in chronic myeloid leukemia) although lower than in healthy controls. The response rates were better in patients with an interval from end of chemotherapy of at least 12 months. They also showed that influenza vaccination was able to induce a T-cell response in approximately 33% of patients compared to 50% in controls.[8]

Patients who have received rituximab as part of their therapy regimen respond very poorly to influenza vaccination for prolonged periods after the end of rituximab therapy. None of 67 lymphoma patients responded to adjuvanted H1N1 vaccine within the first 6 months after the conclusion of rituximab therapy.[10] Furthermore, in lymphoma patients who had completed a rituximab-containing regimen, the response to influenza vaccine was even further impaired if they had been treated with fludarabine.[41]

Studies of influenza vaccination in children with acute lymphoblastic leukemia (ALL) show that the proportion of children reaching "protective" antibody levels varied between 45% and 100% for the different influenza subtypes included in the vaccines.[42–45] A Cochrane review analyzing influenza vaccination in children with cancer summarized that immune responses were weaker in children receiving chemotherapy than in those who had completed chemotherapy and in

healthy controls.[46] In a study of children with solid tumors or lymphoma, who were given one or two doses of vaccine, the overall results regardless of chemotherapy were lower, with only 38% achieving protective antibody levels to all three influenza strains.[47] It has been reported that an antibody titer protective in healthy controls failed to prevent influenza in 24% of children with cancer, but the severity of the influenza infection may have been reduced by the vaccine.[33,48] Vaccination against pH1N1 was studied in a group of 54 children with different types of malignancies.[49] The seroconversion rate was 44%; significantly lower in univariate analysis in children with hematological malignancies compared to those with solid tumors and in children with ongoing chemotherapy. In multivariate analysis, children with ongoing chemotherapy had lower responses ($P = .05$) with a trend for lower responses in children with hematological malignancies ($P = .10$). One way to improve outcome would be to immunize with a vaccine containing higher doses of influenza antigen. A Phase I study was performed in children with ALL and showed safety but no difference in the immune responses, although the study was not powered to assess efficacy.[50]

Despite the lower serological responses in cancer patients, a systematic review showed that vaccinated cancer patients had a reduced risk for influenza-like illness,[51] and a Cochrane review found lower mortality in influenza-vaccinated patients but no difference in infection-related outcomes.[52] Yearly influenza vaccination with the trivalent or quadrivalent inactivated vaccine is recommended for cancer patients.[53,54] Despite these recommendations, the uptake of vaccination is limited and strongly influenced by the attitude of treating oncologists.[55] However, it must be recognized that the protection is likely to be low in those patients who are at highest risk of severe complications. Elting and colleagues reported that influenza in acute leukemia patients undergoing chemotherapy was commonly nosocomially acquired.[56] Immunization of family members and hospital staff is therefore recommended. Only limited data exist with the live attenuated vaccine given to mild to moderately immunocompromised children with cancer.[57] Currently live attenuated vaccine is not recommended in immunocompromised patients.

Tetanus Toxoid, Diphtheria Toxoid, and Inactivated Poliovirus Vaccine

The protection against tetanus, diphtheria, and poliovirus is frequently low in cancer patients undergoing chemotherapy. Hammarström and colleagues showed that 41% of nontransplanted acute leukemia patients were not protected against tetanus.[58] Risk factors for loss of immunity were ALL versus acute myelogenous leukemia, more advanced disease, and increasing age. Lymphoma patients are also likely to have deficient immunity. In children treated for cancer, the antibody titers against tetanus, diphtheria, and poliovirus were lower than in an age-matched healthy population.[59,60] The loss of specific immunity is related to the intensity of given chemotherapy. Children with high-risk ALL were more likely to become nonimmune to tetanus and diphtheria than were patients with low- or standard-risk ALL.[61] In contrast, Nordoy and colleagues reported that treatment of low-grade non-Hodgkin lymphoma patients with radioimmunotherapy did not influence specific immunity to tetanus.[62]

The responses to diphtheria toxoid and tetanus toxoid vaccinations in adult cancer patients have not been systematically studied. Most children respond well to vaccination after chemotherapy for malignancies.[60,63] However, children treated for high-risk ALL had poor responses (22% protected against tetanus; 56% against diphtheria).[61] Stenvik and colleagues reported on 14 children with leukemia who were given a booster dose of vaccine, with 12 of 14 responding.[64] No data exist regarding the vaccination of adults with cancer. Despite the absence of data, it seems logical to recommend an investigation of poliovirus immunity and booster immunization for cancer patients traveling to areas of the world that are still endemic for poliovirus. Inactivated poliovirus vaccine is the only vaccine that should be used in immunocompromised patients as paralytic disease can result in the immunocompromised and transmission of the live vaccine strain has occurred.[64] In the United States and in most countries in Europe, only inactivated polio vaccine is available.

Hepatitis B Virus

Hepatitis B virus (HBV) infection is a major cause of morbidity in many parts of the world. Several studies have been performed regarding the efficacy of HBV vaccination in children[65–71] and these results are summarized in Table 69.2. In general, these vaccines are safe and moderately immunogenic.

Other Nonlive (Nonreplicating) Vaccines

Vaccinations could be considered against pertussis although data are limited regarding if there is an increased risk for severe infection in cancer patients. Age-group–appropriate vaccination against meningococci should be performed according to national guidelines.[24,25] There are also vaccines for cancer patients living in certain areas of the world or traveling to these areas. These include hepatitis A vaccine and vaccines against tickborne encephalitis and Japanese encephalitis. Although there are very limited data regarding serological responses to these vaccines, the risk for significant side effects should be similar to the normal population as they are nonreplicating agents.

The number of long-term survivors after cancer treatment is increasing. These patients might be at increased risk for late complications driven by human papillomavirus infections. The immune response to existing papillomavirus vaccines have not been systematically studied in cancer patients although patients have been vaccinated as part of their age-appropriate routine schedules.[72] Despite this lack of information human papillomavirus vaccination should be considered, especially in survivors after cancer during childhood to be given at the appropriate and recommended age.[24,73]

Live Vaccines

Varicella Vaccine

Primary varicella has previously caused high mortality in children with cancer. With the increased use of acyclovir prophylaxis and treatment, the risk for severe disease seems to have decreased. A recent analysis estimated the mortality to be 0.057% with the mortality varying between regions (0.027% in North America, 0.041% in Asia, and 0.08% in Europe).[74] The existing vaccine is live, attenuated, and based on the Oka strain.[75] The vaccine was shown to be effective and safe in children with leukemia who were in remission,[76] but it should be recognized that the type of leukemia therapy was less intensive in the 1980s. The rate of varicella infection in vaccinees was 8%, but all infected children had mild disease.[76] The frequency of side effects from the vaccine is low, and breakthrough vaccine disease can usually be treated effectively with acyclovir.[76,77] However, there have been fatal vaccine-associated infections reported.[74] Cakir and colleagues investigated single dose versus double dose of varicella vaccine and found a much higher rate of seroconversion (75% vs 29%) and better persistence of specific antibodies in patients receiving a double dose.[78] Furthermore, reinfections in previously vaccinated

TABLE 69.2 Results of Hepatitis B Virus Recombinant Vaccine Studies in Children With Malignancies

Diagnoses	Vaccine	On/Off Therapy	No. of Patients	Vaccine Dose	No. of Doses	Schedule	Booster	Seroconversion Rate	Reference
Leukemia	Engerix-B GenHevac	Maintenance Maintenance	50 44	20 µg 20 µg	3 3	0, 1, 6 mo 0, 1, 2 mo	12 mo 6 mo	32.1% 38.6%	70 70
ALL	Engerix-B	Induction	94	20 µg <10 y 40 µg >10 y	3	0, 1, 2 mo	12 mo	19.5% (10.5% protected)	65
ALL	Engerix-B	Induction	111	20 µg <10 y	5	0,1, 2, 3, 4 mo	12 mo	29.7% (18.9% protective)	66
Lymphoma	GenHevac	Induction	23	40 µg	3	0, 1, 2 mo	12 mo	48% after 3 days; 74% after 4 days	67
Solid tumors	GenHevac	Induction	47	40 µg	3	0, 1, 2 mo	12 mo	77% after 3 days; 94.% after 4 days	67
Leukemia	GenHevac	Maintenance	48	40 µg	3	0,1, 2 mo	12 mo	88% after 3 days; 90% after 4 days	67
Leukemia/ lymphoma	Engerix-B	No maintenance	36 18	20 µg <10 y 40 µg >10 y	4	0, 1, 2, 6 mo	88%	68	
Leukemia	Engerix-B	On Off	64 58	20 µg	3	0, 1, 2 mo	6 mo	26% 88%	69
Leukemia/ lymphoma	Engerix-B	On	60	40 µg	3	0, 1, 2 mo	No	71%	71

ALL, acute lymphoblastic leukemia.

children with cancer have been reported[79] and might become an increasing problem in the future with more adult cancer and leukemia patients having been vaccinated during childhood. The risk for herpes zoster after vaccination is lower than after natural varicella disease.[80,81] In a small randomized study, varicella vaccine was given to children with newly diagnosed cancer before starting chemotherapy. There was a high rate of seroconversion and no severe side effects were found, but the number of included patients was low.[82]

Household exposure to varicella is associated with more-severe varicella disease. An option is to immunize healthy seronegative family members when the child with cancer is undergoing intensive therapy and a live vaccine cannot be given. Diaz and colleagues showed that the attenuated varicella vaccine virus cannot be isolated from oropharyngeal secretions of the immunized siblings.[83] None of the children with cancer showed clinical or serologic evidence of vaccine virus transmission. Immunization with the varicella vaccine is indicated in seronegative patients with cancer when the cancer chemotherapy schedule allows safe immunization, but it would generally not be indicated to interrupt maintenance therapy to allow vaccination considering the relatively low risk for severe and fatal varicella with available antiviral therapy.[74]

Zoster Vaccine

Most cancer patients are elderly and develop zoster more commonly than in age-matched healthy controls.[84] Current recommendations are not to vaccinate during cancer therapy. However, Naidus and colleagues vaccinated 31 patients with hematological malignancies with the zoster vaccine.[85] Eighteen of these patients had previously received cancer therapy and two had ongoing therapy at the time of vaccination; no severe side effects were reported. A case report of a death resulting from dissemination of a vaccine strain occurring 4 years after an autologous stem cell transplant in a patient with untreated lymphoma relapse clearly shows that care is needed in administering live varicella vaccine to immunosuppressed patients.[86] On the other hand, zoster vaccine given before diagnosis and treatment of cancer has a protective effect for cancer patients after chemotherapy.[87]

There are two new inactivated varicella-zoster vaccines in development that offer a solution to the live vaccine dilemma, a subunit vaccine and a heat-inactivated vaccine. The subunit vaccine was reported to be effective in older healthy adults, but has not been studied in elderly cancer patients.[3] A Phase II study with four doses of the heat-treated vaccine in patients with solid tumors and hematological malignancies demonstrated immunogenicity without important side effects.[88] A Phase III study has been fully recruited, but data are not yet available.

Measles, Mumps, and Rubella

Measles disease in cancer patients has a high mortality. Kaplan and colleagues reviewed 27 published cases, among whom 20 (74%) developed pneumonitis and eight (30%) died.[89] The clinical consequences of rubella and mumps infections in cancer patients appear to be of less significance.

Loss of immunity to measles is common in children previously vaccinated with measles, mumps, and rubella (MMR) and then treated for leukemia and other cancers.[61,90,91] Risk factors for loss of measles immunity include younger age and female gender in leukemia patients[61] and younger age in other childhood malignancies.[91] Immunization with the live attenuated vaccine has been contraindicated because of the risk of severe side effects in cancer patients undergoing chemotherapy. Koochakzadeh and colleagues studied MMR vaccination

3 to 12 months after cessation of therapy and reported that it was safe and gave high response rates to mumps (80–100%) but lower (50–71%) to rubella and measles (41–63%).[92] Immunization with live attenuated MMR vaccine should be considered in seronegative cancer patients who are not receiving active chemotherapy and in epidemiologic situations when the risk for measles is high.

Other Live Vaccines

Very limited data exist on immunization with bacille Calmette-Guérin (BCG), yellow fever, or rotavirus vaccines in cancer patients. The use of these live vaccines is not recommended during active cancer therapy.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION PATIENTS

In allogeneic hematopoietic stem cell transplant (HSCT) recipients, four components combine in the production of the immunodeficient state of the patient: (1) the immunosuppressive activity of the primary disease and treatment, (2) the chemotherapy and radiotherapy used to eradicate the host's immune system, (3) the immunologic reactivity between the graft and the host (graft-versus-host disease [GVHD]), and (4) the immunosuppressive therapy given after transplantation to prevent or treat GVHD. The technology of allogeneic HSCT is developing rapidly with the introduction of new stem cell sources (peripheral blood stem cells, cord blood cells, and mesenchymal stem cells), new donor categories (haploidentical donors, unrelated donors), and innovative conditioning regimens using the graft-versus-malignancy reactivity from the donor grafts rather than the chemo- and radiotherapy to clear the recipient's immune system and allow engraftment of the donor's immune system.

Immunity to infectious agents is transferred by the graft and can be detected in the patient early after the HSCT.[93–98] The transferred immunity is usually of a finite duration and over time an increasing number of patients becomes susceptible to infections because of declining immunity to tetanus,[95,99,100] poliovirus,[99,101] and measles.[102,103] The immune status of the donor is important for the short-term transfer of immunity and can be boosted by immunizing the donor before transplantation[97,104–106] combined with early posttransplantation vaccination of the recipient with inactivated polysaccharide–protein conjugate vaccines or protein-based vaccines.[107–109]

The transplantation period can be divided into three distinct phases, each with its unique combination of risks and benefits of immunization. The early phase is characterized by neutropenia, and the characteristic infections are caused by bacteria and fungi. Other infections during this phase are caused by respiratory viruses and herpes simplex virus. The only window of opportunity to vaccinate either the donor or the patient occurs before HSCT. The currently available vaccines that could be of clinical importance are influenza and PCV. The risk for infections during the period from 1 to 6 months after HSCT is strongly influenced by the presence of GVHD and the typical infections during this period are caused by CMV and mold, but influenza and pneumococcal infections can also be important.[6,110,111] During the late phase, infections caused by pneumococci, influenza, and varicella-zoster virus (VZV) are important and potentially preventable by vaccination.[6,110–112] With the reappearance of measles, immunity to this virus is of increased importance. However, long-term protection against other infectious agents such as tetanus, diphtheria, poliovirus, and vaccines to protect travelers is also of importance.

The current recommendations do not take the different types of allogeneic HSCT into account even though we know that the speed of immune reconstitution is different between cord blood graft recipients and recipients of bone marrow or peripheral blood grafts. There is a paucity of studies in the different patient groups. However, certain factors are important when deciding on the timing and schedule of vaccinations. One such factor is the speed and completeness of immune reconstitution. It has been reported that the number of $CD4^+$ cells can be used as a marker for when vaccinations can be started with better responses in patients with $CD4^+$ cells above 200/μL.[113,114] Another important factor to consider is whether patients have GVHD. Patients with GVHD clearly should not be vaccinated with live vaccines because of the risk for severe side effects; additionally, patients with severe GVHD who are receiving intensive immunosuppression should have vaccinations deferred. Existing data support that patients with GVHD have lower response rates or more rapidly lose immunity to some vaccines,[115–119] but there are also studies showing no negative effect of GVHD.[120,121] Patients with GVHD are at risk for severe infections and could benefit from vaccination. The risk for activation of GVHD seems to be low based on the reported safety data from prospective vaccination studies[7,116,122] and clinical experience, can be triggered with adjuvanted vaccines.[123]

Table 69.3 summarizes the recommendations for allogeneic HSCT recipients and is in part based on recently published Infectious Diseases Society of America recommendations.[24,25]

Nonlive Vaccines

Pneumococcal Vaccines

Pneumococcal infections can be severe in HSCT patients especially in patients with chronic GVHD.[124–126] Immunization with the PPSV23 can elicit antibody responses from 6 months after HSCT in patients without GVHD, but was shown to be ineffective in patients with chronic GVHD.[105,115,127,128] In particular, the specific immunoglobulin (Ig) G_2 responses have been poor.[115,129] Children given PPSV23 developed short-lived responses and low avidity,[130] in contrast to adults.[131] Thus, responses to vaccination with PPSV23 are suboptimal in many patients.

PCV7 has been evaluated in several studies in HSCT patients including those with chronic GVHD. Kumar and colleagues conducted a randomized study of one dose of either PPSV23 or PCV7 given to donors before and 6 months after HSCT. PCV7 improved the likelihood of an immune response compared to PPSV23, but neither approach was optimally effective.[132] Molrine and colleagues performed a randomized study with the PCV7 comparing pretransplant vaccination of patients and their donors with no vaccination given pretransplantation. All patients were given three doses of the vaccine at 3, 6, and 12 months after transplantation. The majority of patients (72–100% for the different serotypes) developed protective antibody levels at 12 months after HSCT.[133] There was also an improved response (67% vs 36%) to the first dose of PCV7 when compared to the polysaccharide vaccine. Cordonnier and colleagues compared early (starting at 3 months) with late (starting at 9 months) vaccination with three doses of PCV7 and showed that good protection could be achieved as early as 3 months after HSCT.[116] Furthermore, PCV7 increased the functional antibodies assessed by an opsonophagocytic assay.[134] However, there was a tendency for patients who were immunized early to have a shorter duration of immunity and for patients with chronic GVHD to benefit from a booster dose.[116] It was also shown that a dose of PPSV23 given 9 months after the first PCV7 dose increased the response rates and extended the serotype coverage.[135] Studies also have been

TABLE 69.3 Recommendations for Immunizations in Stem Cell Transplant Patients

Vaccine	Recommendation	Comments
Tetanus toxoid + diphtheria toxoid	Yes	Three doses (DT) starting at 6–12 mo after transplantation
Inactive influenza	Yes	Seasonal, beginning at 4–6 mo after transplantation depending on season
Inactivated poliovirus	Yes	Three doses starting at 6–12 mo after transplantation
Conjugated Hib	Yes	Three doses starting at 6–12 mo after transplantation
Pneumococcal conjugate	Yes	Three doses started at 3–6 mo after transplantation; booster at 12 mo in patients with chronic GVHD
Pneumococcal polysaccharide	Yes	Booster at 12 months in patients without GVHD
Acellular pertussis	Yes	Children <7 y started at 6–12 mo after transplantation
Hepatitis B virus	Yes	In countries where it is recommended to the general population starting at 6 mo after transplantation
Papillomavirus	Can be considered	As in the general population starting at 6 mo after transplantation; three doses
Meningococcal conjugate	Can be considered	As in the general population starting at 6 mo after transplantation; two doses
MMR	Individual consideration	Children and seronegative adults. Not before 24 mo after HSCT; not to be given to patients with GVHD
Varicella vaccine	Individual consideration	Seronegative patients, not before 24 mo after BMT; not to be given in patients with GVHD
Zoster vaccine	Not recommended	

BMT, bone marrow transplantation; DT, diphtheria and tetanus toxoids; GVHD, graft-versus-host disease; Hib, *Haemophilus influenzae* type b; HSCT, hematopoietic stem cell transplantation; MMR, measles, mumps, and rubella.
Modified from Ljungman P, Cordonnier C, Einsele H, et al. Vaccination of hematopoietic cell transplant recipients. Bone Marrow Transplant. *2009;44:521–526.*

performed in children showing good immune responses.[114,136] It also has been shown that the degree of immunological immune reconstitution influenced the immune response, with CD4 cells greater than 200 and normal immunoglobulin levels predicting an improved response.[114]

The current recommendations are to start vaccination with three doses of PCV13 at 3 to 4 months after HSCT followed by either a PPSV23 dose in patients without chronic GVHD or PCV13 in patients with chronic GVHD.[24,25] A prospective, but uncontrolled, trial studied the effect of four doses of the PCV13 and showed very strong immune responses to the fourth dose although associated with more side effects.[122] In this study, there was no beneficial effect of a subsequent dose of PPSV23, but this dose was probably given too early after the fourth PCV dose. It is also most likely necessary to give additional pneumococcal vaccine doses during follow-up to maintain immunity to pneumococci but the exact schedule needs to be defined by further studies.[137] There is also a PCV10 vaccine available but this has not been studied in HSCT recipients.

Haemophilus influenzae Type B Vaccine

Immunization with Hib conjugate vaccines can elicit protective immune responses.[105,128,138] It has been reported that time after transplantation is important for the immune response to Hib vaccine in transplanted children.[114,139] A good immune response could be elicited when the donor was immunized before transplantation and the recipient at 3 months after transplantation.[140] Immunization with a Hib vaccine is indicated in all allogeneic HSCT recipients.[24,25]

Influenza Vaccine

Influenza A and B infections can be severe and life-threatening in HSCT recipients[111,141,142] and can occur up to several years after HSCT.[6,141] The time after HSCT is important for vaccine efficacy, with patients vaccinated more than 6 months after HSCT having better responses in most,[8,117,121,143] but not all, studies.[7]

Ambati and colleagues conducted a prospective, randomized study of the impact of pretransplantation immunization of the donor or the recipient followed by influenza vaccination of the patient at 6 months after HSCT and showed an impact on antibody levels by pretransplantation vaccination of the recipient. It is, however, possible that an earlier post-transplantation dose would have increased the immune response.[109] Machado and colleagues found that influenza vaccination performed at least 6 months after stem cell transplantation (SCT) had an 80% efficacy in preventing influenza.[144] Furthermore, T-cell responses after influenza vaccination can be elicited already at 3 to 4 months after HSCT.[145,146]

During and after the H1N1 pandemic, several studies were performed in HSCT recipients. Issa and colleagues studied 82 patients vaccinated a median of 19 months after transplantation (range: 2.5 to 94 months) with one dose of unadjuvanted influenza vaccine.[121] Protective titers were found in 51% of the patients with better responses seen in patients vaccinated later after HSCT (odds ratio [OR], 1.79/year) and poorer responses if the patient had received rituximab during the last year.[121] There was no effect by conditioning or chronic GVHD. Engelhard and colleagues studied 55 patients vaccinated a median of 27 months after HSCT (1–290 months) with two doses of an AS03-adjuvanted vaccine (Pandemrix, GlaxoSmithKline). The protection rate after two doses was 48.7% and the seroconversion rate 41.9%.[7] Factors influencing seroconversion were the absolute lymphocyte count (OR, 3.04) and donor type (OR, 14.0) with better responses in patients receiving grafts from human leukocyte antigen–identical donors but with no effect of time of influenza vaccination after HSCT.[7] De Lavaillade and colleagues studied 26 allogeneic HSCT recipients receiving two doses of the same adjuvanted vaccine

at a median time from HSCT of 39 months (range, 6 to 127 months).[8] The seroprotection rates were 45% after one and 73% after two doses, but significantly lower than found in healthy controls. The only factor influencing the response was time after HSCT. T-cell responses to influenza were observed in 40% of the patients. Dhédin and colleagues reported comparable immune responses after two doses of adjuvanted pH1N1 vaccine to those seen after natural infection when analyzed 6 months after vaccination.[123] All four studies reported that the vaccines were safe. Dhédin and colleagues reported, however, that four patients developed worsened chronic GVHD.[123]

Influenza vaccination with the inactivated vaccine is recommended for all allogeneic HSCT recipients starting at 6 months after HSCT and starting as early as 4 months after HSCT during a community outbreak. Children 6 months to 8 years of age should be given a second dose. The live attenuated influenza vaccine should not be used in HSCT recipients. Another recommended strategy is to immunize family members and hospital staff, thereby reducing the risk for transmission of the infection.[24,25,147]

Hepatitis B Virus Vaccine

Severe primary HBV infections are rare after HSCT unless an HBV-positive donor is used for a seronegative recipient. Loss of preexisting immunity is quite common and can result in virus reemergence with or without signs of hepatitis. Immunization early after HSCT is likely to be ineffective unless the donor is immunized. Jaffe and colleagues based start of vaccination on grade of immune competence.[118] Of 292 patients who were vaccinated, 187 (64%) seroconverted, a rate lower than in age-matched controls. Older age and history of chronic GVHD were associated with lower responses. In another study, the rate of seroconversion after vaccination was higher when the patient, the donor, or both had been vaccinated before HSCT.[148] The immunization of the marrow donor allows a transfer of immunity to the recipient[104,106] and can be long lasting in at least 50% of patients.[149] Case reports further suggest that transfer of immunity from an HBV-immune donor can clear the virus from an HBV antigen and DNA-positive recipient.[150,151] Another important issue is to prevent HBV seroreversion in seropositive individuals. Vaccinated patients are significantly less likely to experience HBV reactivation compared to unvaccinated controls.[152,153] HBV seropositive patients should receive antiviral prophylaxis until vaccinations can be given after HSCT to prevent seroreversion and viral replication. Vaccination is recommended after allogeneic HSCT.[24,25]

Tetanus Toxoid, Diphtheria Toxoid, and Inactivated Poliovirus Vaccines

Several studies of immunization with these vaccines have been published.[99–101,120,154] A new primary schedule with repeated doses of these vaccines is needed to obtain stable protective immunity.[99–101,120,154] Gerritsen and colleagues immunized children before bone marrow transplantation (BMT) followed by revaccination as early as 6 weeks after BMT. Thirty percent of the patients responded to early immunization.[107] The inactivated poliovirus vaccine should be used. It has been shown that robust and lasting immune responses to poliovirus vaccine can be obtained when immunizations are started at 6 months after HSCT.[154] It was shown that more than 90% of allogeneic HSCT recipients remained protected against poliovirus at 10 years after vaccination with a three-dose schedule.[155] The vaccination of allogeneic HSCT recipients with three doses of tetanus toxoid, diphtheria toxoid, and inactivated poliovirus vaccine is recommended.[24,25] Because posttransplantation patients should be viewed as "never vaccinated" they should receive full doses of toxoids, DT vaccine, and not with the vaccines normally used for booster doses to adults.

HSCT patients might be vulnerable to pertussis although the documentation for severe infections is limited.[156,157] In young children, it is logical to vaccinate against pertussis together with diphtheria, and tetanus since many available vaccines include the pertussis component (diphtheria, tetanus, and acellular pertussis [DTaP]) as well as the Hib and inactivated polio vaccine components. The immune response to tetanus, diphtheria, and pertussis (Tdap) is poorer than to DTaP.[158] Pertussis vaccination can be considered in older children and adults but then it is most likely more effective to use a vaccine with higher pertussis content (P) although these vaccines are not licensed for adults.[24,25]

Human Papillomavirus Vaccine

HSCT patients are prone to develop papillomavirus complications such as cervical dysplasia.[159] The vaccines are safe and efficient in immunocompetent individuals. No study has been performed to show efficacy in HSCT patients, but it is logical to vaccinate HSCT recipients at similar age as in the general population. There are no data supporting vaccination of already human papillomavirus–infected HSCT recipients, but this could be a topic for future studies.

Cytomegalovirus Vaccine

CMV is one of the most important pathogens encountered by patients after HSCT. Vaccines based on new vaccine technologies, such as DNA plasmid–based vaccines, subcomponent vaccines, and vaccines using other viral vectors, are currently in clinical development. A Phase II study of a DNA plasmid vaccine containing plasmids for glycoprotein B and pp65 elicited T-cell responses and reduced the rate of CMV DNAemia[160,161] and a Phase III study is ongoing.

Other Nonlive Vaccines

Vaccination with a meningococcal polysaccharide vaccine can elicit good responses in HSCT recipients against both serogroups A and C.[162] Mahler and colleagues studied the tetravalent conjugated meningococcal vaccine in 46 patients, mainly children, at a median of more than 2 years after HSCT.[163] The response rate to the different serotypes varied between 30% and 52%. Seven of 46 patients (15%) responded to all serotypes and 16 (35%) did not respond to any serotype. Sixteen patients received a second dose and eight (50%) of the 16 responded to responses against all serotypes. Meningococcal vaccination is recommended to older children and teenagers after allogeneic HSCT[24,25] but can be considered in other age groups in countries were vaccination for the general population is recommended.[164]

There are also vaccines for HSCT patients living in certain areas of the world or traveling to these areas. These include vaccines against hepatitis A, tickborne encephalitis, and Japanese encephalitis. There are very limited data regarding serological responses to these vaccines but the risk for significant side effects should be similar to the normal population.

Live Vaccines

Varicella Vaccine

Primary VZV infections can be very severe after HSCT. The existing vaccine is live and attenuated and should not

be used early in the posttransplantation period. A seronegative patient should, if possible, be immunized before transplantation, providing that enough time can elapse from the vaccination to the transplantation procedure. No data exist regarding the interval needed between vaccination and start of conditioning but rash after vaccination has been noted up to 60 days after vaccination in patients with cancer undergoing chemotherapy and in children with acute leukemia in remission.[82,165] Children with acute leukemia who have been immunized with the varicella vaccine have subsequently undergone allogeneic HSCT. The care of varicella-seronegative HSCT recipients is frequently problematic; exposure to varicella-infected individuals is not uncommon and either prophylactic zoster immunoglobulin or antiviral prophylaxis is needed. Vaccination of seronegative family members of allogeneic HSCT recipients is recommended.[164] A few uncontrolled studies have been performed and reported that varicella vaccination is safe and can result in seroconversion if performed more than 2 years after HSCT in patients without chronic GVHD and without ongoing immunosuppression.[113,166,167]

A high proportion of HSCT patients develop herpes zoster that occasionally becomes severe. Redman and colleagues used heat-inactivated varicella vaccine and showed that immunization conferred no reduction in the risk but a reduced severity of the herpes zoster.[168] Inactivated varicella vaccines are currently in development. However, the immune response in allogeneic HSCT recipients was poor in a Phase II study with a heat-treated vaccine.[88] Issa and colleagues gave one dose of live zoster vaccine to 58 allogeneic HSCT recipients and did not note any significant side effects.[169] Because of the proven efficacy and safety of acyclovir prophylaxis,[170] vaccination with the live zoster vaccine is not recommended during at least the first 2 years after HSCT.

Measles, Mumps, and Rubella Vaccine

With the return of measles in many countries, protection against this infection is important because a majority of allogeneic HSCT patients will become seronegative to measles during extended follow-up.[103,171,172] The loss of immunity is more rapid in patients previously vaccinated against measles compared to those having experienced natural infection.[172] There are documented cases of fatal measles in BMT recipients.[89,173] In an outbreak in Brazil, one of eight patients with measles developed interstitial pneumonia but all survived.[171] Immunization can only be considered in a patient without chronic GVHD or without ongoing immunosuppression. Existing data indicate that measles vaccine can be given safely at 2 years after HSCT.[102,174] During the outbreak in Brazil, patients were vaccinated at 1 year after HSCT without serious side effects.[175] The reported effect of vaccination varies between different studies with a seemingly higher response rate in adults than in children.[102,130,175,176]

Rubella vaccine could be indicated in female patients who have retained the potential for becoming pregnant. Existing data indicate that rubella vaccine can be given without severe side effects at 2 years after BMT in patients without chronic GVHD or without ongoing immunosuppression.[102] The effectiveness of the vaccine is high.[102,176] The risk for severe infections with mumps virus in HSCT recipients is likely to be very low, although a case report of a fatal infection in a HSCT recipient has been published.[177] Furthermore, the efficacy of mumps vaccination to induce long-lasting serological immunity is rather poor.[176] Vaccination with MMR is recommended in children and seronegative adults. A second dose of MMR is recommended in children younger than 9 years old.[24,25]

Other Live Vaccines

Other vaccines that can be considered in allogeneic HSCT recipients are BCG and yellow fever. The possible benefits must be weighed against the risk for side effects. Rio and colleagues have reported three patients who were immunized with yellow fever vaccine at 5 years after HSCT without severe side effects[178] and this experience has been expanded to 25 patients without any serious side effects (B. Rio, personal communication, 2001). Immunization could be considered in patients, who live in or must visit areas where yellow fever is endemic at least 2 years after HSCT and if the patient is without GVHD and without ongoing immunosuppression. The BCG vaccine can cause severe infections in patients with depressed T-cell function and is not recommended in HSCT recipients.[24,25]

Autologous Hematopoietic Stem Cell Transplantation Recipients

In autologous HSCT recipients, the immune regeneration is faster than after allogeneic HSCT. Autologous HSCT patients are usually not prone to severe vaccine preventable infections during the early phase after transplant. Several studies have shown that autologous transplant recipients will also lose protective immunity to tetanus, poliovirus, and measles during a long-term follow-up.[101,179–182] Many patients undergoing autologous HSCT for lymphoma will have received rituximab, which negatively influences the responses to vaccination.[8,9]

Nonlive Vaccines

Influenza Vaccine. Influenza virus vaccination is recommended starting at 4 to 6 months after autologous HSCT.[24,25] However, the response is likely to be suboptimal early after transplantation.[7,117,143] It has been shown that rituximab blocks the immune response to influenza vaccine for at least 6 months after HSCT and probably longer in many patients.[9,10,41] No data exist with the live attenuated vaccine since it is contraindicated in this population.

Pneumococcal Vaccine and Conjugated *Haemophilus influenzae* Type B Vaccine. Autologous HSCT patients are less prone than allogeneic patients to develop severe infections with Hib or pneumococci and the infections are more likely to occur early after the transplantation when the response to immunization is poor. The response to a single dose of PPSV23 is poor regardless of the stem cell source,[105,183] and remains decreased compared to controls for several years after transplantation for lymphoma.[182] Vaccination with the PCV7 was shown to be effective and induced protective antibody levels in more than 60% of the patients.[184] Vaccination before stem cell harvest resulted in significantly higher antibody levels at all studied time points up to 12 months after HSCT. Vaccination with PCV13 is recommended starting at 3 to 6 months after autologous HSCT.[24,25] It is likely that treatment with rituximab will decrease the responses, but it has not been systematically studied.

Tetanus Toxoid, Diphtheria Toxoid, Pertussis, and Poliovirus Vaccine. Autologous HSCT recipients have an increased risk compared to the normal population to lose protective immunity to poliovirus,[180,182] diphtheria,[182] and tetanus.[179] Reimmunization with repeated doses of inactivated poliovirus vaccine, diphtheria toxoid, and tetanus toxoid effectively restores protective immunity.[101,180,182,183] There was no difference in response to tetanus toxoid in autologous bone marrow or peripheral blood stem cell graft recipients.[183] The immune

response early after the autologous HSCT can be improved by immunizing the patient before the stem cell harvest followed by tetanus toxoid given repeatedly after the autologous HSCT.[185] Vaccination with reduced-dose pertussis vaccine (Tdap) failed to induce immune responses in most autologous HSCT recipients.[186] Immunization with tetanus toxoid, diphtheria toxoid, and inactivated poliovirus is recommended after autologous HSCT.[24,25] Vaccines included pertussis antigen DTaP is recommended in children.

Live Attenuated Vaccines

Measles, Mumps, and Rubella Vaccine. Individuals who have been immunized against measles before autologous HSCT frequently become seronegative during follow-up, whereas patients having experienced natural measles infection usually remain immune.[181] The risk for side effects after immunization seems to be low if the vaccine is administered later after transplantation.[181] MMR vaccination is recommended for children and seronegative autologous patients not earlier than 24 months after HSCT.[24,25] Two doses are recommended in children younger than age 9 years.

Varicella-Zoster Virus Vaccine. In seropositive patients undergoing autologous HSCT for lymphoma, a randomized study was performed with heat-inactivated varicella vaccine given in four doses: within 30 days of vaccination, and 30, 60, and 90 days after the first vaccination. During the 12 months posttransplantation, the rate of zoster was 13% in vaccinees and 33% in unvaccinated patients, giving an efficacy of 61%.[187] Sasadeusz and colleagues gave one or two doses of the live varicella vaccine 4.5 to 6 months after HSCT and showed that the immune responses were rather poor but no severe side effects occurred.[188] Issa and colleagues vaccinated 52 autologous HSCT recipients with the live zoster vaccine around 2 years after transplantation and did not note any significant side effects.[169] There is, however, one death reported with the vaccine strain 4 years after autologous HSCT in patient with untreated non-Hodgkin lymphoma relapse.[86] The safety of live varicella or varicella-zoster vaccines after autologous HSCT must be balanced against other options such as acyclovir/valacyclovir prophylaxis or the potential receipt of the inactivated varicella vaccine when it is available.

Two studies have reported on inactivated vaccines in development. Stadtmauer and colleagues reported on a Phase I/II placebo-controlled study in which 121 adult patients were vaccinated with three doses of an adjuvanted subunit varicella vaccine.[189] Patients receiving the vaccine had improved $CD4^+$ and antibody responses compared to those given placebo. Mullane and colleagues studied four doses of a heat-inactivated vaccine. The vaccine was able to induce both antibody and cell-mediated immune responses.[88] Phase III studies are ongoing.

SOLID-ORGAN TRANSPLANT RECIPIENTS

The need for immunization in solid-organ transplant (SOT) recipients can arise from three factors, each causing a suppression of the immune system: the immunosuppressive activity of the underlying disease (e.g., chronic renal failure), rejection of the organ graft, and the immunosuppressive therapy given after the transplantation. It is recommended that immunizations be given to candidates awaiting transplantation as the patient is more likely to respond to the vaccine pretransplantation than after the transplantation.[24,25,190] Children awaiting transplantation should complete vaccination schedules appropriate for their age.[24,25]

The optimal time to begin vaccine administration after transplant is not well defined. The immunosuppressive therapy is often most intense during first couple of months and likely will influence the immune response to vaccination. However, this has not been well studied. Table 69.4 summarizes the recommendations.

Nonlive Vaccines

Pneumococci and *Haemophilus influenzae* Type B

Both transplantation candidates and SOT recipients are at an increased risk for invasive pneumococcal infection.[191] Protective titers can be attained after PPSV23 in most candidates,[192] although these titers can wane within 2 years.[193] Children, who are candidates for SOT, should receive pneumococcal vaccine and vaccine against Hib according to their age-appropriate schedules.

There have been conflicting reports regarding the efficacy of the PPSV23 after transplantation. In some studies it was reported to be similar to that in healthy controls after liver,[194] heart,[194,195] and renal transplantation.[196] In other studies, the antibody responses were reported to be suppressed after heart[197] and liver transplantation.[198] Furthermore, the antibody levels declined faster than in controls.[199] Kumar and colleagues conducted a double-blind, randomized study of conjugate and a polysaccharide pneumococcal vaccine in adult renal transplant recipients[200] and found no clear differences in immune responses between the two vaccines. At the 3-year follow-up the antibody concentration declined significantly in both groups.[201] However, adult liver transplant recipients did not have an enhancement in response to PPSV23 when they received a prior dose of PCV7. Similar results were seen in adult renal transplant recipients.[202] Barton and colleagues found that two doses of PCV7 was enough to give twofold or more titer increases in all types of patients, but heart and lung recipients appeared to benefit from a third PCV7 dose.[203] The PPSV23 dose gave significantly higher antibody levels to some PCV7 serotypes. Another study using two doses of PCV7 followed by PPSV23 in children found that rises in serotype-specific antibody occurred after the first dose of PCV7, although at lower titers than in controls.[204] The ACIP recommends one dose of PCV13 followed by a dose of PPSV23 at least 8 weeks later to immunocompromised patients including SOT patients ("prime-boost" strategy).[22,23]

Influenza

Influenza can cause severe infections in SOT patients. In one survey of patients infected with the H1N1 pandemic virus, the risk for pneumonia was reported to be 32% and the mortality 4%.[4] The reported results of immunization vary with the age of the patients, time after transplant, and type of immunosuppression given. Adults recipients of renal,[205] liver,[206,207] heart,[195,208] and lung transplantation[209] have poorer antibody responses than immunocompetent controls. Similar results were found with the pandemic H1N1 vaccine.[210,211] The responses were reported to be better in patients awaiting liver transplantation than in patients after the transplantation.[212] Some but not all studies have noted increased responses with repeat doses of influenza vaccine.[207] In contrast, studies showed normal responses in children after renal[213–215] and liver transplantation.[216] One study showed that the frequency of influenza-specific antibody-secreting cells in peripheral blood was significantly reduced in stable transplant recipients compared to healthy controls.[217]

The immunosuppression given posttransplantation influences the response. In lung transplant recipients, mycophenolate mofetil was associated with a poorer response and

TABLE 69.4 Recommendations for Immunizations in Solid-Organ Transplant Recipients

Vaccine	Recommendations for		Comments
	Candidates	Recipients	
NONLIVE VACCINES			
Pneumococcal polysaccharide	Yes	Yes	Children >2 y and adults
Pneumococcal conjugate	Yes	Yes	Children and adults
Conjugated Hib	Complete schedule	As in the normal population	
Influenza	Yes	Yes	Adults and children; annually
Hepatitis A virus	Yes	Yes	Adults and children undergoing liver transplantation
Hepatitis B virus	Yes	Yes	Adults and children
Inactivated poliovirus	Complete schedule	Complete schedule	Boosters can be considered posttransplantation
Tetanus and diphtheria toxoid	Complete schedule (children)	Complete schedule (children)	Boosters can be considered posttransplantation
Acellular pertussis	Yes	Older children and adults	
Papillomavirus	Yes	Yes	As in the general population
Meningococcal	Yes	Yes	As in the general population
LIVE VACCINES			
MMR	Complete schedule	No	
Varicella	Yes	No	Before transplantation in seronegative patients
Zoster	Age dependent	No	

Hib, *Haemophilus influenzae* type b; MMR, measles, mumps, and rubella.
Modified from Avery RK, Ljungman P. Prophylactic measures in the solid-organ recipient before transplantation. Clin Infect Dis. *2001;33(Suppl 1):S15–S21; and Danzinger-Isakov L, Kumar D. AST Infectious Diseases Community of Practice. Guidelines for vaccination of solid organ transplant candidates and recipients.* Am J Transplant. *2009;9(Suppl 4):S258–S262.*

sirolimus with a better response.[209] It has been suggested that intradermal vaccination can improve the immune response in renal transplant patients,[218] but a randomized study did not confirm the improved immunogenicity in all groups.[219] A Phase I study in children having undergone SOT with high-dose influenza vaccine has been performed. The study showed a higher risk for mild local and systemic side effects and a higher seroconversion rate to one of the antigens although the study was not powered to assess the latter.[220] Also the cellular immune responses to influenza vaccine have been studied and found to be impaired in some,[221,222] but not all, studies.[223]

Despite systematic reviews showing that SOT patients respond more poorly to influenza vaccine than healthy controls,[51,224] vaccinated transplantation patients had a lower risk for influenza-like illness and laboratory-verified influenza compared to nonvaccinated controls.[51] Magnani and colleagues also reported that vaccination of heart transplant recipients reduced the proportion of patients developing influenza-like symptoms.[225] In a retrospective analysis of pediatric liver transplant patients during the H1N1 pandemic, the frequency of infection was 4% in vaccinated patients when compared to 25% in unvaccinated patients.[226]

Several studies have analyzed the risk for graft rejection after vaccination with influenza vaccine and found no increased risk.[195,206,208,225,227–231] However, case reports do exist that report organ rejection after vaccination.[232–234] There are also reports of increased alloreactivity after influenza vaccination.[235] On the other hand, a large cohort study of more than 50,000 renal transplant recipients showed a reduced risk for allograft loss and death during the first year after transplantation in patients receiving influenza vaccine.[236] Given all the available data, the benefits with influenza vaccination are far greater than the possible risks.[190]

Inactivated influenza vaccine is recommended to be given annually to SOT recipients.[24,25] Because influenza can be severe in patients with end-stage organ disease, influenza vaccination with the inactivated seasonal influenza vaccine is recommended also to transplantation candidates.[24,25] No data exist with the live attenuated vaccine and it is contraindicated in this population.[24,25,190]

Tetanus, Diphtheria, and Pertussis Vaccines

Less than 50% of patients with chronic kidney disease have protective titers against tetanus. Although the response rates after vaccination were decreased,[237] vaccination of transplantation candidates is recommended either to complete the primary schedule (young children) or with a booster dose of Tdap (older children and adults).[24,25,190]

In children who had undergone renal transplantation, persistent immunity to diphtheria was found in 38% and to tetanus in 90%.[238] After vaccination, 35% and 32% of patients were protected against diphtheria at 1 and 5 years, respectively.[238] A booster dose gave protective antibody levels to diphtheria in 95% of patients, but at 12 months only 76% remained protected[238] while all patients remain protected against tetanus. Pertussis vaccination after SOT has not been studied, but continuation of the recommended immunization with DTaP is recommended.[24,25]

Hepatitis B Virus Vaccine

Protection against HBV infection can be transferred to a SOT recipient either by an HBV-positive organ graft or through transfusions. HBV vaccination is recommended in HBV-negative candidates because there is an increased risk of severe

HBV infections in transplantation patients. Furthermore, patients receiving grafts from HBV immune donors have a lower risk for posttransplantation de novo HBV infection.[239] The efficacy of HBV vaccine was low in patients on hemodialysis[240,241] and in patients with end-stage liver disease waiting for transplantation.[242,243] Different schedules have been used in attempts to improve the response to vaccinations, including accelerated dose schedules and double-strength doses.[244–247] The seroconversion rates in the different studies varied from 31% to 62%. One study found a higher response rate after repeated high-dose (80 μg) vaccine doses in nonresponders.[248] Factors associated with better responses were young age,[244,249] a diagnosis of biliary atresia,[250] a milder grade of liver disease,[244] and specific human leukocyte antigen types.[244] Besides poor initial responses, the antibody levels decreased rapidly so that up to 35% of the patients who had seroconverted became seronegative after liver transplantation.[244,245,249] Despite the less-than-optimal responses, pretransplantation immunization is recommended.[190]

The efficacy of HBV vaccination was reported as being low with standard vaccine schedules in adults, with response rates between 5% and 15%.[251,252] Reports suggest that pediatric liver transplant patients can achieve better vaccination responses (70% seroconversion).[253] The immunosuppression administered to prevent rejection also influences the vaccination response. Patients receiving triple immunosuppression (cyclosporine, corticosteroids, and azathioprine) responded less well than patients receiving cyclosporine alone.[253] HBV vaccinations with the aim to prevent reactivation in patients transplanted for HBV-induced cirrhosis have given different results. Sanches-Fueyo and colleagues reported good results using a double dose of HBV vaccine,[228] whereas Angelico and colleagues reported poor results with a similar strategy.[254] Another option to improve the immunogenicity is intradermal HBV vaccination. Choy and colleagues reported a 62% response rate to intradermal HBV infection in renal transplant recipients failing intramuscular vaccination.[255] Attempts to prevent HBV reactivation in patients receiving liver transplants for HBV-related liver disease with the combination of lamivudine and a double dose of vaccine have been unsuccessful.[256,257] New vaccine approaches are needed. For example, two studies using experimental adjuvants have given promising results with response rates of 50% to 80% in subjects transplanted for HBV-related disorders.[258,259] A booster with conventional vaccine has also been shown to enhance long-term immunity.[260]

Hepatitis A Virus

Hepatitis A virus can cause decompensation and death in patients with chronic liver disease. Patients with chronic liver or renal disease awaiting transplantation are able to respond to vaccination, although the results are not as good as in healthy individuals.[261,262] Loss of immunity in seropositive patients was reported to occur in 29% of the patients at 2 years posttransplantation.[263] In a large study of kidney transplant recipients in South Korea, 19% were seronegative. Fifty-two patients received two doses of hepatitis A virus vaccine and the seroconversion rate was only 27% with lower serum creatinine and higher hemoglobin being positive predictors for improved response.[264]

Vaccination of transplantation candidates is recommended, particularly those with liver disease.[190] The combined hepatitis A/B vaccine is potentially useful in pretransplantation immunization. Vaccination results after liver or renal transplantation are poorer compared to those in healthy individuals,[249,265] and antibody levels decrease more rapidly.[265]

Papillomavirus Vaccine

SOT patients are at higher risk for human papillomavirus-related genital warts, cervical cancer, and other anogenital malignancies.[266] A small study in adolescent kidney and liver transplant recipients showed that 100% responded to vaccination with the quadrivalent human papillomavirus vaccine.[267] Indications for vaccination against papillomavirus should be the same as for the general population but because of an increased rate of warts, the quadrivalent vaccine might be preferable in SOT candidates and recipients.[24,25]

Cytomegalovirus

CMV is an important pathogen in patients after SOT. A live attenuated virus vaccine based on the Towne strain showed a reduction in the severity of CMV disease and a reduction in graft rejection primarily in seronegative recipients getting organs from seropositive donors,[268,269] but this vaccine is no longer available. In a Phase II study, a glycoprotein B–based subunit vaccine was given before and twice after liver and renal transplantations. The vaccine induced glycoprotein B–specific antibodies and was associated with fewer days of CMV viremia and fewer days of ganciclovir therapy in seronegative patients receiving grafts from CMV seropositive donors.[2] Further studies are necessary to determine if inactivated CMV vaccine will be clinically useful in SOT recipients.

Other Nonlive Vaccines

A study performed in heart transplant patients reported lower vaccine response to tickborne encephalitis vaccine compared to healthy controls.[270] There are no data regarding meningococcal vaccine specifically in transplantation candidates or recipients. Many SOT recipients travel, and there might be indications for "travel vaccines." However, there are no data supporting efficacy for most of these vaccines, making it important to get expert advice before traveling to countries where there is need for specific vaccines.

Live Attenuated Vaccines

Immunization with live vaccines is not recommended after SOT because of the risk of vaccine-associated complications.[24,25] The risk of vaccine-associated disease from pretransplantation administration of live vaccines has not been defined but is likely to be time-dependent with lower risks if the interval from vaccination to transplantation is longer.

Varicella Vaccine

VZV can cause severe and potentially fatal disease in patients after transplantation. It is important to consider the vaccination of seronegative candidates prior to transplantation. Varicella vaccine given to uremic children prior to transplantation was shown to be safe and reduced the posttransplantation risk for varicella.[271] A follow-up showed that the protection was long lasting, with 42% of the patients still having antibodies at more than 10 years after immunization. Furthermore, the risk for varicella was lower and if disease occurred, it was significantly less severe in previously vaccinated subjects.[271] A large study of more than 700 children showed that vaccination before renal transplantation reduced the incidence of both varicella and zoster.[272] A two-dose schedule was given to 50 children awaiting renal transplantation and the seroconversion rate was 98%.[273] Similar results of a single vaccine dose were seen in a study of children with chronic liver disease, with a vaccination efficacy of 100% and no severe side effects.[274]

In another study, 95% of pediatric liver candidates given vaccine prior to transplant seroconverted.[275] A small number of adults who are seronegative to VZV would also benefit from vaccination.

The zoster vaccine decreases the risk for zoster reactivation in elderly individuals for up to 8 years after vaccination.[276] Whether pretransplant zoster vaccination would reduce the risk for posttransplant zoster is unknown but could be considered in candidates fulfilling standard criteria for zoster vaccination.

Vaccination of VZV seronegative patients after transplantation might be contemplated. There have been a number of small studies suggesting safety of varicella vaccine given after transplant.[275,277–281] In one study that included 17 renal transplant recipients, the authors reported an 85% seroconversion rate and only three posttransplant patients developed mild varicella.[277] In another study, booster doses of varicella and MMR vaccine were administered to 15 pediatric liver recipients whose titers had waned, and augmentation of seroresponse was noted without severe adverse effects or rash.[275] However, these patients are likely to have retained some cell-mediated immunity. In a study of 16 pediatric liver and small bowel transplant recipients, 87% of the patients developed both humoral and cell-mediated immunity.[278] Three of the 16 patients were given acyclovir for vaccine induced rash. However, there are also reports of significant varicella virus disease after varicella vaccination in transplant recipients[282,283] and further studies are needed to assess both efficacy and safety of this approach. The availability of inactivated varicella-zoster vaccines will provide new opportunities to evaluate vaccination in transplant recipients.

Measles, Mumps, and Rubella

MMR vaccine has been given to infants awaiting renal transplantation with good responses: 88% of the patients developed immunity to all three components of the vaccine.[284] Analyzing the components separately, 89% developed immunity to measles, 88% to mumps, and 100% to rubella. In another study, 42 seronegative pediatric candidates were vaccinated with measles vaccine with a seroconversion rate of 97% and no significant side effects.[285] Children who are transplantation candidates and not yet vaccinated with MMR should be vaccinated, unless transplantation is imminent.

Other Live Vaccines

There are no data regarding vaccination with other live vaccines such as rotavirus, yellow fever, or BCG either prior to or after transplantation. In general, live vaccines should not be used after solid-organ transplantation.[24,25] However, children awaiting transplantation should receive age appropriate vaccines including that infants waiting for solid-organ transplantation should be vaccinated with rotavirus vaccine starting at 2 months of age.[24,25] It was shown that antibodies to yellow fever were retained in 98% of SOT recipients at a median of 3 years after solid-organ transplantation.[286] However, it is likely that the interval between vaccination and transplantation is important for the risk of postvaccination disease and an interval from vaccination to transplantation of at least 4 weeks is recommended.[24,25]

PRIMARY IMMUNODEFICIENCY DISEASES

Primary immunodeficiency diseases (PIDs) include genetic disorders affecting the functioning of either the innate or the adaptive immune systems. Many different types exist whose causes are now rapidly being detected by molecular genetic analysis. These disorders increase the risk for infections, making it important to vaccinate these children when it is possible and safe. The sensitivity to and spectrum of infections seen in these patients and thereby the need, effectiveness, and risks of vaccination are determined by the genetic defect. The less pronounced of the defects are frequently diagnosed later during childhood and routine pediatric immunizations have been administered to many. Other immune defects, such as disseminated BCG infections, are diagnosed as a result of a vaccination. Most PIDs are rare to very rare and therefore clinical trials in patients with each defect are impossible to perform. The defects can be broadly classified as:

- Defects of the innate immune system including diseases with complement deficiencies, defects in the phagocytic cell functions, and diseases associated with cellular receptors and signaling pathways involving cytokine production.
- Defects of the adaptive immune system including major defects in antibody production such as X-linked agammaglobulinemia, common variable immunodeficiency as well as more minor defects such as IgA deficiency or Ig subclass deficiencies and specific polysaccharide antibody deficiency. The most severe of these patients require lifelong immunoglobulin substitution therapy and are rarely candidates for vaccination.
- Combined defects that involve T-cell function. These include many different disorders such as severe combined immunodeficiency, Wiskott-Aldrich syndrome, DiGeorge syndrome, X-linked hyper-IgM syndrome, severe ataxia-telangiectasia, familial hemophagocytic lymphohistiocytosis with perforin mutations, and several other very rare disorders.

Nonlive vaccines pose no increased risk for side effects, but immunogenicity and efficacy are problematic. Consequently, the recommendation is to give nonlive vaccines according to the same schedule as for healthy children. Regarding live vaccines, the summaries given below must be seen as generalizations and expert advice should be solicited before vaccinating children with specific rare defects. The use of oral poliovirus vaccine has resulted in paralytic disease in some patients with PIDs although it can be argued that many patients have received this vaccine before the diagnosis of the immune defect was made. However, this vaccine is no longer available in many countries including the United States. Family members of immunodeficient patients should also receive the inactivated vaccine.

Defects of the Innate Immune System

Nonlive Vaccines

Pneumococcal, *Haemophilus influenzae* Type B, Meningococcal Vaccine. Patients with primary complement deficiencies are at a very high risk for invasive meningococcal disease.[287] A deficiency of the early complement components C2 to C4 also increases the risk for pneumococcus and Hib disease. Vaccination with the quadrivalent meningococcal polysaccharide vaccine gives good serological responses in patients with complement deficiencies.[288–290] These patients also need to be revaccinated regularly to maintain protective antibody levels.[288,290] There is no study specifically addressing efficacy of the meningococcal conjugate vaccine. Because of its improved immunogenicity, however, it is recommended for use in patients with complement deficiencies.[24,25,291] In ataxia-telangiectasia there is known to be a poor response to PPSV23 in the majority of patients. In three small studies, PCV7 was immunogenic in most ataxia-telangiectasia patients, although not comparable to normal controls.[292–294] The same is suggested for patients with specific polysaccharide antibody

deficiency. Vaccination with PCV13 and Hib conjugate vaccines should be given to patients with defects in the innate immune system.[24,25]

Tetanus Toxoid, Diphtheria Toxoid, Inactivated Poliovirus, and Acellular Pertussis Vaccine. These vaccines should be given to children with complement deficiencies, phagocyte defects, and defects in cytokine production in a similar schedule as in normal children.

Influenza. Inactivated influenza vaccine should be given to children with complement deficiencies, phagocyte defects, and defects in cytokine production.[24,25]

Live Attenuated Vaccines

Varicella Vaccine and Measles, Mumps, and Rubella. Live attenuated varicella vaccine and MMR can be given to children with chronic granulomatous disease and early or late complement defect disorders.[295] A number of molecular genetic changes can result in defects of the antiviral immunity. Consequently, expert advice should be solicited before vaccinating children with phagocytic or cytokine generation disorders.

Other Live Vaccines. There are a number of case reports of disseminated BCG in patients with cytokine generation disorders[296–298] and in patients with other defects in the innate immune system.[299,300] BCG should generally be avoided in patients with PIDs. Patients with cytokine generation disorder are at increased risk for *Salmonella* infections.[301] Therefore, oral typhoid vaccine should also be avoided. There are very limited data regarding the oral rotavirus vaccine in patients with PID.

B-Cell Defects

Nonlive Vaccines

Pneumococcal and *Haemophilus influenzae* Vaccines. Patients with mild to moderate defects in the B-cell function, including IgA deficiency, should be vaccinated against pneumococci and Hib similarly to healthy children. Children with specific polysaccharide antibody deficiency or ataxia-telangiectasia should receive PCV13 and those older than 2 years of age should also receive a dose of PPSV23.[24,25] Patients with more substantial defects in Ig production and on Ig supplementation therapy are unlikely to respond. In cases of specific polysaccharide antibody deficiency, the use of conjugate vaccines might to some extent overcome the defect and produce some antibody response.[302] Studies of patients with common variable immunodeficiency using polysaccharide and conjugate vaccines showed that a subset of patients produce immune responses correlated with the clinical severity of the underlying disease.[303–305]

Tetanus Toxoid, Diphtheria Toxoid, Inactivated Poliovirus, and Acellular Pertussis. These vaccines should be given to children with patients with IgA deficiencies and to other patients with mild to moderate B-cell defects, including subclass deficiencies, although the antibody responses might be suboptimal. Patients with more complete lack of B-cell function are unlikely to respond but there are no risks.

Influenza. Individuals with mild/moderate defects in B-cell function, including IgA deficiency, can receive inactivated influenza vaccine. The efficacy of vaccination has not been studied. Live attenuated influenza vaccine should not be administered to patients with major B-cell defects.

Live Attenuated Vaccines

Varicella and Measles, Mumps, and Rubella. Varicella and MMR vaccines should not be given to patients with significant B-cell deficiencies as it is unlikely that the patients will mount an antibody response and there is a possibility of side effects from the vaccine. In patients with less-severe defects in B-cell function, vaccination with these vaccines can be considered.[295] However, the possibility that patients with B-cell deficiencies also can have defects in the T-cell function, thereby increasing the risk for side effects, should be considered.

Oral Poliovirus Vaccine. Individuals with IgA deficiency can develop vaccine associated poliomyelitis.[306–308] Patients with major B-cell deficiencies should not receive oral poliovirus vaccine.

Combined Immunodeficiency Disorders

Nonlive Vaccines

Pneumococcal, *Haemophilus influenzae*, Inactivated Influenza, Tetanus Toxoid, Diphtheria Toxoid, Inactivated Poliovirus, and Acellular Pertussis. Patients with combined immunodeficiencies can be vaccinated using the same routine immunization schedule as that used for healthy children; however, the more severely affected children are unlikely to respond.

Live Attenuated Vaccines

The data regarding safety and efficacy of vaccinating children with combined immunodeficiencies with live vaccines are limited. Complications from vaccination of T-cell–deficient children receiving live viral vaccines, such as vaccine-associated poliomyelitis[309] disseminated measles infection, and chronic rotavirus infection, have been described.[310–313] Patients with severe combined immunodeficiency, Wiscott-Aldrich syndrome, complete Di George syndrome, and inherited hemophagocytic lymphohistiocytosis should not receive live vaccines, including live attenuated influenza vaccine. In other, less-severe diseases, such as partial DiGeorge syndrome, it has been shown that MMR and varicella vaccines can result in high rates of seroconversion and no severe side effects.[314,315] The number of circulating T cells as well as responses to in vitro T-cell assays have been suggested as markers reflecting the safety of administration of live vaccines in patients with immune defects. Decisions regarding vaccination must be made on an individual basis after assessment of the immune system and consultation with an expert in PIDs.

PATIENTS WITH CHRONIC INFLAMMATORY DISORDERS

Disorders resulting in chronic inflammation include inflammatory bowel diseases (Crohn disease and ulcerative colitis), rheumatological diseases (rheumatoid arthritis, systemic lupus erythematosus [SLE], systemic sclerosis, dermatomyositis), neurological diseases (multiple sclerosis and other autoimmune neurological diseases), and hematologic diseases (autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura). These patients are treated with a wide variety of antiinflammatory agents, including corticosteroids, cytotoxic drugs such as methotrexate and cyclophosphamide, calcineurin inhibitors such as cyclosporine and tacrolimus, and, with increasing frequency, monoclonal antibodies such as tumor necrosis factor (TNF)-blockers or anti–B-cell antibodies including rituximab. Vaccinations of patients with rheumatologic and autoimmune disorders must be

considered from three viewpoints: the response to vaccination during immunosuppressive therapy, the risk for side effects from the vaccines, and the potential risk for flare-up of the underlying disease. Decreased responses to vaccination in rheumatoid arthritis patients treated with rituximab have been shown.[316] In general, patients should be immunized before immunosuppression, if possible, with age-appropriate vaccinations.[24,25]

Nonlive Vaccines

Pneumococcal and *Haemophilus influenzae* Type B Vaccine

The incidence of severe pneumococcal infections is increased 13-fold in patients with SLE.[317] PPSV23 is effective in patients with rheumatoid arthritis and SLE.[318–320] However, Battafarano and colleagues reported that patients on active immunosuppressive therapy had lower immune responses[320] and Elkayam and colleagues demonstrated that 20% to 33% of patients failed to respond to at least one of the analyzed serotypes.[319] PPSV23[318–321] and conjugated Hib vaccines[320] have been shown to be safe and tetanus toxoid and Hib have been shown to be immunogenic in SLE patients.

The effect of TNF blockers on vaccine responses has been studied in patients with rheumatoid arthritis,[322] with no negative impact noted. In contrast, patients treated with methotrexate had a lower response rate to pneumococcal vaccination.[323] Rituximab had a negative impact on the antibody response to PPSV23.[324] There are no reports of disease flares or significant side effects after vaccination with PPSV23 in patients with rheumatoid arthritis. Antibody responses to PPSV23 in 190 adults with rheumatoid arthritis were not adversely affected by treatment with tocilizumab, an anti–interleukin-6 receptor antibody.[325] Nagel and colleagues reported a registry study of the effects of PCV7 and found a nonsignificant reduction in serious pneumococcal infections in patients with rheumatoid arthritis and spondyloarthropathy. The ACIP of the Centers for Disease Control and Prevention has recommended routine use of PCV13 for immunocompromised persons followed by PPSV23 including those receiving immunosuppressive medications.[22,23] However, this regimen has not been studied in patients with inflammatory disorders.

Influenza

Patients with chronic inflammatory disorder (CID) are included in the general recommendations for influenza vaccination of immunosuppressed patients.[24,25] The immunogenicity of influenza vaccine has been studied in patients with SLE. Most patients are able to respond, although the responses tend to be lower in patients compared to healthy controls[326–328] and correlated with the intensity of steroid therapy.[326] Lu and colleagues studied the response to pandemic H1N1 in patients with SLE and found responses similar to controls.[329]

Elkayam and colleagues showed in a study of patients with rheumatoid arthritis and ankylosing spondylitis that patients receiving infliximab responded to vaccination, although the response in patients vaccinated 3 weeks after infliximab had less of an immune response.[330] Van Assen and colleagues studied responses early (1 to 2 months) and late (6 to 10 months) after rituximab and compared them to methotrexate-treated rheumatoid arthritis patients and healthy controls.[331] They found much reduced responses in the rituximab treated patients although there were some increases in geometric mean titer in the group vaccinated later after rituximab therapy. Patients receiving tocilizumab for treatment of rheumatoid arthritis or juvenile idiopathic arthritis had antibody responses to inactivated influenza vaccine that were similar to those of the comparator groups.[332]

Children with rheumatologic diseases respond well to influenza vaccination with seroprotection rates similar to healthy children.[333] Two prospective trials of inactivated influenza vaccine in children with inflammatory bowel disease[334,335] showed that vaccination is safe and effective, although immunogenicity may be decreased in patients treated with TNFα antibodies. Children receiving lower amounts of immunosuppression had seroprotection rates comparable to immunocompetent controls for all three strains in the vaccine. Children treated with TNFα antibodies, however, had normal seroprotection rates to influenza A strains, but lower seroprotection and seroconversion rates to the type B vaccine strain.

In patients with SLE, studies show no increased rate of flare-ups in disease activity in influenza-vaccinated patients than in the controls.[326,327,336,337] Similar results were seen in patients with rheumatoid arthritis and ankylosing spondylitis,[329–331] and in mixed groups of patients with rheumatology disorders.[338,339] As the efficacy is adequate and there are no evident risks, annual influenza vaccination with the inactivated vaccine is recommended for both adults and children with CIDs.

Other Vaccines

Patients with CID respond similarly as healthy controls to tetanus toxoid.[324] A prospective study of HBV vaccination in patients with rheumatoid arthritis demonstrated immune responses in the majority of patients without significant side effects.[340] A large study of meningococcal conjugate vaccine in 234 children and young adults with juvenile arthritis showed that the patients responded well regardless of type and intensity of immunosuppression.[341] Two studies of the quadrivalent papillomavirus vaccine in patients with SLE both showed a high rate of antibody response and no risk for significant side effects.[342,343] Finally, it was shown that two doses of hepatitis A vaccine was required to give protective antibody levels in patients with rheumatoid arthritis treated with TNFα blockers.[344]

Live Attenuated Vaccines

Varicella

Two studies have been published on varicella vaccination in children with CID. Pileggi and colleagues reported varicella vaccination of 25 children with juvenile rheumatoid diseases (20 seronegative children and five children with borderline antibody titers) and compared the results to 18 healthy controls.[345] All 25 patients were receiving methotrexate; 13 were also receiving prednisone and five were also receiving other therapeutic agents. Half the patients responded compared to 72% of the controls, but the responses were not significantly different. The vaccine was well tolerated without signs of dissemination or other side effects. A case series reported results from six children with inflammatory bowel disease where five had received their initial dose of varicella vaccine prior to initiating the immunosuppressive therapy followed by a booster dose after start of therapy. There were no significant side effects seen.[346]

The risk for zoster is increased in patients with CID. Although there are no studies addressing vaccine safety in this population, the Infectious Diseases Society of America guidelines recommend zoster vaccine in patients older than 50 years of age on mild immunosuppression; but zoster vaccine is contraindicated for patients receiving more intensive immunosuppression, including TNFα inhibitors.[24,25]

Other Live Vaccines

There are no data regarding other live vaccines given to adults with CID, but they are not recommended in adults with rheumatoid diseases treated with immunosuppressive therapy. MMR revaccination of patients with juvenile idiopathic arthritis resulted in a good immune response to all three viruses without serious adverse effects despite continued therapy with methotrexate or recent therapy with etanercept or anakinra; however, data are lacking on the safety of primary MMR vaccination in this population. An increase in disease activity was observed in seven patients with multiple sclerosis who were vaccinated with yellow fever vaccine.[347] However, the Committee on Infectious Diseases of the American Academy of Pediatrics states that children who have been given moderate doses of corticosteroids (<20 mg prednisolone in children weighing >10 kg) can receive live vaccines.[295]

HOUSEHOLD CONTACTS OF IMMUNOCOMPROMISED PATIENTS

Household and other close contacts of immunocompromised patients can receive all age-appropriate live vaccines except live oral poliovirus vaccine.[295] Limited data are available regarding the potential for transmission of live attenuated influenza vaccine. However, it is recommended that live influenza vaccine should be avoided in contacts of severely immunocompromised patient groups such as allogeneic HSCT patients early after HSCT or those who are receiving intensive immunosuppression because of GVHD.

FUTURE DIRECTIONS

The number of patients with immunosuppressed states is increasing. The advances in medical technology result in constant changes in groups of patients undergoing intensive immunosuppressive therapy; for example the increase in age of patients undergoing HSCT. At the same time new immunosuppressive agents are being introduced and the types of therapies given to these patients change. Therefore, prospective clinical trials need to be performed to assess the safety, immunogenicity, and efficacy of vaccinations in these new cohorts. These technologies are spreading throughout the world and few if any studies have been performed with vaccines against infectious agents more common in tropical areas. The epidemiology in these countries, and thereby the risks, are different. For example, a high risk for tuberculosis in highly endemic areas, and the risks for yellow fever and hepatitis A virus must be taken into account when assessing the risk-to-benefit and cost-to-benefit of vaccination.[348] The recent outbreak of pandemic H1N1 showed the vulnerability of the immunocompromised populations to new and rapidly spreading infections.[4,6] New vaccines are in development against CMV, varicella, and influenza. These vaccines should be evaluated in immunocompromised hosts because they are vulnerable to both infection and disease.

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70 Vaccination of Human Immunodeficiency Virus–Infected Persons

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In the absence of effective antiretroviral therapy, HIV-1 infection results in a progressive decline in CD4 T lymphocytes, decreased ability to mount protective responses to new antigenic stimuli, loss of prior immunity, and increased risk of complications from infections. As with other causes of serious immune suppression, HIV-1 infection can impair the effectiveness of vaccines and increase the risk of serious adverse events from live vaccines. Fewer HIV-1–infected children and adults develop protective antibody levels after vaccination than do HIV-1–uninfected persons. The magnitude of the antibody response often is inversely correlated with the CD4 T-lymphocyte count.[1,2] In general, HIV-1–infected adults with CD4 T-lymphocyte counts less than 200 cells/mm^3 and HIV-1–infected children with CD4 T-lymphocyte percentages less than 15 have poor serologic[1,2] responses to vaccines. Of those who develop an initial antibody response following vaccination, antibody concentrations frequently wane at a faster rate in HIV-1–infected persons than in HIV-1–uninfected persons. Live viral and bacterial vaccines pose an increased risk to immunosuppressed HIV-1–infected persons because of the potential for disease caused by uncontrolled replication of the vaccine strain, and this risk increases with severe immune suppression. T-lymphocyte proliferation induced by vaccination can transiently increase plasma HIV-1 RNA levels, but an effect of vaccination on HIV-1 disease progression has not been found.[3]

Vaccination is generally safe and effective early in infancy before HIV-1 infection causes significant immune suppression.[4,5] The rate of progression to clinically apparent immune suppression is variable and depends on factors related to the host and the virus. Accordingly, the safety and effectiveness of vaccines in HIV-1–infected children vary with age at vaccination and immune status of the HIV-1–infected child. Partial or protective immunity usually persists until immune suppression becomes severe, although antibody concentrations to some antigens (measles virus, tetanus toxoid, and hepatitis B virus [HBV]) may wane with lesser degrees of immune suppression.[6] In older HIV-1–infected children and adults, the immune response to primary immunization may be impaired, but protective immunity to vaccines received before HIV-1 infection is usually maintained. Antiretroviral therapy can improve immune responses to vaccines in HIV-1–infected children[7,8] and adults,[9] but probably not to levels found in HIV-1–uninfected persons. Antiretroviral therapy, however, does not restore immunity lost prior to treatment. Deferring vaccination in HIV-1–infected persons with advanced immune suppression until HIV-1 replication is controlled and the immune system reconstituted by highly active antiretroviral therapy (HAART) should result in improved responses to vaccination[1,10] and should be considered if they are not at immediate risk of contracting the vaccine-preventable diseases. As an increasing number of HIV-1–infected children start HAART in early infancy, and even prior to vaccination, their immune responses may more closely approximate those of uninfected children. However, serologic responses may wane even in HIV-1–infected persons receiving effective HAART.

Multiple immunologic abnormalities have been described in HIV-exposed but uninfected children, including reductions in the number of naïve CD4 T lymphocytes, although the clinical significance of these changes is unclear.[11] Consistent with these findings, several studies reported lower vaccine responses among HIV-exposed but uninfected infants compared to unexposed infants, including responses to tetanus toxoid, poliovirus, hepatitis B, and bacille Calmette-Guérin (BCG) vaccines.[12–14] Not all studies, however, identified differences in antibody responses to vaccines between HIV-exposed, uninfected children and HIV-unexposed children,[15,16] and the clinical and public health significance of these observations remain unclear. Assessing vaccine efficacy in exposed, uninfected children is complicated by multiple confounding factors. Whether these infants are less likely to develop protective immunity after vaccination remains to be determined.

GUIDELINES FOR USE OF VACCINES IN HIV-INFECTED PERSONS

Guidelines for vaccination of HIV-1–infected persons consider the risks of vaccination, the risks of exposure to diseases, and the risks of complications from the diseases.[17–20] The World Health Organization recommends administering almost all routinely recommended vaccines for asymptomatic HIV-1–infected children and adults.[18] The exception is BCG vaccine, which is not recommended for children known to be HIV-1–infected because of the increased risk of disseminated BCG disease, although the difficulty of identifying HIV-1 infection shortly after birth is recognized.[21,22] When the diagnosis of HIV-1 infection cannot be made in early infancy, BCG vaccine should be administered at birth to all infants regardless of HIV-1 exposure, and infants exposed to HIV-1 should be closely followed for signs and symptoms of disseminated BCG disease.[21] For HIV-1–infected persons who are severely immunocompromised, yellow fever and measles vaccines are not recommended because of the potential for increased risk of complications,[23,24] although additional safety data are needed. In the United States, varicella and zoster vaccines also are not recommended for HIV-1–infected persons who are severely immunocompromised.[19,20] All other vaccines are recommended for HIV-1–infected persons regardless of symptoms. The World Health Organization and the Advisory Committee on Immunization Practices in the United States recommend measles revaccination of HIV-1–infected children after immune reconstitution with antiretroviral therapy because of waning immunity prior to treatment. In settings where the risk from exposure to disease may be less and testing for immunologic status or alternatives to live vaccines are available, more specific recommendations have been made by advisory committees such as the Advisory Committee on Immunization Practices and the Committee on Infectious Diseases of the American Academy of Pediatrics (Table 70.1).

TABLE 70.1 WHO/UNICEF and ACIP Recommendations for Immunization of HIV-1–Infected Children and Adults[17–20]

Vaccine	HIV-1 Infection	Severe Immune Suppression	Comments
WHO/UNICEF			
BCG	Not recommended	Not recommended	HIV-1 infection can be difficult to identify in early infancy
DTP	Recommended	Recommended	
OPV/IPV	Recommended	Recommended	IPV may be used if symptomatic
Measles	Recommended	Not recommended	Administered at 6 and 9 mo; revaccination after immune reconstitution with antiretroviral therapy
Hepatitis B	Recommended	Recommended	
Hib	Recommended	Recommended	
Pneumococcal	Recommended	Recommended	
Rotavirus	Recommended	Not recommended	
Tetanus toxoid	Recommended	Recommended	5 doses
Yellow fever	Recommended	Not recommended	Until safety is further evaluated
Vaccine	**Children With HIV-1 Infection or AIDS**	**Adults With HIV-1 Infection or AIDS**	**Comments**
ACIP			
DTP	Recommended	Not applicable	
OPV	Contraindicated	Contraindicated	
IPV	Recommended	Use if indicated	
MMR	Recommended	Recommended	Not if severe immune suppression (CD4 % T cells <15%) Revaccination with two doses after effective antiretroviral therapy
Hib	Recommended	Considered	Consider risk of disease in adults
Hepatitis B	Recommended	Recommended	
HPV	Considered	Considered	
Pneumococcal	Recommended	Recommended	
Meningococcal	Recommended	Use if indicated	Two doses of conjugate vaccine for all 11–18-year-olds
Rotavirus	Considered	Not applicable	
Varicella	Recommended	Recommended	Not if severe immune suppression (CD4 % T cells <15%)
Influenza, inactivated	Recommended	Recommended	Not for infants <6 mo old
Influenza, attenuated	Contraindicated	Contraindicated	
Td		Recommended	
BCG	Contraindicated	Contraindicated	
Yellow fever	Contraindicated	Contraindicated	Consider if exposure is unavoidable
Rabies	Use if indicated	Use if indicated	
Vaccinia	Contraindicated	Contraindicated	
Anthrax	Use if indicated	Use if indicated	
Plague	Use if indicated	Use if indicated	

ACIP, Advisory Committee on Immunization Practices; BCG, bacille Calmette-Guérin vaccine; DTP, diphtheria and tetanus toxoids and pertussis vaccine; Hib, *Haemophilus influenzae* type b vaccine; HPV, human papillomavirus; IPV, inactivated poliovirus vaccine; MMR, measles-mumps-rubella vaccine; OPV, oral poliovirus vaccine; Td, tetanus-diphtheria toxoid; UNICEF, United Nations International Children's Emergency Fund; WHO, World Health Organization.

NONREPLICATING VACCINES

Diphtheria and Tetanus Toxoids and Pertussis Vaccine

Children

Following primary immunization in infancy, 62% to 100% of HIV-1–infected children not receiving HAART respond to tetanus and diphtheria toxoids by developing protective concentrations of tetanus and diphtheria antitoxins, although geometric mean antibody titers are sometimes lower than in healthy control children and are more likely to wane over time (Table 70.2).[25] However, responses to booster doses are similar to those of uninfected children. Data on the immunogenicity of pertussis vaccines are more limited and interpretation is more difficult because serologic correlates of protection have not been identified. The limited data suggest that the proportion of children who seroconvert and the geometric mean antibody titers to pertussis toxin are lower in HIV-1–infected

TABLE 70.2 Immunogenicity of Diphtheria and Tetanus Toxoids and Pertussis Vaccines in HIV-1–Infected Children and Adults

Vaccine	Reference	Country	No. of Subjects	HAART	Age	HIV-1–Infected Persons Developing Protective Antibody Titers
CHILDREN						
DT	28	United States	5	No	2–6 y	40% to tetanus booster; titers lower than in control subjects
DT	29	France	13	No	4–24 mo	62% to tetanus and 57% to diphtheria; lower titers in children with opportunistic infections
TT	30	Italy	17	No	18–84 mo	77% to tetanus; titers lower than in control subjects
DTP	31	United States	17	No	11–90 mo	60% to tetanus; 18% to diphtheria; CMI in some children without protective antibody titers; titers lower than in control subjects
DTP	32	United States	33	No	<4 y	100% to tetanus; 88% to diphtheria; better responses early in life
DTP	33	Zaire	48	No	<4 mo	96% to tetanus; 71% to diphtheria; titers lower than in control subjects for diphtheria
TT	34	United States	15	Yes	Median, 12.6 y	92%; titers lower than in control subjects
TT	35	Kenya	24	Yes	Median, 4.9 y	75%
DTP	36	United States	11	Yes	3–14 y	90% to tetanus
DTP	37	United States	37	Yes	2–11 y	74% to tetanus; no difference in response by time receiving HAART
DTP	38	United States	46	Yes	3–17 y	94% to tetanus; no difference in response by time receiving HAART
Acellular pertussis	26	Italy	12	No	6–107 mo	75%; titers lower than in control subjects; correlated with CD4 cell count
ADULTS						
TT	39	France	25	No	Adults	77%; no difference in GMT titer from control subjects
TT	40	Switzerland	10	No	Adults	Increase in GMT; correlated with CD4 cell counts
TT	41	Netherlands	47	No	Adults	84%; correlated with CD4 cell count
TT	42	Chile	26	No	Adults	23%; decline in antibody titers at 1 y
TT	42	Brazil	21	No	Adults	Titers lower than in control subjects
TT	42	Brazil	61	No	Adults	Titers lower than in control subjects but increased after booster dose

CMI, cell-mediated immunity; DTP, diphtheria and tetanus toxoids and pertussis vaccine; GMT, geometric mean antibody titer; HAART, highly active antiretroviral therapy; TT, tetanus toxin.

children compared with healthy control children.[26] However, there is no evidence that HIV-1–infected children have higher vaccine failure rates than uninfected children following diphtheria, tetanus, or pertussis immunization.[27]

Because many HIV-1–infected children start antiretroviral therapy at older ages, few data exist on the response to primary immunization among children receiving HAART. In South Africa, infants beginning HAART shortly after diagnosis, and approximately 1 week prior to beginning the primary vaccine series, had similar responses to tetanus (92.3%) and diphtheria (100%) toxoids 1 month after the third dose of vaccine compared to those of HIV-1–uninfected children (tetanus toxoid: 100%; diphtheria and tetanus toxoids–pediatric: 100%). Responses to tetanus, but not diphtheria, toxoids were higher than among HIV-infected infants who started HAART at a later age (tetanus toxoid: 82.7%; diphtheria and tetanus toxoids–pediatric: 100%).[44]

Among previously vaccinated children starting HAART, 74% to 94% responded to a booster dose of tetanus toxoid in the first few months after vaccination,[34–38] although antibody titers waned over time in some children (see Table 70.2).[34,36–38] Responses tended to be higher for children with better immunological recovery and virological control after HAART initiation for tetanus toxoid and pertussis.[34,45] For tetanus toxoid, the age of HAART initiation may be important, as children in whom HAART was initiated in early infancy had responses comparable to those of healthy control children.[46] However, the duration of HAART at the time of revaccination did not influence vaccine responses in one study.[38]

Adults

Mean antibody titers to tetanus and diphtheria toxoids were lower in HIV-1–infected Brazilian women than uninfected women, but titers increased following revaccination.[43] In an uncontrolled study, 48% of 31 HIV-1–infected adults developed a fourfold increase in antibody titers to tetanus toxoid after receiving antiretroviral therapy for at least 48 weeks.[47] Ensuring immunity to tetanus is important for men undergoing voluntary medical circumcision in regions of high HIV prevalence.

Inactivated Poliovirus Vaccine

Despite the global transition to inactivated poliovirus vaccine as part of the Global Polio Eradication Initiative, few studies

have evaluated the immunogenicity of inactivated poliovirus vaccine in HIV-1–infected children and adults. Several early studies measured poor antibody responses in HIV-1–infected children with low CD4 T-lymphocyte counts.[48,49] In a more recent study, intradermally administered inactivated poliovirus vaccine, using a reduced dose, was as immunogenic as intramuscular injection in HIV-1–infected adults.[50]

Hepatitis B Vaccine

Children

HIV-1 shares common risk factors for HBV infection, and HIV-1 infection increases the risk of chronic hepatitis B surface antigen carriage and liver failure, heightening the need for HBV vaccination in persons at risk of HIV-1 infection.[51] Serologic response rates have varied, but only 25% to 70% of HIV-1–infected children not receiving HAART developed protective antibody concentrations following hepatitis B vaccination in most studies (Table 70.3). Attempts to overcome the decreased response by administering higher doses or extra doses of hepatitis B vaccine have not been promising.[52,53] HIV-1–infected children who respond to hepatitis B vaccine have a more rapid decline in antibody titer than do uninfected persons.[54]

Among children previously vaccinated against HBV, HAART did not result in higher antibody levels.[88,89] However, administration of booster doses after HAART initiation improved responses, with serologic response rates ranging from 46% to 100% (see Table 70.3). Waning immunity may occur even after booster doses of hepatitis B vaccine.[64]

Administration of the primary vaccine series after HAART initiation among older children resulted in better antibody

TABLE 70.3 Immunogenicity of Hepatitis B Vaccines in HIV-1–Infected Children and Adults

Reference	Country	Vaccine and Dose	No. of Subjects	HAART	Age	HIV-1–Infected Persons Developing Protective Antibody Titers
CHILDREN						
55	Italy	Engerix-B (20 μg)	18	No	Mean: 1.8 y	78%; titers lower than in control subjects; no correlation with CD4 cell count; poor response to booster dose
56	United States	<11 y: Engerix-B (10 μg) or Recombivax HB (2.5 μg); >11 y: Engerix-B (20 μg) or Recombivax HB (5 μg)	24	No	5–115 mo	25%; correlated with CD4 cell count
57	United States	Recombivax HB (2.5 μg)	17	No	1 day to 11 mo	35%; no correlation with CD4 cell count
58	Spain	Engerix-B (10 μg)	17	No	0–63 mo	41%; response lower than in control subjects; no correlation with CD4 cell count
53	United States	Booster doses: <11 y: Engerix-B (20 μg); >11 y: Engerix-B (40 μg)	14[a]	No	21–129 mo	14% after 1 booster dose; 0 of 7 developed antibody response after second booster dose; correlated with CD4 cell count
54	Italy	Engerix-B (10 μg)	20	No	1–102 mo	45%; not correlated with CD4 cell count; 73% of 11 responded to booster dose
59	Thailand	Engerix-B (10 μg)	14	No	0–12 mo	71% after 3 doses; titers lower than in 3 subjects
60	Poland	10 μg	45	Yes	—	91% >100 mIU/mL
61, 62	Thailand	20 μg	63	Yes	Mean: 10.1 y	92% 1 month after 3 doses; 70% after 3 years; not correlated with CD4 cell count
63	Tanzania	HBVaxPro (5 μg)	48	Yes	Median: 4.7 y	71%
64	United States	Recombivax HB (5 μg)	204	Yes	Median: 9.1 y	46%; correlated with CD4 T-cell percentage
65	South Africa	—	73	No	5–24 mo	78%
66	Brazil	Euvax-B (20 μg)	18	Yes	10–20 y	67% after 1–6 booster doses; correlated with CD4 T-cell percentage
67	Tanzania	DPT-HB	31	No	2–59 mo	26%; lower titers than in control subjects
68	The Netherlands	Ambirix (inactivated HAV and rDNA HBV) (20 μg)	68	89%	1–16 y	Not on HAART: 67% after 2 doses On HAART: 100% after 2 doses
69	Rwanda	Engerix-B (10 μg)	73	Yes	8–17 y	71%; correlated with CD4 count and viral load

Continued on following page

TABLE 70.3 Immunogenicity of Hepatitis B Vaccines in HIV-1–Infected Children and Adults *(Continued)*

Reference	Country	Vaccine and Dose	No. of Subjects	HAART	Age	HIV-1–Infected Persons Developing Protective Antibody Titers
ADULTS						
70	United States	Plasma-derived	16	No	Adults	56%; titers lower than in control subjects
71	Belgium	Recombinant	32	No	Adults	28%; titers lower than in control subjects
72	Spain	Recombinant	21	No	Adults	24%; titers lower than in control subjects
73	United Kingdom	Engerix-B (20 μg)	12	No	Adults	17%; poor response to additional dose
74	Australia	Plasma-derived	14	No	Adults	43% or recombinant
75	France	GenHevac B (20 μg)	20	No	Adults	55% after 3 doses; 78% of 9 nonresponders after 3 additional doses
76	Brazil	Engerix-B (20 μg):		No		% seroconversion by dose and CD4%:
		CD4 <350	38		Adults	26%
		CD4 ≥350	56		Adults	39%
		Engerix-B (40 μg):				
		CD4 <350	42		Adults	24%
		CD4 ≥350	56		Adults	64%
77	United States	Engerix-B or Recombivax (40 μg)	116	Most	Adults	53%; all receiving dialysis; most receiving HAART
78	United States	Engerix-B or Recombivax	57	Most	Adults	37%; most receiving HAART
79	Brazil	Euvax (40 μg)	47	Some	Adults	64%
80	Thailand	Engerix-B (20 μg)	65	Most	Adults	46%
81	Canada	Recombivax (40 μg) intradermal	12	Most	Adults	50%; all previously failed to respond to 6 intramuscular doses
82	Thailand	Engerix-B (20 μg)	28	Yes	Adults	71%
83	United States	Engerix-B or Twinrix (20 μg)	97	Some	Adults	44%; better response in patients receiving HAART
84	United States	Not available	626	Some	Adults	35%; 49% receiving HAART
85	United States	Recombivax (40 μg)	363	Some	Adults	35%; 85% of 40 who received 3 double doses
86	Thailand	Hepavax-Gene:		Yes	≥18 y	% with anti-HBs ≥10 mIU/mL 6 m after last dose:
		3 doses (20 μg)	44			70%
		4 doses (20 μg)	44			86%
		4 doses (40 μg)	44			89%
87	Kenya	Euvax B, Revacc B or Shanvac B (20 μg)	310	No	≥18 y	64% 6 months after last dose; 71% of nonresponders responded to revaccination; lower response than among HIV-uninfected participants

[a]Follow-up study of children previously described by Diamant EP, Schechter CB, Hodes DS, et al. Immunogenicity of hepatitis B vaccine in human immunodeficiency virus infected children. *Pediatr Infect Dis J*. 1993;12:877–878.

DPT-HB, diphtheria, pertussis, tetanus–hepatitis B; HAART, highly active antiretroviral therapy; HAV, hepatitis A virus; HBV, hepatitis B virus; rDNA, ribosomal DNA.

responses.[69] With improvements in early infant diagnosis and initiation of HAART at younger ages, infants may receive the primary vaccine series while receiving treatment. In South Africa, infants initiating HAART approximately 1 week prior to beginning the primary vaccine series had similar serologic responses to HBV (98.8%) as HIV-uninfected (100%) and HIV-exposed-but-uninfected (99.2%) infants.[44]

Adults

Most HIV-1–infected adults have poor responses to hepatitis B vaccine.[90,91] Doubling the dose of hepatitis B vaccine increased the antibody response among HIV-1–infected adolescents and adults,[76,92–96] but may be less effective among persons with lower CD4 counts.[76] In other studies, 85% to 100% of previous HBV nonresponders developed protective antibody titers after three double doses of hepatitis B vaccine.[85,97] HAART was associated with better short-term and long-term responses to hepatitis B vaccine in some, but not all, studies of HIV-1–infected adults.[83,84,98] Higher CD4 counts and lower HIV viral loads at vaccination are also associated with better responses in some, but not all, studies.[70,92,99–103] Other novel strategies to improve responses to hepatitis B vaccine, such as use of adjuvants and intradermal administration, as well as accelerated schedules, have been explored with some success.[93,102,104–106]

Hepatitis A Vaccine

Children

As with hepatitis B vaccine, HIV-1–infected children not receiving HAART are less likely to develop a protective antibody response following vaccination against hepatitis A and have lower antibody titers than do HIV-1–uninfected persons (76% vs 100% 6 months after third dose).[107]

Among children receiving HAART, vaccine response rates were high, with approximately 72% to 100% of children developing protective antibodies to hepatitis A virus 1 month after primary vaccination.[38,108–111] However, immunity waned over time.[38,109] A third dose of vaccine induced higher antibody concentrations than after two doses. Immune status at the time of vaccination was correlated with response to hepatitis A vaccine.[109,110,112]

Adults

HIV-1–infected adults not receiving HAART are less likely to develop a protective antibody response following vaccination against hepatitis A and have lower antibody titers than do HIV-1–uninfected persons. Approximately 90% of immunologically normal adults, but only 48% to 90% of HIV-1–infected adults not receiving HAART, seroconverted following two to three doses of hepatitis A vaccine.[113–123] In one study, the likelihood of seroconversion was associated with a higher CD4 T-lymphocyte count.[120] In an uncontrolled study, 73% of 31 HIV-1–infected adults developed antibodies to hepatitis A vaccine after receiving antiretroviral therapy for at least 48 weeks.[47] Serologic responses persisted 6 to 10 years after vaccination in adults with suppressed HIV viral loads.[124]

Inactivated Influenza Vaccine

Children

Most studies of inactivated influenza vaccine have been conducted among children receiving HAART and found seroprotection rates (as defined by each study) of 57% to 100% for influenza A/H1N1 (including pH1N1 2009),[125] 50% to 100% for influenza A/H3N2, and 56% to 76% for influenza B.[126–131] The proportion of children developing protective immunity seems to be similar in HIV-infected and HIV-uninfected children,[128,129,131] although geometric mean titers were sometimes higher in uninfected children.[128,129,131,132] Few predictors of response were consistently identified.[128–130,133] Influenza vaccine efficacy was only 18% in a double-blind, placebo-controlled trial of influenza vaccine conducted in South Africa among HIV-1–infected children, perhaps attributable to poor immunogenicity and genetic drift of the H3N2 wild-type virus.[134]

Adults

Limited data suggest that influenza vaccine is effective in preventing symptomatic influenza infection in many HIV-1–infected adults.[135–137] In a double-blind, randomized clinical trial of the safety, immunogenicity, and efficacy of trivalent inactivated influenza vaccine in 506 HIV-1–infected South African adults, vaccine efficacy was 76% against confirmed influenza illness and seroconversion rates as measured by hemagglutination inhibition antibody titers (>1 : 40 if baseline titers were <1 : 10 or a greater than fourfold increase in those with baseline titers >1 : 10) were 53% for H1N1, 61% for H3N2, and 54% for influenza B virus.[138] Influenza vaccine was most effective in HIV-1–infected persons with CD4 T-lymphocyte counts greater than 100 cells/mm^3 during an outbreak at a residential facility in New York City.[139] In persons with severe immune suppression, a second dose of influenza vaccine did not result in greater immunogenicity.[140,141] In general, HIV-1–infected adults developed protective antibody titers after receipt of the 2009 pandemic H1N1 influenza vaccine, particularly in conjunction with adjuvants[142–147] and with higher antigen content.[148] A systematic review of influenza vaccination of HIV-1-infected persons concluded that inactivated influenza vaccine is effective in preventing influenza infection in HIV-infected adults but not in HIV-infected young children.[149]

Vaccination of HIV-1–infected pregnant women against influenza is a potential strategy to protect both mother and child. Influenza vaccination of HIV-1–infected pregnant women receiving antiretroviral therapy in the United States is safe and immunogenic[150]; however, a large trial in South Africa failed to demonstrate protection of infants born to influenza vaccinated HIV-1–infected women.[151] HIV-exposed infants had lower antibody levels at birth but similar antibody levels after 8 weeks of life.[152]

Haemophilus influenzae Type B Polysaccharide-Protein Conjugate Vaccines

Children

A wide range of 16% to 86% of HIV-1–infected children not receiving HAART developed protective antibody responses following three doses of *Haemophilus influenzae* type b conjugate vaccines, and geometric mean titers were lower than in HIV-1–uninfected children of similar ages (Table 70.4).[153,154] Vaccine effectiveness was lower in HIV-1–infected South African children (44%) compared with uninfected children (95%),[154] and the estimated risk of vaccine failure was 35-fold (95% confidence interval, 15 to 84) higher in HIV-1–infected than uninfected children.[155] Antibody concentrations waned more rapidly in HIV-1–infected children.[156] However, booster doses of vaccine can induce rapid increases in antibody in some HIV-1–infected children.[157–160]

Few studies of immune responses to *H. influenzae* type b vaccine have been conducted among children receiving HAART. In one study of 18 previously vaccinated children, 78% had detectable antibodies after HAART initiation.[36] In South Africa, HIV-infected infants initiating HAART approximately 1 week prior to beginning the primary vaccine series experienced similar responses 1 month after the last dose (99.4%) compared to HIV-uninfected (97.4%) and HIV-exposed, uninfected (99.2%) infants.[44] HIV-infected infants initiating HAART at older ages also had similar responses (97.3%).[44]

Pneumococcal Polysaccharide and Polysaccharide-Protein Conjugate Vaccines

Children

Antibody responses to pneumococcal conjugate vaccines among children not receiving HAART were high, with the proportion of children with antibody levels above a specified threshold ranging from 63% to 93% by serotype, but were generally lower than among uninfected children (see Table 70.4). The quality of antibodies as measured with an opsonophagocytic assay was deficient among HIV-1–infected children in South Africa,[154] and significant waning of antibody concentrations was found 5 years after vaccination, such that the proportion with antibody concentrations ≥0.35 μg/mL ranged only from 5% to 24%.[174] Provision of a booster dose improved responses for only three of seven serotypes.[174] Vaccine efficacy was lower in a clinical trial of HIV-1–infected South African

TABLE 70.4 Immunogenicity of *Haemophilus influenzae* Type B and Pneumococcal Conjugate Vaccines in HIV-1–Infected Children and Adults

Reference	Country	Vaccine	No. of Subjects	HAART	Age Group	Immunogenicity or Effectiveness
***HAEMOPHILUS INFLUENZAE* TYPE B CONJUGATE VACCINES**						
Children						
157, 159	United States	PRP-CRM_{197}	19	No	15–56 mo	16% immune 4–85 mo after vaccination; 77% after revaccination
153	United States	PRP-CRM_{197}	23	No	1–5 mo	82% of asymptomatic and of 33% symptomatic children immune; similar responses in asymptomatic and control children
156, 161	United States	PRP-tetanus toxoid	56	No	1–13 y	86% with protective titers compared with 100% of uninfected children after 1 mo; 57% at 1 y
158	United States	PRP-CRM_{197}	39	No	Mean: 16.6 mo at booster	46% immune after primary series; 63% after booster; titers lower than in control subjects
162	United States	PRP-CRM_{197}	18	No	6–19 mo	28% immune after primary series; 45% after booster dose
163	United States	PRP-CRM_{197}	35	No	9 mo	74% immune after primary series; 76% after booster dose; no difference with control subjects
155	South Africa	PRP-CRM_{197}	[a]	No		Reduced effectiveness with HIV-1 infection
36	United States	PRP-CRM_{197}	4	Yes	3–14 y	75% with detectable antibodies
164	Thailand	PRP-tetanus toxoid	41	No	24–84 mo	67% with anti-PRP ≥0.15 μg/mL
165	South Africa	PRP-CRM_{197}	66	No	Mean: 3.7 mo at third dose	52% immune compared with 95% in control subjects; correlated with disease stage
Adults						
166	United States	PRP and PRP-CRM_{197}	181	No	Adults	Better antibody response to PRP-CRM_{197} except in people with AIDS
167	Netherlands	PRP-tetanus toxoid	54	No	Adults	Poor antibody response in people with low CD4 cells
168	Brazil	PRP-OMP	60	No	Adults	38% immune; titers lower than in control subjects
PNEUMOCOCCAL CONJUGATE VACCINES						
Children						
169, 170	United States	5-valent CRM_{197}	17	No	≤2 y	78% immune compared with 88% in control subjects
171	United States	7-valent CRM_{197}	30	70%	56–180 days	Variable antibody response by serotype (88% to 100%); good response to booster
172, 173	South Africa	9-valent CRM_{197}	[a]	No	0–3 y	Efficacy 65% compared with 83% in control subjects after 2 y; efficacy 39% compared with 78% in control subjects after 6 y
165, 174	South Africa	9-valent CRM_{197}	30	No	Mean: 3.7 mo after third dose	Variable antibody response by serotype (63% to 93%); similar GMT but lower functional antibodies than control subjects
160	Greece	7-valent CRM_{197}	14	78%	20–163 mo	29% immune compared with 57% in control subjects; no effect of booster dose
175	Spain	7-valent CRM_{197}	56	Yes	3–19 y	Variable antibody response by serotype (29% to 50%)
176, 177	United States	7-valent CRM_{197} + PPV	225	Yes	2 to <19 y	Variable antibody response by serotype (76% to 96%); some waning over 5-year follow-up but good response to booster with either PCV7 or PPV; correlated with immune status and viral load
178	Brazil	7-valent CRM_{197}	40	Yes	2–9 y	65% immune; variable response by serotype (45% to 90%)

TABLE 70.4 Immunogenicity of *Haemophilus influenzae* Type B and Pneumococcal Conjugate Vaccines in HIV-1–Infected Children and Adults *(Continued)*

Reference	Country	Vaccine	No. of Subjects	HAART	Age Group	Immunogenicity or Effectiveness
179, 180	South Africa	7-valent CRM_{197}	249	Yes	6–12 wk	Variable antibody response by serotype (92% to 100%) for infants receiving HAART; similar antibody response for non-HAART status and control subjects; lower functional antibodies for non-HAART
181	United Kingdom	13-valent CRM_{197}	48	Yes	2 mo to18 y	Variable antibody response by serotype (65% to 98%); lower than HIV-uninfected adult controls; correlated with viral load
182	South Africa and Romania	13-valent CRM_{197} + PPV	150	98%	6–17 y	Increase in IgG GMCs for all serotypes
183	Thailand	7-valent CRM_{197}	59	90%	2 mo to 9 y	Antibody response varied by serotype (85% to 98%); similar to HIV-exposed but uninfected children
Adults						
184	United States	5-valent CRM_{197}	141	No	Adults	Similar GMT to polysaccharide vaccine
185	United States	7-valent CRM_{197} + second dose or PPV	67	42%	≥17 y	Increase in GMCs after 1 dose; no increases observed with second dose of either conjugate or polysaccharide vaccine
186	Uganda	7-valent CRM_{197}	109	No	Adults	Antibodies correlated with CD4 cell counts
187	United States	13-valent CRM_{197}	329 (all previously vaccinated with PPV)	95.4%	≥18 y	Increase in GMCs after 3 doses for all serotypes
182	South Africa and Romania	13-valent CRM_{197} + PPV	151	97%	≥18 y	Increase in IgG GMCs for all serotypes
188	Brazil	PPV or 7-valent CRM_{197} or 7-valent CRM_{197} ± PPV	331	77%	18–60 y	Good antibody response to PPV or 7-valent CRM_{197}; no added benefit of second dose of PPV
189	Taiwan	7-valent CRM_{197} or PPV	208	86%	≥20 y	Better antibody response with PCV7 than PPV; correlated with viral load
190	Taiwan	7-valent CRM_{197} or PPV	221 (all previously vaccinated with PPV)	96%	≥20 y	Better antibody response with 2 doses than 1 dose of PCV7 or 1 dose of PPV
191	Taiwan	7-valent CRM_{197}	230	72%	≥20 y	Better antibody response with 2 doses than 1 dose
192	Malawi	7-valent CRM_{197}	219	41%	Adults	74% vaccine efficacy

[a]Population-based study.

GMC, geometric mean concentration; GMT, geometric mean antibody titer; HAART, highly active antiretroviral therapy; IgG, immunoglobulin G; PPV, pneumococcal polysaccharide vaccine; PRP-OMP, polyribosylribitol phosphate–outer membrane protein.

children (65%) and decreased over time (39%) compared with uninfected children (83%).[172,173] Vaccine effectiveness was not demonstrated in a matched case-control study of HIV-1–infected children[193] in South Africa.

Among children receiving HAART, responses to pneumococcal vaccines varied by serotype, ranging from 29% to 100% based on antibody levels above specified thresholds or an increase from baseline, with some evidence of waning immunity.[160,171,177] The benefits of a booster dose are unclear because studies have reported conflicting results.[160,171,175–177] Immune responses by immunological status and viral load suppression were also inconsistent.[154,171,175,176,181] Children in whom HAART was initiated in infancy had responses similar to uninfected children and better responses than children in whom HAART was initiated later in childhood.[46] Another study of early and deferred HAART among infants found similar responses between the two groups and similar to uninfected children, but lower levels of functional antibodies among infants in the deferred HAART group.[179,180]

Adults

Response to 23-valent pneumococcal polysaccharide vaccine is poorer in HIV-1–infected than in uninfected persons, but better in persons receiving HAART.[194–197] However, antibody responses declined even in persons receiving HAART.[198] The 23-valent pneumococcal vaccine reduced the risk of pneumococcal disease in 305 HIV-1–infected adult vaccine recipients

receiving HAART in Taiwan[199] and the risk of pneumonia in a cohort of 23,255 HIV-1–infected adults in the United States.[200] However, in HIV-1–infected Ugandan adults, the 23-valent polysaccharide pneumococcal vaccine was found to be ineffective in preventing first episodes of invasive pneumococcal disease.[201]

The antibody response to a glycoprotein conjugate pneumococcal vaccine was better than that of the polysaccharide vaccine in HIV-1–infected persons, except for adults with CD4 T-lymphocyte counts less than 200 cells/mm^3.[184,202,203] However, the antibody response to specific pneumococcal polysaccharide serotypes varies, with some serotypes eliciting poor antibody responses in HIV-1–infected persons with CD4 T-lymphocyte counts less than 200 cells/mm^3.[204] In a placebo-controlled trial of seven-valent pneumococcal vaccine in predominantly HIV-1–infected adults in Malawi, all of whom had prior invasive pneumococcal disease, vaccine efficacy against invasive pneumococcal disease was 74%.[192] Revaccination with conjugate pneumococcal vaccine resulted in a higher proportion of HIV-1–infected adults with protective antibody levels than adults vaccinated with polysaccharide vaccine, but this difference was not sustained after 6 months.[205] Studies of the benefits of a second dose of conjugate pneumococcal vaccine or polysaccharide vaccine have produced inconsistent results.[206]

Meningococcal Polysaccharide and Conjugate Vaccines

Few data are available regarding responses to meningococcal polysaccharide vaccine in HIV-1–infected children. The proportion of children achieving a fourfold or greater increase in rabbit serum bactericidal antibody titers in a trial (International Maternal Pediatric Adolescent AIDS Clinical Trials [IMPAACT] P1065) of the immunogenicity of a meningococcal polysaccharide diphtheria toxoid conjugate vaccine among 319 HIV-1–infected adolescents 11 to 24 years old, the majority of whom were receiving HAART, were 86% for serogroup A, 55% for serogroup C, 72% for serogroup W-135, and 73% for serogroup Y, although some level of immunity also was detectable at baseline.[207] Response rates were positively correlated with better immunological status and inversely with HIV-1 viral load. In the same trial, vaccine immunogenicity among 59 HIV-1–infected children 2 to 10 years of age was assessed and seroprotective titers were achieved in 78% for serogroup A, 46% for serogroup C, 93% for serogroup W-135, and 91% for serogroup Y by 72 weeks after vaccination.[208] Improved responses were seen with a second dose administered 6 months after the initial dose.[209] Studies of the meningococcal C conjugate vaccine in HIV-1–infected children demonstrated significantly lower serologic responses than those seen in uninfected children.[210,211] The Advisory Committee on Immunization Practices recommends a primary series of two doses of the meningococcal conjugate vaccine for HIV-infected adolescents 11 through 18 years old.[212]

Rabies Vaccine

Antibody responses to an eight-site intradermal rabies vaccination regimen using the purified chick embryo cell rabies vaccine resulted in good antibody responses among 27 HIV-infected adults regardless of CD4 T-lymphocyte count,[213] although another study suggested poor antibody responses to the human diploid cell rabies vaccine in HIV-infected persons with low CD4 T-lymphocyte counts.[214] Antiretroviral therapy may improve the immunogenicity of rabies vaccine but not to levels found in those of uninfected persons.[215]

Human Papillomavirus Vaccines

The human papillomavirus vaccine is recommended for HIV-1–infected and uninfected children.[19] The quadrivalent human papillomavirus vaccine was found to be safe and immunogenic in a study of 126 HIV-1–infected children 7 to 12 years old[216] and stimulated mucosal antibodies and cytotoxic T lymphocytes.[217] Studies in HIV-infected girls and women showed the quadrivalent human papilloma vaccine to be safe and immunogenic,[218–221] with one study finding poorer responses in women with HIV viral loads greater than 10,000 copies/mL and CD4 T lymphocyte counts less than 200 cells/μL.[222]

Japanese Encephalitis Vaccine

HIV-1–infected children have a poor response to mouse brain–derived, inactivated Japanese encephalitis vaccine, but a higher proportion responded to revaccination while receiving HAART[223,224] and levels of protective antibodies persisted for at least 3 years.[225] A cell culture–based vaccine for Japanese encephalitis is available, although no data are available on its use in HIV-infected persons.

Safety of Nonreplicating Vaccines

Nonreplicating vaccines are not associated with increased risks of complications in immunocompromised persons. However, a study of HIV-1–infected Ugandan adults found an increased incidence of pneumonia in recipients of 23-valent pneumococcal polysaccharide vaccine compared with unvaccinated HIV-1–infected adults.[201] The authors hypothesized that immunization resulted in destruction of polysaccharide-responsive B-cell clones, but no specific data to support this hypothesis were provided and this observation has not been corroborated in other studies. Surprisingly, 6-year follow-up of this same population confirmed excess all-cause pneumonia, but an overall survival advantage persisted among vaccinated persons.[226]

Activation of T lymphocytes following immunization could potentially augment HIV-1 replication and result in more rapid progression of HIV-1 disease. Several studies, but not all, described transient elevations of HIV-1 RNA plasma levels lasting several days following immunization with tetanus toxoid,[227,228] influenza,[229–233] pneumococcal,[234,235] and hepatitis B[103,236] vaccines. None of these investigations found prolonged elevation of HIV-1 RNA viral load, decreased CD4 T-lymphocyte counts, or accelerated HIV-1 disease progression following immunization. Although the transient rise in HIV-1 viral load following administration of tetanus toxoid to pregnant women could theoretically affect the risk of maternal-infant HIV-1 transmission, an increased risk of transmission is unlikely if vaccination occurs at least 4 weeks before delivery.[237]

LIVE BACTERIAL VACCINES

Bacille Calmette-Guérin Vaccine

The BCG vaccine is not recommended for children known to have HIV-1 infection because of the increased risk of disseminated BCG disease many months, or even years, after vaccination.[21,22] The effectiveness of BCG in HIV-1–infected persons may be lower than in uninfected persons, and there are numerous reports of regional lymphadenitis, poorly healing ulcers, fistulas, and disseminated disease developing in HIV-1–infected infants, children, and adults.[238,239] HIV-exposed, uninfected infants may have impaired cellular immune responses

to BCG[13] (although findings are not consistent)[240] and may benefit from delayed administration until 8 weeks of age[241] and after HIV infection is excluded.

Typhoid Vaccine

Few studies of live typhoid vaccine in HIV-infected individuals have been published. An increased risk of diarrhea was reported following administration of the oral typhoid vaccine Ty21a to 42 HIV-infected Zambian adults.[4]

LIVE VIRUS VACCINES

Oral Poliovirus Vaccine

Children

The proportion of HIV-1–infected children who responded to three doses of live attenuated poliovirus vaccine was greater than 90% in many studies, although titers tended to be lower compared to those in HIV-1-uninfected children (Table 70.5). Few studies of the immune response to oral poliovirus vaccine have been conducted among children receiving HAART. In addition, no direct estimates of poliovirus vaccine efficacy have been conducted in HIV-1–infected children. However, all three wild-type polioviruses have been successfully eliminated from countries with high prevalence of HIV-1 infection.

Safety of Oral Poliovirus Vaccine in HIV-1–Infected Persons

The risk of vaccine-associated paralytic poliomyelitis (VAPP) is increased in persons with primary B-cell immunodeficiency disorders.[248] Nevertheless, more than 1000 HIV-1–infected children received at least one dose of oral polio vaccine without complications in the United States before it was known that they or their mothers were HIV-1–infected, and millions of HIV-1–infected children in other countries have been vaccinated. Only two HIV-1–infected children have been reported to have VAPP following receipt of oral polio vaccine (Table 70.6): one 2-year-old, HIV-1–infected Romanian girl[246] and one child in Zimbabwe.[247] In Romania, the rate of VAPP in all children was approximately 10-fold higher than the estimated 1 case per 2.5 million doses administered in the United States and Europe, most likely related to simultaneous administration of multiple injections.[249] Thus, HIV-1 infection in these two children with VAPP could be chance associations and not evidence of an increased risk associated with HIV-1 infection. If the risk of VAPP is increased in HIV-1–infected persons, the attributable risk is very low.

Persons with inherited B-cell deficiency disorders can excrete vaccine-derived polioviruses for more than 2 decades.[268] However, prolonged shedding of polioviruses among HIV-infected children[269,270] and adults has not been observed.[269,271,272] A study in Zimbabwe of 92 HIV-1–infected children found

TABLE 70.5 Immunogenicity and Safety of Poliovirus Vaccines in HIV-1–Infected Children

Reference	Country	Vaccine	No. of Subjects	HAART	Age	Safety	HIV-Infected Persons Developing Protective Antibody Titers After ≥3 Doses
29	France	OPV	15	No	4–24 mo	No adverse events	40% to type 2; 33% to types 1 and 3
242	United States	OPV	23	No	1–180 mo	No adverse events	91%; lower titers with advanced disease
243	United States	OPV	180	No	1–132 mo	No adverse events	Immunogenicity not studied
49	Italy	IPV	9	No	3–13 mo	No adverse events	100% to types 1 and 2; 88% to type 3
30	Italy	OPV and/or IPV	12	No	18–84 mo	No adverse events	100% to type 2; 92% to types 1 and 3; decrease in titers over 2 y in 4 children studied
33	Zaire	OPV	48	No	<4 mo	No adverse events	97% to all types; titers lower than in control subjects
244	Zimbabwe	OPV	21	No	2 mo to 2 y	No adverse events	52% to type 1; 62% to type 2; 33% to type 3; lower titers than HIV-exposed but uninfected children
14	Zambia	OPV	17	No	18 mo	No adverse events	65% to type 2; lower titers than HIV-uninfected children
245	Zimbabwe	OPV	92	67%	9 mo	No adverse events	69% to type 1, 74% to type 2, 37% to type 3; lower titers than HIV-uninfected children
246	Romania	OPV	1	No	26 mo	Flaccid paralysis; vaccine strain type 2 in stool	Lacked antibody titers to all 3 types despite receipt of 4 doses of OPV
247	Zimbabwe	OPV	1	No	4 y	Paralysis of right leg	Lacked antibodies to polioviruses types 1 and 3 wk after second OPV despite having received OPV during first year of life

IPV, inactivated polio vaccine; OPV, oral polio vaccine.

TABLE 70.6 Prospective Studies of the Immunogenicity of Measles Vaccine in HIV-1–Infected Children

Reference	Country	No. of Children	HAART	Age	Response to Primary Immunization	Response to Repeat Immunization
250	Zaire	37	No	9 mo	36% of 11 symptomatic; 77% of 26 asymptomatic	NA
251	United States	8	No	11–41 mo	25%	NA
252[a]	United States	35	No	12–194 mo	37%	0%
253	United States	2	No	NA	NA	50%
254	United States	4	No	22–121 mo	NA	0%
255	United States	11	No	72–120 mo	NA	66%
256	United States	7	No	31–120 mo	NA	14%
257	Thailand	16	No	9 mo	57%	NA
258	United States	14	Yes	2–11 y	NA	64%
36	United States	18	Yes	3–14 y	NA	83%
259	Zambia	50	No	2–8 mo	88%	NA
260, 261	Malawi	45	No	6 mo	59%; immunity waned after 12 mo	64% revaccination at 9 mo
35	Kenya	18	Yes	NA	NA	78%
262, 263	Thailand	51	Yes	Mean: 10.2 y	NA	90% after 1 mo; 85% after 3 y
264	United States	15	No	6–12 mo	67% after 1 dose	83% after 2 doses
265	United States	193	Yes	2–19 y	52%	89% at 8 wk and 80% at 80 wk
266	Zambia	169	Yes	9–60 mo	23%	95% of children receiving HAART
267	South Africa	297	Immediate, deferred, or interrupted HAART	6–12 wk	17–42%	NA

[a]Four children received repeated immunization.
HAART, highly active antiretroviral therapy; NA, not available.

significantly higher titers of poliovirus shedding in HIV-1–infected children within 42 days of three or more doses of vaccine, but no evidence of prolonged vaccine virus shedding.[245] Persistent excretion by HIV-1–infected persons is unlikely to be a source of poliovirus transmission to others.

Measles Vaccine

Children

The antibody response to measles vaccine is impaired in HIV-1–infected children not receiving HAART (see Table 70.6).[273] In a metaanalysis of studies of measles vaccination of HIV-1–infected children, levels of serologic response varied.[274] One study of standard-titer measles vaccine at 6 and 9 months of age found 59% of HIV-1–infected children were seropositive after vaccination at 6 months but only 64% after vaccination again at 9 months of age.[260] In contrast, 88% of 50 HIV-1–infected children in Zambia developed antibody levels of 120 mIU/mL or more within 6 months of vaccination compared with 94% of 98 HIV-seronegative children and 94% of 211 HIV-seropositive but uninfected children (P = .3).[259] However, these HIV-1–infected children had lower antibody avidity[275] and experienced waning antibody levels and lower frequency of protective antibody titers by 2 to 3 years of age.[259] The response to a second dose of measles vaccine in five studies of children not receiving antiretroviral therapy was variable, but generally poor.[273] In cross-sectional studies among HIV-infected children, the prevalence of measles antibody after vaccination varied widely, ranging from 17% to 100%.[16,266,273,274,276] An association between lack of measles virus-specific antibodies after vaccination and low CD4 T-lymphocyte count was documented in some studies.[273,274,276]

Placental transfer of maternal antibodies, including antibody to measles virus, is impaired in HIV-1–infected women[277–281] and was associated with high maternal HIV-1 viral load in one study.[282] The lower levels of maternal antibody correlated with an improved response of their HIV-1–infected and –uninfected infants to measles vaccine administered at 6 months of age.

Few data exist on the primary immune response to measles vaccine among children receiving HAART. Initiation of HAART before primary vaccination may result in higher production and maintenance of antibodies and protective immunity. A cross-sectional study in Italy found that children in whom HAART was initiated in infancy, likely before measles vaccination, maintained high levels of protective antibodies (82%) compared with children in whom HAART was initiated later in childhood (40%), and levels were similar to those found in healthy HIV-uninfected control children (100%).[46] Several studies have been conducted on the response to vaccination or revaccination after initiating HAART (see Table 70.6) and suggest that children receiving HAART are more likely to respond to revaccination than are children not receiving HAART.[258] Within the first 3 months after vaccination, the proportion of children responding to revaccination, as defined by each study, was 64% to 90%.[35,36,258,262] A study of 428

HIV-1–infected children 7 to 15 years of age in the United States found that the number of vaccine doses received while on antiretroviral therapy was positively associated with seroprotection.[16] However, antiretroviral therapy does not restore immunity lost prior to treatment. In a prospective study of 169 HIV-1–infected Zambian children, subsequent HAART was not associated with seroconversion.[266] Children vaccinated while receiving HAART may experience waning immunity,[36] although in Thailand the prevalence of protective antibodies to measles virus 3 years after revaccination of HIV-1–infected children receiving antiretroviral therapy was 85% compared with 88% 1 month after revaccination.[263] HIV-1–infected children receiving HAART would likely benefit from revaccination.[283] In 2012, the Advisory Committee on Immunization Practices recommended measles revaccination of HIV-1–infected children who were vaccinated before treatment with two doses of measles vaccine once effective therapy had been established.[284] The World Health Organization made a similar recommendation in December 2015.[284a]

Adults

The majority of HIV-1–infected adults vaccinated as children were seropositive.[285,286] Waning immunity was observed among revaccinated HIV-1–infected, measles-seronegative adults, despite HAART.[287]

Safety of Measles Vaccine in HIV-1–Infected Persons

Prospective studies revealed no increased risk of adverse events in the few weeks following immunization with standard- and high-titer measles vaccines for HIV-1–infected children not receiving antiretroviral therapy compared with uninfected children.[259,260,273] A retrospective survey conducted by the New York City Department of Health found no complications following measles immunization of HIV-1–infected children,[243] and no evidence of persistent measles vaccine virus excretion was found in 10 HIV-1–infected children immunized with measles-mumps-rubella (MMR) vaccine.[254] A systematic review concluded that measles vaccines appear to be safe in HIV-1–infected children, but the evidence is limited.[274]

Only one fatal adverse event has been reported following administration of measles vaccine to an HIV-1–infected person.[288,289] A 20-year-old HIV-1–infected man, who had a very low CD4 T-lymphocyte count at the time he received a second dose of MMR vaccine, developed cough and progressive pulmonary infiltrates 10 months after immunization. An open lung biopsy showed giant cell pneumonitis, and measles vaccine virus was identified in the lung tissue. He died several months later of encephalitis, presumably from cytomegalovirus and progressive pneumonitis attributable in part to measles vaccine virus. An HIV-1–infected child with a CD4 T-lymphocyte count of 340/μL who received MMR vaccine developed rash and interstitial pneumonia, from which he recovered, and vaccine-strain measles virus RNA was present in his blood.[290]

Mumps and Rubella Vaccines

Children

Several studies suggested that HIV-1–infected children not receiving HAART are as likely to develop protective antibody titers to mumps and rubella vaccines as HIV-1–uninfected children.[253,291,292] However, HIV-1–infected children 7 to 15 years of age in the United States were less likely to have serological evidence of protection against mumps (59% vs 97%) and rubella (65% vs 98%) than HIV-exposed uninfected children,[16] with receipt of antiretroviral therapy a strong predictor of immunity. No cases of measles, mumps, or rubella were observed in vaccinated HIV-1–infected children followed in the European Collaborative Study,[27] and no serious adverse events resulting from mumps or rubella vaccines have been reported in HIV-1–infected children. Revaccination has not been found to have a significant effect on mumps and rubella antibody concentrations.[254,291]

Limited data are available on the response to mumps and rubella among children receiving HAART. A study from Brazil evaluated antibodies to mumps among children in whom HAART was initiated in infancy, likely before primary vaccination. HIV-1–infected children had lower responses (80%) compared with HIV-uninfected children (94% to 100%), although responses were higher among children without severe immunosuppression than those with it (100% vs 62%).[293] Another study in Thailand evaluated the response to revaccination among children receiving HAART and found that 100% and 69% of children without detectable antibodies at baseline developed protective antibodies to rubella and mumps viruses 1 month after revaccination, respectively.[262] After 3 years, the proportion of children with protective antibodies decreased to 79% for rubella and 61% for mumps.[263]

Adults

A cross-sectional survey in the United Kingdom found that 12% of HIV-1–infected adults lacked protective antibodies to mumps virus, but the study lacked a comparison group of HIV-uninfected persons.[294]

Rotavirus Vaccines

Few studies have assessed the efficacy, immunogenicity, or safety of rotavirus vaccine in HIV-1–infected children, and limited data suggest rotavirus infection is not more frequent or more severe in HIV-1–infected children.[295] Two multicountry trials of rotavirus vaccine in Africa included HIV-1–infected infants, although estimates of vaccine efficacy or immunogenicity were not reported separately for infected and uninfected the children.[296,297] Three doses of human rotavirus vaccine RIX4414 were found to be safe and immunogenic in a placebo-controlled study of HIV-1–infected infants in South Africa.[298] Serious adverse events were not significantly more common among 37 HIV-1–infected Kenyan children following receipt of the pentavalent rotavirus vaccine than among HIV-uninfected children, although the study power was limited.[299]

Varicella Virus Vaccine

Children

Varicella vaccine was safe and immunogenic (60% with detectable antibodies), as determined by antibody and lymphocyte proliferation assay responses, in mildly symptomatic American HIV-1–infected children.[300] However, disseminated vaccine-strain varicella virus was reported in an HIV-1–infected child with a CD4 T-lymphocyte count of 8 cells/mm^3.[301] Based on these findings, HIV-1–infected children with CD4 T-lymphocyte percentages of 15 or more should receive two doses of varicella vaccine.[19,302]

Varicella vaccine has been found to be safe and immunogenic among children receiving HAART. No serious adverse events have been reported, and 67% to 79% of children had an antibody response within the first 3 months after vac-

cination, although waning immunity was found, with only 30% to 65% having persistent antibody 1 year after vaccination.[303–305] Children receiving HAART were as responsive to vaccination as asymptomatic and untreated HIV-infected children,[304] but they were less likely to respond than HIV-uninfected control children.[306] Providing booster doses to improve immunity did not result in sustained antibody titers among children with a history of varicella infection.[307] In a study that observed children for the development of varicella or herpes zoster in the prevaccine and postvaccine eras, vaccine effectiveness was determined to be 82% against varicella and 100% against herpes zoster.[308]

Adults

In a randomized, placebo-controlled study of two doses of varicella vaccine in 67 HIV-1–infected adults and 15 uninfected adults, the vaccine was found to be safe but only moderately immunogenic as measured by cellular immune responses.[309] Disseminated varicella resulting from the vaccine strain has been reported in HIV-1–infected adults.[310] The herpes zoster vaccine was reported to be safe and immunogenic in HIV-1–infected adults.[311,312]

Yellow Fever Virus Vaccine

Children

Limited data suggest that HIV-1–infected children respond poorly to yellow fever vaccine. Only 17% of 18 HIV-1–infected children developed an antibody response to yellow fever vaccine compared with 74% of 57 HIV-1–uninfected children.[313] No data are available on children receiving HAART or on protection against disease following yellow fever vaccination.

Adults

A Cochrane review of yellow fever vaccination of HIV-1–infected persons identified three cohort studies involving 484 patients and concluded that HIV-1–infected individuals developed significantly lower concentrations of neutralizing antibodies in the first year after vaccination compared to uninfected patients.[314] Lower HIV viral load and higher CD4 T lymphocyte counts were associated with higher antibody titers.[314–316] Within the Swiss HIV Cohort Study, 83% of 78 HIV-1–infected adults seroconverted following receipt of yellow fever vaccine compared with 97% of 66 uninfected adults.[317]

Safety of Yellow Fever Virus Vaccine

Few severe complications have been reported as a result of inadvertent immunization of yellow fever vaccine to immunocompromised persons, but experience is limited.[23,318,319] No serious adverse events were reported among 102 HIV-1–infected adults who received yellow fever vaccine,[317] and no adverse events were observed following yellow fever vaccination of 14 other HIV-1–infected adults.[320,321] However, fatal myeloencephalitis caused by yellow fever vaccine was reported in a 53-year-old HIV-infected man in Thailand who was asymptomatic but had high plasma HIV viral load levels and low CD4 T lymphocyte counts.[322]

Live Attenuated Influenza Virus Vaccine

Children

The trivalent, cold-adapted, live, attenuated influenza vaccine was shown to be safe in studies of HIV-1–infected children receiving antiretroviral therapy.[130,323,324] After two doses of vaccine, 77% of the HIV-1–infected children and 83% of the control children had a fourfold or greater rise in influenza antibody in response to at least one of the three vaccine strains.[323] HIV-infected children receiving the inactivated influenza vaccine tended to have higher antibody responses than children receiving the live attenuated influenza vaccine.[130] Shedding of vaccine virus was detected in 67% of HIV-1–infected and 50% of uninfected children up to 28 days after receiving attenuated influenza vaccine.[325]

Adults

The trivalent, cold-adapted, live, attenuated influenza vaccine was shown to be safe in small studies of HIV-1–infected adults.[126,323,326] Few HIV-1–infected and –uninfected adults developed a seroresponse to the live attenuated influenza vaccine, although most had preexisting titers to the vaccine strains before vaccination.

SUMMARY

HIV-1 infection poses some risk from live vaccines in untreated immunosuppressed patients, and vaccines may be less immunogenic and efficacious than in uninfected persons, but most HIV-1–infected persons can benefit from vaccines.

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71 Vaccines for International Travel

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INTERNATIONAL TRAVEL

People travel for many reasons, including tourism, business, educational experiences, or to flee from war, famine, or other intolerable situations. In 2016, international tourist arrivals exceeded 1.2 billion. The World Tourism Organization predicts that international tourist arrivals may exceed 1.8 billion by 2030.[1] International migrants, a category of traveler not captured in the tourism statistics, included more than 240 million refugees, asylum seekers, migrant workers, illegal migrants, and internally displaced persons in 2015.[2]

Given the growth of international travel, it is likely that many health providers will be called on to offer advice about pretravel immunizations. The International Society of Travel Medicine was started in 1991 to promote healthy and safe travel through education, service, and research activities in the field, and in 2006, the Infectious Diseases Society of America published guidelines on the practice of travel medicine.[3] Although travel health clinics specializing in pretravel advice and immunization are present in many locations, most travelers will not be seen in these clinics before travel. Health professionals of all kinds must be able to provide basic pretravel services and identify patients in need of specialized advice. This chapter focuses on disease risks specific to travelers to destinations where disease risk may be greater than in their home countries and considerations taken in choosing whether a traveler is a candidate for travel-related vaccines. Information about disease epidemiology and vaccine characteristics is presented in detail in other chapters. Because national standards for licensure differ, not all vaccines are available in all countries. Similarly, vaccines against the same disease and recommended vaccine schedules may differ somewhat by manufacturer and national authority.

GENERAL INFORMATION ABOUT IMMUNIZATIONS FOR INTERNATIONAL TRAVEL

Approach to Travel Immunizations

The two steps in immunizing travelers are to update routine immunizations and provide travel-specific immunizations. To do the first, knowledge of a patient's previous immunizations and medical history is necessary. For the second, detailed information about the patient's itinerary, living conditions during travel, mode of travel (e.g., adventure travel or chaperoned luxury tour), and purpose of travel (e.g., medical or veterinary work, tourism, visiting relatives) is needed. Although sometimes mistakenly regarded as a rote selection of vaccines based on destination country, choice of vaccines more often requires thoughtful consideration based on the patient's medical history, vaccine interactions with other vaccines or medications, limitation of time until departure, magnitude of risk of vaccine-preventable diseases, and patient preferences. Cost is a growing factor in a traveler's decision-making process about which vaccines to receive. Travelers with limited means and incomplete insurance coverage may be forced to make decisions about which family members to protect (often choosing children, leaving adults vulnerable) or may choose, because of cost, to limit vaccines to those such as yellow fever vaccine because it is required for entry into some countries. Finally, incremental risk during the voyage needs to be considered. Travelers are originating from countries previously considered only as destination countries, leading to situations in which risk of disease is lower in the destination country; for example, the risk of acquiring typhoid for someone originating in Kolkata, India who plans to visit relatives in East Africa is likely to be lower than at home.

Sources of Information on Travel Vaccines

Sources of health information for international travel include International Travel and Health-Vaccination Requirements and Health Advice; while the print edition has not been available since 2012, electronic updates, especially of the vaccine chapter, are available through the World Health Organization (WHO)[4], Health Information for International Travel (Yellow Book), published by the Centers for Disease Control and Prevention (CDC), Atlanta, GA, and available at the CDC travel health website,[5] and other local and national publications. Many countries publish national guidelines regarding travel health information, and readers are encouraged to base their recommendations on those. Other sources of information are textbooks[6,7] and review articles.[8–16] Many sites on the Internet provide travel health information for health providers and the public, although not all are reliable.

The CDC publishes downloadable vaccine information statements for vaccines available in the United States.[17] These handouts are useful when discussing with patients risk of disease, indications and contraindications for vaccine, adverse events associated with vaccines, and need for and timing of booster doses. Detailed technical recommendations of the Advisory Committee on Immunization Practices (ACIP) for many travel-related vaccines are also available.[18] More comprehensive information on each vaccine can be found in individual chapters in this textbook.

General Considerations Regarding Immunizations

Simultaneous Administration of Vaccines and Immunoglobulin

Scheduling multiple vaccines for the traveler can be challenging, especially when departures are imminent. Although scheduling a consultation at least 4 to 6 weeks before travel is ideal, few travelers allow as long as a month, and many come for consultation only a few days before departure. Whenever the traveler is seen, there is potential for helpful information to be given, and some agents (e.g., hepatitis A vaccine, influenza vaccine when available, updates on routine vaccines), can be given with good effectiveness even on the day of departure. Immunization schedules can be accelerated, or vaccines limited to those most appropriate to the infectious disease risks likely to be faced by the traveler. Although many patients and providers are concerned about the likelihood of adverse reactions to multiple vaccines administered simultaneously, studies performed in travel clinics that evaluated simultaneous

administration of multiple vaccines, including travel vaccines, reported that adverse events were generally minor and not incapacitating.[19,20] The ACIP encourages simultaneous administration of all needed vaccines including settings such as preparation for travel.[21]

Inactivated vaccines can be given concurrently or at any interval before or after other inactivated or live vaccines (see Chapter 9). Live vaccines (such as yellow fever, measles-mumps-rubella [MMR], and varicella) should be administered either simultaneously or at least 28 days apart. When live vaccines are given less than 28 days apart, the second should not be counted as valid and should be repeated at least 28 days from the first administration.[21] Some data exist to support exceptions: oral poliovirus vaccine (OPV), no longer used in most industrialized countries, may be given at any interval before or after parenteral live vaccines; yellow fever vaccine may be given at any interval with respect to monovalent measles vaccine; and Ty21a oral typhoid vaccine may be given at the same time as or at any interval relative to parenteral live vaccines (MMR, varicella, yellow fever).[21,22]

Antibody responses to MMR and varicella vaccines may be impaired if given at the same time as immunoglobulin. MMR and varicella vaccines should be administered at least 2 weeks before immunoglobulin, although they can be administered at the same time, if necessary.[23] If immunoglobulin is given before MMR or varicella vaccines, the appropriate time interval after immunoglobulin for giving these vaccines depends on the dose of immunoglobulin administered; a helpful table detailing appropriate intervals is available.[24] Immunoglobulin has not been shown to interfere with responses to polio, yellow fever, or oral typhoid vaccines.

Immunization of Persons With Altered Immunocompetence

Detailed recommendations regarding immunization of immunocompromised persons are published elsewhere (see Chapters 67 and 68) and are available from the CDC[25] and from the Infectious Diseases Society of America.[21,26,27] Information specific to immunocompromised travelers is available from the CDC.[28] In general, immunocompromised persons should not receive live viral vaccines, but there may be some exceptions. Information specific to travel vaccines is presented in the sections on individual vaccines below.

Effect of Antimalarials and Antimicrobial Agents on Vaccine Response

When antimalarials in the chloroquine/mefloquine family are administered simultaneously with human diploid cell rabies vaccines, they may interfere with immunogenicity of the vaccines.[29] It does not appear that these antimalarials interfere with immunogenicity of oral typhoid vaccine. The combination of antimicrobial agents and oral typhoid vaccine is contraindicated because they would probably interfere with the activity of this live bacterial vaccine.[30,31] Details of these interactions can be found in the sections devoted to individual vaccines below.

Routine Immunizations and Modifications Needed for Travelers

Routine childhood and adult immunizations should be brought up to date as part of preparation for international travel. Information is available from many sources about routine immunizations. In the United States, schedules are updated frequently and are available from the CDC on the Internet.[32] Readers are also referred to country-specific schedules for this information. Some travelers will require accelerated schedules because of imminent departure dates or because of immunization delays. The reader is referred to Chapter 9 for routine and accelerated schedules in the United States and to the chapters on individual vaccines for detailed information on indications, contraindications, precautions, and expected adverse events. Children in the United States are immunized routinely against 16 diseases (Table 71.1). Most diseases for which children are immunized routinely are present worldwide, although risk of contracting them varies markedly depending on travel destination. Additional information about adult immunization is available from several sources.[33–35] Indications specific to travel are given in sections referring to individual vaccines below. All adults should be up to date with routine childhood immunizations, and all (who have no contraindication) in the United States should receive annual influenza vaccination[36]; influenza immunization is recommended from age 60 or 65 years in most other industrialized countries, although the risk of influenza is worldwide. Those 65 years of age or older, or at any age with certain medical conditions, should be immunized against pneumococcal disease, and all adults older than age 60 years without contraindications should receive zoster vaccine.

VACCINES FOR TRAVEL

Selected Routine Immunizations Especially Important for Travelers

Diphtheria

Epidemics of diphtheria in the newly independent states of the former Soviet Union[37] and in Thailand, Algeria, and Ecuador in the early 1990s,[38] along with the continued endemic presence of diphtheria in many developing countries, underscore the need for attention to diphtheria immunization for travelers to these areas and for continuing to provide routine booster doses (supplied as a combination of tetanus-diphtheria toxoids) throughout adulthood. Vitek and colleagues demonstrated a significant relationship between time since the last diphtheria toxoid booster and risk of diphtheria among Russian schoolchildren.[39] Cases of diphtheria, both cutaneous and fatal respiratory disease, have been reported in travelers.[40–43] Migrant populations also may transmit the bacteria into populations in which immunity has waned because of a decrease in natural disease and lack of routine adult vaccine boosters. In the United States, 20% to 60% of adults older than 20 years of age lack diphtheria antibody,[44,45] and in Western Europe, serologic surveys have shown immunity to diphtheria to be poor among adults, particularly women; men may have been given boosters during military service.[46]

Travel consultation is an opportunity to bring every individual up to date with diphtheria immunization. For adults, a booster dose of combined tetanus-diphtheria toxoids every 10 years is recommended in the United States and some other countries, while boosters at 20-year intervals from ages 25 to 65 years, or no boosters after a complete five-dose series, are recommended by health authorities in other countries.

Poliomyelitis

The program for worldwide polio eradication is proceeding rapidly; periodic setbacks have occurred in recent years with resurgence of disease in some countries and importation into previously polio-free countries.[47] The Western Hemisphere was declared polio free in 1994, the Western Pacific region in 2000, the European region in 2002, and the Southeast Asia region, which includes India, in 2014.[48–51] The latest information on

TABLE 71.1 Vaccinations Recommended Routinely for Children and Adults in the United States, With Special Indications for Travelers

Disease	Vaccine	Age Groups	Greatest Areas of Risk	Special Indications
Diphtheria	DTaP or DTP	<7 y	Developing world, countries of former Soviet Union	
	Td	≥7 y		
	Tdap	≥11 y		May be given to those age 7–10 y who are not fully immunized against pertussis
Tetanus	DTaP or DTP	<7 y	Worldwide	
	Td	≥7 y		
	Tdap	≥11 y		
Pertussis	DTaP or DTP Tdap	<7 y ≥11 y	Worldwide circulation	May be given to those age 7–10 y who are not fully immunized against pertussis
Polio	IPV	<18 y and previously unvaccinated adults	Pakistan, Afghanistan, Nigeria	Extra dose of IPV (if previously fully immunized) for persons traveling to areas of risk
Measles	MMR, MR, MMRV, or M[b]	All ages for susceptible persons	Worldwide	2 Doses separated by ≥28 days for those age ≥12 mo; first dose may be given as early as age 6 mo but should not be counted toward the routinely recommended 2 doses for persons on or after their 1st birthday; most persons born before 1957 can be considered immune and do not need vaccination
Mumps	MMR, MMRV, Mu[b]	All ages for susceptible persons	Worldwide	
Rubella	MMR, MMRV, MR, or R[b]	All ages for susceptible persons	Worldwide	Especially for nonpregnant females of childbearing age
Haemophilus influenzae type b	Hib	<5 y	Most of the world	
Hepatitis A	Hep A	≥1-year-old children	Developing world	Children traveling for the purpose of visiting friends or relatives may be at greater risk
Hepatitis B	Hep B	Routine childhood and adolescence; older ages with special risk	Most of the world; see Fig. 71.1	Travel to areas with hepatitis B prevalence ≥2%; occupational or behavioral risk factors for disease
Pneumococcal	Pneumococcal conjugate	All children age 2–23 mo; certain children ages 24–59 mo; high-risk groups of older ages and all adults age ≥65 y	Worldwide	
Pneumococcal[a]	Pneumococcal polysaccharide	All adults ≥65 y; also younger special-risk groups, such as persons with chronic cardiopulmonary disease, asthma, or smoking	Worldwide	
Varicella	Varicella or MMRV	All ages for susceptible persons	Most of the world	
Influenza[a]	Influenza	1 Dose annually in persons age ≥6 mo; 2 doses for the first influenza season for children age <9 y	Most of the world	Northern hemisphere season December through March; southern hemisphere season April through September; tropics all year
Human papillomavirus	HPV	9- to 26-y-old females; 9- to 21-y-old males (HPV4)	Worldwide	
Meningococcal	Conjugate ACYW135	1 Dose for 11- to 12-y-olds; with booster at 16 y; adolescents at high school entry; college students	Meningitis belt in sub-Saharan Africa	Travelers to high-risk areas
Meningococcal B	Serogroup B	>10 y-olds at increased risk for meningococcal disease	Worldwide	
Rotavirus	Rotavirus	All children age 6–32 wk	Worldwide	

The most current ACIP recommended adult and childhood vaccination schedules can be found at www.cdc.gov/vaccines/schedules/hcp/.
[a]Vaccines routinely indicated for adults.
[b]Single antigen measles, mumps, and rubella vaccines are no longer available in the United States.
DTaP, diphtheria and tetanus toxoids and acellular pertussis; DTP, diphtheria and tetanus toxoids and pertussis; Hep A, hepatitis A; Hep B, hepatitis B; Hib, *Haemophilus influenzae* type b; HPV human papillomavirus; IPV, inactivated poliovirus vaccine; M, measles; MMR, measles-mumps-rubella; MR, measles-rubella; Mu, mumps; Td, tetanus and diphtheria toxoids; Tdap, tetanus toxoid, diphtheria toxoid, and acellular pertussis; R, rubella.

the status of global polio eradication can be found at the Polio Global Eradication Initiative website[52]; at the time of writing, the only three countries where polio transmission has never been eradicated are Afghanistan, Nigeria, and Pakistan. Somalia, Cameroon, Iraq, and Equatorial Guinea had cases in 2014. A 2005 case of vaccine-associated paralytic polio in an unimmunized traveler underscores the need to provide immunization against polio to protect not only against wild-type polio but also against vaccine-associated disease.[53]

Travelers to areas of the world where polio continues to occur should ensure that they have completed the recommended age-appropriate vaccine series; adults and children who have completed a basic series of polio vaccine should receive a single lifetime booster of inactivated polio vaccine (IPV). In many European countries, an IPV booster dose is recommended every 10 years if exposure to risk persists. People of all ages staying in a country identified as a poliovirus exporter for longer than 4 weeks may be required to show proof of polio vaccination before departing the country. In 2015, this focused on Pakistan and Afghanistan. The most current list of countries can be found at the Polio Global Eradication Initiative.[52] CDC recommends long-term travelers and expatriates staying in Pakistan and Afghanistan receive a dose of IPV between 4 weeks and 12 months before the date of their planned departure. This dose should be documented on an International Certificate of Vaccination or Prophylaxis (ICVP).[54] IPV is indicated for unvaccinated adults; IPV or OPV can be used for children. Partially immunized children or adults may complete the series with either IPV or OPV. The interval between doses of vaccine may be shortened to 4 weeks to allow the maximal number of doses to be administered before departure. IPV-containing combination vaccines may be used if available. In 2009, the ACIP revised recommendations for the childhood poliovirus immunization to include administration of the final dose of polio vaccine after 4 years of age, regardless of number of previous doses, and extending the interval between doses three and four from 4 weeks to 6 months.[55] Accelerated schedules, however, remain appropriate for imminent travel to polio risk areas.

Hepatitis B

Hepatitis B risk in travelers is associated with longer length of stay, contact with population groups with high carrier rates of hepatitis B (Fig. 71.1), occupations such as healthcare workers or laboratory workers, and behaviors such as injection drug use and having multiple sex partners. Risk for Swiss tourists traveling to developing countries was noted in 1987 to be 39 per 100,000 travelers for a stay of 1 month.[56] Long-stay travelers were at greater risk; unimmunized U.S. missionaries serving in Africa before 1990 had attack rates of 11% during the first 2 years of service and median annual attack rates of 1.2% over the next decade.[57] A prospective study published in 2004 of unvaccinated Dutch missionaries and their families living in rural Nigeria identified seroconversion rates of 1.7 per 1000 person-months in adults and 2.8 per 1000 person-months in children.[58]

A 2015 review of vaccine-preventable disease risk in travelers considers hepatitis B to be of intermediate risk (one to 10 cases per 100,000 travelers per month), with behaviors during travel and contact with the local population likely more important than disease prevalence at destination.[59–62]

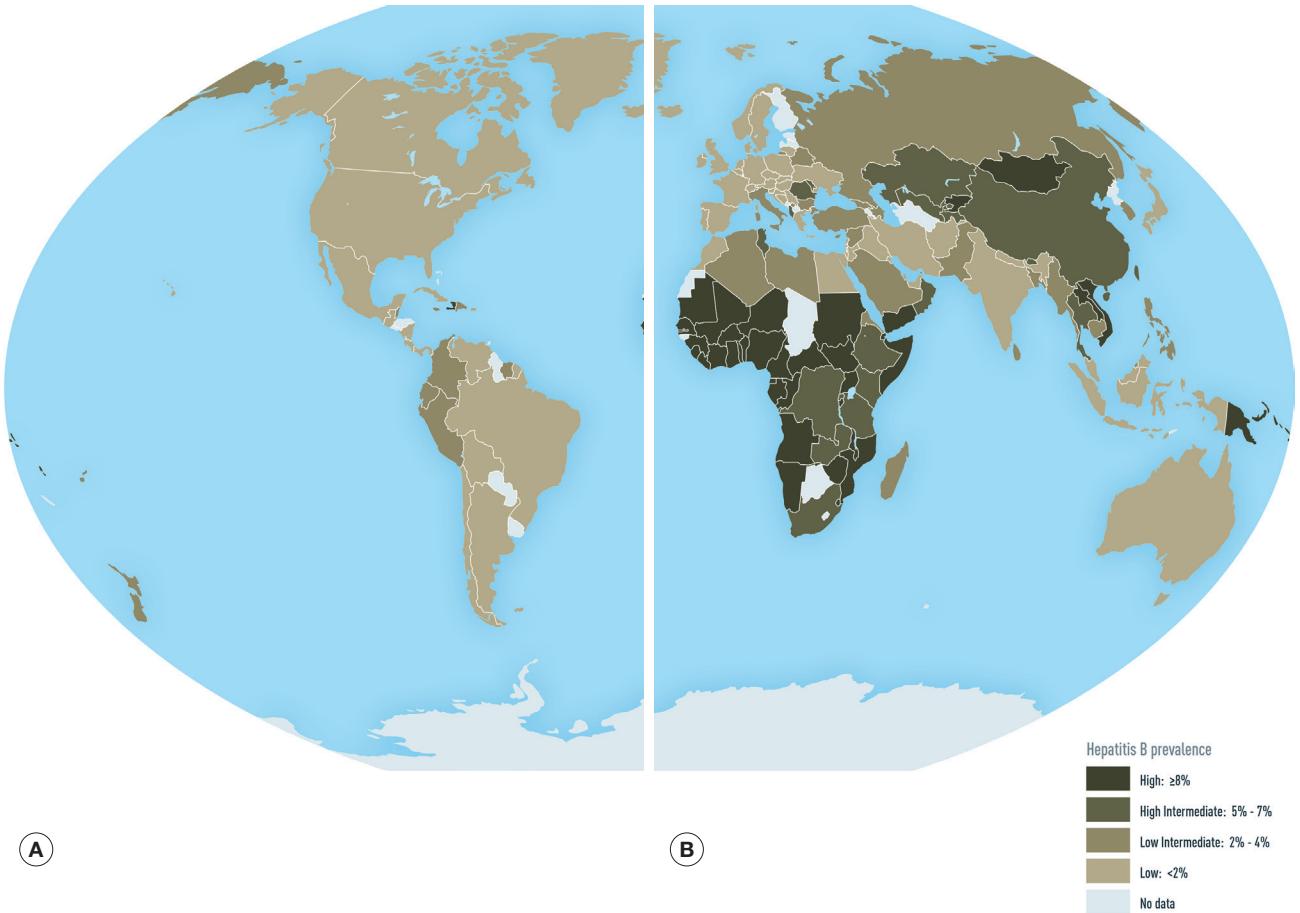


Figure 71.1. Prevalence of chronic hepatitis B virus infection among adults. *(From Centers for Disease Control and Prevention [CDC]. Health Information for International Travel 2016.)*

Immunization against hepatitis B is now a routine immunization for children and adolescents in most countries, except some in Northern Europe. The CDC recommends hepatitis B immunization for travelers to countries with prevalence of hepatitis B infection of ≥2%. The first two doses should be given 4 weeks apart and the third dose 4 to 12 months after the second. If insufficient time is available before departure, rapid seroconversion may be achieved by giving the third dose as early as 4 weeks after the second, with a fourth dose given 12 months from the first to ensure long-term protection. Other accelerated schedules are proposed for hepatitis B and hepatitis A/B combination vaccines and have been recommended and used by travel medicine clinicians, but not all have been approved by the U.S. Food and Drug Administration.

Measles

Measles continues to be an important cause of childhood morbidity and mortality worldwide; cases associated with travel have increased in recent years. In 2014, the United States experienced the largest number of cases since measles elimination was documented in 2000. Multistate outbreaks have been associated with imported cases at sporting events and amusement parks.[63,64] Cases in returning U.S. travelers and foreign visitors predominate and continue to lead to secondary cases and outbreaks primarily among unimmunized individuals.[65] Eighty percent of cases of measles in the United States in 2001 to 2003 were confirmed to be imported, import-linked, or a result of imported virus.[66] Of measles cases in U.S. residents from 2001 to 2004, 55 of 122 (45%) occurred in those who travelled internationally.[67] Outbreaks associated with air travel have been documented; in the summer of 2010, a case of measles was identified in a young unimmunized airline passenger arriving in the United States from Switzerland, exposing 31 persons, one of whom developed measles and in turn exposed 270 persons in the United States.[68] In 2013, a traveler returning from India was associated with direct and indirect transmission to 22 other individuals and investigation of exposures to approximately 1000 individuals.[69,70] Similar outbreaks and exposures have been reported from Australia, Italy, and The Netherlands.[71–73]

Two doses of MMR vaccine are recommended for all international travelers.[32,74] The first dose may be given at 6 months of age; this dose is not counted as part of the routine two dose series. Travelers 1 year of age or older who have received a single MMR vaccine should receive a second, at an interval of 4 weeks from the first, before international travel. Children younger than 6 months of age are likely to be protected by maternal antibody. Adults born in the United States before 1957 can be considered to have natural immunity, but those at increased risk of measles, such as international travelers, may be offered serologic testing or vaccine before travel; if nonimmune, such individuals should receive two doses of vaccine.[74] Mumps, rubella, and varicella vaccines should also be brought up to date, using MMR or MMR with varicella vaccines as appropriate. As seroprevalence of varicella in adult immigrants from tropic countries might be much lower compared with North America or Europe, the latter may be particularly appropriate in this population.

Pertussis

Pertussis continues to be a disease of worldwide importance, with an estimated 16 million cases and 195,000 deaths per year. In the United States there were more than 48,000 cases in 2012, the highest number reported since 1955[75]; more than 28,000 cases were reported in both 2013 and 2014.[76] Attack rates of pertussis are related directly to use of vaccine; after a decline in use of pertussis vaccines in Japan, Sweden, and Britain, a resurgence of disease occurred.[74] Acellular pertussis vaccines are now used in many countries; two vaccines are licensed in the United States.[77] The increased risk for pertussis as a result of international travel is unknown.[78] One study found an increased rate of pertussis among Hajj pilgrims.[79] Children and adolescents should complete as much of a primary series of a pertussis-containing vaccine as possible, or be given any boosters due, before international travel.[77] Adults should be given pertussis-containing vaccine (tetanus, diphtheria, and pertussis [Tdap]) once if due for boosters of tetanus and diphtheria before travel if they have never received a Tdap booster in the past. Pregnant women should receive Tdap vaccine during every pregnancy to provide additional protection to infants.[80]

Influenza

Influenza occurs worldwide; in the tropics transmission occurs throughout the year, whereas peaks of transmission usually occur from December through March in the Northern Hemisphere and April through September in the Southern Hemisphere. Experience with the H1N1 influenza pandemic heightened awareness of influenza risk and travel. Risk to travelers for contracting influenza is increased by travel to a destination during influenza season, and participation in large organized tourist groups, such as those on cruise ships. Risk for serious disease depends on underlying health status. Incidence of influenza in travelers has been addressed by three studies; the rate of serologically confirmed cases was 8.9 per 100 person-months, and rates of symptomatic cases ranged from 0.9% to 1.0%.[81–83] Risk of influenza was sevenfold higher for destinations in East and Southeast Asia compared with other destinations.[84]

Annual influenza vaccination for all persons 6 months of age or older is recommended in the United States[85] and other countries. Challenges specific to travelers are that vaccine may not be available in the traveler's country of origin at the time of travel and available vaccine may not match strains circulating at the destination. In these cases, education about risk of influenza and management of respiratory and influenza-like illness is important. Available influenza vaccines will not protect against avian influenza [A(H5N1) and A(H7N9)], so other precautions (such as staying away from live animal markets) are needed when traveling to areas that might be affected by that virus. Information about circulating virus strains by region can be obtained from CDC[86] or the WHO.[87]

Rotavirus

A three-dose rotavirus vaccine (RotaTeq; Merck, Whitehouse Station, NJ) was licensed in the United States in February 2006; a second two-dose vaccine (Rotarix, GlaxoSmithKline, Research Park Triangle, NC) was licensed in April 2008. Recommendations for use of rotavirus vaccines are available from CDC.[88] The first dose of either vaccine can be given as early as 6 weeks of age, but must be given by 14 weeks and 6 days of age. The minimum interval between doses is 4 weeks, with maximum age at the last dose of the series 8 months 0 days. It would be prudent for infants traveling to areas with high prevalence of rotavirus to complete all doses of vaccine before departure.

Human Papillomavirus Vaccine

Three vaccines against human papillomavirus (HPV) are licensed in the United States at this time. Recommendations for their use are available from CDC.[89] HPV is present worldwide. A travel medicine consultation is an opportunity to offer this vaccine at routinely recommended ages as a part of an

overall strategy to reduce risk of HPV-associated cancers and sexually transmitted diseases.

Travel Vaccines

Bacille Calmette-Guérin

Bacille Calmette-Guérin (BCG) vaccines are available in many countries but vary in efficacy. In the United States and in many European countries, a program of surveillance, using tuberculin skin testing or interferon-γ release assays and early identification and treatment of infected persons, is preferred to universal immunization with BCG.[90]

Estimates of risk for tuberculosis (TB) among travelers are difficult to obtain.[91] Dutch long-term travelers had 3.5 tuberculin skin test conversions per 1000 person-months of travel and 2.8 if healthcare workers were excluded.[92] A study in Peace Corps Volunteers found 1.436 purified protein derivative conversions and 0.084 cases of active TB per 1000 volunteer-months for those serving in "high-risk" countries.[93] Immigrants returning home to visit family are at increased risk of exposure and development of disease.[94–96] Risk to travelers is not thought to be high enough to warrant routine immunization for travel. There are rare circumstances in which BCG might be considered in the United States for long-term travelers (infants or young children) to areas where the incidence of disease is high (more than 30 to 40 per 100,000) and where occupation or living conditions may result in significant exposure. One vaccine, manufactured by Organon Teknika (Durham, NC), is licensed in the United States. Because the protective effect of BCG has been confirmed mainly in infants age 1 year or younger, there would be almost no indication for BCG for adults who are traveling, although some experts believe BCG should be administered to healthcare personnel traveling to work in environments with high TB transmission rates.

Adverse events from vaccine occur in up to 10% of persons and usually consist of local ulceration and inflammatory adenitis. BCG vaccine is contraindicated in immunocompromised persons because of risk of dissemination of infection. Decisions to immunize HIV-infected, but immunocompetent persons are controversial; bacteria are able to remain dormant for years and cause disease if an individual becomes immunocompromised. Risk of infection with TB, such as in infants in developing countries with high rates of TB, must be weighed against risk of vaccination for those who may be at risk for HIV infection. The WHO continues to recommend universal immunization with BCG for infants in areas where risk of TB is substantial, based on potential for reducing miliary and meningeal TB in the first year of life.

Cholera

Cholera cases have increased globally since 2005 and continue to occur in Africa, Southeast Asia, and Haiti.[97] Risk of severe cholera is very low for travelers; the vast majority of infections will be asymptomatic or appear just as travelers' diarrhea. Attack rates are estimated to be one per 500,000 travelers, although higher rates (five per 100,000 travelers) have been reported from Japan, where routine screening for cholera was done in returning travelers with diarrhea.[98] The mainstay of protection against cholera remains drinking and using only water that has been boiled, treated, or supplied in reliable cans or bottles, and avoidance of ingestion of high-risk foods, such as raw shellfish, and the use of precautions when making other food selections. Cholera can be treated by prompt and vigorous oral or intravenous rehydration; antibiotics can be used, especially in hospitalized patients.

Two cholera vaccines are prequalified by WHO and available in various countries outside the United States. A third killed oral cholera vaccine is produced and available only in Vietnam and is 76% protective in the setting of a cholera outbreak.[99] An oral cholera vaccine was approved by the U.S. Food and Drug Administration in 2016 and is indicated for adults aged 18 to 64 years traveling to cholera-affected areas.[99a] Previously produced whole-cell injectable and live oral cholera vaccines are no longer available. Cholera vaccine is not recommended routinely for travelers, and no country requires cholera immunization for entry at this time.[100,101] For the rare person traveling to areas of high risk and poor sanitary conditions (e.g., relief workers in the midst of cholera outbreaks), vaccine may be offered if available. Food and water precautions are the mainstay of prevention of cholera, and appropriate rehydration therapy is the mainstay of treatment.

Oral cholera vaccines available outside the United States are given on a two-dose schedule with a 14-day interval between doses. A booster dose is recommended at an interval of 10 months to 2 years. Mild gastrointestinal side effects have been observed.[100] Immunization is not recommended during pregnancy; specific safety information about the use of these vaccines during pregnancy is not available. The oral cholera vaccine available in the United States is given as single oral dose a minimum of 10 days before potential exposure. Tiredness, headache, and mild gastrointestinal side effects were the most common adverse events. No information is available about booster doses.

Oral cholera vaccine may be given concurrently with yellow fever vaccine, oral typhoid Ty21a vaccine, and OPV.[102] Immunization with cholera vaccine should be completed before initiating malaria prophylaxis with chloroquine or mefloquine because of the potential for these antimalarials to affect immune response to vaccine adversely.[103]

Hepatitis A

Hepatitis A is a common vaccine-preventable disease to affect travelers, but is decreasing because of widespread use of vaccine and improving hygienic conditions at many destinations. In the 1980s, risk was estimated to be as high as 1% for short-term travelers who did not receive immunoglobulin and traveled to highest-risk areas.[104] A study of Canadian travelers reported a rate of one case per 3000 unimmunized travelers.[105] A report from Switzerland documented a reduction in cases of hepatitis A during the years 1988 to 2004 that was variable by country; this was attributed to both improved hygiene and hepatitis A immunization.[106] Risk for disease depends on length of stay and conditions of travel, including frequency of exposure to contaminated food and water. Fig. 71.2 shows the geographic distribution of risk for hepatitis A. Cases of hepatitis A, however, have been reported in tourists staying in luxury accommodations in countries where the risk of hepatitis A is high.[107] Despite availability of both immunoglobulin and an effective vaccine, many travelers do not receive them and therefore are not protected against hepatitis A.

Persons born in Europe, North America, Australia/New Zealand, or Japan since World War II have a low prevalence of immunity to hepatitis A.[108] Rates of immunity are higher with increased age, history of jaundice, and birth or residence in areas with high rates of hepatitis A. As many as 95% of persons born and raised in developing countries with high rates of hepatitis A may be protected by naturally acquired antibody.[109] Some countries, including the United States, have adopted hepatitis A as a routine immunization for children beginning at 1 year of age.

Options for immunoprophylaxis of hepatitis A include intramuscular immunoglobulin and hepatitis A vaccine. Immunoglobulin was the mainstay of protection for many years and is 85% to 90% protective. The major disadvantage

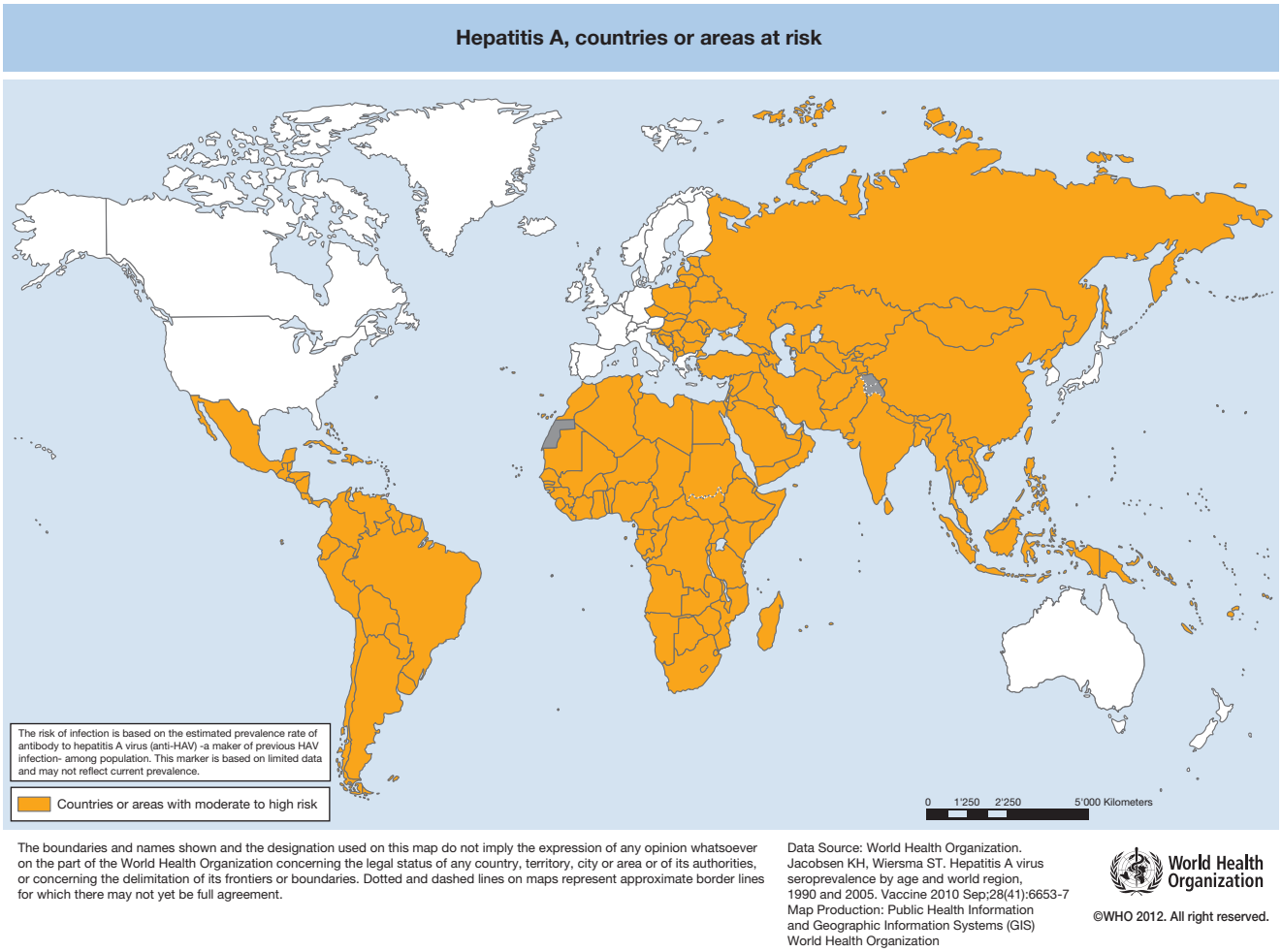


Figure 71.2. Countries or areas with moderate to high risk for hepatitis A. Note: This map generalizes available data, and patterns may vary within countries. *(From World Health Organization (WHO), 2012. http://gamapserver.who.int/mapLibrary/Files/Maps/Global_HepA_ITHRiskMap.png.)*

of immunoglobulin is that it is short acting and must be repeated for subsequent journeys, or additional doses must be given during prolonged residence in endemic areas. It does not offer effective protection against hepatitis B, C, or E. Adverse events are rare, although the large volume required may result in local discomfort. One advantage of this preparation is that it may be given to children younger than 1 year of age. Immunoglobulin has become unavailable in most countries, in part because of concern about the theoretical risk of transmission of variant Creutzfeldt-Jacob disease, and availability is limited in parts of the United States.[110] Hepatitis A vaccines have been marketed in Europe since 1992 and in the United States since 1995. Four products—Havrix (GlaxoSmithKline), Epaxal (PaxVax, formerly Crucell/Berna Biotech, currently unavailable), VAQTA (Merck), and AVAXIM (Pasteur Mérieux Connaught, Lyon, France)—have been manufactured, although AVAXIM and Epaxal, a virosomal, aluminum-free vaccine, are not licensed in the United States. A combined hepatitis A and B vaccine (Twinrix; GlaxoSmithKline) is also marketed widely. The four monovalent hepatitis A vaccines are highly immunogenic in adults, with protective antibody levels achieved in 95% to 100% of adults 1 month after the first injection, using recommended adult doses of Havrix (1440 enzyme-linked immunosorbent assay units [ELU] in 1.0 mL), VAQTA (50 units in 1.0 mL), or AVAXIM (160 units in 0.6 mL). It should be noted that because no international standardized reference exists for hepatitis A antigen content, each laboratory has expressed the antigen content using an in-house reference. One month after the booster dose, given at 6 to 12 months after the first dose, seroconversion rates of 100% were reported with all three vaccines.[111,112] Of importance for travelers with imminent departures, seroconversion rates of 87.1% (Havrix, 1440 ELU) and 95.7% (AVAXIM) were reported at 14 days after a single dose in a comparative trial.[113] Development of antibody occurred more slowly in the elderly, although similar antibody concentrations are achieved.[114] Combination hepatitis A and B vaccine demonstrated immunogenicity comparable to that of the monovalent vaccines.[115] Efficacy studies performed in Thailand (Havrix) and during a hepatitis A epidemic in the United States (VAQTA) demonstrated clinical efficacy of 94% and 100%, respectively. Havrix and VAQTA both have pediatric formulations for children 1 to 17 years of age (720 ELU and 25 units, respectively). Similar protective levels of antibody have been reported in children as in adults.[116,117] A Cochrane Database review documented clinical efficacy of preexposure prophylaxis with hepatitis A vaccines in susceptible individuals.[118]

Travelers to areas of intermediate or high prevalence should be protected against hepatitis A; some providers believe all travelers should be offered vaccine. Ideally, vaccine should be given at least 4 weeks prior to initiation of travel because of the concern that neutralizing antibody is not optimal prior to this time, although most young, healthy vaccinees will have detectable antibody response by 2 weeks, and to date there are

no reports of cases of hepatitis A, even in those immunized the day of departure. For healthy adults younger than 40 years of age, the CDC recommends a dose of hepatitis A vaccine administered at any time before departure; immunoglobulin may be used with vaccine for older persons or those with immunocompromising conditions, chronic liver disease, or other medical conditions.[119] The WHO and most other regional and national expert groups recommend a dose of hepatitis A vaccine at any interval before departure and consider immunoglobulin virtually obsolete for the purpose of prevention of hepatitis A for travelers.[120] A booster dose of vaccine at least 6 months after the initial dose is expected to provide long-term (at least 20 years) protection. Booster doses may be from the same or a different manufacturer, or may be the A/B combination vaccine, without adverse effect on reactogenicity or immunogenicity.[121] Table 71.2 lists the doses and schedules for administration of hepatitis A vaccines.

Generally, hepatitis A vaccine is well tolerated. The most common vaccine side effects include soreness at the injection site, headache, and malaise. Serious adverse events appear to be rare. Vaccine should not be given to persons with allergies to adjuvants or preservatives contained in the vaccine. Although risk from vaccination during pregnancy should be low, risk of disease must be weighed against the theoretical risk of immunization. Hepatitis A vaccines are inactivated and therefore may be used in immunocompromised persons. Simultaneous administration of hepatitis A vaccine and diphtheria, tetanus, oral and inactivated poliovirus, oral typhoid, cholera, Japanese encephalitis, rabies, or yellow fever vaccines does not impair immune response to either vaccine or increase adverse events.[122]

Children younger than 1 year of age should receive a single dose of immunoglobulin (0.02 mL/kg for up to 3 months protection or 0.06 mL/kg for up to 5 months protection), if available, because safety and efficacy of vaccine have not been evaluated adequately in this age group. Doses of immunoglobulin may be given as close as possible to the departure date; the dose depends on weight and length of stay. Serious adverse events following immunoglobulin are rare, although anaphylaxis has been reported in immunoglobulin A–deficient persons; immunoglobulin should not be used in these persons.

Immunoglobulin may interfere with response to certain live virus vaccines, such as measles, mumps, rubella, and varicella. It has been shown not to interfere with the immune response to oral polio, oral typhoid, or yellow fever vaccines. If travelers require both MMR or varicella vaccine and immunoglobulin, the vaccines ideally should be given 2 weeks before immunoglobulin. If immunoglobulin for hepatitis A has been given, subsequent immunization with MMR or varicella vaccine should be delayed at least 3 months.[23,24] If MMR or varicella vaccines and immunoglobulin must be given closer than 2 weeks apart, the MMR and varicella vaccine doses should be repeated 3 or more months after immunoglobulin unless serologic testing shows that antibodies to vaccine were produced.

Testing for susceptibility to hepatitis A before offering vaccine or immunoglobulin may be cost-effective in specific situations. Candidates for testing may include persons born and raised in high-prevalence areas and those with a history of jaundice. Other considerations include cost of testing compared with cost of immunization and assuring that testing will not delay ability to provide immunization if a person is seronegative. It is estimated that if cost of screening is one-third the cost of immunization, and the person's likelihood of immunity is greater than 33%, testing should be cost-effective.[123] Plans-Rubio has described a model to determine whether screening before immunization is cost-effective, based on calculation of a critical prevalence of antibody in the population, in turn based largely on screening and vaccination costs.[124]

Japanese Encephalitis

Japanese encephalitis (JE) is a leading cause of viral encephalitis in Asia. Although disease incidence has decreased in many countries because of immunization, improved hygiene and agricultural practices, and improved standards of living, the predominant picture remains that of expansion of the geographic range of JE over the past four decades.[125,126] JE is transmitted by mosquitoes. Risk of clinical disease in travelers is low, with estimates of risk ranging from less than one per million in civilian travelers to one per 5000 per month of exposure in the military or in the local population in rural endemic areas.

TABLE 71.2 Recommended Doses and Schedules for VAQTA, AVAXIM, Havrix, and Twinrix

Group	Age (y)	Dose	Volume (mL)	No. of Doses	Schedule (mo)[a]
VAQTA[b]					
Children and adolescents	1–17	25 U	0.5	2	0, 6–18
Adults	≥18	50 U	1.0	2	0, 6
AVAXIM[c]					
Adults[d]	≥16	160 U	0.5	2	0, 6–18
HAVRIX[e]					
Children and adolescents	1–18	720 ELU	0.5	2	0, 6–12
Adults	>18	1440 ELU	1.0	2	0, 6–12
TWINRIX[f]					
Adults	>18	720 ELU/20 μg HBsAg	1.0	3[g,h]	0, 1, 6
Children	1–15	360/10	0.5	3	0, 1, 6

[a]Zero (0) months represents timing of the initial dose; subsequent numbers represent months after the initial dose.
[b]Hepatitis A vaccine, inactivated (Merck).
[c]Hepatitis A vaccine (Pasteur Mérieux Connaught).
[d]Hepatitis A vaccine, inactivated (Crucell/Berna Biotech).
[e]Hepatitis A vaccine, inactivated (GlaxoSmithKline).
[f]Hepatitis A, inactivated, and hepatitis B, recombinant (GlaxoSmithKline).
[g]Two doses essential before departure as 720 ELU of hepatitis A would not grant sufficient protection.
[h]In some countries 2 doses of Twinrix Adult may be given to children >1 y up to the 16th birthday, scheduled at 0, 6–12 months.
ELU, enzyme-linked immunosorbent assay unit; RIA U, radioimmunoassay unit; U, unit.

Fifty-five cases of JE in travelers were reported in the literature between 1973 and 2008; none were known to have received JE vaccine.[127] Although there were only eight cases of JE reported in U.S. travelers between 1992 and 2008, the numbers have increased. Although disease in returned travelers is still quite rare, symptomatic illness is typically followed by either severe neurologic sequelae or death.[128] Most travelers have no natural immunity to the disease. Although risk is highest for persons who travel during the season of high transmission and remain in endemic areas for extended periods, many of those few cases have occurred in short-stay tourists.[129–134] Fig. 71.3 is a map of JE risk areas and Table 71.3 provides detailed information about the risk of JE by country, region, and season.[135] JE vaccines are available in many countries. Several vaccines, including a live attenuated vaccine, SA14-14-2, are licensed in Asia but are not available in the United States. The first JE vaccine to be licensed in the United States, JE-VAX, an inactivated, mouse-brain derived vaccine, has not been manufactured since 2006 and is no longer available.

An inactivated, Vero-cell culture-derived JE vaccine (Ixiaro, Jespect in Australia) was licensed for use in adults (age 17 years or older) in many countries and in the United States in 2009, and for use in children in 2013. This vaccine was licensed in the United States on the basis of the ability to produce JE virus–specific neutralizing antibody, a correlate of protection. The vaccine is given as two doses, 28 days apart; it is recommended that both doses be given before travel (Table 71.4). Some experts are using an accelerated schedule of a 1-week interval between doses.[134a] A booster dose may be given 1 year after the primary series has been completed if the traveler will be reexposed to JE. Studies of concomitant administration with other vaccines have been done only for hepatitis A vaccine; no differences were reported in safety or reactogenicity of either vaccine when given together compared with separate administration, although local reactions were reported more often in those who received the vaccines together.[136] The most common adverse events were local reactions, including pain and tenderness at the site of administration. Systemic adverse events were generally mild and included headache (26%), myalgia (21%), influenza-like illness (13%), and fatigue (13%).[127] A recent summary of adverse events reported to the U.S. Vaccine Adverse Events Reporting System (VAERS) suggests that serious adverse events to JE vaccine are infrequent.[137]

Vaccine is most appropriate for persons who will spend 1 month or longer in endemic areas, especially in rural areas during transmission season. Other travelers, such as those traveling to endemic areas during epidemic transmission or those whose occupation or living conditions will place them at increased risk of exposure (those with unprotected outdoor, evening, and nighttime exposure in rural areas, such as campers or bicyclers), may be candidates for vaccine even if traveling for less than 1 month.[138] Protective measures for persons who are not immunized include prevention of mosquito bites through the use of window screens, bed nets, and insect repellents.

Meningococcus

Invasive meningococcal disease occurs worldwide, but major epidemics occur more frequently in the meningitis belt of

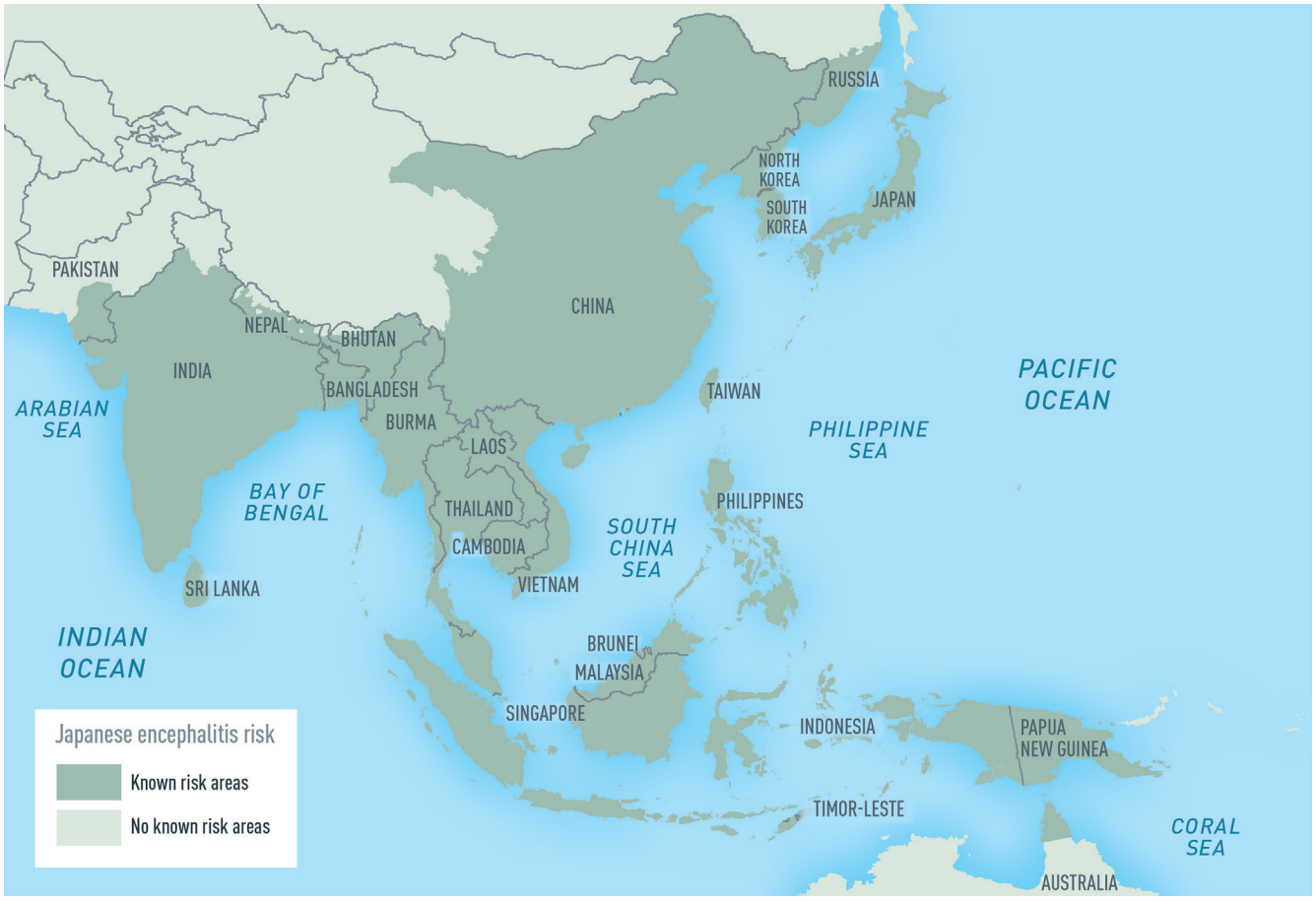


Figure 71.3. Geographic distribution of Japanese encephalitis. *(From Centers for Disease Control and Prevention [CDC]. Health Information for International Travel 2016.)*

TABLE 71.3 Risk for Japanese Encephalitis (JE) by Country[a]

Country	Affected Areas	Transmission Season	Comments
Australia	Outer Torres Strait islands	December–May; all human cases reported February–April	1 Human case reported from north Queensland mainland
Bangladesh	Presumed widespread	Most human cases reported May–October	Sentinel surveillance has identified human cases in Chittagong, Dhaka, Khulna, Rajshahi, Ranjpur, and Sylhet Divisions; highest incidence reported from Rajshahi Division; outbreak reported from Tangail District, Dhaka Division, in 1977
Bhutan	Very rare reports; probably endemic in nonmountainous areas	No data	Proximity to other endemic areas and presence of vectors suggests virus transmission is likely
Brunei	Very rare reports; presumed to be endemic countrywide	Unknown; presumed year-round	Proximity to other endemic areas suggests virus transmission is likely
Burma (Myanmar)	Limited data; presumed to be endemic countrywide	Unknown; most human cases reported May–October	Outbreaks of human disease documented in Shan State; antibodies documented in animals and humans in other areas
Cambodia	Presumed to be endemic countrywide	Year-round with peak season May–October	Sentinel surveillance has identified human cases in at least 15 of 23 provinces, including Phnom Penh, Takeo, Kampong Cham, Battambang, Svay Rieng, and Siem Reap; 1 case reported in 2010 in a traveler who visited Phnom Penh and Angkor Wat/Siem Reap only
China	Human cases reported from all provinces except Xizang (Tibet), Xinjiang, and Qinghai	Most human cases reported June–October	Highest rates reported from Guizhou, Shaanxi, Sichuan, and Yunnan Provinces, and Chongqing City; vaccine not routinely recommended for travel limited to Beijing, Shanghai, Hong Kong City/Kowloon, Macau, or other major cities
India	Human cases reported from all states except Dadra, Daman, Diu, Gujarat, Himachal Pradesh, Jammu and Kashmir, Lakshadweep, Meghalaya, Nagar Haveli, Punjab, Rajasthan, and Sikkim	Most human cases reported May–October, especially in northern India; the season may be extended or year-round in some areas, especially in southern India	Highest rates of human disease reported from the states of Andhra Pradesh, Assam, Bihar, Goa, Haryana, Karnataka, Kerala, Tamil Nadu, Uttar Pradesh, and West Bengal
Indonesia	Presumed to be endemic countrywide	Year-round; peak season varies by island	Sentinel surveillance has identified human cases in Bali, Kalimantan, Java, Nusa Tenggara, Papua, and Sumatra
Japan[b]	Rare sporadic human cases on all islands except Hokkaido; enzootic activity ongoing	Most human cases reported July–October	Large number of human cases reported until JE vaccination program introduced in late 1960s; most recent small outbreak reported from Chugoku district in 2002; enzootic transmission without human cases observed on Hokkaido; vaccine not routinely recommended for travel limited to Tokyo or other major cities
Korea, North	Limited data; presumed to be endemic countrywide	No data; proximity to South Korea suggests peak season is likely to be May–October	
Korea, South[b]	Rare sporadic human cases countrywide; enzootic activity ongoing	Most human cases reported May–October	Large number of human cases reported until routine JE vaccination program introduced in mid-1980s; last major outbreak reported in 1982; vaccine not routinely recommended for travel limited to Seoul or other major cities
Laos	Limited data; presumed to be endemic countrywide	Year-round, with peak season June–September	Sentinel surveillance has identified human cases in north, central, and southern Laos
Malaysia	Endemic in Sarawak; sporadic cases reported from all other states; occasional outbreaks reported	Year-round; in Sarawak, peak season October–December	Most human cases reported from Sarawak; vaccine not routinely recommended for travel limited to Kuala Lumpur or other major cities
Nepal	Endemic in southern lowlands (Terai); cases also reported from hill and mountain districts, including the Kathmandu valley	Most human cases reported June–October	Highest rates of human disease reported from western Terai districts, including Banke, Bardiya, Dang, and Kailali; vaccine not routinely recommended for those trekking in high-altitude areas

TABLE 71.3 Risk for Japanese Encephalitis (JE) by Country[a] *(Continued)*

Country	Affected Areas	Transmission Season	Comments
Pakistan	Limited data; human cases reported from around Karachi	Unknown	
Papua New Guinea	Limited data; probably widespread	Unknown; probably year-round	Sporadic human cases reported from Western Province; serologic evidence of disease from Gulf and Southern Highland Provinces; a case of JE was reported from near Port Moresby in 2004
Philippines	Limited data; presumed to be endemic on all islands	Year-round with peak season July–September	Outbreaks reported in Nueva Ecija and Manila; sentinel surveillance has identified human cases in other areas of Luzon and the Visayas
Russia	Rare human cases reported from the Far Eastern maritime areas south of Khabarovsk	Most human cases reported July–September	
Singapore	Rare sporadic human cases reported	Year-round	Vaccine not routinely recommended
Sri Lanka	Endemic countrywide except in mountainous areas	Year-round with variable peaks based on monsoon rains	Highest rates of human disease reported from Anuradhapura, Gampaha, Kurunegala, Polonnaruwa, and Puttalam districts
Taiwan[b]	Rare sporadic human cases islandwide	Most human cases reported May–October	Large number of human cases reported until routine JE vaccination introduced in 1968; vaccine not routinely recommended for travel limited to Taipei or other major cities
Thailand	Endemic countrywide; seasonal epidemics in the northern provinces	Year-round with peak season May–October, especially in the north	Highest rates of human disease reported from the Chiang Mai Valley; sporadic human cases reported from Bangkok suburbs; several cases reported recently in travelers who visited resort or coastal areas of southern Thailand
Timor-Leste	Sporadic human cases reported; presumed to be endemic countrywide	No data; cases reported year-round in neighboring West Timor	
Vietnam	Endemic countrywide; seasonal epidemics in the northern provinces	Year-round with peak season May–October, especially in the north	Highest rates of disease in the northern provinces around Hanoi and northwestern and northeastern provinces bordering China
Western Pacific Islands	Outbreaks of human disease reported in Guam in 1947–48 and Saipan in 1990	Unknown; most human cases reported October–March	Enzootic cycle might not be sustainable; outbreaks may follow introductions of virus; vaccine not recommended

[a]Data are based on published reports and personal correspondence. Risk assessments should be performed cautiously, because risk can vary within areas and from year to year, and surveillance data regarding human cases and JE virus transmission are incomplete.
[b]In some endemic areas, human cases among residents are limited because of natural immunity among older people or vaccination. However, because JE virus is maintained in an enzootic cycle between animals and mosquitoes, susceptible visitors to these areas still may be at risk for infection.
From Hills SL, Rabe IB, Fischer M; Centers for Disease Control and Prevention (CDC). Traveler's Health. Chapter 3: Infectious Diseases Related to Travel: Japanese Encephalitis. 2014. Available at: http://wwwnc.cdc.gov/travel/yellowbook/2014/chapter-3-infectious-diseases-related-to-travel/japanese-encephalitis.

TABLE 71.4 Japanese Encephalitis Vaccine (Ixiaro, Jespect) Schedule

	Dose (Subcutaneous Route)	Comments
Primary series 1 and 2	0.5 mL for ages ≥3 y 0.25 mL for ages 2 mo to 2 y	Days 0 and 28
Booster[a]	0.5 mL	≥1 y after dose 2 for ages ≥17 y if still at risk; no data for ages 2 mo to 16 y

[a]In vaccinees who have completed a two-dose primary series, the full duration of protection is unknown; consequently, definitive recommendations cannot be given.

sub-Saharan Africa (Fig. 71.4). International travelers may acquire the disease, or may become colonized with epidemic strains of meningococcus and spread the disease to other parts of the world. Disease resulting from *Neisseria meningitidis* infection is caused most often by one of the five serogroups: A, B, C, Y, and W135. Distribution of serogroups may change rapidly. Serogroups C, B, and Y are predominant in the Americas and Europe. Serogroup A predominated in sub-Saharan Africa, but in the 1990s the proportion of cases caused by serogroup W135 increased significantly.[139] Vaccination campaigns conducted in the African meningitis belt with a group A meningococcal conjugate vaccines have led to variable reductions in meningococcal carriage across this region.[140]

Risk to travelers of acquiring invasive meningococcal disease is low, estimated to be less than 0.0001% per month for a stay in a developing country, and up to 2000 per million in pilgrims to Mecca before quadrivalent immunization with

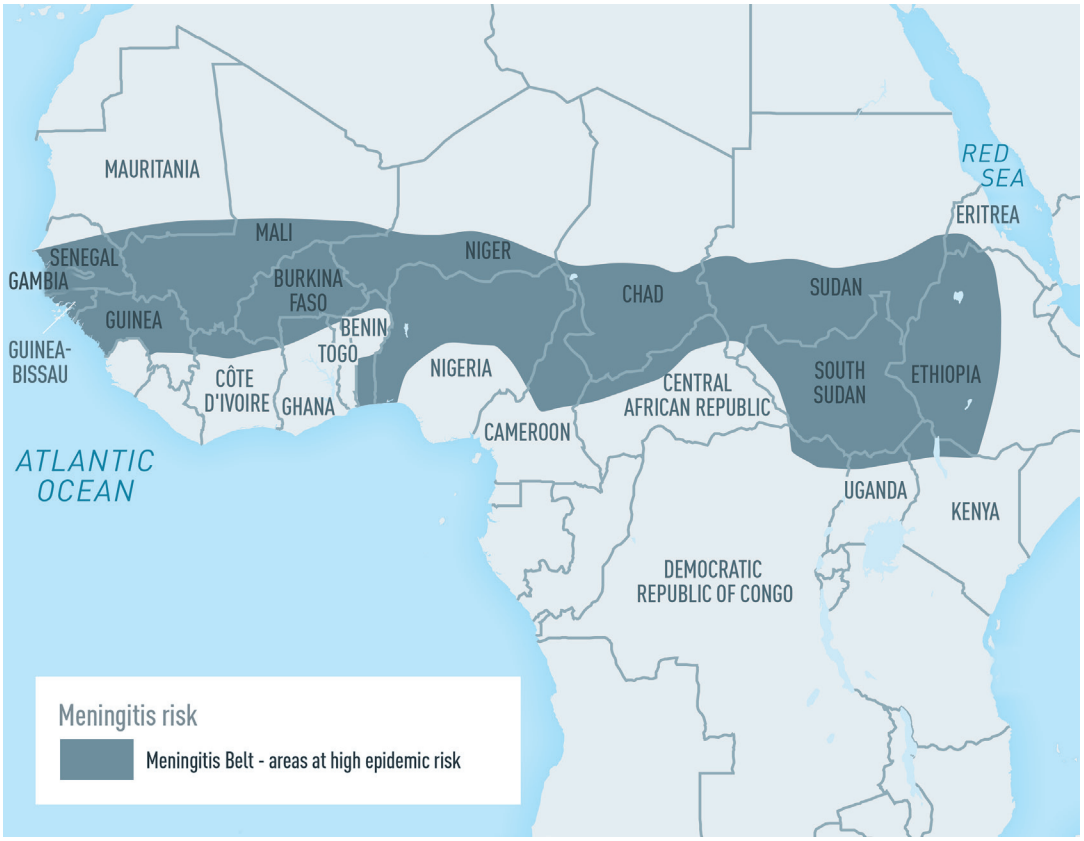


Figure 71.4. Areas with frequent epidemics of meningococcal meningitis. *(From Centers for Disease Control and Prevention [CDC]. Health Information for International Travel 2016.)*

an ACYW135 vaccine became a requirement to obtain a visa.[141,142] Outbreaks have occurred involving travelers, and travelers have become carriers of disease. American pilgrims who returned from Mecca, Saudi Arabia, after an epidemic of group A meningococcal diseases in 1987 were more than 11 times as likely to carry the organism home as travelers returning from other parts of Saudi Arabia.[143] In 2000 and 2001, outbreaks of meningitis caused by serogroup W135 among Hajj pilgrims and their contacts occurred in more than 10 countries.[144,145] Transmission of serogroup W135 strains to household contacts of pilgrims attending the Hajj and returning to Singapore was documented in seven (13%) of 54 contacts.[146] Since 1987, Saudi Arabian health authorities have required immunization against meningococcal disease for pilgrims attending the Hajj. Until 2002, bivalent serogroup A and C vaccine was sufficient; because of outbreaks of disease caused by serogroup W135, the policy was changed for the 2002 Hajj season to require immunization with quadrivalent vaccine (A, C, Y, and W135). A 2003 study of nasopharyngeal carriage of *N. meningitidis* in Burkina Faso identified serogroup W135 as the most common strain carried by the 4- to 29-year-old study population.[147] Although acquisition of meningococcal carriage has been documented in hajj pilgrims who had received polysaccharide vaccine,[147a] this risk would be reduced if the conjugate vaccine would be used.[147b] No additional Hajj-related outbreaks of meningococcal disease have occurred since the Saudi authorities requested quadrivalent vaccine.

Two cases of meningococcal disease thought to be transmitted during an airline flight have been reported.[148] A 2010 paper reviews meningococcal disease and travelers.[149] Persons traveling to countries within the meningitis belt of sub-Saharan Africa and who will have contact with the local population (persons visiting friends and relatives, healthcare workers, and long-term travelers, such as missionaries and volunteer workers) are thought to be at higher risk than tourist travelers and may benefit from immunization. Other vaccine candidates include persons at increased risk of contracting meningococcal disease, such as those with functional or anatomic asplenia or late complement deficiency. Persons who will travel to these areas and stay in tourist accommodations with little contact with the local population are at low risk and may elect not to be immunized. Immunization should be done, if possible, at least 10 to 14 days before departure.

Currently available meningococcal vaccines in the United States and elsewhere include polysaccharide and conjugate vaccines containing the four serogroups A, C, Y, and W135, bivalent A and C polysaccharide and conjugate vaccines, conjugate serogroup C vaccines, a combination vaccine with serogroups C and Y along with *Haemophilus influenzae* type b, and, most recently, serogroup B protein vaccines.[150,151] Conjugate vaccines generate equivalent or higher concentrations of protective antibody and may elicit greater duration of protective immunity than polysaccharide vaccines.[152] Additional benefits include the ability of conjugate vaccines to decrease nasopharyngeal carriage and therefore provide herd immunity.[150] Meningococcal conjugate vaccines also have the potential for greater immunogenicity in infants (and are licensed in the United States for children 2 months of age and older), as well as greater efficacy in those with underlying health problems affecting ability to respond to polysaccharide antigens.[153] It is important to choose vaccines containing serogroup A, and preferably also W135, for travel to the meningitis belt of sub-Saharan Africa. There are as of this writing no specific

recommendations for use of meningococcal vaccines for travel to other destinations, except in the setting of an outbreak. Serogroup B vaccine may be considered if travel to an area with an outbreak is anticipated.[154]

Duration of immunity of polysaccharide vaccine is at least 3 years in persons immunized when at least 4 years of age. Duration of immunity of conjugate vaccine is at least equivalent to that of the polysaccharide vaccine and is expected to be longer lasting; the Saudi health authorities grand validity for 8 years.[152,155,155a] Because of increased evidence of waning immunity, however, recommendations for revaccination have been updated by the CDC. A booster dose is recommended at age 16 years for those who were immunized at age 11 to 12 years, and a two-dose primary series, with doses 2 months apart, is recommended for those at increased risk of meningococcal disease. Children who were immunized at 6 years of age or younger who live in endemic areas or take a subsequent trip to a risk area may be given a booster dose after 3 years, and for those immunized at age 7 years or later, a booster may be given after 5 years.[150] Systemic adverse reactions were reported at similar rates in recipients of conjugate and polysaccharide vaccines; local adverse reactions were more common in those who received the conjugate than those who received polysaccharide vaccine.[150] A possible association of receipt of meningococcal conjugate vaccine and Guillain-Barré syndrome was reported in October 2005; the eight cases reported were not thought to represent an increase in incidence of this condition over what would be expected to occur by chance alone.[156] As of this writing, the CDC continues to recommend use of meningococcal conjugate vaccine for those for whom vaccine is indicated.[157] Adverse effects of immunization with meningococcal polysaccharide vaccine during pregnancy have not been documented; no data are available on the safety of the conjugate vaccine.[150] When considering immunization during pregnancy, the theoretical risk of immunization must be balanced against the risk of disease. In general, conjugate vaccines are preferable to polysaccharide vaccines when available because of superior immunogenicity, potential for reduction of nasopharyngeal carriage resulting in herd immunity, and effectiveness in individuals with conditions resulting in poor response to polysaccharide vaccines.[150,152,153]

Rabies

Human rabies causes approximately 60,000 deaths per year, mostly in Africa and Asia, with more than 95% occurring from exposure to rabid dogs.[158] The WHO estimates that more than 10 million persons receive rabies postexposure prophylaxis each year.[159] Currently there are two to three cases of human rabies per year in the United States, some acquired locally.[161] A review of 60 travel-associated cases worldwide from 1990 to 2012 identified many cases in short-term travelers and those visiting friends and relatives (predominantly immigrants returning to visit their country of origin).[162] Risk of rabies in travelers depends on destination, length of stay, and exposure to animals (especially dogs). A study in Thailand reported rates of animal bites, licks, and scratches of 1.7, 6.9, and 1.8 per 1000 person-months, respectively, in expatriates (non-Thai individuals living in Thailand for extended periods) and 43.1, 136.1, and 33 per 1000 person-months, respectively, in travelers.[163] A 2014 review of rabies in travelers concluded that the incidence of injuries to travelers caused by potentially rabid animals was 0.4% per month of stay.[164] Dogs were associated with 60% of exposures in one global study, and 66% of exposures associated with mammals in a study in Thailand.[163,165] A study of travelers seen during or after travel for rabies postexposure prophylaxis reported Thailand, Nepal, China, India, and Indonesia to be the most frequent countries of exposure for dog bites; other countries were associated with exposures to nonhuman primates (India, Vietnam, Brazil), cats (Algeria and Turkey), and bats (French Guyana, Peru, Mexico, and Surinam).[164,165] In Costa Rica, the first human rabies cases in 31 years were reported in 2001.[166] The 2008 outbreak of rabies in Bali, a popular tourist destination, highlights the importance of speaking with travelers about rabies risk.[167] Travelers should be informed about risk of rabies in the region to which they will travel and be advised to avoid contact with animals that could be rabies carriers, especially dogs but also cats, nonhuman primates, skunks, raccoons, and bats.[168] Cases of rabies in travelers highlight the importance of offering preexposure prophylaxis and counseling travelers about management of exposures while abroad.[169,170] Travelers should be aware of the need for a plan if a bite or exposure occurs because access to treatment is unpredictable globally. Three inactivated rabies vaccines are licensed for use in the United States, with others available worldwide. Vaccines may be used to provide preexposure prophylaxis (termed prophylaxis rather than immunization to emphasize that receiving this product before exposure does not obviate the need for additional injections if an exposure occurs) or postexposure prophylaxis. Travelers to countries where canine rabies is endemic or where exposures to potentially rabid animals could occur are candidates for preexposure vaccination, as are persons whose purpose of travel includes relevant animal or laboratory work; those who are likely to be hiking, bicycling, or riding motorcycles; or those whose itineraries include remote destinations where prompt medical attention is impossible.[171] Preexposure vaccination does not remove the imperative to seek definitive treatment if an exposure occurs but does alter the postexposure prophylaxis regimen. Table 71.5 lists the criteria for preexposure immunization and dosage schedules for vaccines licensed in the United States. Rabies postexposure prophylaxis consists of two doses of rabies vaccine for those who have received preexposure prophylaxis, and rabies immunoglobulin (RIG) plus vaccine for those who have not. Human RIG is necessary for such travelers, and they need to be informed that this preparation may not be available in their destination country.[172–175]

The postexposure prophylaxis series for those who have not had preexposure prophylaxis consists of four doses of vaccine at days 0, 3, 7, and 14 (CDC), and 28 (WHO)[176] The vaccine should be accompanied by a single dose of RIG, given no later than 7 days after the exposure, but ideally accompanying the first dose of vaccine. RIG should be diluted and infused directly into the wound, and vaccine given in a separate injection at a distant site. The WHO recommends intradermal rabies vaccine in places where vaccine is in short supply[177]; this regimen is not approved in the United States or many other countries. Vaccine preparations other than those listed in the table may be available in some countries; these vaccines are produced locally and have unknown safety and efficacy. If bitten by a suspect animal, an individual should receive modern tissue culture vaccine preparations, even if this means traveling to a destination where RIG and vaccine are available, or receiving additional treatment once the individual has returned to the individual's country of origin. Adverse events, such as local pain and swelling, have been reported in 30% to 74% of recipients of human diploid cell vaccine (HDCV). Systemic reactions may occur in up to 40% of recipients. Three cases of central nervous system disease have been reported following HDCV administration, but a causal relationship has not been established. Up to 6% of those receiving booster doses of HDCV may experience immune complex-like reactions. Although vaccines prepared in purified chick embryo cell culture may cause mild erythema, swelling, and pain at the injection site, serum sickness–like hypersensitivity reactions

TABLE 71.5 Rabies Preexposure Immunization[a]

Risk Category	Nature of Risk	Typical Populations	Preexposure Regimen
Frequent	Exposure usually episodic with source recognized, but exposure may also be unrecognized Bite, nonbite, or aerosol exposure possible	Rabies diagnostic laboratory workers, cavers, veterinarians and staff, and animal control and wildlife workers in rabies-epizootic areas	Primary preexposure immunization course; serologic examination or booster immunization every 2 years
Infrequent (but greater than population at large)	Exposure nearly always episodic with source recognized Bite or nonbite exposure	Veterinarians, animal control, and wildlife workers in areas of low rabies endemicity; veterinary students; and travelers visiting areas where rabies is enzootic and immediate access to medical care, including biologics, is limited	Primary preexposure immunization course; no routine booster immunization or serologic examination

[a]Preexposure immunization consists of three doses of human diploid cell vaccine (HDCV), rabies vaccine (adsorbed), or RabAvert, 1.0 mL intramuscularly (IM), one each on days 0, 7, and 21 or 28. Only HDCV may be administered by the intradermal (ID) dose/route (0.1 mL ID on days 0, 7, and 21 or 28). If the traveler will be taking chloroquine or mefloquine for malaria chemoprophylaxis, the three-dose series must be completed before initiation of antimalarials. If this is not possible, the IM dose/route should be used. Administration of routine booster doses of vaccine depends on exposure risk category. Preexposure immunization of immunosuppressed persons is not recommended.

Modified from the Centers for Disease Control and Prevention (CDC) Health Information for International Travel, 2016.

have not been problematic with these vaccines.[178] When given as postexposure prophylaxis, the series of doses should not be interrupted because of mild systemic or local reactions to vaccine; they can be managed with antiinflammatory agents.

Antimalarials such as chloroquine (and possibly mefloquine) may interfere with antibody response to HDCV rabies vaccine.[29] Other drugs (steroids or immunosuppressive agents) or conditions that affect the immune system also may impair efficacy of vaccine. HIV-infected persons, especially those with low CD4 counts, have impaired antibody response following rabies vaccine.[179] Persons taking such agents or experiencing such conditions may be candidates for testing of serum antibody levels following immunization. In addition, immunosuppressed persons who have not received preexposure prophylaxis require specific counseling about the importance of immediate wound care and administration of RIG promptly after any possible exposure, to take into account possible poor response to postexposure immunization.

Pregnancy is not a contraindication to postexposure prophylaxis with rabies vaccine, and no harmful effects to the fetus from immunization have been noted. If risk of exposure is high, preexposure prophylaxis may be offered.[180]

Tickborne Encephalitis

Tickborne encephalitis (TBE) is endemic to Central and Eastern Europe, Russia, and the Far East, and its area of endemicity is expanding.[181,182] In 2012 there were 38 reported cases of TBE in international travelers. The attack rate for an undefined period of time could be roughly extrapolated to be 0.5 to 1.3 per 100,000 population overall in Western/Central European endemic areas for the exposed at-risk population.[142] Disease occurs following the bite of an infected tick or through ingestion of infected unpasteurized milk. Although asymptomatic and mild infections occur commonly, neurologic manifestations and sequelae are significant causes of morbidity and mortality.[183] Most infections are acquired in rural locations; those working or vacationing in such locations between April and November, when tick activity is greatest, are at greatest risk. McNeil and colleagues reported an infection rate of 0.9 per 100 per month of exposure in a study of American service members of a military unit who lived and trained in a highly endemic area in Central Europe.[184] Five cases were reported in U.S. travelers to Europe and Asia from 2001 to 2008.[185]

Active immunization is the most effective means of prevention of TBE. Two vaccines, FSME-IMMUN (Baxter Vaccine AG, Wien, Austria, from 2015 Pfizer) and Encepur (Novartis Vaccine [formerly Chiron Behring], Marburg, Germany, from 2015 GlaxoSmithKline) are used widely in Europe. FSME-IMMUN's efficacy has been reported to be greater than 95%, based on data collected during active surveillance for infection following a mass immunization campaign in Austria. Several vaccines are marketed in Russia; one has been reported to be highly effective.[186] No vaccines are available in the United States or Canada. Travelers who plan extended stays in endemic areas can arrange to receive vaccine locally. TBE vaccination is most appropriate for persons traveling to high-risk TBE regions who plan to take part in outdoor activities.[186] TBE immunoglobulin, once proposed for preexposure prophylaxis, is no longer available.[187] Measures to prevent exposures to ticks, such as use of appropriate clothing and repellents containing *N,N*-diethyl-meta-toluamide (DEET), avoidance of tick habitats, immediate removal of ticks, and avoidance of unpasteurized milk, are additional strategies that can be used to prevent TBE.

The vaccine is given as a three-dose series, with the first two injections at least 4 weeks apart, and a third dose 5 to 12 months later, depending on the vaccine. An accelerated schedule of Encepur, with doses at days 0, 7, and 21 and with a booster 12 to 18 months after the third dose, may be used. Boosters are recommended at 3- to 5-year intervals in most countries, but protection may last much longer (Table 71.6). Fever is the most common side effect of vaccine; mild local and systemic reactions may also occur. Persons with egg allergy should not receive this vaccine. Data are lacking regarding use of these vaccines during pregnancy.

Typhoid

Typhoid is another common vaccine-preventable disease affecting travelers. In the United States, 1393 cases of typhoid fever were reported from 1994 to 1999, with recent travel associated with 74% of cases. In only 36 (4%) of travel-associated cases did persons report receipt of typhoid vaccine.[188] Seventy-nine percent of 1902 cases reported (with adequate information) between 1999 and 2006 were related to foreign travel.[189] There were approximately 400 cases of typhoid per year from 2007 to 2011 in the United States, with approximately 90% occurring in those returning from foreign travel; more than

TABLE 71.6 Recommended Doses and Schedules for Tickborne Encephalitis Vaccines

Group	Vaccine	Dose	No. of Doses	Schedule	Booster
Children 1–15 y old	FSME-IMMUN	0.25 mL SC/IM	3	0, 2 wk to 3 mo	5–12 mo after second dose
Individuals 16 y and older	FSME-IMMUN	0.5 mL SC/IM	3	0, 1–3 mo, 9–12 mo after second dose	3–5 y after last dose
Children 1–11 y old	Encepur	0.75 μg/0.5 mL SC/IM	3	0, 1–3 mo, 9–12 mo after second dose[a]	3–5 y after last dose
12 y and older	Encepur	1.5 μg/0.5 mL SC/IM	3	0, 1–3 mo, 9–12 mo after second dose[a]	3–5 y after last dose

[a]An accelerated schedule of days 0, 7, and 21 also may be used. In this case, a fourth dose should be given 12 to 18 months after completing the three-dose series.
IM, intramuscular; SC, subcutaneous.

TABLE 71.7 Dosage and Schedules for Typhoid Fever Vaccination

Vaccination	Age	Dose/Mode of Administration	No. of Doses	Interval Between Doses	Boosting Interval
ORAL LIVE ATTENUATED TY21A VACCINE					
Primary series (capsule)	≥6 y	1 capsule[a]	3 or 4	48 h	NA
(liquid)	≥3 y	1 packet[b]	3	48 h	
Booster	≥6 y	1 capsule[a]	4	48 h	Every 5 y[c]
VI CAPSULAR POLYSACCHARIDE VACCINE					
Primary series	≥2 y	0.50 mL[d]	1	NA	–
Booster	≥2 y	0.50 mL[d]	1	NA	Every 2 y

[a]Administer with cool liquid no warmer than 37°C.
[b]Dissolve in no more than 4 ounces of cold or lukewarm water.
[c]Every year for those taking the three-dose series.
[d]Intramuscularly.
NA, Not applicable.

75% of these travelers had returned from India, Bangladesh, or Pakistan.[190] Risk of contracting typhoid depends largely on travel destination, with greatest risk associated with travel to the Indian subcontinent.[189,191] Those traveling for the purpose of visiting friends and relatives make up a large proportion of reported cases in travelers.[190] A study showed risk of disease to be related inversely to receiving vaccine prior to travel: incidence rates per visit ranged from 16 per 100,000 in North American tourists, 92% of whom had received typhoid vaccine prior to travel, to 216 per 100,000 in Israeli tourists, 6% of whom had been immunized.[193] A study from Sweden reported rates of enteric fever of 0.42 per 100,000 travelers overall, and this study, along with most others, continues to show the highest risk for typhoid is in travelers to the Indian subcontinent (41.7 per 100,000).[194,195] Steffen and colleagues estimate rates of typhoid to be between 0.01% and 0.1% for travel to South Asia and between 0.001% and 0.01% for other destinations for a 1-month stay.[142] The Canadian Committee to Advise on Tropical Medicine and Travel assesses the risk as 33 cases per 100,000 for travel to South Asia, one to two cases per 100,000 for travel to Africa (with a negligible risk in Southern Africa), the Middle East and South America, and less than one per 300,000 for the Caribbean and Central America.[196]

Two vaccines are available in the United States to protect travelers from typhoid. While the Vi vaccine does not protect against paratyphoid fever, which accounts for a growing proportion of enteric fever in many countries, some claim that the Ty21a vaccine may offer limited protection.[190] Some travel medicine providers are recommending vaccine more frequently as a result of worsening antibiotic resistance profiles of *Salmonella typhi*. Food and water precautions should continue to be used to augment protection afforded by vaccine. Oral live attenuated typhoid vaccine (Vivotif; Crucell) manufactured from the Ty21a strain of *S. typhi* is given in a series of three (Europe) or four (North America) capsules taken every other day and has a vaccine efficacy of 67%.[197] In the United States, it is not licensed for children younger than 6 years of age, and administration of this vaccine may be limited by the ability of small children to swallow capsules.

A capsular polysaccharide vaccine (TYPHIM Vi; Sanofi Pasteur), given in a single intramuscular dose, is available for persons 2 years of age and older. Vaccine efficacy of this product ranges from 55% to 74%.[198,199] Duration of immunity is uncertain; Keddy and colleagues reported persistence of antibody for more than 10 years following immunization with this vaccine in South African schoolchildren, but acknowledge that exposure to disease may have contributed to these findings.[200]

Table 71.7 lists typhoid vaccine schedules. No vaccine is available for children younger than 2 years of age. Mothers of infants may be reassured that exclusive breastfeeding (reducing contact with contaminated food or water) reduces risk for infants when careful hand washing also is done. Booster doses are recommended every 2 years in the United States for the polysaccharide vaccine (3 years in many other countries).[203] In the United States, boosters for the oral vaccine are recommended every 5 years (every 7 years in Canada), and in countries where the three-dose series is used, every year for travelers

from nonendemic to endemic areas, and every 3 years for those living in endemic areas.[203]

Adverse reactions to the capsular polysaccharide vaccine include fever in 1% or fewer of vaccine recipients, headache in up to 3%, and local reactions in approximately 7%. Oral vaccine results in fever or headache in approximately 5% of recipients, and stomach pain, nausea, vomiting or rash occur rarely.[204] Impaired response to capsular polysaccharide vaccine occurs in HIV-infected persons, especially those with low CD4 counts.[205] Oral typhoid vaccine is a live bacterial preparation and should not be given to immunocompromised persons, including those with HIV infection. Concerns have been raised about the immunogenicity of this vaccine in persons receiving proguanil (as in the antimalarial agent atovaquone-proguanil). Growth of the live Ty21a strain is inhibited by some antimicrobial agents and by the antimalarial agent mefloquine, although there are no data to suggest that mefloquine interferes with vaccine efficacy.[30,31] Ideally, the oral typhoid vaccine series should be completed at least 2 weeks before departure. Oral typhoid vaccine should not be taken concurrently with antimicrobial agents; capsules should be taken 24 hours or more after the last dose of the antibiotic (and probably longer if an agent with a long half-life, such as azithromycin, has been taken). Oral typhoid vaccine may be given concurrently with yellow fever vaccine.[102] Concomitant administration of inactivated hepatitis A, Typhim Vi, and yellow fever vaccines resulted in adequate responses to all three vaccines without affecting the adverse event profile.[206] Data are not available regarding concurrent administration of oral typhoid vaccine and other live-virus vaccines. Vaccine may be administered at the same time as immunoglobulin. Safety during pregnancy of vaccines against typhoid has not been established; live vaccines are contraindicated in general, and the injectable vaccine administered only if clearly needed.[190]

Yellow Fever

Yellow fever continues to be a disease of importance in sub-Saharan Africa and South America. Reemergence of disease continues on both continents in 2016–17, resulting in increased attention to measures to reduce transmission and enhance immunization coverage.[207–210] Recent outbreaks occurred in Angola in 2015–16 and in Brazil in 2016–17.[210a,210b] Most cases of yellow fever occur in nonimmune, unvaccinated persons who migrate to or visit areas where transmission occurs.[211] Yellow fever can be a fatal disease, and cases have occurred in unimmunized travelers.[212–215] During the recent Angola outbreak, approximately 12 cases of yellow fever have been exported by nonimmunized workers to China and by other travelers to several other countries within Africa.[215a] Figs. 71.5 and 71.6 show the destination countries for which yellow fever vaccination is recommended. An outbreak of yellow fever in Brazil prompted updating of the yellow fever risk map in Brazil to acknowledge expansion of the risk areas in the states of Bahia, Espirito Santo, Minas Gerais, Rio de Janiero, and Sao Paulo.[215b]

Vaccination against yellow fever offers high levels of protection, with seroconversion rates of greater than 95% in children

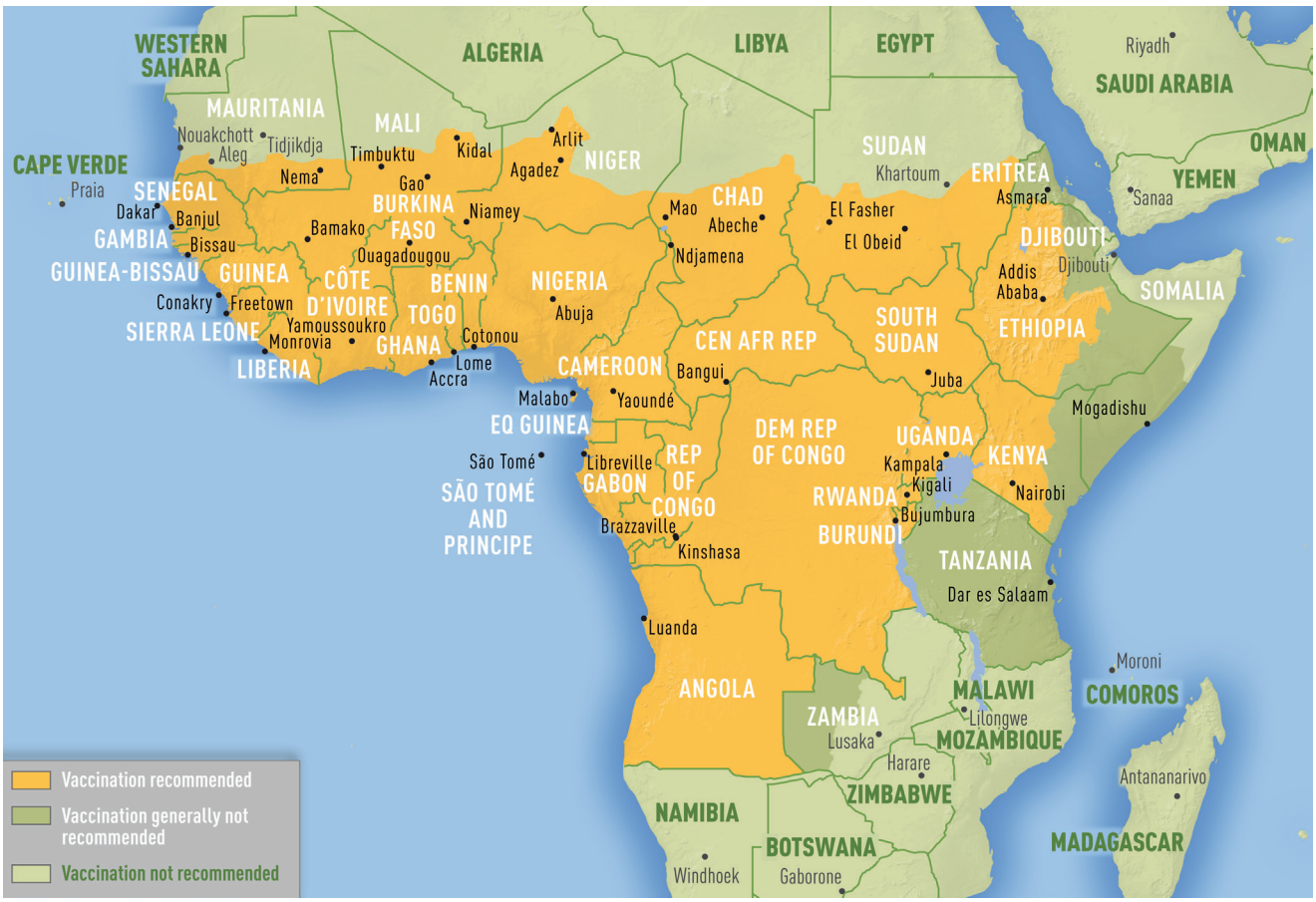


Figure 71.5. Yellow fever vaccine recommendations in Africa. *(From Centers for Disease Control and Prevention [CDC]. Health Information for International Travel 2016. For the most up-to-date version, see https://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/yellow-fever.)*



Figure 71.6. Yellow fever vaccine recommendations in the Americas. *(From Centers for Disease Control and Prevention [CDC]. Health Information for International Travel 2016.)*

and adults and duration of immunity of at least 10 years, and likely lifelong in healthy individuals.[216] Side effects are generally mild and include headaches, myalgia, and low-grade fever occurring 5 to 10 days after immunization in less than 25% of those participating in clinical trials of yellow fever vaccine.[217,218] Immediate hypersensitivity reactions are uncommon and are most likely to occur in those with allergy to eggs.

Serious adverse events to yellow fever vaccine include yellow fever vaccine–associated neurotropic disease (YFV-AND; formerly postvaccinal encephalitis) and yellow fever vaccine–associated viscerotropic disease (YFV-AVD). These reactions have occurred exclusively in persons receiving their first yellow fever vaccine. Encephalitis has been reported in vaccine recipients with higher rates in infants; the vaccine is contraindicated in infants younger than 6 months of age and should be used with caution, balancing carefully risk of disease with risk of severe adverse events, in those 6 to 8 months of age.[219] A syndrome of fever, jaundice, and multiorgan system failure following yellow fever vaccine was reported in 10 patients (ranging in age from 5 to 79 years) worldwide (five in the United States, two in Brazil, and one each in Australia, Switzerland, Germany, and the United Kingdom) from 1996 to 2001.[220–226] This syndrome, initially called febrile multiorgan system failure, ranges in severity from moderate disease with focal-organ dysfunction to severe multisystem failure and death, and may include neurotropic disease. In 2002, two suspected cases of viscerotropic disease and four suspected cases of neurotropic disease were reported in U.S. recipients of yellow fever vaccine.[227] A fatal case reported in 2005 involved a 22-year-old female.[228] A study of reports submitted to the VAERS in the United States identified advanced age as a risk factor for adverse events associated with yellow fever immunization.[229]

Initial estimates of rates of YFV-AND were 0.5 to four cases per 1000 very young infants and fewer than one case per 8 million vaccinees older than 9 months of age in the United States.[230] Initial estimates of incidence of YFV-AND were made based on number of doses distributed to the civilian population, safety monitoring data for doses distributed during 1990 to 1998 in the United States, and number of cases identified using enhanced surveillance of yellow fever vaccine adverse events through the VAERS database. These data yielded an estimated reported incidence of 2.5 per 1,000,000 doses distributed.[231] Limitations of this estimate include possible underreporting of cases and lack of exact information about number of doses administered. The CDC has published current estimates of risk for the three serious adverse events to yellow fever vaccine; these data are printed on the vaccine information statement for yellow fever vaccine and are useful when discussing yellow fever immunization with patients. Risk estimates are as follows: life-threatening allergic reactions: one per 55,000 doses; vaccine-associated neurologic disease: one per 125,000 doses; and vaccine-associated viscerotropic disease: one per 250,000 doses.[232] An update of advanced age as a risk factor for serious adverse events to yellow fever vaccine was published in 2005, confirming the increased risk and documenting a reporting rate ratio of 5.9 (95% confidence interval, 1.6 to 22.2) in first-time vaccinees 60 years of age or older and 10.4 (95% confidence interval, 2.7 to 40.2) in those 70 years of age or older relative to those 19 to 29 years of age (0.7/100,000).[233] Although risk factors for developing severe reactions after yellow fever vaccine are not well understood, one report identified thymus disease as present in a greater proportion of those who developed YFV-AVD after yellow fever vaccination than in the general population.[234] A case of death resulting from YFV-AVD in an individual with undiagnosed thymoma was reported from Oregon, in 2015.[235] Several articles summarize indications for yellow fever vaccine and risk of adverse events in an effort to guide appropriate recommendations for travelers.[236–239] Yellow fever vaccine must be given at official yellow fever vaccine centers and travelers should be given documentation of immunization on the ICVP. Travelers to countries or areas of countries where disease risk is present should receive a single dose of vaccine at least 10 days before departure. As of July 11, 2016, the certificate of vaccination against yellow fever as documented on the ICVP is valid for the life of the person vaccinated per Amendment 7 of the International Health Regulations (2005). The CDC ACIP has issued recommendations for use of yellow fever vaccine booster doses.[240–245] Vaccine is contraindicated in children younger than 6 months of age and should be used cautiously in children 6 to 8 months of age. Vaccine is also contraindicated in HIV-infected persons with CD4 counts less than 200/mm^3 or less than 15% of total lymphocytes for children younger than 6 years of age, and in persons whose immune systems have been altered by immunomodulating agents.[219] Precautions against use of yellow fever vaccine include: age older than 60 years; infants 6 to 8 months of age; pregnant or nursing women; and weakened immune systems. The single most important step in judicious use of yellow fever vaccine is to provide vaccine only to persons traveling to yellow fever–endemic areas: in one report, two of five persons with multiorgan system failure were traveling to areas where yellow fever had never been described.[226] Persons not protected by vaccine should use adequate personal protective measures to avoid mosquito bites.

Serologic response to yellow fever vaccine is not diminished by simultaneous administration of tetanus, diphtheria, pertussis, measles, polio, BCG, hepatitis A, hepatitis B, Vi antigen capsular polysaccharide typhoid, or oral Ty21a typhoid vaccines.[102,246] Immunoglobulin did not decrease the antibody response to yellow fever vaccine when given 0 to 7 days before immunization.[247] The antimalarial chloroquine has been shown not to affect adversely the antibody response to yellow fever vaccine.[248]

Yellow fever immunization may be given during pregnancy if risk of disease is believed to be greater than risk of adverse events to vaccine.[249] Several studies are available to inform these decisions. One documented fetal infection in one of 41 infants exposed to maternal vaccination; the infant was full-term and appeared normal.[250] A mass vaccination campaign in Brazil, in early 2000, resulted in inadvertent immunization of 480 pregnant women with 17DD vaccine; the women and infants were followed until the infants were 1 year of age. Maternal seroconversion was high, and no infants were found to be infected at birth (no immunoglobulin M antibodies detected and no placental or umbilical cord blood found to contain yellow fever vaccine strain virus by polymerase chain reaction).[251] Immunization during pregnancy may result in antibody concentrations that are inferior to those obtained following immunization of nonpregnant women; women immunized during pregnancy may be candidates for reimmunization when next at risk for yellow fever.[252] Immunization with yellow fever vaccine is not recommended for immunocompromised persons or those with symptomatic HIV infection. Vaccine has been administered with good tolerance and efficacy to asymptomatic HIV-infected persons.[253–255] Cases from Brazil and Canada of yellow fever transmitted via breast milk have been reported.[256–258]

COST-EFFECTIVENESS OF TRAVEL IMMUNIZATIONS

Travel medicine experts have long noted the willingness of travelers to accept (and pay for) multiple vaccines for diseases for which they are at minimal risk. Beutels and colleagues, in an economic evaluation of travel medicine interventions, concluded that, except for hepatitis A vaccine in specific circumstances, no travel vaccine has a favorable cost-to-benefit ratio.[259] Costs to prevent travel-related vaccine-preventable disease ranged from US$100,000 to US$300,000 to prevent a case of typhoid, US$4 million to prevent a case of JE, and US$5 to US$29 million to prevent a case of cholera.[260–263] The most studied travel-associated vaccine in terms of cost-effectiveness is hepatitis A. In one study, which also examined cost-effectiveness of typhoid immunization, neither typhoid nor hepatitis A vaccine was found to be cost-effective; societal expenditures of more than £67 million were required to prevent a single death from typhoid fever with either of the two typhoid vaccines.[264] In contrast, a study from The Netherlands suggested that cost-effective choices are available regarding hepatitis A immunization when frequency of travel, likelihood of prior immunity, and product used are taken into consideration.[265] Typhoid vaccination was found rarely to be cost-effective, with the possible exception of travel to very-high-incidence countries when the itinerary involved close contact with the local population.[266] Cost-effectiveness of preexposure rabies prophylaxis has been studied in Canada, with the conclusion that routine preexposure prophylaxis for most travelers is not indicated.[267]

Issues complicating performance of studies of cost-effectiveness of travel vaccines include difficulty estimating risk of vaccine-preventable diseases accurately; difficulties in determining vaccine efficacy, especially for travelers who will have variable exposures in endemic areas; difficulties approximating costs associated with disease; and lack of sufficient experience with some newer vaccines to allow accurate estimation of the incidence of vaccine-associated adverse events.

Finally, some practitioners may be reluctant to omit any vaccine, no matter the risk of disease, for fear of liability. Until these issues are studied in greater detail, each travel medicine provider must continue to individualize recommendations for travel vaccination, taking into account the potential for adverse reactions to immunization and the patient's risk for the disease. Current recommendations by the CDC and the WHO for travel vaccination should be followed, regardless of cost-effectiveness data.

THE FUTURE

Rapid advances in biotechnology promise a potential revolution in the development of new vaccines using diverse approaches ranging from DNA vaccines to transgenic plant vaccines, although significant technical hurdles remain.[268,269] New candidate vaccines show promise in protecting against diverse pathogens that cause diarrheal disease.[270] A dengue vaccine has been licensed in some countries, although there are currently no indications for use in travlers.[270a] Optimism remains for the eventual development of vaccines against Zika virus, Ebola virus, chikungunya virus infection, HIV, norovirus, and malaria.[271–276] In addition to the remaining technical hurdles, however, equally formidable economic hurdles remain in adequate funding of vaccine research and development against diseases that mostly afflict the poorest populations in the poorest countries.[277] The increased interest in travel and travel immunizations by more affluent travelers may provide substantial assistance in making these much-needed vaccines a reality.

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72 Vaccines for Healthcare Personnel

David J. Weber and William A. Rutala

Healthcare personnel (HCP) are commonly exposed to infectious agents. The risks and methods of preventing occupational acquisition of infection by HCP have been reviewed.[1–8] Minimizing the risk of disease acquisition is based on strict adherence to five key recommended interventions: (a) adherence to standard precautions when providing patient care,[9–11] especially the performance of appropriate hand hygiene before and after patient care[12–22]; (b) rapid institution of appropriate isolation precautions for patients with known or suspected communicable diseases[9,10,23,24]; (c) proper use of personal protective equipment, such as masks, N-95 respirators, eye protection, and gowns when caring for patients with potentially communicable diseases[10,11,24,25]; (d) evaluation of personnel who were exposed to communicable diseases for receipt of postexposure prophylaxis[1,23,27–29]; and (e) appropriate immunizations.[1,7,30–32] Laboratory personnel, including hospital personnel working in microbiology laboratories, are also at risk for acquiring infectious diseases.[33–36] Prevention of laboratory-acquired infection requires adherence to recommended administrative protocols (e.g., no eating, drinking, or smoking in areas where microbiologic or pathologic samples are processed), engineering controls (e.g., containment hoods), personal protective equipment (e.g., N-95 masks when culturing *Mycobacterium tuberculosis*), and appropriate immunizations.[37–40] Vaccine-preventable diseases may be classified by their route of transmission and include airborne (e.g., varicella, measles), droplet (e.g., influenza, pertussis, meningococcal infection), contact (e.g., hepatitis A from contact with feces), and parenteral or mucosal exposure to blood or contaminated body fluids (e.g., hepatitis B).

Immunization of HCP should be included by all healthcare facilities as part of a comprehensive occupational health program. Ensuring that HCP are immune to vaccine-preventable diseases is important because it protects HCP from infections that cause potentially serious complications when acquired as an adult (e.g., rubella, varicella, hepatitis B) and prevents the HCP from serving as a source for infecting patients, especially immunocompromised patients, with diseases that may lead to serious morbidity or even death (e.g., varicella, measles).[41,42] For this reason, all new HCP should receive a prompt review of their immunization status against vaccine-preventable diseases. Immunization status also should be reviewed yearly. Other important occupational health interventions include an accessible health service, baseline and periodic testing for tuberculosis (i.e., purified protein derivative skin test or interferon-γ release assay blood test), evaluation of HCP ill with potential communicable diseases to provide appropriate treatment and work restrictions, evaluation of HCP after infectious disease exposures for postexposure prophylaxis and work restrictions, and education of HCP focusing on general infection control guidelines in addition to Occupational Safety and Health Administration (OSHA)-mandated training in the prevention of bloodborne pathogens[43] and tuberculosis.[44]

VACCINES RECOMMENDED FOR HEALTHCARE PERSONNEL

General Guidelines

General recommendations regarding vaccination of HCP have been published by the Centers for Disease Control and Prevention (CDC),[1] the Advisory Committee on Immunization Practices (ACIP),[30,45,46] the American Academy of Pediatrics,[47] the Association for Professionals in Infection Control and Epidemiology,[48] and infectious disease experts.[7,31,49,50] It is recommended that all HCP be immune to mumps, measles, rubella, varicella, pertussis, and influenza (Table 72.1).[1,7,30,31,45–48] Depending on the vaccine, preventable disease immunity may be assured by several different measures. All HCP with potential exposure to blood or body fluids should be immune to hepatitis B. Influenza vaccine should be offered to all HCP yearly. In the past few years, editorials and commentaries have recommended that yearly influenza immunization (unless contraindicated) should be a condition of employment for HCP.[51–55] New policy statements from professional societies have endorsed the requirement for yearly influenza immunization, including the American Academy of Pediatrics,[56] Association for Professionals in Infection Control,[57] Infectious Diseases Society of America,[58] American College of Physicians,[59] and the Society of Healthcare Epidemiologists of America.[60] In February 2012, the National Vaccine Advisory Committee issued a statement which provided recommendations on how to achieve the Healthy People 2020 annual influenza vaccine coverage goal (i.e., 90%) for HCP; for facilities that have implemented the recommended initial strategies but have "not consistently achieved the Healthy People goal for vaccination coverage of HCP in an efficient and timely manner" it was recommended that they should "strongly consider an employer requirement for influenza immunization."[61] However, some of these policy statements allow HCP to opt out if they sign a declination statement, have religious or personal objections, or agree to wear a surgical mask while working in a clinical area during influenza season. Detailed recommendations have been published regarding mumps, measles and rubella[62]; varicella[63]; hepatitis B[64–66]; influenza[67,68]; and Tdap (tetanus toxoid, reduced diphtheria toxoid, acellular pertussis)[69–71] vaccines. All HCP should be subject to these recommendations, including personnel with direct patient care responsibilities (e.g., nurses, respiratory technicians, physical therapists, physicians, students and other trainees), personnel without direct patient care responsibilities (e.g., environmental service workers, security), contract workers, volunteers, and emergency medical personnel. HCP should be provided vaccines that are recommended for adults,[45,46] such as human papillomavirus, herpes zoster, and pneumococcal vaccines, or referred to their local medical provider. In special circumstances, HCP or laboratory personnel should be offered immunization with other vaccines, including polio[72,73]; quadrivalent (A, C, Y, W135) meningococcal (polysaccharide,

conjugate)[74] and meningococcal serogroup B vaccines[75]; rabies[76,77]; typhoid[78]; hepatitis A[79]; anthrax[80]; and vaccinia (smallpox)[81–83] (Table 72.2).

Immunocompromised HCP require special consideration in the provision of immunizations (Table 72.3).[45,46,84,85] First, live virus vaccines (e.g., measles-mumps-rubella [MMR], varicella, live attenuated influenza) may be contraindicated. Second, vaccines not routinely recommended may be indicated (e.g., pneumococcal, meningococcal, *Haemophilus influenzae* type b). Third, higher antigen doses (e.g., hepatitis B vaccine in people with end-stage renal disease), additional doses of vaccine (e.g., rabies vaccine in immunocompromised persons), or postimmunization serologic evaluation may be indicated (e.g., anti–hepatitis B surface antigen titer after hepatitis B vaccine, antibody response to rabies vaccine) because immunization of immunocompromised people may elicit a lower antibody response. Finally, such personnel should be individually evaluated for reassignment (with the consent of the employee) depending on their job duties. Of importance, caring for an immunocompromised patient is not

TABLE 72.1 Vaccines Strongly Recommended for All Healthcare Personnel

Vaccine	Demonstration of Immunity[a]	Indications[b]	Administration[b]	Major Contraindications and Precautions[b]
Influenza	Yearly immunization needed	All healthcare personnel[c]	1 dose 0.5 mL IM (deltoid) inactivated vaccine, 1 dose intradermal, 1 dose live attenuated influenza vaccine (LAIV) via nasal instillation	History of anaphylaxis to eggs; LAIV contraindicated in HCP who work with patients housed in a "protected environment," pregnancy, immunocompromising disorder[d] or medications, ≥50 years of age
Hepatitis B	Laboratory evidence of immunity[e] unless last dose of series provided >6 months prior to evaluation, prior receipt of three doses of vaccine with appropriate schedule	All healthcare personnel with potential exposure to blood or contaminated body fluids	1.0 mL dose IM at 0, 1, 6 mo, additional doses not necessary if adequate response[h]; if response inadequate, test for HBsAg, and if negative, provide additional three doses and retest antibody response (if still inadequate consider HCP a nonresponder)	History of anaphylaxis to common baker's yeast; pregnancy should not be considered a contraindication
Measles	1. Documented administration of two doses of live measles virus vaccine (MMR)[e] *or* 2. Laboratory evidence of immunity *or* laboratory confirmation of disease *or* 3. Born before 1957[f] 4. Physician diagnosed disease with laboratory confirmation	All healthcare personnel	0.5 mL SC, second dose at least 1 mo later	History of anaphylaxis to gelatin or neomycin; pregnancy; immunocompromised disorder,[d] recent (<11 mo) receipt of antibody-containing blood product; need for tuberculin skin testing in next 4 weeks unless performed simultaneously with vaccine administration; moderate or severe acute illness with or without fever
Mumps	1. Documented administration of two doses of live mumps virus vaccine (MMR)[e] *or* 2. Laboratory evidence of immunity or laboratory confirmation of disease *or* 3. Born before 1957[f] 4. Physician diagnosed diseases with laboratory confirmation	All healthcare personnel	0.5 mL SC, second dose at least 1 mo later	As for measles (above) if provided as MMR
Rubella	1. Documented administration of one dose of live rubella virus vaccine (MMR)[e] *or* 2. Laboratory evidence of immunity or laboratory confirmation of disease *or* 3. Born before 1957 (except women of childbearing age who could become pregnant)[f]	All healthcare personnel	0.5 mL SC, no booster	As for measles (above) if provided as MMR
Tdap	Documented administration of one dose of Tdap vaccine	All healthcare personnel	0.5 mL IM	Progressive or unstable neurologic disorder or progressive encephalopathy, Guillain-Barré syndrome <6 wk after a previous dose of a tetanus toxoid–containing vaccine, moderate or severe acute illness with or without fever

Continued on following page

TABLE 72.1 Vaccines Strongly Recommended for All Healthcare Personnel *(Continued)*

Vaccine	Demonstration of Immunity[a]	Indications[b]	Administration[b]	Major Contraindications and Precautions[b]
Varicella	1. Documented receipt of two doses of varicella vaccine *or* 2. Laboratory evidence of immunity *or* 3. Physician diagnosed varicella or zoster[g]	All healthcare personnel	0.5 mL SC, second dose 4–8 wk later	History of anaphylaxis to gelatin or neomycin; pregnancy; immunocompromised disorder,[d] recent (<11 mo) receipt of antibody containing blood product; moderate or severe acute illness with or without fever

[a]Written documentation should be required.
[b]The package insert and Centers for Disease Control and Prevention (CDC)/Advisory Committee on Immunization Practices (ACIP) should always be consulted for specific recommendations regarding indications, storage, administration, precautions, and contraindications. Doses listed are for adults; CDC/ACIP guidelines should be consulted when providing vaccines for children.
[c]Persons who provide healthcare to patients or who work in institutions that provide healthcare (e.g., physicians, nurses, emergency medical personnel, dental professionals and students, medical and nursing students, laboratory technicians, hospital volunteers, and administrative and support staff in healthcare institutions).
[d]Persons immunocompromised because of immune deficiency diseases, human immunodeficiency virus infection, leukemia, lymphoma, or generalized malignancy, or immunosuppressed as a result of therapy with corticosteroids (i.e., >2 mg/kg body weight or 20 mg/day of prednisone for >2 weeks), alkylating drugs, antimetabolites, or radiation. Also see Table 72.2.
[e]The first dose should be administered on or after the first birthday; the second dose of measles and mumps–containing vaccine should be administered no earlier than 1 month (i.e., a minimum of 28 days) after the first dose. Combined MMR vaccine generally should be used whenever any of its component vaccines is indicated.
[f]For unvaccinated personnel born before 1957 who lack laboratory evidence of immunity of measles, mumps, and/or rubella or laboratory confirmation of disease, healthcare facilities should consider vaccinating personnel with two doses of MMR vaccine at the appropriate interval (for measles and mumps) or one dose of MMR vaccine (for rubella), respectively. For unvaccinated personnel born before 1957 who lack laboratory evidence of measles, mumps, and/or rubella immunity or laboratory confirmation of disease, healthcare facilities should recommend two doses of MMR vaccine during an outbreak of measles or mumps and one dose during an outbreak of rubella.
[g]An acceptable history is as follows: history of varicella disease based on healthcare provider diagnosis or self- or parental report of typical varicella disease for non-U.S.–born persons born before 1966 and all persons born from 1966 to 1997. For persons reporting a history of an atypical mild case, healthcare providers should seek either (a) an epidemiologic link to a typical varicella case (e.g., case occurred in the context of an outbreak or patient had household exposure in the previous 3 weeks) or (b) evidence of laboratory confirmation, if it was performed at the time of acute disease. When such documentation is lacking, persons should not be considered as having a valid history of disease because other diseases may mimic mild atypical varicella. For persons born during or after 1998, history of disease is no longer considered as evidence of immunity, unless the illness was laboratory confirmed.
[h]Immunity should be assessed 1 to 2 months after the third dose; if not immune, an additional three doses should be provided and immunity reassessed; if not immune, test for HBsAg, and if HBsAg is absent, provide hepatitis B immunoglobulin as postexposure prophylaxis when indicated. Immunity is indicated by an anti-HBsAg titer of ≥10 mIU/mL.
HBsAg, hepatitis B surface antigen; HCP, healthcare personnel; IM, intramuscularly; LAIV, live attenuated influenza vaccine; MMR, measles-mumps-rubella; SC, subcutaneously.

TABLE 72.2 Vaccines Available for Healthcare and Laboratory Personnel in Special Circumstances

Vaccines	Indications[a]	Administration[a]	Major Contraindications and Precautions[a]
Anthrax (AVA)	Laboratory personnel or researchers engaged in work involving production quantities or concentrations of *Bacillus anthracis* culture or in activities with a high potential for aerosol production. Healthcare personnel exposed as a result of a bioterrorist attack	Preexposure—0.5 mL IM × 5 doses (0 wk, 4 wk, 6 mo, 12 mo, and 18 mo). Annual booster to maintain immunity. PEP (available under an IND), 0.5 mL SC × 3 doses (0, 2, and 4 wk) (in conjunction with 60-day antimicrobial PEP)	History of anaphylaxis to a previous dose of vaccine or a vaccine component. Precautions: latex allergy, history of anthrax, moderate or severe illness. Pregnancy: avoid for preevent use; recommended for PEP
Hepatitis A	Not routinely indicated for healthcare personnel. May be useful in outbreak settings (administer within 2 wk of exposure or use Ig 0.02 mL/kg)	1.0 mL IM × 2 or 3 (follow manufacturer's recommendation for dosing)	History of anaphylaxis to a previous dose of vaccine or a vaccine component. Precautions: pregnancy, moderate or severe illness
Meningococcal quadrivalent (A,C, Y, W135) *and* meningococcal serogroup B (MenB)	Clinical and research microbiologists who may be routinely exposed to isolates of *Neisseria meningitides*. Others: asplenia, persistent complement deficiencies, travel to an endemic area	Microbiologists—one dose MenACWY (adult ≤55 y) or MPSV4 (adults >55 y) 0.5 mL IM. Plus either a 2-dose series of MenB-4C or a 3-dose series of MenB-FHbp. Booster dose every 5 y if still at risk. For other indications (asplenia, persistent complement deficiencies)—appropriate doses	History of anaphylaxis to a previous dose of vaccine or a vaccine component. Precautions: moderate or severe illness

TABLE 72.2 Vaccines Available for Healthcare and Laboratory Personnel in Special Circumstances *(Continued)*

Vaccines	Indications[a]	Administration[a]	Major Contraindications and Precautions[a]
Pneumococcal polysaccharide (PPSV23) and pneumococcal conjugate (PCV13)	Not routinely indicated for healthcare personnel. Indicated for immunocompromised persons 18–65 and adult ≥65 years of age, and residents extended care facilities	Revaccination recommendations depend on vaccine indication	History of anaphylaxis to a previous dose of vaccine or a vaccine component. Precautions: moderate or severe illness
Polio (IPV)	Laboratory personnel or researchers who frequently work with polio. HCP who have close contact with patients who may be excreting wild poliovirus, including HCP who travel to work in areas where polioviruses are circulating	Unimmunized adults—0.5 mL IM or SC × 2 doses (0, 4–8 wk, 6–12 mo after second dose). Immunized—0.5 mL IM or SC × 1 dose	History of anaphylaxis to a previous dose of vaccine or a vaccine component. Precautions: pregnancy, moderate or severe illness
Rabies	Laboratory personnel or researchers who work with rabies virus of potentially infected animals; PEP may be required for potential exposure despite primary immunization	Preexposure—1.0 mL (HDCV, PCECV) IM × 3 doses (0, 7, and 21 or 28 days). Booster doses may be required. Postexposure (unimmunized) —1.0 mL (HDCV, PCECV) in deltoid × 4 (0, 3, 7 and 14 days) (if immunocompromised, provide additional dose on day 28). Also administer HRIG. postexposure (previously immunized)—1.0 mL (HDCV, PCECV) × 2 (0, 3 days)	History of anaphylaxis to a previous dose of vaccine or a vaccine component
Typhoid[b]	Laboratory personnel or researchers who frequently work with *Salmonella* Typhi	Four oral doses (Ty21a; 1 capsule taken every other day) *or* one 0.5 mL-dose IM (ViCPS). Booster doses per manufacturer's recommendations	History of anaphylaxis to a previous dose of vaccine or a vaccine component. Ty21a: receipt of antibiotics or antimalarials (delay for >3 days posttherapy). Precautions: pregnancy, immunocompromised (Ty21a)[c]
Vaccinia	HCP or researchers who directly handle cultures or animals contaminated with recombinant vaccinia or orthopox viruses (monkeypox, cowpox) that infect humans; preexposure or postexposure prophylaxis for bioterrorist use of variola (smallpox)	One dose administered with a bifurcated needle; booster every 10 years	Discuss with public health authorities prior to administration. Contraindications: pregnancy, immunocompromised state in vaccine recipient or household contact, history of eczema in HCP or family contact, other acute or chronic exfoliative skin conditions
Zoster	Not specifically indicated for HCP. Adults ≥50 years of age	One 0.65 mL dose SC	History of anaphylaxis to a previous dose of vaccine or a vaccine component, immunocompromised, pregnant

[a]The package insert and CDC/ACIP should always be consulted for specific recommendations regarding indications, storage, administration, precautions, and contraindications.
[b]Two vaccines available: oral live attenuated vaccine (Ty21a) and Vi capsular polysaccharide vaccine (ViCPS).
[c]Persons immunocompromised because of immune deficiency diseases, human immunodeficiency virus infection, leukemia, lymphoma, or generalized malignancy, or immunosuppressed as a result of therapy with corticosteroids (i.e., >2 mg/kg body weight or 20 mg/day of prednisone for >2 weeks), alkylating drugs, antimetabolites, or radiation.
ACIP, Advisory Committee on Immunization Practices; AVA, anthrax vaccine absorbed; CDC, Centers for Disease Control and Prevention; HCP, healthcare personnel; HDCV, human diploid cell vaccine; HRIG, human rabies immunoglobulin; Ig, immunoglobulin; IM, intramuscular; IND, investigational new drug; IPV, inactivated polio vaccine; PCECV, purified chick embryo cell vaccine; PEP, postexposure prophylaxis; SC, subcutaneous; ViCPS, Vi capsular polysaccharide.

a contraindication to receipt of a live attenuated vaccine, although HCP receiving live attenuated influenza vaccine should not work in a protected environment (i.e., stem cell transplant unit) for 7 days postimmunization.[46,86]

Pregnant HCP also require special consideration in the provision of immunizations. The risks from immunization during pregnancy are largely theoretical.[45] The benefit of immunization among pregnant women usually outweighs the potential risks for adverse reactions, especially when the risk for disease exposure is high, infection would pose a special risk to the mother or fetus, and the vaccine is unlikely to cause harm.[45,46,87–89] Furthermore, newer information continues to confirm the safety of vaccines given inadvertently during pregnancy. Ideally, women of childbearing age, including HCP, should have been immunized against measles, mumps, rubella, varicella, tetanus, diphtheria, pertussis, meningococcus, polio, hepatitis A, and hepatitis B as children or adolescents before becoming pregnant. However, because this may not have occurred, it is especially important that all HCP be screened for rubella immunity (by serology because history is

TABLE 72.3 Recommendations on Immunization of Healthcare Personnel With Special Conditions

Vaccine	Pregnancy	HIV Infection		Immunocompromising Conditions (Excluding HIV)[a]	Diabetes, Heart Disease, Chronic Lung Disease, Chronic Alcoholism	Asplenia and Persistent Complement Component Deficiencies	Chronic Liver Disease	Kidney Failure, End- Stage Renal Disease, Receipt of Hemodialysis
		<200 cells/uL	≥200 cells/uL					
Hepatitis A	UI	UI	UI	UI	UI	UI	R[b]	UI
Hepatitis B	R	R	R	R	R	R	R	R
Influenza	R[c]	R	R	R	R	R	R	R
MMR	C	C	R	C	R	R	R	R
Polio, inactivated[d]	UI	UI	UI	UI	UI	UI	UI	UI
Pneumococcal (PPSV23 and PCV13)	UI	R[b]	R[b]	R[b]	R[b]	R[b]	R[b]	R[b]
Rabies	UI	UI	UI	UI	UI	UI	UI	UI
Tdap	–[e]	R	R	R	R	R	R	R
Typhoid, ViCPS[b]	UI	UI	UI	UI	UI	UI	UI	UI
Typhoid, Ty21a[b]	C	C	C	C	UI	UI	UI	UI
Varicella	C	C	R	C	R	R	R	R
Vaccinia	C	C	C	C	UI	UI	UI	UI
Zoster	C	C	C	C	UI	UI	UI	UI

[a]Severe immunosuppression can be the result of congenital immunodeficiency; leukemia, lymphoma, generalized malignancy, radiation, or therapy with immunosuppressive agents, such as alkylating agents, antimetabolites, and large amounts of corticosteroids.
[b]Recommendation based on the person's underlying medical condition(s) or risks (e.g., travel) rather than occupation.
[c]Recommended for women who will be pregnant during the influenza season.
[d]Immunization with IPV is recommended for unvaccinated healthcare workers who have close contact with persons who may be excreting wild poliovirus. Healthcare workers who have a primary series of OPV or IPV who are directly involved with the provision of care to patients who may be excreting poliovirus may receive another dose of IPV. Any suspected case of poliomyelitis should be investigated immediately. If evidence suggests transmission of wild poliovirus, control measures to contain further transmission should be instituted immediately, including an IPV immunization campaign.
[e]Td vaccine recommended for pregnant women who have not received a Td within the past 10 years; Tdap is specifically recommended for pregnant women more than 20 weeks' gestation (preferred to Td).
C, Contraindicated; HIV, human immunodeficiency virus; IPV, inactivated polio vaccine; MMR, measles-mumps-rubella; OPV, oral polio vaccine; R, recommended; Td, tetanus, diphtheria vaccine; Tdap, tetanus, diphtheria, acellular pertussis vaccine; UI, use if indicated; ViCPS, Vi capsular polysaccharide.

often unreliable) because of the consequences of infection for the developing fetus. Nevertheless, attenuated vaccines (mumps, measles, rubella, and varicella) or live attenuated influenza vaccine should be provided only to nonpregnant HCP and deferred for pregnant women. The ACIP has recommended that "health care personnel should administer Tdap during all pregnancies, preferably during the third or late second trimester (after 20 weeks' gestation)." If not administered during pregnancy, Tdap should be administered immediately postpartum. Women who are pregnant during respiratory virus season should receive inactivated influenza immunization.[45,46,68] There is no convincing evidence of risk from immunizing pregnant women with other inactivated virus or bacterial vaccines, or toxoids. Susceptible pregnant women at high risk for specific infections should receive, as indicated, the following vaccines: hepatitis A, hepatitis B, pneumococcal polysaccharide, meningococcal, rabies, and poliovirus (inactivated).[45] Several vaccines or immunoglobulins (Igs) are recommended as part of postexposure prophylaxis, including hepatitis A (Ig), hepatitis B immunoglobulin (HBIG), and rabies immunoglobulin (RIG). The indications for use are the same in pregnant and nonpregnant women. Pregnant women should be immunized with meningococcal vaccine when there is a substantial risk of infection, such as during epidemics. Breastfeeding does not adversely affect the response to immunization and is not a contraindication for any of the currently routinely recommended vaccines.

Before the administration of any vaccine, the HCP should be evaluated for the presence of condition(s) that are listed as a vaccine contraindication or precaution.[45] If such a condition is present, the risks and benefits of vaccination need to be carefully weighed by the healthcare provider and the HCP. The most common contraindication is a history of an anaphylactic reaction to a previous dose of the vaccine or to a vaccine component. Factors that are not contraindications to immunization include the following: household contact with a pregnant woman; breastfeeding; reaction to a previous vaccination, consisting only of mild to moderate local tenderness, swelling, or both, or a fever of less than 40.5°C; mild acute illness with or without low-grade fever; current antimicrobial therapy (except for oral typhoid vaccine) or convalescence from a recent illness; personal history of allergies except a history of an anaphylactic reaction to a vaccine component (e.g., people with a history of anaphylaxis to neomycin should not receive the MMR vaccine); and family history of allergies, serious adverse reactions to vaccination, or seizures.[45]

Postexposure Prophylaxis

All HCP potentially exposed to a communicable disease should be evaluated by the institution's occupational health service as soon as possible. General guidelines for a postexposure evaluation of medical personnel have been published.[1,30,90] In brief, the following steps should be undertaken:

- Confirm that the source has a communicable disease.
- Determine that potential transmission could have taken place (e.g., close contact for transmission of *Neisseria meningitidis* or *Bordetella pertussis*).
- Determine that the exposed healthcare provider was not protected by the use of personal protective equipment (e.g., N-95 respirator for airborne transmitted diseases).
- Determine that the exposed healthcare provider is susceptible to infection (this may require laboratory evaluation).
- Determine if effective postexposure prophylaxis is available and recommended.
- Determine that the healthcare provider has no contraindications to use of the recommended prophylaxis.
- If the healthcare provider has a contraindication to the recommended prophylaxis, determine if an alternative exists and is safe to use.
- Inform the healthcare provider of the risk of disease transmission, signs and symptoms of infection, and risks and benefits of prophylaxis.
- Obtain informed consent for prophylaxis.
- Obtain baseline laboratory tests, if indicated (e.g., anti–hepatitis B surface antigen [anti-HBsAg] titer).
- Assess whether the healthcare provider's work activities should be restricted or if the provider should be furloughed (e.g., days 8 to 21 after exposure to varicella-zoster in a susceptible employee).
- Arrange follow-up evaluations.

Vaccines that may be indicated for postexposure prophylaxis include tetanus toxoid (Tdap preferred), hepatitis B, and rabies (Tables 72.4 and 72.5). The CDC states that "unvaccinated HCP who have no other evidence of immunity and who are exposed to varicella-zoster virus (VZV; varicella, disseminated herpes zoster, and uncovered lesions of a localized herpes zoster) are potentially infective from days 8 to 21 after exposure and should be furloughed during this period. They should receive postexposure vaccination as soon as possible. Vaccination within 3 to 5 days of exposure to rash might modify the disease if infection occurred. Vaccination more than 5 days postexposure is still indicated because it induces protection against subsequent exposures (if the current exposure did not cause infection)."[30] HCP who have received one dose of vaccine and who are exposed to VZV (varicella, disseminated herpes zoster, and uncovered lesions of a localized herpes zoster in the community or healthcare setting/workplace) should receive the second dose within 3 to 5 days after exposure to rash (provided 4 weeks have elapsed after the first dose).

The ACIP now recommends that for healthy persons 12 months to 40 years of age, single-antigen hepatitis A vaccine at the age-appropriate dose is preferred to immunoglobulin for postexposure prophylaxis for persons exposed to hepatitis A virus because of vaccine advantages that include long-term protection and ease of administration.[79] Currently, there are no data regarding the relative efficacy of vaccine compared with immunoglobulin postexposure prophylaxis for persons older than 40 years of age or for those with underlying medical conditions; consequently, the ACIP states that decisions to use vaccine or immunoglobulin should take into account patient characteristics associated with severe manifestations of hepatitis A, including older age and chronic liver disease.[79] Several vaccines have been provided to persons without known direct exposure to an infected case to help contain communitywide or institutional outbreaks, including hepatitis A, meningococcal vaccine, and pertussis vaccine. Immunoglobulin preparations may be indicated as part of postexposure prophylaxis for exposure to hepatitis A (immunoglobulin), hepatitis B (HBIG), measles (immunoglobulin), rabies (RIG), tetanus (tetanus immunoglobulin), varicella (varicella-zoster immunoglobulin [VariZIG]), and vaccinia immunoglobulin). All HCP who are exposed to a communicable disease or with symptoms of an infectious disease should be evaluated with the consideration of whether work restriction or furlough is required to prevent secondary cases from developing among patients or staff (Table 72.6).

Postexposure Prophylaxis for Bioterrorism Agents

Before the intentional release of anthrax in the United States in 2001, several publications highlighted the concern about the potential for biologic terrorism.[91,92] Since 2001, the continued

TABLE 72.4 Postexposure Prophylaxis for Vaccine-Preventable Diseases

Disease	Definition of Exposure	Prophylaxis[a]	Comments
Hepatitis A	Ingestion of contaminated food; contact with feces from a hepatitis A–infected patient	One dose IM immunoglobulin 0.02 mL/kg given within 14 days of exposure (can also use hepatitis A vaccine)	Ig: avoid in persons with IgA deficiency; do not administer within 2 wk of MMR vaccine or 3 wk of varicella vaccine
Hepatitis B	Contact with HBsAg-positive blood (or body fluid) via percutaneous, mucous membrane, or nonintact skin exposure	See Table 72.5	HCP who have ever demonstrated an anti-HBsAg titer ≥10 mIU/mL do not require postexposure prophylaxis
Influenza A or B	Cohabiting confined air space or face-to-face contact in an open area (nonimmunized HCP)	Zanamivir 10 by inhaler once daily or oseltamivir 75 mg orally once daily may be used; both for 7–10 days	Zanamivir—avoid if asthmatic. Oseltamivir associated with gastrointestinal toxicity (≈ 5–10%)
Measles[b]	Cohabiting confined air space or face-to-face contact in an open area (nonimmune HCP)	Susceptible HCP should receive immunoglobulin 0.25 mL/kg (maximum 15 mL) IM within 6 days of exposure or measles vaccine	Susceptible HCP should be furloughed from days 5–21 postexposure or for 7 days after the rash appears
Meningococcus (invasive)[b]	Direct contact with respiratory secretions (e.g., resuscitating intubating, or closely examining the oropharynx) of infected persons with invasive disease (sepsis, meningitis)	Ciprofloxacin 500 mg orally × 1 dose or ceftriaxone 250 mg IM × 1 dose or rifampin 600 mg orally 2 bid for 2 days Avoid ciprofloxacin if resistant strains reported in the community	Home contacts of exposed HCP do not need to receive prophylaxis unless the HCP develops infection; rifampin and ciprofloxacin not recommended in pregnancy
Pertussis[b]	Direct contact with respiratory secretions or droplets from the respiratory tract of infected persons	Exposed HCP should receive azithromycin orally (500 mg day 1, 250 mg days 2–5) or trimethoprim-sulfamethoxazole orally 1 tablet bid for 14 days	Other acceptable alternatives are erythromycin (14 days) and clarithromycin (7 days)
Varicella[b]	Cohabiting confined air space or face-to-face contact in an open area with a patient with active lesions or within 48 hours before the development of lesions (nonimmune HCP)	For susceptible employees, VariZIG 125 U/10 kg IM (maximum dose 625 U) is indicated for immunocompromised or pregnant adults (within 10 days of exposure but it should be administered as soon as possible after exposure)	Susceptible employees should be furloughed from days 8–21 postexposure (employees who receive VariZIG should be furloughed from days 8–28 postexposure)
Zoster[b]	Cohabiting confined air space or face-to-face contact in an open area with a patient with uncovered active lesions (disseminated zoster or immunocompromised patient) (nonimmune HCP)	As for varicella	Same as varicella exposure

[a]The latest guidelines from the Centers for Disease Control and Prevention should always be consulted.
[b]HCP who was wearing a mask (surgical mask or N-95 respirator) is not considered exposed.
bid, Twice a day; HBsAg, hepatitis B surface antigen; HCP, healthcare personnel; Ig, immunoglobulin; IM, intramuscular; MMR, measles, mumps, and rubella; VariZIG varicella-zoster immunoglobulin (available via an investigational new drug).

TABLE 72.5 Recommended Postexposure Prophylaxis for Exposure to Hepatitis B Virus

Vaccination and Antibody Response of Exposed Workers[a]	Treatment		
	Source HBsAg-positive	Source HBsAg-negative	Source Not Tested or Unknown
Unvaccinated	HBIG[b] × 1 and initiate HBV vaccine	Initiate HBV vaccine series	Initiate HBV vaccine series
Previously vaccinated			
Known responder[c]	No treatment	No treatment	No treatment
Known nonresponder[d]	HBIG × 1 and initiate HBV revaccination or HBIG × 2[e]	No treatment	If known high-risk source, treat as if source were HBsAg positive
Antibody response unknown	Test exposed person for anti-HBsAg 1. If adequate,[c] no treatment is necessary 2. If inadequate,[d] administer HBIG × 1 and HBV vaccine booster	No treatment	Test exposed person for anti-HBsAg 1. If adequate,[c] no treatment is necessary 2. If inadequate,[d] administer HBV vaccine booster and recheck titer in 1–2 mo

[a]Persons who have previously been infected with HBV are immune to reinfection and do not require postexposure prophylaxis.
[b]Dose is 0.06 mL/kg intramuscularly.
[c]A responder is a person who has adequate levels of serum antibody to HBsAg (i.e., anti-HBsAg ≥10 mIU/mL).
[d]A nonresponder is a person who has inadequate response to vaccination (i.e., serum anti-HBsAg <10 mIU/mL).
[e]The option of giving one dose of HBIG and reinitiating the vaccine series is preferred for nonresponders who have not completed a second three-dose series. For persons who previously completed a second vaccine series but failed to respond, two doses of HBIG are preferred.
HBV, hepatitis B virus; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen.

TABLE 72.6 Work Restrictions for Vaccine-Preventable Diseases

Disease	Work Restriction	Duration
Hepatitis A	Relieve from direct patient contact and food handling	Until 7 days after onset of jaundice
Hepatitis B		
Acute	Relieve from direct patient contact	Until jaundice resolves
Chronic	No restriction unless demonstrated to transmit infection to patients or unable to practice appropriate infection control. Restrictions should be considered by SHEA guideline[a] for personnel performing invasive procedures	Counsel regarding need to follow standard precautions. Periodic reevaluation per SHEA guideline[a]
Influenzas A and B	Furlough from healthcare facility	Until fever resolves off antipyretics or 5 days (whichever is longer)
Measles		
Active	Furlough from healthcare facility	Until 4 days after rash appears
Postexposure (susceptible personnel)	Furlough from healthcare facility	From the 5th day after the first exposure through the 21st day after the last exposure or 4 days after the rash appears
Mumps		
Active	Furlough from healthcare facility	Until 9 days after onset of parotitis
Postexposure (susceptible personnel)	Furlough from healthcare facility	From the 12th day after the first exposure through the 26th day after the last exposure or 9 days after the onset of parotitis
Pertussis		
Active	Furlough from healthcare facility	From the beginning of the catarrhal stage through the third week after the onset of paroxysms or until 5 days after start of effective antimicrobial therapy
Postexposure (susceptible personnel)	No restriction, if on prophylactic antimicrobial therapy[a]	—
Postexposure (symptomatic personnel)	Furlough from healthcare facility	Same as active pertussis
Rubella		
Active	Furlough from healthcare facility	Until 5 days after rash appears
Postexposure (susceptible personnel)	Furlough from healthcare facility	From the 7th day after the first exposure through the 21st day after the last exposure or 5–7 days after the rash appears
Varicella		
Active	Furlough from healthcare facility	Until the lesions are dried and crusted
Postexposure (susceptible personnel)[b]	Furlough from healthcare facility	From the 8th day after the first exposure through the 21st day (28th day if VariZIG given) after the last exposure or if varicella occurs, until all lesions are dried and crusted
Zoster		
Localized (nonexposed area of skin); in a normal host	Cover lesions; relieve from care of immunocompromised patients	Until the lesions are dried and crusted
Localized (exposed area of the skin); localized in immunocompromised host; generalized	Furlough from healthcare facility	Until the lesions are dried and crusted
Postexposure (susceptible personnel)	Furlough from healthcare facility	From the 8th day after the first exposure through the 21st day (28th day if VariZIG given) after the last exposure or if varicella occurs, until all lesions are dried and crusted

[a]Data from Henderson DK, Dembry L, Fishman NO, et al. SHEA guideline for management of healthcare workers who are infected with hepatitis B virus, hepatitis C virus, and/or human immunodeficiency virus. *Infect Control Hosp Epidemiol*. 2010;31:203–232.
[b]All personnel should be offered postexposure antimicrobial prophylaxis regardless of receipt of Tdap.
SHEA, Society for Healthcare Epidemiology of America; Tdap, tetanus, diphtheria, acellular pertussis vaccine; VariZIG, varicella-zoster immunoglobulin.

need to be prepared for bioterrorism has been highlighted.[93,94] The CDC has categorized several agents as "high priority" because they can be easily disseminated or transmitted person-to-person, cause high mortality, and are likely to cause public panic and social disruption. These agents include *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), variola major (smallpox), *Clostridium botulinum* toxin (botulism), *Francisella tularensis* (tularemia), filoviruses (Ebola virus disease, Marburg disease), and arenaviruses (Lassa [Lassa hemorrhagic fever], Junin [Argentine hemorrhagic fever], and related viruses).[95] Characteristics of these high-priority agents include the following: they are infectious via aerosol, the organisms are fairly stable in aerosol form, there are susceptible civilian populations, infection is associated with high morbidity and mortality, several exhibit person-to-person transmission (i.e., cutaneous anthrax, pneumonic plague, smallpox, and Ebola, Marburg, Lassa, and Junin viruses), they are difficult to diagnose and/or treat, and most have previously been developed as biologic weapons. General reviews of bioterrorist agents have been published,[93,94] as well as detailed management recommendations for anthrax,[96] botulism,[97] plague,[98] smallpox,[99] tularemia,[100] and viral hemorrhagic fever.[101]

Key aspects of the management of patients infected with bioterrorism agents include decontamination before medical evaluation, proper isolation, rapid diagnosis with appropriate therapy, and postexposure prophylaxis of exposed persons. HCP may acquire infection from patients via contaminated clothes (smallpox, Q fever, plague, and anthrax) or via patient-to-HCP transmission.[23] In the event of an attack with mass casualties and widespread exposures, vaccines likely will play an important adjunctive role in limiting the impact of anthrax[102] and the critical role in containing smallpox.[103] Currently preexposure use of anthrax vaccine is recommended only for laboratory workers or researchers at risk for anthrax exposure (see Table 72.2). Vaccinia vaccine is effective as postexposure prophylaxis after smallpox exposure when used within 4 days.[99] If vaccines now in development for bioterrorist agents (e.g., vaccines for *C. botulinum, F. tularensis, Y. pestis*) become available, HCP may be included in both preexposure and postexposure recommendations.[104]

PROVIDING VACCINES FOR HEALTHCARE PERSONNEL

All HCP new to a healthcare facility should be screened for immunity to vaccine-preventable diseases within 10 working days. Unless immune, the HCP should be appropriately immunized. In general, serologic screening for immunity before immunization is neither necessary nor cost-effective. Each healthcare facility, however, needs to evaluate the cost-effectiveness of screening. Factors that determine the effectiveness of serologically screening HCP for immunity include the cost of the screening test, the cost of the vaccine, and the prevalence of immunity in the population. The prevalence of immunity in the population likely depends on age, gender, race, place of birth, and socioeconomic status. In addition, the sensitivity and specificity of the screening test must be considered.

The immunization status of all HCP should be recorded in their medical record, which should be maintained by the institution's occupational health service. Signed informed consent specific to each vaccine should be obtained before immunization. When vaccines are provided, appropriate information should be recorded in the healthcare provider's medical record (Box 72.1). All institutions should develop a readily available personnel medical record that includes the HCP immune status to vaccine-preventable diseases.

BOX 72.1 Information That Should Be Obtained When Providing Vaccines to Healthcare Personnel

- Personnel name
- Prsonnel identification number
- Date of birth or age
- Date of immunization
- Potential contraindications to vaccine:
  - Anaphylaxis to a previous dose of vaccine
  - Allergy to vaccine constituent
  - For live attenuated vaccines: possible pregnancy, immunocompromising conditions/medications
  - Vaccine-specific contraindications
- Vaccine provided
- Name of vaccine manufacturer
- Lot number of vaccine
- Site of immunization
- Route of immunization
- Date for additional immunizations (if required)
- Complications (if any)
- Name, title, and address of person providing vaccine
- Signed informed consent

IMPROVING VACCINE COVERAGE OF HEALTHCARE PERSONNEL

Despite strong recommendations from the CDC and professional organizations that HCP in the United States be immune to vaccine-preventable diseases, studies continue to demonstrate that substantial numbers of HCP lacked evidence of immunity to these diseases and that many HCP refuse immunization. The proportion of U.S. HCP that have received vaccine has been reported as the following: hepatitis B, 61.7% (2013)[105]; Tdap, 47.0% (2013)[105]; and influenza, 64.3% (2014–15 season).[106] By occupation, influenza vaccine coverage was highest among pharmacists (86.7%), nurse practitioners/physician assistants (85.8%), physicians (82.2%), nurses (81.4%), and other clinical professionals (72.0%). Influenza vaccine coverage was lowest among administrative and nonclinical support staff (59.1%) and assistants or aides (46.6%). By work setting, influenza vaccine coverage was highest among HCP working in hospitals (78.7%) and lowest among HCP working in long-term care facilities (54.4%). Serologic studies of hospital-based HCP indicate that 2% to 9% of those born before 1957 lack antibodies to measles virus.[30] Although most U.S. schools training nurses and physicians required immunity to MMR and hepatitis B (i.e., >95%), less require immunity to varicella (physicians, >95%; registered nurses, ~90%), and even less to Tdap (~89%), and influenza (<20%).[107] Schools training allied HCP (e.g., occupational therapists) are even less likely to require immunity to vaccine-preventable diseases.[108] Vaccination policies in other areas of the world, such as Europe, are less likely than the United States to require or recommend immunity to vaccine-preventable diseases for HCP.[109] A recent review of vaccine coverage of HCP which focused on influenza and hepatitis B vaccine coverage around the world reported, in general, very low rates of coverage.[110]

Barriers to receiving influenza vaccine commonly reported by HCP have included desire to avoid medications, inconvenient vaccine administration, concern about side effects, belief that influenza can be caused by the vaccine, belief that they are at low risk of infection, doubt that influenza is a serious disease, and belief that the vaccine is ineffective.[111–115] Similar concerns are voiced about hepatitis B vaccine, including the desire to avoid medications, perception that the HCP is at low

risk for occupationally acquired hepatitis B virus (HBV) infection, and concern about side effects.[111] Barriers to acceptance of influenza vaccine and measures to improve influenza vaccine compliance among HCP have been reviewed.[115] Interventions that have been demonstrated to improve vaccine uptake have included the use of mobile carts,[116,117] incentive programs,[118] educational campaigns that focus on the seriousness of influenza and address misconceptions about the vaccine,[119–121] providing vaccine at multiple sites and on the wards,[120] and weekly feedback to the staff.[121] An analysis of 50 hospitals during the 2007–08 influenza season revealed that programs with the following components had significantly higher vaccination rates: weekend provision of vaccine, train-the-trainer programs, report of vaccination rates to administrators or board of trustees, a letter sent to HCP emphasizing the importance of vaccination, and any form of visible leadership support.[122] The use of a signed declination form for HCP who refuse vaccination was not associated with an improved frequency of influenza immunization. Although some studies have demonstrated that the use of a signed declination form was associated with improved influenza coverage among HCP,[123] a systematic review found only "modest" benefit.[124] As noted above, multiple professional organizations have endorsed the position that employment for HCP should be conditional on receipt (unless contraindicated) of influenza vaccine. A systematic review of mandatory influenza vaccine for HCP reviewed eight observational studies; influenza vaccine rates in all studies exceeded 94%.[125] The National Vaccine Advisory Committee statement provides an excellent summary of strategies for improving influenza coverage of HCP.[61]

GUIDELINES FOR THE USE OF SELECTED VACCINES

Measles-Mumps-Rubella Vaccine

Epidemiology

The incidence of mumps, measles, and rubella has decreased dramatically since the widespread use of MMR vaccine. Cases reported to the CDC in 2012 were as follows: mumps, 229 (0.07 per 100,000 population); measles, 55 (0.02 per 100,000 population) of which 38% were imported; rubella, eight (0 per 100,000 population), and congenital rubella, three (0 per 100,000 population).[126] In the years between 2013 and 2015, the CDC has reported substantially more cases of measles.[127–129] The vast majority of the cases were import-associated and infected persons were unvaccinated or had an unknown vaccination status. Of note, an outbreak in California which was related to visiting two adjacent Disney theme parks involved 125 measles cases between December 28, 2014 and February 11, 2015.[130]

All three diseases represent an important health hazard for hospital personnel for the following reasons. First, all three are transmitted by the droplet route (measles is also transmitted by the airborne route). Second, in all three diseases, persons become infectious before developing a clinically recognizable illness. Third, a history of prior disease may be unreliable for determining whether HCP actually suffered a vaccine-preventable disease in the past. Hence, many unimmunized HCPs may falsely believe themselves immune.

Rubella is of special concern because of its ability to cause congenital abnormalities in up to 90% of women with confirmed infection in the first trimester of pregnancy. Hospital workers are frequently females of childbearing age. Infants with congenital rubella may be contagious until they are at least 1 year old and therefore can expose HCP.

Lessons From Hospital Outbreaks

Nosocomial outbreaks of mumps have been reported infrequently,[131–138] but transmission from patient-to-patient[131,132,136,137] and from patient-to-healthcare provider has been reported.[13,134—136] In one case, it was suggested that an asymptomatically infected hospital nurse introduced mumps into a children's hospital.[132] Community outbreaks are likely to lead to nosocomial exposures. For example, during the widespread epidemic of mumps in Tennessee from 1986 to 1987, six HCP in three different hospitals developed mumps after nosocomial exposure.[136] Men, including male HCP, are at higher risk of complications from mumps (such as orchitis) than are children.[139] Orchitis has been reported among male HCP who developed mumps as a result of hospital exposure.[140] Because of the threat of mumps outbreaks, the ACIP now recommends that HCP receive two doses of MMR vaccine.[30]

Nosocomial measles is well documented in the literature[141–191] and may aid in the propagation of community outbreaks.[160–162,173,180,189,190] Analyses of measles cases reported to the CDC revealed acquisition in a medical setting for 241 people (1.1% of all cases) between 1980 and 1984[149] and for 1209 people (3.5% of all cases) between 1985 and 1989.[163] Investigations of individual outbreaks, however, have reported that 17% to 59% of cases were acquired in a medical set ting.[144,146,150,153,157,161,165,182,184,191] Acquisition of measles has occurred in outpatient settings, including emergency departments and physician offices, and has involved patient-to-patient, patient-to-staff, and staff-to-patient transmission. In one community outbreak, the relative risk of measles in HCP compared to community adults was 18.6.[182] Transmission in the outpatient setting has occurred even though the index cases had left the waiting or examination room up to 75 minutes earlier.[144,146,147,156,168,171] Case-control studies have demonstrated that persons who visited an emergency department had a 4.9-fold[172] to 5.2-fold[162] higher risk of developing measles one incubation period later compared with those who did not have such visits. In inpatient settings, transmission also has occurred among patients, from patients to staff, and from infected staff to patients. Infected staff most commonly have been nurses, with other groups at high risk of infection being physicians and office or hospital clerical staff. Nosocomial outbreaks have led to hospitalization of infected staff,[164,181,182] severe complications in infected patients,[166] and, occasionally, death of patients.[160,164,174] Multiple generations of nosocomial cases may occur.[184] The cost of controlling a single outbreak has ranged from US$28,000 to more than US$100,000.[160,164] As noted above, although measles is an uncommon disease, there have been an increased number of cases in recent years often import-related and propagated by unimmunized or underimmunized persons.

There has been no documented transmission of rubella to HCP or other hospital staff or patients in U.S. healthcare facilities since the elimination of rubella was announced in 2004. However, as with mumps and measles, nosocomial rubella is well documented in the literature.[192–211] Sources of rubella infection have included not only persons with acute infection, but also infants with congenital rubella.[193,202,210] Hospital-acquired infection of pregnant staff members has led to the termination of pregnancy.[200,206]

Several valuable lessons have been learned from the outbreaks. First, it appears that mumps and rubella are acquired mainly via droplet transmission. Measles, however, may be acquired via airborne transmission. Furthermore, measles transmission may occur for more than an hour after an infected case has left an enclosed area. Second, history of prior disease is unreliable for determining whether

healthcare providers actually contracted measles in the past, and hence many HCP falsely believe they are immune from past illness and fail to take appropriate precautions. Third, failure to institute a mandatory program requiring immunity results in a subpopulation of susceptible HCP capable of propagating epidemics. In multiple outbreaks, three to more than five generations of disease transmission occurred. Fourth, the cost of outbreaks for these diseases is high in both monetary terms and human suffering. Fifth, patients with congenital rubella are capable of transmitting rubella to susceptible adults.

Preexposure Prophylaxis

All HCP should be immune to mumps, measles, and rubella. Immunity may be demonstrated by meeting one of the following criteria: (a) birth before 1957 (rubella except women with childbearing potential who could become pregnant, although pregnancy in this age group would be exceedingly rare), (b) laboratory evidence of immunity (persons with indeterminate levels are considered susceptible), (c) laboratory confirmation of disease, or (d) evidence of appropriate immunizations (see Table 72.1).[30,62] Physician-diagnosed disease is no longer considered adequate evidence of immunity. Appropriate immunization consists of documented administration of two doses of live measles virus vaccine, two doses of live mumps virus vaccine, and one dose of live rubella virus vaccine (use of MMR is preferred). Birth before 1957 provides only presumptive evidence of immunity to measles, mumps, and rubella, although more than 95% of newly hired workers will demonstrate serologic immunity.[212] For unvaccinated personnel born before 1957 who lack laboratory evidence of measles, mumps, and/or rubella immunity or laboratory confirmation of disease, the ACIP recommends that healthcare facilities should consider vaccinating personnel with two doses of MMR vaccine at the appropriate interval (for measles and mumps) and one dose of MMR vaccine (for rubella), respectively.[30,69] If a healthcare facility chooses not to assure immunity of HCP born before 1957 to mumps, measles, and rubella, the ACIP states that healthcare facilities should recommend two doses of MMR vaccine during an outbreak of measles or mumps and one dose during an outbreak of rubella.[30] The American Academy of Pediatrics recommends that all HCP, including those born before 1957 who lack serologic evidence of immunity to rubella, should be immunized regardless of gender. The American Academy of Pediatrics also states that healthcare facilities should consider recommending one dose of MMR for HCP born before 1957 without other evidence of immunity.

Postexposure Management of Exposed Healthcare Workers

No specific prophylaxis is effective for mumps or rubella. Exposed susceptible people should be furloughed from the hospital (see Table 72.5). Patients with incubating or active mumps or rubella should be placed on *droplet precautions.* Patients with measles should be placed on *airborne precautions.* Because of the possibility, albeit low (~1%) of measles vaccine failure in HCP exposed to infected patients, all HCP should observe airborne precautions in caring for patients with measles. Measles vaccine can be used to provide postexposure prophylaxis of susceptible personnel if administered within 72 hours of exposure. However, all exposed HCP without evidence of immunity regardless of whether they receive measles vaccine postexposure, should be excluded from work from days 5 to 21 following exposure.[30] Immunoglobulin, 0.25 mL/kg (maximum dose: 15 mL), may be used for postexposure prophylaxis for susceptible personnel who are exposed to measles but must be provided within 6 days of exposure. Measles vaccine may also be used as postexposure prophylaxis but must be provided within 72 hours. Immunoglobulin postexposure prophylaxis is especially recommended for serosusceptible pregnant women and immunocompromised persons (dose: 0.5 mL/kg; maximum dose: 15 mL).

Varicella Vaccine

Epidemiology

VZV is the causative agent of two diseases: varicella (chickenpox), the primary infection, and zoster (shingles), a secondary infection caused by reactivation of latent VZV.[213–225] The licensure of the varicella vaccine in the mid-1990s allowed primary prevention of chickenpox. With the incorporation of the varicella vaccine into the childhood vaccine schedule, the incidence of varicella disease, hospitalization, deaths, and varicella-related expenditure has declined dramatically in the United States.[226–229] Although varicella is generally a mild disease in children, serious morbidity and mortality are common if infection occurs in neonates, adults, or immunocompromised people. For these reasons, the CDC[9,30] and the American Academy of Pediatrics,[47] have published recommendations regarding the isolation of patients with VZV infection and the management of patients and HCP exposed to VZV.

In most cases, VZV appears to be transmitted from person-to-person by the droplet route, and transmission occurs most efficiently when there is close contact, but true airborne transmission also may occur. The secondary attack rate of varicella among susceptible people in the household setting has ranged from 61% to 87%.[230–233] Vaccinated persons who develop breakthrough varicella are approximately half as contagious as unvaccinated persons with primary infections.[234] Herpes zoster is also infectious, although analysis of households suggests that the risk of transmission is lower than that for varicella.

Lessons From Nosocomial Outbreaks

Control of varicella is important in healthcare facilities because varicella and zoster are highly contagious; infection in adults frequently results in complications, including hospitalization; infection in pregnant women may lead to the congenital varicella syndrome[234–244]; and severe complications, including death, may occur in immunocompromised patients and healthcare providers. Of importance for hospitals, the risk of complications appears to be higher in neonates, adults,[218,245,246] and severely immunocompromised persons.[215,216] Approximately 1% to 2% of adults who develop varicella become ill enough to require hospitalization.[245,246] Patients developing varicella while undergoing chemotherapy for malignancy or undergoing immunosuppressive therapy after organ or bone marrow transplantation have a high risk of complications (30–50%) and death (7–17%).[247–254] Current rates of morbidity and mortality are dramatically lower because of prophylactic use of antivirals, postexposure prophylaxis with VariZIG or antivirals, and availability of antivirals, such as acyclovir, for therapy.[218,219]

Nosocomial transmission of VZV has been well documented in the literature, although few outbreaks have been reported in recent years in the United States.[232,255–276] Varicella may be introduced into the hospital by infected patients, staff, or visitors. Several investigators have noted that the initial source case for an outbreak was in the incubating phase of varicella.[255,266] After nosocomial exposure to VZV infection, 2% to 16% of susceptible staff have developed clinical varicella.[255,266] Nosocomial varicella has occurred among staff and

patients who had no direct contact with the index case, supporting the airborne route as a mode of spread.[256,265,276] Epidemiologic studies using tracers[267] or measurement of VZV DNA[277] have provided definitive evidence to support airborne transmission. By using polymerase chain reaction to detect VZV DNA, Yoshikawa and colleagues[278] demonstrated extensive environmental contamination in the room of a person with dermatomal zoster. Exposure to dermatomal[255,256,261,275] or disseminated[262] zoster in immunocompromised patients and to dermatomal zoster in immunocompetent hosts has led to transmission of VZV to susceptible HCP via the airborne route or droplet route.[255,267]

The CDC/HICPAC (Healthcare Infection Control Practices Advisory Committee),[9] CDC/ACIP,[30] and the American Academy of Pediatrics,[47] have published guidelines designed to aid clinicians in the control of nosocomial exposures. Key components include the following: (1) varicella, disseminated zoster, or dermatomal zoster in immunocompromised persons should be managed using *airborne* and *contact precautions* until all lesions are dried and crusted; (2) immunocompetent patients with dermatomal zoster should be managed using contact precautions until all lesions are dried and crusted; (3) serosusceptible patients exposed to varicella or zoster should be managed using airborne precautions from day 8 after the first exposure through day 21 after the last exposure (if patient has received VariZIG, precautions should extend through day 28) regardless of postexposure vaccination with varicella vaccine; (4) susceptible HCP personnel should not enter the room of patients with varicella-zoster if immune caregivers are available; (5) either a surgical mask or N-95 respirator should be used by susceptible HCP who enter the room; and (6) susceptible HCP who have been exposed to varicella-zoster should be furloughed from day 8 after the first exposure through day 21 after the last exposure (if HCP has received VariZIG, precautions should extend through day 28) regardless of postexposure vaccination with varicella vaccine. The authors are unaware of any nosocomial outbreaks in which transmission was linked to recirculated air of infected patients placed in private rooms with negative pressure. However, it is important to keep the door closed to prevent transient airflow into the corridor, which can lead to infection of susceptible personnel.[273] Given the current incidence of varicella and tuberculosis, it is likely that many hospitals do not have adequate rooms meeting the OSHA tuberculosis requirements to use these rooms to isolate patients with VZV infections.

Preexposure Prophylaxis

The ACIP,[30] the American Academy of Pediatrics,[47] and the American Medical Association[279] have recommended that all HCP should be immune to varicella. HCP should be screened for VZV immunity at the time of initial employment, as is currently recommended for mumps, measles, and rubella. Evidence of immunity includes any of the following: written documentation of vaccination with two doses of varicella vaccine, laboratory evidence of immunity or laboratory confirmation of disease, diagnosis or verification of a history of varicella disease by a healthcare provider, or diagnosis or verification of a history of herpes zoster by a healthcare provider.[30] Birth in the United States before 1980 is not considered presumptive evidence of immunity for HCP.[30] HCP born in tropical countries are more likely to be susceptible to varicella. Healthcare providers without a vaccine contraindication or precaution should be immunized with two doses at least 4 weeks apart of varicella vaccine. The most common test for determining immunity to varicella is the enzyme-linked immunosorbent assay (ELISA) test. Performing postimmunization serology is not recommended because commercial tests (e.g., ELISA) lack the sensitivity to detect the lower antibody levels associated with immunization compared with natural infection.[30] Furthermore, the glycoprotein ELISA test (not commercially available) used by Merck and Co. (West Point, PA) suggests a seroconversion rate of 99% in adults, making postimmunization serologic testing not cost-effective. Decision and/or cost-effective analysis methods have been used to demonstrate that immunization of HCP susceptible to varicella is cost-effective for healthcare facilities.[280–282] The use of varicella vaccine has been demonstrated to effectively protect HCP from varicella, particularly from serious disease.[283]

There appears to be virtually no risk of transmission of the vaccine strain of virus from healthy people who do not develop a rash postimmunization. Furthermore, no transmission has been documented from vaccinated HCP.[30] However, HCP who develop a vaccine-related rash after vaccination should avoid contact with persons without evidence of immunity to varicella who are at risk for severe disease and complications until all lesions resolve (i.e., are crusted over) or, if they develop lesions that do not crust (macules and papules only), until no new lesions appear within a 24-hour period.[30]

Postexposure Management of Exposed Healthcare Personnel

All HCP potentially exposed to varicella or zoster should be evaluated as soon as feasible by the occupational health service. HCP with no history or an uncertain history (e.g., VZV infection in other family members) of varicella should be serologically tested for immunity. HCP with a positive test should be considered immune. Susceptible exposed HCP who are exposed to varicella or zoster (disseminated herpes zoster and uncovered lesions of a localized herpes zoster) are potentially infective from days 8 to 21 after exposure and should be furloughed during this period.[30] They should also receive postexposure vaccination as soon as possible. Vaccination within 3 to 5 days of exposure to rash might modify the disease if infection occurred. Vaccination more than 5 days postexposure is still indicated because it induces protection against subsequent exposures (if the current exposure did not cause infection). For HCP at risk for severe disease for whom varicella vaccination is contraindicated (e.g., pregnant or immunocompromised HCP), varicella-zoster immunoglobulin after exposure is recommended. The varicella-zoster immunoglobulin product currently used in the United States is VariZIG.[284] VariZIG should be administered as soon as possible after exposure and within 10 days. The adult dose of VariZIG is 125 U/10 kg (maximum dose: 625 U or five vials). VariZIG should be administered intramuscularly or as directed by the manufacturer. It should not be administered intravenously. VariZIG has been demonstrated to lead to attenuated disease in pregnant women and immunocompromised people but likely does not prevent congenital infection. VariZIG may prolong the incubation period before disease; hence all HCP treated with VariZIG should be removed from duty from days 8 through 28.

HCP who have received one dose of vaccine and who are exposed to varicella-zoster should receive the second dose as soon as feasible (provided 4 weeks have elapsed since the first dose). HCP should be monitored daily (days 8 to 21 postexposure) for fever, skin lesions, and systemic symptoms suggestive of varicella. HCP can be monitored by the occupational health program or infection control preventionists, or by self-monitoring, with instructions to immediately report fever, headache, constitutional symptoms, or atypical skin lesions. HCP should be excluded immediately from the healthcare facility if symptoms occur. HCP who have had two doses of varicella vaccine should also be monitored as above and

excluded from work if symptoms occur. After vaccination, the management is similar to that of two-dose vaccine recipients.

The American Academy of Pediatrics states that "if VariZIG is not available or more than 96 hours have passed since exposure, some experts recommend prophylaxis with acyclovir (20 mg/kg per dose, administered four times per day, with a maximum dose daily dose of 3200 mg) or valacyclovir (20 mg/kg per dose, administered three times per day with a maximum dose of 3000 mg) beginning 7 to 10 days after exposure."[47] The authors have successfully used acyclovir as postexposure prophylaxis beginning on day 8 postexposure and continuing to day 21 postexposure. The use of acyclovir postexposure prophylaxis in immunocompromised children has been reviewed.[285] The prophylactic use of acyclovir, however, was associated with a decreased rate of the development of varicella immunity as measured by seroconversion. For those that seroconverted, durable protection was produced. Failures of acyclovir prophylaxis have been noted.

All HCP with VZV infection should be evaluated and, after confirmation of infection, should be offered the recommended antiviral therapy, which should ideally be initiated within 72 hours of the onset of clinical infection. There are currently three drugs approved by the Food and Drug Administration for therapy of VZV in the normal adult: acyclovir, famciclovir, and valacyclovir. Although the safety of acyclovir in pregnant women has not been firmly established, adverse fetal events have not been described. The American Academy of Pediatrics states "that some experts recommend oral acyclovir or valacyclovir for pregnant women with varicella, especially during the second and third trimesters. Intravenous acyclovir is recommended for the pregnant patient with serious complications of varicella.[47] HCP who develop varicella may return to work when clinically well and after all lesions are dried and crusted (usually about 5 days).

Hepatitis B Vaccine

Epidemiology

Exposure to bloodborne pathogens via parenteral or mucosal contact remains a major hazard for HCP, especially those performing invasive procedures. Although more than 30 diseases have been transmitted to HCP by sharps injuries or mucosal exposure,[286] the agents of greatest concern are HBV, hepatitis C virus, and HIV.[27–30,287–289] Seroprevalence surveys conducted before the availability of the HBV vaccine in 1981 showed that HCP had prevalence rates of past or present HBV infection threefold to fivefold higher than the general U.S. population.[290–293] The risk of infection was related to both the extent and the duration of blood contact. The CDC predicted that, in 1989, an estimated 12,000 American HCP would become infected with HBV, which would result in 250 deaths.[294] With a decreasing incidence of hepatitis B, the CDC estimated that, in 1994, 1012 HCP became infected with HBV because of occupational exposures, leading to approximately 22 deaths.[295] In a more recent analysis, Mahoney and colleagues reported in 1997 that HBV infection among HCP declined from 17,000 in 1983 to 400 in 1995.[296] The 95% decline in incidence observed among HCP was 1.5-fold greater than the reduction in incidence in the general U.S. population. The decline among HCP was because of the use of HBV vaccine, institution of *universal* (now standard) *precautions*, and other preventive measures such as needleless devices. Hepatitis B acquisition remains a concern for unimmunized HCP because the virus is environmentally stable and has been transmitted by other routes than percutaneous injury, including paper cuts, human bites, and mucosal contamination with blood.

Lessons From Nosocomial Outbreaks

Hepatitis B acquisition represents a major hazard for HCP for several reasons. First, HCP have high rates of percutaneous blood contact. The 2011 EPINet Report from 32 hospitals noted that the percutaneous injury rate was 19.5 per 100 beds.[297] Second, the virus is relatively stable in the environment, as demonstrated by its survival after drying and storage at 25°C and 42% relative humidity for 1 week.[298] Third, HBV is more transmissible than either HIV or hepatitis C virus, with rates of disease transmission after a percutaneous injury with a contaminated sharp reported as between 6% and 30%.[299–301] The risks of disease transmission via mucosal contact or contact with nonintact skin have not been quantitated but appear to be much lower than for percutaneous exposure. HBV infection has been acquired via ocular exposure[302] and has been transmitted to multiple patients by a respiratory therapist with severe exudative dermatitis while obtaining arterial blood gases.[303] The high frequency of hepatitis B among hospital personnel who did not recall a percutaneous exposure in the era before HBV vaccine has been attributed to inapparent inoculation through mucous membranes or small breaks in the skin.[304] Fourth, a significant number of patients are infectious (i.e., HBsAg-positive), with their status unknown to the medical staff. Finally, many HCP remain unimmunized.

Transmission of hepatitis B via contaminated medical instruments and environmental surfaces is well described. Nosocomial outbreaks of HBV infection have been associated with a blood-contaminated jet gun injector,[305] an endoscope,[306] multidose medication vials,[307–310] electroencephalography electrodes,[311] finger-stick (i.e., capillary) blood-sampling devices,[312–322] and reuse of needles and syringes.[323] Contamination of instruments or medication vials resulted in 67 of 243 cardiac transplant patients developing infection during transvenous endomyocardial biopsies.[324] Environmental surfaces in clinical laboratories were demonstrated to be positive for HBsAg in 34% of samples,[325] and contaminated file cards have been reported to lead to transmission among laboratory technicians.[326]

Transmission of hepatitis B in hemodialysis centers, presumably via contaminated environmental surfaces, shared equipment, or common-dose medications, has been an ongoing problem.[327–330] The CDC has described the problem and provided guidelines for preventing HBV transmission in hemodialysis units.[331–334] Unsafe practices that have led to transmission of bloodborne pathogens to patients include: (1) use of a single syringe, with or without the same needle to administer medication to multiple patients; (2) reinsertion of a used syringe into a medication vial or solution container to obtain additional medication for a single patient and then using that vial or solution container for subsequent patients; and (3) preparation of medications in close proximity to contaminated supplies or equipment. Occult HBV infection and reactivation of HBV infection after cytotoxic chemotherapy or immunosuppressive therapy have led to transmission of HBV in hemodialysis centers. Occult HBV infection is defined as long-lasting persistence of HBV genomes in the liver (with detectable or undetectable HBV DNA in the serum) of individuals testing negative for HBV surface antigen (HBsAg).[335] The prevalence of Occult HBV infection in hemodialysis patients has been reported to vary from 0% to 54% according to the diagnostic techniques and HBV endemicity.[336]. Occult HBV infection has been a source of viral spread both to other patients and staff within hemodialysis units.[336] Similarly, reactivation of HBV infection has led to transmission in hemodialysis centers.[337,338] These causes of HBV transmission in hemodialysis centers emphasize why immunity to HBV is so important among hemodialysis patients and staff. The CDC

has noted that since the initiation of strict infection control practices and hepatitis B vaccination, the rate of hepatitis B infection among patients undergoing hemodialysis has declined approximately 95%.[65] In addition to standard precautions, the following hemodialysis-specific infection control practices should be used:

- HBsAg-positive patients should be isolated by room, machine, instruments, medications, supplies, and staff.
- Recommended cleaning and disinfection procedures should be followed.
- Instruments, medications, and supplies should not be shared between any patients. When sharing of multidose medication vials is necessary, medications must be prepared in a clean centralized area separate from areas used for patient care, laboratory work, or refuse disposal. Safe injection practices should be strictly followed.
- Serologic testing should be done for all markers of HBV infection (HBsAg, anti–hepatitis B core antigen antibody [HBc], and anti-HBsAg) on admission of new patients to a hemodialysis center.
- To prevent transmission in dialysis settings, hemodialysis patients should be vaccinated against hepatitis B using the high-dose vaccine and have their anti-HBsAg titers determined 1 to 2 months after the last immunization.
- Vaccine responders on hemodialysis should have their anti-HBsAg titer determined annually and be revaccinated when serum anti-HBsAg titers falls to less than 10 mIU/mL.[331,332]
- Vaccine nonresponders (<10 mIU/mL) should be tested monthly for HBsAg and these results promptly reviewed.

Because hemodialyzed patients have a diminished response to HBV vaccine, higher doses and booster doses are recommended to maintain protection against hepatitis B (see ACIP[65] age-based recommendations for hepatitis B immunization of predialysis and dialysis patients). The Centers for Medicare and Medicaid Services now requires that hemodialysis centers receiving Medicare payment for outpatient dialysis services adhere to CDC guidelines.[339]

More than 40 outbreaks have been described of healthcare provider–to-patient transmission of hepatitis B.[340] The source has most commonly been a dentist, surgeon, or gynecologist performing invasive procedures. The most important risk factors for transmission during an invasive procedure are a hepatitis B *e* antigen–positive source, degree of invasiveness of the procedure, lack of wearing gloves by the infected HCP, or injury to the infected HCP with a sharp object.[339] Guidelines for the management of HBV-infected HCP have been published.[341]

Preexposure Prophylaxis

Because of the risks posed by bloodborne pathogens, OSHA has mandated, since 1991, that HCP undergo annual training in the prevention of bloodborne pathogen acquisition and use of personal protective equipment (gloves, masks, gown, and eyewear) whenever exposure to blood or other potentially infectious body fluids is reasonably anticipated.[43] However, despite the OSHA regulations and the introduction of new technologies, such as needleless devices, percutaneous, mucous membrane, and skin exposures to contaminated body fluids continue to occur at a high frequency. The OSHA standard also required that all healthcare facilities offer HCP hepatitis B immunization. healthcare providers may refuse immunization but must sign a declination form.

Protective serum titers of anti-HBsAg (≥10 mIU/mL) develop in greater than 90% of healthy adults younger than 40 years of age who receive a series of three intramuscular doses of HBV vaccine.[30,342–346] After age 40 years, less than 90% of persons vaccinated with three doses have a protective antibody response, and by age 60 years, protective levels of antibody develop in approximately 75% of vaccinated persons.[30] Independent risk factors for failure to seroconvert in HCP after HBV vaccine include smoking, female gender, higher body mass index, and older age.[347] Protection against symptomatic and chronic HBV infection has been documented to persist for longer than 22 years in vaccine responders.[348] Two single-antigen hepatitis B vaccines, Recombivax HB (Merck & Co., Inc., Whitehouse Station, New Jersey) and Engerix-B (GlaxoSmithKline Biologicals, Rixensart, Belgium) and one combination hepatitis A and hepatitis B vaccine, Twinrix (GlaxoSmithKline Biologicals), are available in the United States.[30] Primary vaccination consists of three or more intramuscular doses of hepatitis B vaccine or of the combined hepatitis A and hepatitis B vaccine. The hepatitis vaccine series does not need to be restarted if the second or third dose is delayed. In adults, hepatitis B vaccine always should be administered into the deltoid muscle. Longer needles (up to 1.5 inches in length) might be required for obese adults. Administering three doses on an accelerated schedule (e.g., 0, 1, and 2 months) results in a more rapid antibody rise but may reduce peak titers.[349,350] Immunogenicity is not reduced when HBV vaccine is given with other vaccines. Pregnancy is not a contraindication to HBV vaccine. Persons with an incomplete series are not considered protected and should complete the three-dose series. Because higher risk has been reported during the professional training period, the vaccination series should be completed before trainees have contact with blood; vaccination should be offered in schools of medicine, dentistry, nursing, laboratory technology, and other allied health professions. Consideration should be given to using a 0-, 1-, and 2-month or 0-, 1-, and 4-month schedule for unimmunized HCP at high risk for HBV acquisition (e.g., hemodialysis workers, cardiac surgeons), but a fourth dose should be given at 12 months to ensure long-term protection.

To determine the need for revaccination and to guide postexposure prophylaxis, postvaccination serologic testing should be performed for all HCP at high risk for occupational percutaneous or mucosal exposure to blood or body fluids. Postvaccination serologic testing is performed 1 to 2 months after administration of the last dose of the vaccine series using a method that allows detection of the protective concentration of anti-HBsAg (≥10 mIU/mL). Persons determined to have anti-HBsAg concentrations of equal to or greater than10 mIU/mL after receipt of the primary vaccine series are considered immune, and the result should be documented. Immunocompetent persons have long-term protection and do not need further periodic testing to assess anti-HBsAg levels. Because approximately half the persons who do not develop a protective level of anti-HBsAg antibodies after a three-dose series do so after additional doses,[351] persons determined to have anti-HBsAg concentrations of less than 10 mIU/mL soon after receipt of the primary vaccine series should be revaccinated. For these persons, administration of a second complete three-dose series on an appropriate schedule, followed by anti-HBsAg testing 1 to 2 months after the third dose, usually is more practical than conducting serologic testing after each additional dose of vaccine.[30] Persons who do not have a protective concentration of anti-HBsAg (≥10 mIU/mL) after revaccination (i.e., after receiving a total of six doses) should be tested for HBsAg and anti-HBc to determine infection status. Those determined not to be infected but who have anti-HBsAg less than 10 mIU/mL (nonresponders) should be considered susceptible to HBV infection and should be counseled about precautions to prevent HBV infection and the need to obtain HBIG postexposure prophylaxis for any known or likely

exposure to HBsAg-positive blood (see Table 72.4).[30] Persons determined to be infected (anti–HBc-positive) and positive for HBsAg should be provided counseling regarding how to prevent HBV transmission to others and referred for further evaluation (e.g., HBV viral load testing), care, treatment, and other services, as appropriate.[332,340,341] Persons who are HBsAg-positive and who perform exposure-prone procedures should seek counsel from a review panel comprised of experts with a balanced perspective (e.g., personal physicians of HCP and infectious disease specialists) regarding the procedures that they can perform safely.[340,341] Persons who were infected in the past (anti–HBc-positive but negative for HBsAg) require no vaccination or treatment.

The CDC has provided guidance for the preexposure evaluation of HCP previously vaccinated with a complete equal to or greater than a three-dose hepatitis B vaccine series who did not have postvaccination testing within 1 to 2 months.[66] For such HCP, an anti-HBsAg quantitative titer should be obtained; if the anti-HBsAg is ≥10 mIU/mL, no action is required for hepatitis B prophylaxis even if in the future the HCP has an exposure to a source patient positive for HBsAg. If the anti-HBsAg is less than 10 mIU/mL then HCP should receive an additional dose of hepatitis B vaccine and have a postvaccination anti-HBsAg titer obtained; if the anti-HBsAg is equal to or greater than 10 mIU/mL, no action is required for hepatitis B prophylaxis. If the anti-HBsAg remains less than 10 mIU/mL after the first booster dose, then HCP should receive an additional two doses of hepatitis B vaccine and have a postvaccination anti-HBsAg titer obtained; if the anti-HBsAg is equal to or greater than 10 mIU/mL, no action is required for hepatitis B prophylaxis. If the anti-HBsAg is less than 10 mIU/mL, the HCP is considered a nonresponder and should be evaluated for HBIG after a bloodborne exposure.

Postexposure Prophylaxis

All HCP with potential exposure to blood or contaminated body fluids should be evaluated. Exposure is defined as parenteral, mucous membrane, or nonintact skin exposure to blood or contaminated fluids. In all cases, the source should be tested for HBsAg, hepatitis C, and HIV. If the source case is HBsAg-positive, postexposure prophylaxis may be indicated (see Table 72.4). HBIG is effective when provided within 7 days. Immunoglobulin should not be used because of lack of efficacy. The simultaneous administration of HBIG and HBV vaccine (at different sites) does not diminish the efficacy of the hepatitis B vaccine.[66]

Influenza Vaccine

Epidemiology

Epidemics of influenza occur nearly every year during the winter months. Influenza causes an estimated 226,000 hospitalizations each year and results in an average of approximately 6300 deaths (range, ≈ 5800 to 7500) because of influenza and pneumonia.[352–354] However, if we take into account influenza-associated deaths with underlying respiratory and circulatory causes, the number of annual deaths rises to approximately 23,000 (range, ≈ 22,000 to 28,000).[252] Older persons (i.e., older than 65 years of age), pregnant women, young children (i.e., younger than 2 years of age) and persons with underlying health problems (e.g., cardiovascular disease, pulmonary disease, certain metabolic conditions) are at increased risk of hospitalization and death. During influenza epidemics, the hospitalization rate for the elderly and persons with chronic health problems may increase twofold to fivefold compared with nonepidemic periods.

Influenza viruses are classified into subtypes on the basis of two surface antigens: hemagglutinin (H) and neuraminidase (N). Widespread human disease since 1977 has been caused by influenza A (H1N1 and H3N2) viruses, and influenza B. Immunity to these antigens, especially to the hemagglutinin, reduces the likelihood of infection and lessens the severity of disease if infection occurs. Infection with a virus of one subtype confers little or no protection against viruses of other subtypes. Over time, antigenic variation (antigenic drift) within a subtype may be so marked that infection or immunization with one strain may not induce immunity to more distantly related strains of the same subtype. The antigenic characteristics of circulating strains provide the basis for selecting the viral strains included in each year's vaccine. During 2009–10 there was a worldwide pandemic because of a novel influenza A H1N1 strain.[355–358]

Influenza virus appears to spread from person-to-person by small-particle aerosol transmission. Although aerosol transmission is well established, nosocomial transmission via fomites and contaminated hands is likely. Influenza virus is shed for at least 5 days beyond the onset of illness in adults, but shedding may occur for longer periods in children and immunocompromised persons. Humans are the primary reservoir of infection, but swine and avian (especially, ducks) reservoirs are likely sources of new human subtypes thought to emerge through mutation and/or genetic reassortment.

Lessons From Nosocomial Outbreaks

Nosocomial acquisition of influenza has been well described.[359–391] Nosocomial transmission most commonly occurs during community influenza outbreaks when patients infected with influenza are admitted to the hospital. Because up to 25% of unimmunized HCP may develop influenza during the winter months, however, infected staff may introduce infection into a healthcare facility.[392] Staff infected by patients have frequently served as the source for secondary transmission of influenza to patients and other staff.[361,362,367,368,381,384,387,390] Acquisition of influenza by HCP may cause absenteeism and significant disruption of in the healthcare system.[362,365,368,391]

Nosocomial outbreaks frequently have involved extended-care facilities for the elderly.[392–424] Such outbreaks may cause significant morbidity and mortality.[396,397,405,424] A metaanalysis assessed studies evaluating whether high rates of influenza immunization among HCP working with persons older than 60 years of age led to a decrease in the attack rate of influenza among patients in extended-care facilities and/or provided other benefits.[425] The metaanalysis included four cluster-randomized clinical trials and one cohort study. Pooled data from three trials demonstrated that HCP vaccination reduced influenza-like illness among the patients and reduced all-cause mortality in persons older than 60 years of age. Even though the coverage among HCP in the intervention facilities only reached approximately 60%, the metaanalysis demonstrated reduced patient mortality. Nosocomial outbreaks also have been reported in other types of long-term care facilities, such as institutions caring for mentally challenged persons.[426]

Important lessons from these outbreaks include the following. First, identification of all patients with influenza is problematic and incomplete. Furthermore, community indicators of influenza activity (e.g., visits to acute ambulatory care centers for upper respiratory illness) cannot be relied on to provide warning of influenza among hospitalized patients.[379] For example, a nosocomial outbreak has been reported during a period without influenza epidemic activity in the community.[381] Second, influenza infection among staff is common during the winter season and results in significant

absenteeism. Attack rates of 25% to 80% often are observed among both patients and staff during outbreaks. Third, high rates of immunization among residents cannot be relied upon to prevent outbreaks.[415] Recommendations have been published for prevention and control of nosocomial influenza in acute-care hospitals[61,67,68,427] and extended-care facilities.[428–432] The CDC guideline for the prevention of healthcare-associated pneumonia contains detailed recommendations for the detection and isolation of patients with known or suspected influenza, management of healthcare workers with known or suspected influenza, and use of antivirals in the management of healthcare-associated outbreaks.[427]

Preexposure Prophylaxis

Influenza vaccine is strongly recommended for any person age 6 months or older, especially those who are at increased risk for complications of influenza because of age or underlying medical condition. Persons at increased risk for influenza-related complications include those age 50 years or older; residents of extended-care facilities or long-term care facilities that house persons of any age who have chronic medical conditions; adults and children who have required regular medical follow-up or hospitalization during the previous year because of chronic metabolic diseases (including diabetes mellitus), renal dysfunction, hemoglobinopathies, or immunosuppression; children and teenagers (ages 6 months to 18 years) who are receiving long-term aspirin therapy and therefore may be at risk for developing Reye syndrome after influenza; and pregnant women.[67,68] In addition, influenza vaccine should be offered to persons who live with or care for persons with certain chronic medical conditions.

Influenza vaccine is also recommended for all HCP because, when they are clinically or subclinically infected, they can transmit influenza virus to people at high risk. In a recent study, Voirin and colleagues assessed the risk of influenza transmission in an acute care geriatric unit in an academic hospital by collecting high-resolution contact data via wearable sensors combined with virological data.[433] Voirin and associates documented three patient- and one nurse-acquired laboratory-confirmed influenza cases and concluded that infectious medical doctors and nurses are potential sources of hospital-acquired influenza for patients. In addition, infectious patients were identified as likely sources for nurses. The CDC specifically recommends immunization for the following HCP: physicians, nurses, and other personnel in both hospital and outpatient care settings; HCP of nursing homes and long-term care facilities who have contact with patients or residents; and providers of home care to persons at high risk (e.g., visiting nurses and volunteer workers).[30] The American Academy of Pediatrics,[56] the Association for Professionals in Infection Control and Epidemiology,[57] the Infectious Diseases Society of America,[58] the American College of Physicians,[59] and the Society of Healthcare Epidemiologists of America,[60] not only specifically endorse this recommendation, but also state that annual influenza immunization should be mandatory for all HCP. Current influenza vaccines may be trivalent (i.e., two A strains and one B strain) or quadrivalent (two A strains and two B strains). In modeling studies quadrivalent vaccines have been demonstrated to prevent an additional 18% to 21% of cases from influenza B strains.[434] Multiple types of influenza vaccine are available including inactivated influenza vaccine (IIV3 or IIV4) that can be administered intramuscularly or intradermally, a cell-culture based IIV (ccIIV3), a high-dose IIV, a recombinant influenza vaccine (RIIV3), and a live attenuated influenza vaccine (LAIV4). HCP providing influenza vaccines should be aware of current age restrictions for each specific vaccine, and whether the vaccine contains thimerosal and/or latex. Although the administration of thimerosal in vaccines has not been demonstrated to result in patient harm, some patients (especially pregnant women) remain concerned about receiving vaccine containing this preservative. Administration of vaccines whose vial or syringe contain latex could precipitate anaphylaxis in persons with latex allergies. Both thimerosal and latex-free influenza vaccines are available. RIIV3 is the only vaccine that is completely free of egg proteins. The CDC does not list any vaccine as being preferential. Importantly, HCP should not receive the attenuated live influenza virus vaccine if they have close contact with severely immunosuppressed persons (e.g., patients with hematopoietic stem cell transplants) during those periods in which the immunosuppressed person requires care in a protective environment.[30] If they receive the vaccine, they should refrain from contact with severely immunosuppressed patients for 7 days after vaccine receipt. An inactivated trivalent vaccine containing 60 μg of hemagglutinin antigen per influenza vaccine virus strain is an alternative inactivated vaccine for persons 65 years of age and older.

Chemoprophylaxis with an antiviral drug (amantadine, rimantadine, zanamivir, oseltamivir) has been used as both seasonal preexposure and postexposure prophylaxis to reduce the likelihood of developing influenza A (amantadine, rimantadine) or influenza A and B (zanamivir, oseltamivir) infection. Amantadine and rimantadine are no longer recommended for either prophylaxis or treatment of viral influenza because of a dramatic increase in resistance that was noted in 2005 to 2006.[435] Specific recommendations for the use of oseltamivir or zanamivir for chemoprophylaxis or treatment of influenza have been published.[435,436] Oseltamivir has been used successfully to provide chemoprophylaxis in extended-care facilities.[412,413,415,417,418] Fewer data are available regarding the use of zanamivir.[411] Zanamivir is associated with bronchospasm, and oseltamivir is associated with gastrointestinal upset. Use of antiviral agents for chemoprophylaxis or treatment of influenza is an adjunct (but not a substitute for) vaccination.

Despite these recommendations, many HCP still choose not to receive the influenza vaccine.[112,113,120,437–448] The knowledge and attitudes of HCP to influenza immunization have been reviewed.[449,450] Institutions should consider introducing innovative methods, such as provision by mobile carts on hospital wards, off-hours clinics, feedback on immunization rates to unit nursing and medical directors, and offering vaccine to medical personnel in clinics and conferences.[451] Only making continued employment conditional on influenza immunization (unless there is a medical contraindication) has succeeded in achieving high rates of influenza vaccine coverage among HCP. The ethics of "mandatory" influenza immunization have been discussed.[57–61] The Society for Healthcare Epidemiology of America has recommended the following methods of improving influenza vaccine coverage of healthcare workers.[451]

- Off-hours clinics
- Use of mobile vaccination carts
- Vaccination at staff and departmental meetings
- Provision of adequate staff and resources
- Provision of vaccine free of charge to HCP
- Targeted education, including specific information to dispel vaccine myths
- Use of live attenuated influenza vaccine for eligible HCP
- Strong and visible administrative leadership
- Visible vaccination of key leaders
- Accurate tracking of individual and unit-based compliance of HCP with vaccination
- Surveillance for healthcare-associated influenza

Postexposure Prophylaxis

During community or nosocomial outbreaks, healthcare institutions should offer and strongly encourage the use of influenza vaccine among HCP. If influenza is causing a community outbreak, consideration should be given to providing chemoprophylaxis for 2 weeks to newly immunized HCP (avoid live attenuated influenza vaccine in such cases) who are at high risk of exposure to infected patients. Prophylaxis should be considered for all HCP, regardless of immunization status, if the outbreak is caused by a variant strain of influenza that might not be controlled by the vaccine. Using antiviral drugs for treatment and chemoprophylaxis of influenza is a key component of influenza outbreak control in institutions.[435,436] When confirmed or suspected outbreaks of influenza occur in healthcare facilities, appropriate chemoprophylaxis should be started as early as possible to reduce the spread of virus. Chemoprophylaxis should be continued until approximately 1 week after the end of the outbreak. Of importance, antivirals are most effective in reducing the severity and shortening the duration of influenza illness among healthy adults when administered within 48 hours of illness onset.

Pertussis Vaccine

Epidemiology

In the United States, the highest recorded annual incidence of pertussis occurred in 1934, when more than 260,000 cases were reported.[452] After the introduction of diphtheria, tetanus, and whole-cell pertussis vaccine, the incidence declined by more than 99%. However, throughout the 1980s and 1990s, the incidence of pertussis increased, peaking in 2004 (8.9 per 100,000 population). Subsequent to this peak, the incidence declined, but is increasing again (8.97 per 100,000 population in 2010, 6.06 per 100,000 population in 2011, and 15.49 per 100,000 population in 2012). The 48,277 reported cases in 2012 is the highest number of cases reported in the United States since 1955 when 62,786 cases were reported. Provisional data for more recent years were as follows: 28,639 cases reported in 2013 and 27,878 cases reported in 2014. The number of cases as reported by the CDC is clearly an underestimate of the true incidence of pertussis as many persons, especially adults with mild disease, are not reported to public health authorities. Infants younger than 6 months of age are at the greatest risk for severe disease and death, and continue to have the highest reported rate of pertussis (124.93 per 100,000 infants younger than 6 months of age). However, children (5 to 14 years of age) accounted for approximately 45% of reported cases in 2012. Since the introduction of the Tdap vaccine for adolescents and adults in 2005, coverage among persons 13 to 17 years of age has dramatically increased, and was approximately 85% in 2014. Most cases occur in adolescents and young adults. Possible explanations for this increase in disease include (a) genetic changes in *B. pertussis*, making vaccine less effective; (b) waning immunity among children, adolescents, and adults vaccinated during childhood, especially those who received acellular pertussis vaccines; (c) lessened effectiveness of acellular pertussis vaccines compared to whole-cell vaccines; (d) greater awareness of pertussis and hence more diagnostic testing; and (e) the general availability of better laboratory tests.[453] Waning immunity after diphtheria, tetanus, acellular pertussis (DTaP) vaccine appears to be the most important reason for the increases in pertussis.[453]

Adolescents and young adults play an important role in transmitting pertussis to susceptible infants because immunization-induced immunity to pertussis wanes with increasing age and because disease in adults frequently is not diagnosed or treated because it is often mild or atypical. Studies using serologic methods have demonstrated that *B. pertussis* is a common cause of respiratory illness, causing cough in adults.[453–455]

Lessons From Nosocomial Outbreaks

At the University of North Carolina Hospitals, pertussis is now the most common source of infectious disease exposure evaluations (Weber, unpublished data, 1994 to 2015). Multiple nosocomial outbreaks have been reported in the literature.[456–485] These most often have involved residential facilities for the mentally or physically impaired or pediatric wards of acute-care hospitals. Although the source case most commonly was an infected patient in whom pertussis was unrecognized,[456,457,461,466,481] infected HCP[459,462,465,468,470,471,481,484] and the infected mother of a child with pertussis[458] also served as sources. HCP often served as vectors for additional nosocomial cases.[456,457,459,462] In several instances, infected HCP also infected members of their households.[457,459,480] Nosocomial outbreaks have occurred for several reasons: (a) failure to recognize and appropriately isolate infected patients, (b) failure to provide antibiotic prophylaxis to exposed staff, and (c) failure to furlough symptomatic staff.[486,487]

Preexposure Prophylaxis

With the licensure of a Tdap for adolescents and adults in 2005, it is now possible to provide preexposure prophylaxis to HCP. The CDC recommends that regardless of age, HCP should receive a single dose of Tdap if they have not previously received Tdap and regardless of the time since their most recent diphtheria and tetanus toxoids (Td) vaccination.[30] The CDC also recommends against HCP receiving any additional Tdap boosters.[488] Importantly, prevaccination serologic testing is not recommended and immunity cannot be demonstrated through serologic testing because serologic correlates of protection are not well established.[30] Hospitals and ambulatory-care facilities should provide Tdap for HCP and use approaches that maximize vaccination rates (e.g., education about the benefits of vaccination, convenient access, and the provision of Tdap at no charge). The benefits of immunizing HCP with Tdap have been reviewed.[488,489]

Postexposure Prophylaxis

B. pertussis is highly susceptible in vitro to erythromycin[490,491] and the newer macrolides, azithromycin, and clarithromycin.[492] It is also susceptible to trimethoprim-sulfamethoxazole.[491] *B. pertussis* is not susceptible to the first-generation cephalosporins. Erythromycin has been shown to decrease the duration of illness when administered early in the course of pertussis and to eliminate *B. pertussis* from the nasopharynx. Erythromycin also has been successfully used to provide chemoprophylaxis to individuals exposed to *B. pertussis* and to aid in preventing secondary spread in households or terminating outbreaks in institutions.[493–495] Azithromycin has been demonstrated to be effective in the prophylaxis and treatment of pertussis.[496] It is now the preferred agent because, compared with erythromycin, it requires a short period of postexposure prophylaxis or therapy (5 vs 7 to 14 days), reduced dosing frequency (one vs four times per day), and is less likely to result in gastrointestinal distress.[496] Trimethoprim-sulfamethoxazole is the recommended alternative for treatment and for chemoprophylaxis of individuals intolerant to a macrolide, although its efficacy as a chemoprophylactic agent has not been evaluated. The evidence of trimethoprim-sulfamethoxazole's efficacy is based on small studies.[497,498]

Erythromycin-resistant clinical isolates of *B. pertussis* have been reported, raising concern about the use of macrolides for therapy or prophylaxis.[499–501] However, surveys of *B. pertussis* strains demonstrate that macrolide resistance is uncommon.[502–504] Immunization of HCP with Tdap vaccine has been used along with chemoprophylaxis to control nosocomial outbreaks.[480]

Data on the need for postexposure prophylaxis in Tdap-vaccinated HCP are inconclusive.[505] Certain vaccinated HCP are still at risk for acquisition of *B. pertussis*. Therefore, Tdap might not preclude the need for postexposure prophylaxis. Postexposure antimicrobial prophylaxis is recommended for all HCP regardless of Tdap status who have unprotected exposure to pertussis and are likely to expose a patient at risk for severe pertussis (e.g., hospitalized neonates and pregnant women). Other HCP regardless of Tdap status should either receive postexposure antimicrobial prophylaxis or be monitored daily for 21 days after pertussis exposure and treated at the onset of signs and symptoms of pertussis.

CONCLUSIONS

All susceptible HCP, unless they have a contraindication to immunization, should be immunized against mumps, measles, rubella, varicella, and pertussis. If HCP have the potential for exposure to blood or contaminated fluids, they should be immunized against hepatitis B. In addition, HCP should be immunized against influenza annually. HCP also should be referred for vaccines recommended for the general public. Selected HCP may be candidates for other available vaccines, including hepatitis A, meningococcal, anthrax, polio, plague, typhoid, vaccinia, and rabies.

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SECTION 5

Public Health and Regulatory Issues

73 Immunization in the United States

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A strong immunization program is critical to ensure vaccines are given at the recommended ages to all children, adolescents, and adults in the United States. The immunization system in the United States is a public- and private-sector partnership, including federal agencies, state and local health departments, healthcare providers, and vaccine manufacturers, that works toward common immunization goals and objectives. The U.S. immunization system has a formal process of establishing national immunization recommendations and uses a set of systems to monitor the health impact, coverage levels, and safety of recommended vaccines. As of 2017, routine immunizations protected the U.S. public against 17 vaccine-preventable diseases. Vaccine access is provided through private and public health insurance for all ages, and financially vulnerable children have a federal government entitlement to vaccines through the Vaccines for Children (VFC) program.

Immunization of young children in the United States has been highly effective. Most vaccine-preventable diseases of children are at record or near record lows in the United States with small or no disparities by race and ethnicity.[1] Measles, rubella, and polio have been, and remain, eliminated in the United States, and the number of young children not immunized at all remains less than 1%. Immunization of adolescents has improved substantially but lags for selected recommended vaccines (e.g., human papillomavirus [HPV]).[2] Immunization of adults continues to be a growing challenge in the United States as vaccination coverage for both older and new vaccines, such as zoster, tetanus toxoid, diphtheria toxoid, and acellular pertussis vaccine (Tdap), and HPV vaccines, is still low, while new vaccines, such as pneumococcal conjugate vaccine, are being introduced.

IMMUNIZATION RECOMMENDATIONS

Vaccines for routine immunization in the United States must be licensed prior to recommendations. The sole authority for licensing vaccines in the United States is the Food and Drug Administration (FDA), which has responsibility for ensuring the safety and efficacy of vaccines before licensure (see Chapter 81). The FDA approves vaccines for specific indications, age groups, dose amounts and schedules, and contraindications and precautions. Information about the safety and efficacy of administering a specific vaccine simultaneously with other vaccines may be obtained before or after licensure of that vaccine, but information about the safety and efficacy of combining several vaccines into one formulation must be obtained before combination vaccines are licensed.

Technical recommendations for the use of vaccines are made by the U.S. Centers for Disease Control and Prevention (CDC) with advice from its Advisory Committee on Immunization Practices (ACIP). The ACIP was chartered in 1964, and members are 15 experts in immunization and related fields selected by the Secretary of the U.S. Department of Health and Human Services to provide advice and guidance on control of vaccine-preventable diseases.[3] In its role as a federal advisory committee, the ACIP develops written recommendations, subject to approval of the CDC director, for administration of FDA-licensed vaccines to children, adolescents, and adults in the U.S. civilian population. The ACIP works closely with several liaison organizations, including the American Academy of Pediatrics, the American Academy of Family Physicians, the American College of Physicians, and the American College of Obstetricians and Gynecologists, to harmonize immunization recommendations among key professional medical organizations in the United States.[4,5] ACIP meetings are conducted three times a year and are open to the public and the media, invite public comment, and are webcast live. Presentations at ACIP are considered in the public domain and are available at the CDC's ACIP website[6] soon after the meeting. Comprehensive descriptions of the structure, role, and procedures of the ACIP are also available in the peer-reviewed literature.[5]

The ACIP is charged to conduct its work in an objective manner, and decisions are to be made without regard for budgetary implications (VFC Statement of Managers[7]). The ACIP recommends vaccines, ages of vaccination, number of doses and dosing interval, and precautions and contraindications for vaccination (Table 73.1). The ACIP considers a careful review of available scientific data, including the epidemiology of the disease in the general U.S. population and in specific risk groups; the licensed indications, safety, efficacy, and cost-effectiveness of the vaccine; and programmatic concerns and related factors. Selection of the age at which a vaccine should be administered depends on the ability of the vaccinee to respond to the vaccine, the risk of exposure to disease, and the age-specific incidence of disease and its complications.[8,9] In general, the approach has been to administer vaccine at the earliest possible age at which the vaccine is reliably effective and has sufficient duration of immunity to protect vaccinees through ages of peak disease risk or until a booster dose is recommended.

In 2010, the ACIP adopted the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) framework for developing evidence-based recommendations.[10,11] In the GRADE framework, key factors include the balance of benefits and harms, type of evidence, values and preferences of the people affected, and economic analyses. Category A recommendations apply for all persons in an age group or for all persons in a risk-factor-based group. Category B recommendations direct individualized clinical decision making; category B recommendations may not apply to all members of an age- or risk-factor-based subgroup of the population, but in the context of a clinician-patient interaction, vaccination may be found to be appropriate for a person.

TABLE 73.1 Selected Major Immunization Policy Decisions, U.S. Advisory Committee on Immunization Practices: 2005–15

New Vaccine/Recommendation	ACIP Vote
Meningococcal A/C/Y/W-135 conjugate (adolescents)	February 2005
Tetanus, diphtheria, and pertussis (Tdap) (adolescents)	June 2005
Measles-mumps-rubella-varicella (MMRV), universal hepatitis A	October 2005
Rotavirus (infants)	February 2006
Quadrivalent human papillomavirus (4vHPV) (adolescent girls); varicella second dose	June 2006
Zoster (shingles)	October 2006
Influenza (universal >6 months), PCV13 children	February 2010
Meningococcal A/C/Y/W-135 conjugate second dose	October 2010
Tdap in every pregnancy	October 2012
Pneumococcal conjugate vaccine (PCV13) in persons age ≥65 years	August 2014
2-dose human papillomavirus for younger adolescents	October 2016
Meningococcal serogroup B (MenB) (adolescents, category B)	June 2015

Evidence tables are used to summarize the benefits and harms and the strengths and limitations of the body of evidence. The ACIP does not have predetermined economic valuation criteria for recommending a vaccine. The CDC provides guidance on the allowable methods for health economic studies presented to the ACIP.[12,13]

Recommendations for the use of a vaccine depend on the balance of benefits of vaccination, duration of protection, risks of disease, and risks of vaccination. This balance must be assessed periodically and when new information is available. As experience is gained with a particular vaccine, recommendations may need to be modified. Examples of modification of recommendations include transitioning from oral poliovirus vaccine to inactivated poliovirus vaccine to eliminate the risk of vaccine-acquired paralytic polio,[14] adding a second dose of varicella vaccine in the face of evidence of limited immunity after one dose of varicella vaccine,[15] withdrawing a recommendation for the rhesus rotavirus vaccine owing to evidence that this particular rotavirus vaccine caused intussusception,[16] adding a second dose of meningococcal conjugate vaccine after evidence of waning immunity,[17] and adjusting Tdap recommendations for women from the postpartum period to the third trimester of each pregnancy.[18]

ACIP recommendations are summarized in two separate immunization schedules: one for children 0 through 18 years of age, and the second for adults (19 years of age and older). There is also a catch-up schedule for children who are not appropriately immunized for their age. There are two adult immunization schedules, one based on age and the other based on underlying conditions. The most current immunization schedules are available from the CDC in a variety of formats.[19] The schedules are updated annually and published at the beginning of each year in the CDC's *Morbidity and Mortality Weekly Report* and in major medical journals, including *Pediatrics*, *Annals of Internal Medicine*, and *American Family Physician*. The schedules provide information for clinicians on routinely recommended vaccines and risk-group recommended vaccines with category A and category B recommendations. The CDC also publishes general recommendations that supplement the schedules with information about vaccination technique and storage and handling of vaccines.[20] Updates to these general recommendations can be found at the CDC website.[21] Vaccines recommended solely for international travel are not included in these schedules, but information on vaccines for travelers is available at the CDC.[22]

Fig. 73.1 shows recommended immunizations for children and adolescents, and Fig. 73.2 shows recommended immunizations for adults as of 2017. The United States currently protects children against 16 infectious diseases, and adults older than age 60 years are additionally protected against zoster. Full protection during the first 18 years of age requires more than 50 doses of vaccines, although combination vaccines can reduce the number of injections needed and may lead to higher coverage rates.[23,24] Lessons learned from making and implementing vaccine recommendations in the United States have been reviewed.[3,25]

Policy immunization recommendations are made by the Department of Health and Human Services' National Vaccine Advisory Committee (NVAC), which was established in 1986 by the National Childhood Vaccine Injury Act to focus on programmatic policies and strategies. Some important NVAC reports have focused on vaccination financing, vaccine supply, standards for immunization practices, the status of vaccine research, and a blueprint for immunization programs.[26–30]

U.S. IMMUNIZATION PROGRAM

History

In the United States, immunizations are provided through the private and public sectors. The public sector consists primarily of health departments, but also includes other clinics, such as community and migrant health centers and public hospital–based clinics supported by public funds. There was no federal support specifically for immunization activities until 1955, when inactivated polio vaccine was licensed. Through the Polio Vaccination Assistance Act, Congress appropriated funds in 1955 and 1956 to the Communicable Disease Center (now the CDC) to help states and local communities purchase and administer vaccine.

In 1962, President John F. Kennedy signed into law the Vaccination Assistance Act. The central thrust of this legislation was to allow the CDC to support mass immunization campaigns and to initiate maintenance programs. The first grants, authorized under Section 317 of the Public Health Service Act, were made in June 1963. During the more than 50 years since the Vaccination Assistance Act was signed into law, this grant program has thrived. There are now 64 grantees under what has become known as the "317" Immunization Grant Program, including all 50 states, six large cities (including the District of Columbia), and eight territories and former territories.[30] The level of grant funding has varied greatly over the years. When the grant program began in 1963, the only vaccines available were diphtheria and tetanus toxoids and whole-cell pertussis (DTP), polio, and smallpox. In 1966, a national effort to eradicate measles began.[31] The focus on measles vaccine administration ultimately led to adoption of many critical components of the program that have had impact on multiple vaccine-preventable diseases[32] and funding has since expanded to support the infrastructure underpinning the addition of new vaccines to the immunization schedule, as outlined in the NVAC report "Protecting the Public's Health: Critical Functions of the Section 317 Immunization Program."[30]

In the early 1970s, immunization programs around the country were in varying states of effectiveness. In 1977, a

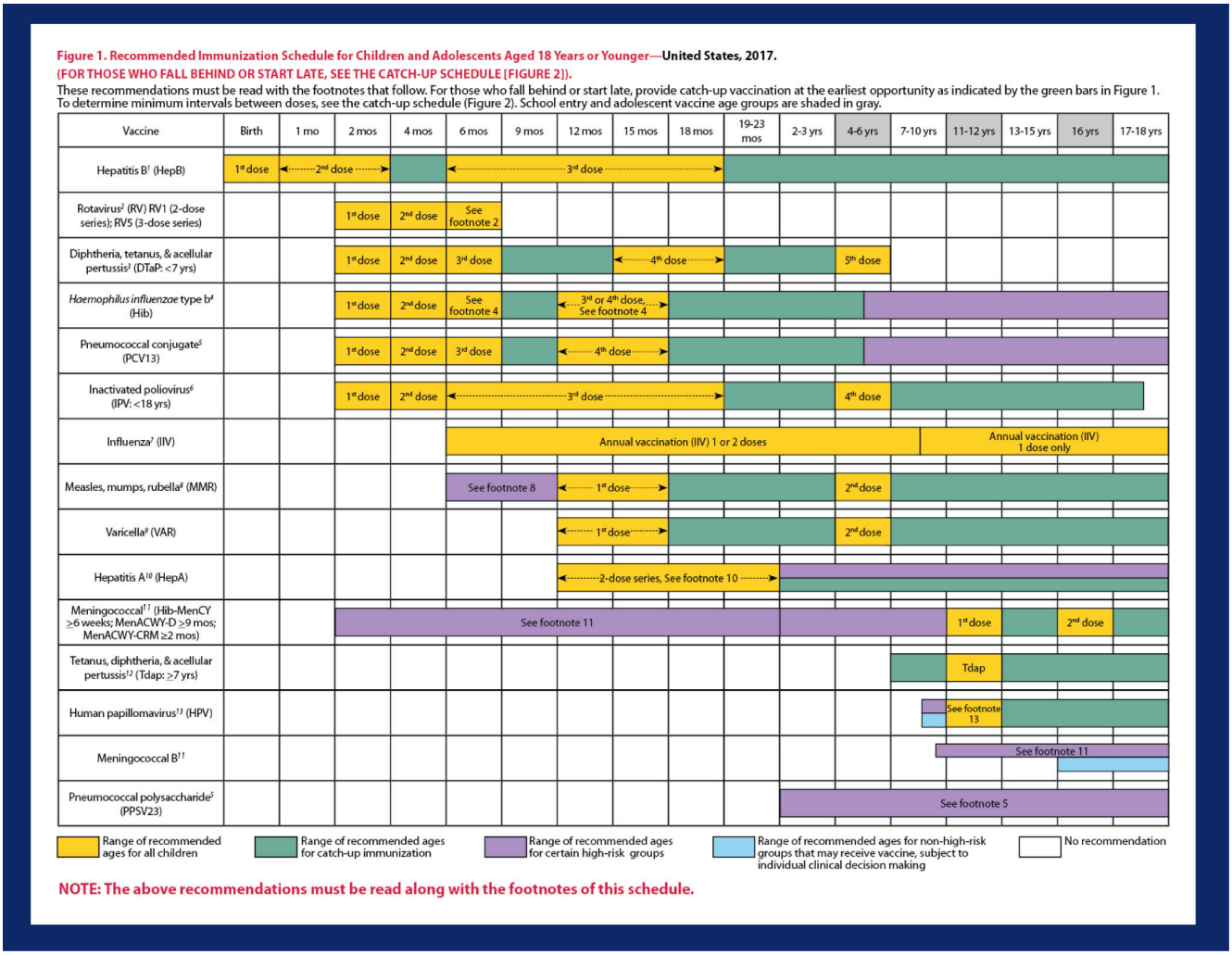


Figure 73.1. 2017 immunization schedule for birth through 18 years of age. Footnotes for each vaccine are not shown in the figure, but can be found at https://www.cdc.gov/vaccines/schedules/hcp/child-adolescent.html.

national childhood immunization initiative was announced with two goals: attainment of immunization levels of 90% in the nation's children by October 1979 and establishment of a permanent system to provide comprehensive immunization services to the 3 million children born annually in the United States at that time.[33] (For comparison, the current U.S. birth cohort is 4.3 million.) At the time, it was estimated that nearly 20 million U.S. children were in need of at least one dose of a vaccine to be fully immunized. The poor and minority populations were disproportionately represented among the persons needing additional vaccine doses. Efforts were expended to enact school immunization requirements in states that did not have them and to enforce those already in existence. As a result of these efforts, all 50 states soon had, and were enforcing, school-entry immunization laws. Since 1981, 95% or more of children entering school have had documented immunizations. Given these levels, even with lower levels in preschoolers, the overall immunization level in children of all ages in this country was 90% or greater. Thus, the first goal of the initiative was met.

Unfortunately, the second goal of the 1977 initiative was not met. Although the overall level of support for immunization grants rose rapidly in the late 1970s and throughout the 1980s (reaching US$126.8 million in 1989), almost all the increase was to meet the increasing cost of vaccines or the addition of new vaccines or additional doses of existing vaccines. In the 1980s, the level of federal support to grantees to carry out maintenance elements did not increase significantly. Over the years, the federal government provided more existing and new vaccines within a delivery system that had remained static in the face of increasing demands. Investigations of the measles epidemics of 1989 to 1991, which especially affected unvaccinated preschool children, made it clear that the public sector delivery system was inadequate for the challenge and that it required substantial assistance.

Part of the problem was that immunization grant funds were only permitted to be used to purchase vaccines and to carry out surveillance, investigation, education, and coordination, but not to support vaccine delivery (e.g., salaries of nurses, clinic supplies, expenses associated with increasing clinic hours). In 1991, President George H.W. Bush announced the federal government's support to accomplish a major health goal—to raise immunization levels by the year 2000 so that 90% or more of the nation's children routinely completed their basic series of vaccinations by their second birthday. The President announced that model

immunization plans would be developed in several areas of the country as a beginning for the national effort to ensure adequate and timely immunization of infants and young children.[34] This began a process that ultimately resulted in the preparation of immunization action plans by all 50 states and 28 metropolitan areas. Although there was great variation in the reported needs around the country, one theme was common to most plans and that was to increase the availability of immunization services. Consequently, for the first time, federal immunization grant funds were allowed to be used for the actual provision of immunization services.

President Bill Clinton's announcement of the Childhood Immunization Initiative in 1993 and the leadership and infusion of funds associated with that initiative, coupled with the VFC program that followed, have brought the United States to the point that it is now, finally achieving 90% coverage in most vaccines recommended for preschool children.[35–37] A critical part of the Childhood Immunization Initiative was to eliminate financial barriers to vaccination and ensure children could be vaccinated at their site of usual care ("medical home"), typically a private provider's office.[38] The Childhood Immunization Initiative also established the National Immunization Survey (NIS) to assess vaccination coverage levels among young children. During the measles resurgence of 1989–91, there was no national mechanism to assess vaccination coverage. NIS was designed to estimate national, state, and selected local area coverage levels of children, 19 to 35 months of age. NIS has been in continuous operation since its establishment in 1994.

Following successful achievement of the 1996 Childhood Immunization Initiative goals, funding for the Section 317 program declined. Concerned about the adequacy of immunization infrastructure in the United States, Congress requested that the Institute of Medicine (IOM) conduct a study on the level of need for federal infrastructure support. The IOM recognized the damaging impact that the ebb and flow of infrastructure funding was having on the nation's immunization system and recommended a combined, 5-year investment of US$1.5 billion, shared between both federal and state resources. Between the publication of the IOM's 2000 report, "Calling the Shots," and 2010, federal infrastructure funding was increased by US$115 million to US$239 million.[39] Colgrove reviewed the history of two critical components of U.S. immunization efforts—public financing and immunization mandates.[40]

Figures 1 and 2 should be read with the footnotes that contain important general information and considerations for special populations.

Figure 1. Recommended immunization schedule for adults aged 19 years or older by age group, United States, 2017

Vaccine	19–21 years	22–26 years	27–59 years	60–64 years	≥ 65 years
Influenza[1]	1 dose annually				
Td/Tdap[2]	Substitute Tdap for Td once, then Td booster every 10 yrs				
MMR[3]	1 or 2 doses depending on indication				
VAR[4]	2 doses				
HZV[5]				1 dose	
HPV–Female[6]	3 doses				
HPV–Male[6]	3 doses				
PCV13[7]	1 dose				
PPSV23[7]	1 or 2 doses depending on indication				1 dose
HepA[8]	2 or 3 doses depending on vaccine				
HepB[9]	3 doses				
MenACWY or MPSV4[10]	1 or more doses depending on indication				
MenB[10]	2 or 3 doses depending on vaccine				
Hib[11]	1 or 3 doses depending on indication				

Recommended for adults who meet the age requirement, lack documentation of vaccination, or lack evidence of past infection

Recommended for adults with additional medical conditions or other indications

No recommendation

Figure 73.2. 2017 immunization schedules for adults, including age-based recommendations and medical and other condition recommendations. Footnotes on each vaccine are not shown in the figure, but can be found at https://www.cdc.gov/vaccines/schedules/hcp/adult.html.

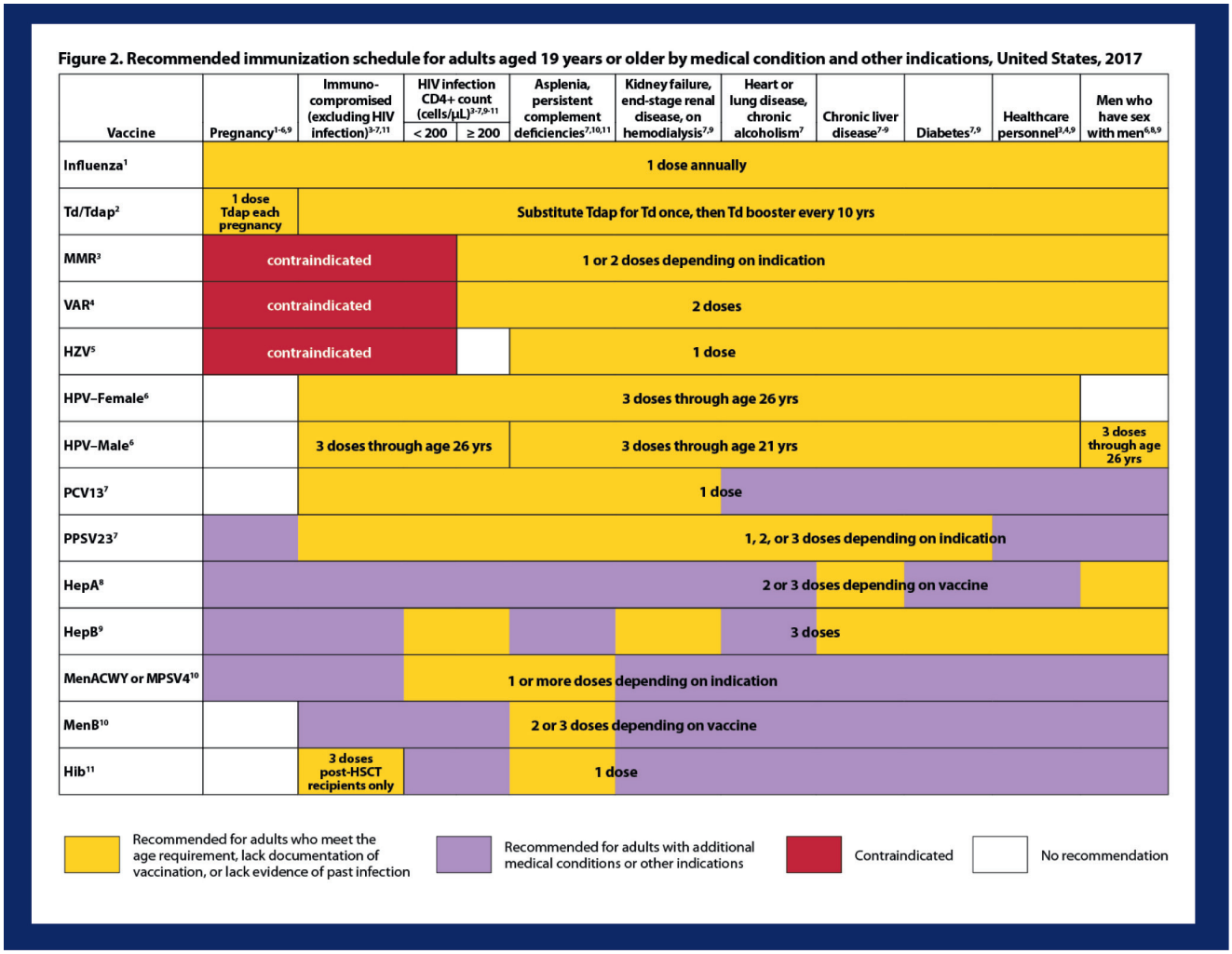


Figure 73.2, cont'd

Three major pieces of legislation have helped solidify the success of the U.S. immunization program: the National Childhood Vaccine Injury Act, the VFC program, and the Patient Protection and Affordable Care Act (ACA). Their role in vaccine successes are summarized below. In 1986, the National Childhood Vaccine Injury Act was enacted, which put in place a no-fault compensation program for persons who had been injured after receipt of a vaccine that was universally recommended for children (no matter the age of the recipient). This reduced the threat of liability for vaccine manufacturers and facilitated the availability of vaccines. The VFC program was created by the Omnibus Budget Reconciliation Act of 1993 and first implemented in 1994. The VFC program entitles eligible children 0 to 18 years of age to receive all ACIP recommended vaccines free of charge. The ACA was passed in 2010 and requires private insurers to cover all vaccines as indicated on the pediatric and adult immunization schedules. A comprehensive review of the *Morbidity and Mortality Weekly Report (MMWR)*'s documentation of the U.S. immunization program has been published.[41]

ROLES OF THE U.S. IMMUNIZATION PROGRAM

The IOM "Calling the Shots" report provided a useful conceptual framework for understanding the complex array of roles of the federal, state, and local immunization programs in collaboration with the nation's healthcare delivery system. The IOM identified five key functions in vaccine implementation: (a) assure purchase of vaccine, (b) assure service delivery, (c) control and prevent infectious diseases and measure disease burden, (d) conduct surveillance of vaccine coverage and safety, and (e) sustain and improve immunization coverage levels.[39] These functions are all needed to ensure the ultimate immunization program goal of controlling and preventing infectious diseases. The impact of immunization policies and practices is assessed through careful disease surveillance, vaccine effectiveness studies, and safety monitoring—an evidence base that is used to adjust immunization recommendations as new evidence emerges. All these activities are supported by a base of immunization finance policies and practices and through the work of health departments and other public sector agencies.

Assuring Vaccine Purchase, Supply, and Distribution

Except for a small amount of diphtheria and tetanus toxoids vaccine, all U.S. vaccines for routine use are manufactured by private sector pharmaceutical companies, with some produced by several companies and others by a single manufacturer.

Manufacturers are the sole decision makers for which vaccines they will develop and bring to the U.S. market, including new vaccines and combinations of existing vaccines.[42] However, the National Vaccine Plan of the U.S. Department of Health and Human Services proposes a government role to encourage the development of vaccines specific to U.S. public health needs.[43] One goal of the National Vaccine Plan is "to create an evidence-based list of new vaccine targets to prevent infectious diseases that are high priorities for development." The IOM, with support from National Vaccine Program Office, developed a new vaccine decision-support tool called the Strategic Multi-Attribute Ranking Tool for Vaccines, or SMART Vaccines tool. National Vaccine Program Office is developing strategies to ensure this multistakeholder decision support tool is useful and available for the public.[44]

Approximately half of the childhood vaccine supply is purchased with federal and state government funding using the CDC's vaccine contracts. These contracts allow state and local immunization programs to obtain vaccines at reduced prices and without having to negotiate separate contracts with the vaccine manufacturers. Current CDC contract prices and comparative private sector catalog prices are available from the CDC.[45] Fig. 73.3 shows the CDC vaccine contract cost to fully vaccinate a child at three points in time. The current policy allows multiple manufacturers to contract for any of the program vaccines. States and local immunization programs are allowed to select which of the vaccines they will use if there is more than one manufacturer for a given vaccine. In turn, states generally permit their providers to select which vaccines they will use to vaccinate program-eligible persons. Vaccine purchased using the CDC contracts is not allowed to be resold, and parents cannot be charged for the cost of CDC-contracted vaccine, although a state-determined administration fee can be charged to parents who can afford this fee. Approximately 90% of vaccine funding is provided by the federal VFC entitlement program, with state/local funding and the federal Section 317 funding making up the remainder. State and local immunization programs are provided a credit line of federal vaccine purchase funding that is awarded through population-based formulas for VFC and Section 317 vaccine.

The management of public-sector vaccine is accomplished with a comprehensive information technology system called VTrckS, for "Vaccine Tracking System." Public sector vaccine is delivered by a CDC-contracted distributor directly to more than 40,000 end-user immunization providers. The providers can order vaccine through VTrckS or through their state's immunization information system that, in turn, places the orders into VTrckS. State and local immunization programs have oversight and control of the vaccine orders placed by providers, and they can also enter the vaccine orders on behalf of their providers. VTrckS and its interfaces to manufacturers, the CDC-funded distributors, and state and local immunization programs now provide a unified, seamless system with complete visibility of all segments of the system. Information about VTrckS and centralized vaccine distribution is available from the CDC.[46]

The CDC maintains stockpiles of childhood vaccine that are intended to serve as a buffer against short- and medium-term vaccine supply disruptions and to control outbreaks of vaccine-preventable diseases.[47] The stockpiles are maintained as storage and rotation inventories of vaccine so that the vaccine that enters the stockpile is used before its expiration. All the vaccines that are routinely recommended for children and are included in the VFC program are included in the stockpiles. The stockpiles include a 6-month supply of each of the CDC-contracted pediatric vaccines purchased through the public sector. Because shortages of vaccine and outbreaks of vaccine-preventable diseases span private and public sectors, the stockpile also supports the private sector vaccine supply system by allowing vaccine manufacturers to borrow vaccine

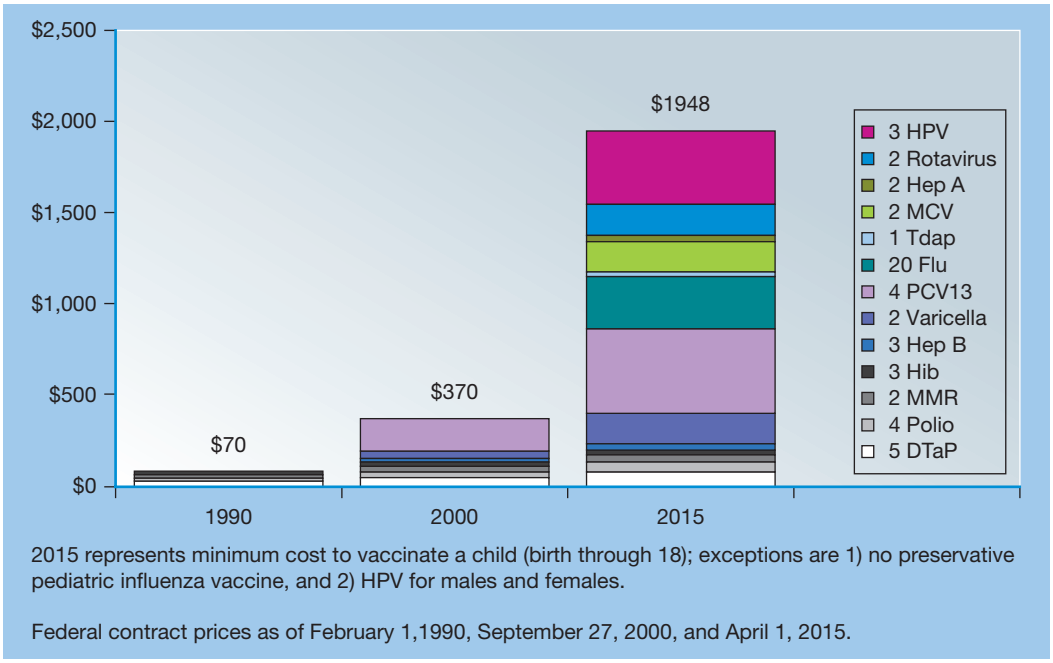


Figure 73.3. Sums of the prices of the Centers for Disease Control and Prevention (CDC) contract prices for vaccines needed to fully vaccinate a child from birth through 18 years of age. The vaccine contract prices are from February 1, 1990, September 27, 2000, and April 1, 2015. DTaP, diphtheria, pertussis, and acellular pertussis; Hep A, hepatitis A; Hep B, hepatitis B; Hib, *Haemophilus influenzae* type b; HPV, human papillomavirus; MCV, meningococcal conjugate vaccine; MMR, measles, mumps, rubella; PCV, pneumococcal conjugate vaccine; Tdap, adolescent-adult formulation tetanus, diphtheria, and acellular pertussis vaccine.

from the stockpile to sell to their private customers. Because the public sector purchases half of the childhood vaccines, CDC's stockpiles can manage a complete vaccine supply disruption of at least 3 month's duration or longer if disruptions are incomplete (e.g., a vaccine with more than one manufacturer).

Assuring an uninterrupted supply of vaccine is especially challenging during manufacturing production problems. A staged response is used to manage such supply disruptions. If there is more than one manufacturer the disruption can often be managed by increasing the use of another manufacturer's vaccine. If there is only one manufacturer, then the shortage is, by definition, a national shortage. The first step in managing such a shortage is to limit vaccine purchase in the public and private sectors so that excessive inventory is halted. If these measures are unable to address the shortage, the next step is to use CDC's vaccine stockpile to maintain the vaccine supply. If the stockpile is exhausted or is insufficient to manage the projected scope and duration of the shortage, temporary recommendations are often made to reduce vaccine use. For example, during the *Haemophilus influenzae* type b (Hib) vaccine shortage from 2007 to 2009, interim recommendations eliminated the booster dose for children unless they were at high risk such as Native American or Native Alaskans. Once the shortage resolved, routine recommendations were reinstated.[48] Following the resolution of a severe vaccine shortage, additional catch-up vaccination is often needed to ensure adequate protection. The much greater visibility of public sector vaccine with centralized vaccine distribution and VTrckS has greatly enhanced the CDC's ability to manage shortages. Current information on U.S. vaccine shortages and delays is available from the CDC.[49]

Assuring Access to Vaccines

A critically important step in the ensuring vaccine delivery is to make vaccines available with as few barriers to immunization as possible and with a minimum of missed opportunities to vaccinate. Cost of vaccines to the patient is a widely studied and recognized barrier, and one that is amenable to government programs that support the purchase of vaccine for financially vulnerable persons and government laws and regulations that require private insurance coverage for immunization—two solutions to the cost barrier that are cornerstones of the U.S. immunization system.

The 2010 ACA requires private health insurers to cover CDC/ACIP-recommended vaccines (category A and category B) on the pediatric and adult immunization schedules without cost sharing (i.e., first dollar coverage) as a benefit for persons of all ages when administered by a provider in the person's health plan network. Vaccines are required to be covered within 1 year of the publication of a new ACIP vaccine recommendation in the *MMWR*. Although health plans not covering immunization before the ACA are exempt from the immunization requirement, their exemption (grandfathered status) disappears if the plan makes any changes in coverage. Before ACA, the general practice of the health insurance industry had been to cover vaccines; 11% of young children and 20% of teens had commercial insurance that did not cover vaccines (underinsured).[50,51] The net effect of the ACA has been a decline in the number of persons with private health insurance that does not cover immunizations and a reduction in the number of persons without health insurance.

The VFC program has been critical in assuring access to vaccine for children by providing financially vulnerable children with government-purchased vaccine up to a child's 19th birthday. The VFC program is an entitlement to eligible children that has mandatory federal funding of ACIP-recommended vaccines. Four groups of children are entitled to VFC vaccine: children who are 18 years of age or younger who are either (a) eligible for Medicaid health insurance (a federal–state means-tested health insurance program), (b) have no health insurance, (c) Native American or Alaska Native children, or (d) not insured for recommended vaccines and are vaccinated at a Federally Qualified Health Center or Rural Health Clinic. The combination of VFC and the ACA's requirement that private health insurance cover ACIP-recommended vaccines ensures that all U.S. children have access to vaccine without out-of-pocket costs for the vaccine.

The VFC program has important characteristics that make it effective at assuring access to vaccine with a minimum of missed vaccination opportunities. First and foremost, VFC is an entitlement with mandatory federal funding that is not subject to the annual appropriations process. State and local VFC immunization programs determine the size of the VFC-eligible population in their jurisdiction, and the CDC uses this information to determine the vaccine purchase funding needed to meet the vaccination needs of all VFC-entitled children.

Second, the ACIP is the sole authority for the list of vaccines that are included in the VFC program. The ACIP indicates to the CDC which vaccines are included by passing a VFC resolution that specifies the number of doses and VFC age groups entitled to VFC vaccine. Unlike ACIP's vaccine recommendations, VFC resolutions are not subject to approval by the CDC director and become effective immediately after a passing vote. For a new vaccine to become available to VFC children, the CDC negotiates a contract with the manufacturers of the vaccine.

Enrolled VFC providers establish eligibility to the program at the point of service by asking parents whether their child meets any of the four eligibility categories. By VFC statute, independent verification is not required. Providers are required to indicate eligibility status in the child's medical record. Having point-of-service eligibility determination reduces missed immunization opportunities because parents are not required to sign their children up for VFC before visiting their child's healthcare provider. This provision is intended to prevent missed opportunities seen in the measles resurgence of 1989 to 1991 where mandatory referrals to public health department clinics were required.[26] VFC providers order and receive their vaccine without charge. The VFC program pays for the vaccine and its distribution to VFC providers, in contrast to the reimbursement model for private insurance where the provider has to pay for vaccine upfront at financial risk.[52]

The CDC also administers the Section 317 program to state and local immunization programs. Although smaller than VFC in total funding, immunization program grantees can use Section 317 vaccine to vaccinate populations that are not captured by private insurance or the VFC program (i.e., uninsured adults) and to vaccinate rapidly in response to a vaccine-preventable disease outbreak. The ACA authorized Section 317 permanently and also added the authority to allow states to purchase vaccine for adults using the CDC's vaccine contracts.

State and local immunization programs are responsible for enrolling providers into VFC. States also enroll non-VFC providers who can receive state or locally purchased vaccine or Section 317–purchased vaccine for the state/local priority populations. These networks of providers are vital to the success of government vaccine programs. The majority of VFC providers are private clinicians who use a combination of VFC vaccine and privately purchased vaccine to immunize children in their practices. VFC has more than 40,000 providers enrolled, and three quarters of these providers are private

providers. In total, VFC providers vaccinate approximately 90% of U.S. children.[53,54]

Maintaining the capacity and quality of their networks of providers is also a responsibility of state and local immunization programs. Federal funding is made available through VFC and Section 317 so that state and local programs are able to enroll new providers and conduct quality improvement activities among existing providers to help the providers practice in accordance with the standards of immunization practices. Section 317 grant funds are also available to provide direct service delivery in public health department clinics.

The community of immunization providers is large and growing in diversity. Many state and local immunization programs are expanding access to vaccines through pharmacists and community vaccinators who can vaccinate in schools and other settings.[55,56]

The governmental program role in assuring access to vaccines for adults has been more limited than the governmental role for assuring access to vaccines for children. Medicare Part B covers pneumococcal and influenza vaccination services for all persons 65 years of age or older enrolled in Medicare Part B (>95% of the older-than-65 population) and hepatitis B vaccine for people undergoing dialysis of any age. State Medicaid programs, supported by federal contributions, may cover recommended vaccines for younger adults enrolled in Medicaid, but there is not a requirement that Medicaid cover vaccines for adults. In 2005, Medicare part D was enacted to cover prescription drugs, including vaccines other than those included in Part B. Thus, all vaccines recommended for the Medicare population are covered by Medicare Part B or Medicare Part D, for persons who pay to participate. Provider billing for vaccines under Medicare Part D is more complicated than under Medicare Part B. The Centers for Medicare & Medicaid Services provides a comparison between Part B and Part D coverage for vaccines.[57]

The clinical work of vaccinating is supported with a fee-for-service payment mechanism in the private and public sectors. Private insurance and Medicaid pay vaccine administration fees with Medicaid paying the administration fee for Medicaid-enrolled VFC children and adults; reimbursement varies from state to state. VFC providers are allowed to charge parents of non-Medicaid VFC children a fee for the administration of VFC vaccine, although the VFC statute prohibits VFC providers from denying VFC vaccine to an eligible child solely because the parent cannot pay the administration fee. The importance of the vaccine administration fee is apparent from its prominence in the NVAC's vaccine financing recommendations. The NVAC made five recommendations to improve the vaccine administration fee, including that "the American Medical Association Relative Value Scale Update Committee should review its relative value unit coding to ensure that it reflects accurately the nonvaccine costs of vaccination, including the potential costs and savings with the use of combination vaccines."[29]

Control and Prevent Infectious Diseases

The desired outcome of immunization is reduction, elimination, or eradication of disease or disability and prevention or control of outbreaks and their associated disruption. The goals of an immunization program determine how intensively a vaccine-preventable disease is monitored (Table 73.2). When programs aim to achieve or sustain disease elimination, intensive efforts are needed surrounding each suspected case. Control programs may also require rapid identification and reporting of cases so that interventions can be offered promptly to reduce complications or limit transmission. Recognition of disease outbreaks is important to limit their scope. Outbreaks may also serve as sentinel events because they may provide the first indication of important program deficiencies or unanticipated limitations in vaccine performance. While some vaccine-preventable diseases manifest as relatively distinct clinical syndromes (e.g., paralytic poliomyelitis, measles, mumps, and congenital rubella syndrome), others may require laboratory testing to differentiate the illness from non–vaccine-preventable causes of the same syndrome (e.g., gastroenteritis or pneumonia). Vaccine-preventable causes of pneumonia (e.g., *Streptococcus pneumoniae* and *Haemophilus influenzae*) are difficult to diagnose even when optimal laboratory testing is available. Monitoring trends in the epidemiology of these diseases relies on targeted tracking of more specific manifestations of the disease, such as bacteremia and meningitis.

Reports of the occurrence of vaccine-preventable diseases have been the means of evaluating the impact of most of the immunization disease programs.[58] Information is obtained through state health departments, which have authority to mandate reporting of selected diseases to the state level by physicians and other healthcare providers.[59] The Council of State and Territorial Epidemiologists, in consultation with the CDC, establishes the list of nationally notifiable diseases. In the United States, most vaccine-preventable diseases of childhood are reported to the National Notifiable Diseases Surveillance System.

For all nationally notifiable diseases, data collected weekly include date of report and locating information (e.g., state and, when appropriate, county). For many of the vaccine-preventable diseases, supplemental information (e.g., date of onset, age, sex, race/ethnicity, vaccination status, whether the case was confirmed by a laboratory, and disease complications) is forwarded to the CDC. Reports by state of cases notified that week and the cumulative total for the year of many of the vaccine-preventable diseases are published in the *MMWR*. Case definitions have been established with the Council of State and Territorial Epidemiologists that detail criteria for confirmation of a given illness as a case of a vaccine-preventable disease.[60,61]

The National Notifiable Diseases Surveillance System is supplemented by other surveillance systems operated by the CDC. Diphtheria surveillance is enhanced through monitoring requests to the CDC for antitoxin, which the CDC has responsibility for distributing.[62] Efforts to collect data beyond the National Notifiable Diseases Surveillance System are made for several vaccine-preventable diseases. Some of these systems involve laboratory-based methods and others rely on death certificate data or other administrative data systems. Where data are not available nationally, special sentinel surveillance systems in selected states and communities may be established. Laboratory-confirmed, population-based surveillance systems in selected geographic areas are used to monitor causes of invasive bacterial disease, including *H. influenzae* type b, meningococcal disease, and pneumococcal disease.[63] The largest of these is the Active Bacterial Core surveillance within the Emerging Infections Program network, which tracks disease and assesses vaccine impact in parts or all of 10 states.[64]

The Emerging Infections Program has also been used to evaluate the incidence of hospitalization for laboratory-confirmed influenza, as well as vaccine effectiveness; the burden of invasive pneumococcal disease, and more recently the Emerging Infections Program network's HPV Impact has conducted active population-based surveillance of cervical intraepithelial neoplasia grades 2 and 3 and adenocarcinoma in situ, as well as associated HPV types in women 18 years of age and older.[65] The New Vaccine Surveillance Network, including population-based surveillance in three sites—Nashville, Tennessee; Rochester, New York; and Cincinnati,

TABLE 73.2 Objectives, Characteristics, and Limitations of Systems for Monitoring Vaccine-Preventable Diseases in the United States

Public Health Goal	Vaccine-Preventable Diseases	Surveillance Objectives	Key System Characteristics	Challenges
Elimination or eradication	Polio, measles, hepatitis B, rubella	Characterize circulating strains Maintain elimination or eradication (i.e., determine source of cases)	Active case-finding; high sensitivity and specificity; timely collection of specimens; verification of vaccination status of cases	Resource intensive; sustainability
Case management and/or intervention(s)	Diphtheria, meningococcal disease, hepatitis A, hepatitis B, invasive *Haemophilus influenzae* type b	Rapidly identify individual cases Trace contacts for prophylaxis	Population-based passive reporting with timely active follow-up; requirements for rapid local reporting	Speed of reporting to health department not always optimal; contact tracing may be incomplete; follow-up is resource intensive
Outbreak detection and control	Pertussis, influenza, hepatitis A, meningococcal disease, mumps, measles, varicella	Determine extent of outbreak Evaluate pathogen characteristics Verify effectiveness of control measures	Passive reporting (population- or facility-based) with rapid, active follow-up; laboratory confirmation of initial cases to confirm diagnosis; specimen collection	Speed of reporting to health department not always optimal; follow-up is resource intensive; specimens collected from limited number of cases; laboratory tests problematic for some vaccine-preventable diseases, especially in highly vaccinated population
Disease control	All	Monitor trends Identify gaps (pockets of need) Characterize populations at ongoing risk	Specific and consistent case definitions (e.g., laboratory confirmation); population-based (sentinel and/or national); adequate case information (demographics, vaccination status); longitudinal data; specimen collection to characterize strains[a] (sentinel laboratory-based reporting)	Routinely collected data may be of poor quality and incomplete; high-quality epidemiologic data and specimens primarily from sentinel active surveillance sites; high-quality systems do not exist in all jurisdictions—limited information to guide local programs

[a]Access to specimens to characterize infecting strain particularly is important when not all disease-causing serotypes/strains are included in the vaccine (e.g., pneumococcus, rotavirus, human papillomavirus, polio, influenza, meningococcus, *Haemophilus influenzae* type b).

From Schuchat A, Bell B. Monitoring the impact of vaccines postlicensure: new challenges, new opportunities. Expert Rev Vaccines. *2008;7:437–456.*

Ohio—provides data on the burden of influenza and rotavirus disease in children and the impact of various vaccines, and has now been expanded to include a total of seven sites.[66,67] As new vaccines are developed, licensed, and made available for use, surveillance and monitoring are critical to address their impact on communities and populations. New Vaccine Surveillance Network supports broad-based surveillance and research projects for acute gastroenteritis in areas with a population base of at least 500,000. A sentinel counties surveillance system was used for tracking hepatitis B infections, and varicella active surveillance has been conducted in three sites (Antelope Valley, California; Travis County, Texas; and Philadelphia, Pennsylvania).[68,69] Administrative data sources have provided information on varicella, rotavirus, and pneumococcal hospitalizations.[70–72] The National Health and Nutrition Examination Survey provides periodic reports on population immunity to various vaccine-preventable diseases, including antibody levels against pertussis, HPV, and other infectious agents.[73,74] Reporting completeness for each of the surveillance systems varies, and the active systems are substantially more sensitive than the National Notifiable Diseases Surveillance System. Completeness of reporting varies by source of report and type of disease, with more complete reporting from hospitals and laboratories than from physicians for diseases such as *H. influenzae* type b.[75]

Surveillance data are analyzed to determine whether reductions of disease occur with increasing vaccine coverage; greater-than-expected reductions and changes in incidence beyond the target age groups may signal important herd effects.[76,77] Surveillance data are also analyzed to evaluate potential changing epidemiology of disease, such as a shift to a predominance of adult cases where there had been a childhood focus or the emergence of disease caused by strains of the pathogen that are not targeted by the vaccine.[78] Surveillance can also determine whether cases are a result of failure of vaccine or of failure to vaccinate.[79,80]

Vaccine Effectiveness

When a substantial proportion of cases of vaccine-preventable disease occurs in persons with a history of vaccination, an investigation is often undertaken to determine whether the rates of vaccine failure are within expected ranges. *Vaccine effectiveness* is the term used for these observational studies, to contrast with *vaccine efficacy*, which is usually measured prospectively in randomized, double-blind, placebo-controlled

trials. Several methods are used to evaluate vaccine effectiveness in the postlicensure setting, including cohort, case-control, secondary attack rates in families, and other techniques.[76,81] Vaccine effectiveness is calculated as where ARV is the attack rate in vaccinated persons and ARU is the attack rate in unvaccinated persons. When case-control studies are used, the odds ratio is used to approximate the relative risk of disease in the vaccinated compared with the unvaccinated population (ARV/ARU).

Of particular note has been a relatively simple screening technique to help determine whether a more rigorous investigation of vaccine effectiveness is warranted. The screening analysis requires data on the vaccination status of cases and the vaccination status of the populations from which the cases are drawn. These data are often available from existing population-based estimates from surveys and other methods. Data must always be broken down into dichotomous variables, with unvaccinated persons compared with vaccinated individuals. For example, for a vaccine recommended in three doses, the population of cases vaccinated must be calculated excluding persons who received only one or two doses. Similar adjustments must be made for coverage assessment. Fig. 73.4 allows approximation of vaccine effectiveness using a nomogram.

Public health investigation of vaccine effectiveness can be facilitated in areas where comprehensive immunization information systems exist. One study of pentavalent rotavirus vaccine reported a vaccine effectiveness of 82% using data from an immunization information system (IIS) compared with 82% to 88% using data from providers.[82] Vaccine effectiveness studies may use population-based control subjects obtained through telephone surveys or household visits, as well as control subjects obtained through inpatient or outpatient healthcare facilities who have conditions different from the vaccine-preventable disease under investigation. For example, influenza vaccine effectiveness studies often use control subjects who have sought medical care for respiratory illness but have laboratory tests negative for influenza virus.[83] When vaccine effectiveness is within expected limits but disease transmission persists, new immunization strategies may be considered. For example, investigations showed that measles transmission could persist despite a vaccine effectiveness of greater than 90% and high levels of coverage with one dose of vaccine.[84] This information was critical in the decision to universally recommend a second dose of measles vaccine. When vaccine effectiveness is lower than expected, investigations also are triggered into potential causes such as failure to maintain vaccine at the proper temperature (i.e., deficiencies in the cold chain).

When most cases occur in unvaccinated persons, investigations can identify geographic and demographic characteristics to help guide future vaccination efforts. For example, a resurgence of measles in the United States during 1989 to 1991 was found to be caused by a failure to vaccinate young preschool children and led to a major national initiative to improve coverage.[26,32] In contrast, relatively small outbreaks of measles in 2008 and 2011 were focused in communities where personal beliefs led parents to exempt their children from vaccination required for school entry.[85,86] In 2013 and 2014, several measles outbreaks were linked to local religious and cultural communities where immunization rates were very low.[87–89]

Conduct Surveillance for Immunization Coverage

The U.S. immunization program uses a set of coverage surveys to monitor implementation of CDC/ACIP vaccination

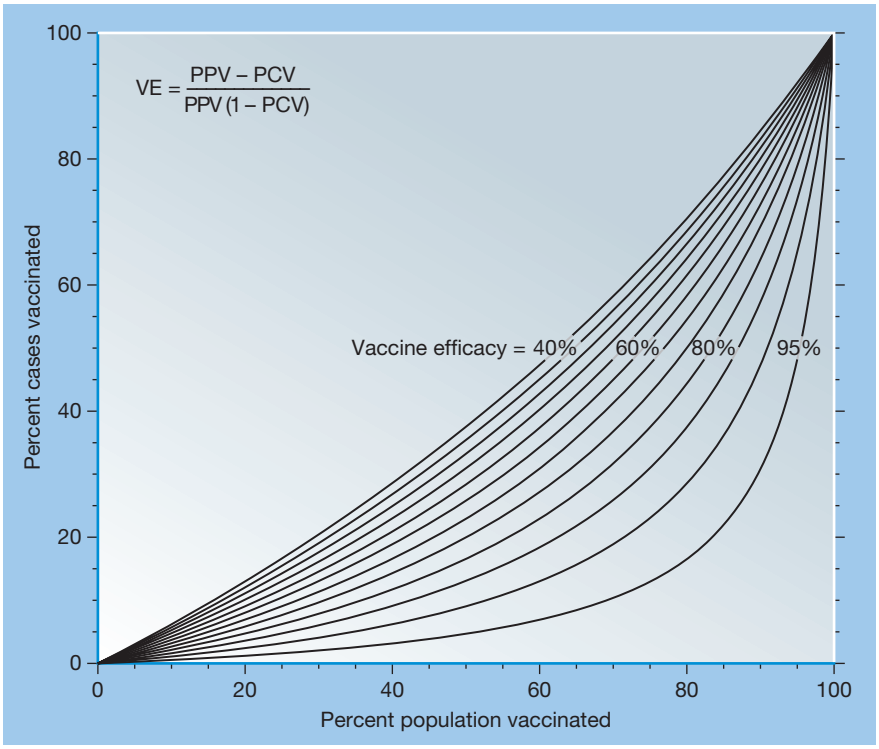


Figure 73.4. Percentage of cases vaccinated (PCV) per percentage of population vaccinated (PPV) for 12 values of vaccine efficacy (VE), varying from 40% through 95% at 5-percentage-point increments. Embedded formula uses proportions rather than percentages. *(From Orenstein WA, Bernier RH, Dondero TJ, et al. Field evaluation of vaccine efficacy.* Bull World Health Organ. *1985;63:1055–1068.)*

recommendations. These surveys provide an indirect measure of the population protection from vaccine-preventable diseases while assessing performance of the federal, state, and urban area immunization programs.

Coverage among young children has been comprehensively measured for the longest period. Measurement of childhood coverage has been measured since 1960, except for 1985 to 1991.[90,91] Beginning in 1994, immunization of preschool children has been assessed through the NIS, which consists of random-sample telephone surveys measuring immunization coverage of 19- to 35-month-old children (median age: 27 months). In 2006, the NIS was expanded to the NIS-teen to include children 13- through 17-years-old to assess coverage of vaccines recommended for 11- and 12-year-olds. Descriptions of this family of national immunization surveys is available at the CDC website.[92] The website provides information for the public and for healthcare professionals on the purpose, methods, instruments, and results of the surveys.[92]

The NIS and NIS-teen provide national estimates of coverage, usually with 95% confidence intervals of less than 3 percentage points, and comparable, statistically valid estimates for each of the 50 states, the six urban area Section 317 awardees, and the U.S. Virgin Islands, Guam, and Puerto Rico.[93,94] Although response rates vary by survey area, the methods are identical, allowing for comparison among the survey areas. In addition to the jurisdictions sampled annually, states are allowed to purchase from the CDC additional coverage surveys for geographic areas of interest within their jurisdictions.

Since parental recall of vaccination is known to have poor concordance with provider-verified vaccination status,[95] the NIS and NIS-teen surveys only report verified vaccinations by the immunization provider who administered the vaccine to the sampled child. Parents are interviewed about their demographics and access to vaccines, and they are asked permission to contact their child's providers to report the child's immunization history. Survey results are adjusted for telephone nonresponse. Because of the increasing use of cell phones and a concomitant decline in landline-only households, a cell phone sample frame was added in 2011 for adjustment of telephone access.[96] The NIS and NIS-teen results are reported annually in the CDC's *MMWR* and on the CDC's website. The CDC makes NIS and NIS-teen public-use data files available to researchers and others who request access to deidentified raw data. The CDC evaluates its immunization surveys for evidence of bias or nonrepresentativeness, and conducts research on methods to improve representativeness and value of the surveys.

The CDC estimates annual influenza vaccination coverage for the United States by using data from several nationally representative surveys: the Behavioral Risk Factor Surveillance System, the National Health Interview Survey, the NIS-flu survey, and Internet panel surveys of adults, healthcare personnel, and pregnant women. The CDC also provides ongoing vaccine effectiveness assessment during and after each influenza season through the Influenza vaccine effectiveness unit. The CDC's website provides reports, interactive figures, and data tables for these influenza coverage surveys at http://www.cdc.gov/flu/fluvaxview/index.htm. Coverage among adults for vaccines other than influenza is assessed using Behavioral Risk Factor Surveillance System and National Health Interview Survey. Vaccines include pneumococcal, HPV, zoster, and Tdap.[97]

Surveillance for vaccination coverage among new kindergarten students is conducted by the states and reported annually to the CDC. These data are published on the CDC's SchoolVaxView website and in the MMWR.[98,99] Managed care health plan performance on immunization is measured annually by the National Committee on Quality Assurance using the Health Plan Employer Data and Information Set (HEDIS). The HEDIS currently estimates immunization coverage for 2-year-old children, 13-year-old adolescents, and 50-year-old and older adults. Separate measures are made for commercial plans and Medicaid or Medicare plans. Results of HEDIS measures are published annually in a report called "The State of Healthcare Quality."[100]

Techniques for measuring immunization coverage at the provider level were developed during the 1990s and have been integrated into the VFC program since 2000. Additionally, immunization programs can use their IISs for determining provider-level coverage.[101] IISs are also an important way for immunization programs to assess coverage at the local level and target areas of undervaccination for outreach.

Sustain and Improve Immunization Coverage Levels

Sustaining and improving vaccination coverage levels requires a system that actively promotes vaccination of individuals targeted by recommendations. Simply making vaccines available will not result in coverage levels high enough to achieve immunization program goals and objectives. This section describes barriers to vaccination, key program activities to promote vaccination, and evidence-based strategies to improve coverage.

Barriers to Vaccination

In the United States, barriers to vaccination vary by vaccine and target population. For example, providers of vaccines recommended only for adults with behavioral or health risk factors must identify those for whom the vaccine is recommended; implementation of new vaccines must overcome the barrier of unfamiliarity; and seasonal vaccination against influenza must overcome time constraints associated with an annual seasonal vaccination schedule. However, common barriers to vaccination exist. These include lack of a provider recommendation to be vaccinated; missed opportunities to immunize during visits to immunization providers; lack of affordable access to vaccines; lack of knowledge that a vaccine is due or overdue to be given; lack of confidence in the safety or effectiveness of vaccines leading to hesitancy to vaccinate; and lack of belief in the necessity of vaccination.

For advice about vaccines and immunization, parents trust the advice of their children's healthcare providers more than any other source of information.[102] Thus, lack of a provider's recommendation to vaccinate can be a significant barrier to vaccine receipt as seen with HPV vaccination of adolescents.[103] Provider endorsement increases vaccine acceptance rates and lack of endorsement decreases acceptance.[104] Recommendations from providers serve not only as professional advice, but they also serve as reminders that a vaccination is due or overdue, thus helping parents and patients navigate a complicated vaccination schedule.

During a visit to a healthcare provider, failure to give all recommended vaccines constitutes a missed opportunity to immunize. Epidemiological investigation shows that missed opportunity to immunize was a prominent root cause of the 1989–91 measles resurgence in the United States.[105] Missed opportunities to immunize can be caused by use of false contraindications to vaccination, for example not administering influenza vaccine out of concern for mild illness at the time of vaccination; by deferring some vaccines to decrease the number of injections at a single visit; or by referring to another provider such as the public health clinics out of concern for

the costs to vaccinate. Supporting evidence that missed opportunities to immunize constitute barriers to achieving coverage goals is found in studies showing that if all opportunities were taken to vaccinate, coverage would improve. Fu and colleagues showed that 45% of undervaccinated children would have been up-to-date on their infant vaccinations had all age-appropriate immunization opportunities been taken,[105] and Stokley and colleagues showed that more than 90% of adolescent females could have been up-to-date with HPV vaccine had all vaccination opportunities been taken.[106]

Out-of-pocket costs for vaccination are a well-known barrier to vaccination. Indeed, many of the missed opportunities to immunize during the 1989–91 measles resurgence were caused by referral from a private provider to a free vaccine provider because of cost considerations, a finding that, in part, led to the creation of the VFC program. Currently, in the United States, government support and private health insurance largely cover the costs of vaccination of most children up to 18 years of age, and a combination of private and government insurance covers vaccination costs of adults older than 65 years of age and younger adults with health insurance coverage. Uninsured adults between 19 and 64 years of age comprise the largest group of individuals lacking coverage for vaccination services. Supportive evidence that out-of-pocket costs constitute a barrier to vaccination comes from studies demonstrating that provision of affordable access to vaccines increases coverage. In 2007, the Community Preventive Services Task Force (TFCPS) reviewed studies on the impact of reducing out-of-pocket costs on vaccination coverage levels. The TFCPS found that in 11 studies published between 1997 and 2009, the median increase in vaccination coverage by reducing cost barriers was 22 percentage points.[107]

Vaccination recommendations are updated at least annually as new vaccines are recommended or previous vaccine recommendations are changed with new evidence. The complexity and changing nature of the routine immunization schedule makes it unrealistic to assume that parents or patients track their child's or their own vaccination status. Supportive evidence that parents need reminders when vaccinations are due or past due comes from intervention studies showing the positive impact of reminder/recall systems on vaccine uptake. The TFCPS reviewed 62 qualified studies of reminder/recall systems that showed median increases in vaccination coverage of 12 percentage points among studies conducted in the 1980s and 1990s and 6 percentage points in studies conducted after 1997.[108] The TFCPS also reviewed evidence for impact of reminder systems for targeted adult vaccines. In nine studies, reminder systems increased coverage by a median of 18 percentage points.[109]

Parent or patient concern about the safety, effectiveness, or need for a vaccine can lead to hesitancy or outright refusal of vaccination. Vaccine hesitancy is a global phenomenon that has been studied by a working group of the World Health Organization's Strategic Advisory Group of Experts (SAGE) on immunization. In the definition of the World Health Organization's Strategic Advisory Group of Experts, vaccine hesitancy refers to "delay in acceptance or refusal of vaccination despite availability of vaccination services."[110,111]

In the United States, vaccine refusal among parents of young children is monitored indirectly through the NIS by assessing the number of young children with zero doses of vaccine, a figure that has remained below 1% nationwide since 2006. Evidence that vaccine hesitancy is a problem in the United States can be seen in surveys of parents and studies on exemptors from school vaccination requirements.[112] In a nationally representative survey of parents of children younger than 18 years of age conducted in 2009, Freed and colleagues found that more than half of parents had fears of serious adverse events from vaccines and that 12% of parents had refused to have their children vaccinated with at least one vaccine.[113] Objective measures of vaccine hesitancy show a relation between vaccine hesitancy and undervaccination of young children and adolescents.[114,115]

A 2015 NVAC report discusses the importance of vaccine confidence to maintain high immunization coverage levels in the United States. In this report, vaccine confidence refers to the trust that parents or healthcare providers have (a) in the recommended immunizations, (b) the provider(s) who administers vaccines, and (c) the process that leads to vaccine licensure and the recommended vaccination schedule. This report outlines current measures of vaccine confidence and recommends healthcare providers, immunization programs, and those involved in promoting recommended vaccinations actively reinforce that vaccination according to the ACIP recommended schedule is the social norm and not the exception.[116]

Key Program Activities

Reporting and Monitoring Adverse Events Following Vaccination. It is important to ensure that the vaccine recipient (or parent or guardian) is adequately aware of the risks and the benefits of vaccination and that the recipient has a record of all immunizations received. The National Childhood Vaccine Injury Act of 1986 and subsequent changes (section XXI of the Public Health Service Act) require that all vaccine providers formally notify patients and parents or guardians of the risks and benefits of vaccines covered by the National Childhood Vaccine Injury Act. Formal notification is accomplished through the use of standardized vaccine information statements.[117] Printable versions of these forms are available for downloading at the CDC website.[118]

The National Childhood Vaccine Injury Act also established a no-fault compensation mechanism for persons with injuries from the vaccines covered by the act. Persons wanting further information about this program should contact the National Childhood Vaccine Injury Compensation Program at 800–338–2382 or at the Program's website (see Chapter 83).[119] The National Childhood Vaccine Injury Act also requires providers to note in the patient's permanent medical record the date the vaccine was administered, the vaccine manufacturer, the vaccine lot number, and the name, address, and title of the person administering the vaccines, in addition to noting the provision of vaccine information materials. Finally, the Act requires that providers report selected adverse events occurring after vaccination and events that would contraindicate further doses of vaccines to the Vaccine Adverse Event Reporting System.[120] Providers are encouraged to report all serious adverse events following all vaccines, regardless of whether they believe that a vaccine caused the event. The Vaccine Adverse Event Reporting System forms can be obtained by calling 800–822–7967 or through the Internet.[121] (See Chapter 82 for further discussion of vaccine safety.)

Operating Immunization Information Systems. IISs have the potential to strengthen the system and raise and maintain immunization levels.[101] The IISs are confidential, computerized information systems that contain information about immunizations provided to individuals. The IISs are capable of monitoring the immunization status of individuals and population groups, generating reminder/recall notices, and generating official immunization records.[122] The Healthy People 2020 objectives call for 95% of children younger than 6 years of age to be enrolled in a fully operational population-based IIS.

Registries have demonstrated usefulness in sending reminder/recall notices, increasing coverage, monitoring

implementation of policy changes, generating official immunization records, assessing immunization levels for healthcare quality indices, reducing missed opportunities, preventing unnecessary immunization, vaccine inventory management, and public sector vaccine ordering. They have also been used to identify and recall children who did not receive vaccines during temporary, national shortages and to identify children in need of vaccination when new vaccines are introduced. The TFCPS recommends the use of IISs based on strong evidence of their effectiveness.

In 2015, all 50 states have active, functioning IISs. As of December 31, 2012, results from an IIS annual survey indicate that 86% (19.5 million) of U.S. children younger than 6 years of age had two or more vaccinations recorded in an IIS, and 25% (57.8 million) of U.S. adults had one or more vaccinations recorded in an IIS.[123] An important functional component of an IIS is that it receives information from an electronic health record. The percentage of IISs meeting Functional Standards 7 (i.e., meeting basic international standards for exchange of clinical data between systems) increased from 58% in 2011 to 77% in 2012, and 37% of IISs met more advanced functionality.[123] Thus, full interoperability between electronic health record systems and IISs is closer to reality. Another critical function of an IIS to improve accuracy of records and to reduce missed opportunities is for data to be exchanged between jurisdictions. A 2015 NVAC report promotes ongoing efforts to address the technical and legal barriers to cross-jurisdictional IIS data exchange.[117]

In 2012, the CDC launched a strategic plan to achieve the vision for the future of immunization information management that real time, consolidated immunization data and services for all ages would be available for authorized clinical, administrative, and public health users, and consumers, anytime and anywhere.[124] Table 73.3 summarizes the goals and challenges facing IISs. Strategic investments are being made at the federal level to support state and local IISs in meeting these challenges as a community.

Enforcing Laws and Regulations for Immunization. Laws requiring immunization in the United States date from the early 19th century.[125–127] Massachusetts enacted a law in 1809 that required smallpox vaccination of the general population, and other jurisdictions followed during the course of the century. In 1905, the Supreme Court affirmed the right of states to pass and enforce compulsory immunization statutes and in 1922 the Supreme Court also affirmed laws requiring vaccination before school entry. School entry laws were variably enforced, and the vaccines covered varied considerably. After measles vaccine was introduced in the mid-1960s, school entry requirements for measles vaccination became common because measles was a disease primarily of school-age children. It became apparent that states without laws were

TABLE 73.3 Immunization Information Systems Strategic Plan Focus Areas and Challenges, Centers for Disease Control and Prevention, 2012[a]

Focus Area	Long-Term Goal	Challenge
National Leadership	There is a national immunization and immunization information management program vision, strategy, policy and metrics.	Policies and standards to support immunization management are not coordinated nationwide across all immunization stakeholders. There is no overriding nationwide consent policy for interstate data exchange. There is no nationwide policy for interstate data exchange.
Sustainability	The national immunization information management program has sufficient funding and permanent informatics, epidemiology, economics, computer science, and information technology resources providing immunization-centric analytical services.	There is no IIS line item in VFC or section 317 funding programs; state-level support of IIS is variable and although CDC-provided Prevention and Public Health Fund awards have helped, these funds are short in duration and cannot be used to support ongoing operations to maintain the IIS technology and appropriately skilled staff to support it.
Provider Services	Providers transmit immunization event data automatically and receive consolidated patient history and clinical decision support within the same application.	Some IIS integration standards for providers and EHR-S vendors are applied in a variable way across jurisdictions due to local statutes and business rules. Immunization-centric data, functionality, usability, and interoperability standards are applied in a variable way for immunization data collection at the point of care.
Public Health Services	IIS collect and display real-time, accurate immunization coverage analytics for all ages and vaccine inventory status for the jurisdiction.	There is a lack of informatics, epidemiology, economics, computer science, and information technology knowledge, skills, and roles within some sectors of the immunization information management community. Some IIS functions are redundant while others are variable across jurisdictions due to local statutes and business rules. Some IIS interoperability standards for immunization programs and IIS vendors are implemented in a variable way across jurisdictions because of local statutes and business rules. Methods and standards for analyzing IIS data (e.g., developing coverage rates) vary from IIS to IIS, resulting in potential misinterpretation by public health officials and scientists.
Interoperability	Data exchange among immunization information management systems is automatic and transparent regardless of location.	Challenges remain to ensure immunization data in IIS are complete, accurate, and acquired in a timely manner.

[a]Available at https://www.cdc.gov/vaccines/programs/iis/strategic.html

CDC, Centers for Disease Control and Prevention; EHR-S, electronic health record systems; IIS, Immunization Information System; VFC, Vaccines for Children.

experiencing a higher incidence of measles than those with laws and, with the Immunization Initiative of 1977, considerable emphasis was placed on enactment and enforcement of school entry laws.

Laws requiring immunization before entry in school or daycare are the safety net for the U.S. immunization program. All 50 states and the District of Columbia have laws in effect, although the precise vaccines, doses, and schedules vary. The impact of these laws on the decreased incidence of measles and mumps in states with laws versus states without laws was apparent. A major resurgence of mumps during 1986 was shown to occur almost exclusively in states without comprehensive laws requiring mumps vaccination.[128] A comprehensive review of the impact of school laws was done by Orenstein and Hinman.[126]

Although the vast majority of parents comply with school immunization requirements, a substantial—and possibly growing—number of parents exempt their children from school laws.[112] All states allow medical exemptions from school immunization requirements, and by July 2016, all but three states will allow religious or philosophical exemptions from school immunization requirements. The process of obtaining an exemption from school immunization requirements varies widely by state. Rota and colleagues showed that the difficulty of the exemption process was inversely related to the percentage of children obtaining exemptions, indicating that many exemptions are likely to be exemptions of convenience rather than deeply held convictions.[129]

Exemptions from school immunization requirements have consequences. Exemption was associated with a 35-fold higher risk of measles in a 1999 study.[130] Feikin and colleagues showed that exemption was associated with a sixfold higher risk of pertussis.[131] Omer and colleagues showed that the ease of obtaining exemptions was associated with a nearly threefold increase in pertussis outbreaks in Michigan, primarily owing to clustering of exemptors.[132] Parents who exempt their children from school immunization requirements hold measurably different beliefs about vaccines than parents complying with school immunization requirements. For example, Salmon and colleagues have shown that exempting parents are much more likely to believe that vaccines weaken the immune system and that immunization does more harm than good.[133] A recent review on exemption from school immunization laws summarizes much of the current literature.[134]

Using the Standards of Immunization Practices. In 1993, the NVAC recommended a set of 18 standards to improve the immunization performance of pediatric providers, and in 2002, these standards were updated to incorporate advances in knowledge of best immunization practices (Table 73.4).[27] Many of the standards specifically address improving immunization coverage in children, including removing barriers to immunization, taking advantage of all opportunities to vaccinate, simultaneously vaccinating and using only valid contraindications, establishing tracking systems to identify children who are underimmunized and to facilitate taking corrective actions, and semiannually auditing patients served by a given clinic or practice to evaluate performance and to determine whether improvements are needed. The Infectious Diseases Society of America has established 46 implementation standards that apply to adults, adolescents, and children.[122] These 46 standards are graded according to the quality of their supportive evidence.

In 2013, NVAC released revised Standards for Adult Immunization Practice, recognizing the importance of the healthcare provider recommendation for patients to receive needed adult vaccines, the current low vaccination rates among U.S. adults, and the changing environment within which adult vaccines are now given.[135] These standards are a call for all healthcare professionals to take the following steps to ensure that adult patients are fully immunized and have maximum protection from serious diseases:

- ASSESS immunization status of all patients in every clinical encounter.
- SHARE a strong recommendation for vaccines that patients need.
- ADMINISTER needed vaccines or REFER to a provider who can immunize.
- DOCUMENT vaccines administered or received by your patients.

The National Adult and Influenza Immunization Summit (NAIIS) is a partnership of more than 130 private and public

TABLE 73.4 Standards for Pediatric Immunization Practices

1. Immunization services are readily available.
2. There are no barriers or unnecessary prerequisites to the receipt of vaccines.
3. Immunization services are available free or for a minimal fee.
4. Providers utilize all clinical encounters to screen and, when indicated, vaccinate children.
5. Providers educate parents and guardians about immunization in general terms.
6. Providers question parents or guardians about contraindications and, before vaccinating a child, inform them in specific terms about the risks and benefits of the vaccinations their child is to receive.
7. Providers follow only true contraindications.
8. Providers administer simultaneously all vaccine doses for which a child is eligible at the time of each visit.
9. Providers use accurate and complete recording procedures.
10. Providers co-schedule immunization appointments in conjunction with appointments for other child health services.
11. Providers report adverse events following vaccination promptly, accurately, and completely.
12. Providers operate a tracking system.
13. Providers adhere to appropriate procedures for vaccine management.
14. Providers conduct semi-annual audits to assess immunization coverage levels and to review immunization records in the patient populations they serve.
15. Providers maintain up-to-date, easily retrievable medical protocols at all locations where vaccines are administered.
16. Providers practice patient-oriented and community-based approaches.
17. Vaccines are administered by properly trained persons.
18. Providers receive ongoing education and training regarding current immunization recommendations.

From Centers for Disease Control and Prevention. Standards for pediatric immunization practices. MMWR. *1993;42(RR-5):1-13.*

organizations that focuses on increasing adult and influenza immunization rates and on increasing implementation of the adult standards.[136] NAIIS has active workgroups on topics such as provider outreach, access and collaboration, patient education, policy and decision makers, and quality and performance measures. NAIIS has an annual meeting to bring together partners to discuss opportunities to improve adult immunization.

Educating and Updating the Immunization Workforce. The U.S. immunization schedule is complicated and changes at least once each year. Temporary changes in the schedule owing to vaccine supply disruptions can happen at any time. Specific vaccines can have safety concerns or issues that require temporary or permanent changes in vaccination practices. One example is the temporary halt in 2010 of the use of one of the licensed rotavirus vaccines because of the presence of porcine circovirus and the recommendation against using one of the licensed influenza vaccines owing to concerns about febrile seizures. This dynamic environment, coupled with the massive number of vaccinations given each year in the United States, require a means to educate and update the immunization workforce.

Continuously updated authoritative material is available at government sites listed later under "Sources of Additional Information." Professional organizations and other immunization education organizations also have continuously updated material, and several of these are listed in that section. Professional organizations, like the American Academy of Pediatrics, offer continuing medical education courses focused on immunization practices. The VFC program provides funding for state and local immunization programs to educate their VFC providers on all aspects of immunization practices, especially those required by law (use of the vaccine information statement and documenting immunizations properly), storage and handling of vaccines, and updates to the VFC program and immunization schedule.

State and local immunization programs communicate directly with their networks of immunization providers. To reduce the need to create multiple versions of immunization materials, the CDC now offers a new Web Content Syndication service that allows partners, including federal public health agencies, state and local public health departments, nonprofit organizations, academic institutions, and commercial organizations to syndicate CDC content directly to their sites. Public health partners can gain direct access to CDC Web content without having to monitor and copy updates, control which content from CDC.gov to use on their site, or integrate CDC content with localized content while keeping visitors on their sites. Information about content syndication is available at the CDC's Public Health Media Library.[137]

Communicating With the Public. Communicating with the public requires an understanding of the questions and concerns of the public and mechanisms to address them. With NVAC, the CDC developed a parental concerns module for the NIS that was administered in 2010. This module asked parents whether they have refused or delayed a vaccination, and, if so, which vaccine and why.[138] Using the NIS as the sample frame has the advantage of being able to link parent responses to immunization status of their child. The CDC also uses other survey and polling mechanisms to learn of parental concerns, and is currently conducting a prospective study looking at changes in vaccine attitudes among the same group of mothers over 5 years.[139]

Official communication with individual parents or guardians about vaccines and vaccine-preventable diseases is required on an individual basis for every vaccination with the vaccine information statement. These two-page information sheets are used by immunization providers to address questions and concerns and to bring important information to the attention of parents, guardians, and vaccinees. Broader communication with the public includes informational Internet sites as listed in this chapter and strategic communication with media channels. CDC launched an Internet site in 2011 dedicated to providing vaccine information to parents.[140]

Evidence-Based Strategies to Improve Coverage

The evidence of what works to raise and sustain immunization coverage levels has been systematically reviewed by the TFCPS[141] and other independent reviewers. The TFCPS is an independent body that reviews the evidence base of community preventive services and makes recommendations for interventions based on the quantity and quality of evidence regarding their effectiveness. The TFCPS has reviewed a number of interventions addressing approaches aimed at enhancing access to vaccination services, increasing community demand for vaccinations, and provider- or system-based interventions and updates these reviews regularly. Table 73.5 summarizes the Task Forces' recommendations.

Supporting Provider-Based Interventions. Physicians and nurses need to ensure that their patients are immunized. However, when queried, they tend to overestimate the coverage of the children they serve. Studies report that physicians overestimated coverage by 10% to more than 40%.[142] The purpose of assessment and feedback is to alert providers to the actual coverage in their patient populations and to help motivate them to improve that coverage. The VFC program funds state and local immunization programs to conduct assessment and feedback on providers enrolled in VFC using the AFIX method. The AFIX method includes four components:

- *A*ssessment of the immunization coverage of the preschool children served by a given clinic.
- *F*eedback of the results to persons in the clinic with the authority to make changes. Such feedback often includes potential problems identified during the review, such as failure to administer vaccines simultaneously.
- *I*ncentives for improved performance. In the public sector, this often involves community recognition, plaques, and/or dinners rather than financial incentives.
- e*X*change of information or comparing the performance of one clinic with that of others to stimulate competition to improve performance.

Assessment and feedback has been shown to increase immunization coverage for children in a provider's office and is a recommendation of the TFCPS. Many immunization programs are also implementing AFIX to provide assessment and feedback to providers on adolescent immunization coverage in their practice. While evidence supporting the impact of AFIX on adolescent coverage is limited, adolescent AFIX is an opportunity to engage providers and encourage a strong recommendation for HPV immunization in their practice.[143]

Many studies show that reminders for immunizations that are due or recall systems for immunization appointments that are missed can significantly improve immunization coverage in a variety of settings. In a health maintenance organization setting, computer-generated letters to families with children who had not received an measles-mumps-rubella (MMR) vaccine by 20 months of age improved coverage by 19 percentage points compared with similar children whose parents did not receive reminders (54% vs 35%), at a cost of US$4.04 per

TABLE 73.5 Effective Strategies to Improve Immunization Coverage

ENHANCING ACCESS TO VACCINATION SERVICES	
Home visits to increase vaccination rates	Recommended—March 2009
Reducing client out-of-pocket costs	Recommended—October 2008
Vaccination programs in schools and organized childcare centers	Recommended—June 2009
Vaccination programs in WIC settings	Recommended—March 2009
INCREASING COMMUNITY DEMAND FOR VACCINATIONS	
Client or family incentive rewards	Recommended—May 2015
Client reminder and recall systems	Recommended—May 2015
Client-held paper immunization records	Insufficient evidence—March 2010
Clinic-based education when used alone	Insufficient evidence—May 2015
Communitywide education when used alone	Insufficient evidence—March 2010
Community-based interventions implemented in combination	Recommended—October 2014
Monetary sanction policies	Insufficient evidence—April 2011
Vaccination requirements for child care, school, and college attendance	Recommended—June 2009
PROVIDER- OR SYSTEM-BASED INTERVENTIONS	
Healthcare system-based interventions implemented in combination[a]	Recommended—October 2014
Immunization information systems	Recommended—July 2010
Provider assessment and feedback	Recommended—February 2008
Provider education when used alone	Insufficient Evidence—May 2015
Provider reminders[b]	Recommended—June 2008
Standing orders	Recommended

[a]Combination refers to the situation in which an intervention is applied in concert with another intervention rather than as a standalone intervention. For example, combining client-based education with reducing out-of-pocket costs for vaccination.
[b]Provider reminders serve as prompts to vaccinate a patient being seen who is in need of vaccination.
From The Guide to Community Preventive Services. Increasing Appropriate Vaccination. Available at: https://www.thecommunityguide.org/topic/vaccination.

additional immunized child.[144] Provider-based recall systems have been shown to be effective at the community level in reducing racial and ethnic disparities in immunization coverage levels.[145] Provider reminders to inform those who administer vaccinations that individual clients are due for specific vaccinations are also effective. Immunization providers can use reminders and recalls in concert with other effective interventions to ensure the best protection possible for their patient population.

TABLE 73.6 Comparison of Prevaccine Era and 2016 Counts of Cases for U.S. Vaccine-Preventable Diseases, Excluding Invasive Meningococcal Disease and Human Papillomavirus–Related Diseases

Disease	20th Century Estimated Annual Cases[a]	2016 Reported Cases[b]	Percent Decrease
Smallpox	29,005	0	100%
Diphtheria	21,053	0	100%
Measles	530,217	69	>99%
Mumps	162,344	5311	97%
Pertussis	200,752	15,737	92%
Polio (paralytic)	16,316	0	100%
Rubella	47,745	5	>99%
Congenital rubella syndrome	152	1	99%
Tetanus	580	33	94%
Haemophilus influenzae	20,000	22[c]	>99%

[a]Data from Rousch SW, Murphy TV. Vaccine-preventable disease table working group. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. *JAMA*. 2007;298(18):2155-2163.
[b]Data from Centers for Disease Control and Prevention. MMWR January 9,2015/63(53);ND-733-ND-746. (MMWR 2014 provisional week 53 data).
[c]*Haemophilus influenzae* type b (Hib) <5 years of age. An additional 12 cases of Hib are estimated to have occurred among the 226 reports of Hi (<5 years of age) with unknown serotype.

IMPACT OF IMMUNIZATION PROGRAMS

Occurrence of Disease

The occurrence of most vaccine-preventable diseases is at or near record low levels. Table 73.6 shows the representative annual levels of disease reported or estimated for selected vaccine-preventable diseases during the 20th century. Most often, these are the years immediately preceding vaccine licensure. Most diseases with mature vaccination programs have been reduced by 95% or more from representative 20th-century morbidity. In March 2000, a group of experts concluded that endemic transmission of measles in the United States had ceased,[146] and in 2003, rubella was declared to be eliminated from the United States.[147] Although measles outbreaks continue to occur around imported cases,[85] the elimination of indigenous transmission has been sustained. Maintaining elimination status is especially important in an era in which measles remains uncontrolled in many countries. Substantial reductions are also evident for several of the diseases for which vaccines have been introduced more recently, such as rotavirus and pneumococcal disease.[148–150] Vaccination in the United States has led to substantial reductions in hospitalizations attributed to pneumococcus, rotavirus, and varicella.[70,151,152]

Immunization Coverage

Preschool Immunization

The United States is currently experiencing record-high or near-record-high vaccination coverage levels among 2-year-old

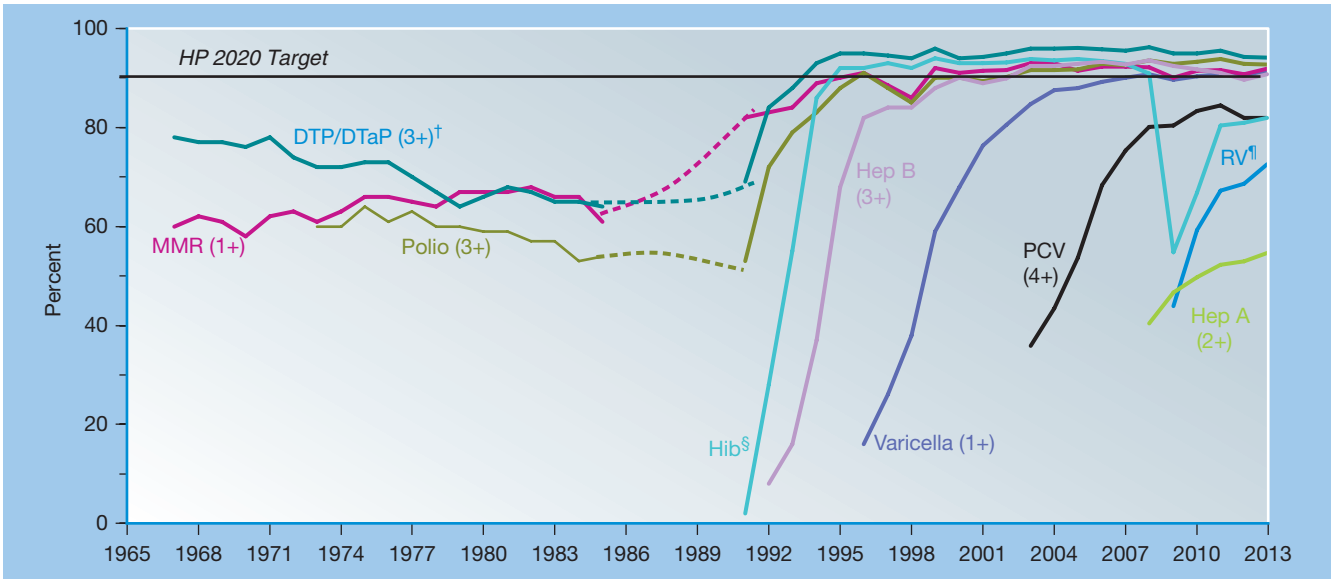


Figure 73.5. Vaccine-specific coverage among children 19 to 35 months of age, National Immunization Survey, 1994 to 2014. The *Healthy People 2020* (HP 2020) target for coverage is 90% for all these vaccines except rotavirus (80%) and HepA (85%). DTP/DTaP, diphtheria, tetanus toxoids, and pertussis vaccine/diphtheria, tetanus toxoids, and acellular pertussis vaccine; HepA, hepatitis A vaccine; HepB, hepatitis B vaccine; Hib, *Haemophilus influenzae* type b vaccine; MMR, measles, mumps, and rubella vaccine; PCV, pneumococcal conjugate vaccine. *(From CDC. For Immunization Managers. Figure Depicting Coverage with Individual Vaccines from the Inception of NIS, 1994 Through 2014. http://www.cdc.gov/vaccines/imz-managers/coverage/nis/child/figures/2014-map.html.)*

children, although there is moderate variation in coverage levels across states (Fig. 73.5). In 2014, the *Healthy People 2020* target of 90% was met for three or more doses of poliovirus vaccine (93.3%), one or more doses of MMR (91.5%), three or more doses of hepatitis B vaccine (91.6%), and one or more doses of varicella vaccine (91.0%). However, the targets were not met for four or more doses of diphtheria, tetanus, and pertussis vaccine (DTaP); the full series of Hib; four or more doses of pneumococcal conjugate vaccine; two or more doses of hepatitis A vaccine; the hepatitis B vaccine birth dose, and the full series of rotavirus vaccine.[1] The NIS monitors the number of young children who are completely unvaccinated—the "zero-dose" children. Since 2006, the number of zero-dose children has remained consistently below 1%. Coverage with vaccines scheduled to be given in the second year of life is lower than for vaccines given during the first year. Most recent immunization coverage tables for children by state, race, ethnicity, and poverty status can be found at the CDC's For Immunization Managers web page.[153]

The United States has made substantial progress in reducing vaccination coverage level disparities by race in the past three decades. Measles disease and measles immunization serve as an example. In the 1970s, the white–nonwhite disparity in MMR vaccine coverage was 18 percentage points, and attack rates for measles among children younger than 5 years of age were four to seven times higher in nonwhites than in whites. During the 1990s, the white–black coverage disparity was reduced to 2 percentage points, and the disease disparity was eliminated. Zhao and Luman analyzed series-complete data and found that during the time from 2000 to 2008, the series-complete coverage levels (four DTaP, three inactivated poliovirus vaccine, one MMR, three hepatitis B, three Hib, and one varicella vaccines) showed relatively little black–white coverage disparities. In 2000, the disparity was approximately 4 percentage points, and by 2008, it was reduced to approximately 2 percentage points.[154] Current disparities by race and ethnicity have remained relatively small, but disparities remain for doses given in the second year of life.

Teens

All U.S. adolescents are recommended to receive HPV, Tdap, and meningococcal conjugate vaccine-4 vaccines at 11 to 12 years of age. Coverage among 13- to 17-year-old teens has steadily climbed for Tdap and meningococcal conjugate vaccine-4 to over the Healthy People 2020 coverage goal of 80%, with 2014 coverage of 87.6% and 79.6% respectively.[2] However, coverage for HPV series initiation and completion continues to be low, with initiation rates of 60% among girls and 41.7% among boys in 2014 (Fig. 73.6). There is wide variation in coverage rates by state for all three vaccines, but disparities by race, ethnicity, and poverty status are small. The most recent coverage estimates for teens are available online at the CDC.[155]

Adult Immunization

Vaccination coverage levels among U.S. adults are substantially lower than coverage for young children.[97] Table 73.7 shows adult coverage for influenza, pneumococcal polysaccharide, and zoster vaccines, all three of which are recommended routinely for age-appropriate adults. Adult vaccination coverage levels reveal a much larger disparity by race and ethnicity than the disparity seen among young children and teens, with coverage for pneumococcal vaccine in adults 65 years of age and older, diphtheria and tetanus toxoids vaccine, and zoster vaccine lower among blacks and Hispanics compared to white non-Hispanics.[156,157] Influenza vaccination coverage varies by year and data from each influenza season can be found at the CDC's FluVaxView interactive website.[158]

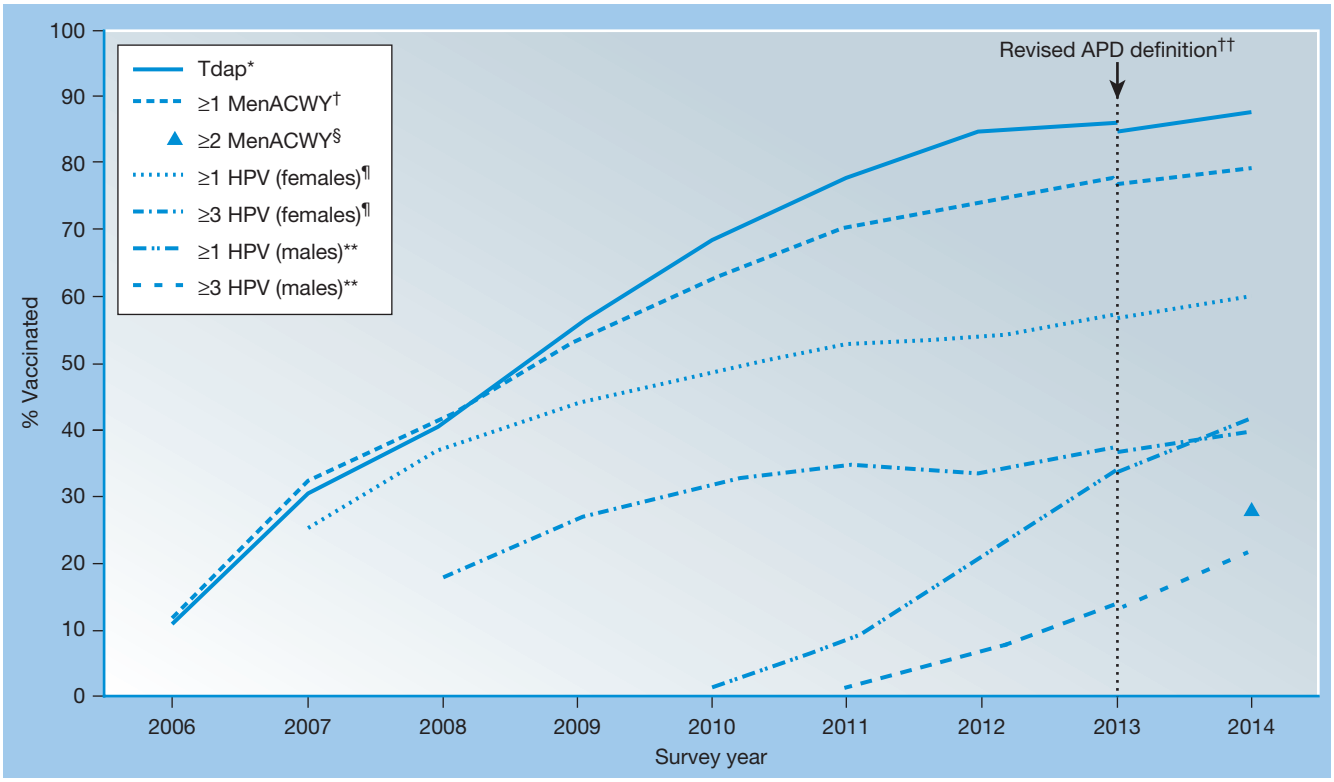


Figure 73.6. Vaccination coverage with selected vaccines and doses among adolescents age 13 to 17 years, by survey year—National Immunization Survey-Teen, United States, 2006 to 2014. HPV, human papillomavirus; MenACWY, quadrivalent meningococcal conjugate vaccine; Tdap, tetanus, diphtheria, and pertussis.

TABLE 73.7 Vaccination Coverage Levels of Adults, 2013

Vaccine and Age Group	National Average	White, Non-Hispanic	Black, Non-Hispanic	Hispanic
Pneumococcal polysaccharide, 19–64 y	21.2%	22.3%	21.2%	17.9%
Pneumococcal polysaccharide, >64 y	59.7%	63.6%	48.7%	39.2%
Td, 19–49 y	62.9%	69.0%	54.1%	52.5%
Td, 50–64 y	64.0%	67.3%	54.4%	55.0%
Td >64 y	56.4%	59.6%	40.3%	45.3%
Tdap, 19–64 y	18.4%	21.6%	13.6%	10.5%
Hepatitis A, 19–49 y	12.3%	12.6%	11.0%	10.6%
Hepatitis B, 19–49 y	32.6%	35.2%	30.5%	23.7%
Herpes zoster, >59 y	24.2%	27.4%	10.7%	9.5%
HPV, females 19–26 y	36.9%	41.7%	30.6%	30.3%

HPV, human papillomavirus; Td, tetanus and diphtheria vaccine.
From Williams WW, Lu PJ, O'Halloran A, et al; Centers for Disease Control and Prevention (CDC). Vaccination coverage among adults, excluding influenza vaccination—United States, 2013. MMWR Morb Mortal Wkly Rep. *2015:64;95–102.*

Impact of the U.S. Program

The immunization schedule, taken as a whole, saves lives, prevents suffering, and saves money for the healthcare system and for society. In 2014, a model was conducted to assess the impact of 20 years of the VFC Program (Table 73.8). This analysis was based on a modeling approach that assessed the economic impact of each of the recommended vaccines individually and combined into the routine program. Among 78.6 million children born during 1994 to 2013, routine childhood immunization estimated that 322 million illnesses (averaging 4.1 illnesses per child) and 21 million hospitalizations (0.27 per child) were prevented over the course of their lifetimes and 732,000 premature deaths from vaccine-preventable illnesses were averted. Vaccination will potentially save $402 billion in direct costs and $1.5 trillion in societal

TABLE 73.8 Estimated Number of Illnesses, Hospitalizations, and Deaths Prevented by Routine Childhood Immunization for Selected Vaccine-Preventable Diseases Among Children Born During the Vaccines for Children Era—United States, 1994–2013

Vaccine-Preventable Disease[a]	Cases Prevented (in Thousands)		
	Illnesses	Hospitalizations	Deaths
Diphtheria	5073	5073	507.3
Tetanus	3	3	0.5
Pertussis	54,406	2697	20.3
Haemophilus influenzae type b	361	334	13.7
Polio	1244	530	14.8
Measles	70,748	8877	57.3
Mumps	42,704	1361	0.2
Rubella	36,540	134	0.3
Congenital rubella syndrome	12	17	1.3
Hepatitis B	4007	623	59.7
Varicella	68,445	176	1.2
Pneumococcus-related diseases[b]	26,578	903	55.0
Rotavirus	11,968	327	0.1
Total	**322,089**	**21,055**	**731.7**

[a]Vaccines were considered as preventing disease for birth cohorts born in all years during 1994–2013 except for the following, which were only in use for part of the 20-year period: varicella, 1996–2013; 7-valent and 13-valent pneumococcal conjugate vaccines, 2001–13; and rotavirus, 2007–13.
[b]Includes invasive pneumococcal disease, otitis media, and pneumonia.

costs because of illnesses prevented in these birth cohorts. After accounting for $107 billion and $121 billion in direct and societal costs of routine childhood immunization, respectively, the net present values (net savings) of routine childhood immunization from the payers' and societal perspectives were US$295 billion and US$1.38 trillion, respectively.[159]

Immunization has consistently ranked as one of the most effective and cost-effective clinical preventive services available.[160]

CONCLUSION

Development and use of vaccines in the United States has had a profound effect on the occurrence of vaccine-preventable diseases. The success represents a blend of public-private and federal, state, and local partnerships. Measles, rubella, and polio remain eliminated as indigenous diseases in the United States, and large declines in other diseases for which routine vaccination is recommended have been seen. Childhood vaccination coverage levels have remained high for many years with small or no disparities by race and ethnicity. The number of children receiving no vaccines has remained less than 1% since 2006.

There are numerous challenges. During the past 20 years, the number of vaccine-preventable diseases of childhood has doubled, from eight to 16. Since 2004, vaccines against influenza, hepatitis A, meningococcal disease, rotavirus, and HPV have been routinely recommended for children and/or adolescents. In addition, an adolescent booster has become available for pertussis, a second dose of varicella vaccine has been recommended, and a new vaccine for zoster is recommended for all adults older than 60 years. Assuring financial barriers to access are removed, sustaining high coverage among children, and attaining high levels of coverage among adolescents and adults will be major challenges for the next several years as the U.S. immunization system works to achieve the Healthy People 2020 immunization objectives.

Sources of Additional Information

There are numerous sources of information about U.S. vaccines, immunization recommendations, and immunization systems, including the following:

- The CDC has a comprehensive website that includes information about ACIP, vaccines, vaccine-preventable diseases, immunization programs, communication and health education materials, and data and statistics about disease and coverage rates: https://www.cdc.gov/vaccines.
- Official package inserts. Manufacturers provide product-specific information with each vaccine; some of these circulars are reproduced in their entirety in the *Physicians' Desk Reference* (a new edition published annually can be obtained at http://www.PDRBookstore.com).
- *Morbidity and Mortality Weekly Report*. This report is published weekly by the CDC. ACIP statements are usually published in separate *Recommendations and Reports* supplements to the weekly publication. Current and back issues are available at https://www.cdc.gov/mmwr.
- *Red Book*: The Report of the Committee on Infectious Diseases of the American Academy of Pediatrics. The full report containing recommendations on all licensed vaccines is usually updated every 2 to 4 years, and updates are provided on the Red Book's Internet site: http://aapredbook.aappublications.org.
- *Health Information for International Travel* (the Yellow Book). This book is published every other year by the CDC as a guide to requirements and recommendations for specific immunizations and health practices for travel to various countries. It can be obtained by telephone at 800–545–2522 or from the publisher at http://www.us.elsevierhealth.com. A comprehensive and frequently updated resource for travel information is available at https://www.cdc.gov/travel. This site includes an online version of the Yellow Book. Advisory notices for international travel are also available at https://www.cdc.gov/travel.
- *Control of Communicable Diseases Manual*. This manual is published by the American Public Health Association at approximately 5-year intervals; the 19th edition (2008) is currently available. The manual contains valuable information concerning infectious diseases, their occurrence worldwide, immunization, diagnostic and therapeutic issues, and up-to-date recommendations on isolation and other control measures for each disease presented. It is available through Internet booksellers.
- State and local health departments, medical schools, and large hospitals. Many of these agencies or institutions provide routine immunizations, immunization cards, and schedules to patients. They may also send out routine reports of disease incidence. Internet sites for state and local immunization programs are available at https://www.cdc.gov/vaccines/partners.htm.
- The TFCPS (http://www.thecommunityguide.org) has published structured reviews of evidence for interventions to improve public health. These reviews cover universally recommended immunizations for children and adults and

targeted vaccination of children and adults at high risk of vaccine-preventable diseases or their complications.

- The CDC National Center for Immunization and Respiratory Diseases (NCIRD) has established toll-free numbers for answering questions from the general public and providers: 800–232–4636. Inquiries can also be addressed to the CDC by e-mail (nipinfo@cdc.gov).
- The Internet serves as a rich source of immunization information for providers, health officials, and the general public. The CDC maintains a list of immunization partner websites at https://www.cdc.gov/vaccines/partners.htm.
- The Department of Health and Human Services provides Internet resources for the public concerning vaccines and influenza at http://www.vaccines.gov and http://www.flu.gov, respectively.

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74 Immunization in Europe

Kari Johansen, Dina Pfeifer, and David Salisbury

OVERVIEW

Europe is composed of a diverse group of countries and the extent of diversity depends on the definition of the boundaries of Europe. The European Union (EU) comprises 28 Member States of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom. The EU together with the three Member States of the European Economic Area (EEA) (Iceland, Lichtenstein, and Norway), has a population of approximately 500 million people (birth cohort ≈5 million and population under 15 years ≈75 million). The World Health Organization (WHO) European Region has a population of approximately 900 million (birth cohort ≈11 million and population under 15 years ≈160 million) and includes the EU/EEA countries and 23 others: Albania, Andorra, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Georgia, Israel, Kazakhstan, Kyrgyzstan, Monaco, Republic of Moldova, Montenegro, Russian Federation, San Marino, Serbia, Switzerland, Tajikistan, Former Yugoslav Republic of Macedonia, Turkey, Turkmenistan, Ukraine and Uzbekistan. While Lichtenstein is not a Member State of the WHO European Region, it along with other territories participates in elimination and eradication activities for vaccine-preventable diseases. The European Region covered in this chapter is defined by the countries from which the WHO collects annual data through the WHO and the United Nations Children's Fund (UNICEF) (WHO/UNICEF) Joint Reporting Form (JRF).[1]

The diversity of the European Region is reflected not only in the cultures and languages but also by economies and health systems. The countries of Europe have some of the highest per capita incomes in the world but in 2014 also include two lower-middle income countries with gross national income per capita of less than US$1500 (Kyrgyzstan (GNI US$1250) and Tajikistan (GNI US$1060).[2] This results in substantial diversity in the health systems among European Region countries and has contributed to the wide variation of immunization programs including which vaccines are offered and at what age. This makes it difficult to share experiences on best practices, but also imposes obstacles when individuals move between countries. Examples of variations in schedules are shown in the recommendations for when pertussis- or measles-containing vaccines are offered (Figs. 74.1 and 74.2).[3]

Vaccines for routine immunization in the European Region must have a Marketing Authorization prior to introduction into national immunization programs. Vaccines may be licensed nationally by respective national regulatory agencies or centrally by the European Medicines Agency (EMA) that was created in 1995 and serves the 31 EU/EEA countries. In the EU/EEA, most vaccines licensed since 1995 are authorized through the centralized procedure. Furthermore, batch testing of vaccines is controlled through EU legislation (for details, see Chapter 80).[4] Where the regulatory capacity for medicines is limited or when vaccines are procured through the UNICEF mechanism, countries are encouraged to engage in an assessment and accelerated national registration of WHO-prequalified pharmaceutical products and vaccines.[5]

All countries in the European Region have national mechanisms for setting immunization policy. However, in a number of countries, local and regional governments or health insurance providers have policies and programs that differ from those recommended at the national level.

To date, immunization policy or practice has not been subject to EU legislation for harmonization and EU/EEA countries do not have common immunization policies although there are agreed targets for influenza vaccination coverage of the elderly population.[6] Recommendations at the national level are supported by infectious disease surveillance and by the WHO Regional Office for Europe in collaboration with the European Centre for Disease Prevention and Control (ECDC), as well as with provision of scientific advice from the two organizations. Increased collaboration between the two organizations is being taken forward.

POLICYMAKING PROCESSES IN EUROPEAN COUNTRIES

In 2014, 26 Member States of the EU/EEA had National Immunization Technical Advisory Groups (NITAGs). Austria, Cyprus, Italy, Norway and Sweden did not. Of the non-EU/EEA countries in the Region, 17 countries had a NITAG. Monaco, Russian Federation, San Marino, Serbia, Tajikistan and Turkmenistan did not report having a NITAG in 2014. Efforts are underway to establish such independent recommendation-making entities in all countries in the region. The majority of the existing committees do have a legislative basis for making vaccine recommendations to the government. The effect of the recommendations varies according to how immunization programs are organized (centralized or decentralized) and the balance between public and private sector provision of services. The following examples demonstrate how immunization policy is made and implemented in selected countries with markedly different systems.

In Sweden, the national immunization schedule is recommended centrally by the government but local health authorities have the freedom to select among products having valid market authorization and may introduce vaccines before national recommendations are adopted. The cost of immunization services and vaccines are covered from regional public funds. Vaccines are offered through public well-baby clinics up to the age of 6 years, and thereafter through school-based preventive clinics.

In some countries, such as Germany and Spain, federal states (Germany) or the autonomous communities (Spain) are responsible for protecting public health. Although these countries have national advisory committees, their recommendations can be modified at the regional level and the vaccines actually provided depend on the choice of private practitioners and the reimbursement arrangements with governments and/or insurance companies.

The health system in Germany is decentralized with most responsibilities for public health resting with the sixteen federal states. Recommendations on vaccine use are made by the Standing Committee on Vaccination, which reports to the Ministry of Health through the Robert Koch Institute, a federal agency that oversees national surveillance and prevention

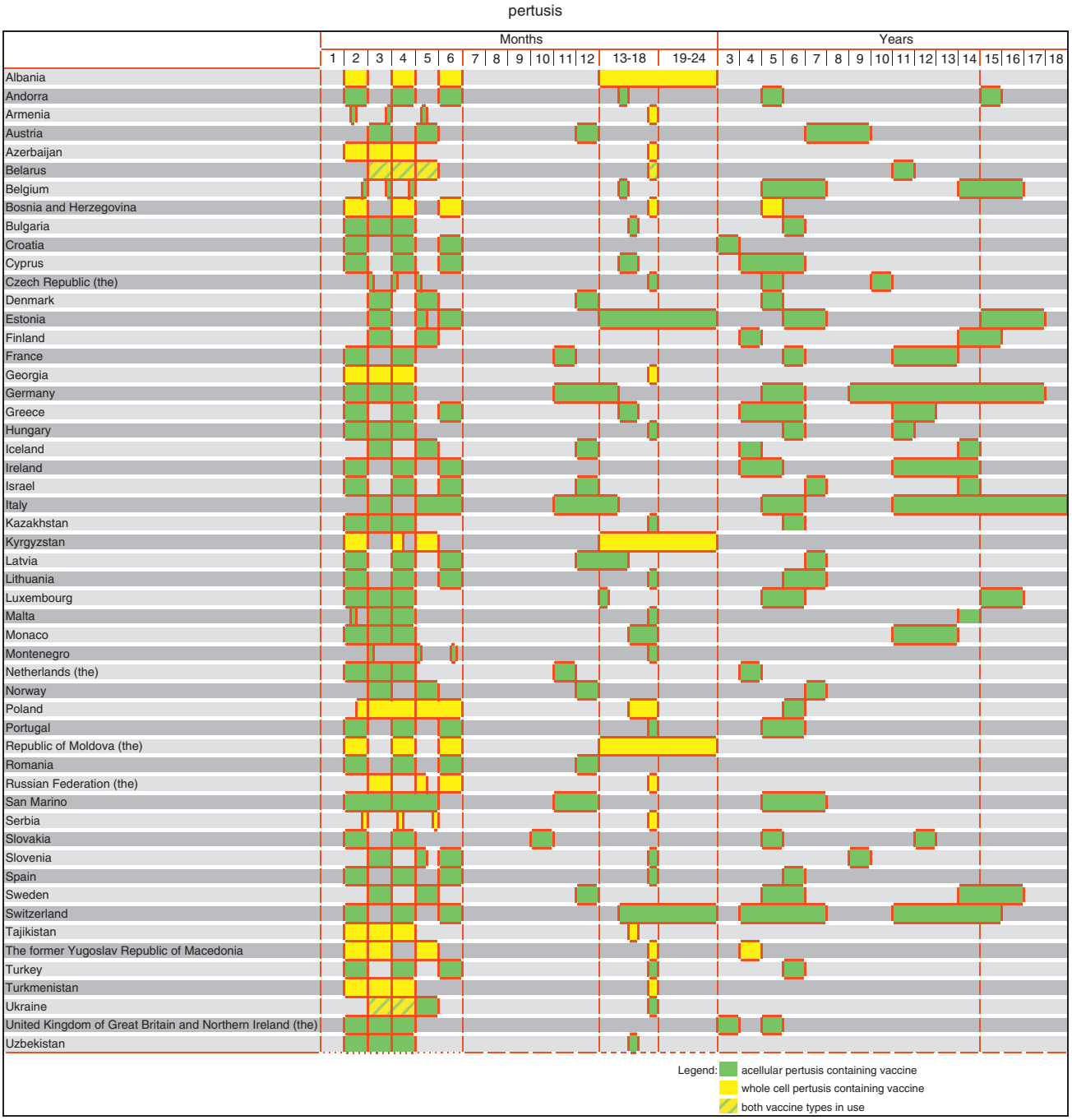


Figure 74.1. Pertussis-containing vaccine immunization schedules up to 18 years of age per country in region of Europe in 2014. *(From WHO vaccine-preventable diseases: monitoring system. 2016 Global summary. Available at: http://apps.who.int/immunization_monitoring/globalsummary/schedules.)*

activities for communicable diseases.[7] However, Standing Committee on Vaccination recommendations need to be approved by each federal state before being implemented.

Decisions regarding reimbursement for vaccination in Germany are made by each of the more than 400 insurance plans, almost all of which are statutory and predominantly financed through payroll taxes. Physicians bill the patient's insurance plan for services provided. Only 10% of vaccines are paid for by the federal states through special programs. The other 90% are primarily covered by the statutory insurance plans. The federal government sets requirements for which health services are mandatory, but vaccinations are not currently among these mandatory services.

France has a national level immunization technical advisory committee, Comité technique des vaccinations, set up by decree. It has voting and nonvoting members. This committee proposes the immunization schedule annually to the Directorate General for Health at the Ministry of Social Affairs, Health and Women's Rights that is responsible for management of the committee and implementation of its recommendations. Some vaccinations remain mandatory (polio, diphtheria, tetanus). Approximately 85% of childhood vaccinations are

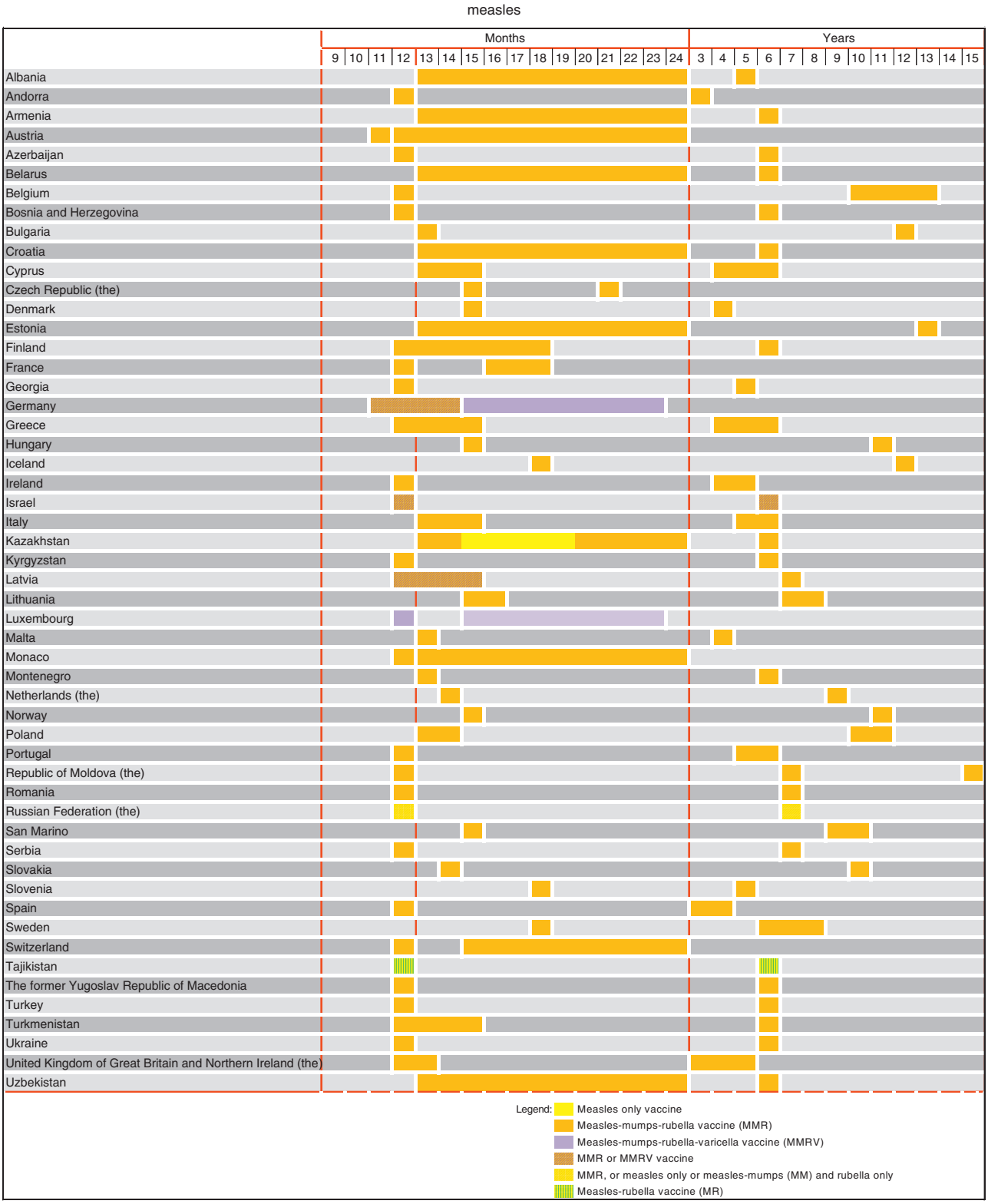


Figure 74.2. Measles-containing vaccine immunization schedules up to 15 years of age per country in region of Europe in 2014. *(From WHO vaccine-preventable diseases: monitoring system. 2016 Global summary. Available at: http://apps.who.int/immunization_monitoring/globalsummary/schedules.)*

performed by private sector pediatricians with the remainder provided by public maternal and child health clinics where most vaccinations are free. Parents claim reimbursement for paid-for vaccinations in the private sector either through their voluntary complimentary health insurance or through the Social Security Scheme. Measles-mumps-rubella (MMR) and influenza vaccinations for those in recommended groups are free, irrespective of where they are provided. Physicians in the private sector choose which products to offer to patients.[8] In 2012, the Directorate developed a national strategy to improve the immunization program in France over a 5-year period. The foci of the new policies are simplifying the immunization schedule, facilitating access to immunization services, and improving monitoring and evaluation of the program performance.[9]

The United Kingdom has a centralized health system in which the National Health Service (NHS) provides universal comprehensive health services, including immunization. The Chief Medical Officers within the health departments of England, Wales, Scotland, and Northern Ireland are responsible for coordinating and providing health-related advice to the government. Routine immunization services are provided by general practitioners in primary care or by nurses in school settings.

In the United Kingdom, the Joint Committee on Vaccination and Immunisation (JCVI), a statutory committee independent of the Department of Health, makes recommendations to the Secretary of State for Health for England and to the devolved administrations in Wales, Scotland, and Northern Ireland; thus policy is implemented similarly in each of these countries. There is a joint procurement process so that the same vaccines are provided in all of the United Kingdom. JCVI recommendations must have the approval of the Chief Medical Officers before they are submitted to the respective health minister and/or secretary of state for approval for implementation. In England, under the provisions of the Health Protection (Vaccination) Regulations 2009,[10] which implements the NHS Constitution in England, following a request for advice by the Secretary of State, the JCVI must make recommendations relating to new provision for vaccination (other than relating to travel or occupational health) under a national program. It also makes recommendation to change existing provisions under such a program, based on an assessment that demonstrates cost-effectiveness. The Secretary of State for Health is obliged to fund such recommendations. Once national funding has been agreed upon, implementation arrangements are taken forward, with all four countries implementing the new policy in the same way and almost always at the same time. Efforts are made to ensure that all localities make changes to their immunization programs simultaneously, facilitated by the existence of national conformity of immunization computing arrangements that call up children for their immunizations, chase defaulters, pay providers, and measure coverage. Local providers have very little discretion in whether to accept or modify national recommendations. Before implementation, consultation takes place with the representatives of the primary care providers because changes in policy can have consequences for the remuneration of physicians.

For many years, some vaccinations under national immunization schedules were considered mandatory under respective infection control legislations. These regulations are now being modified in a number of countries. In 2010, the Vaccine European New Integrated Collaboration Effort (VENICE) network conducted a survey in the EU/EEA to learn whether national vaccination programs were recommended or were mandatory.[11] Information was collected from all 29 EU/EEA Member States. Fifteen countries did not have any mandatory vaccinations; the remaining 14 had at least one mandatory vaccination included in their program. Vaccination against polio was mandatory for both children and adults in 12 countries; diphtheria and tetanus vaccinations were mandatory in 11 countries and hepatitis B vaccination in 10 countries. Some Member States consider mandatory vaccination to be a way of improving compliance with vaccination programs. However, compliance with recommendations is high in many programs in Europe without being mandatory. For countries with mandatory vaccination there is little evidence of any punitive action being taken against those who choose not to accept vaccination although the law may allow for it. Orders not to attend school during outbreaks are implemented in, for example, Germany, to contain spread of vaccine-preventable diseases, a strategy proven effective to increase uptake in some communities refraining from vaccination.[12]

There are fewer differences in the immunization policymaking processes in the non-EU/EEA countries of the region. Most countries have centralized policymaking processes, public sector programs and state procurement. In the eastern part of the region, in Albania and Armenia, for example, the national immunization schedule is also recommended centrally by the government, based on the recommendation of the NITAG and implemented through the immunization system managed by the government. Vaccines are centrally procured and paid from the national budget. In Georgia, the immunization policy is also set by the government but the services are delivered through privatized health insurance schemes.

In the absence of a national immunization technical advisory group, and if there are no identified experts in a country, the national immunization program's manager makes recommendations on vaccine introduction, primarily based on WHO recommendations, and the Ministry of Health makes the final decision. The WHO Regional Office for Europe is working with such member states to establish or enhance NITAGs to strengthen the policymaking process.

In addition, the European Region is experiencing an increasing involvement of the private sector in pediatric and adult immunization programs, for example, in Georgia, Greece, Spain and Turkey. The same is true for most European countries with regard to travel vaccines.

ORGANIZATIONS WORKING IN IMMUNIZATION IN THE EUROPEAN REGION

Box 74.1 lists the organizations and institutions working on immunization in the European Region.

WHO Regional Office for Europe

The WHO Regional Office for Europe (located in Copenhagen, Denmark) has overarching responsibility for developing immunization policies suitable for all 53 member states. The first WHO European Technical Conference on the control of infectious diseases through vaccination programs was held in Rabat, Morocco in 1959.[13] When the Expanded Programme on Immunization (EPI) was launched by WHO in 1974, most European Region countries had already successfully prevented outbreaks of serious childhood diseases such as poliomyelitis and diphtheria, through national immunization programs. Thus, the European Region considered participation in the EPI to be a low priority. This changed in the early 1980s when the member states were all signatories to the regional Health for All Strategy by the Year 2000.

In 1984, the second Conference on Immunization Policies in Europe was held in Karlovy Vary, Czechoslovakia. Participants reviewed the status of immunization programs,

BOX 74.1 Organizations and Institutions Working on Immunization in the European Region

WORLD HEALTH ORGANIZATION (WHO) REGIONAL OFFICE FOR EUROPE

The WHO Regional Office for Europe is responsible for developing immunization policies for 53 member states of the European Region. The WHO Vaccine-Preventable Diseases and Immunization Programme (VPI) coordinates regional immunization and vaccine-preventable diseases activities by providing normative guidance, policy advice, and technical support to the 53 member states ensuring that populations are protected from vaccine-preventable diseases, disability, and death through strong and sustainable immunization systems. European Technical Advisory Group of Experts on Immunization (ETAGE) serves as an advisory body to provide independent review and expert technical input to the WHO European Region's Vaccine-Preventable Diseases and Immunization Programme, with the objective of facilitating and accelerating achievements of the regional targets.

UNICEF REGIONAL OFFICE FOR CENTRAL AND EASTERN EUROPEAN AND COMMONWEALTH OF INDEPENDENT STATES (UNICEF CEE/CIS)

UNICEF CEE/CIS provides support to 22 countries and entities in Central and Eastern Europe, Caucasus, and Central Asia. It works with partner agencies to strengthen country health systems to achieve and sustain universal, high-quality, equitable, and sustainable immunization services. UNICEF advocates for the maintenance of immunization as a public health intervention available to all, particularly for the vulnerable and marginalized populations.

EUROPEAN UNION (EU)

European Commission (EC)

Directorate General for Health and Food Safety (DG SANTE). Directorate General for Health and Food Safety (DG SANTE) support EU countries in maintaining or increasing rates of vaccination against vaccine-preventable diseases and encouraging EU countries to ensure that as many children as possible receive the main childhood vaccines. According to article 168 of the treaty establishing the European Community, the EC may address proposals on measures to protect and improve human health to the council, which in turn may adopt the recommendations made by a qualified majority.[28]

Directorate General for Research and Innovation (DG RTD). The Directorate General for Research and Innovation coordinates European-funded health research. It has prioritized funding in the fields of infectious diseases: poverty-related diseases (HIV/AIDS, tuberculosis, malaria), neglected infectious diseases, antimicrobial drug resistance, and potentially new or re-emerging infectious diseases. Research on new vaccines and adjuvants has been funded under this group.

Joint Undertaking—Innovative Medicine Initiative (IMI). The Innovative Medicines Initiative (IMI) is working to improve health by speeding up the development of, and patient access to, innovative medicines, particularly in areas where there is an unmet medical or social need. It does this by facilitating collaboration between the key players involved in healthcare research, including universities, the pharmaceutical and other industries, small and medium-sized enterprises (SMEs), patient organizations, and medicines regulators. IMI is a partnership between the European Union (represented by the European Commission) and the European pharmaceutical industry (represented by EFPIA, the European Federation of Pharmaceutical Industries and Associations) co-funded through the Horizon 2020 program and the member manufacturers of the EFPIA.

EU Agencies

European Centre for Disease Prevention and Control (ECDC). ECDC identifies, assesses, and communicates current and emerging threats to human health posed by infectious diseases. To achieve this mission, ECDC works in close partnership with national public health bodies across the European Union to strengthen and develop disease surveillance and early warning systems and to produce authoritative scientific opinions about the risks posed by new and emerging infectious diseases. Through projects, with additional scientific advice panels, ECDC provides policy makers in the EU/EEA Member States with solid and scientifically sound evidence for Member States' decision-making processes.

European Medicines Agency (EMA). The European Medicines Agency is responsible for the scientific evaluation of medicines developed by pharmaceutical companies for use in the European Union. Under the European centralized procedure, companies submit a single marketing-authorization application to the European Medicines Agency (EMA). Once granted by the European Commission (EC), a centralized marketing authorization is valid in all EU/EEA Member States. All medicines for human and animal use derived from biotechnology and other high-tech processes, including vaccines, must be approved via the centralized procedure. The European Medicines Agency monitors vaccine safety through a pharmacovigilance network (Eudravigilance) and takes appropriate actions if adverse reaction reports suggest that the benefit-to-risk balance has changed since it was authorized. The Pharmacovigilance Risk Assessment Committee (PRAC) is responsible for assessing all aspects of the risk management of medicines, including vaccines, for human use. The Agency's Committee for Medicinal Products for Human Use (CHMP) prepares scientific guidelines in consultation with regulatory authorities in the EU/EEA Member States, to guide marketing-authorization applications as well as to provide harmonisation of quality, safety and efficacy requirements.

Consumers, Health, Agriculture and Food Executive Agency (CHAFEA). The CHAFEA implements the EU Health Programme, the Consumer Programme and the Better Training for Safer Food Initiative. The third Health Programme is the European Commission's main instrument for implementing the EU health strategy (http://ec.europa.eu/chafea/health/). The Health program funds projects and other actions (conferences, operating grants) aimed at improving the level of health and well-being of EU citizens and reducing health in equalities throughout the European Union including projects in the field of vaccine programs.

OTHER ORGANIZATIONS

Global Alliance for Vaccines and Immunization (GAVI)

Global alliance for Vaccines and Immunization supports GAVI-eligible countries in the European Region in the introduction of new vaccines, including *Haemophilus influenzae* type b, rotavirus, and pneumococcal vaccines. Funding has also been provided to GAVI-eligible countries for strengthening immunization services, conducting burden-of disease studies, strengthening injection safety, and developing comprehensive multiyear plans for immunization programs.

established immunization targets, determined necessary actions, and reinforced the commitment of member states to the goals and activities of the EPI. The conference became a turning point for strengthening region-wide coordinated immunization activities. The recommendations of the conference also strengthened the responsibility of the Regional Office.

The dramatic political and socioeconomic changes that occurred before, during, and after the dissolution of the Soviet Union compromised the ability of the newly independent states to produce or provide vaccines for disease-control and immunization programs through transforming health systems and hence WHO has prioritized the countries in the eastern part of the European Region for special attention. The reemergence of diphtheria in the 1990s, widespread measles outbreaks during the early 2000s, and an outbreak of wild poliovirus in 2010 are examples of the special needs in these countries.[14–17]

The European Advisory Group on the Expanded Programme on Immunization (EAG) was created in 1986 to facilitate the development of the regional immunization program. It effectively guided the program and facilitated and accelerated the achievements of the regional targets specified in the Health21 policy framework.[18] The EAG particularly provided direction for the revision of immunization policies in the newly independent states, reviewed diphtheria control strategies, and supported poliomyelitis elimination from the European Region. The EAG's terms of reference were revised to respond to major changes of member states' health systems as well as to the evolution of vaccine-preventable diseases epidemiology and the European Technical Advisory Group of Experts (ETAGE) was established in 2003. A further revision was made to ETAGE's operations in 2011 in order to better align them with that of the parent advisory committee at the global level, the Strategic Advisory Group of Experts on Immunization (SAGE), and with regional policies such as Health 2020.[19] Members of ETAGE are individuals acknowledged for their expertise in immunization and who are independent of WHO.[20] They are chosen from across the European Region through an appointment process similar to that used by SAGE. ETAGE meets at least once a year.

ETAGE serves as a forum for regular review of immunization services' performance in the Member States of the European Region, with special attention to the progress being made toward the vaccine-preventable disease control targets and objectives, especially identifying any constraints, obstacles, and threats. ETAGE engages in technical discussion and formulates recommendations on operational aspects of immunization policies and strategies, as well as incorporating new scientific developments. It provides technical support to NITAGs, and offers a forum for discussion on implementation of SAGE recommendations within the European Region context.[21]

The Regional Certification Commission for Poliomyelitis Eradication (RCC) is an independent panel of international experts established in 1996. Since the certification of the European Region as polio free in 2002,[22] the terms of reference of the Commission have been modified with a focus on sustaining the polio-free status, providing national risk assessments, and ensuring rapid action following any wild polio virus introduction or identified shortfalls of national polio programs. The RCC also monitors poliovirus containment and contributes to global polio elimination certification in line with objective 3 of the Global Polio Eradication and Endgame Strategic Plan 2013–2018.[23] The RCC meets annually or whenever necessary and its reports are publically accessible.[24]

The Regional Verification Commission for Measles and Rubella Elimination is also an independent panel of experts, established in 2012, tasked with determining whether endemic transmission of measles and rubella continues or has been interrupted in each Member State, based on annual updates submitted by national verification committees. Regional and national verification committees use a standardized review methodology and reporting requirements.[25] Annual assessments consider information about measles and rubella epidemiology, virological surveillance data, supported by molecular epidemiology, analyses of vaccinated population cohorts, the quality of surveillance and the sustainability of the relevant national immunization program.

WHO immunization-related activities in the European Region are encompassed in three strategic areas: strengthening of immunization systems, accelerated control of vaccine-preventable diseases, and associated laboratory-based surveillance. Crosscutting activities include performance monitoring, communications, data collection, and data management. The Vaccine-preventable Diseases and Immunization Programme publishes guidelines and technical documents, ensures routine interactions with national immunization program managers, and facilitates sharing of experiences, achievements, and constraints in attaining the regional targets. The Regional Office has developed surveillance guidelines for selected vaccine-preventable diseases. It collects information on acute flaccid paralysis cases (weekly, all member states); measles and rubella cases (monthly, all member states); diphtheria cases (monthly, 16 member states); rotavirus (quarterly, 8 member states); invasive bacterial diseases (quarterly, 5 member states); and influenza (weekly, 53 member states).

Since 1998, the WHO and UNICEF jointly collect annual disease surveillance and immunization data using a standardized questionnaire, the WHO/UNICEF JRF. National immunization programs complete the JRF and describe various aspects of their program, including vaccine schedules, vaccine financing, coverage rates achieved for each of the vaccines, reported vaccine-preventable disease incidence, and indicators of program performance. WHO and UNICEF jointly produce the best estimates of coverage from the data reported in the JRF as well as using other sources provided by the member states. All member states of the WHO European Region use this form to report annual vaccine coverage rates and incidence for vaccine-preventable diseases to the WHO Regional Office for Europe. Comparisons of immunization schedules by disease, antigen, and age for the WHO European Region countries are available from WHO/UNICEF JRF website.[1]

All the reported data are used for monitoring and evaluation and are disseminated through the region using the Centralized Information System for Infectious Diseases (CISID) and are aggregated for global reporting through WHO Headquarters. EU/EEA member states' data are submitted through the ECDC platform "European Surveillance System (TESSy)" and forwarded to the CISID platform to avoid double reporting. Feedback is provided monthly to member states on polio, measles, and rubella data, and quarterly reports are prepared for rotavirus and invasive bacterial diseases (meningitis/pneumonia/sepsis caused by infections caused by *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*). A monthly publication, "WHO EpiData," and a quarterly bulletin, "WHO EpiBrief," provide feedback to all member states on trends and surveillance performance indicators toward elimination or maintenance of eradication status.[26] "Flu News Europe" is a weekly online publication on influenza surveillance jointly produced by ECDC and the Regional Office throughout the northern hemisphere winter season, from week 40 until week 20 of the following year.[27]

The Regional Office works closely with GAVI, the Global Alliance for Vaccines and Immunization, which has been an important partner for introducing new vaccines in GAVI-eligible countries in the European Region. Eleven countries

received support during the first 4 years of GAVI. In 2006, the number of GAVI-eligible countries decreased to eight (Armenia, Azerbaijan, Georgia, Kyrgyzstan, Republic of Moldova, Tajikistan, Ukraine, and Uzbekistan); in 2014 there are two GAVI-eligible countries (Kyrgyzstan and Tajikistan) and six graduated countries (Armenia, Azerbaijan, Georgia, Republic of Moldova, Ukraine, and Uzbekistan). WHO and GAVI provide technical support following graduation, with special focus on strategic planning, and strengthening national vaccine procurement and regulation systems to enable access to quality assured vaccines at affordable prices.

UNICEF Regional Office for Central and Eastern European and the Commonwealth of Independent States

The UNICEF Regional Office for Central and Eastern Europe and the Commonwealth of Independent States (UNICEF CEE/CIS) provides support to 21 countries and one territory in Central and Eastern Europe, the Caucasus, and Central Asia through its field offices. While working towards attaining regional immunization coverage and disease control targets, UNICEF's focus areas are generating evidence on immunization programs through field assessments toward ensuring adequate and reliable vaccine supplies and effective vaccine management while building capacity and strengthening country health communication systems to maintain and restore public trust in immunization.

European Union Institutions

Several EU institutions play a role in immunization. The European Commission, through the Directorate General for Health and Food Safety (DG SANTE, after the French denomination) can provide support to the implementation of national vaccine programs. DG SANTE is supported in this area by three European Agencies: the ECDC, the EMA, and the Consumers, Health, Agriculture and Food Executive Agency (CHAFEA).

According to Article 168 (ex art. 152) of the treaty establishing the EU,[28] the European Commission may address proposals on measures to protect and improve human health to the Council of the EU, which, in turn, may adopt the recommendations made by a qualified majority. The first example of such a legislative initiative in the area of human immunization is represented by the *Council Recommendation 2009/1019/EU of 22 December 2009 on seasonal influenza vaccination*.[6] In this recommendation, the EU Parliament and the EU Council have encouraged Member States to adopt and implement action plans or policies aimed at improving seasonal influenza vaccination coverage with the aim of reaching a vaccination coverage rate of 75% in elderly and other high-risk groups, and to improve vaccination coverage among healthcare workers. During the 2009 influenza pandemic, DG SANTE (at that time DG SANCO) played an active role in developing a common EU vaccination strategy and coordinating the overall public health response through the influenza subgroup of the Health Security Committee. This first Council recommendation was followed by a council conclusion on *Childhood immunisation: successes and challenges of European childhood immunisation and the way forward*.[29] Of importance going forward for all vaccination programs in the EU are the new *Council conclusions on vaccinations as an effective tool in public health* of 1 December 2014[30] addressing, among other things, the changes in the epidemiology of childhood diseases preventable by vaccination, and the observed shift towards older age groups, the importance of vaccination providing life-long protection, and not only vaccination for children. Furthermore, EU Member States are invited to conduct catch-up campaigns to improve the control of specific diseases. Finally, the Conclusions call upon the European Commission with ECDC and the EMA to (a) identify guidance and methodologies that might strengthen Member States' immunization programs, and (b) support the evaluation of risk communication and improving vaccine uptake strategies by Member States by putting forward appropriate research methods.

The ECDC, based in Stockholm, Sweden, became operational in 2005, with the aim of strengthening Europe's defences against infectious diseases, including vaccine-preventable diseases. The ECDC and the WHO Regional Office for Europe have established a close working relationship, with reciprocal participation on management and technical advisory committees.

To strengthen surveillance within the EU, a network for the epidemiologic surveillance and control of communicable diseases was established by Decision 2119/98/EC of the European Parliament and the Council of Europe in 1999.[31] This led to the creation of a number of surveillance networks funded by the European Commission to coordinate national data into an EU-wide system: the European Laboratory Working Group for Diphtheria, the EU Invasive Bacterial Infections Surveillance, which included *H. influenzae* and meningococcal infections; and the Surveillance Community Network for Vaccine Preventable Infectious Diseases, which included measles, rubella and pertussis. All the tasks previously conducted under these networks have since 2012 been transferred to the ECDC. Currently, 49 notifiable infectious diseases from 31 EU/EEA countries are reported to the ECDC TESSy database. Surveillance data are published in ECDC annual reports and increasingly on the website in almost real-time.[32,33] Eighteen of the communicable diseases are vaccine-preventable: cholera; diphtheria; *H. influenzae* type b causing invasive disease; hepatitis A; hepatitis B; *N. meningitidis* types A, B, C, W135, and Y causing invasive disease; influenza (seasonal, avian, and pandemic); measles; mumps; pertussis; poliomyelitis; rabies; rubella; smallpox; *S. pneumoniae* causing invasive disease; tetanus; tick-borne encephalitis; and tuberculosis. As of 2015, ECDC is not yet conducting surveillance for the following vaccine-preventable diseases: human papillomavirus (HPV), rotavirus, and varicella infections.

The ECDC has an established program on vaccine-preventable diseases running specific projects in partnership with European experts and national public health institutions. Priority areas and criteria for funding specific actions are set out in a work plan that is published every year and is followed by specific calls for proposals. The following network projects have been funded from 2008 and onwards.

The *VENICE II project* was launched in 2006 with the general aim of collecting and sharing information on national vaccination programs, initially through a network of professionals, and later through official nominations by EU/EEA countries. The VENICE network had been initiated earlier but was transferred in 2006 to ECDC. VENICE has for example provided vaccine coverage data by country in support of the above mentioned Council Recommendation 2009/1019/EU of 22 December 2009 on seasonal influenza vaccination, and timely updates on pandemic influenza vaccine coverage by country.[6,34] Another activity is documenting which countries have introduced new vaccines and, as mentioned earlier, to document which vaccines are mandatory and recommended in individual countries.[11] Their reports are available to the public and most data collected are published in the scientific literature.[11,35]

The *I-MOVE project* (Influenza—Monitoring Vaccine Effectiveness) was established to estimate seasonal and pandemic influenza vaccine effectiveness in 19 EU/EEA Member States

using test-negative design case-control and cohort studies. Vaccine effectiveness estimates have been provided since the 2008–09 season and presented to the annual WHO strain selection meeting and reported in the scientific literature.[36,37]

A further network, *SpiDNet*, was created for surveillance of invasive pneumococcal disease in the EU and to assess the impact of the conjugate vaccines on the incidence and severity of the disease among children younger than 5 years of age; it was initiated in 2012.[38] The project aims to measure pneumococcal vaccine effectiveness in Europe.

A small network for surveillance of rotavirus disease in the EU was created in 2011 to assess rotavirus vaccine effectiveness in Europe.[39]

The *VAESCO network* (Vaccine Adverse Events Surveillance and Communication) was initiated in 2009 with the aim of improving the quality of vaccine safety information in the EU by standardizing methods for association studies, facilitating data comparability, and building collaborative networks. The network has not received funding since 2013. The VAESCO network included nine EU/EEA countries that are able to conduct studies using large databases (e.g., hospital records, vaccine registries) that can be pooled and crosslinked. The ultimate goal of the project was to be able to provide background rates of adverse events of interest, such as Guillain-Barré syndrome, and to perform specific epidemiologic studies following detection of a vaccine safety signal using the VAESCO platform. Vaccine safety signals can either be raised by a Member State or through reports of national medicines regulatory agencies to the Eudravigilance database, a pharmacovigilance platform of EMA. The EMA Pharmacovigilance Risk Assessment Committee (PRAC) formally assesses any vaccine safety signal, and should pharmacoepidemiological studies be needed, the network may provide support. During and after the 2009 influenza pandemic, background rates for Guillain-Barré syndrome were developed and analyzed in a multicountry study[40] and after a safety signal of narcolepsy following vaccination with Pandemrix was detected, an incidence study using data linkage of the participating databases and a case-control study were conducted to assess the safety signal.[41,42] The future of such studies is currently under evaluation.

Through all of these projects, ECDC has built a large resource base that is complemented by additional scientific advice produced with internal and external expertise (ad-hoc scientific panels). Several guidance documents have been produced on influenza, HPV, varicella and diphtheria-tetanus-pertussis (DTP) vaccination.[43–46] In addition, risk assessments of threats to public health in the EU/EEA by vaccine-preventable diseases are conducted.[47] ECDC conducts risk assessments regularly and for vaccine-preventable diseases has published risk assessments on wild poliovirus when identified in environmental samples (2014), a pertussis-containing vaccine shortage (2015), as well as a shortage of diphtheria anti-toxin antibodies for treatment of toxigenic diphtheria (2015).[48–50]

ECDC hosts a website named *Vaccine Schedule*.[51] The Vaccine Schedule is an interactive platform of vaccination schedules of individual EU/EEA countries and is available for use by both health professionals and the general public.

ECDC publishes *Eurosurveillance*, a journal devoted to the epidemiology, surveillance, prevention, and control of communicable diseases.

An older EU network, the European Sero-Epidemiology Network (ESEN) established in 1996 through funding from the European Commission to coordinate and harmonize serologic surveillance of immunity to vaccine-preventable diseases, continued its work until 2014, eventually involving 18 Member States. Immunity to diphtheria, hepatitides A and B, measles, mumps, pertussis, rubella, tetanus, and varicella was assessed in selected populations.[52] This network showed significant immunity gaps in European populations against diseases such as diphtheria, measles, and rubella.[53–55]

The EMA is located in London, United Kingdom and became operational in 1995; it is responsible for the scientific evaluation of medicines including vaccines developed by pharmaceutical companies for use in the EU. The EMA works closely with national regulatory agencies; vaccine products can still be authorized nationally and can be recognized in other EU/EEA Member States through a mutual recognition procedure.[56]

The Agency's Committee for Medicinal Products for Human Use prepares scientific guidelines, in consultation with regulatory authorities in the EU/EEA Member States, to guide marketing-authorization applications as well as to provide harmonization of quality, safety, and efficacy requirements. Examples of particular importance are guidelines for dossier structure of influenza vaccines or vaccine adjuvants for human use.[57,58] More detail on the functions of the EMA can be found in Chapter 80.

The EMA monitors vaccine safety through a pharmacovigilance network (Eudravigilance database). The PRAC is responsible for assessing all aspects of the risk management of medicines, including vaccines, for human use. This includes the detection of signals, risk assessment, mitigation, and communication relating to the risk of adverse reactions, while taking the benefit-to-risk balance into account. It also has responsibility for the design and evaluation of post-authorization safety studies and pharmacovigilance audits. PRAC monitors effectiveness of risk-management systems that generate information for regulatory decision making. The PRAC also generally provides recommendations to the Committee for Medicinal Products for Human Use, the Co-ordination Group for Mutual Recognition and Decentralised Procedures-human (CMDh), the EMA secretariat, the Management Board, and the European Commission, as applicable.

The CHAFEA is located in Luxembourg and became operational in a smaller format in 2005. CHAFEA in its current form was created on January 1, 2015. CHAFEA implements the EU Health Programme, the Consumer Programme, and the Better Training for Safer Food initiative. The Third EU Health Programme is the European Commission's main instrument for implementing the EU health strategy and has four overarching objectives: (a) promote health, prevent diseases, and foster supportive environments for healthy lifestyles taking into account the "health in all policies" principle; (b) protect Union citizens from serious cross-border health threats; (c) contribute to innovative, efficient, and sustainable health systems; and (d) facilitate access to better and safer healthcare for Union citizens. The program has provided funding to support the development of prevention, vaccination, and immunization policies and to improve partnerships, networks, tools, and reporting systems for immunization status and adverse events monitoring.[59]

The EU supports research and development in health. European-funded health research is coordinated by the Directorate General for Research and Innovation. Horizon 2020 is an EU Research and Innovation program with nearly €80 billion of funding available over 7 years (2014 to 2020), in addition to the private investments that this money will attract. The goal is to support European research institutions reaching scientific breakthroughs, remove barriers to innovation, and make it easier for the public and private sectors to work together in delivering innovation. Research collaborations on new vaccines and adjuvants such as ADITEC, universal influenza vaccines, and Ebola vaccines have been funded under this stream.

Horizon 2020 contributes funding to the Innovative Medicine Initiative research program, a public and private partnership (joint undertaking) equally cofunded by the European Commission and the member manufacturers of the European Federation of Pharmaceutical Industries and Associations.[60] The main priority of the Innovative Medicine Initiative research agenda is to tackle the major challenges in drug development—including vaccines—with a specific focus on evaluating safety and efficacy. According to Vaccine Europe (earlier called the European Vaccine Manufacturers Association), 79% of their global production comes from the European Region, with a 47% growth in research and development investment over the past 8 years, and approximately 50% of current research and development projects based in the EU.[61,62] Five large vaccine-related projects have been funded in the last few years; PROTECT (Pharmacoepidemiological Research on Outcomes of Therapeutics funded in 2009), ADVANCE (vaccine benefit-risk assessments funded in 2013), Correlates of Protection for influenza funded in 2014, support to Ebola vaccine development funded in 2014, and pertussis vaccination research funded in 2015.

PROCUREMENT

Procurement of vaccines in EU/EEA countries may be undertaken by governments as public sector provision of vaccines—that are usually free to recipients; through private sector arrangements—where there may be full or partial reimbursements of costs; or through a mix of these arrangements. In non-EU countries, nearly all procurement of vaccines used under national immunization schedules is through government or public sector central arrangements. The role of the private sector in supporting immunization services is reportedly increasing in areas such as logistics and service delivery as countries move to decentralize public administration and health services, and phase out immunization as vertical standalone programs, anchoring it within general medical practice. The vaccine supply systems vary from very centralized public procurement and distribution systems to completely decentralized systems where each immunization site contracts with a private supplier for vaccines. Provision of paid private immunization services has significantly increased in central and eastern Europe and in the newly independent states since the turn of the present century, picking up an increasing role in meeting the demands of specific population groups.

National budget lines for routine immunization vaccine procurement exist in 42 of the European Region countries, most covering full costs of the recommended routine schedule.[3] Member states reporting less than 100% government funding for vaccines procurement reflect either financial or in kind contributions of GAVI or funding though insurance schemes. Immunization as per national schedule is available free of charge or against reimbursement through health insurance in all Member States of the Region, with some optional vaccines provided against copayment or full reimbursement by health insurance.

UNICEF plays a critical role in vaccine procurement in almost all GAVI-supported and GAVI-graduated countries (Armenia, Azerbaijan, Georgia, Kyrgyzstan, Republic of Moldova, Tajikistan, and Uzbekistan), as well as some other countries in the European Region that have specific considerations or difficulties in procuring vaccines. Some national legislation on public procurement allows contracting for the current calendar year only, which, in turn, may lead to difficulties securing stable vaccine supplies. The vaccines procured by UNICEF are not necessarily produced and registered in Europe, but all have been prequalified by the WHO.

There has been increasing conformity of the vaccines being used in European countries, diminishing the differences that used to occur between EU and former Soviet Union countries. However, there are considerable differences in the prices that are paid.[63] The EU countries tend to pay full industrialized country prices, subject to national procurement arrangements where centralized contracts might achieve better prices than private-sector purchasing. Countries with smaller to mid-range size of relevant cohorts frequently report problems negotiating prices and a lack of interest from suppliers to apply to vaccine procurement tenders, which often results in vaccine prices that exceed considerably the ones achieved by EU member states. Recently, the WHO Regional Office for Europe has been working closely with middle-income countries (those with a gross national income [GNI] per capita of more than US$1045 but less than US$12,736 in 2014) to ensure equitable access to vaccines at affordable prices. There is an ongoing review of different schemes, including tiered pricing and pooled procurement.

The 2009 influenza pandemic highlighted the differences in procurement arrangements for vaccines in Europe. Based on assessments of risks of H5N1 influenza viruses being the likely cause of the next pandemic, one half of the EU countries had made arrangements with vaccine-producing companies for access to pandemic vaccines through advance-purchase agreements. Under these arrangements, as soon as a pandemic was declared by WHO, production of seasonal vaccines would stop and production would switch to pandemic vaccine. Countries with advance-purchase agreements in place would then benefit from the first deliveries of production of the pandemic vaccine. Countries that had contracted for advance-purchase agreements paid monthly fees to vaccine companies; these fees were to cover ongoing research by the companies and to reserve production capacity when a pandemic occurred. When the 2009 H1N1 pandemic began, countries that did not have advance-purchase agreements were obliged either to wait for the second phase of pandemic vaccine production after the advance-purchase agreements had been fulfilled or to apply to WHO for access to its influenza A (H1N1)pdm09 vaccine supplies. Azerbaijan, Georgia, Republic of Moldova, and Tajikistan received vaccine through WHO donations.

There were considerable differences in the quantities of vaccine that were ordered through advance-purchase agreements. Austria, Sweden, Switzerland, and the United Kingdom committed to two vaccine doses per head of population and other countries had smaller orders. Poland chose not to have an advance-purchase agreement and not to seek supplies of influenza A (H1N1)pdm09 vaccine after the onset of the pandemic. Eastern European countries received their vaccine supplies much later than the western countries. Following the 2009 pandemic, EU member states asked the European Commission to investigate mechanisms that would permit a more equitable sharing of access to vaccine in a future pandemic so that less-affluent countries did not have to be as disadvantaged as they had been in 2009. The Commission investigated the opportunities ranging from central procurement for EU countries to joint contracting arrangements by like-minded countries and production of purchasing guidelines to help countries doing their own procurement. The European Commission issued an assessment report on EU-wide pandemic vaccine strategies in August 2010. The efforts have since continued and an EU/EEA-wide joint procurement mechanism for all countermeasures, including pandemic vaccines, is now in place, supported by legislation in the event of serious cross-border threats.[64] This should provide stronger negotiating power, lower costs, and equitable access, and would help to create a common understanding of liability issues. The triggering mechanisms of switching from seasonal influenza vaccine to

pandemic vaccine is currently being further defined by the WHO, and will include risk assessments of the severity of clinical cases, transmissibility in human populations, the epidemiological situation, and the characterization of the emerging influenza virus. Other issues to be addressed include dealing with excess doses, economic sanctions for delayed delivery of vaccines, liability should a safety concern arise, and conditions under which a contract becomes void.

SURVEILLANCE OF VACCINE-PREVENTABLE DISEASES IN THE EUROPEAN REGION

The responsibility for surveillance of vaccine-preventable diseases lies with individual countries. Within the countries, some vaccine-preventable diseases are statutorily reportable (measles in the United Kingdom, Germany, Kazakhstan), some are collected nationally on a voluntary basis (pneumococcal infection in the United Kingdom and the Netherlands), and others are kept under surveillance through sentinel systems (influenza in the United Kingdom and France, rotavirus in Ukraine, invasive bacterial diseases in Azerbaijan and Uzbekistan).

The ECDC and the WHO Regional Office for Europe have undertaken a review of diseases under epidemiologic surveillance and the case definitions used. This has resulted in strengthened surveillance systems for vaccine-preventable diseases within the European Region through the availability of information on a broader range of diseases, using mutually agreed upon case definitions and reducing the duplication of reporting by Member States.

For polio surveillance, 43 of 53 member states of the European Region, including 21 of 30 EU/EEA countries, conduct surveillance for acute flaccid paralysis (AFP) and report weekly to the Regional Office. The number of countries abolishing traditional AFP surveillance is increasing in an era of widespread use of advanced imaging techniques and molecular biology technologies that help establish a diagnosis in much shorter timeframe than is required for virus isolation. Thirty-nine countries conduct enhanced enterovirus surveillance and 22 conduct environmental surveillance through sewage collection (Fig. 74.3). A WHO polio laboratory network of 48 laboratories located in 37 countries undergo annual accreditation. In addition, four regional reference laboratories (Berlin, Bilthoven, Paris, and Rome) and three of the global polio reference laboratories (Bilthoven, London, Moscow) are located in the WHO European Region.[65]

Measles is notifiable in all 53 member states. Countries report clinically diagnosed measles cases to the WHO annually through the WHO/UNICEF JRF. In accordance with regional surveillance guidelines, 38 countries report case-based information to WHO monthly. The Regional measles and rubella laboratory network was established in 2002 and consists of 72 laboratories: one global (London), three regional (Luxembourg, Berlin, and Moscow), 48 national, and 20 subnational laboratories.

Fifty-one of the 53 member states, including all EU/EEA countries, conduct measles surveillance. All data are stored and disseminated using the CISID (the WHO European Region database).[66] National and regional reference laboratories provide a WHO measles and rubella regional laboratory network for genotyping viruses and sharing these data for identifying circulating viruses and monitoring progress toward elimination.[67]

Reporting of rubella cases at the national level is mandatory in 43 of the 53 Member States. Of these, 34 have case-based surveillance at the national level, and the reporting scheme is similar to the one described for measles. Belgium, Denmark, and France do not have mandatory notification systems covering the total population for reporting rubella cases. Germany introduced a national rubella notification system in 2013, which became operational in 2014. Intense efforts are under way to strengthen rubella surveillance in the region. Some countries face difficulties justifying the additional expense of comprehensive surveillance for measles and rubella given the current scarcity of funding for health and the nature of national health funding priorities.[68,69]

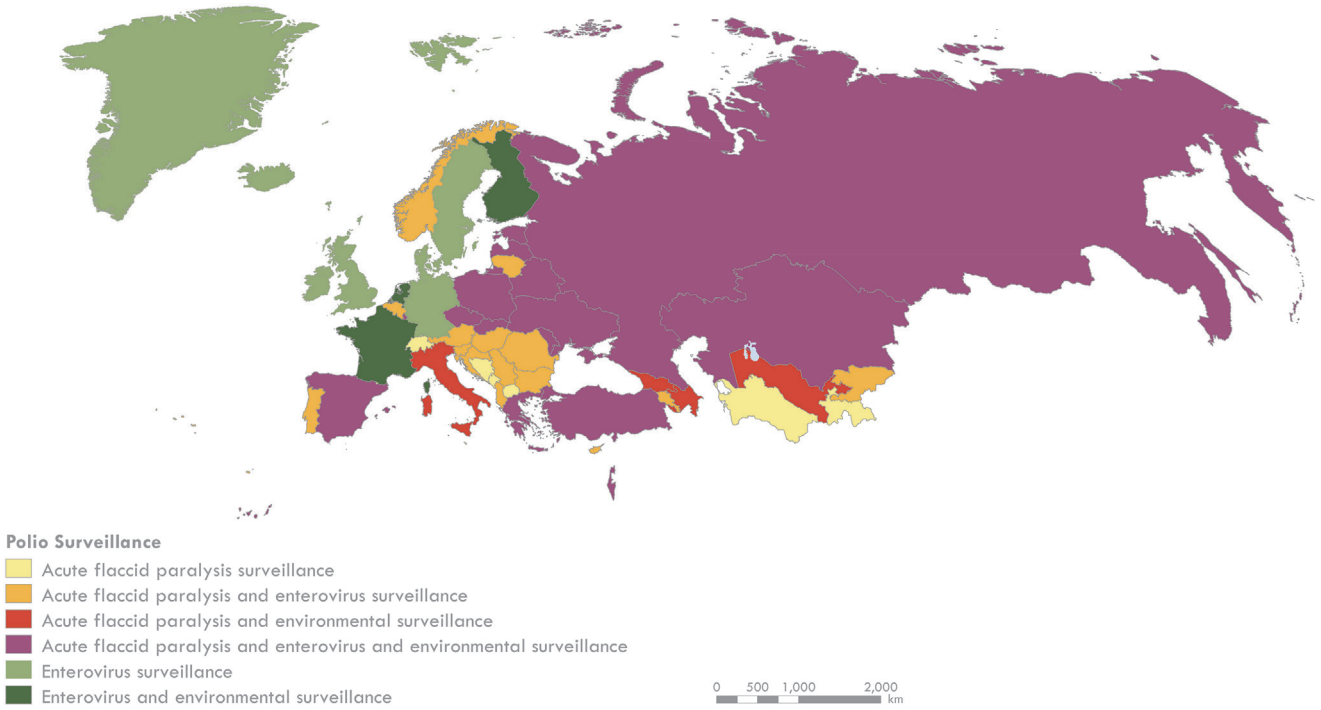


Figure 74.3. Types of polio virus surveillance in WHO Region of Europe in 2014.

All EU/EEA member states have surveillance for invasive bacterial diseases caused by *N. meningitidis, S. pneumoniae,* and *H. influenzae.* The WHO European Regional Office is working with eastern European countries and newly independent states to establish and strengthen the invasive bacterial diseases surveillance network, currently comprising laboratories in seven countries to monitor circulating serotypes.

The WHO European Regional Office has also established a rotavirus surveillance network for eastern European countries with a regional reference laboratory based in Minsk, Belarus. Surveillance for rotavirus in the western part of the region already exists through a regulatory requirement on the two manufacturers (Eurorotanet).[70,71] Rotavirus accounts for a substantial proportion of hospitalizations for diarrhea in the seven countries in the WHO network.

European member states had a target to eliminate indigenous diphtheria by 2000 (the absence of indigenous cases caused by toxigenic *Corynebacterium diphtheriae* strains). ECDC has since assumed diphtheria surveillance for the EU/EEA countries along with 16 non-EU/EEA countries, including Belarus, Republic of Moldova, Turkey, Ukraine; all eastern European countries report monthly to the Regional Office.

VACCINES USED AND IMMUNIZATION COVERAGE IN THE EUROPEAN REGION

The vaccines and immunization schedules used in the 53 countries of the European Region are undergoing continuous change with the introduction of new antigens and the increasing use of combined-antigen vaccines. Annual information is collected from WHO Member States on immunization programs and vaccine-preventable diseases using the WHO/UNICEF JRF as part of the global data collection jointly conducted with UNICEF.[1] The information for a given year is collected during the spring of the following year and usually is available by the autumn. The data referred to in this chapter are those available to the Regional Office on immunization programs for 2014.[3] For information on specific countries, the reader is encouraged to access the WHO website.[3] Table 74.1 provides summary information on childhood vaccines provided through routine national immunization systems. Other vaccines may be in selective use, either for specific risk groups, in selected geographical areas, by parental choice, for outbreak management or in relation to travel. Vaccines that have obtained NITAG recommendation but are pending introduction are not reflected in the overview.

The measurement of immunization coverage in the European Region is challenging because of the wide range of techniques employed for data collection in individual countries resulting in issues with data quality and comparability. ECDC, through the VENICE network, started the European Vaccine Coverage Project (EVACO) in 2011.[72] The main aim of this project is to provide reliable and standardized vaccine coverage data in the EU/EEA. A standard for data collection has been agreed on and a piloting phase has been concluded.

The methods for assessing coverage include collecting data manually or electronically by the administrative method (doses reported administered divided by estimates of the target population), conducting surveys at regular time intervals of selected populations, or use of electronic immunization registers. All three methods are used in the European Region and the number of countries with electronic immunization registries is increasing. In Denmark, Finland, Ireland, Israel, the Netherlands, Norway, and the United Kingdom, public health data are fully computerized and actively managed; thus coverage is calculated by comparing the number of children who have completed immunization by a specified age with the number of children of the same age residing in the particular community—coverage by birth cohort. Another 13 countries are known to have local registries: Belgium, Estonia, Germany, Hungary, Iceland, Italy, the Netherlands, Portugal, Romania, Spain, and Sweden.

In Austria, Belgium, France, Greece, and Spain, where the private-sector providers give many immunizations, coverage is calculated by comparing the number of doses of vaccine imported or distributed with the estimated target population. In these countries, allowances for wastage and inaccuracies in the target population make these coverage estimates unreliable.

In non-EU countries, administrative coverage is usually reported as the number of doses administered in each locality divided by the estimated target population. Inaccuracies can occur in the estimates of the numerators and denominators because of inaccurate census data and facilities incorrectly determining their catchment population. Many countries also routinely conduct household surveys, such as the Demographic Health Surveys or Multiple Indicator Cluster Surveys. These data are used in calculating the best estimate by WHO and UNICEF.

In most countries, coverage is measured at 2 years of age, but in Germany it is measured at school entry, usually at 5 years of age, through data held by insurance providers. Because countries use such different techniques for estimating coverage and with variable accuracy, comparisons among countries' coverage reports should be made with caution.

There are three schedule patterns for the primary immunizations in the region. The Netherlands and the United Kingdom use a three-dose early-onset schedule with vaccination at 2, 3, and 4 months of age, as do Azerbaijan, Bulgaria, Georgia, and Kazakhstan. Belgium, Ireland, and Switzerland used a three-dose middle-onset schedule at 3, 4, and 5 months of age or 2, 4, and 6 months of age. Most of these three-dose priming schedules offer a booster dose during the second year of life. France and Slovakia use a two-dose early schedule at 2 and 4 months of age with a third dose as booster administered at 10 to 11 months of age. Austria, Denmark, Finland, Norway, Sweden, Iceland, and Italy use a two-dose late schedule, whereby doses are recommended at 3 and 5 months of age and a third dose as a booster is given around the first birthday (see Fig. 74.1).

Diphtheria, Tetanus, and Pertussis Vaccines

Thirty-nine (74%) of the 53 countries in the European Region use acellular pertussis antigens in their DTP vaccines for primary childhood immunization; the remaining 14 countries use whole-cell pertussis vaccines (see Fig. 74.1).

The weighted WHO/UNICEF estimated vaccine coverage in 2014 for the third dose of DTP by 1 year of age was 95% among the 45 countries that reported. The eight countries not reporting coverage estimates in 2014 were Austria, Czech Republic, Finland, Germany, Israel, Monaco, Poland, and Ukraine.

Recognition of the increasing pertussis burden in young infants has prompted provision of acellular containing vaccines to pregnant women.[72,73] Routine maternal immunization against pertussis has been initiated in Ireland, Portugal, Spain, Switzerland, and the United Kingdom.[74]

Haemophilus influenzae Type B Vaccine

Fifty-two countries (98%) have national programs for *Haemophilus influenzae* type b (Hib) vaccine. Belarus has not introduced the vaccine into the routine schedule; however the vaccine is available as a monovalent product for risk groups.

TABLE 74.1 Vaccines Included in Routine National Immunization Schedules Up to 18 Years of Age in 2014[a]

Country	BCG	Diphtheria	Tetanus	Pertussis	Polio	Measles	Mumps	Rubella	Varicella	Hepatitis B	Hepatitis A	Hib	Pneumococcal	Meningococcal	Rotavirus	HPV	Influenza	TBE
Albania	YES	YES	YES	wP	IPV+OPV	YES	YES	YES		YES		YES	YES					
Andorra		YES	YES	aP	IPV	YES	YES	YES		YES		YES	YES	MenC conj		YES		
Armenia	YES	YES	YES	aP	OPV	YES	YES	YES		YES		YES	YES		YES			
Austria		YES	YES	aP	IPV	YES	YES	YES		YES		YES	YES	MenACWY	YES	YES		YES
Azerbaijan	YES	YES	YES	wP	OPV	YES	YES	YES		YES		YES	YES					
Belarus	YES	YES	YES	wP+aP	IPV+OPV	YES	YES	YES		YES							YES	
Belgium		YES	YES	aP	IPV	YES	YES	YES		YES		YES	YES	MenC conj	YES	YES		
Bosnia and Herzegovina	YES	YES	YES	wP	IPV+OPV	YES	YES	YES		YES		YES						
Bulgaria	YES	YES	YES	aP	IPV	YES	YES	YES		YES		YES	YES					
Croatia	YES	YES	YES	aP	IPV	YES	YES	YES		YES		YES						
Cyprus		YES	YES	aP	IPV	YES	YES	YES	YES	YES		YES	YES	MenC conj				
Czech Republic		YES	YES	aP	IPV	YES	YES	YES		YES		YES				YES		
Denmark		YES	YES	aP	IPV	YES	YES	YES				YES	YES			YES		
Estonia	YES	YES	YES	aP	IPV	YES	YES	YES		YES		YES			YES			
Finland		YES	YES	aP	IPV	YES	YES	YES				YES	YES		YES	YES	YES	YES[b]
France		YES	YES	aP	IPV	YES	YES	YES	YES	YES		YES	YES	MenC conj		YES		
Georgia	YES	YES	YES	wP	OPV	YES	YES	YES		YES		YES	YES		YES			
Germany		YES	YES	aP	IPV	YES	YES	YES	YES	YES		YES	YES	MenC conj	YES	YES		
Greece	YES	YES	YES	aP	IPV	YES	YES	YES	YES	YES	YES	YES	YES	MenC conj + MenACWY	YES	YES		
Hungary	YES	YES	YES	aP	IPV	YES	YES	YES		YES		YES	YES			YES		
Iceland		YES	YES	aP	IPV	YES	YES	YES				YES	YES	MenC conj		YES		
Ireland	YES[b]	YES	YES	aP	IPV	YES	YES	YES		YES		YES	YES	MenC conj		YES		
Israel		YES	YES	aP	IPV+bOPV	YES	YES	YES	YES	YES	YES	YES	YES		YES	YES	YES	
Italy		YES	YES	aP	IPV	YES	YES	YES	YES	YES		YES	YES	MenC conj		YES		
Kazakhstan	YES	YES	YES	aP	IPV+OPV	YES	YES	YES		YES	YES[b]	YES	YES			YES		
Kyrgyzstan	YES	YES	YES	wP	OPV	YES	YES	YES		YES		YES						
Latvia	YES	YES	YES	aP	IPV	YES	YES	YES	YES	YES		YES	YES			YES	YES	
Lithuania	YES	YES	YES	aP	IPV	YES	YES	YES		YES		YES	YES					
Luxembourg		YES	YES	aP	IPV	YES	YES	YES	YES	YES		YES	YES	MenC conj	YES	YES		
Malta		YES	YES	aP	IPV	YES	YES	YES		YES		YES				YES	YES	

Monaco		YES	YES	aP	IPV	YES	YES	YES		YES		YES	YES	MenC conj		YES	
Montenegro	YES	YES	YES	aP	IPV+OPV	YES	YES	YES		YES		YES					
Netherlands		YES	YES	aP	IPV	YES	YES	YES		YES		YES	YES	MenC conj		YES	
Norway		YES	YES	aP	IPV	YES	YES	YES				YES	YES		YES	YES	
Poland	YES	YES	YES	wP	IPV+OPV	YES	YES	YES		YES		YES					
Portugal	YES	YES	YES	aP	IPV	YES	YES	YES		YES		YES		MenC conj		YES	
Republic of Moldova	YES	YES	YES	wP	IPV+OPV	YES	YES	YES		YES		YES	YES		YES		
Romania	YES	YES	YES	aP	IPV	YES	YES	YES		YES		YES					
Russian Federation	YES	YES	YES	wP	IPV+OPV	YES	YES	YES	YES[b]	YES	YES[b]	YES	YES			YES	YES
San Marino		YES	YES	aP	IPV	YES	YES	YES		YES		YES				YES	
Serbia	YES	YES	YES	wP	OPV	YES	YES	YES		YES		YES					
Slovakia		YES	YES	aP	IPV	YES	YES	YES		YES		YES	YES				YES
Slovenia		YES	YES	aP	IPV	YES	YES	YES		YES		YES				YES	YES
Spain		YES	YES	aP	IPV	YES	YES	YES	YES	YES		YES		MenC conj		YES	
Sweden		YES	YES	aP	IPV	YES	YES	YES				YES	YES		YES[b]	YES	
Switzerland		YES	YES	aP	IPV	YES	YES	YES	YES	YES		YES	YES	MenC conj		YES	
Tajikistan	YES	YES	YES	wP	OPV	YES	NO	YES		YES		YES					
The former Yugoslav Republic of Macedonia	YES	YES	YES	wP	OPV	YES	YES	YES		YES		YES				YES	
Turkey	YES	YES	YES	aP	IPV+OPV	YES	YES	YES	YES	YES	YES	YES	YES				
Turkmenistan	YES	YES	YES	wP	OPV	YES	YES	YES		YES		YES					
Ukraine	YES	YES	YES	wP+aP	IPV+OPV	YES	YES	YES		YES		YES					
United Kingdom of Great Britain and Northern Ireland		YES	YES	aP	IPV	YES	YES	YES				YES	YES	MenC conj	YES	YES	YES
Uzbekistan	YES	YES	YES	aP	OPV	YES	YES	YES		YES		YES			YES		

[a]Risk group recommendations not included.
[b]Part of country.
aP, acellular pertussis vaccine; BCG, bacillus Calmette-Guérin; Hib, *Haemophilus influenzae* type b; HPV, human papilloma virus; IPV, inactivated polio vaccine; MenACWY, meningococcal ACWY vaccine; MenC conj, meningococcal C conjugated vaccine; OPV, oral polio vaccine; TBE, tick-borne encephalitis; wP, whole-cell pertussis vaccine.
From WHO vaccine-preventable diseases: monitoring system. 20165 global summary. Available at: http://apps.who.int/immunization_monitoring/globalsummary/schedules.

In the past, not all the countries using Hib vaccine had used a three-dose primary series followed by a booster in the second year of life. Both the United Kingdom and Ireland initially used only a three-dose primary series; both subsequently introduced a booster dose and undertook catch-up programs for children younger than 5 years of age following detection of an increase in cases of invasive Hib disease among children older than 1 year.[75,76]

In 2006, the United Kingdom augmented its routine schedule to include Hib and meningococcal type C (Hib/MenC) combination vaccine, offered at 13 months of age, at the same time as the first MMR immunization. This provided a booster for both Hib and MenC primary vaccinations. The introduction of the Hib/MenC booster allowed the reduction of the number of primary series MenC vaccine doses from three (2, 3, and 4 months of age) to two doses (3 and 4 months of age). The first two doses of pneumococcal conjugate vaccine (PCV) are given at 2 months and 4 months of age with a third dose at 12 to 13 months of age. From summer 2014, only a single dose of MenC vaccine is given in the primary series at 3 months of age.

In 2014, 30 countries had a three-dose Hib primary series with a booster in the second year of life. Of the remaining countries, 12 gave three doses of Hib containing vaccine by 6 months of age and no booster; nine gave the two-dose primary series and a booster at 12 months of age; and the Ukraine's schedule recommends Hib-containing vaccines at 3, 4, and 18 months of age.

Hepatitis B Vaccine

Among the 53 European Region countries, 48 have universal immunization programs for hepatitis B and 18 have universal administration of a birth dose. Malta provides a series of monovalent hepatitis B vaccinations in the second year of life; Slovenia provides hepatitis B vaccine as an option during infancy but the immunization is considered mandatory by school entry; Hungary and Switzerland immunize adolescents. The remaining countries (Denmark, Finland, Norway, Sweden, and the United Kingdom) recommend hepatitis B vaccine in programs for high-risk groups. As a consequence of a change of the multicomponent vaccine product used in the Danish national immunization program in 2014, hepatitis B vaccine was temporarily provided with the primary series in infancy. Iceland does not use hepatitis B vaccine.

Human Papillomavirus Vaccine

HPV vaccine is recommended for use in 31 European Region countries including 23 EU/EEA countries (Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Luxembourg, Malta, the Netherlands, Norway, Portugal, Romania, Slovenia, Spain, Sweden, United Kingdom) and eight non-EU/EEA countries (Andorra, Israel, Kazakhstan, Monaco, Russian Federation [part of the territory], San Marino, Switzerland, and the former Yugoslav Republic of Macedonia) (Fig. 74.4).

There is no preference for type of vaccine: eight countries use both bivalent and quadrivalent vaccine. Only Austria recommends the inclusion of males for routine immunization. HPV vaccination is provided in schools in the United Kingdom, in nine of the 19 autonomous communities in Spain, Israel, Ireland, Kazakhstan, Sweden, and in the former Yugoslav Republic of Macedonia. The highest coverage rates are reported from countries using school-based immunization. Most countries have offered the vaccine in catch-up programs, although there are differences in the upper ages covered. Costs to vaccinees range from free for all female vaccinees through school-based programs in Denmark, to reimbursement through insurance in Germany, and with copayment in Belgium and France. Monitoring for impact on cervical cancer is being established in some countries.

The introduction of HPV vaccination into national schedules in Europe was reviewed in a VENICE II survey in 2010.[46,77]



Figure 74.4. Human papillomavirus vaccination in routine immunization program. *(Modified from WHO/UNICEF Vaccine-preventable Diseases Monitoring System Global Summary 2016. Available at: http://apps.who.int/immunization_monitoring/globalsummary/schedules.)*

Of the countries that reported coverage in 2009, two (Portugal and the United Kingdom) reported more than 80% for the third dose; Denmark, France, Italy, Luxembourg, and Norway reported coverage from 17% to 58%. France reported that 23% of the primary target group had received three doses of vaccine by December 2010. HPV vaccination was introduced in Spain in 2007 with immunization provided in schools or in health centers; higher coverage was reported from the schools and third-dose coverage was 77%. Health center–based immunization was implemented in the Netherlands, but only 53% of the 13- to 16-year-old girls of the 2009 cohort were vaccinated.

Over the period of 6 years starting from introduction of HPV vaccine in 2008, the annual coverage in the United Kingdom for the third dose ranged from 77.5% to 85.9%; the Netherlands reported coverage of 60.7% with three doses in 2014 and Germany estimates coverage with three doses to be just below 40% with higher uptake in older teenage cohorts.[78–80] Conversely, Ireland had higher uptake in first-level secondary schools age 12 to 16 years of age (84.9%) compared to sixth grade 16 to 18/19 years of age (44.6%) in 2013–14.[81] While public health authorities are addressing obstacles to achieving higher uptake, early surveillance data from Denmark and Sweden are noting substantial reduction of cervical lesions and genital warts respectively at the currently achieved coverage levels.[82,83]

Influenza Vaccine

The WHO Regional Office for Europe, in partnership with the VENICE Collaboration and the ECDC, conducted a comprehensive survey on seasonal influenza vaccine policies and vaccination uptake (2008–09 and 2009–10 seasons) among all 53 Member States of the Region.[84] Forty-seven countries (89%) responded to the survey. Of these, 37 countries (79%) had influenza vaccine recommendations for the elderly and eight countries recommended vaccination for all adults without specific reference to elderly populations. Of the 37 countries that recommended vaccination of the elderly, 29 (78%) had mechanisms in place to monitor uptake for this specific population group.[84] Of the 47 countries responding to the survey, 18 countries recommended vaccination for children, 41 recommended influenza vaccination for all or some healthcare workers, and 29 recommended vaccination of pregnant women.[84] In an updated survey conducted following the 2012–13 season, no significant changes were observed in EU/EEA countries.[85]

Vaccination coverage for elderly populations for the seasons 2008–09 and 2009–10 ranged from 0.2% to 83%, with only one country reaching a 75% coverage target for the elderly.[84] A more recent report by ECDC for the 2012–13 season showed vaccination coverage rates between EU/EEA varied from 1% to 77.4%, with only the Netherlands having reached the 75% target, followed closely by the United Kingdom.[85] Finally, the VENICE III study outcomes on influenza vaccination provided for 2013–14 and 2014–15 show little change in coverage.

Most countries used either administrative coverage measurement or surveys to determine coverage rates; however, coverage by the different target groups is not available in all countries. The coverage among healthcare workers was known in 13 countries, ranging from 9.5% in Poland to 45.6% in England with a median of 28%.[85] The coverage for clinical risk groups was reported by nine countries ranging from 28% in Portugal to 80.2% in Northern Ireland, with a median of 50%.[85] The coverage for pregnant women in a subset of 10 countries ranged from 0.2% in Lithuania to 64.6% for all pregnant women in Northern Ireland (median: 16%).[85] The coverage for residents of long-stay care facilities was available in three countries: 71.1% in Slovakia, 73% in Ireland, and 89% in Portugal. In England, two-thirds of primary care physicians (general practitioners) report influenza vaccination coverage weekly using automated reporting systems that provide near real-time data by risk group. One hundred percent of general practitioners provide monthly data. National coverage data are also available by vaccination of type of healthcare worker and by locality.[86]

The United Kingdom introduced routine influenza vaccination of all children using live attenuated influenza vaccine in 2013. The program started with 2- and 3-year-old children and was extended to 4-year-old children the following year. Pilot programs of vaccination of school-age children have been implemented in a variety of settings (inner city, outer city, urban, and rural). Early results suggest that there have been reductions in influenza-like illnesses and respiratory infections in nonvaccinated groups in pilot localities.[87]

Measles, Mumps, and Rubella Vaccines

All countries in the European Region have routine two-dose measles vaccination programs.[3,88] By the end of 2014,47 countries (89%) used combined MMR vaccine; four used MMRV; the Russian Federation used MMR or measles only/measles-mumps with monovalent rubella vaccine; and Tajikistan used measles-rubella vaccine (see Fig. 74.2). Austria, France, Germany, Monaco, and Switzerland provide a second dose of measles-containing vaccine by 24 months of age. For 2014, the WHO/UNICEF estimated vaccine coverage for the first dose of measles-containing vaccine was 94% and 84% for the second dose in the European Region. Despite this high estimate of first-dose measles-containing vaccine coverage, there remain considerable local variations with pockets of low coverage in a number of countries. These coverage variations and consequent cohorts of susceptible individuals account for the continuing reports of measles outbreak, and to some extent rubella and mumps from European countries.

Meningococcal Vaccines

Sixteen Member States use meningococcal group C conjugate vaccines in their universal childhood immunization programs. Austria and Greece provide polysaccharide meningococcal ACYW vaccine to their adolescent populations. Cyprus, Finland, Israel, Kazakhstan, Latvia, Russian Federation, Serbia, Slovenia, the United Kingdom, and Uzbekistan use these vaccines for high-risk groups. The United Kingdom offers conjugate ACYW vaccine to teenagers and university students following a rise in group W135 infections.[89] The Czech Republic and United Kingdom introduced meningococcal B vaccine in 2015. Three doses are given in the United Kingdom at 2, 4 and 12 months of age. No catch-up campaign was implemented at the time of the launch of the program.

Pneumococcal Conjugate Vaccines

Thirty-six countries (68%) include PCVs in the national immunization schedule (Fig. 74.5). Those countries have been switching progressively from seven-valent vaccine to 10- or 13-valent vaccines. Some countries such as Denmark, Italy, and the United Kingdom use a three-dose schedule with doses at 2 to 3 and 3 to 6 months of age and a booster at 1 year of age; Bulgaria, Germany, Greece, and Turkey use a four-dose schedule. There does not appear to have been any difference in reported impact from the reduced dose schedules. The United Kingdom has reported high impact against strains of pneumococci contained in the seven-valent conjugate vaccine using a two-plus-one schedule, but has also reported



Figure 74.5. Pneumococcal conjugate vaccine (PCV) vaccination in routine immunization program. *(Modified from WHO/UNICEF Vaccine-preventable Diseases Monitoring System Global Summary 2016. Available at: http://apps.who.int/immunization_monitoring/globalsummary/schedules.)*

considerable increases in nonvaccine strains coincident with the immunization program. The United Kingdom switched to 13-valent conjugate vaccine in 2010 following a rise of the nonvaccine strains that emerged after the introduction of seven-valent vaccine. Eight years of PCV use in England and Wales has reduced the overall incidence of invasive pneumococcal disease by more than 50%. The herd protection induced by the seven-valent conjugate vaccine is continuing, and similar indirect protection is occurring from the additional serotypes covered by the 13-valent conjugate vaccine. There is, however, evidence of increasing invasive pneumococcal disease resulting from non–13-valent conjugate vaccine serotypes, particularly in children younger than 5 years of age in 2014.[90,91] Belarus, Croatia, Czech Republic, Iceland, Malta, Serbia, Slovenia, and Spain have recommendations only for vaccination of risk groups. Hungary introduced 13-valent vaccine in 2014 and Spain plans to introduce it in 2016.[51]

Polio Vaccine

The WHO/UNICEF estimated vaccine coverage for the third dose of polio vaccine (inactivated polio vaccine [IPV]/oral polio vaccine [OPV]) by 1 year of age was 95% for the European Region. Nine countries (17%) used OPVs for routine immunization of infants in 2014, compared to 16 in 2011. Eleven countries (21%) have a sequential IPV-then-OPV strategy and 33 (62%) use only IPV.[3,92] Following silent circulation of wild polio virus type 1 in Israel in 2013, the national immunization schedule that previously included IPV-containing vaccines at 2, 4, 6, and 12 months of age was amended with two additional doses of bivalent OPV (bOPV) at 6 and 18 months of age.[93] Countries that were using trivalent oral polio vaccine (tOPV) switched to inactivated polio vaccine (IPV) alone (Poland and Belarus) or bivalent oral polio vaccine (bOPV) in April 2016 and destroyed any residual tOPV stocks. Countries that had been using only tOPV were encouraged to introduce at least one dose of IPV into their schedules. Due to supply shortages, IPV introduction has been delayed in Moldova, Kyrgyzstan, Tajikistan, Uzbekistan, and Turkmenistan.[93a]

Rotavirus Vaccine

Rotavirus vaccine is recommended for routine use in Austria, Armenia, Belgium, Estonia, Finland, Georgia, Germany, Greece, Israel, Italy, Luxemburg, Norway, Republic of Moldova, the United Kingdom, and Uzbekistan (Fig. 74.6). Sweden has introduced the vaccine in two of 21 counties. Albania, Latvia, and Tajikistan introduced the vaccine in 2015. Impact data from Austria, Belgium, Finland, Spain and the United Kingdom all confirm high effectiveness as observed in non-European countries.[94,95]

Varicella Vaccine

Cyprus, Germany, Greece, Latvia, Luxemburg, Spain, Switzerland, and Turkey have recommendations for the use of varicella vaccine in their universal childhood immunization programs.[3,96] Iceland, France, Slovenia, and the United Kingdom use it among specific groups. To date, reliable data on coverage are not available.

Zoster vaccines for older individuals have been introduced in Austria, France, and the United Kingdom. Coverage in the first vaccinated cohorts of 70- and 79-year-old individuals in England are approximately 52%.[97]

CHALLENGES TO IMMUNIZATION IN THE EUROPEAN REGION

Vulnerable Groups

All countries in the European Region have persons and groups who are difficult to vaccinate because they are isolated from routine health services for reasons of culture, language, ethnicity, or frequent change of place of residence. These groups

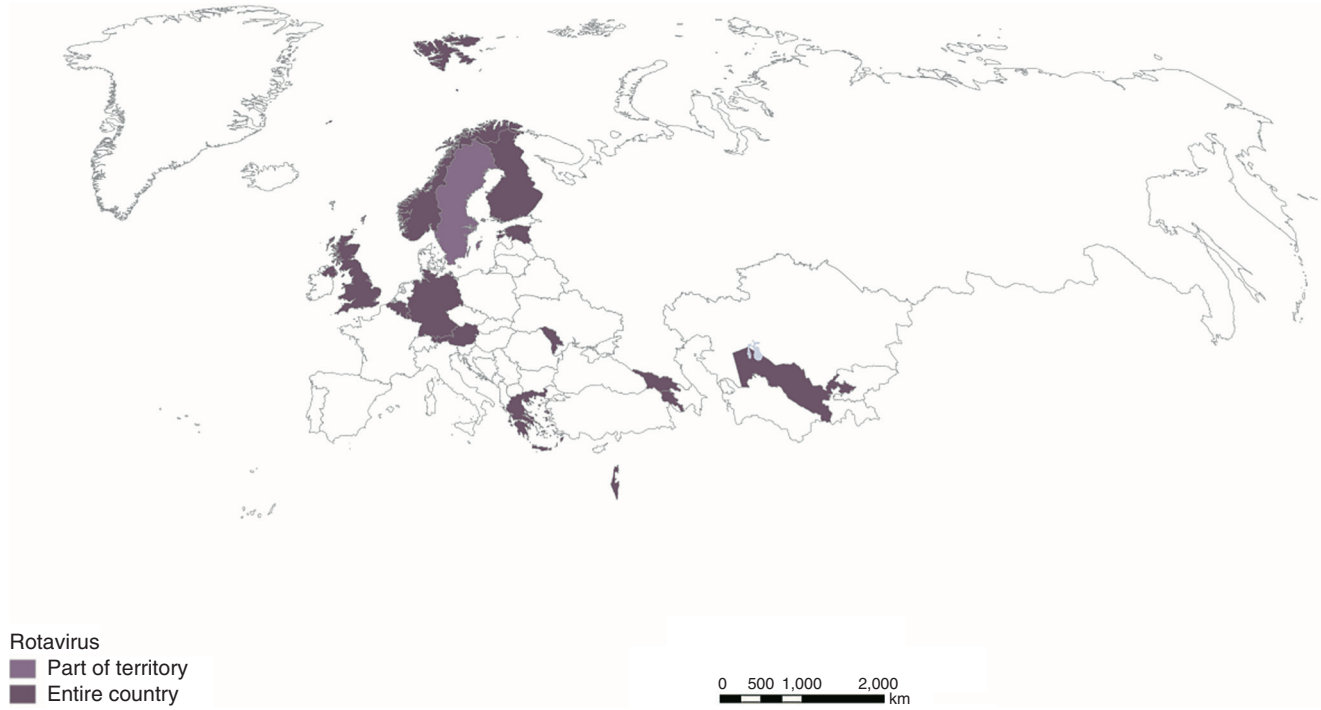


Figure 74.6. Rotavirus vaccination in routine immunization program. *(Adapted from WHO/UNICEF Vaccine-preventable Diseases Monitoring System Global Summary 2016. Available at: http://apps.who.int/immunization_monitoring/globalsummary/schedules.)*

include immigrants, asylum seekers, refugees, persons characterized as Irish Travelers, Roma, or gypsies, as well as migrant workers and their family members.[98] Outbreaks of measles and rubella have occurred among these groups in Greece, Germany, United Kingdom, Serbia, Spain, and a number of other countries in the European Region.[99–102]

It has been estimated that 6.8 to 8.9 million of the 12 to 15 million Roma living around the world live in Europe.[19] Most of the Roma are thought to live in Central and Eastern Europe but some Western and Northern European countries also have sizable populations. It is thought that many of these Roma might not have adequate access to or appropriately use health services available within the countries in which they live for a number of reasons, including personal knowledge and understanding, cultural practices, geographic isolation, and more systemic barriers within the health system.[19]

In 2009–11, the measles virus Hamburg D4 spread primarily within the Roma populations in a number of countries in the Region, including Bulgaria, Romania, Spain, and Turkey.[103] The ensuing nationwide outbreak in Bulgaria resulted in more than 24,000 cases and 24 deaths from measles-related complications.[104,105] This outbreak underscores the need to strengthen access to services among vulnerable populations, bringing vaccine to these populations through extended routine immunization or specifically targeted services. It is of concern that these groups also might not be adequately immunized against polio, tetanus, and diphtheria.

Vaccine Hesitancy

Clear trends are emerging related to vaccination coverage in Member States of the whole Region. After the creation of national immunization programs in the 1980s and 1990s, there was high acceptance of immunization as evidenced by the high immunization coverage rates reached (>90%) for most vaccines. However, in recent years the trend has been affected by fears over vaccine safety, public acceptance of mandatory immunizations, as well as other behavioral factors and determinants. The United Kingdom has had to deal with a long legacy of claims, not supported by scientific evidence, that MMR vaccine induced autism, resulting in over a decade of suboptimal MMR uptake.[106] In 2006, four deaths in Israel in temporal association with seasonal influenza vaccination led to a 25% decrease of vaccination coverage in the elderly compared to previous years and a marked increase of pneumonia deaths during the 2006–07 influenza season.[107] Anecdotal reports of multiple sclerosis occurrences following hepatitis B vaccination in France resulted in very slow uptake of the vaccine from 51% in 2009 at its introduction to 83% in 2014.[3] Suspicions of an association between HPV immunization and sudden death (Austria, Germany, Kazakhstan, and Spain) or infant vaccines and sudden death (Bosnia and Herzegovina, Germany) were never confirmed, but have threatened to disrupt vaccination programs.

As a consequence of the perceived absence of vaccine-preventable diseases in Europe and shifts to a more safety-oriented and risk-averse public, antivaccination sentiment has been growing in many European countries. However, in the United Kingdom, where there has been a long experience of tracking of parental attitudes to vaccination there has been a progressive and encouraging shift of parental opinion away from weighing up the pros and cons to automatically vaccinating when the immunization was due.[108–110] Studies show the importance of accurate information being available for parents and healthcare workers whose influences play a major role in determining immunization coverage.[108,111–113]

Vaccine Resistance Because of Religious Beliefs

Anthroposophic Communities in German-Speaking Countries and Elsewhere

Between 2001 and 2014, Germany reported 21,498 cases of measles, of which the highest incidence over the last 2 years was reported from the states of Berlin, Bayern, and

Brandenburg.[114] In spite of reported immunization coverage of greater than 90% by the age of school entry, numerous pockets of low vaccination coverage remain, some of which were linked to the anthroposophic beliefs of parents and some physicians. In November 2001, an outbreak in Coburg, Bavaria, which started in an anthroposophic school opposed to vaccination, infected 1100 people, of whom 398 developed complications such as pneumonia and 21 were hospitalized.[115] Further outbreaks occurred in 2005 and 2008 where there were concentrations of unvaccinated children in some communities, certain schools, and childcare facilities (most of which had connections with the anthroposophic teachings of Rudolf Steiner).[115,116] Outbreaks from similar settings have been repeatedly reported from Austria and Switzerland.[117–120] People of these communities are selective about the medical services they use and often refuse vaccination, especially for measles. Outbreak assessments in 2013 of measles outbreaks in Waldorf schools of Bremen, Erftstadt, and Landsberg indicated that less than half of students attending the schools had been vaccinated against measles. In 2014, upon return of a student with measles from abroad, half of school children attending an anthroposophic school in Finland were placed under quarantine because they were unvaccinated against measles.[120] The German Gesellshaft Antroposophischer Ärtze in Deutschland e.V. has regularly published the leaflet "Merkblatt Masern." In the issue published August 2015 a change in the view of measles can be noted; measles is no longer regarded a childhood disease only because it may cause severe disease in older children and pregnant women.[121] Among other consequences of nonvaccination mentioned are risks to exposed infants when older siblings are ill, the obligation to avoid exposure of others, and the willingness to accept an extended absence from kindergarten or school. It should be noted that the "Merkblatt Masern" cannot be compared to issued guidelines in conventional medicine and it is unknown if this change in views will have an impact on immunization coverage long term among the anthroposophic communities in Germany and elsewhere.

Religious Groups in the Netherlands Rejecting Vaccination

In 1992, the *New Scientist* reported "Dutch Panic over Polio Outbreak."[122] At the time of the report, only one case of polio—in a 14-year-old boy—had occurred. However, this child came from a community that rejected immunization and outbreaks of polio had occurred previously in the same community, notably in 1978. The *New Scientist* report noted that "the affected boy and his family were members of a strict Calvinist church (Dutch Orthodox Reformed Church) that rejects vaccination on the grounds that acquiring disease is an 'act of God.'"

The outbreak in the end affected 71 individuals (age range: 10 days to 61 years of age; median: 18 years), of whom two died and 59 developed paralysis. None of the patients had been vaccinated and all but one belonged to a socially and geographically clustered group of people who refuse vaccination. Despite the cases occurring in the group that rejected immunization and the widespread transmission in that community, no cases were detected in the remainder of the Dutch population.[123] Although there was no evidence for ongoing transmission within the Dutch population, there was evidence that the poliovirus responsible for the Netherlands outbreak had been exported into Canada with circulation among a community that shared the beliefs of the Netherlands group.[124]

Measles and rubella outbreaks are also repeatedly recorded among individuals of the Orthodox Dutch Reformed Church denomination, including measles related deaths or cases of congenital rubella syndrome.[125–127] The Netherlands is making efforts to work with the religious groups to prevent such vaccine-preventable outbreaks and to ensure that cases do not spread outside of the identified communities.[128]

Availability of Vaccines

Vaccine procurement by member states or through UNICEF Supply Division has ensured stable access to vaccine supplies. However, a total of 16 products have been reported to be in short supply in 2014, the most frequently being bacille Calmette-Guérin (BCG), and acellular and whole-cell pertussis–containing vaccines.[3,49,129] Bosnia and Herzegovina, Bulgaria, Croatia, Montenegro, Romania, and Slovakia reported "stock-outs" leading to interruptions of immunization programs,[3] and Serbia reported a delay with IPV introduction in 2014 because of selection of a combination product that is in short supply. Similarly, difficulties with BCG vaccine availability have been reported because of a reduction of number of vaccine manufacturers producing the vaccine. In 2015, a large number of EU/EEA Member States reported stock-outs of acellular-containing vaccines and vaccine shortage was the subject of the European Commission's Health Security Committee meetings under the new serious cross-border threats legislation.[31,130]

Low Vaccine Uptake Leads to Outbreaks

A number of countries have faced periods of low vaccine uptake as a result of economic, political, or safety-related concerns. During the war in Bosnia and Herzegovina in the 1990s, a switch from MMR to measles-rubella (MR) and difficulties with access to immunization services rendered cohorts of children unimmunized or underimmunized. Coverage in later catch-up campaigns in the post-conflict period left the population susceptible to measles and mumps outbreaks.[131] The situation was further aggravated by significant antivaccination sentiments following media coverage of infant deaths in temporal association with routine immunization and reports of increased rates of reactions to the mumps component of MMR vaccine administered to older children and adolescents. A nationwide outbreak of measles in Bosnia and Herzegovina with more than 6,800 cases reported from January 2014 through December 2015, largely involving cohorts born during the conflict years and the immediate postwar period (1990–2000).[132,133] Most cases are among unimmunized persons or those with unknown immunization status.

During 2000–11, more than 65 million persons received measles-containing vaccines through supplemental immunization activities (SIAs) in 21 of 53 member states of the European Region.[3,134] Azerbaijan (2001, 2002) and Kyrgyzstan (2004, 2006) immunized more than 5 million persons 7 to 23 and 7 to 25 years of age, respectively, in addition to women of childbearing age up to 35 years of age. Lot quality assurance coverage surveys confirmed high coverage.[134] Georgia planned a nationwide SIA in 2008 targeting 980,136 individuals 6 to 27 years of age. Vaccination safety concerns raised during Ukraine SIAs interfered with vaccination uptake in Georgia, resulting in coverage of 50.3% in 2008.[134] Despite the SIAs, Georgia, Kyrgyzstan, and Ukraine reported more than 21,000 cases of measles from January 2014 until April 2015, prompting further SIAs.[135] Likely causes of these recent outbreaks are the gradual accumulation of susceptible individuals born after earlier measles and rubella supplementary immunizations activities were undertaken, presence of suboptimally performing immunization

programs, migration, changes of immunization systems, and instances of nosocomial transmission. The three countries organized a variety of SIAs between 2013 and 2014 and administered more than 2.5 million doses of measles-containing vaccine. Coverage in Azerbaijan and Kyrgyzstan was greater than 95%, while Georgia achieved only 14% of the targeted population 2 to 29 years of age.

Ukraine experienced a return of epidemic diphtheria in 1991 affecting all oblasts by 1995. It has been hypothesized that the diphtheria epidemic in Ukraine started among children, who had been left vulnerable as a result of inadequate childhood immunizations, and then quickly spread to inadequately protected adults.[136] At the peak of the epidemic, more than 5000 cases and more than 200 deaths were reported.[136] Selective target-group immunization was ineffective in controlling the outbreak, requiring a change of strategy. Campaigns conducted in all of the 27 oblasts and administrative regions of the country over an 18-month period led to a decrease of reported cases of diphtheria by 1996. The campaigns were affected by inadequate logistic and strategic preparation, unanticipated resistance from healthcare workers, and expression of distrust by a small, but significant, proportion of the population.[136] Between September 2005 and mid-June 2006, more than 50,000 measles cases were reported in Ukraine. Outcomes of a case-control study in undertaken in the Dnepropetrovsk region suggested vaccine effectiveness with one dose of measles vaccine of 63.0% (95% confidence interval [CI], –92.3% to 93.9%) and two doses of 92.0% (95% CI, 79.4% to 97.2%) when matched by school or university.[137] The level of population immunity achieved with two doses of vaccine still permitted sufficient numbers of susceptibles to accumulate over time and was estimated to be approximately equal to 440,000 in 2008.[137] A national MR campaign targeting the population 15 to 29 years of age, regardless of prior vaccination status, was planned for May 2008 but encountered obstacles similar to those experienced during the diphtheria campaign a decade earlier.[134,136,138] Within days of the campaign start, a 17-year old boy died within 13 hours of vaccination of an unrelated cause.[139] This event led to widespread concerns, a wave of hospitalizations among vaccinees, cancellation of the campaign, and extremely intense widespread antivaccination sentiments which persist. Since 2008, Ukraine has faced further major measles outbreaks and a significant decline of public trust in the routine immunization program, with major concerns about vaccine safety in particular. Vaccine procurement problems, major vaccine stock-outs, and erosion of the public health system are further reasons for the steep decline of vaccination coverage and accumulation of a significant cohort of children susceptible to vaccine-preventable diseases. In 2014, only 50% of children in Ukraine were immunized against polio (three doses of polio containing vaccine by 12 months of age) compared to 99% in 2004.[3] In 2015, Ukraine reported two cases of circulating vaccine-derived poliovirus type 1, both from the Zakarpatskaya oblast, in southwestern Ukraine, bordering Romania, Hungary, Slovakia, and Poland.[140]

Failure to maintain high coverage through routine immunization services over long periods present a serious challenge to maintaining polio-free status.[17,141] In 2010, importation of wild-type 1 polio virus resulted in a large-scale outbreak of poliomyelitis in Tajikistan with 460 laboratory-confirmed cases of poliomyelitis, including 29 deaths. The virus also spread to neighboring countries and in 2010 the Russian Federation reported 14 laboratory-confirmed cases of poliomyelitis and one death, Turkmenistan reported three cases, and Kazakhstan reported one case, all resulting from wild poliovirus type 1 from Tajikistan.

Silent Wild Poliovirus Circulation in Israel

Environmental surveillance for enteroviruses was established following the 1988 polio outbreak in Israel. Composite sewage samples have been collected monthly from sentinel sites covering 30% to 40% of the Israeli population using computerized automatic inline samplers located at the mouth of sewage treatment facilities.[142] In April 2013, wild poliovirus type 1 of the South Asia (SOAS) lineage was recovered from sewage in southern Israel.[143] The virus was detected in sewage only; no case of paralytic polio has been reported for the duration of the outbreak. The Israeli routine immunization schedule had recommended three doses of IPV by the age of 6 months and two boosters at 12 months and 7 years of age. The use of OPV had stopped in 2005. IPV3 coverage has been maintained at between 94% and 96% over the last decade. A stool sample study in 2013 indicated that majority of shedders belonged to a cohort of children immunized with IPV vaccine only.[143] The outbreak response included intensification of environmental, enterovirus, and AFP surveillance; a catch-up campaign using IPV vaccine at the epicenter of the outbreak was followed by a national vaccination campaign using bOPV vaccine. The first bOPV round achieved coverage of 79% (range by district: 65% to 92%); a second round was conducted only in the Southern district and reached coverage of 52%.[93] Public health authorities faced several challenges communicating the need for a mass campaign in the context of asymptomatic wild polio virus transmission.[144] Based on a review by the Israeli NITAG and the National Polio Certification Commission, the Israeli routine immunization schedule has been modified by reintroducing OPV (bOPV) vaccine into the routine schedule for infants born after July 2013; the schedule is now IPV at 2, 4, 6, 12 months, and 7 years of age, in addition to bOPV at 6 and 18 months of age. The last wild poliovirus type 1 isolation from an environmental source in Israel was in March 2014.

Vaccine Safety Issues

Autism and Measles-Mumps-Rubella Vaccine

Starting in 1993, a number of scientific articles were published reporting studies of the relationship of measles or measles-containing vaccines and chronic inflammatory bowel disease and autism. Most of the studies originated from one group working in the United Kingdom. The studies attempted to show that Crohn disease and, variably, ulcerative colitis was associated with either early measles infection or measles immunization. The studies were based on either epidemiologic or virologic data.[93,142–150] These claims for infection or vaccine-linked causes of inflammatory bowel disease received much media attention. As each new claim appeared, work was subsequently published that failed to confirm the earlier findings.[151–167]

The publication of each report that failed to confirm the earlier claims attracted less media interest than the original claims, leaving the impression that the putative associations with vaccination were robust.[168] In 1998, new claims were made of an association between MMR immunization and bowel disease and autism.[169] Although the authors were careful to say that their findings did not confirm a causal association, the media reported the findings as if the study had implicated the vaccine. At a press conference timed to coincide with the publication, one of the paper's authors stated that he believed that autism and bowel disease might be preventable by separating MMR and giving its ingredient vaccines singly with year-long intervals between doses. There remains no published epidemiologic evidence that confirms a causal association between MMR vaccine and autism. Numerous studies using different populations and different analytic techniques

have failed to confirm any increased risk of autism or bowel disease in association with MMR.[170–181]

Virology studies had reported as showing evidence for the presence of measles virus genetic material in bowel tissue, blood, and cerebrospinal fluid. These reports have come from one group, or workers associated with that group, and there has been no fully independent confirmation of these claims.[182] Three separate other groups have studied peripheral blood lymphocytes from autistic children and failed to find evidence of persistence of measles infection.

After 1997, there often was high media interest in MMR, culminating in early 2002, when the public and political focus became intense, with calls for either withdrawal of the vaccine or replacement with single-component vaccines for MMR. Vaccine coverage and public confidence in the safety of MMR had both been adversely affected, with coverage falling from 92% to 93% in the late 1990s to 83% in 2002.[183,184] During 2001 and 2002, it became clear that those opposed to the use of MMR vaccine were adopting a new strategy: The results of studies that challenged the safety of MMR were released to the media before exposure to the scientific community, exacerbating the public perception of risk from the vaccine without any opportunity to evaluate the quality of the work. More recently, and closely linked to the disappearance of media support for the MMR–autism story, coverage has risen, with matching returns in assessments of parents' confidence in the safety of MMR. The loss of media support for the proponents of the MMR–autism story followed newspaper and television investigations into the events surrounding the research of those proposing the link.[185] Additionally, two detailed studies, undertaken by journalists, revealed the impact of unbalanced reporting on a major public health issue.[186,187]

In 2010, following a protracted investigation by the General Medical Council, the body responsible for the registration of physicians in the United Kingdom, the names of two of the authors of the 1998 *Lancet* paper were removed from the UK Medical Register, preventing them from practicing medicine in the United Kingdom. In 2011, the *British Medical Journal* published a series of linked articles by an investigative journalist that demonstrated that in addition to the charges found proved by the General Medical Council, there had been extensive research fraud.[188–190]

Despite the decline in coverage, cases of measles remained rare until 2006, with fewer than 100 confirmed cases in 2005.[191] However, from 2006, confirmed cases started to rise, with many cases being part of a linked series of outbreaks across the United Kingdom. Many of the cases occurred in the Travelers community, often originally Irish, non-Roma gypsy, mobile groups.[192,193] Current small outbreaks have been linked to travel to Europe.

The Department of Health in London adopted a clear strategy to respond to the threats to the MMR immunization program. New information materials were disseminated widely to health professionals, and matching materials were prepared for parents. Immunization coordinators with local responsibility for implementing immunization services were briefed with all new materials as they became available. Newspaper and radio advertisements were used to direct parents to sources for these information materials. In addition to making information available on existing Department of Health and allied Web sites, a site dedicated to MMR was set up, with a facility for parents to send in their MMR-based questions.[194] Despite pressure from the media and parents for single vaccines to be made available to allow parents to choose whether their children would have MMR or single vaccines, single vaccines remained available only outside of the National Childhood Immunization Programme, and many of the products were unlicensed. Throughout the time of the campaign against MMR, there was strong political support for the vaccine, despite intense media-driven pressure.

The results from repeated media stories linking MMR vaccine with autism and other adverse outcomes led to progressive loss of confidence in the safety of MMR vaccine among health professionals and parents.[109,183,184] As a consequence, MMR vaccine coverage fell from greater than 90% in 1994 to approximately 80% nationally in 2003–04, but with much lower figures in some parts of the country, especially in London and South Wales.[195,196] Since 2005, however, MMR coverage has progressively increased as anti-MMR stories faded from the media, and as local campaigns improved vaccination uptake.[197]

As a result of these activities, coverage started to rise in 2005, with consistent increases in coverage at 16 months of age and with commensurate increases in 2-year coverage (Fig. 74.7). In 2015, MMR coverage in the United Kingdom exceeded

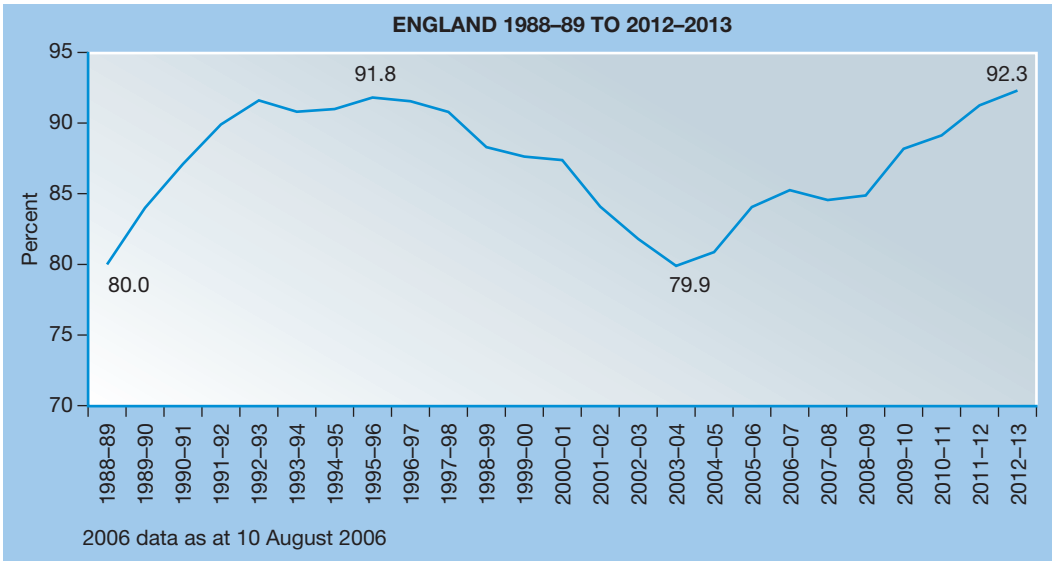


Figure 74.7. Measles-mumps-rubella (MMR) coverage at 24 months of age. *(From Mason BW, Donnelly PD. Impact of a local newspaper campaign on the uptake of the measles mumps and rubella vaccine.* J Epidemiol Community Health. *2000;54:473–474.)*

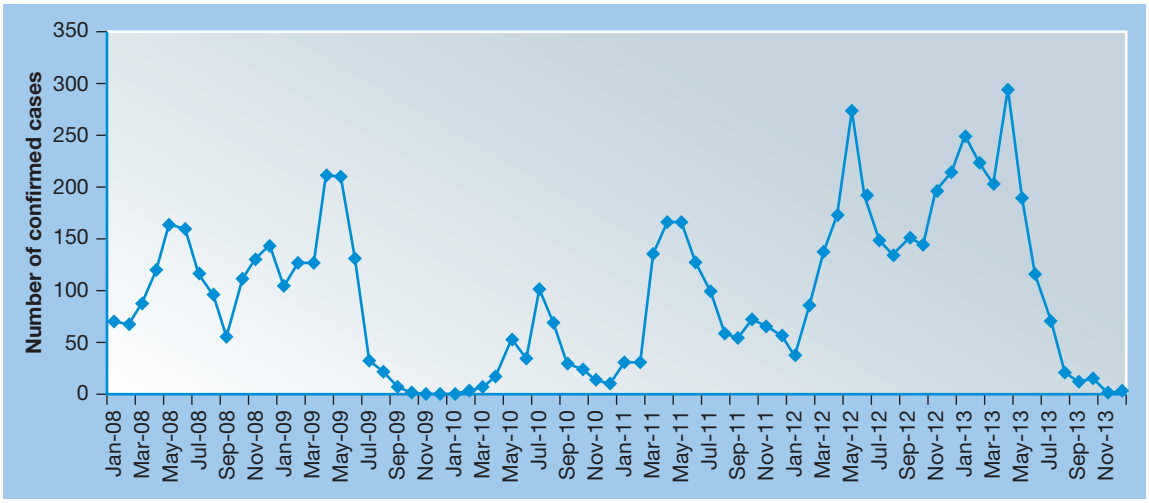


Figure 74.8. Number of confirmed measles cases by month of onset, England 2008–2013. *(From Ramsay ME, Jin L, White J, et al. The elimination of indigenous measles transmission in England and Wales.* J Infect Dis. *2013;187 Suppl 1:S198–S207.)*

90% by the third birthday and reached that level by the second birthday.

Measles had been virtually eliminated from England and Wales between 1994 and 2001, but by 2006 there were resurgences of localized measles outbreaks (although no national level outbreaks occurred).[198] Despite the apparently reassuring increases in MMR coverage, exceeding 90% by 2011, there was still a high risk of measles resurgences, owing to the cohorts of undervaccinated children born during the years when MMR coverage was lowest. As these cohorts began to attend school, they started to come into contact with many more children, raising the risk of measles transmission. In 2012, measles cases rose to levels that had not been seen in England and Wales since 1989, reaching a peak of 1912 confirmed cases (Fig. 74.8).[199] Multiple outbreaks were detected, especially in the North East, the North West and in South Wales. Cases continued to rise up to the first quarter of 2013.

The highest numbers of cases were in children and young people who had been born during the years of lowest MMR vaccine coverage, when anti-MMR rhetoric was at its height. A national MMR vaccine catch-up campaign was therefore rolled out, targeted at those who had had no MMR vaccine (those 10 to 16 years of age were the highest priority), followed by those who had only had a single dose of MMR. By the midpoint of the 2013 campaign, coverage in the 10- to 16-year-old group was estimated to have reached more than 95%. Since the completion of the campaign, measles cases have reverted to extremely low levels.

Narcolepsy Associated With Use of an AS03-Adjuvanted Influenza A (H1N1)PDM09 Vaccine Produced and Used in Europe

The AS03-adjuvanted influenza A (H1N1)pdm09 vaccine (Pandemrix) was the most used vaccine in Europe for protection against the 2009 influenza A pandemic. It has been estimated that more than 30.5 million Europeans received Pandemrix.[200] Although at least 20 EU/EEA countries are known to have administered Pandemrix to their population in vaccination campaigns conducted in 2009–10, the amount of vaccine used per unit population was highly variable and there were also important differences in the age groups that received the vaccine. The highest overall vaccine coverage was obtained in Finland, Ireland, Norway, and Sweden, where school-based immunization targeting children and adolescents were common.

Pandemrix contained the WHO-recommended flu strain A/California/7/2009 (H1N1) v-like strain (X-179A), an immunostimulating adjuvant (AS03) that included α-tocopherol (vitamin E) and squalene, and the often-used preservative thiomersal. Pandemrix was produced in Dresden, Germany. A very similar AS03-adjuvanted influenza A (H1N1)pdm09 vaccine (Arepanrix) was produced in Quebec, Canada by the same manufacturer. This vaccine was used extensively in Canada, including in children and adolescents.

Spontaneous adverse drug reaction reports of narcolepsy in children and adolescents previously vaccinated with Pandemrix started to appear in August 2010,[201] after vaccination campaigns had long ceased. Subsequent formal epidemiological studies confirmed an epidemiological association. The first study came from Finland in 2012 and showed a 12.7-fold increased risk for development of narcolepsy in children and adolescents between 4 and 19 years of age observed within 8 months after vaccination when compared with unvaccinated individuals in the same age group.[202,203] A series of further studies conducted in France, Ireland, Norway, Sweden, and the United Kingdom confirmed the epidemiological association. Similar studies conducted in Canada suggest a significantly smaller increased risk.[42,204–208] By January 2015, a total of 1379 reports of narcolepsy following vaccination with the Pandemrix vaccine had been received in the EMA Eudravigilance database.

Narcolepsy is a chronic disorder presenting with excessive daytime sleepiness often combined in young individuals with cataplexy (a transient loss of muscle tone triggered by strong emotional stimuli such as laughter or sadness) and is linked to the HLA-DQB1*0602 haplotype and a dysregulation of the hypocretin ligand-hypocretin receptor pathway. Investigations of narcolepsy patients have shown that loss of hypothalamic cells results in deficiency of the neuropeptide hypocretin in the cerebrospinal fluid. However, the mechanism leading to this loss of hypothalamic cells is unknown.

Recently, a possible biological mechanism for development of narcolepsy following Pandemrix vaccination was proposed.[209] The study compared antibody reactions to the influenza nucleoprotein and the hypocretin receptor 2 protein. It showed that a significant proportion of sera from HLA-DQB1*0602 haplotype-positive narcoleptic patients with a

history of Pandemrix vaccination and antibodies to nucleoprotein contained cross-reactive antibodies to the hypocretin receptor 2 protein when compared to sera from nonnarcoleptic individuals with a history of either a 2009 influenza A (H1N1) infection or MF-59 adjuvanted influenza A (H1N1) pdm09 (Focetria) vaccination. Although this study provides a first hypothesis for a possible biological mechanism, further studies are urgently needed.

An earlier study noted higher levels of antibodies to nucleoprotein in children with DQB1*06:02 risk allele and in DQB1*06:02 transgenic mice immunized with Pandemrix when compared to controls.[210]

Adolescent and Adult Immunization Programs

School-based programs providing vaccines to adolescent populations are reported by 25 of 53 member states[3] providing mainly tetanus toxin, diphtheria and tetanus toxoids or tetanus, diphtheria, and pertussis boosters, HPV, and to a lesser extent meningococcal vaccine, primary series of hepatitis B, MMR or BCG following purified protein derivative testing. Twenty-three Member States report schedules involving adolescents; however vaccination services continue to be provided by pediatricians, general practitioners, or other means. Adult immunization programs are often organized for special groups; for example, occupational groups, pregnant women, those older than 65 years of age, and individuals with specific conditions. There are extensive data to show that some adult groups are underimmunized. Measles nosocomial outbreaks provide testimony of unimmunized or underimmunized healthcare workers.[211] In 2014, a 47-year-old index case with a history of travel to India during the incubation period was diagnosed with measles in Masaryk Hospital in the Czech Republic. Subsequently, over a period of 4 months, 305 suspected measles cases were reported, of which 186 cases were laboratory-confirmed. Of the laboratory-confirmed cases, 88 were healthcare workers: 54 were nurses, 12 medical doctors, and 22 other hospital workers in emergency, infectious disease, and dermatology departments.[212] Also in 2014, nosocomial infections were reported in three Latvian hospitals involving 13 healthcare workers, while Spain reported an outbreak in Catalonia of which 24% of cases were healthcare workers, most of whom were unimmunized or had received only one dose of measles-containing vaccine.[211]

Reaching adults for immunization is challenging, but recommendations for adult immunization differ widely across the region.[213,214] Accurate data on uptake are not readily available.

Vaccinology Training

There are few data available on the amount of training on vaccines and vaccination throughout European countries. One EU project, VACSATC (Vaccine Safety, Attitudes, Training and Communication), performed a benchmarking study on undergraduate training in vaccinology in Europe.[215,216] Results showed that training on vaccines and immunization is disseminated over a wide range of courses over several academic years during medical education and in nursing and midwifery schools. Topics such as immunology and vaccine-preventable diseases were well covered during the training, but major gaps in knowledge and competencies were identified in understanding vaccine safety, communication with parents, addressing antivaccine arguments, and performing practical skills. This survey underlined the rationale for adequate training, identified opportunities for improving training, and indicated that the medical and nurse undergraduate training in Europe is not sufficiently equipped to ensure that all future healthcare workers are able to deliver such responsibilities. Undergraduate immunization training should not replace specialization courses or postgraduate training, but it should ensure that every healthcare worker, after basic training, can and will effectively communicate about vaccination, based on knowledge, competence, and skills.

In the United Kingdom, an advisory group hosted by the then Health Protection Agency produced National Minimum Standards for Immunization Training to offer consistency in the training provided across the country and to aid in those areas where training is not yet established.[217] The authors observed that undergraduate courses rarely include much training about immunization and that the provision of (postgraduate) immunization courses for healthcare workers varied considerably between localities. Mid-level and higher-level training courses on vaccinology are increasingly available in different localities. Students were accepted from a wide range of related disciplines on a global basis.[218]

The WHO Regional Office for Europe in collaboration with the European Society for Paediatric Infectious Diseases (ESPID) has developed vaccinology training materials under a continuous medical education scheme.[218a]

The World Wide Web is a widely used source of information on various topics; however, some of the information provided is of dubious credibility and is at times misleading. This is particularly true in the area of vaccine safety, where many websites have been developed that provide unbalanced and misleading information. Often these websites are linked and thus can create undue concerns among health professionals and the general public.

In response, the WHO has supported the development of a network of websites that meet criteria of good information practices as approved by the Global Advisory Committee on Vaccine Safety.[219,220] Launched in 2004 by the WHO Geneva and the WHO Regional Office for Europe, the vaccine safety network now includes 53 WHO-approved websites globally, including 26 websites in 12 languages within the European Region. The listed sites are reevaluated for their adherence to credibility and content every 2 years. Further work is under way within the European Region to increase the number of approved websites as well as those available in major European languages, particularly Russian.

EUROPEAN REGION STRATEGY FOR STRENGTHENING NATIONAL IMMUNIZATION SYSTEMS

European Vaccine Action Plan

The European Vaccine Action Plan 2015–2020 (EVAP) is a regional interpretation of the Global Vaccine Action Plan developed to address the specific needs and challenges related to immunization in the WHO European Region. Aligned with Health 2020 and other key regional health strategies and policies, EVAP was formulated through a consultative process, reviewed by Member States and endorsed by the ETAGE on Immunization before submission to the 64th session of the Regional Committee for Europe in September 2014.[18,19,221] EVAP's aim is to guide European Member States towards a Region free of vaccine-preventable diseases. It establishes six goals (sustain polio-free status, eliminate measles and rubella, control hepatitis B infection, meet regional vaccination coverage targets at all administrative levels throughout the Region, make evidence-based decisions on introduction of new vaccines, and achieve financial sustainability of national immunization programs) and outlines a path to achieve them through defined objectives, priority action areas, proposed

actions, and an evaluation and monitoring framework. Relevant elements of EVAP progress are addressed in the following sections.

Measles and Rubella Elimination Goal

In 2005, the WHO Regional Committee for Europe recommended rubella elimination as part of a resolution on strengthening national immunization systems.[222] A revised strategic plan was published in 2005 and included five key strategies[88]:

- Achieve and sustain very high coverage (>95%) with two doses of measles vaccine and at least one dose of rubella vaccine through high-quality routine immunization services.
- Provide a second opportunity for measles immunization through SIAs to populations susceptible to measles.
- Provide rubella vaccination opportunities, including SIAs, to all rubella-susceptible children, adolescents, and women of childbearing age.
- Strengthen surveillance systems by rigorous investigation of cases and laboratory confirmation of suspected cases.
- Improve the availability of high-quality, valued information for health professionals and the public on the benefits and risks associated with immunization against measles and rubella.

With the widespread use of measles- and rubella-containing vaccines across the Region, reported measles and rubella cases have dramatically declined. During 2007 to 2009, measles incidence in the region declined to historically low levels of less than 10 cases per million. The decline in cases was a result of high immunization coverage with two doses of measles- and rubella-containing vaccines and implementation of SIAs. The latter were implemented in more than 10 countries targeting remaining susceptible age cohorts and reached more than 55 million persons during 2003 to 2009.[223] Most countries that implemented SIAs conducted them in less than 1 month; some countries, such as the Russian Federation, implemented them using a phased approach. Rubella-susceptible women who have immigrated into the European Region have been identified as an important target group and programs to immunize them will be necessary even after the elimination target for rubella has been met.[224]

Along with the decline of incidence and the reduction of outbreaks in the eastern part of the Region, the proportion of reported cases has increased in the western part of the region. In 2009, outbreaks in five countries in Western Europe accounted for 89.6% of reported cases. The major challenges to achieving the regional measles and rubella elimination goal by 2010 included ongoing outbreaks caused by a failure to vaccinate, rather than the failure of measles vaccine: nearly 90% of the reported measles patients eligible for vaccination had not received two doses of a measles-containing vaccine.[225] Although national measles immunization coverage estimates remain high, coverage at the subnational level and among vulnerable populations in many countries reveal coverage to be much lower than the recommended 95% or higher for two doses. In some instances, the outbreaks led to reestablished endemicity (Switzerland and the United Kingdom). Marginalized and vulnerable populations with limited access to immunization services and decreased demand for vaccines because of an increased focus on measles vaccine safety by parents have also been major challenges to achieving the elimination goal.

The existence of geographic areas with coverage less than 95% has resulted in pockets of susceptible individuals, sufficient to support transmission and lead to explosive outbreaks, as has been seen with the recent resurgence of measles cases in western and central European countries.[226,227] In 2009, the likelihood of eliminating measles by 2010 was assessed for all 53 member states. Although the regional elimination goal had been thought to be attainable, it was determined that it would not be met by the target date of 2010.[225] Therefore, in September 2010, the European Regional Committee revised the target date for elimination to 2015.[228]

Despite substantial progress made by many Member States since 2010, the regional target of measles and rubella elimination remains under threat. In 2012, there were 27,134 measles cases and 29,601 rubella cases reported in the Region.[229,230] In 2013, these figures were 31,520 and 39,367 respectively. Most measles cases in 2013 (96%; $n = 30,178$) were reported by nine countries: Georgia (7830), Germany (1773), Italy (2216), the Netherlands (2499), Romania (1074), Russian Federation (2174), Turkey (7404), Ukraine (3308), and the United Kingdom (1900). Most cases were among unvaccinated persons and more than one in three patients were 20 years of age and older. Gaps in population immunity were reconfirmed by reports of 16,156 measles cases in 2014, of which greater than 41% of cases were adults 20 years of age or older.[135] That year, more than 75% of European measles cases were reported from Bosnia and Herzegovina, Georgia, Italy, Russian Federation, and Ukraine. The highest incidence rates per 1 million population were in Bosnia and Herzegovina and Georgia at 5762 and 7379, respectively.[231] The number of cases for 2014 appears to have dropped to the lowest level since 2010 ($n = 30,604$).[135] However, measles transmission continued in 2015 with large-scale outbreaks reported in Bosnia and Herzegovina, Germany, Kyrgyzstan, and Serbia.

In 2013 and 2014, most Europe-wide rubella cases were reported by Poland ($n = 38,585$; 98% in 2013; $n = 6516$; 91% in 2014). In 2013, the incidence of rubella in boys and men was significantly higher than the incidence in girls and women (181.4 vs 23.9), as a result of the immunization policies (1987 to 2003) that provided single doses of rubella vaccine to 13-year-old girls only, and where no catch-up campaigns were implemented where two-dose MMR schedules were introduced.[232]

The Regional Verification Commission for Measles and Rubella Elimination, based on annual reports provided by national verification commissions, concluded that as of the end of 2013, there were 22 countries in which endemic measles transmission had been interrupted, and 23 countries in which endemic rubella transmission was interrupted. Of those, nine countries were at high risk of reestablishing endemic transmission of one or both diseases, mainly because of significant immunity gaps in the population. Thirteen Member States were considered to have endemic measles transmission, and of these, nine were considered to also have endemic rubella transmission.[233]

To stop both endemic and imported measles virus circulation, all efforts continue to be directed toward reaching very high immunization coverage and population immunity for these diseases in all countries. The "Package for Accelerated Action" for measles and rubella elimination identifies priority areas in which the Regional Office provides technical support to Member States as they seek to eliminate these vaccine-preventable diseases, and sets indicators and milestones by which progress resulting from the efforts of all stakeholders can be measured.[234] Political commitment and accelerated action by Member States and partners remain the most crucial factors in achieving elimination of measles and rubella in the WHO European Region.

Polio Eradication Goal

In 2002, the Regional Certification Commission for Polio Eradication reviewed evidence from all countries in the European Region and announced that transmission of wild polio viruses had been interrupted, based on the evidence presented.[22] The continuing priorities were maintenance of certification level surveillance, laboratory containment, and sustainability of polio immunization programs.

Each country has its own method for implementing polio surveillance (see paragraph on surveillance of vaccine-preventable diseases above). Nine countries in the Region reported AFP surveillance as their only polio surveillance system in 2014, and an additional nine reported exclusive use of other types of surveillance in the absence of AFP surveillance. The remaining 34 countries (except San Marino) reported using AFP surveillance together with a variety of supplementary surveillance systems or activities. For those countries continuing to report AFP surveillance, the AFP index of surveillance quality remains high in Member States from the eastern part of the Region and poor in those in the southwestern part of the Region. Five countries claiming to maintain AFP surveillance failed to detect any AFP cases in 2014.[235]

Sustained indigenous transmission has not recurred since polio elimination was certified in 2002. However, the risk of importation of wild polioviruses into the European Region became a reality in 2010 when type 1 wild poliovirus genetically related to virus from Uttar Pradesh, India, was imported into Tajikistan, and in 2013 when silent transmission of wild polio virus type 1 of South Asian genotype was confirmed in Israel through environmental surveillance. In 2010, Tajikistan conducted six rounds of national SIAs with two rounds in children younger than 6 years of age and four rounds in children younger than 16 years of age. Uzbekistan conducted four national SIAs and one round of subnational immunization days. SIAs were conducted also in Turkmenistan (three national SIAs), Kyrgyzstan (two national SIAs), Kazakhstan (one national SIA and one subnational immunization day), and the Russian Federation (two subnational immunization days). The effective response measures ensured that wild poliovirus transmission was interrupted within 6 months after detection of the first case. In 2011, the five Central Asian republics, the Russian Federation and Azerbaijan conducted coordinated SIAs to close any immunity gaps. In total more than 45 million doses of monovalent OPV1 and trivalent OPV were used for outbreak response immunization activities in the Region. Israel public health authorities conducted two immunization rounds in 2013 using bOPV vaccine.[144] Given special consideration because of the outbreak of polio in Syria in 2013 and significant cross-border movements of refugees, Turkey implemented four rounds of subnational SIAs targeting resident, refugee and "hard-to-reach" populations[236] in 2014.

Although high routine poliovirus immunization coverage has been maintained in most countries and good performance indicators for polio surveillance are reported by many member states, data suggest that the quality of AFP surveillance has been slowly declining since 2002. Vulnerable subpopulations and underserved areas remain.

To ensure an adequate level of preparedness for responding to introduction of wild polio viruses, the WHO Regional office for Europe regularly organizes polio simulation exercises with a focus on member-states considered to be at high risk of a polio outbreak following introduction of polio virus.[237–240] To date, 14 countries have participated in the exercises.

The global strategy for minimizing risks associated with laboratories and healthcare facilities includes destruction of poliovirus-containing materials in all but a few essential facilities and strict adherence to required safeguards by such facilities. Each country must establish a national poliovirus policy prohibiting retention of poliovirus materials in all nonessential facilities, including promulgating enforceable legislation or regulations. Twenty-three member states of the European Region reported in 2014 that 69 facilities are storing wild poliovirus infectious materials. This represents a decrease from 25 countries with 111 laboratories in 2006. Of the vaccine production facilities that handle wild-type and vaccine polioviruses in the world, 85% are located in 10 countries, eight of which are Member States of the WHO European Region. Early in 2015, the Regional Office informed all affected Member States that they should update and finalize an inventory of facilities holding wild and Sabin (vaccine and vaccine-derived) polioviruses and prepare for the destruction or containment of all type 2 strains and/or samples. Essential poliovirus type 2 materials will be contained in or transferred to designated facilities that have obtained national certification in compliance with Global Action Plan (GAP) III.[241]

Diphtheria Elimination Goal

Operational targets for diphtheria immunity and surveillance were established toward achieving the 2000 elimination goal of less than one case per 1,000,000 population. Since 1995 these included 95% coverage of the primary vaccination course (DTP3) by 2 years of age and include a booster dose/doses of diphtheria-containing vaccine in school-age children in all countries in the region. Every country in the region should have achieved either 95% coverage for this dose or an immunity rate of 90% for children and 75% for adults.[242] By 1997, no district in any country should have reported less than 90% coverage for the primary course at 2 years of age. Operational targets for surveillance included effective diphtheria surveillance to ensure that every case is identified, as well as access to laboratories to differentiate toxigenic from nontoxigenic strains by 1994 in all countries. By 1995 all reported cases of diphtheria were to be classified as indigenous or imported.

Significant progress has been made in reducing diphtheria in the European Region. Indigenous transmission has been interrupted in much of the region. The regional decrease in reported cases since 2005 has been dramatic: from more than 200 cases and carriers in 2005 to fewer than 40 in 2015.[3] There have been sporadic cases (less than one per 1 million population) in a number of countries over the last decade with Georgia, Latvia, Russian Federation, and Ukraine reporting sporadic cases most frequently. To ensure elimination, high coverage must be sustained, surveillance and laboratory capacity must be maintained, the coverage rates for adult booster doses must be increased, and research needs to be conducted to better understand the remaining risk groups.

Immunization Registries

For the vision of a European Region free of vaccine-preventable diseases established in the EVAP, where all countries provide equitable access to high-quality, safe, affordable vaccines and immunization services throughout the life course tools such as immunization registries or immunization information systems could be useful. Such systems could among many tasks monitor the agreed EVAP targets such as: all countries should obtain high-quality immunization coverage data, all 53 countries should have less than a 5% dropout rate between first and third doses of DTP-containing vaccine, and all countries should have sustained coverage with DTP-containing vaccines of greater than 90% for 3 or more consecutive years. The number of countries with electronic immunization registries/

immunization information systems is increasing in the region. ECDC and WHO EURO are collaborating to support countries wishing to establish or expand their capacity to monitor all vaccination, including travel and occupational vaccines, in immunization information systems building upon best practices in countries that have functioning and advanced registries.

Tailoring Immunization Programs

The WHO Regional Office for Europe has developed a framework "Tailoring Immunization Programmes (TIP)" that helps to identify and prioritize vaccine hesitant populations and subgroups, allows diagnosis of the demand and supply-side barriers to vaccination in these populations, and designs evidence-informed responses to vaccine hesitancy appropriate to the setting, context, and hesitant population.[112] The framework was tested in in Bulgaria (to profile and target Roma populations and healthcare providers working within these underserved communities) and Sweden (to improve vaccination coverage among immigrants, the anthroposophic community, and undocumented migrants).[243,244] It was also used in Montenegro to better understand seasonal influenza vaccine uptake by healthcare workers.[245] Subsequently, TIP is being rolled out in several countries across the Region. A TIP project in the United Kingdom will determine demand- and supply-side barriers to vaccination among the Haredi Jewish population of North London. Facing recurrent outbreaks of vaccine-preventable diseases in sections of the population, immunization programs of Belgium, Israel, and the United States are also considering using the European TIP framework to address the barriers to immunization. Kazakhstan, Germany, and Switzerland are preparing TIP projects to be implemented in 2015.

European Immunization Week

European Immunization Week (EIW) is a regional initiative, led and coordinated by WHO Europe and implemented by Member States to address their particular immunization-related issues and to share common immunization themes. Partner organizations, including UNICEF and the ECDC, contribute at regional and national levels. EIW aims to help member states advance plans to ensure universal immunization by using the power of advocacy and targeted communication to boost awareness and increase the success of immunization programs. These efforts aim to reach people who have not been immunized or did not receive all recommended vaccinations and to contribute to building confidence in immunization among those who have participated in EIW. The effort began in 2005 as a small pilot initiative designed to spotlight promulgation of the regional goals for measles and rubella elimination by 2010. Since its beginnings with only a handful of participating countries, the initiative has spread across the region and has become an effective tool for sharing and reinforcing its central message: "Prevent. Protect. Immunize." In 2011, 52 of the 53 Member States participated in EIW with the theme "Shared solutions to common threats." In 2014 the focus of the initiative was "Are you up to date?," looking at immunization across the life course from infancy to old age. Celebrating its tenth anniversary in 2015, the campaign focused on closing immunization gaps and reaching equity in immunization.

EIW brings awareness to immunization strategies and efforts implemented by member states, WHO and its partners. Activities during the week in 2015 have included information and media outreach campaigns using traditional communication methods, but they have also branched out into more innovative tools such as online tests and smartphone apps to track immunization status.[246] Several countries conducted immunization campaigns that provided access to vaccines for more than 10 million people during the month of April. Other countries focused their efforts on organizing meetings, conferences, and training.

CONCLUSIONS

Although the European Region is composed of a diverse group of countries with different immunization policies, the immunization programs offered to different age groups have successfully reduced the burden of vaccine-preventable diseases. However, there are vaccines that are not available in countries that have, so far, decided not to recommend them; there are also countries with hesitant subpopulations that reject recommended vaccines when they are available. Some remaining gaps can be identified with tools such as the newly developed TIP tool that could help identify and respond to vaccine hesitant populations and subgroups, as well as identifying other barriers to vaccination decision making or access to immunization services. Using the outcome of such assessments can facilitate targeted training of the general public and healthcare workers. Increasing numbers of countries are putting registries in place to obtain the benefits that such data systems offer. Meanwhile, reaching and sustaining Regional elimination goals remain challenging, especially for measles and rubella.

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75 Immunization in the Asia-Pacific Region

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The Asia-Pacific region, spanning one-third of the globe's circumference and containing more than half of the world's population, is highly diverse culturally, politically, economically, and in other dimensions that impact vaccine development and implementation. The region includes China and Japan, the second and third largest national economies globally but, at the same time, also encompasses nine Gavi (The Vaccine Alliance)–eligible countries, as well as others that meet Gavi low-income, but not other, eligibility criteria.[1] In a region this diverse and experiencing breakneck economic development, all stages of vaccine innovation, production, and consumption are represented, along with paradoxical overlaps of reliance on aid for global vaccination programs by countries that also are vaccine exporters. While China, India, Indonesia, and Vietnam are Gavi-graduate or Gavi-eligible countries, eight manufacturers located there and in Korea are among the 12 Developing Country Vaccine Manufacturers Network (DCVMN) suppliers that produce 75% of United Nations (UN) agency-procured vaccine doses.[2] Asian manufacturers have evolved from providers of downstream vaccine processing, to developers of lower-cost production solutions for previously licensed vaccines, such as hepatitis B (HepB), oral polio, diphtheria-tetanus-pertussis (DTP) combination vaccines incorporating *Haemophilus influenzae* type b (Hib) antigen, measles-containing vaccines, and more recently, group A meningococcal glycoconjugate vaccine. At the same time, they have become innovators of novel products such as an oral cholera vaccine, virus-like particle hepatitis E and live-attenuated hepatitis A vaccines, typhoid glycoconjugate vaccine, rotavirus vaccines, and inactivated enterovirus A71 (EV-A71) vaccine—representing, in fact, a continuation of local innovation to meet public health needs to control regionally important diseases such as Japanese encephalitis (JE), hemorrhagic fever with renal syndrome (HFRS), and Kyasanur Forest disease (KFD; see "Kyasanur Forest Disease Vaccine" later).

While investment in biomedical research and development (R&D) from 2007 to 2012 declined in the United States, Canada, and Europe, expenditures by countries in Asia-Oceania increased from 18.2% of the global share to 23.8%, led by China.[3] Interestingly, inflation-adjusted R&D spending by the U.S. public sector remained flat in this interval, while expenditures of U.S. industry declined by US$12.9 billion even as Asia-Oceania regional industry expenditures increased by US$15.1 billion, enlarging its global share from 19.0% to 26.5%. The trend may reflect industry recognition of the greater growth potential of vaccine opportunities in Asia, which as a component of other emerging-country markets, has been projected to exceed that of developed countries by 2020.[2,3] The trends in R&D expenditure also are reflected in growth rates of scientific publications and patents from Asian institutions that now exceed those from the United States and Europe.[4]

Manufacturers are entering the global network of vaccine production and supply, not only by providing basic Expanded Programme on Immunization (EPI) vaccines but also by commercially exporting products to Africa, Latin America, and within the region (e.g., meningococcal polysaccharide vaccine from China to Latin America and Africa; live attenuated and inactivated Vero cell–derived JE vaccines from China, Japan, and Korea, regionally; live attenuated hepatitis A vaccine from China, regionally; live attenuated and inactivated pandemic and seasonal influenza vaccines from India and China, internationally; and oral cholera vaccine from Vietnam, internationally). Previously, Asian manufacturers did not themselves market novel vaccines in Europe or the United States, choosing to distribute their innovative products, such as acellular pertussis and live attenuated varicella vaccines, through multinational companies. However, an increasing global integration is taking place, as multinational companies acquire Asian manufacturers (e.g., Sanofi-Aventis, France, acquired Shantha Biotechnics, India); Asian companies acquire or obtain technologies and distribution rights from European countries (e.g., inactivated polio vaccine by Serum Institute of India Ltd. acquiring Bilthoven Biologicals, Netherlands; Astellas, Japan, acquiring recombinant influenza hemagglutinin from Protein Sciences, U.S.; Thai Government Pharmaceutical Organization acquiring chimeric JE vaccine from Sanofi-Pasteur, France; and Biological Evans, India, acquiring JE vaccine from Intercell AG, Austria); and vaccine codevelopment is agreed between entities in developed and Asian countries (e.g., genetically modified, inactivated HIV vaccine codeveloped by Sumagen, Korea, and the University of Western Ontario, Canada; mycobacterial proteinAg85A candidate tuberculosis vaccine codeveloped by Tianjin CanSino Biotechnology, China, and McMaster University, Canada; universal influenza vaccine codeveloped by Xiamen Wantai and Sanofi-Pasteur, France; and novel pneumococcal conjugate vaccine codeveloped by SK Chemicals, Korea and Sanofi-Pasteur, France).[2,4,5]

The role of Asian companies as developers and providers of neglected and improved vaccines for the region and, for developing countries more generally, is an emerging trend as illustrated by the joint research activity agreement between the National Research Council, Canada, and the Chinese National Biotec Group that covers development of *H. influenzae* type a and Hib bivalent conjugate vaccine, novel mucosal adjuvants and therapeutic vaccines against *Helicobacter pylori* infection, and cell culture manufacturing platforms for viral and vectored vaccines. A Korean-manufactured biosimilar (generic) biological, infliximab, now is licensed in Europe, a step toward commercial expansion of Asian region-manufactured biologicals to developed countries. The emergence of Asia as the base of new multinational vaccine companies with broad development, production, and distribution capabilities is on the horizon, even as consolidation of existing companies occurs elsewhere.[6–8]

The broad income range within countries in the region results in large population segments that have sufficient means to pay for vaccines out-of-pocket. Even among countries that otherwise qualify economically for Gavi funding (e.g., India), substantial numbers of families can avail themselves of vaccines not covered by the national EPI, resulting in a two-tiered system of vaccination, paralleling the public–private dichotomy of healthcare delivery in general. Practitioners serving these and expatriate families generally follow current U.S., European, or Australian vaccine recommendations, or some modification of those schedules.

Perceptions of the value of vaccines and their risks also range widely, regionally and within individual countries, from largely enthusiastic acceptance and even demand for additional routine vaccinations (e.g., for JE vaccine in southern and Southeast Asia) to a degree of skepticism equal to, if not more deeply and widely held, than vaccine hesitancy in Europe and the United States. Within the last 20 years, Japan discontinued routine childhood vaccine programs for combination measles-mumps-rubella, influenza, and JE, and withdrew recommendations for the human papillomavirus (HPV) vaccine for adolescents, owing in several of instances, to incorrectly thinking that coincidental adverse events were causally related. The requirement for subcutaneous, as opposed to IM administration for all vaccines, exemplifies the misattribution of adverse reactions, arising in this case from an extrapolation of muscle contractures resulting from repeated IM administration of antibiotics, to other intramuscularly administered products, including vaccines.[9,10] The extrapolation has had unintended consequences of impeding the licensure of vaccines with newer adjuvants with mechanisms of action that require IM administration. With the global spread of information, concerns over the thimerosal content of childhood vaccines and vaccine-associated autism have been as active a parental concern among middle-class families in developing countries as elsewhere. Parental refusal of routine JE vaccination in Korea and significant declines in vaccine coverage occurred in a different context after seven cases of temporally related cases of anaphylactic shock and neurological disease, including five deaths, occurred in 1994. The cases could not be excluded as causally related to administration of the mouse-brain–derived vaccine, prompting a national debate and establishment of a vaccine adverse events reporting scheme, a national vaccine injury compensation system, and introduction of a live attenuated JE vaccine derived from a nonneural tissue substrate.[11]

From this mosaic, we describe some common themes, highlighting representative approaches and unique issues that hold a wider interest. Because they are covered elsewhere in this volume, we have not reviewed specific vaccines of regional concern (e.g., pandemic influenza and JE vaccines) or vaccination topics common to developing countries (e.g., initiatives surrounding injection safety, measles and neonatal tetanus elimination, and polio eradication, nor financing mechanisms). We concentrate, instead, on other aspects of vaccine development and implementation, organized by the steps of vaccine development, approval, production, recommendation, and delivery. We also focus on childhood vaccines and vaccination and on selected countries in the region.

VACCINES DEVELOPED IN AND FOR THE ASIA-PACIFIC REGION

Japan is acknowledged as the innovator of several vaccines now used internationally, including acellular pertussis and live attenuated varicella vaccines, but other novel vaccines have been developed by Japan, China, India, Australia, and Vietnam for region-specific needs (Table 75.1). These include vaccines for JE, Hantaan (HTN)- and Seoul (SEO) virus-related HFRS, Russian spring–summer encephalitis, KFD, cholera, severe acute respiratory syndrome, and Q fever. In addition, novel attenuated strains of measles, mumps, hepatitis A, rotavirus, and intranasally delivered pandemic H1N1 virus have been derived for products distributed principally within the region. Additional novel vaccines for hepatitis E and EV-A71 have potential for broader use internationally, an indicator of the region's transition from a provider of fill-finish and manufacturing capacity to a full-fledged participant in biotechnology research and clinical development.

Japanese Encephalitis Vaccines

Five JE vaccines have been developed and licensed in Asian countries. The widely used first-generation inactivated suckling mouse brain (SMB)–derived vaccine is being replaced rapidly in economically disadvantaged countries by the Chinese developed and manufactured live attenuated or inactivated vaccine (SA14-14-2 strain) grown in primary baby hamster kidney (PHK) cells and in higher-income countries with Vero cell–derived inactivated vaccines (licensed in the United States, Australia, Canada, and Europe, as well as several Asian countries) or a replicating chimeric yellow fever–JE virus recombinant vaccine (manufactured in Thailand). Details are provided in Chapter 33.

Tickborne Encephalitis Vaccine

To control cases and occasional outbreaks of the Far Eastern subtype of tickborne encephalitis virus in northeastern China, the Changchun Biologicals Institute developed a formalin-inactivated vaccine, derived from a human isolate, Senzhang strain, and grown in PHK cell cultures. Related vaccines prepared from Central European strains and distributed in Europe are described in Chapter 59.

Kyasanur Forest Disease Vaccine

The Kyasanur Forest disease virus (KFDV) is a highly pathogenic member of the family Flaviviridae causing a zoonosis, KFD, that is transmitted by the bite of infective ticks (*Haemaphysalis spinigera*) primarily in its nymphal stage, and characterized by acute febrile illness with severe hemorrhagic manifestations.[12] It was first described from outbreaks centered in Karnataka State, India, among herders and villagers with forest exposure and was considered to be localized in the Shimoga district area of the state. However, since first being reported in 1957, the virus has been found in other areas of India including the Kutch and Saurashtra parts of Gujarat state, Andaman Islands and West Bengal. It is estimated that close to 500 cases of KFD occur in India every year and, from 2003 to 2012, among 823 confirmed cases, 28 were fatal.

Following the outbreak in India various vaccines including a formalin inactivated Russian Spring Summer Encephalitis virus, a Russian Spring-Summer Encephalitis virus–based mouse-brain vaccine, and a live attenuated vaccine that was serially passaged in tissue culture were tried but with limited success.[13] Finally, a formalin inactivated vaccine with the KFD virus grown in chicken embryo fibroblasts was tested in a large field trial from 1990 to 1992 among inhabitants of 72 affected villages. The disease attack rates reported were 0.15% (14/9072) among persons receiving one dose and 0.047% (10/21,083) among recipients of two doses, respectively, compared to an attack rate of 0.870% (325/37,373) in unvaccinated persons, for vaccine efficacies of 82.4% and 94.8%, respectively.[14] The vaccine was subsequently commercialized and is produced by the state Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore, and has been central to KFD prevention efforts in the state of Karnataka. Annual vaccinations have been done since 1990 in the Shimoga and adjacent districts wherein two doses of the vaccine were administered in individuals 7 to 65 years of age at an interval of 1 month. Periodic boosters were also administered after 6 to 9 months.

However, recent observations suggest a lower field effectiveness than had been reported previously, especially following a single dose, while overall coverage has also been low. Between 2005 and 2010, effectiveness among individuals

Text continued on p. 1472

TABLE 75.1 Pediatric Immunization Recommendations by Antigen and Jurisdiction, Asia-Pacific Region, 2014[a]

Jurisdiction	BCG	HBV	DTP Combinations	Hib	Polio	PCV/PnPS	Rota	Dengue
Australia		B	DTaP-Hib-HBV-IPV 2, 4, 6 mo; DTaP 18 mo; DtaP-IPV 4y; dTap 10–15 y	2, 4, 6 mo; Hib-Men C, 12 mo		PCV 2, 4, 6, mo; *PCV 12-18 mo*; PCV 12 mo (medical risk); PPS 4 y (medical risk)	2, 4, 6 mo	
Bangladesh	B		DTP-Hib- HBV, 6,10,14 wk; TT, 15 y		OPV, 6, 10, 14, 38 wk; IPV 14 wk	6, 10, 18 wk		
Bhutan	B	B	DTP-Hib- HBV, 6, 10, 14 wk; DTP, 24 mo; Td, 6, 12 y		OPV, B, 6, 10, 14 wk IPV, 14 wk			
Brunei Darussalam	B	B, 1, 6 mo	DTP-Hib, HBV, IPV, 2, 4, 6 mo; DTaP, IPV, 5 y	Hib, 1 y	IPV, 2, 3, 4 mo			
Cambodia	B	B	DTP-Hib, HBV, 6, 10, 14 wk		OPV, 6, 10, 14 wk	6,10,14 wk		
China	B	B,1,6 mo	DTaP, 3, 4, 5, 18 mo; DT 6y; *DT 4 y*		OPV 3, 4, mo; 4y IPV 2 mo; *IPV 2, 3 mo*			
Democratic People's Republic of Korea	B	B	DTP-Hib- HBV, 6, 10, 14 wk; TT, 3–4 y		OPV, 6,10,14 wk; IPV, 14 wk			
Hong Kong	B	B, 1, 6 mo	DTaP-IPV, 2, 4, 6 mo, 1.5 y, Primary 1; Primary 6, dTap-IPV			2,4,6 mo, 1.5y, Primary 1		
India	B	B, **6–10 wk, 6 mo**	DTP-Hib-HBV, 6, 10, 14 wk; DPT 16–24 mo, 5 y; TT, 10–16 y; **DTaP, 6, 10, 14 wk, 16–18 mo, 4–6 y; TdaP, 10–12 y**	**6, 10, 14 wk; 16–18 mo**	OPV B, 6, 10, 14 wk, 16–24 mo; *IPV, 14 wk;* **IPV, 6, 10, 14–18 wk, 16–18 mo; OPV 6, 9 mo, 4–6 y**	*6, 10,14 wk; 12–18 mo;* ***PnPS, 2–18 y***	**6, 10, 14 wk**	
Indonesia	1 mo; **B– 2 mo**	0–7 d	DPT-Hib-HBV, 2, 3, 4, 18 mo; Td 7–8, 8–9 y; **DTP, 2, 3, 4, 18 mo, 15–18 mo, 5 y**	**2, 3, 4, 15–18 mo**	OPV, B, 1, 2, 3, 4 mo; *IPV, 2, 3, 4 mo;* **IPV, 2, 4, 6 mo, 1.5–2, 5 y**	**2, 4, 6, 12–15 mo**	**2,4,6 mo**	9 mo + × 3
Japan	7 mo	2, 3, 7 mo	DTaPIPV 3, 4, 5, 18 mo	2, 3, 4, mo 1 y		2, 3, 4, mo, 1 y	**Dose 1, <15 wk × 2 or 3**	
Korea	B	B, 1, 6 mo	DTaP, 2, 4, 6 mo; 15–18 mo, 4–6 y; Td/ Tdap, 11–12 y	2, 4,6, 12–15 mo	IPV, 2, 4, 6 mo; 4–6 y	2, 4, 6, 12–15 mo	**2,4, or 2,4,6 mo**	
Lao People's Democratic Republic	B	B	DTP-Hib-HBV, 6, 10, 14 wk; Td 15 y		OPV, 6, 10, 14 wk IPV 14 wk	4, 10, 14 wk		

ANTIGENS									
MCV-rubella	**Varicella**	**Men**	**JE**	**HPV**	**Influenza**	**HepA**	**Typhoid**	**Rabies**	**HFRS**
MMR, 12 mo; MMRV 18 mo	10–15 y in schools	Hib-MenC, 12 mo	*1 y+, × 3; boosters every 3 y*	12–13 y in schools	*6 mo+ (at medical risk); 6 mo–5 y; 15 y+ (at-risk ethnic groups)*	*1–2 y × 2*			
M 15 mo; MR 38 wk, 15 y									
MR, 9, 24 mo				12–18 y × 2 in 6th grade girls					
MMR, 12, 18 mo				13 y × 3	*< 2 y*				
9, 18 mo			SA14-14-2, 9 mo						
MR, 8 mo; MMR, 18 mo; *MMR, 4–5 y, M college entry*		A, 6–18 mo (polysaccharide); AC, 3, 6 y (conjugate)	SA14-14-8 mo, 2 y; *inactivated, 8 mo × 2, 2, 6 y*		6 mo to 8 y, × 2	Live, 18 mo; *inactivated, 18 mo, 2 y*			*16–60 y × 3*
M, 9, 15 mo			*SA14-14-2, 1 y*						
MMR, 1 y; MMRV, Primary1	1 y	1 y			*6 mo, 6 y*				
9, 16–24 mo; **MMR, 9, 15–18 mo, 4–6 y**	**15 mo, 4–6 y**	***2–18 y***	*SA14-14-2, 9, 16–24 mo*	**10–12 y/9–26 y**	***6 mo + annually***	**12, 18 mo, inactivated; 12 mo, live**	**TCV, 9–12 mo, 2–3 y**	***B+ × 3***	
M, 9 mo, 2, 6 y; **M, 9 mo; MMR, 12 mo, 5 y**	**12 mo +**		12 mo, 24–36 mo	**10–18 y × 3**	**6 mo +**	**24 mo+, × 2**	**24 mo + × 3**		
MR, 1 y, 5–7 y; **mumps, 1, 5–6 y**	12, 18 mo		36, 37 mo, 4, 9 y		6 mo–12y, × 2 annually; 13y+, 1 × annually	**1 y+ × 3**			
MMR, 12–15 mo; 4–6 y	12–15 mo		1–3 y × 3, 6 y, 12 y; SA14-14-2, 1–3 y × 2	12 y × 2	6 mo +, IIV, 24 mo + LAIV	1–3 y × 2	PS, 2 y +; oral, 4–6 y × 3–4		10 y + × 2 + 1 booster
MR, 9 mo			live vaccine, 9 mo	*10 y + 6 m girls, 5th grade*					

Continued on following page

TABLE 75.1 Pediatric Immunization Recommendations by Antigen and Jurisdiction, Asia-Pacific Region, 2014[a] *(Continued)*

Jurisdiction	BCG	HBV	DTP Combinations	Hib	Polio	PCV/PnPS	Rota	Dengue
Malaysia	B	B, 1, 6 mo	DTaP-Hib-IPV 2, 3, 5, 18 mo; DT, 7 y; TT, 15 y			**2–6 mo × 1–3, 12 mo**	1.5–5 y × 2 or 3	
Mongolia	B	B	DTP-Hib-HBV, 2, 3, 4 mo; Td, 7, 15 y		OPV, B, 2, 3, 4 mo; IPV, age TBD	TBD		
Myanmar	B– 2 mo	B, 6, 10, 14 wk	DTP-Hib-HBV, 2, 4, 6 mo		OPV, 2, 4, 6 mo; IPV, 4 mo			
Nepal	B		DTP-Hib-HBV, 6, 10, 14 wk		OPV, 6, 10, 14 wk; IPV 14 wk	6, 10 wk, 9 mo		
New Zealand	*B (high risk)*		DTaP-IPV-Hib-HBV, 6 wk, 3, 5 mo; DTaP-IPV, 4 y; dTap, 11 y	15 mo		6 wk, 3, 5, 15 mo; *PPS (high risk)*	6 wk, 3 mo	
Pakistan	B	B, 6, 10, 14 wk	DTP-Hib-HBV, 6, 10, 14 wk		OPV, B, 6, 10, 14 wk; *IPV, 14 wk*	6, 10, 14 wk		
Papua New Guinea	B	B	DTP-Hib-HBV: 1, 2, 3 mo; TT 7, 13 y		IPV, 3 mo; OPV 1, 2, 3 mo	1, 2, 3 mo		
Philippines	B	B	DTP-Hib- HBV, 6, 10, 14 wk; Td, 6, 10 y; **DTaP-Hib-HBV, 6–8, 10–16, 14–24 wk; DTaP-IPV-Hib, 12–18 mo; DTaP-IPV, 4–6 y; Tdap/Td, 7–18 y**		OPV, 6, 10, 14 wk; IPV 14 wk; **OPV/IPV, 6–8, 10–16, 14–24 wk**	*6, 10, 14 wk;* **6–8, 10–16, 14–24 wk, 12–15 mo**	*6–15, 32 wk;* **6–32 wk, 2 or 3**	*9 mo + × 3*
Singapore	B	B, 1, 5–6 mo	DTaP, 3, 4, 5 mo; 1.5 y; TdaP, 10–11 y	3,4,5,mo, 1.5 y	IPV, 3, 4, 5 mo; 1.5 y; OPV 10–11 y	3, 5, 12 mo		
Sri Lanka	B		DTP-Hib-HBV, 2, 4, 6 mo; DTP, 18 mo; DT, 5 y; Td, 12 y		OPV, 2, 4, 6, 18 mo, 5 y; IPV, 4 mo			
Taiwan	5 mo	B, 1, 6 mo	DTaP-Hib-IPV, 2, 4, 6 mo, 1.5 y; Tdap, 5 y			1, 2, 12–15 mo		
Timor-Leste	B		DTP-Hib-HBV, 6, 10, 14 wk		OPV, 6, 10, 14 wk; IPV, 4 mo			
Thailand	B	B, 1 mo	DTP-HBV, 2, 4, 6 mo; DTP 1.5, 4 y; Td, 12 y in 6th grade; **DTaP, 2, 4, 6, 18 mo; TdaP/DTaP, 4–6 y; TdaP/dT11–12 y**	**2, 4, 6, 18 mo**	OPV, 2, 4, 6, 18 mo, 4–6 y; **IPV, 2, 4, 6, 18 mo, 4–6 y;** IPV, age TBD	**2, 4, 6 mo, 1–2 y**	**2, 4, 6 mo;** *2, 4 mo*	**9 y + × 3**
Vietnam[b]	B	B	DTP-Hib-HBV, 2, 3, 4 mo; DTP 18 mo	9 mo	OPV, 2, 3, 4 mo			

[a]Schedules recommended as of April 2015. Sources: surveys of local governments and academic societies. MMR or various combinations are used as replacement for M in different provinces depending on vaccine availability: Guangxi, MR at 8 and MMR at 18–24 months; Hebei, MR at 8 mo and MM (measles + mumps) at 18–24 months; Zhejiang, M at 8 months, MMR at 18–24 months, and MR at 15 years; Shanghai, M at 8 months, MMR at 18–24 months, and MMR at 4 years. *Italic type* indicates recommendations for certain geographic areas or groups; **bold type** indicates optional vaccines in national schedule or recommendations of academic or practitioner societies.

[b]Oral cholera vaccine: 2 doses at 2–5 years in Thua Thien-Hue province.

aP, acellular pertussis; B, birth; BCG, bacille Calmette-Guérin; DTP, diphtheria, tetanus, pertussis; HBV, hepatitis B vaccine; HepA, hepatitis A; Hib, *Haemophilus influenzae* type b; HPV, human papillomavirus vaccine; IPV, inactivated poliovirus vaccine; JE, Japanese encephalitis; M, measles; MCV, measles-containing vaccine; Men, meningococcal; MMR, measles, mumps, rubella; OPV, oral poliovirus vaccine; PCV, pneumococcal conjugate vaccine; PnPS, pneumococcal polysaccharide; R, rubella; Rota, rotavirus; TBD, to be determined; TCV, typhoid conjugate vaccine.

ANTIGENS									
MCV-rubella	**Varicella**	**Men**	**JE**	**HPV**	**Influenza**	**HepA**	**Typhoid**	**Rabies**	**HFRS**
MMR, 9, 12 mo; MR, 7 y; *M, 6 mo*	**12 mo– 9 y, × 2**	**ACWY 2 mo+ × 1**	*Live, 9, 21 mo*	13 y + 6 mo, girls	**6 mo+ (chronic disease)**	**12 mo+ × 2**			
MMR, 9, 12 mo; MR, 7 y					6 mo– 14 y, chronic illness	14 mo, 2 y			
MR, 9 mo; M, 18 mo									
MR, 9, **15 mo**			*SA14-14-2, 12–23 mo*						
MMR, 15 mo, 4 y	15 mo, 4 y	*C (high risk)*		9–14 y × 2; 15–26 y × 3	*6 mo+ (at risk)*	*× 2 (high risk)*			
M, 9, 15 mo									
MR, 6, 9, 18 mo									
M, 9 MMR 12 mo; MR, 6 y; **M, 9–12 mo; MMR, 12–15, 16–72 mo**	12–15 mo; 18m-6 y		**9 mo, 18 y**	*× 2, age not specified;* **9–18 y × 3**	**6 mo +**	**1–2 y × 2**			
MMR, 12, 15–18 mo				HPV, females 9–26 y × 3					
MMR, 1, 3 y			SA14-14-2, 12 mo						
MMR, 12 mo, 5 y	1 y		15 mo × 2; 27 mo, 5 y		6 mo +	*12, 18 mo*			
MR, 9, 18 mo									
MMR, 9–12 mo, 2.5 y	**MMRV, 1–2, 2.5–6 y**		9–12 mo, × 2, 2.5 y; **9–18 mo × 2, 2–2.5 y**	**9–12 y × 2;** *girls, grade school 5–6 × 2*	**6 mo–12 y**, 6 mo to 2 y	*1–12 y × 2*			
M, 9 mo; MR, 18 mo			12 mo × 2, 2 y				*3 y*		

who received two primary vaccine doses was 62.4% (95% confidence interval [CI], 26.1 to 80.8), and 82.9% (95% CI, 71.3 to 89.8) among those who received two doses followed by a booster.[9] Moreover, vaccination during the months of April and May 2011 did not confer protection in an outbreak in Shimoga during the months of December 2011 to March 2012.[15] The reasons for low vaccine efficacy and coverage rates need to be investigated and the appropriate vaccine regimen for effective control requires further definition. Newer vaccine approaches (e.g.., chimeric or virus protein subunit vaccines) are being investigated to potentially replace the current vaccine.

Elsewhere, a nearly identical strain to the KFDV was isolated from a patient suffering from acute febrile illness from Yunnan province, China in 1985. Seroprevalence studies indicate that KFDV (or the Nanjianyin virus or a related tickborne flavivirus) may be present in various parts of southwestern China.[16] In 1995 a virus similar to KFDV called Alkhurma hemorrhagic fever virus was isolated from patients with febrile illness in Saudi Arabia.[17] Overall, 10 cases with two deaths occurred in sheep and camel handlers exposed to a tick *Ornithodoros savignyi*. The disease has now been confirmed to be more widespread in the country than previously considered.[12]

As tickborne diseases are "diseases of place," KFD virus itself, if it spreads, is likely to disseminate locally. Nevertheless, the discovery of antigenically related viruses elsewhere, such as Alkhurma hemorrhagic fever virus, suggests a potential for more widespread use of KFD vaccine, depending on public health needs.

Hantavirus Vaccines

HFRS, a widespread rodent-borne bunyaviral zoonosis in Asia, is a pantropic infection with prominent capillary hemorrhages, interstitial nephritis, and a 3% to 10% case-fatality ratio that, until the last decade, caused more than 1000 annual cases in the Republic of Korea and more than 100,000 cases in China.[18] Although the disease had been well known in parts of Russia and Asia as a sporadic and occasionally epidemic disease among farmers, soldiers, and others exposed to campestral and sylvatic habitats, it was largely unknown in the West until thousands of military cases and deaths occurred during the Korean war, when the disease was described as Korean hemorrhagic fever. The etiologic agent eluded investigators until 1976, when a novel bunyavirus, HTN virus was isolated from the striped field mouse, *Apodemus agrarius*, which proved to be the principal viral reservoir in most areas of Asia. Later, antigenically related SEO virus was isolated from *Rattus rattus* and *Rattus norvegicus*, explaining the occurrence of sporadic HFRS cases and outbreaks in urban areas. Subsequently, Sin Nombre and related hantaviruses were discovered in the Western hemisphere, where rare encounters with infected rodents lead to small numbers of cases that feature prominent pulmonary involvement. A multitude of hantaviruses now have been described globally.

The widespread impact of HFRS in China led public health authorities in the 1980s and 1990s to pronounce the disease second only to HepB as a public health menace, and, beginning in 1991, several Chinese vaccine manufacturers used SMB, primary baby gerbil kidney cells (GKCs) or PHK cells to produce inactivated, monovalent vaccines against HTN or SEO viruses. The GKC vaccine was inactivated by β-propiolactone and the other two by formalin. Subsequently, Vero cell line-derived vaccines have been developed. These vaccines were evaluated in nine Chinese provinces hyperendemic for HFRS during 1994 to 2000.

The GKC-derived vaccine against HTN virus produced seroconversions to putatively protective titers of neutralizing antibody in 70.0% of subjects after three primary doses at 0, 7, and 28 days, the proportion rising to 91.2% after a booster at 1 year, and declining to 59.0% at 2 years and 38.9% at 3 years.[1] Similar immunogenicity results were reported for the PHK-derived vaccine and the purified SMB vaccine.[19]

In a randomized, controlled, three-arm trial of GKC vaccine in which vaccinated subjects received three primary doses and a booster at 1 year, 18 HFRS cases were observed in the 7866 age-, sex-, and residence- matched controls, and 23 cases in the 10,196 unvaccinated subjects of similar age (16 to 60 years), compared with none in 7866 vaccinees during 76 months of follow-up, for a protective efficacy of 100% (95% lower confidence limit of 81.94%, $P = .00003$, cumulative binomial probability).[20] Efficacy of the three primary doses alone was shown in the year between administration of the three primary dose series and the booster dose: with zero cases in the vaccinated, and nine and 10 cases in the unvaccinated and control groups respectively. Among 41 cases in the control and unvaccinated groups, 24 were caused by HTN virus, 13 were caused by SEO virus, and four by a virus of indeterminate serotype. Thus, the monovalent GKC-derived HTN virus vaccine was protective not only against the homologous virus, but also cross-protective against SEO virus. No vaccine-related serious adverse event was reported during the trial, and mild local and systematic reactions were reported in 3.78% of vaccinees.

The efficacies of the PHK vaccine and the purified SMB vaccine were similar: in nonrandomized trials, one HFRS case was found in 40,757 recipients of PHK vaccine, compared with 53 in 47,313 unvaccinated subjects, a reduction of 97.81%; for the purified SMB vaccine, the rates were 3.71 per 100,000 (1/26,942) versus 97.98 per 100,000 (34/34,699) for vaccinees and unvaccinated subjects, respectively, a reduction of 88.45%. The observed reductions were maintained through 6 years of follow-up. Nonsevere reactions were found in 1.57% of PHK vaccine recipients and in 3.26% of SMB vaccine recipients.[21,22]

Bivalent HTN and SEO GKC- and PHK-derived vaccines were developed and improved by purification procedures through gradient density ultracentrifugation or chromatography to be more immunogenic and less reactogenic. The purified bivalent GKC vaccine induced neutralizing antibody seroconversion against HTN virus and SEO viruses in 95.4% (83/87) and 93.1% (81/87) of volunteers, respectively, after two doses with an interval of 14 days, and 96.3% (78/81) and 95.1% (77/81), respectively, after a booster dose at 6 months. Only mild reactions were observed; local reactions in 1.72% (14/812) and systemic reactions in 2.83% (23/812) of the vaccinees.[23] The purified bivalent PHK vaccine induced neutralizing antibody seroconversion against HTN virus and SEO virus in 87.4% (90/103) and 89.3% (92/103) of subjects, respectively, after two doses separated by 14 days, and 93.3% (84/90) and 92.2% (83/90), respectively, after a booster dose at 6 months. No systemic reaction was found among 396 vaccinees and mild local reactions were observed in two (0.5%).[24]

The purified, bivalent GKC vaccine was tested for protective efficacy in a nonrandomized trial among 225,576 subjects, 16 to 60 years of age; 112,143 persons received the two primary doses with an interval of 14 days and a booster dose at 6 months; 113,433 persons were unvaccinated. The two groups were similar in age distribution. During 3 years of follow-up, 22 HFRS cases were found in 337,812 person-years among the unvaccinated, a rate of 6.51 per 100,000, compared with none in the vaccinated 334,086 person-years, a reduction of 100%.[25]

Several manufacturers have adapted their processes from primary gerbil or hamster cells to continuous Vero cells. The purified, bivalent Vero cell–derived vaccine administered in two doses separated by 14 days, induced neutralizing antibody against HTN virus and SEO viruses in 90.12% (73/81) and

91.35% (74/81) adult volunteers, respectively.[7] Mild systemic reactions were observed in 3.66% (12/328) and mild local reactions in 1.83% (6/328) of vaccine recipients.[26] The immunogenicity and safety profiles of the Vero cell–derived, purified bivalent vaccine were similar in children and older adults.[27]

Based on the above data, a schedule of two primary doses with an interval of 14 days, plus a booster at 6 months, has been recommended for the purified bivalent GKC-, PHK-, and Vero-cell–derived vaccines.

A postlicensure, retrospective study was conducted to measure the long-term effectiveness of the GKC vaccine among 24,556 adults 16 to 60 years of age, in 21 villages located in a hyperendemic area of Shaanxi Province.[28] HFRS incidence rates were compared between the vaccinated and the unvaccinated adults: 0.06% (4/6828) versus 3.09% (27/875), respectively, for the first 5 years after vaccination; 0.18% (10/5707) versus 1.53% (28/1827) in years 6 to 10; 0.11% (5/4673) versus 0.96% (26/2719) for years 11 to 14; and 0.29% (5/1713) versus 2.80% (6/214) at 15 to 17 years. The vaccine's effectiveness was thus estimated at 98.06%, 88.24%, 88.54%, and 89.64%, respectively, for the four study periods. The effectiveness was underestimated because the year of onset of HFRS was unknown for 36 cases, all of whom belonged to the unvaccinated group and were not included for analysis.[10] The overall HFRS attack rate was 0.13% (24/18921) in the vaccinees and 2.18% (123/5635) in the unvaccinated subjects, a reduction of 94.04%.[28]

A long-term study of the monovalent PHK-derived SEO virus vaccine also was conducted among adults 15 to 60 years old in a SEO virus-predominating area, from 1995 through 2005.[11] Only three primary doses were given at 0, 7, and 28 days without a booster. Seven HFRS cases were found in 1,467,188 subjects in the vaccine group, a rate of 0.48 per 100,000, and 412 cases were found in 6,379,278 controls, a rate of 6.46 per 100,000, with an overall reduction of 92.61% (95% CI, 87.09% to 98.13%) during the 11 years of the study. The vaccine's effectiveness was estimated at 100% for the first year, 95.56% for the second year, and 92.61% for the 11th year. The rate reductions in other years were approximately 92%.[29]

A SMB-derived HTN virus vaccine also was developed in the Republic of Korea and is available for at-risk individuals. The incidence of HFRS in China and Korea has declined in the last 10 years with the introduction of vaccination and probably, more importantly, because of urbanization, rural economic development leading to improved (cement) houses, and grain harvesting and storage practices, resulting in reduced exposures to the rodent reservoir. This trend has been most evident in rapidly developing areas of southeastern China and likely will continue in other regions, leading to a diminution of disease incidence and, potentially, discontinuation of routine vaccination in endemic provinces.

Other Novel Vaccines Developed in the Asia-Pacific Region

See Table 75.2.

Two similar live attenuated hepatitis A vaccines, based on the H2 and LA-1 strains, and measles vaccines, based on the Shanghai S-191 and Changchun-47 strains, have been licensed in China and are used domestically and exported. The National Institute of Hygiene and Epidemiology in Vietnam developed an oral bivalent O1-O139 killed whole-cell cholera vaccine that now is produced and distributed by VABIOTECH, Company for Vaccine and Biological Production No.1, in Hanoi; another oral bivalent O1-O139 vaccine based on the VABIOTEC vaccine but with improved production design is produced in India by Shantha (Sanofi, France). Both vaccines are used domestically and also exported. Similarly, live rotavirus vaccines based on local strains have been developed in India and China for local use. EV-A71 and related enteroviruses have emerged in major seasonal epidemics in Asia and Australia, leading to millions of cases and extensive social disruption as daycares and schools are closed. The extent and impact of seasonal outbreaks stimulated vaccine development in China, Taiwan, Malaysia, Singapore, and Japan, with government prioritization and support in some countries, analogous to mechanisms that facilitated pandemic influenza vaccine development. An *Escherichia coli*–expressed capsid peptide virus-like particle hepatitis E vaccine, approved by the China Food and Drug Administration, is the first novel recombinant vaccine developed and licensed in Asia. Its potential use in Africa, South Asia and, possibly, even in developed countries in immunocompromised or other risk groups could be envisioned.

CLINICAL DEVELOPMENT AND REGULATORY APPROVAL

Implicit in the region's progress toward novel vaccine development is a maturing capacity to conduct clinical trials and improvements toward more robust regulatory processes and capacity, including pharmacovigilance systems. In addition, multinational companies increasingly have turned to countries in Asia to conduct clinical trials because of lower costs and more streamlined regulatory approvals of clinical trial applications. International contract research organizations operate in many countries, and a growing local infrastructure to conduct clinical trials in compliance with the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use and good clinical practices standards will improve clinical research conducted in the region.

Unlike Europe, Asian countries are not unified in a central regulatory approval process. Nor is there a regional public health presence as in Latin America, where the Pan American Health Organization (PAHO) leads regional vaccination programs and also provides central purchasing of certain qualified vaccines. However, the 10-nation Association of Southeast Asian Nations (ASEAN; includes Brunei-Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand, and Vietnam) in 1992 initiated efforts for a subregional regulatory harmonization scheme to reduce differences in technical requirements and regulatory procedures for pharmaceuticals.[30] A 1999 harmonization initiative, under auspices of a Pharmaceutical Product Working Group, aimed to remove barriers to regional commerce and to eliminate technical barriers to trade without compromising product quality, efficacy, and safety. Eventually, a subregional central or mutual-recognition procedure similar to that of the European Union could be envisioned. Importantly, local clinical trials are not required for registration under abbreviated pathways specified by the ASEAN Common Technical Dossier if the vaccine was approved and licensed by a benchmark regulatory agency, resulting in a certificate of pharmaceutical product. By contrast, the national regulatory authorities of China, India, Japan, Korea, and Taiwan have required local clinical trials before or after registration, and in other countries, while data in local populations may not be required for registration, those data are important in deliberations on a vaccine's inclusion in the national schedule. For dengue, a disease of special public health urgency regionally, the global debut of candidate vaccines in the region is being considered, with individual country vaccine registrations ahead of approval by a benchmark agency and provision of a certificate of pharmaceutical product. The

Text continued on p. 1478

TABLE 75.2 Vaccines and Selected Vaccine Manufacturers in Asia[a]

Manufacturer	Viral	Bacterial and Pediatric Combination
AUSTRALIA		
Seqirus	Influenza, inactivated split	*Coxiella burnetii,* inactivated
CHINA		
Beijing Minhai	Inactivated poliovirus vaccine (Sabin strains)	*Haemophilus influenzae* (Hib)-tetanus toxin (TT) glycoconjugate Diphtheria, tetanus, and acellular pertussis (DTaP)-Hib
Beijing Lvzhu		Meningococcal (Men) A and C, glycoconjugate Men A, C, Y, W135 polysaccharide (PS); Men A, C + Hib glycoconjugate
Beijing Sanroad (Xiangrui)		Men AC glycoconjugate
Changchun Baike (BCHT)	Varicella, live attenuated (Oka strain) Rabies, Vero cell-derived	
Changchun Changsheng	Influenza, inactivated seasonal split H1N1 inactivated pandemic Hepatitis A virus (HAV), live attenuated, 2BS-cell, lyophilized (LA-1) Rabies, inactivated, Vero cell–derived, lyophilized Varicella, live attenuated, lyophilized (Oka strain)	DTaP, adsorbed; Men AC PS Men ACWY PS
China National Biotech Corporation comprising Beijing Tiantan Co, Changchun Institute of Biological Products (IBP), Changchun Keygen Co, Chengdu IBP-Rongsheng, Lanzhou IBP, Shanghai IBP, Wuhan IBP	Oral polio virus (OPV), trivalent, live attenuated Inactivated poliovirus vaccine (Sabin strains) Influenza, inactivated seasonal Influenza H1N1 pandemic inactivated Measles, mumps, rubella (MMR) combined, live attenuated Measles and mumps (MM) combined, live attenuated Measles and rubella (MR) combined, live attenuated Measles (M), live attenuated (S-192 or Chang-47 strains) Mumps, live attenuated (S79 strain) Rubella, live attenuated (BRD II strain) Hepatitis B (HBV), recombinant yeast HAV, live attenuated, 2BS cell, lyophilized (H-2 strain) HAV, live attenuated, 2BS cell, lyophilized (La-1 strain) HAV, live attenuated, liquid (H-2) HAV, live attenuated, liquid (La-1) HAV, inactivated Varicella, live attenuated, MRC-5 cell-derived, lyophilized (Oka strain) Japanese encephalitis (JE), live attenuated, PHK cell–derived, (SA14-14-2) Rotavirus, live, newborn calf kidney cell, oral (LLR strain) Tickborne encephalitis (TBE), inactivated, PHK cell–derived (Senzhang strain)	Diphtheria toxoid (DT), adsorbed TT, adsorbed DTaP, adsorbed Diphtheria and tetanus toxoids combined with whole-cell pertussis (DTwP), adsorbed DT, adsorbed Typhoid, Vi PS Meningococcal A PS, lyophilized Meningococcal A and C, PS lyophilized Leptospirosis, inactivated, bacille Calmette-Guérin (BCG) Brucellosis, live attenuated, lyophilized BCG PS + nucleic acid–therapeutic use Plague, live attenuated, lyophilized Anthrax, live attenuated, lyophilized Hib tetanus toxoid conjugate Pneumococcal PS, 23v
Chengdu Kanghua	Rabies, inactivated	Men ACWY PS
Chongquing Zhife		Hib glyconjugate Men AC PS Men AC glycoconjugate Men AC+ Hib glycoconjugate
Dalian Hissen (Hanxin)	Influenza seasonal inactivated HBV, recombinant yeast	
Dalian Aleph	Influenza, seasonal inactivated Influenza H1N1 pandemic, inactivated	
Guangzhou Promise (Nuocheng)	Rabies, inactivated, Vero cell-derived	
Huabei Jintan	HBV, recombinant Chinese hamster ovary (CHO) cell	
Hualan Bio	Influenza seasonal inactivated Influenza pandemic H1N1, inactivated HBV, recombinant, yeast	Men ACWY PS
Jiangsu Simcere Vaxtec	Influenza seasonal inactivated	
Jilin Maifeng	Rabies, inactivated, Vero cell–derived	
Jilin Yatai	Rabies, inactivated, PHK cell–derived	
Kunming Medical Biology Institute	Oral polio vaccine (OPV), live attenuated HAV, live attenuated, KMB17 cell, liquid (Lv-8 strain) HAV, live attenuated, KMB17 cell, lyophilized (H-2 strain) HAV, inactivated, KMB17 cell, liquid (Lv-8 strain)	

TABLE 75.2 Vaccines and Selected Vaccine Manufacturers in Asia[a] *(Continued)*

Manufacturer	Viral	Bacterial and Pediatric Combination
Liaoning Chengda	Rabies, inactivated, Vero cell–derived, (PV2061 strain) JE, inactivated, Vero cell–derived (P3 strain)	
Ningbo Rongan	Rabies, inactivated, Vero cell–derived (aGV strain)	
Shanghai United Biotech		Cholera rB subunit-whole cell, oral
Shenzhen Kangtai	HBV, recombinant yeast	
Shenzhen sanofi-pasteur[b]	Influenza seasonal, inactivated	
Sinovac	Influenza, seasonal inactivated Influenza, pandemic H1N1, inactivated Influenza, avian H5N1, alum adjuvanted [NIBRG-14 A/VietNam/1194/2003(H5N1)RG strain] HAV, inactivated (Hm175 strain) HAV + HBV, inactivated, recombinant EVA71–inactivated	
Sinovac (Dalian)	Mumps, live attenuated	
Walvax Biotechnology		Hib-TT glycoconjugate Men A polysaccharide Men AC polysaccharide
Wuxi Royal (Luoyi)	HFRS, inactivated bivalent, Vero cell–derived	Men A and C, glycoconjugate, lyophilized
Xiamen Innovax	Hepatitis E, recombinant, *Escherichia coli*	
Yunnan Walvax (Wosen)		Hib-TT, conjugate (58534) Men A and C, glycoconjugate, Men ACWY PS
Zhejiang Pukang	HAV, live attenuated	
Zhejiang Tianyuan[b]	Influenza, seasonal inactivated Influenza pandemic H1N1, inactivated HFRS, inactivated bivalent, primary Mongolian gerbil kidney cell (Z10,Z37 strains) JE, inactivated, PHK cell (SA14-14-2)	Men A and C, PS Men AW135 PS
Zhejiang Vacin (Weixin)	Mumps, live attenuated (S79 strain) HFRS, bivalent inactivated, Vero cell derived	
Zhongke	Rabies inactivated PHK cell-derived (aGV strain)	
INDIA		
Bharat Biotech	Rabies, Vero cell–derived OPV, trivalent HBV, recombinant Live attenuated oral rotavirus vaccine (neonatal human strain 116E) JE inactivated Vero cell-derived (821564-XY Indian strain) H1N1 inactivated pandemic vaccine, Madin-Darby canine kidney (MDCK) cell-derived	TT DT DTP DTP-Hib DTP-HBV DTP-HepB-Hib Hib-TT glycoconjugate Typhoid Vi PS glycoconjugate
Biological E	HepB JE inactivated Vero cell-derived	TT Td DTP HBV-Hib-TT glycoconjugate
Biomed	OPV, trivalent	Typhoid Vi PS and glycoconjugate Hib glycoconjugate Men ACYW135 PS
Coonoor, Pasteur Institute of India	Rabies, Vero cell–derived	DPT TT DT
Haffkine	OPV 1 OPV 1, 3 OPV, trivalent, MRC5 or primary monkey kidney cell culture–derived	
Green Signal BioPharma		BCG
Indian Immunologicals Ltd.	HBV, recombinant Rabies, inactivated, Vero cell–derived	TT DT DTP

Continued on following page

TABLE 75.2 Vaccines and Selected Vaccine Manufacturers in Asia[a] *(Continued)*

Manufacturer	Viral	Bacterial and Pediatric Combination
Panacea Biotech Ltd.	OPV 1 OPV 3 OPV, trivalent HBV	DTP DTP-HBV DTP-Hib-CRM_{197} glycoconjugate DTP-HBV-Hib-CRM_{197} glycoconjugate
Serum Institute of India	Influenza, live attenuated H1N1 pandemic HBV, recombinant Measles, live attenuated (EZ strain) Rubella, live attenuated (RA27/3 strain) MR, live attenuated (EZ, RA27/3 strains) MMR, live attenuated (EZ, L-Zagreb, RA27/3 strains) Inactivated polio	BCG TT, adsorbed DT, adsorbed DTP, adsorbed DTP-HepBV DTP-Hib DTP-HepB-Hib Td Hib-TT Meningococcal A-TT glycoconjugate
Shanta Biotech Ltd[b]	HBV, recombinant	TT Hib-TT glycoconjugate DTP-HBV DTP-Hib DTwP-HBV-Hib Oral cholera, whole cell, heat and formalin inactivated (O1 classical and El Tor biotypes and O 139 strains)
Zydus Cadila	Influenza, inactivated H1N1 pandemic Rabies, primary duck embryo cell–derived Influenza, inactivated seasonal, quadrivalet	
INDONESIA		
Biofarma	Influenza, inactivated seasonal OPV, trivalent OPV, type 1 OPV, types 1, 3 HBV, recombinant Measles, live attenuated (CAM-70 strain)	BCG Tetanus toxoid, adsorbed Td DT DTP DTP-HepB DTP-HepB-Hib
JAPAN		
Daiichi Sankyo-Kitasato	Influenza, inactivated split (seasonal egg-derived tetravalent); H1N1 and H5N1pandemic/prepandemic) H5N1 H5N1 pandemic, MDCK cell-culture derived Measles, live attenuated (AIK-C strain) Rubella, live attenuated (Takahashi strain) Mumps, live attenuated (Hoshino strain) MR, live attenuated	Tetanus toxoid, adsorbed DTaP, adsorbed DTPIPV (Salk strains, imported)
Denka-Seiken	Influenza, inactivated split (seasonal egg-derived tetravalent); H1N1 and H5N1 pandemic/prepandemic)	TT, adsorbed Leptospirosis polyvalent
Handai-Biken	Influenza, inactivated split (seasonal egg-derived tetravalent); H1N1 and H5N1 pandemic/prepandemic) Measles, live attenuated Tanabe strain Rubella, live attenuated Matsuura strain MR, live attenuated Varicella, live attenuated (Oka strain) JE, inactivated Vero cell–derived (Beijing strain)	TT, adsorbed DT, adsorbed DT toxoid, adsorbed DTaP, adsorbed Adsorbed diphtheria-purified pertussis-tetanus-inactivated polio (Sabin strains)
Japan BCG		BCG
Kaketsuken	Influenza, inactivated split (seasonal egg-derived tetravalent); H1N1 and H5N1 pandemic/prepandemic) H5N1 pandemic/prepandemic EB66 cell-derived HBV, recombinant, yeast-derived HAV, inactivated Rabies inactivated, cell culture–derived Smallpox, LC16m8 strain JE, inactivated Vero cell–derived (Beijing strain)	Tetanus toxoid, adsorbed DT toxoids, adsorbed DPT, adsorbed DTaP, adsorbed DPT, adsorbed IPV (Sabin strains)
Takeda	Measles, live attenuated Rubella, live attenuated Mumps, live attenuated MR, live attenuated H5N1 pandemic–Vero cell-derived	TT, adsorbed DT

TABLE 75.2 Vaccines and Selected Vaccine Manufacturers in Asia[a] *(Continued)*

Manufacturer	Viral	Bacterial and Pediatric Combination
KOREA		
Berna Biotech, Korea	HBV, recombinant HAV, virosomal	DTwPHibHepB
Boryung Biopharma	JE, inactivated, Vero cell-derived (Beijing Handai strain) JE, inactivated, SMB-derived	DTaP Typhoid, polysaccharide Vi (oral)
CJ Healthcare	Smallpox, cell culture-derived	
Daewoong	IPV	DTP, adsorbed
Green Cross	Influenza, inactivated split (seasonal trivalent and H1N1 pandemic) Influenza, inactivated split, MF59-adjuvanted H1N1 pandemic HFRS, inactivated SMB-derived (ROK84–105 strain) Varicella, live attenuated (MAV/06 strain) JE–SMB-derived JE, inactivated, Vero cell-derived (Beijing-Handai strain)	
Il-Yang Pharm	Influenza, inactivated, seasonal, quadrivalent	
LG Life Sciences	HBV, recombinant	DTaP Hib-TT glycoconjugate
SK Chemical	Influenza, trivalent, inactivated subunit Influenza, quadrivalent, MDCK cell-derived, inactivated subunit HBV, recombinant	DTaP Td
MYANMAR		
Department of Medical Research	HBV, recombinant Rabies, Vero cell–derived	
PAKISTAN		
CIRIN	Rabies, Vero cell–derived	
TAIWAN		
Adimmune	Influenza vaccine, inactivated split (seasonal and H1N1 pandemic) JE, inactivated, SMB (Nakayama strain)	TT, adsorbed
CDC		BCG TT, adsorbed DT, adsorbed dT, adsorbed
THAILAND		
Government Pharmaceutical Organization[b]	LAIV H1N1 pandemic JE inactivated SMB-derived, Beijing strain Rabies, Vero cell-derived OPV, trivalent Measles, MMR DTwP-HepB JE-YF17d chimeric live recombinant (Sanofi technology transfer)	
Queen Saovabha Memorial Institute		BCG
VIETNAM		
IVAC	Severe acute respiratory syndrome (SARS) virus, inactivated	BCG TT, adsorbed Td, adsorbed DTP, adsorbed
POLYVAC	OPV Measles MR (Daiichi-Sankyo technology transfer)	
Vabiotech	JE, inactivated, SMB-derived (Beijing-1 and Nakayama strains) HBV, recombinant yeast-derived HAV, inactivated Varicella (Green Cross technology transfer) Rubella (India Serum Institute technology transfer)	Oral cholera, whole cell, heat and formalin inactivated including toxin coregulated pilus (O1 classical and El Tor biotypes and O 139 strains) Men B-C (Finlay Institute technology transfer)

[a]Information obtained through surveys of local governments and manufacturers, through April 2015.
[b]Major investment/partnership with multinational company.

initiative is a collaboration among the nongovernmental organizations, the Dengue Vaccine Initiative, and the World Health Organization (WHO) Developing Countries Vaccine Regulatory Network (DCVRN). The WHO through the DCVRN has been actively working toward harmonizing procedures in affiliated countries, including China, India, and Indonesia, to bring those regulators under the WHO prequalification umbrella and to facilitate approval and supply of their products for Gavi and United Nations Children's Fund (UNICEF).[31]

The requirement of some national regulatory authorities for clinical data in local populations is based on a concern that racial, ethnic, or environmental differences could affect responses of the local population, both immunologically and in their risk for adverse events. Genetically based differences in drug pharmacokinetics and pharmacodynamics, as well as disease risk, increasingly have been recognized, including immune responses to vaccines. Studies of antibody responses to pneumococcal conjugate vaccines in Asia, for example, have found higher prevaccination and postvaccination antibody titers among Philippine and Taiwanese infants compared with European or historical control subjects, and in Korea, a considerably higher proportion of subjects were seropositive to meningococcal serogroup W135 polysaccharide at baseline than in the United States.[32–34] While the basis for these differences may be an earlier exposure in life to cognate or cross-reacting antigens (e.g., because of regional differences in host microbiomes), genetically restricted responses, as have been observed with HepB, measles, vaccinia, rubella, Hib, and other antigens, or, in the case of oral rotavirus vaccine, in genetically determined viral attachment or receptor binding molecules, have been described.[35–38] From the perspective of adverse events following immunization, the example of narcolepsy occurring in some recipients of an adjuvanted pandemic H1N1 vaccine illustrates the role of genetic background as a cofactor in risk.

In many examples, regulatory systems and processes in the region have had the effect of markedly slowing or effectively blocking the introduction of novel vaccines developed externally. In China, the introduction of an internationally registered and otherwise widely used product nevertheless necessitates recapitulating the entire clinical development program in China, including Phase I studies, despite an abundance of previously scrutinized evidence. This requirement introduces a delay of a decade or more for registration of internationally developed, as opposed to domestically developed, vaccines. Specifications in national pharmacopeias that deviate from established compendia, for example, exclusion of well-accepted excipients or methods, also have seriously impeded or prevented registration of foreign products or, when imposed with a revision of the pharmacopeia, have led to withdrawal of a previously registered product. Clinical trial processes also have hindered local introduction of established or novel products (e.g., a 2013 Indian Supreme Court ordered suspension of ongoing clinical trials and reexamination of previously approved trials was followed by a wholesale revision of clinical trial guidelines, leading to a temporary cessation of all industry-sponsored clinical trial activity). The potential inclusion of video recording of the informed consent process, newer insurance requirements and further proposed but unclear amendments to the Drug and Cosmetics Act that could impose criminal penalties against trial investigators for poorly defined violations may further limit trial activity.[39] China and Indonesia place severe restrictions on the exportation of clinical samples from study subjects, thereby requiring that validated laboratories and procedures are established locally, adding a barrier that has led to delays of or avoidance of clinical studies in those countries. Whether resulting from inexperience, a dearth of trained personnel, trade protection, or other reasons, administrative mechanisms in various countries in the region have had the effect of delaying the registration of proven vaccines that otherwise could have prevented significant morbidity and mortality with more timely introductions.

Descriptions of individual regulatory requirements for clinical trial applications and new product approvals are beyond the scope of this chapter; see the previous edition for a more detailed introduction.

VACCINE PRODUCTION

Governments have had a greater role in vaccine manufacturing in the region than elsewhere, although devolution toward privatized or state-owned enterprises (i.e., government-owned corporations) has occurred (e.g., Commonwealth Serum Laboratories in Australia was privatized, and the six major government vaccine institutes in China now operate as a state-owned enterprise, China National Biotech Group; see Table 75.2). Although a growing number of private manufacturers have emerged, especially in China and India, in other countries, national and local government manufacturers continue to be important sources of certain vaccines and biologicals for domestic needs (e.g., the Government Pharmaceutical Office in Thailand, Research Institute for Tropical Medicine in the Philippines, Biofarma in Indonesia, the National Institute of Hygiene and Epidemiology in Vietnam, and the Central Research Institute and local government institutes in India). These and other facilities also fill and distribute bulk vaccines supplied by international manufacturers. Several private and state-owned enterprise manufacturers in the region are members of the DCVMN, a consortium that seeks to identify and develop solutions to common challenges faced by manufacturers in developing countries.[2,4,8,40]

A number of manufacturers (including 12 in five Asia-Pacific countries) operate under practices and procedures that have prequalified them to produce certain vaccines for UNICEF, PAHO, and Gavi purchases (e.g., pentavalent DTP combinations, oral polio vaccine, inactivated polio vaccine, HepB, rabies, influenza, oral cholera, and measles-containing vaccines) or that allow them to export vaccine to other countries in the region. A reliable supply of inexpensive diphtheria and tetanus toxoids combined with whole-cell pertussis (DTwP)-Hib-HepB combination vaccines, made possible largely by Indian and Korean manufacturers, has facilitated the introduction of Hib antigen into schedules of economically disadvantaged countries that otherwise would not have adopted the monovalent vaccine. Similarly, provision of measles and measles containing vaccine by Indian manufacturers was key to the elimination of that disease in Latin America and the current state of polio elimination could not have been achieved without supplies from Asian regional manufacturers. The provision of oral cholera vaccine for outbreak control in Haiti, Pakistan and other countries is an important example of the increasing ability of and global dependence on these manufacturers.

WHO prequalification requires that the manufacturers and plants not only must satisfy WHO good manufacturing practices inspections, but, in addition, that national notifications of adverse events following immunization are captured and analyzed satisfactorily. This last requirement has been the principal impediment to prequalification of products from some countries and prequalification aided by WHO Blueprint and other vaccine safety-related guidelines have facilitated the improvement of vaccine-related pharmacovigilance in the region.

In China, the state-owned China National Biotec Group is the dominant supplier of vaccines in the country, providing 82% of doses used in the public program and 28% taken up

privately. The Group comprises 12 manufacturing sites, which produce some 40 products, including the first WHO prequalified vaccine produced in China (SA14-14-2 JE vaccine). Other private companies compete principally to provide vaccines for out-of-pocket sales at local centers for disease control and prevention and hospitals.

Within the ASEAN community, comprised principally of low- and middle-income countries, regional vaccine security has been a focus of discussion, reflected in the establishment of the ASEAN-Network for Drug, Diagnostics and Vaccines Innovation that focuses on a broad agenda of health technology development and collaborations on vaccine manufacturing and plans for regional vaccine purchasing—similar to PAHO's revolving fund. Similarly, the eight-nation South Asian Association for Regional Cooperation includes biotechnology in its agenda for cooperative research. A goal to achieve self-reliance in vaccine supply also has been articulated in Korea, in its 2020 horizon-setting.

To a growing extent, multinational companies are acquiring or partnering with local companies in the region, with the result that manufacturing standards and their regulation should improve toward meeting international specifications.[29]

Table 75.2 lists the region's principal vaccine manufacturers and their licensed products. The list is not intended to be comprehensive, as the sometimes rapid emergence or disappearance of pharmaceutical and vaccine companies in China and elsewhere is difficult to track. Vaccines that are manufactured elsewhere and refilled and distributed by local manufacturers are not listed.

VACCINE POLICY AND SCHEDULES

Countries in the region can be divided broadly into countries with a single national schedule and countries in which a basic schedule of free EPI vaccines is supplemented by recommendations of a professional organization (such as the national pediatric society) for additional antigens that are paid for out-of-pocket. Countries in the first group include, on the one hand, mainly developing countries offering a basic EPI schedule and, on the other, countries like Australia, New Zealand, and Taiwan that provide a universal vaccination program that includes an array of antigens or combination vaccines paralleling those of European and U.S. schedules.

The continued introduction of new and frequently expensive vaccines is an ongoing tension for vaccine recommending and funding entities that must weigh the relative value of such innovations against other preventive and therapeutic health expenditures. Even for low-middle-income countries in the region, the total per capita expenditure for all healthcare may be less than the cost of a full course of a novel vaccine! On the other hand, national schedules in the region can be as comprehensive as to include the HPV vaccine (Australia) and influenza and varicella vaccines (e.g., Korea, Taiwan). At the same time, Hib vaccine still is not recommended in some jurisdictions with high per capita income (Hong Kong, Singapore). To some degree, the seemingly paradoxical recommendations of relatively high-income countries in the region reflect different social expectations of personal responsibility in healthcare purchases (see subsequent text). As shown in Table 75.1, some national schedules provide optional recommendations for some antigens; in many countries where government tenders choose specific manufacturer products, specific combinations are recommended in the national schedule. In addition, for some antigens, provincial-specific recommendations address regional differences in risk (e.g., for routine group AC meningococcal vaccine in China; for JE vaccine in Sarawak, Malaysia, and for the Torres Straits, Australia; and for rabies vaccine [preexposure] in areas of the Philippines).

In most countries in the region, public health authorities now draw on external advisors to help formulate national vaccine recommendations in National Immunization Technical Advisory Groups (NITAG), resulting, in part, from activities of the Supporting Independent Immunization and Vaccine Advisory Committees Initiative (at the Agence de Médecine Préventive).[41–44] The Advisory Committee on Immunization Practices (ACIP) in Taiwan and Korea, Expert Committee on Immunization in Singapore, Chinese Expert Committee on EPI, Hong Kong Scientific Committee on Vaccine Preventable Diseases, Immunization Committee of the Indonesian Pediatric Society, National Technical Advisory Group for Immunization in India, and the Australian Technical Advisory Group on Immunization are examples of such medical advisory groups. In China, vaccine recommendations are made through the National Centers for Disease Control based on recommendations of the Chinese Expert Committee on EPI under the Ministry of Health and Family Welfare; however, provincial or local Centers for Disease Control may issue independent recommendations for specific vaccines or modify the national recommendation for routine vaccines (see Tables 75.1 and 75.3).

The issues considered by Asian NITAGs in formulating vaccine recommendations parallel those of other NITAGs, focusing on medical need, vaccine safety and efficacy, national resources, as well as implementation issues, including supply, cold-chain, fit within the national schedule, vaccine presentation, etc. Health economic analyses are considered in the deliberation of some committees or are provided by an independent body (e.g., the Health Intervention and Technology Assessment Program in Thailand); although, in general, the use of health technology assessments in the region lag behind the United States and United Kingdom. In some cases, industry sponsors, in providing such analyses to NITAGs in their justifications to include new vaccines into national programs, have played a role in introducing cost-to-benefit analyses to the recommendation process. In Indonesia and Malaysia, the halal status of vaccines is an important factor in public acceptance of a product and also is a consideration in the vaccine recommendation process, although there is movement to remove this consideration from debate.

In certain Asian countries, as well as in Latin America, the approval process to include a new vaccine into the national program is used to leverage multinational companies to foster local manufacturing expertise. In Brazil, technology transfer of the vaccine production process is required in turn for the vaccine's inclusion into the national schedule while, in Indonesia, all EPI vaccines are locally produced by BioFarma, and no new vaccine has been introduced into the national schedule unless it was produced locally. Technology transfer of some element of the manufacturing process also is a factor in introduction of new vaccines to Thailand and Malaysia. Such requirements may be tested as costly vaccines manufactured by more complex technologies are introduced to the region.

The recommendation process in Japan illustrates how, even after registration, organizational and administrative processes can result in a lengthy interval before a new vaccine is introduced to the national schedule.[44] Although, since 2009, Japan has recovered from a "vaccine gap"—the self-acknowledged interval during which antigens such as Hib and pneumococcal conjugate, rotavirus, HPV, inactivated polio and various combination vaccines were not introduced into Japan despite their widespread use in other developed countries—adoption of new vaccines into the National Immunization Program after their registration still lags several years. A number of sequential approvals lengthens the process: the Immunization Policy and Vaccination Committee provides an initial recommendation whether the newly registered vaccine should

TABLE 75.3 Adult Immunization Recommendations by Antigen and Jurisdiction, Asia-Pacific Region[a]

Jurisdiction	Tdap Variations[b]	Seasonal Influenza	Pneumococcal	MMR
Australia	DT 45, 54 y	65 y+, pregnant women, *>15 y+*	PPS 65 y+, *50 y+,* 15–49 y high-risk	
Brunei Darusalaam		Chronic illness, pregnancy, HCW, Hajj travelers		
Cambodia	TT pregnant women ×3 + 2 boosters			
China[c]		60 y+, high-risk groups, pregnant women	PPS 60 y+ (Shanghai)	
Hong Kong		50 y+, 6 mo–64 y with risk conditions, pregnant women, healthcare workers, poultry workers, pig farmers, and abattoir workers, BMI 30+	65 y+	
India	TT pregnant women	**>50 y**	>65 y	
Indonesia	**19–64 y: 3 primary doses, TdaP, Tdx2; 19 y+: Td every 10 years;** TT 15–39 y child-bearing-age women	**50 y+; 19–49 y (at-risk)**	**65 y+; 19–64 y (at-risk)**	**Varicella: 19–49 y, 2 primary doses; zoster: 50 y+ (at-risk)**
Japan		65 y+, 50–64 y with chronic disease	PPS 65 y	
Korea	**Tdap × 1; Td every 10 y**	**50 y+, pregnant women, high-risk groups**	**65 y+ and high-risk groups, 1 dose**	**Unvaccinated high-risk groups**
Lao People's Democratic Republic		50 y+, chronic illness, pregnant women, HCW		
Malaysia	**19 y+, every 10 y**	Chronic illness, HCW, Hajj and other travelers, other high-risk groups; **19 y+**	**PPS 65 y+**	
New Zealand	Td, 45, 65 y TdaP–pregnant women 28–38 wk	65 y+, pregnant women, chronic disease, HCW, Hajj and other travelers, other high-risk groups		Rubella-susceptible women of childbearing age
Mongolia		15 y+, chronic illness, HCW, Hajj and other travelers		
Myanmar	TT pregnant women ×2			
Nepal	TT pregnant women			
Pakistan	Pregnant women ×3 + 2 boosters			
Philippines	Pregnant women ×2; **dT, primary vaccination for susceptible persons, every 10 y**	**50 y+, high-risk groups, healthcare workers and workers in essential services, all wanting to reduce risk, including travelers;** chronic disease, pregnancy, HCW, Hajj and other travelers, other high-risk groups	PPS, 60 y+, high-risk groups	**All, particularly high-risk groups**
Sri Lanka	TT pregnancy, schedule not specified			Rubella, 15–44 y not previously vaccinated
Thailand		>65 y, HCW, at risk		
Timor-Leste	TT pregnant women ×3 + 2 boosters			
Vietnam	TT pregnant women ×3 + 1 booster			

[a]**Bold type** indicates recommendations of academic or practitioner societies: italic type indicates recommendations for certain geographic areas or groups.
[b]Tetanus toxoid vaccine for pregnant women is recommended in nearly all Expanded Program of Immunizations schedules within the region. In the region, only Cambodia, Indonesia, Pakistan, and Papua New Guinea have not eliminated neonatal tetanus.
[c]Hepatitis E vaccine is recommended for food handlers in Xiamen.
HCW, healthcare workers.

Zoster	Hepatitis B	Hepatitis A	JE	Meningococcal	HPV	Typhoid	Rabies
70 y	Catch-up, all nonimmune						
>60 y							
60 y+	**19 y+: 3 doses (at-risk)**	**19 y+: 2 doses (at-risk)**		**19 y+: (at-risk)**	**19–49 y: 2 doses**		
Unvaccinated and seronegative	**Seronegative**	**30- to 39-y-olds**		**Military recruits; dormitory residents**	**Unvaccinated up to 29 y old**		
60 y+	**19 y+: 3 doses (at-risk)**	**19 y+: 2 doses (at-risk)**		ACWY, Hajj travelers	**females 19–26 y; males 19–21 y**	Food handlers	
	Contacts of hepatitis B surface antigen carriers						
All, particularly high-risk groups	**All, particularly high-risk groups**					**Food handlers, healthcare workers and trainees, laboratorians, contacts of case**	**Healthcare workers, veterinarians and trainees, laboratorians, field workers**
						Food handlers	

be classified as either "routine" or "voluntary," based on available data; the technical recommendation is considered by the Tuberculosis & Infectious Diseases Control Division which makes the administrative decision for the vaccine's inclusion in the national schedule; however, that decision requires additional legislative approval whether the disease (category A or B) qualifies for full or partial vaccine funding (up to circa 70%), respectively. The recommendation process is even lengthier than appearances suggest, as the Immunization Policy and Vaccination Committee does not convene a deliberative vaccine working group until after the product is registered, unlike the parallel activities of the U.S. ACIP and Food and Drug Administration. Only then does the committee assemble a dossier (Fact Sheet) that establishes the epidemiology of the disease and its local burden; if insufficient data are available, de novo studies might be required to establish need. The overall interval between vaccine approval and issuance of a recommendation typically is 3 years.

Other Asian countries have similar or even lengthier intervals between vaccine registration and full EPI implementation. In Thailand, for example, after a preliminary NITAG recommendation, a new vaccine is implemented in a pilot program to establish effectiveness and to collect additional safety experience. Such a program may be gradually extended to other localities over a period of as long as a decade before the antigen is provided nationally. For diseases with regional differences in disease burden, high-risk provinces may be covered first (e.g., JE vaccine initially was introduced in Thailand to eight high-incidence provinces and progressively, from 1990 to 2000, to all 76 provinces, while local production was established and expanded). For new, often costly vaccines, phased introduction provides a mechanism to accommodate their full EPI coverage costs over time. In the interim, local governments of wealthier provinces or municipalities have issued their own recommendations for vaccines to be reimbursed (e.g., Shanghai provides pneumococcal polysaccharide vaccine free of cost to older adults and Bangkok established a school-based HPV vaccination program, while neither vaccine is included in respective national schedules).

Innovative financing mechanisms have played an important role in the introduction of vaccines to low-income countries, and their extension to graduating Gavi will enable more rapid adoption of new vaccines in those countries. At the same time, tiered pricing, negotiated between sponsors, local government and other entities will aid middle income countries to accelerate vaccine adoption, as exemplified by introductions of PCV and rotavirus vaccines.

During the interval between a vaccine's registration and its inclusion in the national schedule, after which it is available without cost, out-of-pocket sales still may result in considerable uptake. While rotavirus vaccine is still considered a voluntary vaccine in Japan, coverage among infants is estimated to be approximately 50%. In Korea, although almost all pediatric vaccines are self-paid by parents, vaccine coverage for antigens such as Hib and pneumococcal conjugate vaccine rapidly reached coverage rates of approximately 90% that, with herd effects, led to disappearance of the respective diseases as quickly as in other countries. Although in Japan, the "voluntary" vaccine recommendation emanates from a government committee, in other countries, academic societies play the principal role in recommending vaccines that are not included in the EPI schedule. The Malaysian Pediatric Association, The Pediatric Society of Thailand, and The Philippines Foundation for Vaccination not only advise their respective ministries and NITAGs in formulating national recommendations, but also promulgate recommended schedules of administration for other approved but not EPI-covered antigens, emulating in large part or entirely from U.S., Australian, or European schedules. Table 75.1 distinguishes schedules of such bodies from respective national EPI schedules.

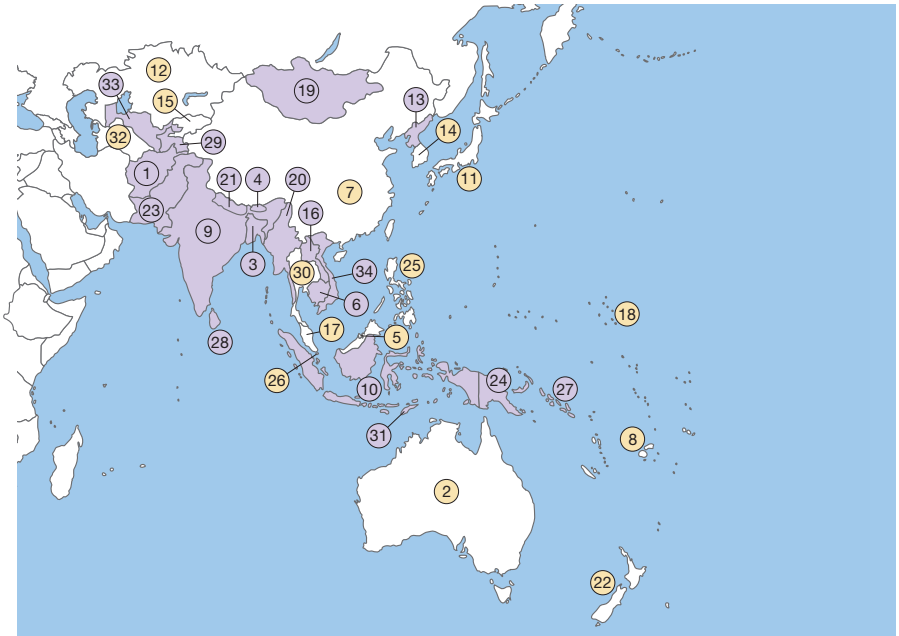
VACCINE DELIVERY AND COVERAGE

Vaccines are delivered in varying proportions through public or private channels, depending mainly on local income levels and accessibility to private practitioners. In general, vaccines on national schedules are available at no cost in primary health centers or their equivalent (e.g., puskesmas in Indonesia; polyclinics or government hospital clinics in Singapore, Malaysia, and Thailand; village and county level Centers for Disease Control in China; village communes in Vietnam; public health centers and clinics in India and Japan; and at general practitioner offices in Japan, and Australia). As vaccines generally are available free in public clinics, even in affluent countries, families may obtain them in government clinics or hospitals (e.g., in Singapore, ≈60% of families obtain vaccines through the government system of polyclinics and hospitals). However, to avoid long waiting times and rotating staff at public clinics, many families opt to obtain these otherwise free vaccines privately and to pay out-of-pocket at pediatric, general practitioner, or other private clinics. In addition, as newer vaccines may be delayed in their introduction to the national reimbursement scheme, it is common for parents to pay voluntarily for these vaccines (see earlier). As might be expected from the distribution of income, the proportion of children vaccinated in government primary health centers is higher in rural areas. Overall, approximately 90% of children in Thailand and 70% in Malaysia are vaccinated through public channels. In China, all vaccinations are under control of centers for disease control and prevention; therefore, nearly all Chinese children receive free EPI vaccines, as well as payable optional vaccines (e.g., Hib, pneumococcal conjugate vaccines, varicella, rotavirus, and others) at public clinics. Fig. 75.1 summarizes the coverage for EPI vaccines for selected countries.[45]

Supplementary immunization activities have played a critical role in the elimination of polio from the WHO Southeast Asian Region that was achieved in 2014, and in ongoing efforts to eliminate measles and congenital rubella syndrome. Routine and supplementary immunization activities tetanus vaccinations have eliminated maternal and neonatal tetanus in all but four countries in the region: Cambodia, Indonesia, Papua New Guinea, and Pakistan.

FUTURE TRENDS AND CHALLENGES

Economic growth and development in Asia and secular trends in population structure and the evolution of healthcare systems are forces that inevitably will change various aspects of immunization in the region, if in as-yet unforeseeable ways.[31,32] The population of Asia, as in other regions, is aging and shifting toward a structure with a larger proportion of adults and elderly persons. Between 2005 and 2025, the birth cohort of Asia will decrease slightly from 76.1 to 72.2 million, and the population of children 0 to 4 years old will hold nearly constant while the number and proportion of adults from 15 to 64 years will increase dramatically, and the number of people older than 65 years of age will nearly double, from 250.6 to 480.6 million. A demographic crossover point with more adults 60+ years of age than children younger than 15 years of age was reached in Europe in the 1990s, and will occur within another generation in Asia (Fig. 75.2).[46] With the exception of almost universal EPI programs of tetanus toxoid vaccination of pregnant women, adult vaccination has been viewed mainly in the context of travel, as in group A meningococcal vaccine for the Hajj, and in tropical Asia, influenza vaccine



Country	2013 WHO-UNICEF estimated coverage, %*					
	DTP3	HepB3	Pol3	Hib3	MCV	MCV2
1 Afghanistan	71	71	71	71	75	40
2 Australia	91	91	91	91	94	92
3 Bangladesh	97	97	97	97	93	91
4 Bhutan	97	97	97	97	94	89
5 Brunei Darussalam	90	99	90	90	99	96
6 Cambodia	92	92	77	92	90	63
7 China	91	99	99	–	99	99
8 Fiji	99	99	99	99	94	94
9 India	72	67	70	20	74	42
10 Indonesia	85	85	86	4	84	79
11 Japan	98	–	99	–	95	93
12 Kazakhstan	98	99	98	98	99	99
13 Korea, DPR	93	93	99	32	99	99
14 Korea, Republic of	99	99	99	–	99	95
15 Kyrgystan	97	97	97	97	99	97
16 Lao, PDR	87	87	86	87	82	95
17 Malaysia	97	96	97	97	95	99
18 Marshall Islands	36	41	36	21	70	42
19 Mongolia	98	98	98	98	97	97
20 Myanmar	75	72	76	72	86	80
21 Nepal	92	92	92	92	88	–
22 New Zealand	92	93	92	92	92	86
23 Pakistan	72	72	72	72	61	58
24 Papua New Guinea	68	68	69	68	70	–
25 Philippines	94	94	88	94	90	53
26 Singapore	97	97	97	–	95	95
27 Solomon Islands	83	83	85	83	76	–
28 Sri Lanka	99	99	99	99	99	99
29 Tajikistan	96	96	97	96	92	92
30 Thailand	99	99	99	–	99	94
31 Timor Leste	82	82	82	82	70	–
32 Turkmenistan	98	98	98	–	99	99
33 Uzbekistan	99	99	99	99	97	99
34 Vietnam	59	59	93	59	98	86

*Estimates may not include coverage achieved through private channels

Figure 75.1. Estimated coverage of basic childhood vaccines by country, 2015. DPT3, at least 3 doses of DPT vaccine; Hep3, at least 3 doses of hepatitis B vaccine; Pol3, at least 3 doses of polio vaccine; Hib3, at least three doses of *Haemophilus influenzae* type b-containing vaccine; MCV, at least one dose of a measles-containing vaccine; MCV2, second dose of a measles-containing vaccine. Many Global Alliance for Vaccines and Immunization–eligible countries (shaded) have similar coverage rates of basic vaccines as countries at higher levels of economic development, illustrating the success of Expanded Programme on Immunization.

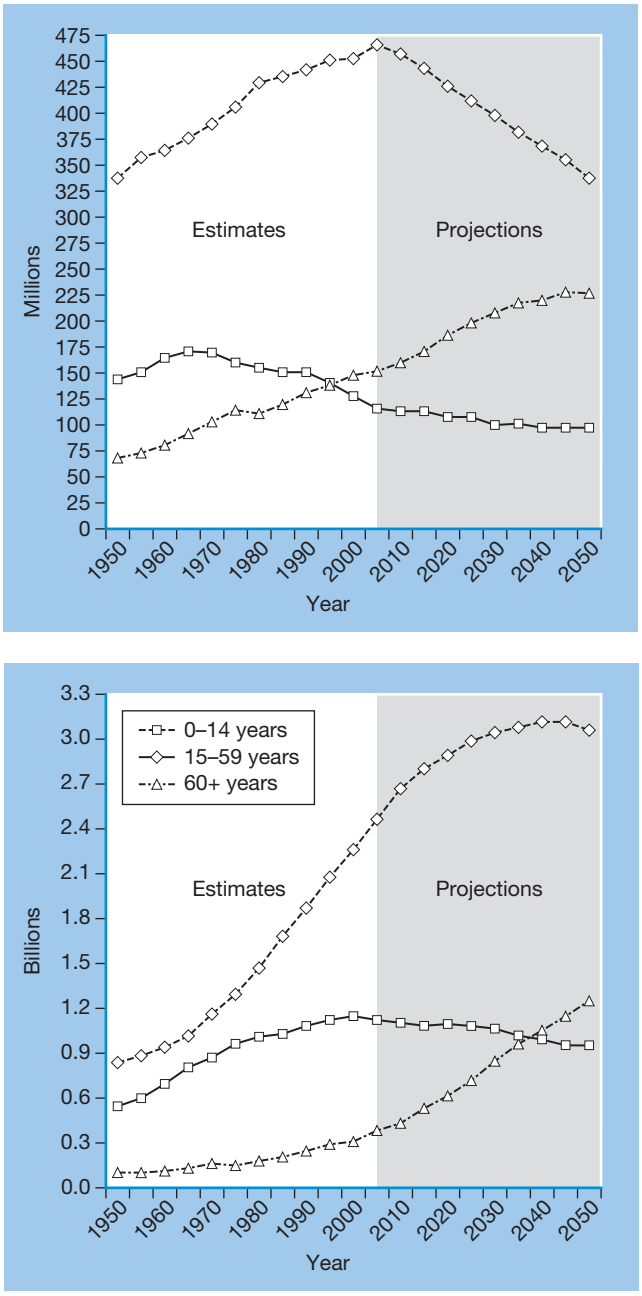


Figure 75.2. Population strata by age in Europe (top) and Asia (bottom), 1950 to 2050 (projected). The crossover point when the population of adults older than 60 years of age exceeded the population of children younger than 14 years of age was crossed in Europe around 1995; that crossover is projected to occur in Asia around 2037, within a generation from now.

for travelers to temperate locations. However, the severe acute respiratory syndrome and pandemic H1N1 outbreaks and the regional threat of H5N1 influenza have focused attention on routine seasonal influenza vaccination for the first time in many countries, beginning with elderly populations, and the role of children in influenza transmission is being recognized while it is rediscovered in Japan. As a result of high pediatric vaccination coverage in developed countries in the region, JE has become almost exclusively a disease of adults older than 45 years of age, reflecting the intrinsic biological susceptibility of older adults to neurotropic flaviviruses and suggesting a future need for adult vaccination.[47] Hepatitis A, interestingly, is now principally a risk in the cohort of young adults who were raised in an era of economic development and improved sanitation and who therefore lack natural immunity but were born before routine childhood vaccination was implemented.[48] A catch-up program to address this epidemiological shift has been recognized by adult vaccination recommendations in some countries (see Table 75.3). In China, adult measles vaccination is under discussion, as more than 100,000 cases have occurred annually in recent years, in equal proportion in adults older than 20 years and in infants who had not received their first vaccine dose. Growing awareness of adult vaccination is reflected in an increasing number of countries with adult vaccination recommendations (see Table 75.3).

Two other population trends that will influence the demand for vaccines and channels for their delivery are urbanization and income disparity.[37] The urban-dwelling population in Asia is projected to increase by almost a billion persons between 2005 and 2025, from 1.5 billion to 2.4 billion, while the rural population will decline only slightly. Urban crowding is likely to affect the transmission patterns of certain person-to-person transmitted diseases and even of infections acquired from environmental sources. Dengue, for example, is transmitted by mosquito vectors that are more prevalent in urban environments; the already great need for a dengue vaccine will almost certainly increase with the growth of urban centers.[49] While the growing size and number of large cities may increase transmission of certain infections, delivery of vaccine and of healthcare in general is better organized in cities than in rural areas. Specific interventions are needed to ensure that the existing disparity in access to healthcare between urban and rural dwellers does not widen.

Associated with urbanization is the increasing income gap in many countries that, in the health arena, has translated into a two-tiered system of healthcare, including preventive medicine. While vaccines are regarded by many as a public good to be provided as a government service, as mentioned, access to the increasing number of new vaccines is likely to be stratified by income level and ability to pay, as governments must choose among increasingly costly vaccines and other health interventions. As shown in Table 75.1, pediatric societies in a number of countries promulgate recommendations emulating those of the U.S. ACIP, and these schedules, aimed at practitioners serving private-paying families, may diverge increasingly from the national EPI schedules benefiting the majority of children in those countries. How the public and governments will respond to an increasing disparity of what has been perceived as a basic medical service remains to be seen.

In coming years, more novel vaccines are likely to be developed in Asia or licensed first in Asia for a regional, developing world, or international market. Governments and ASEAN have expressed increased interest in providing for national and regional vaccine security. The collaboration of industry sponsors with nongovernmental organizations and government in public private partnerships for new product development has been highlighted by the successful introduction of vaccines and drugs for several neglected diseases, for which the DCVMN view a responsibility. For example, the Japan International Cooperation Agency and Kitasato Daiichi Sankyo provided technical assistance to establish domestic measles-rubella vaccine production in Vietnam's public corporation, Center for Research and Production of Vaccines and Biologicals, POLYVAC. At the same time, the entry of nongovernmental organizations as actual sponsors of novel vaccine development for certain target diseases introduces competition with DCVMN manufacturers and multinational companies that might also consider similar development programs. Asian academic institutions and companies possess elements

of the scientific and technical expertise needed to develop vaccines for current and emergent needs and, seemingly, the will to establish themselves on the global stage and contribute to their development. Regional institutions responded rapidly to threats of Middle East Respiratory Syndrome virus and Ebola virus with candidate vaccine development even when transmission was geographically remote. Further participation of regional institutions in global responses in the future is likely.

Trends toward increasing local development and manufacturing in the region and the accompanying need to strengthen respective regulatory agencies have been recognized by the WHO and local national regulatory authorities. Revising and harmonizing guidelines and procedures to international standards and enforcing procedures in a consistent and predictable manner will improve the timely regional introduction of vaccines developed internationally. As important, compliance with international standards will be required of regional manufacturers hoping to license locally developed vaccines more broadly. Indian and Chinese manufacturers currently export a limited number of vaccines, mainly regionally and to African and Latin American countries, but their horizons undoubtedly will expand.

In the six-component framework of product development capability—manufacturing; national and international distribution systems; private and public R&D capabilities; intellectual property system; and drug and vaccine regulation—regional manufacturers are at different stages of maturation.[50] In its ascendance to an advanced country producing complex biologicals as well as other high technology products, Korea followed a path that might be emulated by others in the region, highlighted by its arrival at a stage with a national system of innovation in science and technology, linking government, universities and industry, a strong regulatory system and observation of intellectual property rights, including adherence to Trade-Related Aspects of Intellectual Property Rights (TRIPS agreement).

A specific area of regulatory control needing particular attention is the strengthening of national control laboratories. Many countries lack the laboratory capacity to test samples for lot release, and because manufacturing and testing technologies change rapidly, keeping up with new procedures and purchasing needed equipment are ongoing challenges. Continuous support also is needed to produce working quantities of reference standards, validation of new assays, staff training, and proficiency testing. As resources are unavailable in many countries to establish and maintain a fully functioning national control laboratory, a regional network has been proposed as an approach to share expertise and to divide workload, while at the same time standardizing methods and criteria. Field surveillance of adverse events following immunization is another area requiring strengthening. Investigations of adjuvanted H1N1 and H5N1 pandemic and prepandemic vaccines administered in Korea and Taiwan, respectively, illustrated the interest in and epidemiological capacity of local investigators but also the limitations of existing systems and databases. Japan is establishing a database of clinical encounters that if linked to immunization records could be used as a future adverse events surveillance system.

Regulatory oversight of clinical trials and human subjects protection are other areas that are under growing pressure for improvement. Multinational companies have increased the number of clinical trials in Asian countries to reduce costs and to obtain local registration of products. Their activities serve an important role in strengthening local compliance with Good Clinical Practices, as many groups conducting trials in the region have limited experience with these precepts and procedures. Countries in the region have an interest to establish and enforce clear guidelines, not only as hosts to an increasing number of trials but also because their manufacturers, as future sponsors of new products, will be accountable internationally to uphold recognized standards.

Acknowledgments

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76 Immunization in Developing Countries

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Since the late 1970s, childhood immunization has been one of the most effective and cost-effective public health preventive measures in developing and developed countries.[1–4] Immunization is estimated to prevent 2 to 3 million deaths annually across all age groups.[5]

The history of immunization in low-income countries can be traced back to the birth of the Expanded Programme on Immunization (EPI) in 1974. At that time less than 5% of the infants in low-income countries were receiving basic immunization. The success of the smallpox eradication program, the recognition of the enormous potential of vaccination to control communicable diseases even in the most resource-poor settings, and the fact that in many regions and countries children had almost no access to this lifesaving intervention, led the World Health Assembly to pass a resolution (WHA27.57) establishing EPI in May 1974. Thus, EPI was created as the legacy of the smallpox eradication program for the purpose of expanding large-scale delivery of vaccines from a single antigen (i.e., smallpox) to include vaccines against an additional six life-threatening or disabling diseases, namely, tuberculosis, diphtheria, tetanus, pertussis, poliomyelitis, and measles. The principles and lessons learned from the smallpox eradication program were key to the successful implementation of EPI.[6]

Global policies for immunization, with a goal for providing universal access to immunization to all children by 1990, were established in 1977, through a World Health Assembly resolution (WHA30.53). Concern about the slow progress in increasing coverage led to the establishment of the Universal Childhood Immunization initiative led by United Nations Children's Fund (UNICEF) in collaboration with the World Health Organization (WHO) in 1984, with the aim of increasing coverage with three doses of diphtheria-tetanus-pertussis (DTP)-containing vaccines (DTP3) to 80% globally by 1990.[7] This initiative was extremely successful in rapidly increasing immunization coverage. Estimated DTP3 global coverage rose from approximately 20% in 1980 to close to 80% in 1990; coverage in low-income countries rose from less than 10% in 1980 to 51% in 1990 (Fig. 76.1).

This early success with increasing immunization coverage and its consequent impact on the diseases targeted, led to the establishment of ambitious disease eradication (poliomyelitis), elimination (measles, rubella, and maternal and neonatal tetanus), and control (hepatitis B and Japanese encephalitis) goals. Surveillance capacity to document progress with eradication and elimination goals provided a platform for communicable disease surveillance that has served to detect and respond to a range of diseases, including those that are not vaccine-preventable.

However, despite these early successes, immunization coverage stagnated in the 1990s. In addition, none of the new lifesaving vaccines that were becoming available were reaching children in the poorest countries. Gavi, the Vaccine Alliance, was established in 2000 to improve the access to new and underused vaccines for children living in the world's poorest countries and to reduce the historical time lag between the time when such innovations became available to rich but not to poor countries.[8] In its initial phases, available new and underused vaccines—hepatitis B, *Haemophilus influenzae* type b (Hib), yellow fever, pneumococcal, and rotavirus vaccines—were selected on the basis of the estimated burden of disease in Gavi-eligible countries and a WHO recommendation for use. In subsequent years, as the number of new and underused vaccines increased, selection of vaccines for support was determined through a Vaccine Investment Strategy, an evidence-informed and consultative process that is undertaken every 5 years.[9]

Eligibility for Gavi support is based on the Gross National Income (GNI) per capita. Countries with GNI of US$1580 or less (according to the World Bank data for the latest available year) are eligible to seek support for the introduction of new or underused vaccines, or for strengthening of health systems. Countries enter into a "graduation" process and start phasing out of Gavi support once their per capita GNI exceeds the prescribed threshold. Countries entering graduation have an additional year to apply for new vaccine support and can access time-limited catalytic investments to support their graduation plans. Table 76.1 lists the countries were eligible for new vaccine support in 2015.

Support from Gavi catalyzed the introduction of new and underused vaccines in low- and low-middle income countries. As a result, most countries, including the low- and middle-income countries have nine or more vaccines in their national schedules, compared to the six when EPI was established (Fig. 76.2). With the addition of vaccines targeted to preadolescents and adolescents (e.g., human papillomavirus vaccines), pregnant women and the elderly (e.g., seasonal influenza vaccines), as well as additional doses of existing vaccines in the second year of life or later to enhance disease control, EPI evolved from being a program primarily targeting infants to one targeting individuals across the life span.

With newer, more expensive, vaccines rapidly becoming available, guidance is required to enable developing countries to make informed choices on the introduction and use of these vaccines in their national programs. Most developing countries, as well as their development partners, including Gavi, look to the WHO to provide such guidance. The WHO Strategic Advisory Group of Experts (SAGE) for immunization was established in 1999 to advise the WHO on vaccine policy. In 2005, the SAGE was restructured and its working mechanism adjusted to meet the emerging needs of the program.[10] The group functions in an open and transparent manner and provides policy and strategy recommendations on the use of vaccines in national programs, which form the basis of the WHO position papers published in the *Weekly Epidemiological Record*.[11] The latest addition is the publication of tables that assess and grade the quality of the evidence underlying the recommendations using the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) approach.[12]

Despite the remarkable success of the global immunization program, the full potential of immunization is still to be realized. Increasing immunization coverage above 80% to reach the "fifth child" with the required doses of vaccines has proved to be difficult for many developing countries (Fig. 76.3). As a result, an estimated 19.4 million children still fail to receive all three of the required doses of DTP-containing vaccines[13] and an estimated 1.5 million children still die each year from vaccine-preventable diseases, most of them in low- and

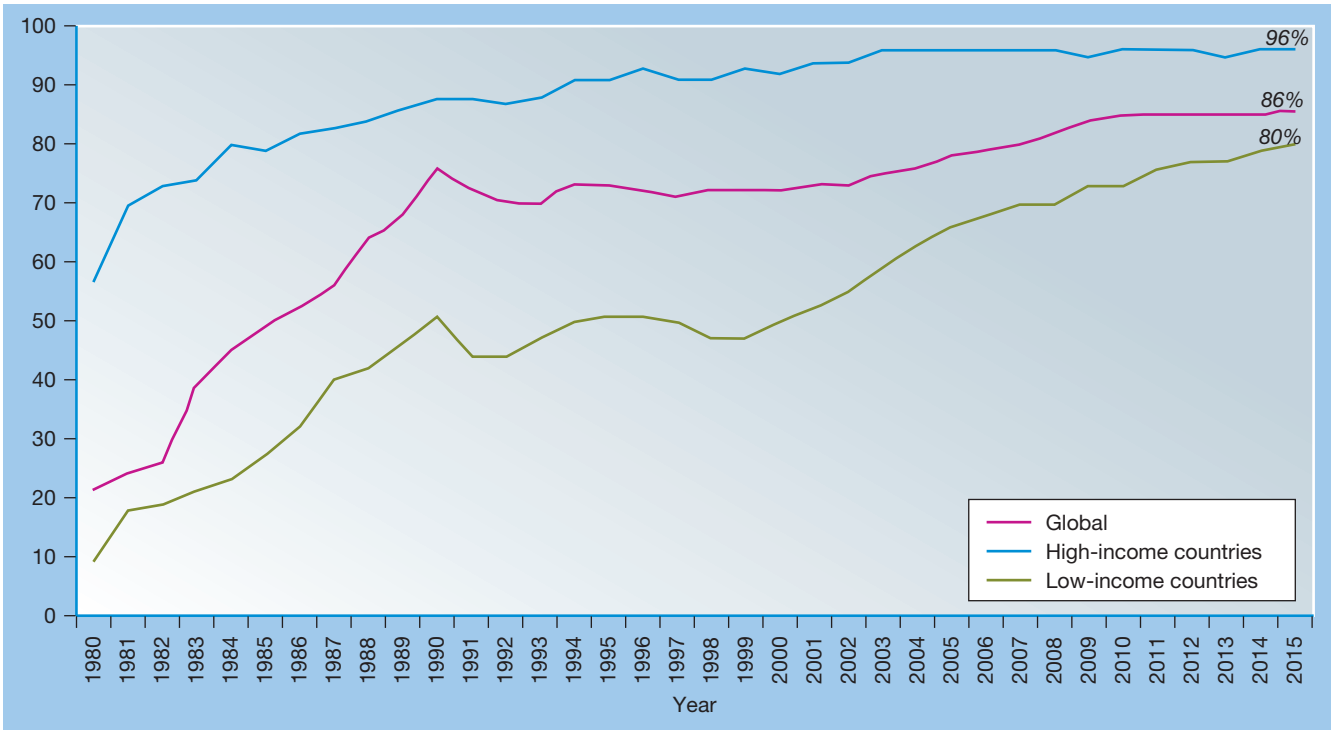


Figure 76.1. Coverage with three doses of diphtheria, tetanus, and pertussis (DTP)-containing vaccines (DTP3) by country income levels, 1980–2015. *(From WHO/UNICEF coverage estimates 2015 revision. July 2016 and Country Income Categories [World Bank] as of July 2016 [2015 GNI per capita]. Income classification not available for Cook Islands and Niue. Map production: Immunization Vaccines and Biologicals (IVB), World Health Organization.)*

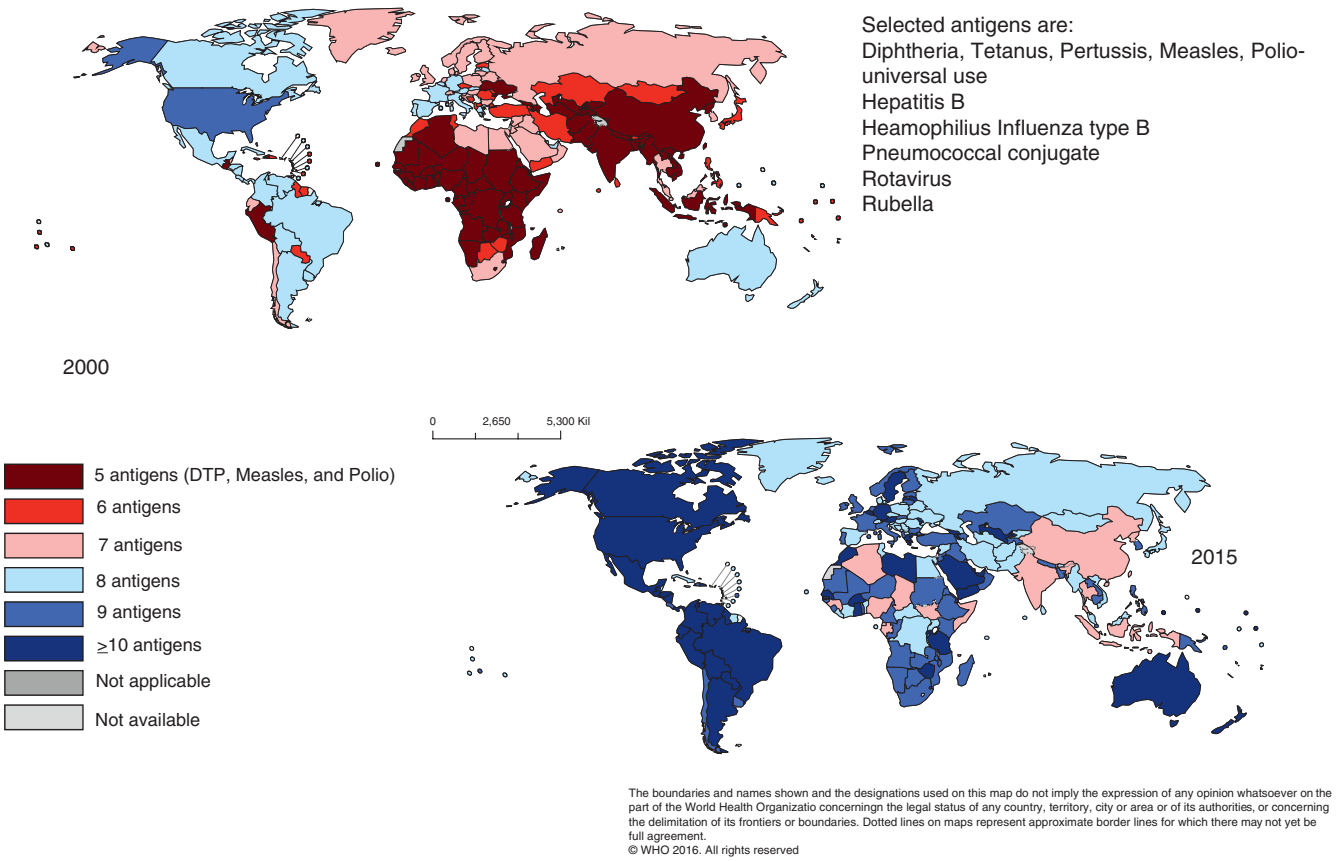


Figure 76.2. Number of vaccines/antigens introduced in national immunization schedules—2000 compared to July 2015. *(Data from WHO/IVB Database, as of September 29, 2016. Map production: Immunization Vaccines and Biologicals [IVB], World Health Organization.)*

TABLE 76.1 Countries Eligible for Gavi Support in 2016

Afghanistan	Madagascar
Bangladesh	Malawi
Benin	Mali
Burkina Faso	Mauritania
Burundi	Mozambique
Cambodia	Myanmar
Cameroon	Nepal
Central African Republic	Niger
Chad	Nigeria
Comoros	Nicaragua
Côte d'Ivoire	Pakistan
Democratic Republic of Congo	Rwanda
Djibouti	Sao Tomé e Principe
Eritrea	Senegal
Ethiopia	Sierra Leone
Gambia	Solomon Islands
Ghana	Somalia
Guinea	Republic of Sudan
Guinea Bissau	South Sudan
Haiti	Tajikistan
India	Tanzania
Kenya	Togo
Korea, DPR	Uganda
Kyrgyz Republic	Vietnam
Lao PDR	Yemen
Lesotho	Zambia
Liberia	Zimbabwe

From Gavi–The Vaccine Alliance. Countries eligible for support. http://www.gavi.org/Support/Apply/Countries-eligible-for-support/.

middle-income countries (Fig. 76.4). The increasing number of vaccines in national programmes has exposed the fragility of the immunization systems and stretched the immunization budgets in many low- and middle-income countries, leading to concerns about their long-term sustainability. Even though the quantum of immunization data being collected and reported has increased exponentially, the quality and use of the data has remained suboptimal and has not resulted in timely action. Armed conflict, insecurity, and other humanitarian disasters, including disease outbreaks, have led to sharp declines in immunization coverage in countries that had well-performing immunization programs.[14]

GLOBAL VISION FOR VACCINES AND IMMUNIZATION

Recognition of the substantial achievements of EPI since its inception, coupled with the realization that the full potential of immunization was yet to be tapped and the unprecedented opportunities to make a greater impact on the health of populations through the use of the new array of lifesaving vaccines led to the call for a Decade of Vaccines in 2010.[15] In response to this call, the Global Vaccine Action Plan (GVAP) was developed through a broad consultative process that involved more than 1100 individuals from 140 countries, representing more than 290 different organizations, to provide a framework for achieving a common vision of a world in which all individuals and communities enjoy lives free from vaccine-preventable diseases.[16]

The GVAP, which was endorsed by the World Health Assembly in May 2012, replaced the existing Global Immunization Vision and Strategy. While the GVAP reiterated several of the goals and principles of the Global Immunization Vision and Strategy, it also included some shifts in emphasis, namely: (a) country ownership and the need for better governance of the program; (b) equal emphasis on community demand and service delivery; (c) the need to make immunization programs an integral part of the broader health systems and the need for better coordination and synergy with other primary healthcare programs; (d) moving beyond equity in coverage at the district level to include other parameters of equity and to reach every community with immunization services; and (e) greater focus on research, not just for development of new vaccines, but also for development of delivery technologies and for the social science research to enhance access to and delivery of immunization. Beyond these changes in content, in what is considered a game changer, the GVAP is accompanied by a monitoring and accountability framework and an independent assessment of progress that is presented annually to the WHO governing bodies. The first three assessments of progress were presented to the WHO Executive Board and the World Health Assembly in 2014, 2015, and 2016.[17,18]

PROGRESS WITH ACHIEVING GLOBAL GOALS

The GVAP includes the following five global goals for immunization: (1) achieve a world free of poliomyelitis; (2) meet global and regional elimination targets for measles, rubella, and neonatal tetanus; (3) meet vaccination coverage targets in every region, country, and community; (4) develop and introduce new vaccines and technologies; and (5) exceed the Millennium Development Goal 4 target for reducing child mortality. In 2016, the WHO SAGE for immunization, the body charged with making an independent assessment of progress as per the monitoring and accountability framework, reviewed progress against these goals. Based on a review of data from 2015, the SAGE concluded that at the mid-point of the Decade of Vaccines, the implementation of the GVAP has been patchy and slow.[14] The assessment report noted that there were six immunization targets for 2014 or 2015, of which only one, the target for the introduction of new vaccines in low- and middle-income countries, appeared to be on track; the remaining were missed. While the targets related to different diseases and vaccines, two common threads ran throughout: failure to extend vaccination services to those who cannot access them at all, and failure to strengthen the healthcare systems so that all the required doses are reliably provided. In a sense, the fundamental changes that the GVAP aimed to bring about are not being realized. The progress against the goals are briefly summarized below.

Polio Eradication

The interim goal for polio eradication was the interruption in transmission of wild virus by 2014. This goal was missed. Although there was substantial progress in 2011–12 with India and the Southeast Asia region being declared wild virus–free, the situation deteriorated in other regions, with an increasing number of cases in Pakistan and the subsequent spread of the virus to the conflict-affected countries in the Middle East and the horn of Africa. Despite the failure to achieve the 2014

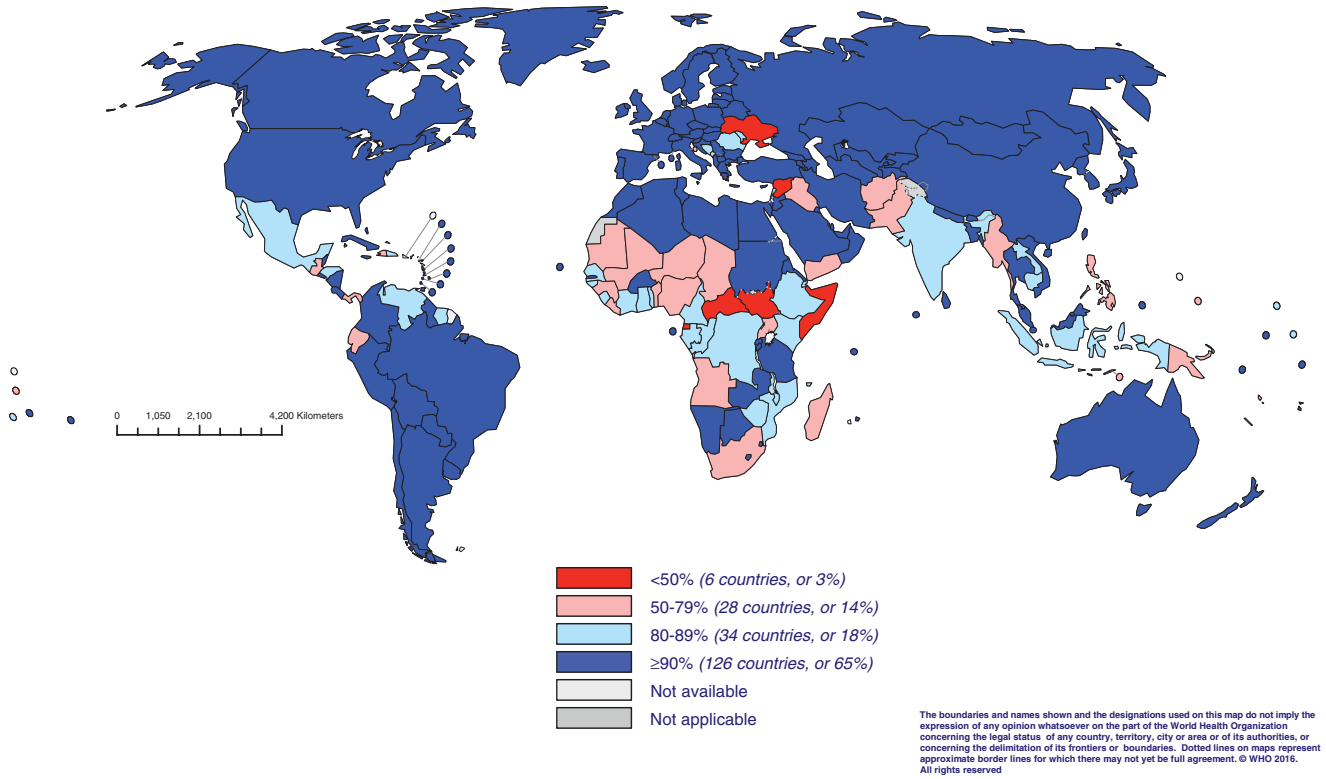


Figure 76.3. Immunization coverage with three doses of diphtheria, tetanus and pertussis (DTP)-containing vaccines in infants in 2015. *(From WHO/UNICEF coverage estimates 2015 revision, July 2016. Map production: Immunization Vaccines and Biologicals [IVB], World Health Organization.)*

target, there was progress. In 2013, for the first time in history, all cases of wild poliovirus disease were caused by a single serotype, type 1.[19] For 2016 (as of October 19), 27 cases of wild poliovirus disease had been reported in three countries (Pakistan, Afghanistan, and Nigeria). In 2015, only two countries—Pakistan and Afghanistan—reported wild poliovirus; progress in these two countries is impeded by insecurity, including the killing of health workers.

Measles and Rubella Elimination

Worldwide, the estimated mortality from measles has been reduced by 79% since the year 2000 (Fig. 76.5). In 2015, 85% of children globally were estimated to have received their first dose of measles-containing vaccines (MCV1), 61% received a second routine dose of measles containing vaccines and more than 100 million children were reported to have received additional doses each year through supplementary immunization activities. However, elimination of measles is thought to require coverage exceeding 95% with two doses of measles-containing vaccines. The interim global milestone for 2015 to achieve greater than 90% coverage with MCV1 and reducing measles incidence to less than five per 1 million population was not met. By 2014, all six WHO regions had established goals for eliminating measles by 2020 or earlier. The region of the Americas was declared to have eliminated measles in 2016, but remains under constant threat of spread of disease following importation from other regions, highlighting the challenges of maintaining elimination in the face of ongoing transmission in other regions and competing health priorities. The remaining three regions that had established goals to eliminate measles by 2015, namely the Eastern Mediterranean, European, and Western Pacific regions, did not achieve their target. The African and South-East Asian regions have adopted goals to eliminate measles by 2020 but face significant challenges to achieving their goals. Despite sustaining high routine coverage, some countries have experienced large outbreaks that pick out pockets of susceptible individuals. The experience in the Americas shows that elimination is possible, but requires strong political commitment, financial resources, and sustained efforts to achieve and sustain elimination.

Elimination of rubella and congenital rubella syndrome (CRS) from at least five regions by 2020 is one of the goals in the GVAP. This ambitious goal was based on the assumption that if all countries used measles- and rubella-containing vaccines for achieving and sustaining measles elimination, rubella and CRS elimination would follow. However, only three regions, namely the American, European, and Western Pacific regions, have established regional elimination goals; the South-East Asia region has established a control goal, while the African and Eastern Mediterranean regions have not yet established goals. By December 2015, 47 countries, mainly those in regions without elimination goals, had not introduced rubella containing vaccines in their national immunization programs. These include 40 countries who are eligible for and could receive Gavi support for introduction of rubella-containing vaccines; some of these countries have partially introduced the vaccine or have plans to introduce the vaccine. Rubella and CRS surveillance systems are weak and cases remain underreported, particularly in countries that have not yet introduced rubella-containing vaccines and/or do not have rubella control or elimination goals. Hence, global rubella and CRS surveillance data do not reflect the true burden of these diseases. Failure to fully integrate prevention of rubella and CRS with measles elimination activities represents a major missed opportunity for immunization.

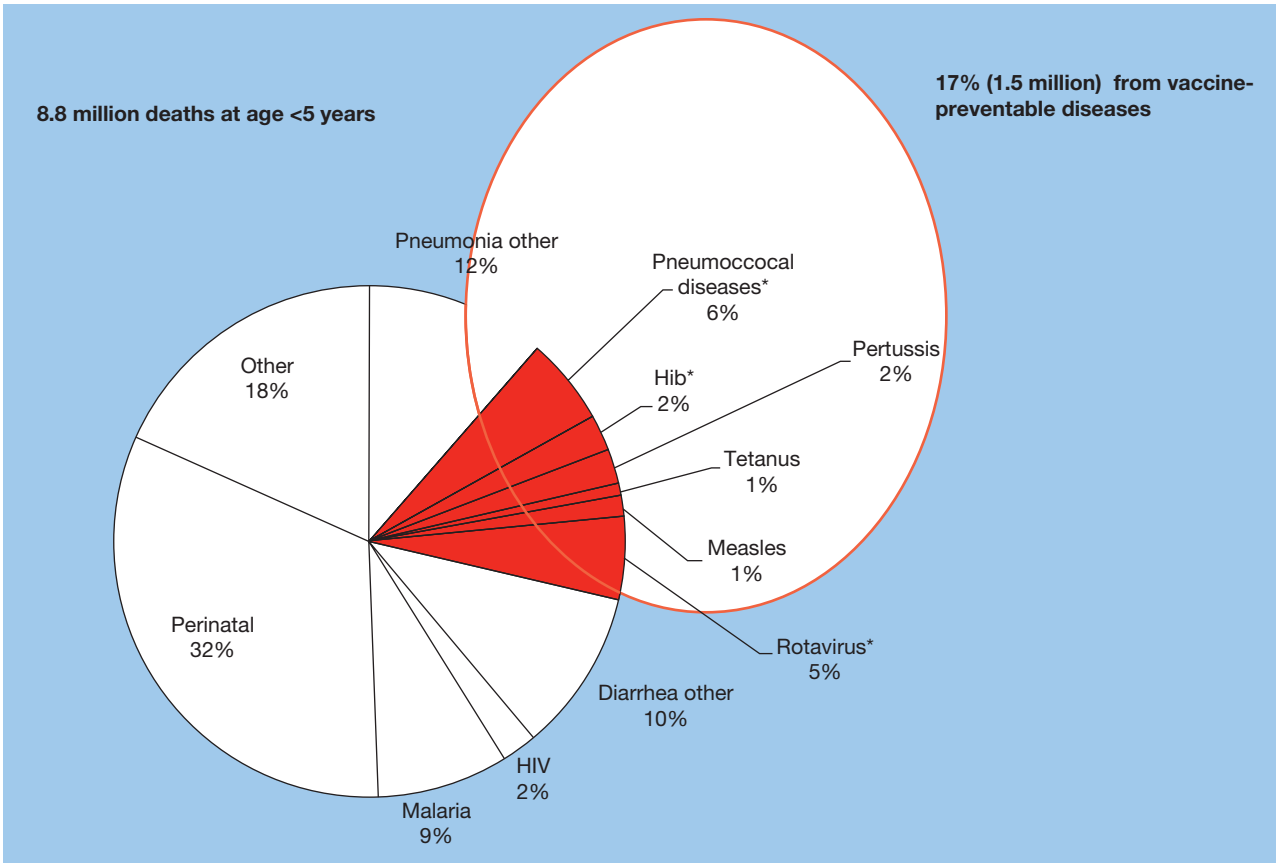


Figure 76.4. Global mortality of children age <5 years: role of vaccine preventable diseases (estimates for the year 2008). *(From World Health Organization estimates; and Black RE, Cousens S, Johnson HL, et al; Child Health Epidemiology Reference Group of WHO and UNICEF. Global, regional, and national causes of child mortality in 2008: a systematic analysis.* Lancet. *2010;375[9730]:1969–1987.)*

Maternal and Neonatal Tetanus Elimination

In 1988, the WHO estimated that 787,000 newborn infants died of neonatal tetanus each year, which led to the adoption of a goal to eliminate neonatal tetanus (defined as <1 death/1000 live births) by 1995. This goal was later shifted to 2005 and then to 2015, as each target was missed. Elimination efforts were focused on 59 countries that carried almost all the burden of disease. In 2015, the estimated deaths from neonatal tetanus were 34,019, representing a 96% reduction compared to 1988. However, by December 2015 only 38 of 59 priority countries had been validated as having achieved elimination (Fig. 76.6), leaving 21 countries yet to achieve elimination. Thus, the target to achieve elimination of maternal and neonatal tetanus by 2015 was missed yet again.

Neonatal tetanus represents an important consequence of health inequity. The disease occurs mainly in communities that lack access to basic health services, including immunization, antenatal care, and safe and clean deliveries. Bridging this equity gap remains a challenge. The GVAP assessment report noted that maternal and neonatal tetanus elimination was "embarrassingly underfunded."[14] Success in many countries indicate that the existing strategies work and what is required is the political will, the required finances, and the provision of the vaccine in compact, prefilled, autodisable devices that will make it easier to deliver vaccine to pregnant women and women of childbearing age living in remote areas, inaccessible to routine health services.

Immunization Coverage and Equity

DTP3 coverage is often used as a proxy measure for the overall performance of an immunization program and has sometimes been used as an indicator of the performance of the broader health system. In 2005, a goal for achieving 90% or greater DTP3 coverage nationally and 80% or greater in every district or equivalent administrative unit in the country by the end of 2015 was established. Sadly, the estimated global DTP3 coverage has remained stagnant for the past 5 years. In 2015, only 126 (65%) of countries had achieved national coverage of 90% or greater, although the number of countries achieving and sustaining DTP3 of 90% or greater for 3 or more years has been steadily increasing (Fig. 76.7). In 2015, DTP3 was below 50% in six countries; some of these countries, such as the Central African Republic, Somalia, South Sudan, and the Syrian Arab Republic have been affected by conflicts.

In many countries that are failing to achieve national DTP3 coverage of 90% or greater, high dropout rates between the first and third doses of DTP-containing vaccines have remained a persistent problem. Large countries like India, Nigeria, Pakistan, Indonesia, and Ethiopia have substantial dropout rates and reducing this rate could have a large impact on the global DTP3 coverage (Fig. 76.8). Addressing the causes of undervaccination, including missed opportunities for vaccination, could have a large impact in reducing the number of such children.

Assessment of equity in coverage is affected by the lack of valid data. Only 112 (58%) of the 194 WHO Member States

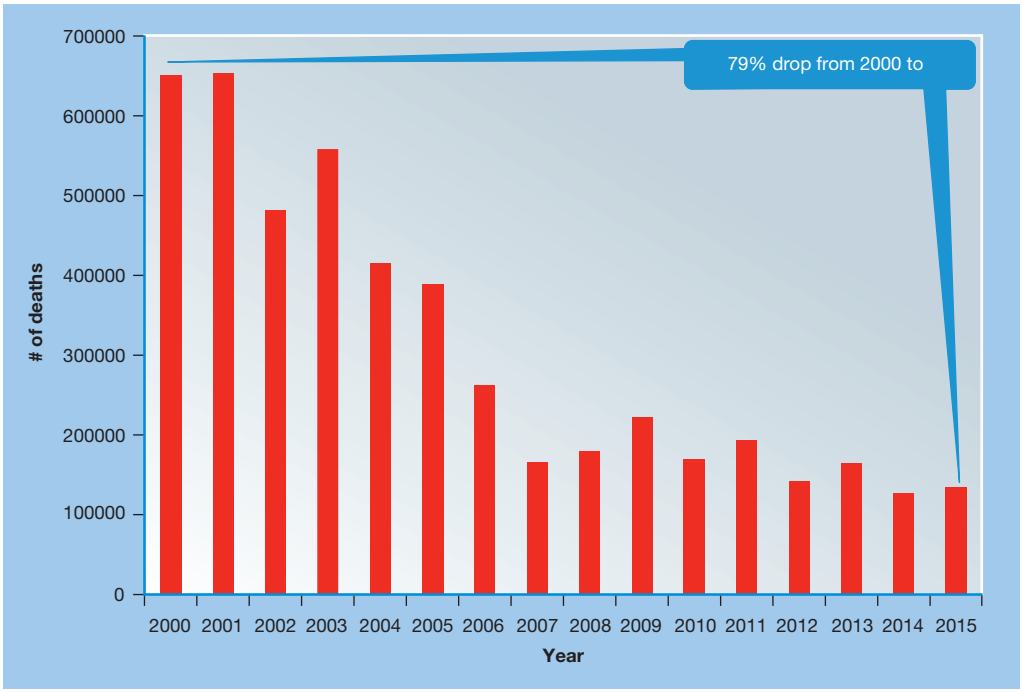


Figure 76.5. Reduction in estimated measles deaths, 2000–2015. *(From Weekly Epidemiological Record No. 45, 2016:525–536.)*

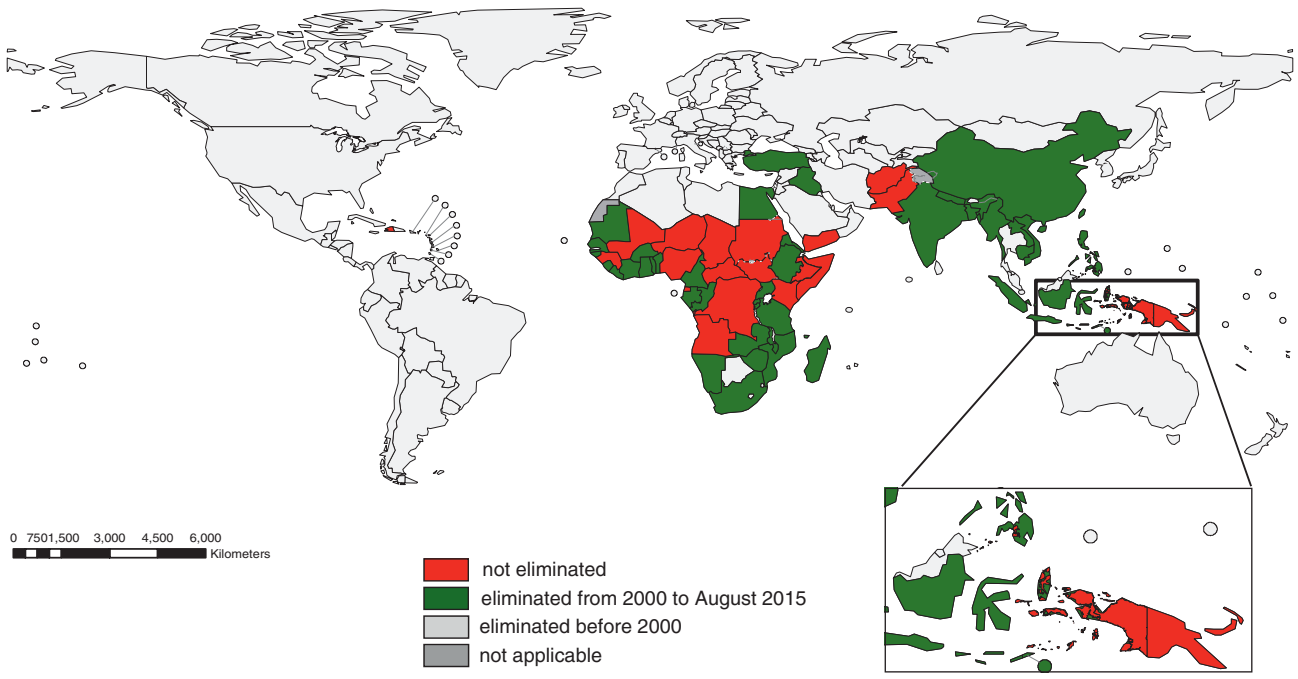


Figure 76.6. Status of maternal and neonatal tetanus elimination. *(Data from WHO-UNICEF database, July 2016. Map production: Immunization Vaccines and Biologicals [IVB], World Health Organization.)*

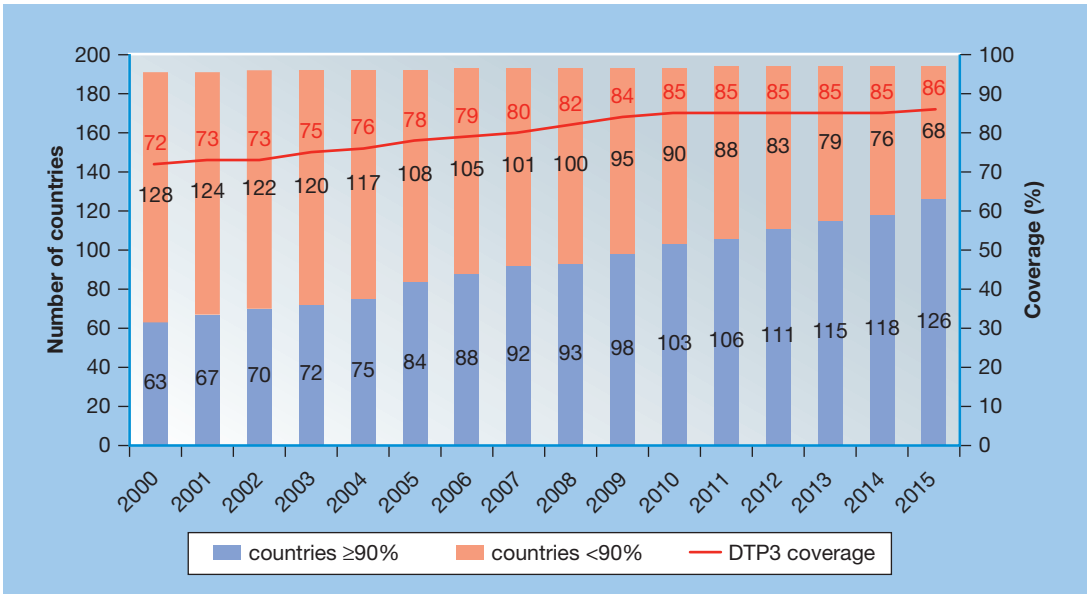


Figure 76.7. Number of countries that reached and sustained 90% or greater coverage with three doses of diphtheria, tetanus, and pertussis (DTP)-containing vaccines and global DTP3 coverage from 2000–15. *(From WHO/UNICEF coverage estimates, 2015 revision. July 2016. Map production: Immunization Vaccines and Biologicals [IVB], World Health Organization.)*

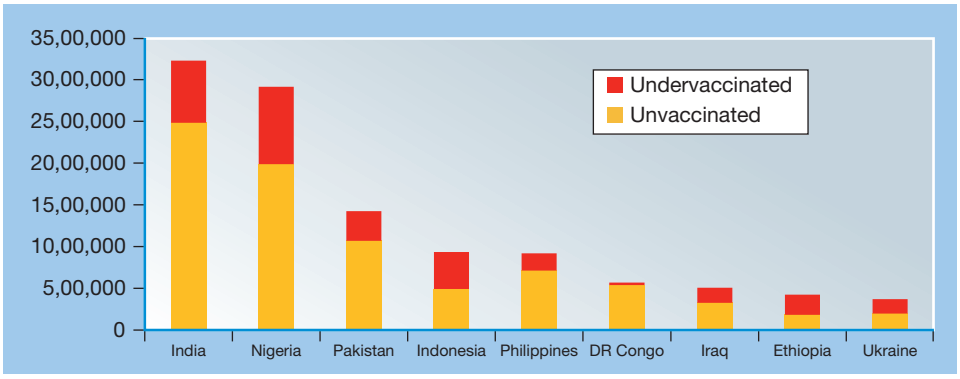


Figure 76.8. Ten countries with the most undervaccinated and unvaccinated children (based on coverage with the first and third doses of diphtheria, tetanus, pertussis [DTP]-containing vaccines), 2015. *Unvaccinated*, those who have not received any dose of DTP-containing vaccines; *undervaccinated*, those who have received one but not all three doses of DTP-containing vaccines. *(From WHO/UNICEF coverage estimates, 2015 revision. July 2016.)*

provided valid district-level coverage data in 2015, with only 52 (27%) having DTP3 coverage of 90% or greater nationally and of 80% or greater in all districts. Only 64 countries had coverage data from recent surveys, of which 31 had a difference of 10% or more in coverage between the richest and poorest wealth quintiles; the difference was 20% or more in 20 of these 31 countries. Thus, even though two-thirds of countries are estimated to be achieving high national coverage (i.e., ≥90%), inequity in coverage is prevalent.

Introduction of New Vaccines in Low- and Middle-Income Countries

There has been significant progress in recent years in the introduction of new vaccines in national immunization programs. A total of 160 new vaccine introductions took place in 99 low- and middle-income countries in the period 2010 to 2015.[20] The vaccines introduced include vaccines against Hib, *Streptococcus pneumoniae*, human papillomavirus, rubella, and Japanese encephalitis. Among these 99 countries, 64 benefited from support from Gavi. Among 62 middle-income countries not eligible for Gavi support, 35 (56%) have introduced a vaccine during the period 2010 to 2015, compared to 64 of 73 (88%) of Gavi-eligible countries, indicating a lag in countries that do not benefit from Gavi support.

Innovation and Research

Beyond the full use of the vaccines available today, it must be recognized that vaccine science has enormous future potential, including vaccines and technologies that would specifically address the needs of developing countries. The assessment of progress with the GVAP implementation included an assessment of progress with research and innovation.[14]

The assessment concluded that there is a robust pipeline of new or improved vaccines under development, including candidate vaccines against tuberculosis (13 candidates), malaria

(30 candidates), and HIV (40 candidates), all of which potentially address a large burden of disease in developing countries. However, the report also noted that there were a number of bottlenecks that needed to be addressed, including the following: (1) lengthy and expensive clinical evaluation processes; (2) failure or delays in reporting clinical trial results that could potentially lead to biased decision making; (3) complex development pathways and regulatory processes, with inadequate capacity in many developing countries to deal with these complexities; and (4) lack of coordination between different agencies and research groups conducting vaccine research.

While vaccine research is conducted in all regions in the world, the quantum of research is very unevenly distributed. Of the 624 vaccine clinical trials registered in different clinical trial registries during the period May 2015 to April 2016, only 37 were in the African region and 10 in the Eastern Mediterranean region.[20] This suggests the need for strengthening capacity in developing countries to conduct the relevant research to serve their needs.

With the increasing costs of vaccines and the vaccination programs, and with ever-increasing and competing health and development priorities, national decision makers and ministries of finance are likely to demand more evidence in support of the increased budgetary requests. This will include proper documentation of the impact of vaccination. Even though some of the data may be generated through improved disease surveillance, as described later in this chapter, some of the information required will need specifically designed and well-implemented research studies. This further underlines the urgent need to strengthen epidemiology and research capacities in developing countries. Failure to do so may result in inadequate investments and continued dependency on support from development partners and international agencies.

As vaccines are added to the national programs in developing countries, their immunization schedules are likely to become more crowded, straining the immunization systems and requiring additional human resources with appropriate training to properly deliver the vaccines. A number of new technologies are available that could simplify delivery and address some of these bottlenecks. However, these products have cost implications, making the demand uncertain and, thus, not providing incentives for investing in the development and commercialization of vaccines to be delivered using these technologies in developing country markets. This calls for a better assessment of the trade-off between the increased costs of the product, the cost savings from the method of vaccine delivery, and the increased impact through expanded coverage to inform decisions, stimulate demand, and lead to greater investments in further developing and using these technologies.

Developing countries face a number of managerial, health systems, sociobehavioral, and communications challenges for achieving and sustaining high immunization coverage and introducing new vaccines. Implementation research can generate information to support evidence-informed solutions. Once again, many developing countries may lack the capacity to identify research needs and to design and implement the required research. The WHO is developing a prioritized implementation research agenda in consultation with key stakeholders and offers to provide technical support to enhance country capacity to design and conduct such research and provide a platform for researchers to share their results.[21]

OVERCOMING THE CHALLENGES

The assessment of progress clearly indicates that we are not on track to achieving the goals established for the Decade of Vaccines. The shortfalls are mainly in developing countries, which also stand to gain the most from the use of vaccines. To overcome some of the challenges that these countries face, several broad areas need to be addressed particularly these: (a) enhancing country ownership and governance; (b) strengthening health systems, including human resource capacity, access to sustained supply of vaccines at affordable prices, vaccine supply chains, and information systems; and (c) strategies to improve the quality and coverage of service delivery.

Country Ownership, Management, and Financing of Immunization Programs

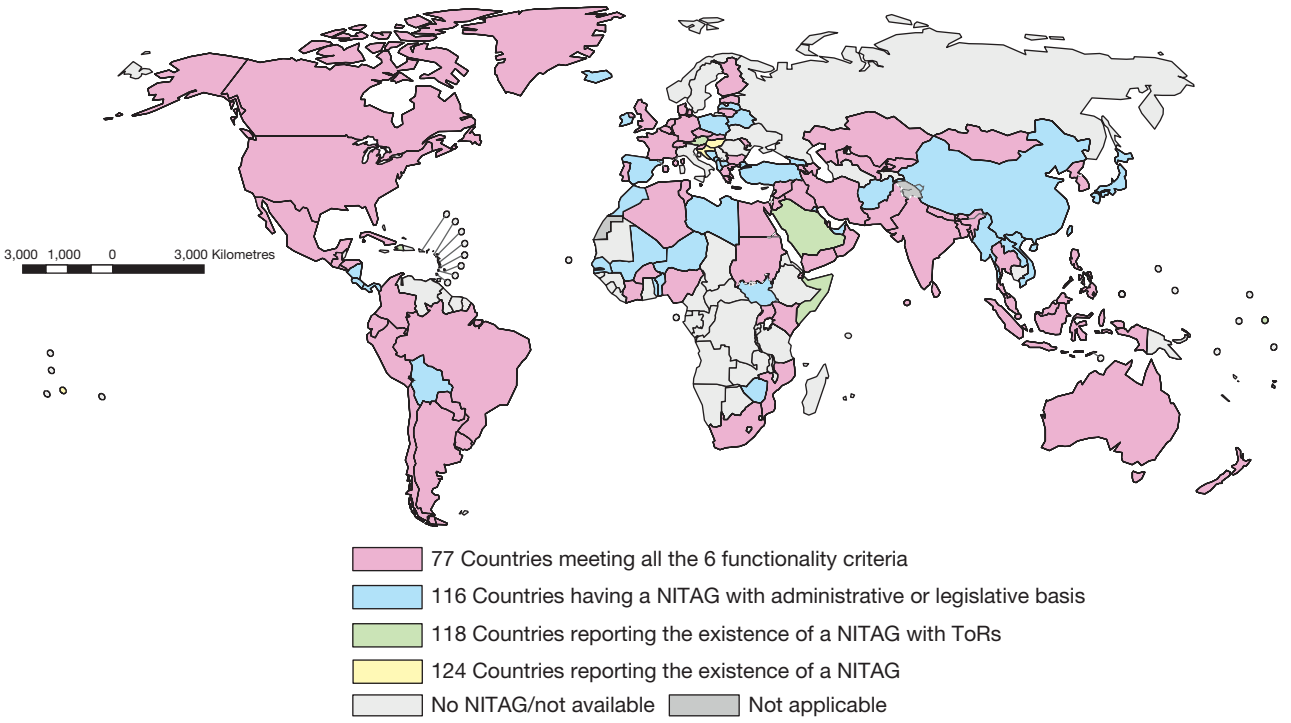
Lack of ownership and good governance of the immunization program constitutes an important barrier to its successful implementation in several countries, particularly those with the largest numbers of unimmunized children. In these settings, weak capacity for developing evidence-based national policies and insufficient or poor quality of data required for planning have often led to poor management of national immunization programs.

Ensuring effective immunization programs requires collaboration among multiple stakeholders, including policymakers, and is facilitated through comprehensive national multiyear plans (cMYPs) for immunization.[22,23] Annual work plans should be generated from and aligned with cMYPs to ensure that programs are working toward established longer-term goals. Evidence-based decisions must be made about the goals of the vaccination program, including the introduction of additional vaccines and technologies and the integration or improved coordination with other programs.

The WHO and its partners encourage countries to develop national immunization technical advisory groups,[24] which serve a key role in supporting government decision making. The existence and functionality of these groups is being used as an indicator of country ownership in the GVAP monitoring and accountability framework.[16] An increasing number of countries are now reporting the existence of a National Immunization Technical Advisory Group (NITAG). In 2015, 124 of 194 (64%) countries reported the existence of a NITAG, of which 77, including 49 developing countries, reported a NITAG that met the six indicators for functionality (legislative or administrative basis for the advisory group; formal written terms of reference; at least five different areas of expertise represented among core members; at least one meeting per year; circulation of the agenda and background documents at least 1 week prior to meetings; mandatory disclosure of any conflict of interest) established by the WHO (Fig. 76.9). The number of NITAGs meeting the six process indicators for assessing functionality increased from 43 in 2010 to 77 in 2015.

Technical guidelines and standard operating procedures must be established regarding vaccine delivery, including selection of the optimal schedule and recommendations regarding contraindications to vaccines. National interagency coordinating committees or equivalent structures, which should include all key government ministries (e.g., health, education, and finance), international partners, civil society organizations, and the private sector, can provide a key mechanism to facilitate coordinated planning, financing, advocacy, capacity strengthening, and community mobilization, as well as to promote a cohesive effort to enhance and sustain the performance of the national immunization programs.[23]

Immunization programs in developing countries traditionally have been organized under a centralized system, with services delivered predominantly through the public sector. Ministries of Health are responsible for setting policies and norms and for managing programs. Donor support is given



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.
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Figure 76.9. Countries reporting the presence of National Immunization Technical Advisory Groups (NITAGs), 2015. The six process indicators for functionality are (1) legislative or administrative basis for the advisory group; (2) formal written terms of reference; (3) at least five different areas of expertise represented among core members; (4) at least one meeting per year; (5) circulation of the agenda and background documents at least 1 week prior to meetings; and (6) mandatory disclosure of any conflict of interest. *(From WHO-UNICEF, 2015. Map production: Immunization Vaccines and Biologicals (IVB), World Health Organization.)*

through the ministries of health that control the budget for immunization.

This structure is evolving, however, with several cross currents of change creating great diversity among countries and regions. Under health sector reforms implemented in some countries, there is an increasing trend toward decentralization of health services and a burgeoning private sector.[25–27] The growing private sector is often disconnected from the public sector with an unclear role in achieving national goals.[28] At the same time, international support has increased substantially for specific disease-control initiatives (e.g., Global Polio Eradication Initiative) as well as for the health sector as a whole (e.g., sector-wide approaches and the International Health Partnership). Countries vary in the degree to which health services have been decentralized and in the proportion of funding provided for specific programs and under the broader health umbrella. With decentralization, health services are under local government control, budgets are often managed at the district level, while the central ministries of health focus on policy making, procurement of vaccines and injection supplies, and surveillance and monitoring of program impact. There is also a trend toward greater involvement of private and civil society organizations in health services delivery, including national and international not-for-profit agencies and private for-profit practitioners.[28–30] These changes have increased the need for effective national leadership and coordination of the different service providers and international funders and for the dissemination of clear and practical policies.

The costs of immunization programs in developing countries vary widely according to the vaccines used, source of vaccine and its price, immunization coverage, the level of a country's economic development, and, importantly, the delivery strategies used to reach more children. The costs of a national routine immunization program typically include vaccines, human resources (salaries of the immunization team, support staff, and supervisors), cold chain equipment and maintenance, vehicles and transportation, diseases surveillance, health worker training, and social mobilization. The growth of EPI and the addition of several new more-expensive vaccines has led to a severalfold increase in the price of a full vaccination course for a child.

Before 2000, human resources accounted for the greatest share of a national immunization program. Since 2000 and with the increased availability and use of a wider range of newer vaccines, vaccines are now the single largest cost driver of routine immunization programs in developing countries. In Africa from 2006 to 2012, government spending on vaccines increased from $35 million to $65 million, but during this same time period, there was a decrease in percentage of

governments spending on vaccines from 54% to 36%.[31] In general, the cost to fully immunize a child decreases as the number of children immunized increases,[32,33] although there may be a U-shaped curve as the marginal costs of reaching the last 10% of the population may be higher. However, the cost-effectiveness of different strategies varied widely in different geographic locations, and the number of high-quality studies is limited.[34,35] The cost per child immunized needs to be balanced against the effectiveness of the strategy in accessing all children in need of vaccination.

Access to sufficient and predictable financing is essential to the ability of an immunization program to improve the health of children through vaccination, with continuous increases in coverage, quality, and access to traditional and newer vaccines.[16] In developing and developed countries, the management and financing of immunization services are considered core functions of the state and need to be considered in the overall context of a country's health sector financing, planning, and budgeting.[23,36,37]

After many years of being almost entirely reliant on external financing for their immunization programs in the 1970s and 1980s, developing country national governments are increasing the allocations of domestic resources for their immunization programs, although there is considerable variation from region to region and country to country. The poorest countries are still largely dependent on external support for their immunization programs and are projected to continue to remain financially dependent in the coming years.[38–40] Government financing for immunization seems to be related to national income, so as a country's economic development improves, so does its allocation to immunization.[23] With the advent of more expensive, lifesaving vaccines and efforts to increase coverage and meet disease control and eradication goals, ensuring sustainable immunization financing for all countries has become an important challenge for national and donor governments.[16]

In the 1970s and 1980s, external donors made major investments to help countries attain the 1990 goal of universal childhood immunization. However, in the 1990s, donor funding for routine immunization programs declined, with funding for vaccines and immunization targeted to disease control and eradication initiatives.[7,41] Unfortunately, the withdrawal of external funding for immunization was not matched by a commensurate increase in national financing, and immunization coverage slipped in the 1990s.[41] Since the mid-1990s, the Global Polio Eradication Initiative, accelerated disease-control programs such as the measles mortality reduction initiative and neonatal tetanus elimination, and Gavi have contributed significant resources to immunization programs. Gavi has emerged as a pivotal and important financier of immunization programs for the world's poorest countries. In 2006, it was estimated that Gavi contributed 37% of immunization program expenditures in poor countries, the majority toward the procurement of new vaccines. While it was previously estimated that, on average, donors finance 21% of immunization programs in low-income countries,[23] there is still a 57% projected funding gap of low and middle income countries through 2020.[38]

There are three widely used indicators of national financing capacity: the presence of a national budget line item for vaccines and for immunization operational costs; the percentage of government funding of expenditures on vaccines used in routine immunization; and the percentage of government funding of overall expenditures on routine immunization. Globally, the proportion of countries that have a line item in the national budget for routine immunization increased from 82% in 2006 to 89% in 2012; among low-income countries, the proportion increased from 71% in 2006 to 94% in 2012. The proportion of expenditures on vaccines used in the routine program financed by national governments decreased from 80% in 2006 to 71% in 2012; in low-income countries this proportion decreased from 30% in 2006 to 18% in 2012. However, in absolute amounts, government expenditures on immunization has increased in low- and middle-income countries, although not as rapidly as donor contributions, largely Gavi support for new vaccine introduction, have increased. There was also wide variation in routine immunization financing by governments in the different regions of the world in 2012, ranging from 50% in the African region to 92% in the Americas.[31] Countries in the African region are still heavily dependent on external financing to support their immunization programs.

With the increasing attention to financial sustainability of health and immunization programs, a number of new tools have been developed to help countries evaluate the cost, cost-effectiveness, financing, and sustainability of their immunization programs. To support financial planning, the WHO in collaboration with other Gavi alliance partners developed a cMYP costing tool and maintains an immunization financing resource database complete with analyses of cMYPs and financial sustainability, which are required as part of the country applications for Gavi support.[42] National financial sustainability planning is increasing, with governments taking the lead in working with partners to identify long-term resources for immunization through multiyear planning; in some developing countries, this has resulted in increased national financing for purchase of new vaccines and autodisable syringes. In 2005, the Pan American Health Organization (PAHO) initiated the ProVac Initiative (Promoting Economic Analysis for Vaccine Introduction in the Americas) to strengthen national capacity to make evidence-based, informed decisions on new vaccines, including conducting and using cost-effectiveness analyses.[43] With help from the Sabin Vaccine Institute, this initiative is now being rolled out to other regions through the formation of the ProVac International Working Group.

Strengthening Health and Immunization Systems

To realize the full potential of vaccines, health and immunization programs in all countries need the systems and people in place to reach all populations, including the hard-to-reach populations. This section explores some of the critical deficiencies in the health systems in developing countries that need to be addressed to reach the goals for the Decade of Vaccines.

Human Resources

The role of trained health workers in the strengthening of health systems is well recognized.[44] The relationship between healthcare worker density and immunization coverage has been well documented in studies from several developing countries[45–48]; one of these studies in Turkey also found that the relationship between healthcare worker density and immunization coverage is stronger in rural provinces.[47] Despite the recognition of the problem, there has been little progress in addressing the human resource crisis. However, it is recognized that the solutions are not straightforward and considerable investments in time, effort, and money are required to train and retain health workers. In the World Health Report 2006, 57 countries, 36 of which are in Africa, were identified as having a health-worker-to-population ratio below the threshold of 2.3 per 1000 population, which is considered to be necessary for meeting the health-related Millennium Development Goals.[47] This implies that below this critical level countries would be unable to increase their immunization coverage to greater than 80%, among other health indicators.

A review of progress published in 2011, 5 years after the publication of the World Health Report, indicates that progress has been patchy, with a diverse picture of country efforts, challenges, and successes, and suggests that success in meeting the goal set by the World Health Assembly in 2008 to reduce the number of crisis countries by 25% by 2015 will be determined by the level of investments made in this area in the interim.[49]

As immunization programs in developing countries increase in complexity as a result of adding new vaccines and the need to extend coverage to reach 90% or more of their target populations, both the number of health workers and their skill levels will need to be addressed. Specialized skills will be required for managing the different components of the health systems, such as supply chains, information, and regulatory systems. Addressing the human resource constraints remains an area where appropriate guidance for countries is either not available or is not being fully implemented. No benchmarks exist for the number of healthcare workers required to deliver immunization services and there is no guidance on what increments may need to be made when countries add new vaccines to their national programs. As a result national programs demand more from their health workers without providing the commensurate training or resources. While training materials and tools exist, program reviews and evaluations reveal gaps in health worker knowledge and practice. Supportive supervision has been shown to improve program performance, but there is still a lack of recognition of the need for and the knowledge and tools to implement supportive supervision in many developing countries.[50] As is the case with health systems in general, human resource development for immunization remains an underinvested area. Failure to address this critical bottleneck is likely to be a barrier to achieving national goals in developing countries and global goals as a consequence.

Sustainable Supply of Vaccines at Affordable Prices

Another key challenge is to ensure a reliable, sufficient, and affordable global vaccine supply. Ensuring an adequate supply of safe and effective vaccines for developing countries, as well as to each vaccination session in these countries, continues to be a global priority in a world that is seeing unprecedented changes in the vaccine industry and market.[51–53] In the 1980s, the vaccine market was in serious decline, with many companies in developed and developing countries exiting the market, viewing vaccines as high-volume, low-margin products.[54] The exit of vaccine producers led to shortages in key products and reliance on a small pool of source suppliers for key vaccine products.[55]

Beginning in 2000 and intensifying since 2005, the vaccine industry is experiencing a major turnaround, driven in part by blockbuster sales of pneumococcal conjugate and human papillomavirus vaccines in developed countries and the prospects for growth in lower- and middle-income country markets. Developing country manufacturers are now fulfilling an increasing role in vaccine supply, and their entrance into the market is stimulating price declines of key vaccines.[56] More than 60% of traditional EPI vaccines are supplied by developing-country manufacturers.[57] The Serum Institute of India in Pune, India, supplies more than 80% of measles vaccine to developing countries. Developing-country manufacturers are increasingly entering into joint development agreements with developed-country vaccine manufacturers to produce new vaccines, and many now participate in a developing country vaccine manufacturers' network.[58]

The WHO continues to focus on strengthening national regulatory authorities (NRAs) in vaccine-producing countries as weak NRAs have been consistently identified as a major barrier to entry for new suppliers. Multiple criteria in seven basic functions have been developed to determine whether each NRA is able to guarantee that a vaccine is of "assured quality" (national regulatory system; market authorization and licensing; NRA lot release; laboratory access; postmarketing surveillance, including adverse events following immunization [AEFIs] surveillance; regular inspections; and authorization/approval of clinical trials). In June 2016, 43 countries were documented to be producing vaccines for human use, of which 37 had a functional NRA. Twenty-two of these countries were producing one or more vaccines prequalified by WHO for procurement by United Nations agencies, the Russian Federation being the latest addition to the list of countries producing prequalified vaccines.[20]

To sustain the national, regional, and even global supply of EPI vaccines, the most important areas of technology transfer for developing countries are those relevant to achieving and maintaining good manufacturing practices standards in vaccine production, establishing independent and credible national quality-control laboratories, and instituting national (or regional) regulatory capability. Facilitating successful and sustainable technology transfers is a challenge facing the international organizations that support the global EPI.

Affordability of the newer vaccines, especially for middle-income countries who are not eligible for or will soon lose Gavi support, is becoming an increasing concern.[59] Available information suggests that there is a wide variation in the prices paid by middle-income countries for the same products. However, the solutions are not simple and issues beyond just access to affordable prices need to be considered to support the introduction of new vaccines in middle-income countries not eligible for Gavi support. These issues include strengthened decision-making processes, increased political and financial commitment from national governments, and enhanced public demand for vaccines. These issues have been considered in earlier sections of the chapter. This section specifically deals with the issues to be considered to secure a sustained supply of vaccines at optimal prices, including the following: (1) efficient procurement processes; (2) efficient regulatory and product registration processes; (3) access to price and contract information; (4) access to revolving funds; and (5) pooled procurement and access to external procurement services.

Previous studies have identified inefficient procurement as a barrier to obtaining competitive prices for vaccines.[60] The support provided to countries in improving their procurement practices has been patchy and remains an area where more needs to be done to secure access to affordable prices.

Inefficient and lengthy processes for the registration of vaccines can drive up the cost for suppliers and consequently the price of vaccines. The barrier that the onerous and lengthy processes pose are likely to have a greater effect on lower cost manufacturers who may not have in-country representatives and fewer resources, thus limiting the choice of products for the countries concerned and resulting in higher prices from a lack of competition. At least 30 middle-income countries not eligible for Gavi support could benefit from more streamlined and efficient regulatory processes.[61]

Available data suggests that increasing price transparency can result in more favorable outcomes from contract negotiations and better prices.[62] While international agencies such as UNICEF and the PAHO have started publishing the prices of vaccine procured by them, data on prices paid by self-procuring countries is sparse. The WHO has established the Vaccine Product, Price and Procurement (V3P) web-based platform with a goal of increasing vaccine price transparency to inform contract negotiations and decisions on vaccine procurement.[63]

Lack of assurance that suppliers will be paid on time adds to their risk and hence to higher prices. Many middle-income

countries face uncertainties about allocation and release of funds for procurement. Others may have legal restrictions about prepayment for goods or face barriers related to currency fluctuation. Revolving funds, such as the PAHO revolving fund and the UNICEF Vaccine Independence Initiative,[64–66] provide solutions to some, but not all, of these problems by providing a line of credit for countries not able to produce the funds at the time when they are needed. However, the existing funds are available only to a limited number of countries in the regions they serve. Expanding the access of other middle-income countries to revolving funds may contribute to their being able to access lower prices.

The use of external procurement services, such as through UNICEF, could benefit a number of middle-income countries, especially those with small populations and limited bargaining power. When coupled with a revolving fund, such pooled procurement through external agencies has been shown to lead to lower prices.[64] However, many countries have legal barriers to using external procurement services.

Immunization Supply Chains

Situation After Years of Neglect. The success of immunization systems in developing countries over the past decades can be attributed to a large extent to its backbone—the supply chain and logistics systems that strive to ensure the uninterrupted availability of vaccines up to service delivery levels as efficiently as possible. These supply chains have fairly successfully supported the flow of vaccines through a quality cold chain system that safeguard their potency from heat or freeze damage from the moment they arrive in a country until vaccination.

Unfortunately, during the past 2 decades these supply chain systems have been largely neglected and taken for granted. With few exceptions, immunization supply chains in developing countries continue to face chronic difficulties in providing uninterrupted availability of potent vaccines up to service delivery levels, and many government-managed systems remain crippled by inefficiencies in vaccine storage, distribution, vaccine management, and stock control.[67–69] An increasing number of new vaccines with different characteristics and immunization schedules, using a variety of service delivery strategies to cover an expanding target population are just a few of the new realities that immunization supply chain systems are being confronted with today.[70] The existing systems in many developing countries were designed to manage fewer, less-expensive, and less-bulky vaccines and related supplies.

Faced with the mounting concerns, the WHO and UNICEF have been supporting developing countries in the implementation of the Effective Vaccine Management (EVM) initiative that allows for an end-to-end, in-depth diagnosis of the strengths, weaknesses, and bottlenecks of an in-country immunization supply chain and to develop comprehensive plans for improvements based on the outcomes of the assessment.[71] Since 2010, more than 80 developing countries have conducted EVM assessments reviewing nine critical dimensions of an effective system in terms of vaccine arrival procedures; cold chain storage adequacy; efficiency in temperature control throughout the cold chain; compliance with vaccine management policies; vaccine distribution and handling best practices; information systems for forecasting, stock management, and stock control; and adequate infrastructure and maintenance services for the supply chain. The EVM assessment sets a minimum standard to reach for each of these dimensions, based on WHO norms and guidance. The available evidence from the 80 countries indicate that none reached the minimum standard on each dimension at each level of the supply chain, from the national to the service delivery point (Fig. 76.10).

Key findings from these assessments include the following: (1) adequate temperature control for vaccines is achieved in only 40% of countries, which suggests that vaccine potency is

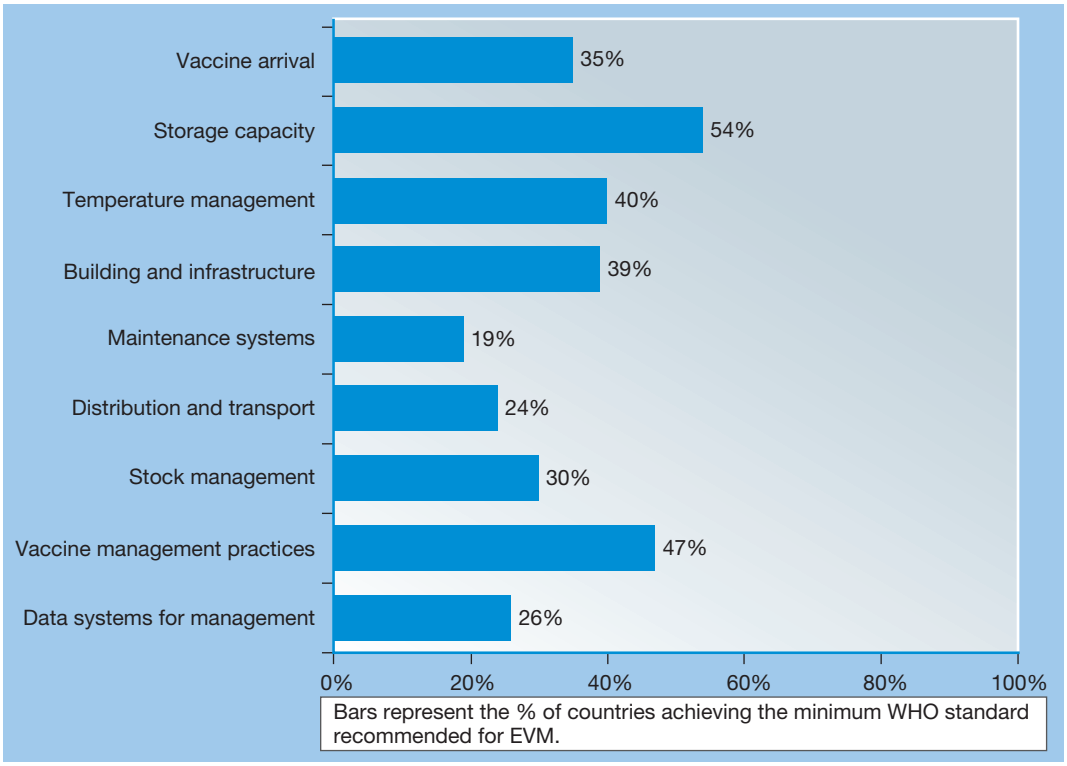


Figure 76.10. Supply chain performance in 80 developing countries that have conducted Effective Vaccine Management (EVM) assessments, 2010–15. *(Source: WHO EPI.)*

at risk in the cold chain systems of many countries; (2) sufficient storage capacity for existing vaccines and related supplies is available in only 54% of countries, which suggests that infrastructure is generally insufficient for existing vaccines, let alone for introducing new vaccines; (3) functional vaccine stock management is attained in only 30% of countries with the resultant increased risk of vaccine stock out or unopened vial wastage; (4) effective distribution of vaccines is compliant with the established standards in only 24% of countries, compromising the timely availability of vaccines at relevant subnational levels; and (5) adherence to vaccine management policies and practices are reached in only 47% of countries, which leads to inefficiencies and poor vaccine handling practices.

More recent evidence has brought to light that many countries report regular shortages of vaccines at the national level; consequently, 38% of developing countries in 2015 experienced at least one stockout incident for one vaccine for 1 month (Table 76.2). The extent to which this has led to interruptions of services at lower levels remains an open question, but this additional evidence points to the chronic difficulty of securing an uninterrupted flow of vaccines—one of the key goals of an effective immunization supply chain system. A root-cause analysis of national level vaccine stockouts conducted between 2010 and 2015 identified poor forecasting, stock management, or procurement issues being the cause of 41% of national-level stockouts and confirms country weaknesses in basic vaccine management practices.[72]

Coupled with these persistent challenges of ensuring that fundamentals are in place like EVM, good forecasting, and stock control for vaccines, new challenges resulting from the rapid evolution and growth of national immunization programs add to the pressure on currently fragile and strained systems. Compared to a decade ago, many immunization programs in developing countries now manage six times as many doses of vaccines in the supply chain; store and transport four times more vaccine volume per fully immunized child; safeguard the potency of vaccines when the value of supplies at risk in the cold chain has increased fivefold; and have doubled inventory levels and buffer stocks that have implication on the cold chain infrastructure.

New challenges include the growing number of vaccines, along with their formulation, thermostability, and packaging. These add more layers of complexity that have a tremendous impact on in-country supply systems. In the initial years of EPI, most of the basic six antigens used in national immunization programs had more standardized multidose presentations (usually 10 to 20 doses per vial). The vaccine management rules were much simpler, especially with the introduction of the Vaccine Vial Monitor more than 2 decades ago. The Vaccine Vial Monitor is a temperature indicator that measures the cumulative exposure to damaging heat. Not only did this help healthcare workers identify if vaccine potency had been compromised by excessive exposures to heat, the Vaccine Vial Monitor was instrumental in the implementation of the open vial or multidose vial policy—an approach to reduce vaccine wastage and reduce the overall impact on cold chain capacity requirements.

Today, however, many of the new vaccines ready to be introduced in developing countries come in liquid formulations that are sensitive to freezing. Yet, there is no equivalent of a Vaccine Vial Monitor to indicate if a vaccine has lost its potency from being exposed to undetected but damaging freezing temperatures. With the knowledge that at least 35% of vaccines are exposed to extended periods of freezing in the cold chain of developing countries,[73] safeguarding the potency of the newer, more expensive, vaccines will require measures for much stricter temperature control in the cold chain to let health workers know if the vaccine has been exposed to freezing temperatures (see "Game-Changing Solutions to Renew Systems" for technological solutions). In the coming years, wastage of vaccines because of freezing can have substantial financial consequences and will no longer be acceptable. Moreover, some of these new vaccines lack preservatives and, therefore, come in single- or two-dose vials to reduce wastage, which lead to markedly increased cold chain storage and shipping volumes.

The combination of a lack of attention and funding has meant that immunization supply chains have failed to evolve and adapt, resulting in delays to introduce new vaccines, stockout of existing vaccines, avoidable wastage, or loss of vaccine potency from storage in weak cold-chain systems unable to protect vaccines from excessive heat or freeze damage—all of which have implications in terms of increased costs and lower immunization coverage. These inefficiencies not only hinder the ability to meet desired immunization targets, they lower the return on health outcomes for those investing in improving immunization programs in developing countries.[70]

TABLE 76.2 Summary Statistics for Developing Countries[a] Reporting National Vaccine Stockout[b]

	2015	2014	2013	2012	2011	2010
Number of developing countries reporting stockouts	31	25	36	33	34	38
% Developing countries reporting stockouts	38%	31%	44%	41%	42%	47%
Number of stockout events[c–e]	57%	55%	79%	71%	72%	99%
BCG vaccine	42%	38%	39%	42%	38%	35%
DTP-containing vaccines	37%	29%	33%	32%	33%	48%
Measles-containing vaccines	5%	16%	13%	8%	15%	6%
OPV/IPV vaccines	16	16	15	17	14	10
Average number of stockout events[d,e]	1.84	2.20	2.19	2.15	2.12	2.61
Average duration of a stockout event (days)[d]	47	62	49	40	36	70

[a]Developing countries are defined as the 81 low- and lower-middle income countries based on the 2015 World Bank classification.
[b]For BCG, DTP-, measles-, and polio-containing vaccines.
[c]Some countries reported multiple stockouts in a given year, resulting in stockout events being greater than number of countries reporting stockout.
[d]Number of stockout events/number of countries reporting stockouts.
[e]For 81 developing countries reporting stockout events.
From WHO Expanded Programme on Immunization (2016).

Game-Changing Solutions to Renew Systems. Fortunately, a range of technological and supply systems solutions exist that hold the promise of addressing the majority of the current and new challenges faced by immunization supply chains in developing countries. There are several promising and transformational solutions ready for wider scale adoption in countries by 2020.

Leveraging the True Thermostability of Vaccines. Several vaccines commercialized for use in developing countries are, in fact, quite heat stable. Unfortunately, irrespective of the actual thermostability of a vaccine, almost all vaccines used today are

licensed for storage at 2°C to 8°C at all points in the immunization supply chains. In November 2012, a meningococcal A conjugate vaccine, MenAfriVac (Serum Institute of India, Pune, India), became the first product to be relicensed for use up to 40°C for up to 4 days in campaign settings. As there is no associated loss of safety, potency, or efficacy, this regulatory approval opened the possibility of keeping doses of this vaccine in a relatively simple controlled temperature chain for a few days rather than in a standard cold chain system. Several other vaccines are equally heat resistant and have the potential to be used in a controlled temperature chain. This comes with important benefits since reaching hard to reach areas and people is no longer bound by the reach of the cold chain if vaccines can be kept in a controlled temperature chain even for relatively short periods. In addition, the incidence of freeze damage for freeze sensitive vaccines like hepatitis-B could be drastically reduced if this heat stable vaccine could be used in a controlled temperature chain. Moreover, this approach has the potential to alleviate the burden on the traditional 2°C to 8°C cold chain storage space, and has been documented to reduce by up to 50% the overall cold chain and logistics cost of conducting immunization campaigns.[74–76]

Extending the Reach of Vaccines With New Cold Chain Technologies. Large areas of many developing countries cannot make vaccines available to the communities in need because energy requirements to run the cold chain are nonexistent, either because areas do not have electricity or cannot reliably provide fossil fuels for the main alternatives to electrically powered cold chain (kerosene- and gas-driven refrigerators). Even when the supply of fossil fuels is within reach, these types of equipment are plagued by problems with gas supply interruptions, low efficiency, high cost, poor temperature control, and frequent/expensive maintenance needs. Since 2010, important technological breakthroughs in cold chain equipment have occurred. The first is the availability of battery-free solar cold chain equipment whose performance, reliability and life-cycle costs are significantly better than nonelectrical models available (kerosene or gas).[77,78] The second is the availability of long-lasting passive cooling containers for health centers and "last mile" usage. These superinsulated containers can store vaccines for more than 30 days using regular ice. Whether it's the new generation of battery-free solar refrigerators or long-lasting passive cooling containers, these new technologies allow vaccine storage in areas where cold chain infrastructure was previously impossible to have. Extending the reach of the cold chain much further down the supply chain not only increases the availability of vaccines at the point of delivery, it can respond to and decongest the storage capacity constraints further up the chain.

Improving the Quality of the Cold Chain With Continuous Temperature Monitoring Technologies. As an increasing proportion of the value of vaccines now being procured is comprised by vaccines that are freeze-sensitive,[79] accidental freezing of vaccines is a growing threat. Today, an increasing number of inexpensive devices exist for continuous temperature monitoring. The use of these devices can mitigate the risks of potency loss of vaccines from damaging temperature excursions in the cold chain during both storage and transport. Studies have shown that the use of electronic continuous temperature monitoring and freeze prevention technologies can help reduce the incidence of inadvertent freezing of vaccine by 55%.[80] Continuous temperature monitoring has the additional advantage of helping improve vaccine handling quality, detecting malfunctioning cold chain equipment, and mitigate damaging temperature fluctuations and freeze events even when health workers are not present.

Moving Away From a Paper-Based Data Management System. Supply chain managers in countries seldom have information on vaccine stock balances beyond the central stores; do not have knowledge of avoidable vaccine wastage in the system from breakage, leakage, or expiration; have incomplete understanding of current cold chain capacity or what equipment is not functioning; and can never be sure if vaccine stockouts are occurring at the lower levels of the supply chain. The lack of reliable and timely data for accurate vaccine forecasting, immunization supply chain planning and to adequately respond to vaccine orders within a country is often cited as a key bottleneck. The lack of visibility of vaccine stocks and flows within an immunization supply chain is a detriment to ensure timely availability of vaccines and lead to inefficiencies in the supply chain (e.g., need to have idle buffer stocks throughout the chain; not delivering enough vaccines leading to stock out; delivering too much vaccine leading to expired vaccines that need to be discarded).

One root of the problem is that many developing countries continue to manage their supply chain with a "paper-based" logistics management information systems that do not permit real-time tracking and tracing of vaccines and visibility of stock levels throughout the supply chain. Yet, the rate of innovation in the field of information and communications technology to respond to this has been staggering in recent years. Many cost-effective information and communications technology solutions are available to developing countries. These range from mobile phone networks and devices that can now connect even the most remote locations to the Internet and cloud-based server hosting, making it possible to operate networked systems between the central vaccine store and subnational stores. In addition, barcode systems are more widespread allowing their printing onto vaccine packaging to enable tracing of vaccine lots down to the district level and beyond. The widespread availability of mobile and cloud-based information and communications technology enable the establishment of networked systems for ordering vaccines, managing stocks and equipment, monitoring consumption and vaccine use, and help vaccine supply managers make sure that vaccines are being kept in the right conditions and made available when and where they are needed.[81,82] These technologies could be expanded to other low- and middle-income countries to improve stock management and to ensure reliable and constant supplies of vaccines at the service delivery points.

Optimizing the Supply Chain Network Design. Many developing country immunization supply chains were designed more than 35 years ago and few have adapted their distribution network to the needs of today's national immunization programs. Most government-run vaccine supply chain networks replicate the administrative structure of the health system rather than being designed with the objectives of ensuring availability of vaccines, making rational use of the cold chain infrastructure, or optimizing distribution circuits for more efficient vaccine deliveries. With the surge in the volume of vaccines, some of the storage capacity constraints and bottlenecks can be overcome by redesigning the supply chain network. This is particularly true in countries where vaccine supplies have to transit through an inordinate number of levels from the point of entry at the national level to the service delivery points. Each storage point and distribution route along the way poses a risk to the vaccines (from mishandling to breaks in the cold chain). In addition, each point continuously requires infrastructure expansion of the cold chain capacity. Improved distribution methods and "level-skipping" could alleviate some of the cold chain capacity constraints in the system by reducing handling touch-points in the supply chain.

In recent years, there is increasing evidence that redesigning supply chain networks have the potential to improve vaccine availability, reduce stockouts, speed up delivery time, increase

storage capacity downstream, and reduce costs. One study indicates that without any change to the network design, timely vaccine availability at lower levels of the system, following the introduction of new vaccines, could drop by 71% because of bottlenecks in storage capacity.[83] Network redesign has also been shown to reduce the logistics cost of delivering a dose of vaccines to the service delivery point by up to 60%.[83–85]

Synergies With Other Health Commodity Supply Chains. Since 2010, there has been a rising trend in developing countries where central medical stores and health supply chains are government-managed to explore how to rationalize the use of both dry and cold storage infrastructure, and how to leverage economies of scale from supply chain integration. Historically, vaccines were among the handful of essential commodities that required their own specific supply chain—most vaccines needing to be kept in a strict 2°C to 8°C cold chain, unlike other essential medicines. Today, this is no longer the case. There are a growing number of pharmaceutical products needed at the more peripheral healthcare facilities that require strictly controlled temperature storage, particularly at the last mile. One example is oxytocin, an injectable drug to prevent postpartum hemorrhaging in women, the efficacy and shelf life of which deteriorate when exposed to heat. As immunization supply chains struggle to meet current and upcoming challenges, and with other temperature-sensitive lifesaving health commodities competing for cold chain space, opportunities for supply chain integration of time and temperature-sensitive health products is an emerging solution. When implemented correctly, it has the potential to address storage constraint and to reduce redundancies, complexities, and costs to the health system as compared to operating parallel and vertical supply chains.[86]

Private Sector Engagement in Immunization Supply Chains. Many developing country immunization programs are recognizing that government-managed logistics functions that were once ancillary now require important investments and tie up significant resources should they be scaled up appropriately to meet service delivery targets and to introduce new vaccines. Many developing countries have seen the emergence of private sector logistics providers who have made significant advances with the use of technology and information management, and capable of providing better and more efficient services than what is possible in the public sector. Rather than attempt to keep pace with these advances, more and more countries are recognizing the benefits of engaging the private sector in supply chain and logistics functions by outsourcing the physical storage and handling of vaccines to specialized private sector operators when the right expertise is available in country. In many instances, the private sector can complement governments' efforts in improving health logistics in specific aspects such as procurement, storage and in-country distribution. Outsourcing supply chain function to the private sector can enhance operating performance and leverage managerial efficiencies, allowing governments to focus on core expertise. Although outsourcing can help improve the performance of the vaccine supply chain, it has the potential to do the reverse if done incorrectly.[87]

Strengthening Human Resources for Effective Vaccine Management. Immunization supply chain systems critically depend on the skills and commitment of a strong and sufficient supply chain and logistics health workforce. The availability of skilled cold chain logisticians has eroded over the past decade as part of the overall neglect and inattention given to immunization supply chains in developing countries. With more valuable inventories of vaccines to handle, store, and distribute, this has put increased pressure on country supply-chain managers to ensure that EVM best-practices are implemented, and that vaccine management policies are strictly followed. As the evidence shows, there are important shortcomings in this regard (see Fig. 76.10).

The EVM initiative has established management standards for the vaccine supply chain and logistics system and benchmark these against best-practice standards.[71] The EVM is first and foremost a continuous quality improvement process to stimulate supply chain managers to strive toward the highest quality management practices for their vaccine supply chain. With the acceleration of new vaccine introduction, greater cold storage capacity is now required at every level of the supply chain, and countries must strive to maintain lower stock levels, reduce closed vial wastage, improve their logistics management information systems to accurately forecast vaccine requirements, and prevent cold chain equipment failures so that vaccines are not damaged by heat or freezing. Today, vaccine logisticians in countries need to be trained and imparted with the right skills to handle new vaccine products, use and monitor new cold chain equipment and cooling technologies, and learn how to establish and use new data and information management systems to forecast needs, allocate stock, act rapidly where cold chain equipment becomes dysfunctional, and recommend policy changes in support of network optimization, supply chain integration, or engagement with the private sector. The EVM initiative builds on the well-established principles of quality management to ensure the skills of supply-chain managers and vaccine logisticians are raised and that a holistic process to assess, plan and implement change is built as part of a continuous quality improvement process for reaching recommend standards for EVM.[71]

Future Prospects and Opportunities. While these systems have shown extreme resilience to deal with the growing challenges, they have done so by resorting to ad hoc solutions and unsustainable coping strategies. The limits of these approaches have now been reached, to the point where in-country immunization supply chains have become a bottleneck to achieving future immunization goals and aspirations in developing countries. In the absence of systematic, coherent, and planned improvements for both current and future challenges, and without significant investments to upgrade immunization supply and logistics systems through the wide-scale adoption of known game-changing solutions, the aspirations to strengthen routine immunization as a whole by 2020 will remain quixotic at best.

The level of investment required is substantial. However, compared to the investments being made in vaccine products themselves, these investments are relatively marginal.[38] Estimates suggest that investing annually up to 10% of the value of vaccines being procured will be sufficient to upgrade in-country immunization supply chain systems in developing countries by 2020 and ensure EVM best practices.[88] While investments for strengthening developing country supply chains are desperately needed, these investments need to be directed toward the right strategies and implementing frameworks. In response to this, recent global initiatives have begun to revitalize immunization supply chain efforts beginning with the development of a 2020 Vision and action plans[89]; moving toward securing international resources to invest in country systems as part of the Gavi Alliance Immunization Supply Chain strategy[90]; and establishing a global mechanism for supporting developing countries to implement change and to adopt game-changing solutions through the recently established WHO-UNICEF immunization supply chain hub. These opportunities hold the promise to facilitate the equitable access and availability of effective vaccines at service delivery points over the next decades and ultimately support immunization programs to reach the estimated 19.4 million children who remain unimmunized or underimmunized.

Information Systems: Monitoring and Evaluation of Immunization Programs

The availability of *good quality* data is fundamental to achieving the goals and objectives of the GVAP. The term *good quality* when applied to immunization data can be interpreted in many different ways, depending on the intended use of the data. In 2013, the WHO SAGE on immunization highlighted the importance of the need and use of high-quality data both to improve performance and to monitor the results. Data may be considered to be of *good quality* if it contributes to a **complete, unambiguous, meaningful,** and **correct** inference of the status of an immunization system. Data should also be available, analyzed, and interpreted in a **timely** way, to allow managers to take actions to optimize the performance and impact of the program.

Data required to monitor the performance and impact of immunization programs include the following:

- *Data to monitor indicators of program activities and inputs*—to detect potential problems with the availability and quality of vaccination services and to identify appropriate solutions;
- *Data to monitor indicators of program outputs*—coverage of each dose of each vaccine in the national schedule and related indicators of dropout and missed opportunities; these are intermediate indicators that are more easily measured than the measurement of disease impact through surveillance and allow monitoring of progress toward GVAP and country goals;
- *Data on the burden and trends of vaccine-preventable diseases (VPDs)*—reduced disease incidence and mortality are the ultimate indicators of program success[91];
- *Data on AEFIs*—must be monitored to ensure that any such events are detected and investigated, and that an appropriate response is mounted.

The WHO and UNICEF collect and compile information annually for all countries on key program indicators through the joint reporting form.[92] The WHO also collects information through reports from the various program assessments as well as reports of household surveys. However, beyond reporting to WHO and UNICEF, it is more important that these data and indicators be monitored and used both nationally and at the district (or other appropriate) administrative level at which the program is managed. Provision of regular feedback to health workers at all levels of the health system is a powerful way to motivate health workers to improve.[93–95] In addition to the above groups of indicators, vaccine effectiveness can be monitored through a number of methods described in Chapter 73.

Monitoring Program Inputs and Activities. It is important to ensure that the program is adequately resourced and that activities are conducted as planned, both in terms of their quantity and their quality. Routine reports provide data on the availability of micro plans and on vaccine supply. These should be supplemented by periodic assessments of vaccination activities, for which a number of tools and opportunities exist. The immunization system as a whole is reviewed periodically either by comprehensive program reviews often involving external assessors,[96,97] or during a "postintroduction evaluation," which is a systematic method for evaluating the impact of the introduction of a vaccine on the existing immunization system in a country.[98] In both of these, onsite observation and interviews are performed at national, regional, district, health center, and community levels in selected areas, using a series of questionnaires and checklists produced by the WHO. Following data analysis, a list of major issues is prepared. For each issue, achievements and problems are listed and an action plan is formulated. At the local level, district managers are encouraged to conduct health facility–based assessments of missed immunization opportunities,[99,100] data quality self-assessments,[101] and cold chain performance.[102] These provide quick information at the local level on program performance that should lead to timely remedial action.

Monitoring Vaccination Coverage. At the global level, vaccination coverage is a tracer condition for results-based financing,[103] an indicator of eligibility for Millennium Challenge Account assistance,[104] and a criterion for support from the Gavi for the introduction of new vaccines.[105] At national and subnational levels, monitoring coverage of different vaccines can provide insight not only into the overall program success in providing all vaccines to the target population (percentage of children vaccinated with each vaccine and fully vaccinated with all vaccines in the national program), but also into areas of program weakness. High coverage with DTP1 and the first dose of polio-containing vaccine (POL1) indicates high access to primary healthcare facilities. If DTP1/POL1 coverage is high by age 2 months but coverage for the respective third doses or for the MCV1 is low, further investigation to determine the cause of the dropout is required. High coverage of one vaccine (e.g., DTP) but lower coverage of another scheduled concurrently (e.g., pneumococcal conjugate vaccine) may show missed opportunities to administer vaccines when the child attends a vaccination session, because of vaccine stockouts, real (rare) or perceived (more common) contraindications, or reluctance to administer multiple vaccines at the same visit.

Coverage and the related indicators of dropout, missed opportunities, and timeliness of vaccination are monitored through several methods, each having advantages and disadvantages (Table 76.3).[106,107]

Register-Based Coverage Estimates. Ideally, vaccination coverage should be monitored continuously using registries or administrative reports.[107] Electronic immunization registries aim to document all vaccinations of each individual in each birth cohort.[108–110] Denominators may derive from the same registry or from a separate vital statistics system. When well implemented, electronic immunization registries can provide data for coverage measurement and for program-management activities such as monitoring vaccine supply and requisitions and sending vaccination reminders. Challenges facing such registries include accounting for migration within and between countries, ensuring complete and timely birth registration and vaccination reporting, avoiding record duplication,[111] and ensuring continuity after organizational changes.[112] As access to information and communications technology expands, the use of electronic registries is increasing and lessons learned from pilot studies as well as national experiences are being exchanged among countries and regions to improve their use.[110] However, substantial time and resources are required to conceptualize, design, pilot, and scale-up the use of these registries countrywide.

Administrative Reports. So-called administrative coverage is calculated using aggregate reported data on the number of doses of each vaccine administered to children in the target age group in a given time period and target population estimates and projections from censuses or, in some cases, from the number of registered births. All countries have such systems in place for vaccination of infants (national coverage data are reported annually to the WHO and UNICEF), and an increasing number also monitor booster doses and vaccination of

TABLE 76.3 Advantages and Disadvantages of Methods to Measure Vaccination Coverage

Method	Advantages	Disadvantages
Register-based (electronic)	• Can give complete and accurate information on cumulative vaccination status of individual persons and populations • Can be used to set appointments and issue reminders and recalls • Use of electronic systems could reduce time spent on paper registers that are widespread in low-income countries and often not used	• Need good computer access • Need complete birth registry for true denominator • Need unique ID number throughout life • If held locally, difficult to track vaccination of migrants • If held nationally, feedback/use at local level may be slow • Requires adequate funding and human resources • Need secure procedures to maintain confidentiality
Routine reports of vaccinations delivered	• Simple in conception • Continuous information allows monitoring of cumulative coverage through the year and by district/health facility • Can be used at local level to track coverage and dropout rates	• Population denominators often inaccurate • Private sector often does not report • Exaggeration of doses administered common (e.g., double counting of same child if home-based record mislaid, inclusion of children outside target age group, or purposeful exaggeration) • Transcription errors at each health system level when paper-based systems used
Surveys	• If well-conducted, can provide accurate information • Other indicators (e.g., missed opportunities, caretaker knowledge) can be assessed • Involvement of health workers can be training opportunity • Large-scale surveys for multiple programs can reduce costs • Lot quality sample surveys can be used to identify health facilities and low coverage population subunits	• Accuracy of data depends on adequate survey design, training, supervision, and quality control • Sampling frame often based on outdated census information • High-risk subgroups (e.g., migrants, street children) may be missed • Home-based records may be missing or incomplete, and accuracy of verbal history of vaccination varies • Participation rate affects reliability of results • Often long delays until results are known • Small samples give imprecise results; large samples are expensive and more time-consuming

other age groups, such as human papillomavirus vaccine to school-age girls.

The administrative method has the advantages of providing continuous information at the local and district levels and being relatively inexpensive. Each facility and district can track achievement of coverage goals and dropout using standard immunization monitoring charts and take immediate action to correct any problems identified. This approach is central to the district approach recommended in the Reach Every District strategy. From routine reports, the doses administered to children also can be compared with total doses distributed to estimate wastage rates.[113]

Despite these advantages, "administrative coverage" is often unreliable as a result of incomplete, inaccurate, or delayed reporting of numerator data and out-of-date information on population denominators.[106] There are often additional difficulties in accounting for vaccinations of individuals from outside the catchment area or from other countries.

Community Surveys. Household surveys are complementary ways to determine coverage.[106] The most common surveys have been the EPI cluster survey[114–117] and the larger UNICEF Multiple Indicators Cluster Survey (MIC) and Demographic and Health Surveys (DHS).[118,119] In the past, the EPI cluster survey method typically did not use a probability sample (one in which each individual has a known and nonzero probability of being included in the survey) and did not always implement or document strict quality control measures.[106] The WHO updated the EPI survey guidelines so that surveys now use probability sampling and excellent quality control is required.[120]

Data from surveys are often considered to be more reliable (closer to the "true" vaccination coverage) than those from administrative reports, but this is not necessarily the case. The accuracy of survey data is affected by factors such as selection bias and information bias, which cannot be solved by increasing the sample size.[106] Common causes of selection bias are exclusion of certain population groups from the sampling frame (e.g., for security reasons or homeless populations); poor field practices such as substituting a sampled household with one that is easier to reach or not revisiting households where respondents were absent on the first visit. These factors most often inflate coverage estimates. Information bias occurs when a child's vaccination status is misclassified because of mistakes on the vaccination record, transcription of data from the record, or the guardian's recall for children without a written record, and may lead to over- or underestimation of coverage in surveys.

When well conducted and of adequate size, surveys may provide more accurate and nuanced information than other sources. If designed appropriately, coverage at subnational level can be measured or classified and clusters in the survey that had extremely low numbers of vaccinated children can be highlighted, pointing to the need for further investigation in those geographic areas. Analyses can assess whether vaccines are received as close to the scheduled ages as possible—not too early or with too short an interval between doses ("invalid doses"), which might reduce vaccine effectiveness, but as early as recommended to shorten the period that a child remains at risk of contracting the relevant disease.[106,121] Missed immunization opportunities through the failure to administer vaccines simultaneously can also be measured. Questionnaires can also assess reasons for failure to vaccinate [122–124] and the occurrence of adverse events, such as abscesses after vaccination.[125] Coverage surveys can be extended to include indicators of other programs, especially if home-based records include dates of visits to healthcare facilities for curative care or growth monitoring.[94,126]

Lot Quality Sample Surveys. Originating from the manufacturing industry, lot quality assessment sampling (LQAS) methods classify results found in a survey as acceptable or unacceptable, based on predefined upper and lower cutoffs. In its classical form, LQAS uses simple random samples. In many instances, however, this is not feasible for field surveys even with small overall sample sizes, and a clustered LQAS

design has been developed whereby within each lot (e.g., district), a certain number of clusters are selected with probability proportional to estimated size (e.g., from a list of all villages with their respective populations).[126,127] In each cluster, a sample of the required number of children is selected, most often using nonprobability sampling methods. For evaluation of polio campaigns, commonly six clusters of 10 children each are sampled per lot,[128,129] but ideally a larger number of clusters would be included and children would be selected by random sampling within each cluster.[130]

It is important to recognize that LQAS surveys inevitably have a central range of coverage (the "gray area") that is not excluded by either the "adequate" or the "inadequate" classification.[131] That is, neither classification excludes the medium category. As a consequence of small sample sizes, quantitative estimates for each subunit are not available, although under certain circumstances, results from multiple subunits can be pooled to derive an overall estimate. LQAS has been used to identify health service units, neighborhoods or villages, that are not meeting predefined standards, such as coverage in an immunization campaign,[132–134] immunization coverage among clinic attendees, cold chain maintenance, safe injection techniques, and use of all opportunities to vaccinate.

Sero-Surveys. Although biomarkers are under consideration for estimating coverage, they have several limitations for this purpose[106,135] and are not currently included routinely in surveys measuring childhood vaccination coverage. For most vaccines, the presence of antibody following vaccination cannot be distinguished from that following "natural" infection. Exceptions are the presence of tetanus antibody (infection does not generate lasting immunity) and the presence of antibodies to antigens included in subunit vaccines such as hepatitis B (anti-HBs [anti-surface antibody]) but not to other antigens found in the whole organism (anti-HBc [anti-core antibody]), which are considered to be an indication of vaccine-induced immunity. Even for these vaccines, detection of antibodies does not indicate reliably how many doses have been received.[136] Furthermore, absence of antibody means either that the child is unvaccinated, that vaccines have lost their potency, or that antibody levels have waned if a long period has elapsed since vaccination. Biomarkers are therefore potentially useful to estimate population-level protection,[137] but not necessarily to validate coverage measurements or vaccination program performance. The development of antibody assays on oral fluid samples for tetanus[136] and measles[138] may make surveys with repeated sample collection more acceptable and may facilitate evaluation of vaccination campaigns.[139,140]

Triangulating Coverage Data From Different Sources: WHO-UNICEF Estimates of National Immunization Coverage. Since June 2000, the WHO and UNICEF have done annual reviews of routine immunization coverage worldwide.[92] Administrative coverage and any available national coverage survey data are reviewed and survey data ranked in terms of perceived quality. Draft estimates are determined and sent to national authorities, modified in light of comments received, and published as the WHO-UNICEF estimates of national immunization coverage in July or August of the year following the end of the reporting period.[141] Country-specific estimates are updated annually. If a new survey becomes available that contains information for previous years, retrospective adjustment of coverage estimates is made for relevant years. This process has helped improve the quality of administrative reports from many countries. There are, however, several constraints,[141] including the quality and availability of empirical data from routine reports or surveys, and delays before obtaining results from surveys which mean that major retrospective changes in WHO-UNICEF estimates of national immunization coverage may occur. To account for the availability and limitations of empiric data, an indicator of the WHO's confidence in the estimates is shown for each country on the individual report.

To improve coverage data quality, the following merit consideration:

- Prioritizing the improvement of the quality and availability of administrative data as the most effective, representative, and penetrative source of information on program performance;
- Promoting the regular use of data to inform policies, strategies, and corrective actions to enhance program performance at all administrative levels (because if data are not used, there will be no incentive to improve quality);
- Promoting a systematic approach to data quality assessments and corrective actions (reviews of data quality can be useful training tools);
- Promoting the strategic and cost-effective use of household surveys to provide supplementary data, through clear definitions of survey objectives and designing survey methods to meet the objectives;
- Strengthening the human resource capacities for improving data quality and meeting program needs; and
- Advocating for investments in sustainable solutions to improve the availability and quality of data.

Acting on Coverage Data. Improving the quality of coverage data is only useful if the results lead to action. Program managers should investigate reasons for low immunization coverage at national or subnational levels, by addressing the following questions[142]:

- Is the present vaccination delivery system inadequate?
- Are there characteristics of incompletely vaccinated children and their families that can help identify them?
- Do parents lack accurate information about the "where, when, and why" of vaccination?
- Do unfavorable attitudes (including rumors about perceived adverse events) among communities and families outweigh even a good vaccination system and good information?

Available information should be collected from secondary data sources (such as reports of previous program reviews, analysis of coverage data by health center) and by direct observation of vaccination practices and clinic organization during supervisory visits. Program inputs and activities should be compared between areas having high and those with low coverage, together with data on the catchment populations (size, demographics, socioeconomic and educational status, migration patterns, security issues, etc) to look for reasons for the differences in coverage and identify solutions. Program managers need to develop strategies to reduce barriers at health services and to reach the families and populations who have characteristics that make them harder to reach—for example, one of the most consistent findings is the association of low coverage of vaccination and other health interventions with low levels of education, particularly female education.[143] Discussions with experienced healthcare workers and community discussions will help identify programmatic barriers to immunization and/or vaccine hesitancy at the community or individual levels.[144–147] Often these practical investigations and sharing of experience will generate ideas for action to improve coverage.

Occasionally, supplementary studies may be needed to answer specific questions that remain after the above

investigations. A number of methods are useful for studies of reasons for incomplete vaccination, including any combination of the following:

- Health facility–based surveys (e.g., "health-facility assessments"), which include a mixture of observation of practices, interviews with providers to assess their knowledge and practices, and exit interviews with mothers.[94,148] These inexpensive studies can easily be coupled with home visits to adjacent neighborhoods to investigate reasons for undervaccination in households with easy access to health services.[148,149]
- Qualitative methods such as key informant interviews and focus groups to evaluate healthcare professional knowledge and attitudes relating to vaccination and family and community demand for vaccination.[150–152] These methods may be used alone,[152] as preliminary work to develop a quantitative data collection instrument,[153] or after quantitative surveys to examine how risk factors operate and to develop interventions.[154]
- Interviews of mothers of incompletely vaccinated or of fully vaccinated children to describe their characteristics (case studies or case series).[155]
- Cross-sectional studies with interviews of a random sample of participants to assess attitudes to vaccination and describe vaccine confidence and vaccine hesitancy.[147]
- Quantitative studies of factors associated with incomplete vaccination which compare characteristics of "vaccinated" with "unvaccinated" groups in cross-sectional "knowledge, attitudes, and practice" surveys,[122,125,156] case-control studies,[157] or cohort studies.[158]
- Intervention studies, in which communities are randomized to the intervention compared with a control group[124] or in which a whole population receives the intervention and "before and after" comparisons of coverage are made.[35,150,159–161]

Disease Surveillance. Surveillance is fundamental to measure the impact of immunization programs on reducing VPDs and to generate information to inform policy decisions and support sustained financing for the immunization program. It is also important to identify changes in disease epidemiology which may require modifications of vaccination schedules (e.g., addition of booster doses) and/or adaptation of program strategies (e.g., implementation of supplementary immunization activities of different age ranges). Early identification of outbreaks allows rapid response to avert as much morbidity and mortality as possible.

Different methods of surveillance are used at different stages of immunization programs and for varying objectives. No single approach may be sufficient to meet the epidemiological information needs for planning or for the comprehensive documentation of the impact of immunization. Hence, a variety of approaches are used to monitor the epidemiology of diseases targeted by vaccines. One or more approaches are used for any particular disease. These include the following:

- Passive countrywide aggregate reporting of VPDs and outbreak investigation;
- Active countrywide surveillance (usually case-based) with laboratory confirmation;
- Sentinel site surveillance; and
- Special studies and sero-surveys (e.g., for estimating hepatitis B vaccine impact).

For all disease surveillance, standard case definitions and minimum data elements should be used.[162,163] Surveillance systems should be monitored through the use of quality indicators, the main three being the timeliness and completeness of reporting, the proportion of reported cases and outbreaks that are investigated in a timely manner (including laboratory confirmation of diagnosis, where appropriate), and the proportion of investigated cases and outbreaks that are followed by an appropriate response.[164]

Passive Countrywide Reporting of Vaccine-Preventable Diseases and Outbreak Investigation. Since its inception, the EPI has promoted the reporting of aggregate numbers of cases of selected VPDs. The WHO has also published surveillance standards and case definitions for reporting cases.[162] Currently, almost all Member States report aggregate numbers of cases of these diseases through the WHO/UNICEF joint reporting form. However, the quality of these reports is highly variable and the disease trends in some countries, especially the low- and middle-income countries, are often difficult to interpret.[163] While most countries report clinically suspected or confirmed cases, others may also report laboratory confirmed cases and a few only report laboratory confirmed cases. Currently, most countries do not have mechanisms for data verification and the WHO does not have a way to assess the quality of the reported data.

Active Countrywide Surveillance, With Laboratory Confirmation. This surveillance platform builds on the acute flaccid paralysis and poliomyelitis surveillance network. The platform has been used to establish or strengthen case-based surveillance, with laboratory confirmation of cases, for measles and rubella. The resources available through this platform, along with the associated laboratory networks, have also been used to investigate outbreaks of other diseases, both vaccine-preventable as well as those that are currently not vaccine preventable.

Critical elements of case-based reporting include active case-finding (a systematic approach to identify and report every eligible case, e.g., as used for acute flaccid paralysis surveillance in the polio eradication initiative), negative (or "zero") reporting (i.e., submitting a report of zero cases if there are no cases during a specified reporting period), prompt investigation of each reported case of disease to collect relevant risk-factor and program information, laboratory confirmation, and program response. Case-based reporting requires substantially more human resources than routine reporting and should be initiated only in programs with high vaccine coverage and disease reduction and/or elimination initiatives.

Most countries now report case-based data for polio and measles to the WHO weekly and detailed guidance on surveillance and laboratory methods is available.[165] An increasing number of countries are now using the same platform to also report rubella cases.

Although active countrywide surveillance is likely to provide more useful information than passive aggregate reporting, there are still many challenges with interpreting data. Measles, for example, remains grossly underreported.[166] Globally, in 2013 there were 194,139 reported cases, compared to 122,000 estimated measles deaths in 2012—this would imply an implausible case fatality rate of 62%.[167] Insensitive surveillance may be used to evaluate trends if sensitivity remains constant but it is more difficult to assess trends when reporting completeness changes over time, and insensitive surveillance is inadequate for planning actions to sustain measles elimination. Surveillance of rubella and CRS is even more limited, even in some industrialized countries in Europe; Belgium, France, and Germany do not have rubella surveillance systems covering the total population.[166]

Sentinel Site Surveillance. Sentinel site surveillance (a system where surveillance is conducted in a limited number of reporting units, usually health facilities, as opposed to conducting countrywide surveillance), as a platform to generate information to support the introduction of Hib, pneumococcal conjugate, and rotavirus vaccines, was established in a few regions through the Paediatric Bacterial Meningitis and Rotavirus Diarrhoea surveillance networks. With the establishment of the PneumoADIP (Pneumococcal vaccines Accelerated Development and Introduction Plan), Rotavirus Vaccine Program, and the Hib Initiative, these networks were expanded to include all regions. In 2008, these networks transitioned to WHO coordination.[168] Recognizing technical, financial and human resource limitations, the WHO and partners proposed a layered approach for surveillance for invasive bacterial diseases, whereby the intensity of surveillance and number of disease syndromes reported varied by country. In addition, centers of excellence were identified to conduct specialized epidemiologic studies to complement surveillance. Following an external strategic review by an expert panel,[169,170] the surveillance objectives are being refined and the performance management framework has been updated.

This surveillance platform lends itself for the inclusion of other potentially VPDs, including acute encephalitis, typhoid fever, and CRS.

Special Studies. Special surveys have been used to determine disease burden in areas where access to healthcare facilities is low, particularly in the early years of immunization programs, and more recently for certification of neonatal tetanus elimination.[133,171,172] For some diseases, serologic surveys may be suitable to determine disease burden and measure impact on disease (e.g., hepatitis B).[173] Serological surveillance is a potentially important tool for elimination programs, especially once disease incidence is very low, as serology can potentially indicate problem areas where population susceptibility is increasing and there is the potential for an outbreak. Serological surveys may help identify which age groups should be included in campaigns, and evaluate the contribution of campaigns to reducing population susceptibility.[106,166]

Use of Mathematical Models to Estimate Burden of Vaccine-Preventable Diseases and Impact of Vaccination. The choice of vaccination strategy requires an understanding of the dynamic effects of vaccination on disease transmission and knowledge of age-related changes in severity of disease, vaccine complications, and the probability of transmission of the infection.[174,175] Mathematical modeling is useful to estimate the burden of disease for which surveillance data are insufficient (e.g., rubella and hepatitis B). Mathematical models can assist with comparing the merits of different strategies and improving our understanding of the observed impact of vaccination programs.[176–179]

Dynamic simulation models attempt to describe the dynamics of infections in populations and to predict their behavior under the conditions of vaccination programs.[174,176,180,181] Modeling has been used to explore thresholds for elimination or eradication of infections from populations[174,182–184] and to examine the immunologic response to vaccines and the minimum number of vaccine doses necessary to induce long-term protective immunity.[185]

The potential to combine economic with dynamic modeling to predict the effects and cost-effectiveness of different vaccination strategies has increasingly been recognized,[186,187] particularly to take into account herd-immunity externalities when assessing the cost-effectiveness of vaccines that prevent infection. With the growing complexity of the vaccine schedule, vaccine presentation (e.g., single-dose or multidose vials; prefilled syringes), and vaccine procurement and distribution, modeling is now being used to investigate how to optimize the supply chain in different settings.[83,188] Multiple factors are assessed, including vial size and type, storage patterns (degree of decentralization), transport patterns, methods for determining the frequency and size of vaccine shipments to peripheral levels (e.g., based on estimated or actual consumption), and integration or not of vaccine distribution with that of other commodities. With the availability of global positioning system maps, models can assess a variety of supply chain dynamics according to the actual geography and demography of a country or district.

Monitoring Adverse Events Following Immunization. Although modern vaccines are well tolerated and efficacious, no vaccine is totally safe. The more successful vaccination programs are in controlling disease, the higher the attention attracted by adverse events, often reported in the media. An inadequate response to such events can erode public trust, lead to the reduction in vaccination coverage, with the resultant risk of reemergence of VPDs. Developing countries are increasingly facing such situations and many have had to interrupt vaccination temporarily in response to AEFIs. When disease has been eliminated, the acceptance of even very rare adverse events may become politically untenable. The WHO and individual countries have greatly strengthened immunization safety activities, convening a Global Advisory Committee on Vaccine Safety,[189] which has issued reports on controversial vaccine safety issues; supported the Brighton Collaboration in the development of common definitions for adverse events; established the Vaccines Safety Net project; and required enhanced AEFI reporting as a critical function of national vaccine regulatory authorities.[190,191]

Reported adverse events can either be true adverse events (i.e., resulting from the vaccine or immunization process) or coincidental events that are not caused by the vaccine or the immunization process but are temporally associated with immunization. In 2012, the existing classification regarding cause-specific categorization of AEFIs was revised by the Council for International Organizations of Medical Sciences and the WHO, and a new categorization was introduced (Table 76.4).

All countries should establish immunization safety monitoring activities.[191] The WHO has published guidance on the investigation of AEFI, how to analyze surveillance data, causality assessment and how to respond to serious AEFI, including communication.[192] The guidance document also includes a description of the causes and classification of vaccine reactions, the most common reactions, and updated references in vaccine pharmacovigilance.

National surveillance for AEFIs should emphasize reporting and follow-up of cases caused by program error. Such errors include reconstitution with the wrong diluent; administration of dangerous drugs mistaken for vaccines; contamination of multiple-use vials, leading to abscesses or sepsis; and use of contaminated needles or syringes, which may transmit bloodborne viral pathogens such as HIV and hepatitides B and C.[193–195] Single serious events or clusters of unusual events should be appropriately investigated and an independent review committee should review serious cases and assess causality. Surveillance should also monitor and investigate any serious events perceived to be potentially caused by vaccines. Training on adverse events surveillance is critical to remind healthcare workers of the potential for adverse events and the need for constant vigilance when biologic preparations are administered. The WHO has developed an e-learning course on vaccine safety, which is available online.[196]

TABLE 76.4 Cause-Specific Categorization of Adverse Events Following Immunization

Cause-Specific Type of AEFI	Definitions
Vaccine product-related reaction	An AEFI that is caused or precipitated by a vaccine owing to one or more inherent properties of the vaccine product
Vaccine quality defect-related reaction	An AEFI that is caused or precipitated by a vaccine that is owing to one or more quality defects of the vaccine product, including its administration device as provided by the manufacturer
Immunization error-related reaction (formerly "programmatic error")	An AEFI that is caused by inappropriate vaccine handling, prescribing, or administration and thus by its nature is preventable
Immunization[a] anxiety-related reaction	An AEFI arising from anxiety about the immunization
Coincidental event	An AEFI that is caused by something other than the vaccine product, immunization error, or immunization anxiety, but a temporal association with immunization exists

[a]"Immunization" is used in these definitions to mean the use of a vaccine for the purpose of immunizing individuals. "Use" includes all processes that occur after the vaccine product has left the manufacturing/packaging site (e.g., prescribing and administration of the vaccine).
AEFI, adverse events following immunization.
From Council for International Organizations of Medical Sciences and the World Health Organization, 2012.

Enhancing Service Delivery

Overview of Vaccines, Schedules, and Delivery Strategies. As discussed earlier, during the past decade most developing countries have added new vaccines to their national immunization schedules and extended immunization activities to also include older age groups. In particular, many developing countries have added or scaled up the use of vaccines in the second year of life (for second routine measles doses and other booster doses of vaccines administered in infancy) and during school age. With the recent introduction of human papillomavirus vaccine, several developing countries have also included vaccination of the preteen and adolescent age groups in their national immunization schedules. As a result, the fairly simple table with the recommended immunization schedule developed at the inception of the EPI have now been transformed into fairly complex tables that reflect the WHO recommendations for vaccination as stated in the WHO vaccine position papers (Table 76.5).[197] The recommendations in the tables provide countries with the flexibility to tailor national schedules to suit their needs based on the local disease epidemiology and their program characteristics.[198]

The tables are designed for use by national immunization managers and key decision makers, chairs and members of national advisory committees on immunization to guide the development of the respective national schedules, and for the information of partner organizations, including the vaccine industry. They were not intended for direct use by health workers.

As developing countries increased their immunization coverage from the historically very low levels to reach close to the 80% mark, they found coverage levels stagnating as the programs found it difficult to identify and deliver services to the remaining smaller segment of their target populations. Thus, many developing countries are faced with the dual challenge of dealing with the increasing complexity of the program, with several new vaccines and additional doses being added to the immunization schedule, as well as with tracking and delivering services to the so-called fifth child. Strengthening immunization and health systems (referred to in the previous section of this chapter) is one part of the solution. However, this needs to be coupled with strategies to reach those who are consistently missed by the system.

In developing countries administration of vaccine is most often done in a fixed site such as a health post, a clinic, or a health center. Birth doses tend to be given in the postnatal care clinic or ward. However, as health facilities in some countries are not accessible by populations living further away, across difficult terrain or are on the move because of the seasons or other influences, many countries augment their fixed site routine vaccination delivery services with various mobile and outreach services; in some countries mobile and outreach services have become the primary mode of delivering immunization. Community-level service delivery may be accomplished in several different ways:

- *Outreach services*: These are generally held regularly at the same site but are manned only when the outreach team arrives. They may be held at a formal health site or be held in community gathering places such as schools, places of worship, or markets. Generally, the outreach sessions are held regularly at least every month, and the attendant health workers would normally return to their home base on the same day of the service. In some countries, such as Bangladesh, approximately 80% of immunizations are delivered through outreach services.
- *Multiday outreach services*: In this case, the health worker would not return to their home base between outreach sessions, but would continue to the next outreach sites before returning home. This is clearly logistically more challenging, requiring lodging for the health worker and ability to maintain the vaccines in the cold chain throughout the outreach period.
- *Mobile teams*: These services would be provided in very sparsely populated areas, where the team would provide services in many places during the course of a day, moving from one vaccination post to the next.
- *Pulse vaccinations*: In this case, services are provided to far-flung areas requiring extensive and complicated logistical support (e.g., boats, helicopters, multiday walking excursions). Normally these would be done quarterly, half-yearly, or annually, as the resources required for this approach can be considerable.
- *Periodic intensification of routine immunizations)*: These are held on an ad hoc basis to catch-up routine vaccinations that have been missed, and are frequently combined with other appropriate preventive interventions, and may be promulgated as "Child Health Days" or the like.[199]

In addition to scheduled, routine vaccination doses, national immunization programs may try to reach the children left susceptible by inadequate routine vaccination services through supplementary immunization activities or vaccination campaigns. Whereas routine vaccination doses depend on the health worker making a decision to vaccinate each individual child that is seen by reviewing the previous vaccination history and eligibility for a dose, the doses given to children during supplementary immunization activities are given to every age-eligible child without contraindications

TABLE 76.5 Recommended Vaccination Schedule for All Immunization Programs Based on the World Health Organization's Vaccines Position Papers

Antigen	Type	Age of 1st Dose	Doses in Primary Series	Interval Between Doses			Booster Dose	Considerations (see footnotes for details)
				1st to 2nd	2nd to 3rd	3rd to 4th		
RECOMMENDATIONS FOR ALL CHILDREN								
BCG[1]		As soon as possible after birth	1					Exception: HIV
Hepatitis B[2]	Option 1	As soon as possible after birth (<24 h)	3	4 wk (min) with DTP1	4 wk (min) with DTP3			Premature and low birth weight; coadministration and combination vaccine; high-risk groups
	Option 2	As soon as possible after birth (<24 h)	4	4 wk (min) with DTP1	4 wk (min) with DTP2	4 wk (min),with DTP3		
Polio[3]	OPV + IPV	6 wk (see footnote for birth dose)	4 (IPV dose to be given with OPV dose from 14 wk)	4 wk (min) with DTP2	4 wk (min) with DTP3			OPV birth dose; transmission and importation risk criteria
	IPV/OPV Sequential	8 wk (IPV 1st)	1–2 IPV, 2 OPV	4–8 wk	4–8 wk	4–8 wk		
	IPV	8 wk	3	4–8 wk	4–8 wk		See WHO website footnote	IPV booster needed for early schedule (i.e., first dose given <8 wk)
DTP[4]		6 wk (min)	3	4 wk (min)-8 wk	4 wk (min)-8 wk		1–6 y of age (see WHO website footnote)	Delayed/interrupted schedule; combination vaccine
Haemophilus influenzae type b[5]	Option 1	6 wk (min)	3	4 wk (min) with DTP2	4 wk (min) with DTP3		See WHO website footnote	Single dose if >12 mo of age; not recommended for children >5 y; delayed/interrupted schedule; coadministration and combination vaccine
	Option 2	59 mo (max)	2–3	8 wk (min) if only 2 doses 4 wk (min) if 3 doses	4 wk (min) if 3 doses		At least 6 mo (min) after last dose	
Pneumococcal (conjugate)[6]	Option 1	6 wk (min)	3	4 wk (min)	4 wk		See WHO website footnote	Initiate before 6 mo of age; coadministration; HIV+ and preterm neonates booster
	Option 2	6 wk (min)	2	8 wk (min)			9–15 mo	
Rotavirus[7]	Rotarix	6 wk (min) with DTP1	2	4 wk (min) with DTP2				Not recommended if >24 mo old
	Rota Teq	6 wk (min) with DTP1	3	4 wk (min)–10 wk with DTP2	4 wk (min) with DTP3			
Measles[8]		9 or 12 mo (6 mo min, see WHO website footnote)	2	4 wk (min) (see footnote)				Combination vaccine; HIV early vaccination; pregnancy

Continued on following page

TABLE 76.5 Recommended Vaccination Schedule for All Immunization Programs Based on the World Health Organization's Vaccines Position Papers *(Continued)*

Antigen	Type	Age of 1st Dose	Doses in Primary Series	Interval Between Doses			Booster Dose	Considerations (see footnotes for details)
				1st to 2nd	2nd to 3rd	3rd to 4th		
Rubella[9]		9 or 12 mo with measles-containing vaccine	1					Achieve and sustain 80% coverage; combination vaccine and coadministration; pregnancy
HPV[10]		As soon as possible from 9 y of age (females only)	2	6 mo (min 5 mo)				Target girls age 9–13y ; pregnancy; older age ≥15 y = 3 doses; HIV and immunocompromised
RECOMMENDATIONS FOR CHILDREN RESIDING IN CERTAIN REGIONS								
Japanese encephalitis[11]	Inactivated Vero cell-derived vaccine	6 month	2 generally	4 wk (generally)				
	Live attenuated	8 mo	1					Vaccine options and manufacturer's recommendations; pregnancy; immunocompromised
	Live recombinant vaccine	9 mo	1					
Yellow fever[12]		9–12 mo with measles-containing vaccine	1					
Tickborne encephalitis[13]		≥1 y FSME-IMMUN and Encepur ≥3 y TBE-Moscow and EnceVir	3	1–3 mo FSME-IMMUN and Encepur 1–7 mo TBE-Moscow and EnceVir	5–12 mo FSME-IMMUN and Encepur 12 mo TBE-Moscow and EnceVir		At least 1 every 3 y (See WHO website footnote)	Definition of high-risk; vaccine options; timing of booster
RECOMMENDATIONS FOR CHILDREN IN SOME HIGH-RISK POPULATIONS								
Typhoid[14]	Vi PS	2 y (min)	1				Every 3 y	Definition of high risk
	Ty21a	Capsules 5 y (min) (see WHO website footnote)	3 or 4 (see footnote)	1 day	1 day	1 day	Every 3–7 y	Definition of high risk

Cholera[15]	Dukoral (WC-rBS)	2 y (min)	3 (2–5 y)	≥7 days (min)	≥7 days (min)	Every 6 mo	Minimum age
			2 (≥6 y)	<6 wk (max)	<6 wk (max)	Every 2 y	Minimum age
	Shanchol and mORCVAX	1 y (min)	2	14 days		After 2 y	Definition of high risk
Meningococcal[16]	MenA conjugate	9–18 mo (5 μg)	1				Definition of high risk; vaccine options; 2 doses if <9 mo with 8-wk interval
	MenC conjugate	2–11 mo	2	8 wk		After 1 y	Definition of high risk; vaccine options
		≥12 mo	1				
	Quadrivalent conjugate	9–23 mo	2	12 wk			Definition of high risk; vaccine options
		≥2 y	1				
Hepatitis A[17]		1 y	At least 1				Level of endemicity; vaccine options Definition of high-risk groups
Rabies[18]		As required	3	7 days	14–21 days	See WHO website footnote	Definition of high risk, booster
Recommendations for Children Receiving Vaccinations From Immunization Programs With Certain Characteristics							
Mumps[19]		12–18 mo with measles containing vaccine	2	1 mo (min) to school entry			Coverage criteria >80%; combination vaccine
Seasonal influenza (inactivated tri- and quadrivalent)[20]		6 mo (min)	2 (<9 y), 1 (≥9 y)	4 wk		Revaccinate annually: 1 dose only (See WHO website footnote)	Priority risk groups, especially pregnant women; lower dosage for children 6–35 mo
Varicella[21]		12–18 mo	1–2	4 wk to 3 mo per manufacturer recommendations			Achieve and sustain ≥80% coverage; pregnancy; coadminister with other live vaccines

Accompanying tables and footnotes to this table may be found at: http://www.who.int/immunization/policy/immunization_tables/en/

attending the campaign, irrespective of previous vaccination. This also means that only the aggregate number of doses given in supplementary immunization activities is recorded at the site of vaccination, and these doses are not counted in estimating routine immunization coverage.

Strategies and Practices to Strengthen Routine Immunization Systems and Improve Immunization Coverage. The term "routine immunization" is understood in two distinct ways that relate to the health system components required for delivering the services and to the specific strategies and activities to improve coverage with the scheduled vaccination doses, as described in Table 76.6. It is important to distinguish between these perspectives, as many routine immunization systems strengthening activities may not result in short-term or rapid improvements in vaccination coverage. Equally, many activities specifically designed to rapidly increase routine coverage may not result in stronger delivery systems but rather may use short-term solutions to overcome deficiencies in the delivery system.

Following the development of the GVAP, which provides a framework to guide immunization actions in the current decade (2011–20), a companion document was developed to provide more specific guidance on strategies and practices to achieve the immunization coverage goals in the GVAP. The Global Routine Immunization Strategies and Practices outlines four strategic directions to strengthen the delivery of scheduled immunization doses and improve coverage, namely:

- *Maximize Reach*: strategies and activities to ensure that vaccination services use all means at their disposal to reach beyond the currently reached populations and seek out and vaccinate those that remain unvaccinated. This includes new approaches to detect and identify those that remain unvaccinated, designing the services to reach them, building the capacity of vaccinators and peripheral managers, assuring the availability of vaccines and using all opportunities to vaccinate;
- *Manage the Program*: strategies and activities that support and maintain the managerial functions of the program, including the political commitment to the program, proper planning and budgeting processes, leadership and the competency of the program management team, and the process by countries to set national policies and guidance;
- *Mobilize People*: strategies and activities to engage with communities to create the demand for vaccination, mobilizing and communicating activities to promote vaccination, and dealing with vaccine hesitancy, false perceptions, and negative consequences of perceived AEFIs;
- *Monitor Progress*: strategies and activities to be able to direct and steer the program through monitoring program performance and disease occurrence and evaluating the program through surveys, assessments, and reviews at each of the various levels of the program from the national level to the service delivery point.

The Global Routine Immunization Strategies and Practices also identifies nine key investments that should be made to build and sustain the program. These investments are meant to highlight those key areas where the national governments of developing countries and their development partners should focus in the coming years. While several of these investments are for strengthening the health systems, others relate to investments in strategies to identify and specifically target populations who remain outside the reach of the health system. These investments should target the following areas:

- A capable and sufficiently resourced national program management team in each country;
- Tailored vaccination strategies to identify the undervaccinated and the unvaccinated and provide them with all needed vaccinations regularly;
- A comprehensive planning and monitoring process, from comprehensive multiyear plans and annual operational plans to the quarterly monitoring of implementation of these plans;
- Processes that ensure that sufficient funds reach the operational level of the program in a timely fashion;
- Enhance the performance of vaccinators and district managers by regularly and systematically strengthening their technical capacity, and improve their performance through supportive supervision;
- Modernization of vaccine management and supply chains to make sure that each vaccination session has sufficient amounts of the right and potent vaccines available;

TABLE 76.6 Distinguishing Features Between the Concepts of Strengthening Routine Immunization Services and Accelerating Routine Immunization Coverage

Securing the Foundation; Strengthen Routine Immunization Systems	Reaching Universal Immunization Coverage; Accelerate Routine Immunization Activities
"Routine immunization" refers to the part of the health **system** that allows vaccination to occur.	"Routine immunization" may also describe the process to regularly **deliver** vaccines according to the national vaccination schedule.
The term encompasses system components such as:	Routine vaccination coverage is the percentage of the target group that is provided with vaccination, and is a measure of the ability to reach the population.
• Program communication	Specific activities can be designed to boost routine immunization coverage rapidly, to reach stated coverage or disease control goals, but may frequently lack long-term sustainability.
• Vaccine supply and management with quality assurance	In addition to disease-specific activities and special projects such as the introduction of a new vaccine, a country's routine immunization program should implement tailored activities to increase routine immunization coverage where it is lagging. Within GRISP, "Reaching Universal Immunization Coverage" is reflected in strategies and activities such as:
• Service implementation and monitoring	• Detecting and reaching marginalized populations
It is the foundation on which the entire program is built and is a part of the overall health system of a country.	• Improving coverage equity, creating synergy with accelerated disease control activities
Many components of the immunization system are not unique to the immunization program but shared with all programs and components of the health system.	• Mobilizing and communicating for vaccination, and addressing vaccine hesitancy
Within GRISP, "Securing the Foundation" is reflected in the strategies and activities such as program leadership, human resource capacity building, vaccine availability and quality, political commitment, program planning and financing, policy guidance and monitoring and surveillance.	

GRISP, Global Routine Immunization Strategies and Practices.

- Good interactions and collaborations between communities and the immunization services;
- An information system that identifies and tracks the vaccination status of everyone targeted; and
- Sustainably expand the routine vaccination schedules to cover the entire life course.

Increasingly, the first investment in the national program leadership team to have competency and authority to direct the program is acknowledged as the one key factor that distinguishes good national vaccination programs from their poorly performing counterparts in other countries.

Dropouts and Missed Opportunities. In addition to the issues of accessibility (i.e., the location and timing of service delivery so that they are accessible and suitable for clients), the immunization program should also pay close attention to those clients who initially access services but then do not return for subsequent vaccinations. These clients are termed "dropouts" and are measured through the calculation of the difference between the doses administered at the two scheduled visits. In well-running programs, the dropout rate between the first dose and the third dose of DTP-containing vaccine (DTP3–DTP1 dropout rate) should be less than 10%. This measurement, together with other dropout rates such as the Measles-BCG dropout rate, gives a better insight into program performance than the coverage rate alone. A high dropout rate may point to utilization factors, such as the quality of the service, the affability and competence of the health staff, knowledge of the benefits of vaccinations, and the awareness among the clients or their guardians of the need for multiple vaccinations. While the DTP3–DTP1 dropout rate measures the utilization of health facility services more narrowly, the Measles-BCG dropout rate measures the dropout rate over a longer period.

To improve coverage it is equally important that missed opportunities to vaccinate are minimized. A missed opportunity is defined as "an occasion when a person eligible for immunization and with no valid contraindication visits a health service facility and does not receive all recommended vaccines." Two basic types of missed opportunities are defined:

- Giving overdue doses at vaccination visits scheduled for other vaccine doses. It has been estimated that the median DTP3 coverage could be improved by 8% globally (C. Sanderson, personal communication, 2015) and coverage of the first dose of measles could be improved by 7% if one or both vaccines were administered to children if they had not yet received these vaccines, but were age-eligible to receive them; and
- Giving overdue doses to eligible child or other persons when they visit a health facility for seeking care for conditions that do not contraindicate the provision of vaccination. The rigorous review of vaccination cards at every such opportunity, and the provision of any missed doses of vaccination, would boost coverage considerably. In addition, if infants accompanying adult patients seeking healthcare were considered for vaccination further gains could be made, and vice versa for tetanus toxoid coverage for adult women.

Use of Financial Incentives for Increasing Immunization Coverage. There is growing interest in the use of financial incentives to achieving higher immunization coverage and improved health outcomes.[103] Results-based financing is a broad term that includes a variety of incentive schemes to provide resources to individuals, households, healthcare providers, and district or local-level governments, whereby payment is conditional on the achievement of agreed-on and measurable outcomes. Although there is potential to improve coverage with such programs,[200] the overall quality of evidence is still relatively low.[201] Countries with weaker management and monitoring and evaluation systems in place can face challenges in implementing results-based financing mechanisms, and the impact of results-based financing in countries with weaker health systems is unclear. Other forms of incentive approaches to improving immunization coverage include national vaccine legislation that mandates immunization and often includes the provision of a budget line for vaccine procurement. Vaccine legislation is widely used in the Americas and is a prerequisite to gaining access to the PAHO Revolving Fund.

Fostering Integrated and Coordinated Approaches

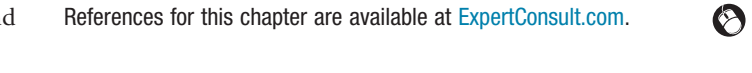
Integration or better coordination in the delivery of immunization and other primary healthcare services provides opportunities to improve the coverage of both immunization as well as the other healthcare interventions by reducing missed opportunities for immunization and using immunization visits for counselling to improve care-seeking behaviors for other interventions; for example, care-seeking for diarrhea and pneumonia. As the health workers delivering the different services are often the same in many developing countries, coordination between the different programs in terms of health worker training, program planning, supervision, and monitoring can lead to efficiencies and improved coverage with all the relevant interventions.

The Global Action Plan for Pneumonia and Diarrhoea, developed jointly by the WHO and UNICEF[202] and the Comprehensive Cervical Cancer Control Guide to Essential Practice[203] are two examples of such coordinated and comprehensive approaches to controlling pneumonia, diarrhea, and cervical cancer.

CONCLUSION

Immunization is one of the most cost-effective health interventions. Through its use 2 to 3 million deaths are estimated to be averted each year. Developing countries that carry the disproportionate burden of disease stand to gain the most from immunization and since the establishment of the EPI more than 40 years ago have realized significant health gains by scaling-up the coverage with vaccination. With the expanding portfolio of vaccines, targeting diseases that are still important causes of death and disability in many developing countries, immunization has a large untapped potential for saving lives and improving the health of populations in developing countries. An estimated 25 to 26 million future deaths could be averted in low- and middle-income countries through the introduction and scale-up of currently available vaccines.[16] However, the addition of an increasing number of vaccines to the immunization programs of developing countries has exposed the fragility of their health systems. Fortunately, there are solutions to the problems that these countries face, which, if applied, can lead to improving the health of their populations.

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77 Community Protection

Paul E.M. Fine, Kim Mulholland, J. Anthony Scott, and W. John Edmunds

Vaccines are given to people to protect them against disease. They are also given to reduce transmission of infection, thereby preventing disease even in nonvaccinated persons and protecting communities. The extension of perspective from individuals to their communities is the business of public health. The use of an increasing number of different vaccines, in all populations of the world, represents a major, evolving, and highly complicated intervention into human ecology.

For the past several decades, this subject has been discussed largely with reference to the concept of *herd immunity*, a term that refers to the prevalence or proportion of immune persons in a population, but that is often used with reference to indirect protection of nonimmune persons, attributable to the presence and proximity of immune persons (in effect by the vaccine-derived protection of those who would otherwise infect them).[1–3] Much of the discussion has concentrated on the issue of indirect protection of nonimmune persons and its implication of threshold levels of immunity, which, if achieved and maintained, should lead to the elimination of an infection from a population. Although this is clearly of theoretical interest and public health importance, the emphasis on such thresholds has turned attention away from other, more important, community implications of vaccination programs. In recognition of this, the editors of this volume suggested a broadening of the scope of this discussion, under the term *community protection*. The phrase is appropriate insofar as individuals can be protected by vaccines without being themselves immune, through reduced community transmission. In this context it is important to acknowledge, at the outset, that both individual and community protection may also be achieved from the direct and indirect effects of other interventions, including drugs, education, and environmental changes, which are beyond the scope of this chapter and book.

The shift in perspective from the individual to the community raises many programmatic issues about vaccination, including schedules, consent, contraindications, legal enforcement and exemptions, access, public versus private providers, fixed versus mobile services, routine versus targeted programs, campaigns, coverage, monitoring, and evaluation. Many of these issues are covered with respect to particular vaccines and programs elsewhere in this volume. Here we consider more general issues concerning the distribution and implications of vaccine-derived immunity in communities. In simplest terms, one might expect disease incidence to be reduced by a factor equal to the product of vaccine coverage and vaccine efficacy. But the real world is not that simple, and, to predict a program's impact, we must also consider other issues, such as (1) the distribution of vaccines and of disease risk in communities (neither of which is ever uniform or random); (2) the nature of the immunity induced by the vaccine (measured in terms of partial or complete protection against infection, or reinfection, or against disease, or even against transmission, as well as its natural boosting or waning over time); and (3) the indirect protection of nonimmune persons by the presence of immune persons (the traditional focus of herd immunity discussions).

The issue of indirect protection of nonimmune persons is central to discussions of community effects of vaccination. Such protection applies to important segments of society: to children too young to have been vaccinated, to immunocompromised or elderly persons who cannot make an adequate immune response, to persons with contraindications to vaccination, to persons in whom vaccine-induced immunity was incomplete or has waned, and to those who are unvaccinated, either by choice or because they do not have adequate access to vaccines. The social benefits of such indirect protection have important policy implications, including provision of a rationale for mandating immunization. It provides a justification for the vaccine injury compensation program in the United States: a child who is vaccinated is not only deriving direct protection but is also helping to protect the community; hence, society has some obligation for any injuries incurred by the vaccination. In addition, it is important in determining the full costs and benefits of immunization policies; as new vaccines have become increasingly costly, issues of indirect protection benefits become increasingly important in determining whether to introduce new vaccines and to whom they should be given. The community implications of indirect protection are indeed broad.

After a brief historical review, this chapter summarizes the theory and experience that have evolved to describe various aspects of community immunity, and then considers these principles with reference to major vaccines in use today.

HISTORICAL BACKGROUND

Although both Jenner[4] and Pasteur[5] recognized explicitly the potential of vaccines to control and even to eradicate specific infections from populations, neither is known to have considered the practical issues involved in the application of vaccines to populations. Smallpox vaccination was introduced widely in Europe and North America during the early 19th century, with increasing success over time. Its enforcement through legislation would have long-lasting effects on public acceptance of vaccines, including the appearance of the first antivaccination sentiment opposed to this intrusion by states into the bodies of their citizens. This remains a major issue today.[6] Close observers noted interesting implications of vaccination—for example, William Farr commented as early as 1840, "The smallpox would be disturbed, and sometimes arrested, by vaccination which protected a part of the population," probably the first recorded recognition of indirect protection by vaccines.[7]

The early years of the 20th century saw important advances in our understanding of the dynamics of infections in populations as a function of the balance of infectious, susceptible, and immune individuals. Several factors contributed to this—in particular, the increased understanding of disease patterns in communities, which arose from the introduction of morbidity (in addition to mortality) notification schemes, the growth of immunology as a discipline, and the development of a theoretical framework linking these data and disciplines together.

The first published use of the term *herd immunity* appears to have been in a paper published in 1923 by Topley and Wilson, entitled "The spread of bacterial infection: the problem of herd immunity."[8] The paper described one of a series of classical studies on epidemics of various infections in closely

monitored populations of laboratory mice, and introduced the term in this manner: "Consideration of the results obtained during the past five years … led us to believe that the question of immunity as an attribute of the herd should be studied as a separate problem, closely related but in many ways distinct from, the problem of the immunity of an individual host." They went on to pose an "obvious problem to be solved: Assuming a given total quantity of resistance against a specific bacterial parasite to be available among a considerable population, in what way should that resistance be distributed among the individuals at risk, so as best to ensure against the spread of the disease, of which the parasite is the causal agent"?

Wilson later recalled that the phrase "herd immunity" had first arisen in the course of a conversation with Major Greenwood (G.S. Wilson, personal communication, 1981), and Greenwood employed it in his 1935 textbook, *Epidemics and Crowd Diseases*.[9] Although these authors did not distinguish explicitly between direct and indirect protection stemming from vaccine-derived immunity, later authors picked up the phrase and applied it in particular to the indirect protection afforded to nonimmune persons by the presence and proximity of immune persons.

The population implications of immunization programs became a major issue during the last quarter of the 20th century, in association with the widespread introduction of vaccines in all populations of the world. Discussion was stimulated in particular by the success of the global smallpox eradication program, as well as by the creation in 1974 of the World Health Organization's (WHO's) Expanded Programme on Immunization, and the setting of targets to "eliminate" or to eradicate several infectious diseases through vaccines: global targets to eradicate polio and to eliminate neonatal tetanus, and national and regional goals to eliminate measles and rubella.[10] These massive efforts encouraged a body of theoretical literature exploring the population implications of immunization programs, including much discussion of the extent of indirect protection by vaccines, and of coverage criteria for eradication of infection. Although much of the initial theory assumed that immunity was a simple property, that individuals were either fully immune or fully susceptible, and that the protection was solid and permanent against both infection and disease, it became increasingly clear during the 1980s and 1990s that this was incorrect, and that different infections and vaccines could induce different degrees of protection against infection, against disease, and even against infectiousness, and that these different sorts of immunity could wane or be boosted over time. More recent research has attempted to measure these different forms of immunity and to incorporate these subtleties into evaluations of immunity at the community level.[11]

THEORETICAL ARGUMENTS

The focal point of much of the theoretical work on community immunity has been the recognition that, if an infection or vaccine induces some degree of immunity against infection, then some nonimmune people will be protected indirectly, by the presence and proximity of immune persons, and transmission should stop in a population prior to the infection of all susceptible individuals. This insight encourages the estimation of threshold numbers or proportions of immune persons necessary for this cessation to occur. An elegant body of theory has evolved around this issue, developed initially to describe the implications of introducing fully immune people into randomly mixing populations, but subsequently extending to more realistic scenarios.

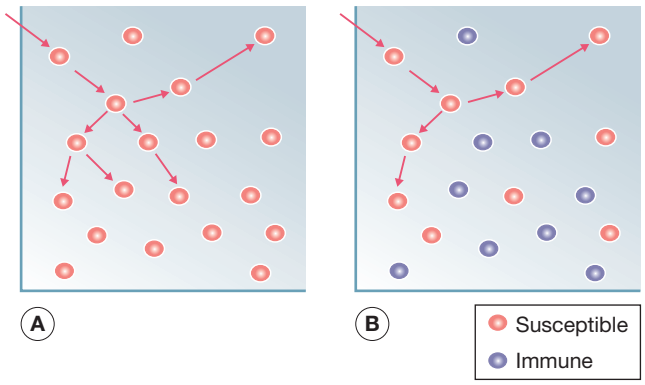


Figure 77.1. Simple diagram illustrating three generations of transmission after introduction of an infection into a fully susceptible population **(A)** and into a population containing immune persons **(B)**. Although it is clear from this diagram that some people may be shielded from infection in population **B**, similar shielding can also occur in **A**.

Fig. 77.1 illustrates (very simply) the principle of indirect protection by showing susceptible and immune individuals in a population, with arrows indicating the spread of an infection. Actual transmission patterns depend on the distribution of susceptible persons in space and time (social mixing patterns), and some susceptible persons may be shielded from infection by being surrounded by immune persons. However, representations such as Fig. 77.1 fail to show that, even if there were no immune individuals at the start of an outbreak, infections may *generate* immune persons who can in turn block transmission and lead to termination of an epidemic before all individuals become infected. We thus need to consider dynamics.

Mass Action Approach

The crucial argument was introduced in 1906, by William Hamer, in the context of a discussion of the dynamics of measles in London.[12] He argued that the number of new cases ($\mathbf{C}_{t+1}$) in a time period equal to the serial interval of the infection (thus one generation of cases, or approximately 2 weeks for measles) is a function of the product of the numbers of infectious cases and of susceptible persons in the previous time period (C_t and S_t, respectively; Fig. 77.2). In simple terms,

$$\mathbf{C}_{t+1} = \mathbf{C}_t \times \mathbf{S}_t \times r \qquad \text{[Eq. 1]}$$

where r is a transmission parameter, or contact rate—in effect, the proportion of all possible contacts between susceptible and infectious individuals that lead to new infections (so, $r \ll 1$). For example, if there are 10 cases and 90 susceptibles, there are 900 possible connections between cases and susceptible persons. The transmission parameter r is the proportion of those possible contacts resulting in transmission and new cases. To simulate successive changes over time, the number of susceptible persons is recalculated for each new time period as

$$\mathbf{S}_{t+1} = \mathbf{S}_t - \mathbf{C}_{t+1} + \mathbf{B}_t \qquad \text{[Eq. 2]}$$

where S_{t+1} is the number of susceptible persons in the next time period and B_t is the number of new susceptible persons added (e.g., born into) the population per unit time. (Note that **bold** symbols are used in this chapter to denote **numbers** of individuals, and nonbold symbols to denote proportions or other descriptive statistics [Table 77.1].) The relationship in Eq. 1, that future incidence is a function of the product of current prevalence and current numbers susceptible, is known

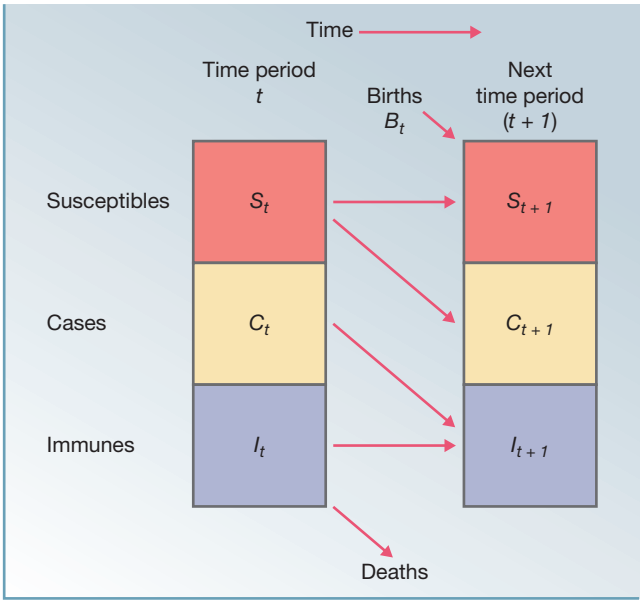


Figure 77.2. Relationship between susceptible persons *(S)*, infectious cases *(C)*, and immune persons *(I)* in successive time intervals (*t*, *t* + 1) in the simple discrete time mass action model. In each time period, some ($\mathbf{C}_{t+1}$) susceptible persons become cases, and the others remain susceptible. Each case is assumed to remain infectious for no more than a single time period (= serial interval). $\mathbf{B}_t$ individuals enter as susceptible births during each time period (e.g., Eq. 2 in the text).

TABLE 77.1 Variables in Theoretical Arguments Concerning Infection Dynamics and Herd Immunity Thresholds

Symbol[a]	Variable Definition
$\mathbf{C}_t$, $\mathbf{C}_{t+1}$	Numbers of cases in successive serial intervals
$\mathbf{S}_t$, $\mathbf{S}_{t+1}$	Numbers of susceptible individuals in successive serial intervals
$\mathbf{I}_t$, $\mathbf{I}_{t+1}$	Numbers of immune persons in successive serial intervals
$\mathbf{B}_t$	Number of new susceptible persons (i.e., births) per serial interval
r	Transmission parameter for the simple mass action model, interpretable as the proportion of all possible contacts between cases and susceptible persons that lead to new infections
$\mathbf{S}_E$	Epidemic threshold number of susceptible persons (if exceeded, incidence increases)
$\mathbf{T}$	Total population size
H	Herd immunity threshold (proportion of immune persons in a population required to prevent large outbreaks from occurring): $H = 1 - \mathbf{S}_E/\mathbf{T} = 1 - 1/\mathbf{R}_0$
$\mathbf{R}_0$	Basic reproduction number (average [expected] number of secondary cases attributable to a single case introduced into a totally susceptible population)
$\mathbf{R}_n$	Net reproduction number (actual number of transmissions per case)
S	Proportion of susceptible persons in a population
A	Average age at infection
L	Average life expectancy in years
C	Vaccine coverage
C_H	Vaccine coverage to achieve herd immunity threshold
P	Protective efficacy of vaccine (proportion protected among vaccinees)
P_i	Protective efficacy of vaccine in terms of susceptibility to infection (proportion of vaccinees who are rendered nonsusceptible to infection)
P_t	Protective efficacy of vaccine in terms of transmissibility (proportion of vaccinees who fail to shed organisms despite becoming infected)

[a]**Bold** symbols represent numbers of individuals; nonbold symbols represent proportions or other descriptive statistics.

as the epidemiologic law of mass action, by analogy with the physicochemical principle that the rate of a chemical reaction is a function of the product of the concentrations of the reactants. Often expressed as a differential (continuous time) rather than a difference (discrete time) equation, as here, this relationship underlies much theoretical work on the dynamics of infections in populations.[13] Note that Eqs. 1 and 2 include no explicit term for immune persons. By implication, deaths prior to infection are not considered in this simplest model (i.e., in each period, the same number of immune persons die as susceptible people are born into the population). The introduction of terms for immune persons; for individuals incubating the infection; for population growth and selective mortality of susceptible, infected, and immune persons; and for age is explored at length in the literature, but the terms do not change the basic message emphasized here.[13]

Fig. 77.3 shows what happens when Eqs. 1 and 2 are iterated, and it serves to illustrate several principles of the epidemiology of those acute immunizing infections, such as measles, mumps, rubella, chickenpox, poliomyelitis, and pertussis, that affect a high proportion of people in unvaccinated communities. First, the model predicts cycles of infection incidence, which are well recognized for many of the ubiquitous infections of childhood (Fig. 77.4). The incidence of infection rises above and dips below the birth rate, or the rate of influx of new susceptible persons. Second, the number of susceptible people also cycles, around a number that is sometimes described as the epidemic threshold (S_E). Rearrangement of Eq. 1 to $C_{t+1}/C_t = S_t \times r$ reveals that this threshold number of susceptible persons is numerically equivalent to the reciprocal of the transmission parameter r, as incidence increases (i.e., $C_{t+1} > C_t$) when, and only when, $S_t > 1/r$, and thus $S_E = 1/r$. In the example illustrated in Fig. 77.3, $r = 0.0001$ and S_E is thus 10,000. The correspondence between the case and susceptible lines in the figure illustrates this relationship: whenever case incidence exceeds the birth rate, the number of susceptible people falls (as it must), and vice versa. Also, whenever the number of susceptible people rises above the epidemic threshold, incidence rises, and vice versa.

This epidemic threshold implies a simple herd immunity criterion. If the *proportion* immune is so high that the *number* of susceptible people is below the epidemic threshold, then incidence will decrease. We can express this threshold algebraically as follows:

$$\mathrm{H} = 1 - \mathrm{S_E}/\mathrm{T} = 1 - 1/\mathrm{r}\,\mathrm{T} \qquad \text{[Eq. 3]}$$

where T is the total population size, S_E is the epidemic threshold number of susceptible people for the population, S_E/T is the proportion susceptible at the threshold, and H is the herd immunity threshold—that is, the proportion of immune persons that must be exceeded if incidence is to decrease. If the total population in the Fig. 77.3 example were 100,000, this would imply a herd immunity threshold (H) equal to $1 - 10{,}000/100{,}000 = 1 - 1/10$, or 90%.

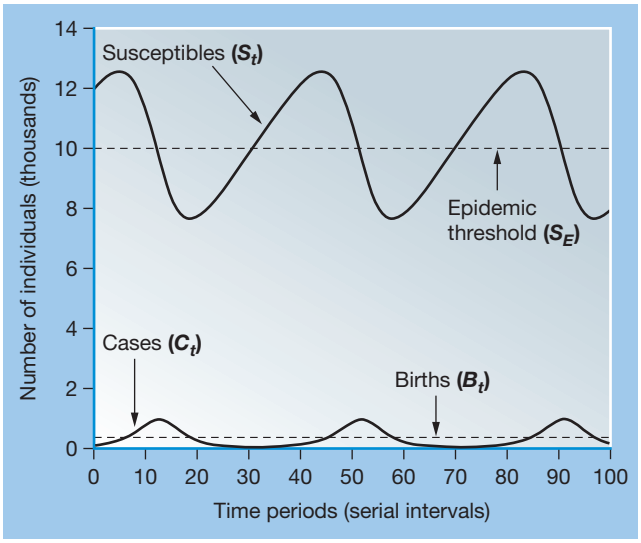


Figure 77.3. Results obtained on reiteration of Eqs. 1 and 2. The illustrated simulation was based on 12,000 susceptible persons and 100 patients at the start, $r = 0.0001$, and 300 births per time period. Note that the incidence of cases cycles around the birth rate, and that the number of susceptible persons cycles around the epidemic threshold: $\boldsymbol{S_E} = 1/r = 10{,}000$. If the total population were $\boldsymbol{T} = 100{,}000$, this implies a herd immunity threshold of 90%, and $\boldsymbol{R_0} = 10$.

Case Reproduction Numbers

We can approach this herd immunity concept from an alternative, and equally informative, perspective. If an infection is to persist, each infected individual must, on average, transmit the agent to at least one other individual. If not, incidence will decline and the infection will disappear progressively from the population. The number, or distribution, of actual onward transmissions per case thus describes the spread of an infection in a population, and it is a function of four things: (1) the duration of infectiousness; (2) the likelihood of transmission per "contact" between infectious and susceptible individuals; (3) the rate and pattern of contact between members of the host population; and (4) the proportion susceptible in the host population. Its value under any set of circumstances is known as the reproduction number of the infection, by analogy with standard demographic measures (the average number of progeny per individual per generation). This average number of actual transmissions should be at a maximum if all members of the host population are susceptible—in which circumstance it is known as a basic reproduction number $(\boldsymbol{R_0})$, defined formally as the average number of transmissions expected from a single primary case introduced into a totally susceptible population.[14,15] This definition *can* be translated directly into the mass action formulation (Eq. 1) by setting $C_t = 1$ and $S_t = T$, to represent the single case introduced into a fully susceptible population. The number of secondary cases, C_{t+1}, is then equivalent, by definition, to the basic reproduction number (R_0):

$$\mathbf{C_{t+1} = T \times r = R_0} \qquad \text{[Eq. 4]}$$

This basic reproduction number describes the maximal spreading potential of an infection in a population. Continuing with the example in Fig. 77.3, the introduction of a single primary case into the population of $T = 100{,}000$ susceptible persons should lead to 10 secondary cases (C_{t+1}=100,000 × 0.0001 = 10 = R_0). Table 77.2 shows examples of numerical values of this statistic that are applicable to different infections and derived by methods described later. Fig. 77.5A is an illustration of the concept.

TABLE 77.2 Approximate Basic Reproduction Numbers (in Developed Countries) and Implied Crude Herd Immunity Thresholds[a] for Common Vaccine-Preventable Diseases[b]

Infection	Basic Reproduction Number (R_0)	Crude Herd Immunity Threshold, H (%)
Diphtheria	6–7	83–85
Influenza[c]	1.4–4	30–75
Measles	12–18	92–94
Mumps	4–7	75–86
Pertussis[d]	5–17	80–94
Polio[e]	2–20	50–95
Rubella	6–7	83–85
Smallpox	5–7	80–85
Tetanus	Not applicable	Not applicable
Tuberculosis[f]	?	?
Varicella[g]	8–10?	?

[a]Herd immunity threshold (H), calculated as $1–1/R_0$.
[b]The values given in this table are approximate and crude; they do not properly reflect the tremendous range and diversity among populations, nor do they reflect the full immunologic complexity underlying the epidemiology and persistence of these infections. See text for further discussion.
[c]R_0 of influenza viruses probably varies greatly between subtypes.
[d]Note that immunity to *Bordetella pertussis* infection is not solid, and it wanes.
[e]Complicated by uncertainties over immunity to infection and variation related to hygiene standards.
[f]Protective immunity not defined.
[g]Immunity not sterile; herd immunity threshold not defined.
From Fine PEM. Herd immunity: history, theory, practice. Epidemiol Rev. *1993;15:265–302; and Anderson RM, May RM.* Infectious Diseases of Humans: Dynamics and Control. *Oxford, UK: Oxford University Press; 1991.*

If immune individuals are present in a population, then some of the contacts of infectious individuals will be with these immune persons, and hence will fail to lead to transmission. As a result, the average number of actual infection transmissions per case will be less than the basic case reproduction number, and this has been defined as the net, or actual, or effective, reproduction number (R_n).[13,14] The actual number of transmissions (R_n) should be equivalent to the basic case reproduction number (R_0) times the proportion susceptible in the population (S):

$$\mathbf{R_n = R_0 \times S} \qquad \text{[Eq. 5]}$$

By this expression, if the proportion susceptible (S) were equal to the reciprocal of the basic reproduction number of the infection $(1/R_0)$, the average number of transmissions per case (R_n) should be 1, and thus incidence should remain constant over time. Fig. 77.5B illustrates this, and it once again leads us directly to the herd immunity threshold (H). Because the proportion immune is just the complement of the proportion susceptible $(H = 1 - S)$, we have

$$\mathbf{H = 1 - 1/R_0 = (R_0 - 1)/R_0} \qquad \text{[Eq. 6]}$$

The same expression can also be derived just by combining Eqs. 3 and 4. As long as the proportion immune is maintained above this threshold, incidence should decrease, ultimately to the point of eradication of the infection from the population. Fig. 77.6 shows the relationship graphically, which shows the

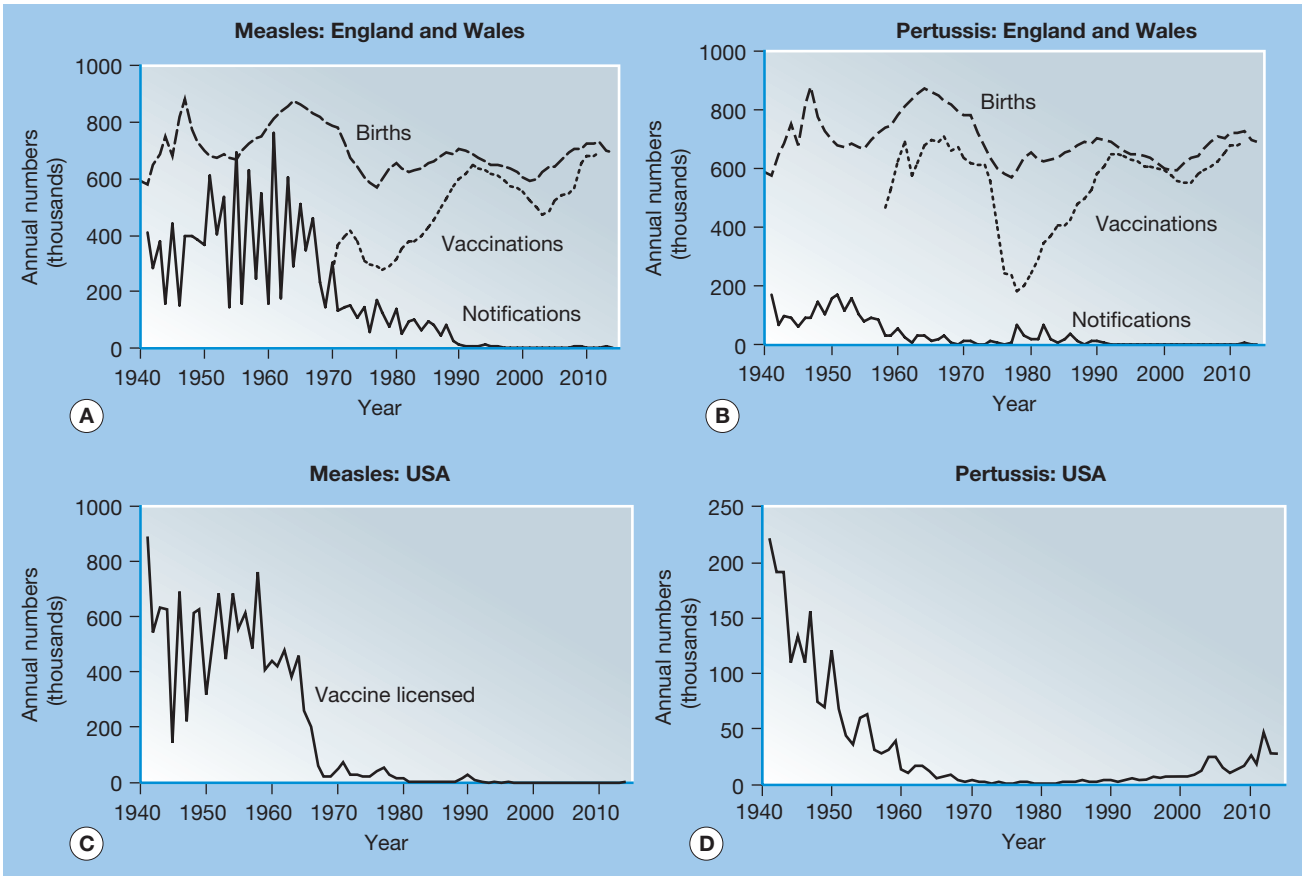


Figure 77.4. Reported incidence of common childhood vaccine-preventable diseases. Measles showed a tendency to biennial epidemics in England and Wales prior to vaccination **(A)**. This pattern was less dramatic in data for the entire United States **(C)**, because of the size and heterogeneity of the population (not all areas were in phase with one another). All areas showed a strong seasonal oscillation, in addition to the biennial cycle. Pertussis shows a 3- to 4-year cycle, with little obvious seasonality, in the United Kingdom **(B)**. This cycling is also seen in national data for the United States, in particular prior to 1970 **(D)**. Notification efficiency for measles was approximately 60% in England and Wales prior to vaccination, but it was considerably lower for pertussis, and for both diseases in the United States. Indigenous measles and pertussis persist in the United Kingdom (2503 and 1850 notified cases, respectively, in 2014); in the United States all measles cases since 2002 have been attributable to importation, and there were 28,660 pertussis notifications in 2014.

implications for persistence or decline of an infection depending on its basic reproduction number and the proportion of immune persons in the population.[1,13,16]

Estimation of R_0

Given this theoretical relationship between the basic reproduction number of an infection in a population and the herd immunity threshold, the estimation of R_0 becomes an important challenge. If the infection is so common that it affects virtually everyone in unvaccinated populations, as is the case with many of the acute immunizable diseases of childhood (e.g., measles, mumps, rubella, polio), one derivation of R_0 turns out to be relatively straightforward. According to the logic described earlier, R_0 is equivalent to the reciprocal of the average proportion susceptible in the population over an epidemic cycle (see Eq. 5 and Fig. 77.5B; note that the proportion susceptible cycles around $S_E/T = 1 - H$, as in Fig. 77.3 and Eq. 3). Dietz[16] pointed out that for immunizing infections the proportion susceptible is approximated by the average age at infection (A) divided by the average life expectancy in years (L), and thus $R_0 \approx L/A$. He went on to show that, for populations with exponential age structures (typically found in low-income countries), a better estimate of R_0 can be obtained with the following equation:

$$R_0 \cong 1 + L/A \qquad \text{[Eq. 7]}$$

Another method of estimating R_0, used in particular for influenza, is based on an analysis of the rate of increase in incidence in the early phase of an epidemic with a new virus subtype. In theory, incidence should increase by a factor equal to R_0 each generation, or serial interval (the time between successive cases in a chain of transmission, approximately 3 days for influenza). The effective reproduction number can also be estimated by this approach, if there is prior immunity in the population—and with knowledge of the fraction susceptible, it may then be possible to estimate R_0, although this latter step is typically more difficult.[17–19] Detailed discussion of the estimation of R_0 by these and other methods can be found in several references.[13,15,16] The herd immunity thresholds cited in Table 77.2 are based on these values, and provide crude estimates of the proportions of immune persons required, *in theory*, to eliminate these infections. As discussed below, and emphasized repeatedly in this chapter, these thresholds, even though widely cited, are based upon unrealistic assumptions (solid immunity, homogeneity, random

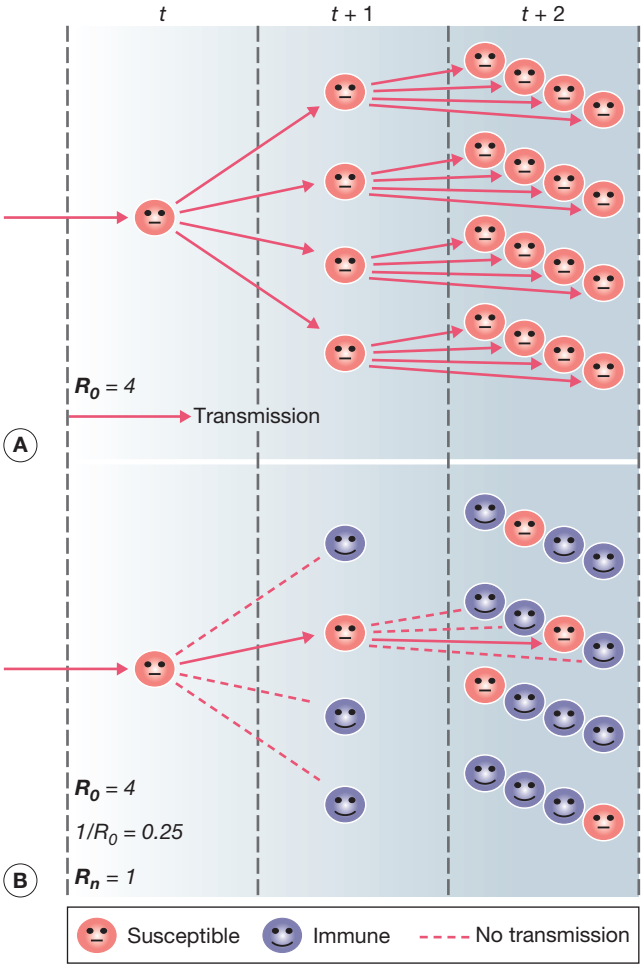


Figure 77.5. Implications of a basic reproduction number $R_0 = 4$. In each successive time (serial) interval, each individual has effective contact with four other individuals. If the population is initially entirely susceptible **(A)**, incidence increases exponentially, fourfold each generation (until the accumulation of immune persons slows the process). If 75% of the population is immune **(B)**, then on average only $S = 25\%$ of each set of four contacts lead to successful transmissions, and the net reproductive number $R_n = R_0 \times S = 1$.

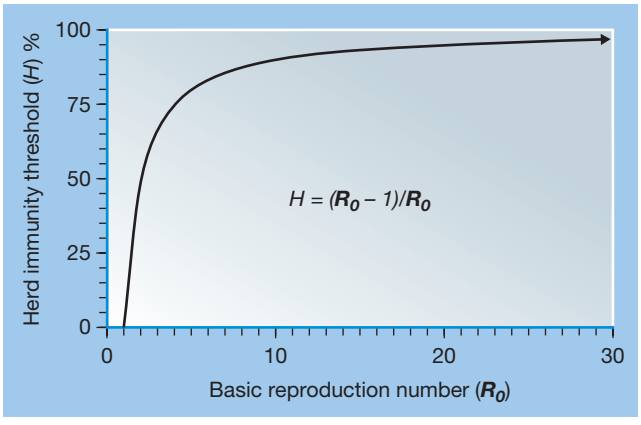


Figure 77.6. Relationship between herd immunity threshold *(H)* and basic reproduction number R_0, as in Eq. 6: $H = 1 - 1/R_0$.

mixing) and should not be thought to apply in real populations.

R_0, Rapidity of Spread, and Herd Immunity Thresholds

R_0 is a standard measure of the transmissibility of an infection, and hence of the potential rapidity of its spread in a population, per generation, or serial interval, of the infection. This can have very different real time implications, depending on whether the serial interval is relatively short (e.g., approximately 3 days for influenza) or relatively long (e.g., approximately 15 days for a measles-like infection).[17] Although the R_0 of influenza is appreciably smaller than that for measles (i.e., about 2 vs about 14) (see Table 77.2), influenza can go through 10 generations within a 30-day month whereas measles can go through only two generations, implying $2^{10} = 1024$ new cases of influenza at the end of the month compared with only $14^2 = 196$ for measles, if the populations were 100% susceptible at the start of each outbreak. (These are just illustrative numbers; of course, the increase in the number of immune persons would slow down the spread.) Despite this more rapid spread of influenza in a virgin population, consideration of the R_0 values shows that influenza should be more easily brought under control than measles: the herd immunity thresholds being H = 50% for influenza versus H = 93% for measles infection, assuming an R_0 for influenza of 2 and for measles of 14. This distinction has received much attention in recent discussions of the feasibility of controlling pandemic influenza.[18,20]

Implications of Population Heterogeneity

The elegant theory just summarized is built on extreme assumptions: that all individuals mix at random, that individuals are either fully susceptible or fully immune, and that the population is uniform (i.e., that all individuals behave the same way and all infected individuals are equally infectious). These assumptions are unlikely to hold for any infection in any human population. In recent years, there has been an effort to adapt the theory to more realistic assumptions, including heterogeneity of mixing patterns and of susceptibility and infectiousness.

The first attempt to consider the implications of population heterogeneity for herd immunity appeared in a 1971 paper by Fox and colleagues, entitled "Herd immunity: basic concept and relevance to public health immunization practices."[2] This paper, for many years the most frequently cited article on herd immunity, took a very different approach from that reviewed above and was based on simulation rather than on analytic argument. The authors set up a population of 1000 people, divided into family, school, and social groupings, and assigned different—but realistic—contact rates within and between these groups. Importantly, they did not consider age, nor did they consider an influx of susceptible persons into the population (the B_t term in Eq. 2). Their approach was thus applicable only to closed populations, and they were unable to address the larger issues of infections in dynamic populations.[1] Although the approach precluded estimation of a herd immunity threshold, it provided a first glimpse of the epidemiologic complexity inherent in heterogeneous populations, and it led the authors to be skeptical of simplistic notions of herd immunity.

Subsequent workers have used a variety of computer simulation and analytic approaches to explore more realistic assumptions than those underlying the simple mass action theory. In particular, there has been exploration of the important implications of age, recognizing the need to vaccinate as early in life as possible after maternal immunity has waned

and before exposure to wild-type infections.[12,20–22] These studies have allowed exploration of the effects of various vaccination strategies on the age distribution of infection—in particular, how low or moderate levels of vaccine coverage among infants or young children will change the dynamic equilibrium, slowing down transmission, and thereby leading to an increase in the average age at infection among those who fail to be immunized by the vaccine. These effects are particularly important for infections whose seriousness increases with age, such as rubella and, to a lesser extent, mumps, chickenpox, or polio.[1,12,23,24] For any vaccination program that targets one age group (often young children) but may impact others (e.g., the elderly) the pattern of contact between groups will be critical. To help predict these effects, a number of studies of contact patterns have been performed, the largest and most influential to date being the POLYMOD study, which documented contact patterns across eight European countries.[25] This study found remarkable consistency across Europe in the patterns of age-dependent mixing, with contacts being typically assortative (like with like), particularly in children and young adults.

There are many other forms of heterogeneity in human populations—in particular, geographic and social groupings—all of which have subtleties and are likely to confound one another, because different social groups tend to have different age and spatial patterns. Social groups may be separate or may overlap (e.g., neighborhood and occupational or school groupings) in different ways in different societies, and they may be associated with environments or behaviors that either encourage or discourage the transmission of infection. Consideration of such heterogeneities in simulation models has demonstrated that it is generally more efficient to target high-risk versus low-risk groups with vaccines, in the sense that the number of cases of disease prevented is greater per single vaccination in a high-risk than in a low-risk population.[26,27] However, a real vaccination program might be unwise to emphasize such a strategy, because of issues of social equity, and also because evidence shows that, in the absence of eradication, susceptible people continue to accumulate in the less-served areas and population mobility continues to seed virus into such populations.[28–30] Table 77.3 summarizes the implications of these several heterogeneities for the simple herd immunity threshold values as shown in Table 77.2.

On a more practical level, the nonuniformity of populations is used commonly either to target vaccines at high-risk groups or to facilitate widespread vaccine coverage. Thus the selective use of varicella vaccine in nurses or family members reduces the likelihood of varicella transmission to immunosuppressed patients. The targeting of hepatitis B vaccine in prisons provides protection to a population at high risk through intravenous drug use. Several European countries now recommend bacille Calmette-Guérin (BCG) vaccine only to high-risk communities. And school-based programs have been widely used in many countries to access large numbers of children.

Heterogeneity in Immune Status

Thus far, our discussion has implicitly assumed that immunity is one face of a simple binary property—that individuals either are susceptible or they are immune and protected from both infection and disease. For most infections, the real world is not like this at all. We know that immune responses are highly complex, involving humoral and cellular pathways, as well as a variety of cell types and chemical mediators, and that these vary among infections, among individuals, and over time. The epidemiologic implications of this heterogeneity are important.

The conventional epidemiologic measure of immunity induced by vaccination is known as *vaccine efficacy*, defined as the percent reduction in incidence of disease among vaccinated persons as compared with nonvaccinated, equally exposed controls. It is now recognized that this basic measure of vaccine-derived immunity is itself ambiguous, insofar as 80% efficacy could mean either that 80% of vaccinees are totally protected, by some all-or-none mechanism of protection, or that the vaccine-derived immunity is only "partial," such that the level of susceptibility in all vaccinated individuals has been reduced by 80%.[31] This distinction has profound implications both for measurement of vaccine effects (e.g., whether it is more appropriate to use risks or person-time rates in calculations) and for our understanding of immunity (e.g., whether certain immune responses are fully protective or only partially so, perhaps more effective against low-dose than against high-dose challenge, or more protective against closely related compared with more distantly related antigenic types of the pathogen). The combined epidemiologic and immunologic implications of the distinction between all-or-none and partial protection are not yet well understood.

It is known that immunity can wane with time, and thus boosters are recommended for many vaccines. The rate at which this waning occurs differs between infections and

TABLE 77.3 Implications of Various Assumptions for Theoretical Estimates of the Herd Immunity Threshold

Variable and Assumption	Implications for Herd Immunity Threshold *(H)*	References
Maternal immunity	If vaccines are not effective until maternal immunity wanes, crude *H* estimates are too low. This can be corrected by considering that a child is not born until maternal immunity disappears.	13
Variation in age at vaccination	Herd immunity effect is greatest (*H* threshold lowest) when vaccination occurs at the earliest possible age. Delayed vaccination implies that threshold coverage level will be higher than simple estimates.	13, 51
Age differences in contact rates or infection risk	Implications vary with relationship between age and contact rate. Falling contact rates with age implies that true *H* may be lower than simple global estimate.	21, 22
Seasonal changes in contact rates	Seasonality may imply lower herd immunity threshold if seasonal change is marked, and fadeout can occur during low transmission period.	21, 250
Geographic heterogeneity	In theory, geographic differences in contact rates may permit elimination with lower overall vaccine coverage than that implied by *H* based on total population, by targeting high-risk groups.	30
Social structure (nonrandom mixing)	Social structure can have complicated implications because it implies group differences in vaccination uptake and infection risk. Existence of vaccine-neglecting high-contact groups means true *H* will be higher than simple estimates. This effect can be very large.	2 (and others)

vaccines, being relatively slow for measles, for example, and higher for pertussis. The term *boosting* is interesting in this context, as there is increasing recognition that immunity to several infections (e.g., varicella, pertussis) is enhanced through repeated natural exposure throughout life.[32] In the absence of natural boosting reexposures, the risk of reinfection or reactivation disease may increase with time, many years after the first encounter.

Beyond these quantitative differences in immunity, there are also important qualitative differences, dependent on whether, and to what level, any protection acts against infection, against reinfection, against severe or mild disease, or against infectiousness (transmissibility). For some infections (e.g., measles), vaccine-induced immunity may protect similarly against infection and against disease, whereas for others (e.g., polio, pertussis, diphtheria, rotavirus), the vaccine-induced protection appears stronger against disease. It also may reduce shedding of the agent by infected persons (e.g., polio, pertussis). We understand the biologic mechanisms for some of these differences—for example, live oral poliovirus vaccines induce local enteric immunity and hence are more effective against poliovirus infection than are killed, parenterally administered poliovirus vaccines. It is in theory possible to measure these different forms of protection epidemiologically, by collecting data on both infection and disease status of people, and by estimating vaccine efficacy conditional on the vaccination status of both the source and the recipient of potential transmission. Such data are sometimes available,[33] in particular in the context of household secondary attack rate studies, but their rarity means that most of our epidemiologic measures of immunity overlook these distinctions. This oversight does not mean that the distinctions are unimportant.

Implications of Heterogeneity in Immune Status for Vaccine Effects

Vaccines reduce disease by direct protection of vaccinees and by indirect protection of nonimmune persons. Indirect protection depends on a reduction in infection transmission, and hence on protection (immunity) against infection, not just against disease. If a vaccine were to protect only against disease, and not at all against infection, then it would have no influence on infection transmission in the community and there would be no indirect protection (vaccination of one person would have no influence on any others in the community). It would be possible to reduce disease with such a vaccine but not to eradicate the infection. This is an extreme scenario (although it has been discussed in the context of tetanus, diphtheria, pertussis, and inactivated poliovirus vaccines), but we can explore more plausible circumstances.

We first assume that vaccine-induced immunity is a simple all-or-none affair, such that a proportion (P) of vaccinees are fully protected against an infectious agent. If vaccine coverage is C, then a proportion $P \times C$ of the total population will be rendered fully immune. Under these circumstances, from Eq. 6, the coverage required to reach the herd immunity threshold (C_H), and thereby bring about a sustained reduction in incidence, will be as follows:

$$C_H = (R_0 - 1)/(R_0 \times P) \qquad \text{[Eq. 8]}$$

If, in contrast, a vaccine can protect to some degree against infection (a proportion P_i of vaccinees are rendered no longer susceptible to infection) and can also reduce transmissibility, that is, infectiousness (a proportion P_t among those vaccinated persons who do become infected do not shed, or shed fewer, organisms and hence cannot transmit infection), then, as shown by Longini and colleagues,[33] the coverage required to reach the herd immunity threshold (i.e., to reduce incidence) should be as follows:

$$C_H = (R_0 - 1)/(R_0[P_i + P_t - P_i \times P_t]) \qquad \text{[Eq. 9]}$$

This expression is a way of illustrating the importance of vaccine-mediated reductions in infectiousness, as well as in reducing susceptibility to infection. If protection induced by the vaccine against infection (P_i) and transmissibility (P_t) are both 100%, this equation reduces to Eq. 6, and the critical herd immunity coverage threshold is just $(R_0 - 1)/(R_0)$. However, if, for example, the vaccine induced only 60% protection against infection, and 40% protection against transmissibility among those who were infected, the critical coverage threshold to reduce infection incidence should be $(R_0 - 1)/(R_0 \times [0.6 + 0.4 - 0.24]) = (R_0 - 1)/(R_0 \times [0.76])$, thus about 4/3 times, or 33% higher, than the simple $(R_0 - 1)/(R_0)$ estimate. One should not expect precision in such estimates, but this result makes intuitive sense. The overall effect of a vaccine in reducing *disease* incidence in a community will combine these two mechanisms: protection against infection transmission, and direct protection of vaccinees against disease. These properties are important for understanding the effects of several vaccines in common use (e.g., polio, pertussis, diphtheria) and are likely to be even more important for several vaccines currently under development (e.g., malaria, hookworm, HIV).

THE REAL WORLD

We turn now to compare the observed impact of community vaccination programs with what was and is predicted by theory, and to highlight a variety of particular issues raised by individual vaccine-preventable diseases. Readers are referred elsewhere in this text for further details on the infections and vaccines used here as examples.

Smallpox

The initial WHO encouragement toward global eradication of smallpox came in a resolution passed by the 12th World Health Assembly in 1959, which stated that "eradication of smallpox from an endemic area can be accomplished by successfully vaccinating or revaccinating 80 percent of the population within a period of four to five years, as has been demonstrated in several countries."[34] The wording is of interest because of its explicit stipulation of a herd immunity threshold, and also because of its implication that waning vaccine-derived immunity might pose an obstacle to achieving the threshold (thus the call for revaccination).

The disappearance of smallpox from many populations, despite the continued presence of large numbers of unvaccinated susceptible persons, was evident from the historical record (as was noted by Farr more than 150 years ago).[7] This is consistent with relatively low estimates of basic reproduction numbers and hence relatively low herd immunity thresholds for the interruption of smallpox transmission (see Table 77.2).[35] The 1959 WHO recommendation implied an R_0 of 5. Although this is consistent with more recent estimates,[36] the 80% target was based originally on experience alone, having been made before the development of the theory described earlier. The validation of such estimates, however derived, remains difficult. The actual value must have varied among populations and been higher in crowded urban than in rural settings. Beyond that, the severity of smallpox, in particular of variola major, was such that outbreaks typically encouraged active intervention (different forms of quarantine, contact tracing, and ring vaccination), so it is not always clear to what extent the course of any particular epidemic was determined

by the general levels of immunity or by targeted emergency measures.

Arita and colleagues assembled data on crude population densities and smallpox vaccination coverage in African and Asian countries during the late 1960s and early 1970s.[37] Despite problems of nonuniform distributions of populations and of vaccinations, let alone the vagaries of the vaccination statistics themselves, these data indicate that smallpox disappeared early from countries in which the crude density of susceptible persons (unvaccinated individuals) fell below 10 persons/km^2 (corresponding to 80% immunity in populations with crude population density less than 50 persons/km^2). The infection persisted in more densely populated regions, however, especially in Nigeria (54 persons/km^2), Pakistan (83 persons/km^2), India (175 persons/km^2), and Bangladesh (502 persons/km^2). Whether continued reliance on routine populationwide vaccination programs might ultimately have been sufficient to eliminate smallpox from the more densely populated nations of Africa and Asia is now a moot point. If the threshold of 10 susceptible persons per square kilometer is a guide, then 98% vaccination coverage would have been necessary for Bangladesh—and such coverages were impracticable. However, it was recognized by 1970 that variola virus could be eliminated from populations more efficiently by a policy of active case detection, contact tracing, and the breaking of individual chains of transmission by quarantine and ring vaccination, than by relying on overall coverage from routine or mass vaccination programs.[38] In effect, the focus of prevention activity shifted from the population back to the individual, and the heterogeneity of the population was itself exploited to achieve eradication of the disease. The success of this policy is now a matter of record.[35]

Among the major lessons from the smallpox program was the inadequacy of relying on reported vaccine uptake statistics and herd immunity predictions in planning and managing an eradication program. Many experiences illustrated that reported data could be extremely unreliable, and high coverage statistics often obscured the fact that important segments of a population were inadequately vaccinated and could serve to maintain and transport the infection for long periods and distances.[35,38]

The disappearance of smallpox from many populations before the intensive campaigns of the final eradication program is consistent with the idea that indirect protection of unvaccinated susceptible persons contributed importantly to the overall decline of this disease. Beyond that, the persistence of the disease in densely populated third-world countries, despite apparent vaccination coverages far in excess of the WHO's recommended 80% herd immunity threshold, reflects the importance of population heterogeneities in vaccine coverage and in transmission risk. The smallpox experience thus demonstrates both the validity and the limitations of herd immunity in practice. It should also be appreciated that several features of the natural history of smallpox favored the shift in strategy toward targeted vaccination—in particular, the high case-to-infection ratio and characteristic pathology (which facilitated detection of cases), the long incubation period (7 to 19 days), and the relatively low transmissibility (which coincided with clinical lesions and hence facilitated control by identification of contacts and ring vaccination). Without these features, much greater emphasis would have had to be placed on raising the communitywide prevalence of immunity to achieve eradication of this disease.

By an unfortunate irony, questions of community immunity to smallpox remain topical, decades after its eradication. Routine smallpox vaccination was discontinued everywhere by 1980, and more than 80% of the world's population is probably now susceptible to the infection (although vaccinia-induced protection wanes with time, and boosters were recommended every 5 years, there is evidence that 90% of individuals vaccinated 25 to 75 years ago still retain some humoral or cellular immune responsiveness[39,40]). Although smallpox (variola) virus was supposed to have been preserved in only two high-security laboratories (in Atlanta, Georgia, and in Novosibirsk, Russia), the fate of virus produced in biological warfare laboratories (especially in Russia) is uncertain, and it is now possible to recreate the virus de novo.[41,42] Given the low level of immunity in contemporary populations, the release or escape of such virus could lead to rapid and widespread dispersal, with devastating consequences. Concern over such an event has led to much discussion, to the enactment of mock outbreaks to test control approaches, to the decision on the part of several governments to replenish national supplies of smallpox vaccine, and to contingency plans for emergency vaccine deployment.[43–45] These discussions raise many important issues concerning community protection, such as whether healthcare workers or workers in other essential services should be vaccinated routinely or voluntarily; to what extent the vaccine can be diluted without seriously compromising its efficacy; and the extent to which resources should be deployed in any emergency for mass vaccination as opposed to targeted case finding, contact tracing, and ring vaccination.[46–49]

Measles

No disease has been studied more intensely with reference to community immunity than has measles.[1,2,12,13,21,50–55] There are two reasons for this. One is that measles has long been a favorite subject for theoretical modeling, because of its frequency, its easy recognition in epidemic form, its predictable behavior, and the high quality of available data. The second is that there has been serious discussion ever since 1967 of the possibility of eliminating measles both nationally and internationally.[56–62] Many of these discussions have touched on estimates and interpretations of herd immunity thresholds.

Published estimates of herd immunity thresholds required to eradicate measles have ranged from 55% to 96%, depending on the modeling approach and the assumptions employed (e.g., whether age or seasonality of transmission was included; the 55% estimate was published before development of the theory described previously).[56] The logic and the flaws underlying various estimates have been discussed elsewhere.[1,13,21,63] Measles is among the most highly transmissible of all directly transmitted infections (see Table 77.2), and thus its crude herd immunity threshold is likely to be very high, at least in urban populations—on the order of 95%. Given that the efficacy of measles vaccine even under good conditions is approximately 95%, that vaccination coverage can never be 100%, and that poor coverage tends to cluster in particular communities, this means that a single-dose measles vaccination policy is unlikely to be sufficient to eliminate measles from any large population. Recognition of this fact has led to recommendations for two-dose measles vaccination policies in many countries, with the first dose administered at approximately 1 year of age (after maternal immunity has declined), and the second usually at school age, in an effort to immunize those children who escaped vaccination as infants or in whom the initial vaccination failed to induce protective immunity (primary vaccine failures).[64] In addition, many countries in recent years have employed campaigns to deliver measles vaccines to all children up to the age of 5 years, or 12 to 15 years, in an effort to protect those who had escaped natural infection or successful vaccination.[65] These efforts have been pursued longest in Latin America, and more recently in Africa, where catch-up (initial mass campaign up to age 15 years), keep-up (enhanced

routine vaccination at 12 to 15 months), and follow-up (repeated campaigns among those younger than age 5 years) vaccination policies have succeeded in reducing measles incidence to very low levels.[66]

The reduction or absence of endemic measles in highly vaccinated communities allows the gradual accumulation of susceptible individuals (those who escaped vaccination or in whom the vaccine failed). This may lead to delayed epidemics after several years of little or no measles, known as "post–honeymoon-period epidemics," which typically involve older age groups than were traditionally involved in measles. Dramatic examples of such delayed epidemics have been observed in several countries, including the United States in 1990 (see Fig. 77.4 C) and Brazil in 1997.[67] Routine serologic surveillance in the United Kingdom revealed a gradual accumulation of susceptible persons in the early 1990s, and led to the prediction of such a delayed epidemic. A nationwide campaign was implemented in 1994 to vaccinate all children 5 to 16 years of age to remove this threat.[68] Continued measles virus circulation in several European countries reflects low vaccination coverage in particular populations and associated accumulation of susceptible persons.

Among the remarkable observations about measles is that immunity induced by natural infection appears to remain strong for life: thus, Panum observed that individuals exposed to measles in 1781 in the Faroe Islands were still immune when the virus was next introduced, 65 years later, in 1846.[69] However, the immune response to measles vaccine is weaker than that to natural infection, and antibody levels decline among vaccinated people no longer exposed to frequent boosting reexposure.[70] Among the implications of this change is a decline in average levels of maternal antibody in infants, which means that infants born to mothers who never experienced wild measles infection become susceptible to infection—and can be vaccinated successfully—at an earlier age than was the case in the past.[71,72]

The American Region of the WHO set a target of regional measles elimination in 1994 and succeeded in arresting continued transmission in November 2002, since which time all measles has been attributable to importations (given that measles virus has now circulated for more than a year in Brazil, the Americas are now again considered endemic for measles). This has confirmed the theoretical feasibility of measles eradication through a combination of high routine two-dose coverage supplemented in some countries by campaigns, along with intensified surveillance and aggressive outbreak response. All WHO regions, have now set measles elimination targets, and there is discussion of setting a global measles eradication target.[62] This raises a variety of population immunity problems, including measures to combat increasing antivaccine sentiment (a particular issue in Europe),[73] and policies relating to vaccination in a posteradication era. Some of the arguments relating to smallpox will be raised again, with the complications that measles is far more transmissible than smallpox and that it would spread more rapidly in a population without very high levels of immunity.

Rubella

Although the basic transmission dynamics of rubella are similar to those of measles, it raises very different questions relating to community immunity. Public health concern with rubella is concentrated on congenital rubella syndrome (CRS) and thus on infections occurring in women in their reproductive years.[24] Control can in theory be brought about either by reducing the proportion of women who are susceptible or by reducing their risk of infection. Vaccination of adolescent girls, as practiced in the United Kingdom between 1971 and 1988, emphasized the reduction of susceptible women by ensuring that a maximum percentage of women and girls would acquire either natural or vaccine-derived immunity prior to their reproductive years. In contrast, vaccination of boys and girls in their second year of life, as practiced in the United States since 1969, in the United Kingdom since 1988, and in many other countries, also leads to reduction of circulating rubella virus and hence to the reduction of risk of infection for any remaining susceptible persons in the population. In theory, the latter policy carries a risk if coverage is not high, because low vaccination coverage of young children of both sexes can reduce the transmission of rubella virus to such a degree that the proportion of reproductive-age women still susceptible to the virus, and the numbers of consequent cases of CRS, actually increase. Indeed, even if coverage is high, but no catch-up campaign is performed, the cohorts of children who would ordinarily have been infected during childhood may pass into young adulthood before being infected.

Several investigations have concluded that, for the incidence of CRS to decrease in the long term, the minimal vaccination coverage that must be achieved and maintained in young children of both sexes is in the region of 50% to 80%.[24,50,74,75] The higher the initial intensity of transmission in the population, the higher is the threshold of vaccination coverage required among young children to avoid increasing the incidence of CRS. Given that vaccination uptake rates in the early 1970s in the United States and the United Kingdom were on the order of 90% and 50%, respectively, each nation's strategy was probably appropriate under the circumstances of the time. In contrast, it appears that the situation in Greece was unfortunate, and that low levels of childhood vaccination during the 1980s may have actually increased the incidence of congenital rubella.[76] Because of the possibility that low childhood vaccination coverage could result in an increase in CRS, several countries have implemented supplementary programs to reduce adult susceptibility (e.g., postpartum vaccination of identified susceptible women, and routine testing of women at the time of marriage) at the same time that they began childhood rubella vaccination programs. Clearly, there needs to be caution about the use of combined measles-rubella vaccines in countries that are unable to achieve high and sustained coverage, and it needs to be recognized that immunity levels may be affected by selective use of rubella vaccines in the private sector in some populations.

Demographic factors can also influence rubella and CRS epidemiology. Small island states pose a particular problem. If the population is of insufficient size to maintain endemic circulation of rubella virus, susceptible persons may accumulate in older age groups between periodic introductions, leading to high rates of CRS, when rubella virus is introduced.[77] A different demographic effect may be underway in China, where the one-child policy is likely to have reduced rubella spread, leading to an increase in the average age at infection and increased CRS risk.[78] Rubella vaccination has now been introduced in China.[79]

Rubella is less transmissible than measles, and thus a lower prevalence of immunity should be required for its elimination (see Table 77.2). Indeed, rubella may disappear as a consequence of measles elimination activities in those countries that use combined measles-rubella vaccines, with little additional effort. Thus a target was set in 2003 to eliminate rubella virus transmission in the Americas by 2010, as a concomitant of their measles elimination initiative, and this appears to have been achieved in February 2009.[80] Rubella elimination has been discussed in other global regions, in particular in Europe, but rubella-containing vaccines are still not widely used in Africa or Asia. There will be much discussion and concern over rubella immunity levels in years to come in

association with debates over global measles eradication and whether to include rubella in any such initiative, and the related issue of maintaining high immunity levels in populations from which it has been eliminated.

Mumps

Mumps is similar to measles (both are paramyxoviruses maintained by respiratory spread), but it is less transmissible in household settings and appears to have a lower crude R_0 and hence a lower crude herd immunity threshold, than either measles or rubella (see Table 77.2). On the other hand, there is evidence that vaccine-induced immunity to mumps may decline more rapidly than immunity to measles or rubella (see Chapter 39), which must be considered when inferring actual levels of immunity in populations. The disease resembles rubella in being more important in adults (in men because of orchitis) than in children, and hence there is the theoretical potential for low vaccine coverage to increase morbidity on a population scale by delaying infection to older ages. Although mumps vaccine is widely used in wealthier countries (combined with measles and rubella), it is not recommended in the basic Expanded Programme on Immunization schedule and thus mumps has not been influenced by vaccine in much of the world. Mumps notifications fell by more than 95% after introduction of the vaccine in the United States (it was licensed in 1967 and recommended universally for children in 1977), which is appreciably greater than the product of coverage and efficacy[64,81,82] and hence a clear indication of indirect protection.

The United Kingdom experienced a major post-honeymoon epidemic of mumps in 2004 to 2006 (more than 40,000 cases reported in 2005), concentrated among young adults,[83] reflecting the accumulation of susceptible persons in cohorts born too early to have received mumps vaccine (first dose introduced in 1988, and second dose [at school entry] introduced in 1996) and too late to have been exposed as children to extensive circulation of wild virus. A similar epidemic occurred in the United States in 1987 to 1994, 10 to 15 years after mumps vaccine was introduced nationally in 1977, and another in 2006. Each of these events illustrates the dynamic effect of vaccination leading to reduced incidence risk, to accumulation of susceptible persons, and to delayed epidemics in older age groups.

Because mumps vaccine is often given in combination with measles and rubella, there may be discussion of the possible implications of inclusion of mumps vaccines in association with any global effort to eradicate measles. This has received little attention to date.

Chickenpox (Herpes Varicella-Zoster Virus)

Herpes varicella-zoster virus is common in all human populations. Primary infection usually occurs in childhood and is manifested clinically as chickenpox. In common with many herpesviruses, varicella-zoster virus establishes a persistent "latent" infection, typically in the dorsal root ganglia, from which site it may recrudesce many years later to cause herpes zoster (shingles). Chickenpox is more severe in infants and adults than in children, and was responsible for some 11,000 hospitalizations and 100 deaths in the United States annually before the introduction of vaccination.[84] Because of this, varicella vaccine (a live attenuated virus) was introduced into the routine childhood immunization schedule in the United States in 1995, and its introduction is under consideration by several other countries. Surveillance data show a major decline in chickenpox incidence in the United States as a consequence of this program, including evidence for indirect protection among infants and adults, outside the vaccinated target age groups.[84,85]

Unlike immunity to variola, measles, rubella, and mumps viruses, immunity to varicella-zoster is not sterile following vaccination or natural infection, but it serves to suppress or to contain the persistent latent infection. Although it may provide some degree of protection against reinfection (or, better, superinfection), there is evidence that this protection wanes with time and that superinfections can occur.[86] This waning of naturally acquired protection is associated with increased risk of clinical episodes of herpes zoster. There is conflicting evidence over the extent to which exposure to children with varicella boosts immunity and reduces the risk of zoster in adults (see Chapter 61).[87,88] This has important implications.

Several authors have warned that reduction in the circulation of varicella virus, as a consequence of widespread vaccination of children, could lead to an increase in the incidence of herpes zoster.[89–91] This implies a perverse result—vaccination of one segment of a population (children) leading to reduced transmission and hence to reduced boosting of immunity, and ultimately to increased incidence of disease in another segment of society (adults). Although evidence for such an increase in zoster in the United States is inconsistent,[92,93] interest has turned to the possible need to vaccinate adults to artificially boost their immunity and prevent zoster in the absence of natural boosting. A trial has confirmed the efficacy of a zoster vaccine in adults older than 60 years of age (60% reduction in burden of illness).[94] This vaccine is now recommended for universal use starting at age 60 years in the United States[95] and for those 70 to 79 years of age in the United Kingdom.[96] A large-scale trial of a second-generation adjuvanted subunit herpes zoster vaccine demonstrated very high levels of protection, even in older age groups.[97]

The ecology of varicella-zoster virus and its related immunity (different levels of protection against infection and transmissibility in the presence or absence of boosting exposures) is complicated. Although the age distribution of chickenpox would suggest an R_0 of 8 to 10 in developed countries, this number should not be interpreted as implying an 85% to 90% herd immunity threshold for eradication, given that immunity is not sterile and zoster cases are infectious. It is now recognized that the immunity associated with varicella vaccine is not high, and that it wanes with time,[98] leading to a recommendation for two-dose schedules. Beyond this, the long-term risk of zoster associated with vaccine strains is still not clear. The situation will need close monitoring in the coming years to ensure an overall public health benefit from this intervention.[90] As a consequence of this uncertainty, several countries (e.g., the United Kingdom) still prefer to use varicella-zoster vaccine selectively—for example, in high-risk patients and healthcare workers, as well as the elderly[99]—although these policies may change if the U.S. policy proves successful and cost-effective in the long term.

Pertussis

Responsible for considerable morbidity and mortality in unvaccinated populations in the past, pertussis has been a target for routine vaccination programs in all countries for several decades. These programs have been successful in reducing greatly the burden of disease caused by pertussis, and there is good evidence of indirect protection, in terms of reduced disease risks among unvaccinated as well as vaccinated individuals.[100,101] The vaccines, schedules, and patterns of immunity have changed importantly in recent years.

The cyclical pattern of pertussis epidemics provides a classic example of mass action dynamics (compare Figs. 77.3, 77.4B,

and 77.4D).[102,103] Published estimates of the crude basic reproduction number for *Bordetella pertussis* range from 5 to 17 for developed countries in recent decades (see Table 77.2).[104] The higher values are similar to measles and imply a crude herd immunity threshold in excess of 90%, which is higher than most estimates of the protective efficacy of a complete course of any pertussis vaccine,[105,106] let alone that there is evidence of waning vaccine-derived protection[105,107] and of reinfections in adults.[108] Eradication of this infection is thus not possible by childhood vaccination alone.[109]

Immunity to pertussis is difficult to define, either in individuals or in populations. There is no good serologic correlate for protective immunity; and there are controversies over the effectiveness of available pertussis vaccines and the extent to which they protect better against disease than against infection.[105,106,110–112] Many industrialized countries have introduced acellular vaccines because of safety concerns with the older whole-cell vaccines, and several of these countries (e.g., United States, United Kingdom, Australia) subsequently experienced major resurgences of pertussis, apparently linked to more rapid waning of protection postacellular than post–whole-cell vacines.[102,105,108,113] Increased awareness of pertussis infections and reinfections in older individuals, and the recognition that protection induced by acellular vaccines is weaker and shorter lived than protection by whole-cell vaccines has led some countries to recommend routine (acellular) pertussis boosters for adolescents[114] and pregnant women.[115] Routine (acellular) pertussis boosters for pregnant women provides passive protection to young infants, in whom the disease is most severe.[115–120] This has been extended to selective vaccination of parents and others in contact with young infants, a strategy known as *cocooning*, yet another approach to manipulating the community distribution of immunity.[115,121,122]

Pertussis is thus another example of an infection for which the immunity profile in many populations has changed greatly in recent decades, from a predominance of immunity attributable only to natural infection and boosted by frequent reexposure (now restricted to older persons), to immunity attributable largely to killed whole-cell vaccines, which waned as natural reexposure became less frequent (in a high proportion of individuals born since 1950); and now, in wealthy countries, to immunity attributable largely to various acellular vaccines (in younger individuals). Each of these forms of immunity is likely to have different implications for protection against infection, disease, and infectiousness. The long-term implications of these changes are as yet unclear.

Diphtheria

Diphtheria was a major cause of morbidity and mortality in Europe and North America during the 19th century. Incidence fell from the early years of the 20th century, and the decline accelerated with introduction of widespread toxoid vaccination in the United States, the United Kingdom, and other countries during the 1940s. Because vaccination of less than 90% of children was associated with a more than 99.99% fall in disease, it might be argued that a herd immunity threshold was achieved. But a closer look reveals a complicated story of community protection.

Published estimates of a crude herd immunity threshold for diphtheria have ranged from 50% to 90%.[1] Estimates aside, the proportion of the population actually immune to diphtheria today is elusive. Vaccine coverage is difficult to define because it has varied over time, and because at least three doses are recommended, although one or two provide some protection. The protection against disease imparted by diphtheria toxoid vaccines has never been evaluated in formal trials, although observational studies provide estimates ranging from 55% to 90%.[123–125] Serologic studies show that vaccine-induced antitoxin titers decline with time, and that titers in some populations may be lower among persons born in recent decades, perhaps because they have not been boosted by exposure to natural infections.[126] Surveys done in industrialized countries have shown a wide range in prevalence of protective antitoxin levels among adults, from 29% to 80%, leading to recommendations that adults should receive booster doses of diphtheria vaccine.[126–129]

An important question concerning community protection against diphtheria relates to the nature of the immunity induced by toxoid vaccines, and how it may differ from infection-attributable immunity. In the sense that herd immunity implies indirect protection, it requires immunity against infection or against transmission. Given that diphtheria toxin is not a constituent of *Corynebacterium diphtheriae* per se, but exists as a consequence of bacteriophage infection, the immunity induced by toxoid vaccination may not provide protection against infection at all.[130] However, toxoid vaccines do protect against toxin-mediated disease, and transmission of the diphtheria bacillus is more efficient from clinical patients than from subclinical carriers[131]—thus the toxoid vaccines may protect against infectiousness and infection transmission, but not (or more than) against infection receipt ($P_t \gg P_i$, according to the logic of Eq. 9). This may have been an important contributor to the disappearance of diphtheria in vaccinated populations.

Skin and respiratory tract infections with nontoxigenic corynebacteria are common in many populations, in particular in the tropics, and these probably impart some cross-protection against infection with toxigenic *C. diphtheriae*.[132] Because this protection is independent of antitoxin, the emphasis on antitoxin antibodies in surveys of immunity to diphtheria may miss an important element in a community's protection against this disease.

Haemophilus influenzae Type B

Haemophilus influenzae type b (Hib) is a major cause of invasive bacterial disease in young children, with disease occurring at a median age of 18 months in northern Europe and Australia, before vaccine introduction, but as low as 6 months in developing countries with a high incidence of disease. Neonatal disease is extremely rare, but by 2 months children start to become susceptible. Although disease can occur at any age, in most of the world 85% to 90% of disease is in children younger than age 2 years. The absence of disease in the neonatal period is a result of maternal antibody, passed to the infant either transplacentally or via breast milk. As infant antibody levels fall, susceptibility rises, only to fall after 2 to 3 years as naturally acquired antibody levels rise associated with natural exposure. The source of these exposures is not entirely clear. Nasal carriage of Hib occurs in 4% to 12% of young children in unvaccinated communities and is the major source for transmission. Carriage also appears to provide immunity against disease. Interestingly, antibodies to *Escherichia coli* can be cross-reactive against Hib infection, although it is unknown whether these have any role in immunity to human infections.[133] What is clear is that immunity to Hib is conferred by antibodies to the polysaccharide capsule, which are specific for serotype b.

Polysaccharide-protein conjugate vaccines are immunogenic in early infancy, providing high protection (>90%) against invasive disease and some protection against carriage. The latter effect has been sufficient to reduce transmission and to provide appreciable indirect protection to nonvaccinated groups. This has been described in children too young to be vaccinated in Israel[134] and The Gambia,[135] and in those too

old to be vaccinated in Scandinavia.[136] This was responsible for substantial indirect effects demonstrated in the United States when the conjugate vaccine was introduced for children at 15 months of age, and in the United Kingdom where the vaccine was introduced in 1992 in infancy. In both settings, Hib disease fell to extremely low levels in all age groups. The United Kingdom was the only industrialized country to introduce Hib vaccine without a booster dose, having started with a catch-up campaign covering children younger than 5 years of age. After initially disappearing, Hib incidence increased in all age groups after 7 years. This indicates that, in the absence of the boosting effect of carriage, or a booster dose of vaccine, community immunity wanes.[137] Interestingly, while the United Kingdom was the only industrialized country to introduce Hib vaccine without a booster dose, the WHO has recommended that the vaccine be given without a booster dose. The Gambia, which was the first African country to introduce Hib vaccine in 1997 (in a three-dose schedule without a booster) recently reported a transient increase in cases, suggesting that, for this vaccine, a later booster might be required in similar settings.[138]

Streptococcus pneumoniae (Pneumococcus)

The pneumococcus is one of the most important pathogens of humans, causing more deaths in children than any other organism and probably a similar number of deaths in adults. Pneumococcal vaccines have been available for many years, but the poor immunogenicity of polysaccharide vaccines in infants, the importance and prevalence of noninvasive carriage in maintaining these organisms in populations, and the multiplicity of circulating serotypes raise special problems of community immunity.

There are nearly 100 immunologically distinct pneumococcal serotypes that cause disease in humans, but most are rare. Up to 23 serotypes have been included in polysaccharide vaccines, covering most disease-causing strains in adults, and the 13 serotypes incorporated in the largest conjugate vaccine currently available include most strains causing carriage or disease in young children. Pathogenicity varies with serotype, and different serotypes are more or less likely to affect different age groups, although all can cause disease in all age groups. To further complicate issues, the duration of carriage and the ability of a carriage episode to immunize an individual is also variable and dependent on age and serotype.[139]

The introduction of a seven-valent pneumococcal conjugate vaccine in the United States in 2000 had a dramatic impact. Although the substantial fall in infant invasive pneumococcal disease rates was as expected, it was associated with significant falls in pneumococcal disease among adults, more than doubling the overall benefits to the community.[140] The greater-than-expected protection accorded to U.S. adults by infant pneumococcal vaccination revealed the extent to which this infection was naturally transmitted from children to adults in this population. By 2007, rates of invasive pneumococcal disease caused by serotypes in the vaccine had fallen by 94% in all age groups.[141] A metaanalysis of 19 surveillance systems in industrialized countries confirmed the generalizability of this effect, with a mean reduction of vaccine-serotype invasive pneumococcal disease among adults of 75% to 83% 5 years after introduction of seven-valent vaccine. The benefit is enhanced by the fact that the vaccine includes, and has greatly reduced the appearance of, the five serotypes most associated with antibiotic resistance.[140,142]

Despite these powerful effects, wherever carriage has been studied, vaccination has not had a lasting effect on pneumococcal carriage prevalence overall, but has led to a reduction in the carriage of vaccine serotypes, with a compensatory rise in carriage of nonvaccine serotypes.[142,143] To a variable extent, there has also been an increase in disease associated with the replacement nonvaccine serotypes, and this has reduced the initial impact of the disease reduction by the vaccine in both children and adults.[142,144,145] The herd protection observed in adults in the general population indicates that either the replacement nonvaccine serotypes are less likely to cause disease in adults, or there are qualitative differences in carriage in vaccinated children, rendering them less likely to transmit the organism. Whatever the explanation, the net balance is beneficial in the majority of settings, despite variation in the magnitude of serotype replacement disease.[146] In the United Kingdom, after only 3 years of use of the seven-valent vaccine, serotype replacement forced authorities to move to a 13-valency preparation. The indirect effect of the 13-valent vaccine against disease in adults caused by the residual six serotypes has subsequently mirrored that of the seven-valent vaccine against the original seven serotypes.[147] The indirect effects of vaccine are so powerful that it is arguable they are sufficient for population control of disease, even among children. This, in turn, raises the question whether community immunity could be sustained, in a mature vaccine program, by a childhood schedule with fewer doses of vaccine, optimizing effectiveness against carriage.[148]

How all these effects will play out in low-income countries is unclear, but introduction of a 10-valent vaccine incorporating a catch-up campaign for children younger than 5 years of age in Kenya, was followed within 6 months by a sustained two-thirds reduction in carriage prevalence of vaccine serotypes among children younger than 5 years of age and among older children and adults.[149] Given the variety of epidemiological settings, patterns of circulating serotypes and comorbidities, especially HIV, it is difficult to predict whether indirect effects will enhance the direct effect of the vaccine (by generating community protection) or reduce it (as a consequence of serotype replacement).

Meningococcal Disease *(Neisseria meningitidis)*

Meningococcal disease is important everywhere, with different epidemiological patterns associated with different serotypes in different parts of the world. The introduction of conjugate meningococcal vaccines in recent years has provided some of the most spectacular evidence of the importance of community protection.

In the late 1990s, several high-income countries observed an increase in meningococcus type C (Men C) disease, with highest incidence in infants and teenagers. The United Kingdom's strategy (implemented in 1999–2000) was to introduce the newly licensed Men C conjugate vaccine into the infant schedule at 2, 3, and 4 months of age, with a single-dose catch-up campaign for those 1 to 18 years of age. This rapidly reduced the incidence of Men C disease across all age groups,[150,151] as well as nasopharyngeal carriage (which was only measured in teenagers[152]). Continuing surveillance demonstrated the long-term impact of the program.[150] This was all the more remarkable, given that the three-dose infant program seemed to offer only short-term protection (up to approximately 1 year).[151] The sustained reduction in incidence in the routinely vaccinated cohorts appeared to derive from the longer-term protection afforded by the one-dose campaign in the older cohorts, which presumably provided continued reduction in carriage.[153] In an attempt to maintain this community protection, the United Kingdom subsequently made two changes to its schedule: in each case, doses in infancy were dropped and replaced by doses later in life, initially in the second year of life but now also in teenagers. When The Netherlands introduced the Men C conjugate vaccine in 2002, it

also introduced a campaign for 1- to 18-year-olds, but did not vaccinate infants. Instead, the routine aspect of the program, which was put in place at the same time, consisted of a single dose at 14 months of age. High-quality surveillance over the first decade of the program confirmed the effectiveness of this strategy; with a 99% reduction in incidence in age groups eligible for vaccination and a 93% reduction in those that were not.[154]

Meningococcus serogroup A (Men A) has been the major cause of repeated, devastating meningococcal epidemics in the Sahel region of Africa (sometimes referred to as the "meningitis belt"), despite the widespread use of polysaccharide vaccines.[6] The situation has been altered dramatically by development and introduction of a conjugate Men A vaccine for Africa (MenAfriVac).[155] This vaccine has been rapidly rolled out across the meningitis belt in very large-scale campaigns that have led to the near elimination of Men A disease[156,157] and carriage.[158] The reduction in carriage has provided important indirect (or community) protection. For instance, in Burkina Faso, which was the first country to initiate a nationwide program, individuals 1 to 29 years of age were targeted for vaccination in 2010. In 2011 there was a 62% decline in the incidence of meningococcal meningitis (of any serogroup) in the targeted age groups, and a 55% reduction in those too old or young to be vaccinated.[156] In fact, in the year after the campaign only one case of Men A disease was identified from Burkina Faso—a 99.8% decline from the previous year.[156]

Tetanus

Clostridium tetani is not communicable between human hosts, and thus vaccination cannot lead to indirect protection in the sense implied in many definitions of herd immunity. Certainly, there is no threshold proportion of immune persons, below 100%, that can ensure total absence of tetanus from a community.

There is little doubt that the introduction of routine tetanus toxoid vaccination in the 1940s had an impact on trends and patterns of the disease. However, because the incidence of tetanus was declining prior to widespread vaccination, as a result of decreasing exposure (fewer people in contact with soil and animal feces, which are the main reservoirs of the tetanus bacillus), and because of the widespread use of tetanus toxoid in wound management, it is difficult to assess the extent to which routine prophylactic vaccination contributed to the decline in tetanus morbidity.

Despite the noncommunicability of *C. tetani* between people, there is a special sense in which vaccination does impart indirect protection. Antitetanus immunity of mothers is transmitted across the placenta, and two doses of toxoid during pregnancy can protect a woman's offspring against neonatal disease.[159] This is extremely important in that a major contributor to the public health importance of tetanus on a global scale is neonatal disease. In 1989, the World Health Assembly declared an initiative to "eliminate" neonatal tetanus (defined as less than one case of neonatal tetanus for every 1000 live births in each administrative district throughout the world). Although the initiative includes efforts to improve birth practices, it relies largely on provision of tetanus toxoid to girls and women in antenatal clinics. In this sense, the critical population for community immunity to tetanus consists of girls and women in their reproductive years. In developing countries with strong vaccination programs, the necessity for full tetanus toxoid vaccination during pregnancy should decline as more and more young women have received the vaccine in infancy. In this instance, the monitoring of community immunity may enable the winding back of an adult vaccination program in years to come.

Poliomyelitis

The issue of community protection against polioviruses is of particular importance today, because of the global polio eradication program. The subject is enriched by more than 5 decades of debate relating to different types of immunity induction by different types of polio vaccines.[160,161] Serologic surveys done long ago have been used to estimate the prevalence of immune persons in unvaccinated communities, suggesting that the basic reproduction number of wild-type polioviruses ranges from 2 to 20, implying crude herd immunity thresholds ranging from 50% to 95%, these numbers being inversely related to the level of hygiene.[161] Nevertheless, even this is far too simplistic.

The polio immunity discussion has focused upon the relative advantages of inactivated poliovirus vaccine (IPV) versus live oral poliovirus vaccine (OPV). Among the arguments favoring the live vaccines (in addition to low cost and ease of administration) has been the claim that they provide greater "herd immunity" than do IPVs, for two reasons. The first is that live vaccines impart greater intestinal (local, mucosal, immunoglobulin A–mediated) immunity, and hence impart greater protection against infection, than do the killed vaccines (which induce an immunoglobulin G response which is effective against tissue invasion and disease). The second is that OPV viruses are transmissible to contacts of vaccinees, and hence, in theory, one can immunize more people than are directly vaccinated.[162] This is a special property of the oral polio vaccines and may be responsible for substantial levels of indirectly acquired immunity in populations.[162] Both of these arguments are complicated by the fact that the effectiveness of OPV varies between different settings, being appreciably lower in tropical low-hygiene populations than in temperate high-hygiene populations, partly as a result of interference in the gut by various other enteric agents.[163] Furthermore, most of the traditional measures of vaccine effectiveness relate to protection against disease, whereas it is protection against infection, and against transmission, which are absolutely crucial for the eradication program, and it is known that seropositivity does not equate to solid protection against enteric infection. The importance of this distinction was exemplified in Israel in 2013–14, where environmental surveillance revealed extensive circulation of type 1 wild virus over more than a year, without a single clinical case.[164] This observation is in part a reflection of the low case-to-infection ratio of polioviruses, and the intensity of environmental surveillance in that country, but also, and importantly, by the fact that Israel had shifted from routine OPV to routine IPV in 2005, thus shifting its immunity profile from emphasis upon protection against infection toward protection against disease.[164]

Whereas OPV has been favored by the global program, it has two disadvantages. First, it causes paralytic polio in a small proportion of recipients and their contacts.[165] Because of this risk, most wealthy countries have shifted from OPV to IPV vaccines over the past 15 years, thus changing their population immunity profiles. Second, the transmissibility of OPV viruses is such that they can circulate by continued human to human transmission and revert to wild-type transmissibility and virulence. Such viruses, known as circulating vaccine-derived polio viruses have been responsible for outbreaks of clinical polio in more than 20 countries since their first recognition in 1999, typically in populations with poor hygiene and relatively low coverage of OPV, thus with relatively large numbers of individuals susceptible to infection.[166–168] The ultimate success of the global polio eradication initiative requires that transmission of these viruses, as well as the wild viruses, be stopped.

The eradication program is currently attempting to manipulate population levels of immunity to polioviruses on an

unprecedented scale. Given the complexities of low seroconversion post-OPV in tropical countries, and the fact that mucosal immunity is insufficient to completely stop infection with polioviruses, the strategy has been to keep vaccinating until wild virus is no longer found. This has entailed repeated massive OPV campaigns (up to 10 times per year, for children younger than 5 years of age, in northern India), producing the most heavily vaccinated populations in history.[169] At time of writing, this strategy has proven successful in terms of reducing wild virus cases to lowest ever numbers (only 37 cases in 2016). The global program is thus entering the "endgame."[170–172]

Because type 2 wild virus had not been seen for more than 15 years, type 2 OPV was withdrawn, globally, in April 2016, by substituting bivalent 1–3 OPV for trivalent OPV.[172] The main concern with this shift has been the need to ensure than type 2 vaccine-derived viruses do not continue to circulate, as they are known to cause vaccine-associated paralysis in rare circumstances and to revert toward wild-type transmissibility and virulence.[166–168] Thus all countries were requested to introduce at least one dose of (trivalent) IPV into their routine schedule by the end of 2015, in addition to continuing (bivalent) OPV.[172] IPV vaccines alone were sufficient to stop transmission of wild polioviruses in The Netherlands, Sweden, and Finland, but these countries have high levels of sanitation (and low herd immunity thresholds).[173,174] There is evidence that IPV can boost intestinal immunity in individuals with history of prior OPV[175]; but whether the introduction of IPV will be sufficient to raise community immunity levels high enough both qualitatively (i.e., in terms of mucosal immunity) and quantitatively (in terms of proportions of individuals) to stop circulation of type 2 vaccine-derived viruses in developing countries with poor hygiene standards and high herd immunity thresholds remains to be seen.

Rotavirus

Rotavirus is the commonest cause of severe diarrhea in infants and young children worldwide, estimated to cause 453,000 deaths in children younger than 5 years of age in 2008, 90% of these in low-income countries.[176] In the United States, before vaccine introduction, it principally affected children from 6 months to 4 years of age, and followed a marked seasonal pattern migrating from a winter peak in the Southwest to a spring peak in the Northeast, probably owing to differential birth rates and more rapid accumulation of susceptibles in the Southwest.[177]

Two oral live attenuated vaccines are in widespread use, one containing five strains given in three doses (RotaTeq), the other a monovalent vaccine given in two doses (Rotarix), from 6 weeks of age. The vaccines were efficacious against all episodes (74%) and severe episodes (85% to 100%) of rotavirus gastroenteritis in trials in United States, Finland, and Latin America.[178,179] After RotaTeq was introduced into the childhood immunization program in the United States in 2006, the seasonal rotavirus epidemic was delayed, shortened, and diminished in size,[180] and the rate of rotavirus-coded hospitalizations in children fell by 89%.[181] The average age at infection increased and the decline in the rate of rotavirus hospitalizations in infants and young children substantially exceeded the estimated vaccine coverage[182]; both features suggest that vaccination of infants had reduced virus transmission in the population. Although rotavirus vaccine was not given to older children, because of anxieties regarding the risk of intussusception, impact studies have demonstrated a reduction in the incidence of all-cause and rotavirus gastroenteritis hospitalizations among children too old to be vaccinated, and in adults, in the United States and other high-income countries following introduction.[183–185]

Rotavirus vaccine may induce herd protection by reducing infection among the infant population, who are at the center of transmission networks in the community, or by disseminating vaccine-type virus in stool leading to immunizing exposure among unvaccinated populations. To date there is no evidence of disease caused by vaccine strains. Dynamic models exploring indirect protection predicted an alternating pattern of yearly peaks among partially vaccinated populations, and this has subsequently been observed.[186] This emphasizes the need to evaluate impact over a longer time frame as, disconcertingly, disease rates may be higher in the second season after vaccine introduction than the first.[182]

Circumstances in low-income countries are different. Efficacy of the oral vaccines was estimated to be 39% to 56% in randomized controlled trials in several countries in Africa and South Asia.[187–190] The lower efficacy in these settings may be a consequence of higher titers of transplacental or breastmilk antibodies in infants, micronutrient deficiencies, interfering gut flora, intercurrent infections, or varying pathogenic strains. Rotavirus vaccine has now been introduced in several low-income countries. Although early evidence from Mexico is promising,[191] given their lower efficacy against disease (and presumably transmission) and the higher force of infection, it remains an open question whether current rotavirus vaccines will have an appreciable herd protection effect in low-income countries.

Influenza

Type A influenza viruses present yet another set of problems relating to immunity in the community. Given the genetic lability of these viruses, as manifested in periodic major (shift) and more frequent minor (drift) antigenic changes of their hemagglutinin and neuraminidase antigens, and their persistence in many different vertebrate species, there is no prospect of their total eradication. The large majority of humans have been infected by several antigenically different influenza viruses and thus have antibodies to a variety of hemagglutinin and neuraminidase epitopes; and the concept of herd immunity has been invoked as an explanation for the changing profile of influenza viruses in human populations and the successive disappearance of particular antigenic subtypes. The argument is that increasing proportions of the population immune to each individual influenza subtype, and varying degrees of cross-protection provided between subtypes, provide selective pressure favoring the spread of new antigenic variants. Although such a mechanism appears to fit the available evidence, it does not lend itself to precise numeric description and prediction because of the complicated immunologic relationships between virus subtypes, and the unpredictable nature of the antigenic changes of these viruses.

Influenza control by immunization is an important public health activity in all the wealthier countries. Much concern focuses on the threat of a global pandemic, attributable to some new shift strain of virus, but even without such an event, there is need for continuous control because of the changing antigenic nature of the viruses and their rapid spread, explosive epidemics, and serious impact in terms of hospitalization of infants, sickness absences among the employed adults, and mortality among the elderly.[192] Although the favored strategy of most countries has been to target high-risk groups, an alternative is to reduce community spread by concentrating on vaccination of schoolchildren, because transmission in crowded classrooms leads to rapid dispersal throughout the community and into the homes where susceptible adults reside. The effectiveness of this approach in providing a measurable degree of indirect protection to nonvaccinees in the community has been demonstrated under trial conditions in

Michigan,[193] Russia,[194] Texas,[195] and Canada.[196] Partly as a result of such evidence, different countries have recommended very different strategies. The United States recommends annual vaccination to all ages older than 6 months, but fails to achieve high coverage.[197] Ontario shifted to influenza vaccine to all ages in 2000, achieving marginally higher coverage than in the United States, a policy that has been supported by cost utility analysis of data through 2004.[198] In contrast, the United Kingdom now recommends annual vaccination to schoolchildren 2 to 16 years of age and the elderly and risk groups, based on a detailed evidence synthesis and modeling analysis.[199] Even more extreme, Japan for several years had a policy of vaccinating only schoolchildren. The Japanese strategy was unique in explicitly directing vaccination at one segment of the population (schoolchildren) to protect another (the elderly). Though this was national policy from 1962 and enforced by law between 1977 and 1987, the policy was discontinued in 1994 because of skepticism as to its effectiveness and public concern over adverse reactions to the vaccines.[200,201] The success of these different policies depends on several important parameters including demographic structure, contact rates between the various groups, coverage achieved and vaccine cost, as well as upon actual vaccine effectiveness and the indirect effects actually achieved.

Memories of the great Spanish influenza pandemic of 1917–20 continue to haunt the public health community. Concerns have been heightened over the past decade, first by the appearance of a highly pathogenic avian H5N1 strain carried by migrating birds and then the appearance of a new H1N1 swine virus which met the WHO's criterion of pandemic spread on June 11, 2009.[202] Rapid spread of influenza viruses is possible despite relatively low R_0 (on the order of 2) because of their short serial interval (3 or 4 days)—thus, three or four generations of influenza virus spread may occur in the same time as a single generation of measles. This rapidity of spread has important implications for the feasibility of developing, testing, producing, and administering vaccines at the scale required to control a new influenza strain. On the other hand, the low R_0 means that, in theory, the herd immunity threshold should be relatively low for influenza viruses (see Table 77.2).[18,19,203–205] Pandemic contingency planning in many countries includes a role for vaccines (e.g., targeted to essential service personnel or to schools) in an effort to reduce spread in the community, in addition to the use of antivirals and measures to reduce contact.[204,206] Longer-term strategic thinking includes recommendations for repeated (annual) routine vaccination of large segments of populations, perhaps with multivalent live attenuated vaccines, which may provide broader protection against influenza viruses than do inactivated vaccines.[195,207] The extent to which this would be beneficial in building up broad immunity against influenza virus antigens is unclear, but it would enhance influenza vaccine production and delivery infrastructure, which would be useful in implementing a vaccine response to a pandemic, if and when the need arises.[205]

Human Papillomaviruses

Humans are host to more than 100 different human papillomavirus (HPV) types, spread by different means and affecting different tissues. Most important for public health are several types spread predominantly by sexual contact, including high-risk oncogenic types 16 and 18, which are responsible for approximately 70% of cervical cancer (the second most common cancer of women worldwide), as well as many cancers of the anus, penis, and oral cavity, and types 6 and 11 that are associated with anogenital warts. Three vaccines are available: a bivalent vaccine that has been developed against types 16 and 18, a quadrivalent vaccine that also protects against types 6 and 11, and a nine-valent vaccine that also includes the rarer cancer-causing types 31, 33, 45, 52, and 58. Clinical trials and postimplementation surveillance studies show that the two- and four-valent vaccines offer good protection against persistent infection and precancerous stages,[208,209] although because of the long lag between infection and cancer there is no direct evidence of effectiveness against end-stage cancers. Both vaccines also appear to offer a degree of cross-protection against other high-risk types, such as types 31, 33, and 45,[209] and postvaccination surveillance from England even suggests that the bivalent vaccine may offer some protection against anogenital warts.[210] Many countries now recommend the routine use of these vaccines among (typically preadolescent) females. Some industrialized countries have also included catch-up programs up to 25 years of age as part of the introduction.

Two of the remaining controversies regarding these vaccines are whether they should be offered to boys, and whether the program should be extended to older women. Both of these issues hinge on indirect, or community protection. If an organism depends entirely on heterosexual contact, it can in theory be eliminated from a population by preventing infection of, or transmission by, a sufficient proportion of individuals of only one sex (e.g., females in the case of HPV 16/18 or 6/11). Thus, heterosexual men might be expected to benefit from vaccination of women, and older women might also benefit as the level of infection in their sexual network declines. There is growing evidence that this does, indeed, occur. Australia was one of the first countries to adopt HPV vaccination, introducing the quadrivalent vaccine for adolescent girls in 2007, with a catch-up (at lower coverage) for women up to 25 years of age. An analysis of national surveillance data found large (92%) declines in genital warts in women younger than 21 years of age, but also similar levels of declines in heterosexual males in the same age range (82%), even though they were not vaccinated.[211] Large declines in warts incidence were also observed in 21- to 30-year-old women and men. A recent systematic review and metaanalysis has lent weight to these findings.[209] The Australian study also made one further, important observation—no effect was seen in men who have sex with men—which led to the introduction of HPV vaccination of boys in Australia starting in 2013. Indeed, one of the arguments put forward for vaccinating all boys (as well as girls) is that it is not easy to target young men who have sex with men before they become exposed to the virus.

Policy decisions on the use of HPV vaccines are further complicated by the fact that these are among the most expensive current vaccines, coupled with the recognition that there will be a lag phase of many years (decades) before their introduction has a substantial effect in terms of cancer reduction, and the fact that there are alternative control approaches in place to reduce cervical cancer (large screening programs and associated surgical intervention). Several modeling studies that attempt to provide a rational basis on which to make decisions on the use of HPV vaccines are based on predicted impact and cost-to-benefit ratios associated with vaccinating males as well as females.[212,213] These models suggest that vaccination of males is in general not cost-effective under current circumstances, unless coverage in the female population is low (e.g., three-dose vaccine coverage <50% by age 12 years).[214] This is because females have a much higher burden of HPV-related disease than males, and so, other things being equal, it is much more cost-effective to raise coverage in girls than to start to vaccinate boys. Low coverage in the U.S. female population (only 32% with three doses among 13- to 17-year-olds in 2001) led the United States to recommend vaccination of males in 2011. Much more needs to be learned about the

natural history of the infections, in males as well as in females, before we can be confident of predictions of the long-term impact of HPV vaccination programs.

Tuberculosis (and Leprosy)

BCG vaccination is an important element in tuberculosis control in most countries of the world, and it raises still different questions about immunity at the population level. An appreciable proportion of the global human population—up to 32 % by some estimates[215]—is currently infected with *Mycobacterium tuberculosis*. As fewer than 10% of these infected individuals will ever manifest disease, this implies widespread immunity against tuberculosis disease. Although there is a consensus that it involves the T-cell compartment, the nature of this immunity is not well understood.

Even though BCG vaccines have been shown to reduce risks of disease, at least to some degree, in a large number of studies,[216] we have no measurable correlate of this protective immunity, and the most widely used measure of cellular immunity to the tubercle bacillus (i.e., delayed-type hypersensitivity to tuberculin) is associated with relatively high, rather than relatively low, risk of disease![217] This paradox arises because a high proportion of infections with the tubercle bacillus persist in the human host, and an appreciable proportion of all cases of disease reflect a breakdown of immunity in older, long-infected (and tuberculin-sensitive) persons. This breakdown of immunity may be attributable to several different factors, such as age or intercurrent disease, or, very importantly, to immunosuppression as a consequence of HIV infection. Several authors have commented that there is little evidence that BCG reduces infection risks in endemic communities, in part because most transmission is attributable to cavitary disease in older individuals,[218] whereas BCG's action is mainly against primary disease in younger individuals, which is less likely to be infectious (there is, however, a report of BCG's protection lasting for 50 years[219]). The argument is consistent with evidence that BCG works by preventing hematogenous spread of tubercle bacilli, thus against disease, rather than against primary infection itself,[220] but has recently been challenged by studies using in vitro assays with specific *M. tuberculosis* antigens, which suggest that BCG can reduce infection risks in child contacts of cases, by approximately 20 %.[221]

Despite the immense scale of BCG use over the past 50 years, its overall contribution to protection in endemic populations is unclear. Although there is indisputable evidence for its effectiveness in some populations, in particular against severe disease of childhood but also against pulmonary disease in young adults in northern temperate populations,[222] there is also indisputable evidence of the failure of some of the same BCG vaccines to provide any detectable protection against pulmonary tuberculosis in other populations, in particular in tropical environments.[223–225] The most likely explanation for these differences is that they reflect variations in exposure to different environmental mycobacteria in different parts of the world, the effect of which is either to obstruct or to mask any protection induced by BCG.[223,226] Among the lines of evidence are data from experimental animals and from human populations indicating that exposure to bacteria such as those of the *Mycobacterium avium-intracellulare* complex can induce a degree of cross-reactivity to tuberculin and can impart measurable protection against *M. tuberculosis*.[223] Related to this observation of cross-immunity is the fact that BCG provides variable, but substantial, protection (from 20% to 80% in different populations) against other diseases related to mycobacteria, in particular leprosy (*Mycobacterium leprae*), and it is one of the factors responsible for declines in leprosy in many parts of the world.[226,227] The mycobacteria thus provide examples of geographically variant, heterologous protective immunity, which acts differently against infection and different forms of disease, and is derived from environmental exposure, a group of vaccines derived from a bovine pathogen (*Mycobacterium bovis*), and different human pathogens. The overall picture is far from simple!

"Nonspecific Effects"

Most of the data and discussion of vaccine effects relate, understandably, to the target diseases against which the vaccines were designed. However, immunologists are aware of many examples of cross protection between different infections in animal models (e.g., BCG can protect mice against vaccinia virus)[227] and human vaccinologists can cite examples of vaccines acting beyond their target disease (an obvious example being BCG vaccine's protection against leprosy and its use in treating bladder cancer). Given that many vaccines contain a large number of antigens, let alone adjuvants, it should not be surprising that there are examples of vaccine effects on nontarget conditions. It is appropriate to consider the potential population implications of these nonspecific, or nontarget, effects of vaccines.

The subject is complicated by its very open-endedness and has a controversial history. Logic might lead to an expectation that nontarget effects would be most likely against closely related organisms (consistent with protection by BCG against *M. leprae*), but the complexity of vaccine antigens, of the immune system, of the human microbiome, and of the potential pathogens confronted in different environments is such that one may conceive a wide variety of possible effects. The controversy arises in part because the phrase "nonspecific effects" arose first in the context of a totally unexpected observation of apparent increase in all-cause mortality among (in particular female) recipients of high titer measles vaccines in trials done in the 1980s.[228] This observation led to discontinuation of research on these vaccines, but was never completely explained, and spawned a series of hypotheses attributing the observed effects not just to the measles vaccines but to their timing and sequence relationship to other vaccines, in particular diphtheria, tetanus, and pertussis.[229,230] Most of the studies were perforce observational, and many were open to criticism given that vaccinated and nonvaccinated individuals differ in many ways, and hence comparisons are open to bias. However a substantial body of data and methodological discussion has now accumulated which provides a basis for guiding more definitive research.

Much of the work on this subject has been done by Aaby and colleagues in West Africa, in particular in Guinea Bissau, thus under difficult conditions and in populations with high background rates of infectious disease morbidity and mortality. Because of the absence of diagnostic facilities in such populations, most of the studies have examined all-cause mortality as an outcome; thus mortality may be attributable to both target and nontarget diseases. Independent systematic review of this literature in 2014, supported by the WHO, provides evidence that (standard titer) measles and BCG vaccines are associated with reduced all-cause child mortality in several populations.[231] The effects look to be greater than can be explained in terms of prevention of measles or tuberculosis (range: 8% to 89% for BCG; 0% to 90% for measles). The extent of apparent protection varies considerably between populations, as might be expected, given not only many sampling factors but also the variation between infection exposures and hence causes of mortality between populations. For this reason it is inappropriate to provide single estimates of nonspecific protective effects.

A smaller body of work has examined possible nonspecific vaccine effects in wealthy countries. Analyses of linked data in Denmark have provided evidence that receipt of measles-mumps-rubella vaccine may be associated with low rates of hospital admissions for nontargeted infections and specifically for respiratory syncytial virus.[232,233] Interpretation of these findings is complicated by a longstanding argument over the extent to which measles disease is associated with, or can lead to, increased susceptibility to a variety of other infections, and hence whether an effect of measles-mumps-rubella vaccine or other nontargeted infections might be attributed indirectly to the prevention of measles per se.[234,235] This issue may become of importance in the future, if measles is ultimately eradicated, as there might be a discussion whether measles vaccination should be continued because of its potential "direct" protective effects on other ("nontarget") causes of morbidity and mortality.

The complexity of this subject, and attendant controversies have meant that it has not to date captured mainstream attention of the vaccine community. However, the accumulation of substantial data and recent independent systematic reviews mean that it is likely to be taken more seriously in future. Among various implications this means that there may be calls to consider the collection of data on all-cause morbidity and mortality effects of particular vaccines, and in assessment of different vaccine schedules, in the future.

DISCUSSION

This brief review reveals many complexities to the measurement and interpretation of immunity at the community level. Though not discussed in this chapter, the issues of vaccine coverage and effectiveness are obviously fundamental to protecting communities through vaccination programs. Maximizing coverage raises many practical problems relating to services, access, logistics, targeting, schedules, consent, enforcement, health education, and cost.[236] Increasing effort is now devoted to combatting antivaccine sentiment or "vaccine hesitancy," which is on the rise in many societies.[73] The definition and measurement of vaccine coverage are themselves complex issues, and are discussed elsewhere in this volume.

Beyond issues of coverage, to say that x% of a population has been vaccinated, or is "immune" to some infection can be misleading without detailed qualification of the nature of the immunologic states and the distribution of the various sorts of individuals in the population, in particular by age and area. For some infections, exemplified by measles, immunity to infection, to disease, and to infectiousness appear to be quite similar in nature and magnitude (although titers are lower postvaccination than after natural measles), but for many infections they are different. There are important differences between protection against infection, reinfection, carriage, disease, and infectiousness in varicella, pertussis, Hib, diphtheria, polio, HPV, and tuberculosis; immunity is not sterile in varicella and tuberculosis; heterologous immunity is important in influenza, diphtheria, and tuberculosis, and perhaps Hib; and both waning and boosting are important for natural or vaccine-derived immunity to many infections, including smallpox, varicella, pertussis, Hib, and pneumococcus. The step from description of immunologic states in individuals to their implications at the population level is also complex: serotype replacement occurs with both pneumococcus and influenza; vaccination of certain subsets of populations has important effects on risks in other subsets for rubella, pertussis, tetanus, varicella, pneumococcus, meningococcus, HPV, and influenza, and delayed post-honeymoon epidemics illustrate important temporal dynamics in measles, rubella, mumps, varicella, pertussis, and Hib. The acknowledgment of nonspecific or nontarget effects of vaccines introduces a considerable complexity that awaits further research.

Ironically, a major problem affecting the subject of community protection by vaccines is the word immunity itself, which too often is taken to mean solid protection against both infection and disease, thus overlooking the many subtleties of actual immune responses. These subtleties are likely to be even greater for some of the vaccines currently under development, for conditions such as malaria, HIV, and helminthiases, than for many of the vaccines in use today. The fact that these vaccines have been so difficult to develop reflects complexities in the biology of the target organisms and in the immune responses they generate. Distinctions between solid and partial immunity and between protection against infection, reinfection, carriage, disease, and transmissibility are thus likely to be particularly important for these new vaccines and will determine their overall effects in communities.[237–241]

Immunological mechanisms aside, the interpretation of measures of vaccine-derived protection in individuals can depend on the vaccine's distribution in populations. If vaccinated persons are clustered in community groups (as is often the case), then they benefit both directly, from individual receipt of the vaccine, and indirectly, from reduced transmission in their neighborhoods. In such circumstances, the vaccinated and unvaccinated groups are not equally exposed to infection, and crude measures of vaccine efficacy will overestimate the "immunizing" capacity of the vaccine among individual recipients.[105,242] This is a particular problem in observational studies of vaccines, but it also may affect trials in which randomization is by group and not by individual.[243–245] Related to this issue is the fact that cluster randomized trials provide a measure of overall benefit of vaccine distribution (including both direct and indirect effects), which may be appropriate in predicting overall impact and cost-effectiveness of a vaccine program.

This discussion has referred repeatedly to the *indirect protection* of nonimmune individuals brought about by the presence and proximity of immune persons. That such protection should occur is evident both intuitively and through theoretical argument, and it has been demonstrated repeatedly and convincingly in the context of many vaccination programs. Of the infections and vaccines discussed here, the only examples in which indirect protection has not been demonstrated are tetanus (though there is the argument relating to passive antibody transfer from mothers to their neonates) and tuberculosis (though the recent recognition that BCG can impart some protection against *M. tuberculosis* infection implies the possibility of some indirect effects[221]). In several examples, the indirect protection from vaccination has been obvious (e.g., smallpox, rubella, mumps, chickenpox, Hib, pneumococcus, meningococcus, diphtheria, influenza, HPV).

There are several important features to this indirect protection. One is that it applies to all individuals in the population containing the immune persons—including those too young to have been vaccinated, and those who may be still susceptible because of immune deficiency, or because they refused or otherwise failed to be successfully vaccinated, perhaps as a result of contraindications or lack of access to vaccination services or simple vaccine failure, or because their immunity has waned. Both pneumococcus and influenza illustrate how targeted vaccination of children can protect the elderly who are more susceptible to serious disease, and perhaps less able to respond to the vaccine. A second important point is that indirect protection is not a form of immunity; thus a person indirectly protected (shielded) from infection remains still susceptible, and may in fact contract infection at a later date and age. Under certain circumstances, this delay in infection may actually be hazardous for that person, or for society (e.g.,

with rubella or mumps, or perhaps polio). In this sense, indirect protection by vaccines is the opposite of a traditional approach, practiced in many societies, of exposing children to infectious cases of, for example, rubella or measles or chickenpox at a young age when they are most likely to contract a mild form of the disease. In addition, we have noted that indirect protection can have another potentially detrimental effect insofar as it may reduce boosting of immunity through repeated exposure to infection and may thereby lead to greater waning of immunity and increased susceptibility in older persons. This is a particular concern with regard to pertussis and perhaps varicella-zoster. In this context, an increase in one or another pattern of susceptibility in adults need not necessarily be detrimental to society—for example, if the use of vaccines in the young is sufficiently intense to greatly reduce transmission among the groups with the highest levels of contact, so that there is greatly reduced exposure among adults. However, such shifts in the age distribution of immunity and susceptibility are potentially hazardous and need to be monitored closely.

Appropriate comparisons between groups with different proportions of vaccinated individuals may allow estimates of both direct and indirect protection attributable to vaccines,[33,242] as shown in analyses of cholera vaccines in Bangladesh.[244] Despite the elegance of the methods, it is likely that measures of direct protection will be far more generalizable than are measures of indirect protection, given that the former depend only on the immunologic response of individual vaccine recipients, whereas the latter will be a function of the social and spatial distribution of vaccinated persons, and of social mixing patterns, which may differ greatly between populations. For example, the role of schoolchildren in community dispersal of influenza viruses (and hence in the indirect protection to adults that may be provided by their vaccination)[193–196,203] is a function of the age and family size distribution of the population (which determine what proportion of families have children in school) as well as the size and physical environment of the schools (a single, large, crowded school being more likely to serve as a distribution center for the community than a large number of small and well-ventilated schools).

This influenza example exemplifies one of several interesting and important ethical issues in community immunity: to what extent is it justifiable to protect—or even to increase risk in—unvaccinated individuals indirectly, by directly vaccinating and protecting other individuals (e.g., the vaccination of males against rubella)? What are the implications of such indirect effects for informed consent, or compulsion, or targeting of vaccines, or compensation of vaccinees should they suffer any adverse effects? To what extent can cost-effectiveness analyses incorporate such considerations? Children vaccinated with live attenuated influenza vaccines may be protected themselves, but under what circumstances is it justifiable to direct a program at them when the greater benefit (and perhaps even the intention) is to protect their grandparents? The fact that Japan's program to target schoolchildren with influenza vaccines was discontinued[200,201] gives one example of one nation's perception, at one point in time. These are among the difficult and important implications of the community effects of vaccination programs. They are addressed more fully in the chapters on economics, vaccine safety, legal issues and ethics within this volume.

Much of the literature on community, or herd, immunity to various infections emphasizes threshold proportions of immune persons that, if reached and sustained (e.g., by vaccination), should lead to progressive elimination of the infection from the population. Although such estimates (see Table 77.2) may provide a rough ranking of the levels of natural and vaccine- derived immunity required for control or eradication of these infections, they should not be accepted uncritically, as their validity depends on the various assumptions underlying their derivations, and even the most elaborate derivations typically omit important features of the immune response (e.g., implications of protection against infection, disease or infectiousness, or waning) and important features of the practical logistics and nonuniformity of real populations and of real vaccination programs. Although these thresholds are often referred to in the context of discussions of eradication targets, the practical experience of the major programs to date, as against smallpox, measles, and polio, has found them of little practical use. In addition, their relevance is mitigated by the fact that most public health programs aim at *control* rather than elimination or eradication of infections. Even if there is an eradication goal, the practical approach will not be to just attain some threshold, but to aim for and sustain the highest possible coverage, in theory 100%, because this will maximize the rapidity of the disappearance of the infection in question. Merely achieving a herd immunity threshold does not mean immediate disappearance of the infection; it only starts a downward trend.

Such caveats do not mean that herd immunity is not a valid concept. That indirect protection occurs is obvious, both in logic and in observation. Prevention of a communicable infection in any one person reduces by one the potential sources of infection— and hence the potential risk of infection—for that person's peers. That is indirect protection and a form of "herd effect."[246] The observation of apparent exceptions, small communities in which infections appear to be transmitted despite very high levels of vaccination coverage (e.g. because of chance or unusual clinical presentation or behavior or environmental circumstances), do not refute this principle,[247] just as the failure of a particular vaccination in some individual recipients need not refute an overall high effectiveness of a vaccine.

The herd immunity threshold concept provides an epidemiologic attribute with which to characterize particular infections. Although precision may not be possible because of population heterogeneities or because of variability in the immune status of individuals, and although they may be naïvely optimistic in practice, even crude estimates can be of use in giving a rough guideline for predicting the impact of a vaccination program and at least a hint as to the potential for eradication. As experience grows, and models become more sophisticated, we will come to appreciate better how the various subtleties of the immunology and epidemiology of different infections—for example, those attributable to the nature of the immune response and to the social structure of populations—imply greater or lesser biases in the estimates derived from simple models. The concept of a threshold is also useful in the context of teaching. It is part of the basic science of infectious disease epidemiology, and it provides an essential background for understanding the behavior of infections in populations.

The emphasis on elimination thresholds in the literature has distracted attention from important and complicated implications of changing patterns and levels of immunity in populations over time. A vaccination intervention entails a complex disruption of the previous natural balance, and it can destabilize epidemiologic patterns for many years. The introduction of an effective vaccination program among children may reduce infection incidence to such a degree that a large number of susceptible persons can accumulate among those born just too early to receive the vaccinations, and who thus escape both the natural infection and the benefits of vaccination. The accumulation of such susceptible groups may lead to changes in the age distribution of cases in subsequent years,

as has been described for many infections. Discussion of such changes is sometimes confused by presentation in terms of proportions of cases in different age groups, because it is possible for the proportion of (e.g., measles or pertussis) cases among adults to increase dramatically even though their absolute number decreases. Thus a shift to older cases need not necessarily be harmful overall. Prediction of such effects requires simulation with models that take into account differences in contact within and between age groups, using data such as generated in the POLYMOD-related projects.[248] The only way to develop convincing descriptions is by the accumulation of detailed analyses of age-specific data over time, preferably before and after vaccine interventions.

Although vaccines are given to increase levels of immunity in populations, we also need to consider immunity loss as an important issue. The loss of protective immunity may be attributable to waning of an immune response with time, which may in turn be a function of the nature and strength of the initial response, or to a reduction of boosting by natural exposures, as is important in contemporary trends in pertussis, Hib, and herpes zoster. Immunosuppression is also important in this regard, for the elderly and infirm and in particular as a consequence of HIV infection. HIV-attributable immunosuppression is a major force behind the global epidemic of tuberculosis today. Age itself is an important determinant of immune competence and status, and the increased numbers of older persons in many populations may lead to declines in overall levels of various aspects of immunity in many communities in the future. The mechanisms underlying the persistence of immunologic memory are poorly understood but of great importance to communities as well as to individuals.

The growth of vaccination programs and the recognition of the complexity of their implications highlight the importance of immunologic monitoring of populations. Only by accumulating such data will we ultimately be able to understand the dynamics of community immunity and the full effects of vaccine interventions and to optimize future interventions. Such monitoring should enable detection of accumulating pockets of susceptible persons, and hence the prediction of delayed epidemics such as have been observed after a period of vaccine program–attributable low incidence. The monitoring of increasing numbers of susceptible persons to measles in the United Kingdom in the early 1990s through routine serosurveillance, and the subsequent nationwide mass campaign, provides an example of effective monitoring and response—although, ironically, the omission of mumps antigen from the campaign allowed the massive resurgence of mumps in the United Kingdom starting in 2004.[68,83] Serosurveillance of antibody levels to rubella and diphtheria have also been used to plan immunization strategies, and such approaches are likely to increase in the future. Effective serosurveillance requires knowledge of appropriate humoral (preferably) or cellular correlates of protective immunity, such as we have for some vaccine-preventable diseases (e.g., measles and rubella) but not for others (e.g., pertussis and tuberculosis). Defining such correlates, and the development of appropriate assays for them, will be an important area of research in coming years.[249]

This review has avoided emphasis on any simple interpretation of herd immunity, instead accepting the varied uses of the term by different authors. This is in keeping with the first published use of the term, which posed the problem of "herd immunity" as the problem of how to distribute any given amount of immunity (e.g., antibodies, vaccinations) so as best to protect a population from disease.[8] The mechanisms will be several—direct protection of vaccinees against disease or transmissible infection, and indirect protection of nonrecipients by virtue of surreptitious vaccination, passive antibody, or just reduced sources of transmission and hence risks of infection in the community. The solutions likewise will depend on many factors—the nature of the population, the infection, the vaccine, and the health services. The population and the infection are generally given, the vaccine we may try to improve, but the distribution of that vaccine is up to the public health community. How to optimize that distribution for the betterment of society remains, in the broadest sense, the real problem of community protection.

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78 Economic Analyses of Vaccine Policies

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Vaccination programs have greatly reduced morbidity and mortality worldwide. While new vaccines and the strategic use of existing vaccines offer the prospect of further reductions in disease, policy makers are often constrained by limited public health resources* and require assessments of a vaccine's value to justify and optimize its use. Newer vaccines tend to cost more than older products and those not under patent protection. Studies involving the integration of epidemiologic and economic data are critical to identify the most efficient use of public health resources, to optimize health benefits, and to help define research agendas.

Different quantitative techniques have been used to analyze policy decisions related to health outcomes, including those related to vaccine-preventable diseases. Economic analyses, such as **cost analysis**, **cost-benefit analysis**, **cost-effectiveness analysis (CEA)**, and **cost-utility analysis (CUA)** (Table 78.1), help estimate potential financial requirements and program impact for direct comparisons among vaccination strategies. A systematic assessment of policy options facilitates the creation of policies that are based on a set of established criteria and provides transparent accountability for the choices made.

These approaches share the characteristics of explicit delineation of possible alternatives, estimation of probabilities and costs associated with each of these alternatives, various health outcome metrics and values, and development of a summary statement of the implications of alternative courses of action. They also involve sensitivity analyses, in which estimated probabilities or costs are varied to determine how sensitive the conclusions are to particular variables.

Cost analysis is the basis of all economic studies because it quantifies the costs associated with a given intervention. The costs of the program may include, but are not limited to, vaccines, vaccine administration, efforts to address adverse events, and public education. Cost-benefit analysis is an extension of cost analysis that further accounts for the monetary benefits from a policy or program. Benefits are usually calculated as a function of the total cost of a disease in the presence and absence of an intervention. In a cost-benefit analysis, results are usually presented as ratios of the benefits from the intervention to the costs of conducting the program (B:C ratio) or the differences between the two, expressed as a net benefit (cost). By convention, the ratio is expressed as benefits to a cost of 1. If the B:C ratio is greater than 1.0, the intervention is considered to be cost saving, because 1.0 is the breakeven point at which costs and benefits are equal.

Because of the difficulties of assigning economic values for certain outcomes, such as averted deaths, or the macroeconomic impact of outbreaks, a full accounting for a cost-benefit analysis is challenging. However, because it can give a summary statement about whether the benefits of a program exceed its costs, irrespective of the character of the outcomes, it can be used to compare health programs with nonhealth programs. CEA and CUA are more useful for comparing different health programs and for avoiding the subjective valuation of intangibles such as death.

In CEA, results are presented in terms of the cost required to achieve a particular health outcome (Table 78.2). Usually no attempt is made to assign an economic value to a prevented death. In general, interventions are considered highly cost-effective (good buys) by financial authorities if the cost per year of life saved is less than or equal to the annual per capita gross domestic product (GDP), which can range from US$500 for low-income countries to greater than US$30,000 for high-income countries (the GDP per capita in the United States was US$47,400 in 2010).[1] The relative cost-effectiveness of a death prevented can depend on the age of the individual and the consequent number of years of life saved. For example, an intervention that prevented death at an early age is generally considered cost-effective even if the cost per death prevented is several times higher than the per capita GDP. By contrast, a public health intervention with the same cost per death prevented might not be considered cost-effective in an elderly person with fewer potential years of life saved. Ethical considerations are critical, because society must decide on the value of life-years saved at different ages.

CEA is particularly useful when there are several options to achieve a common outcome (e.g., delivering a second dose of measles vaccine as part of a campaign or as part of a routine schedule to prevent outbreaks). Despite its advantages, CEA is not useful when comparing investment in alternative health programs with disparate outcomes. In this situation, CUA is much more useful, because the units of measurement are standardized, albeit with subjective and therefore sometimes controversial, metrics.

CUA is a specific form of CEA in which health outcomes are reduced to a common denominator such as a quality-adjusted life-year (QALY)[2] or a disability adjusted life-year (DALY). The units of each of these outcomes are standardized through a process by which panels of ethicists, researchers, and decision makers weigh the value of different morbid states relative to full health for use in studies using either of these metrics. For example, a consensus group might find that two-thirds of a year living with full sight is equivalent to 1 year living with blindness, effectively equating the quality of the time spent with blindness as 67% of the quality of the time with full sight. The relative proportion of time preference with and without a specific condition allows the formulation of a common health metric unit that can offer direct comparisons of a variety of morbid conditions. There are more than 30 such health outcome metrics that integrate morbidity and mortality. Table 78.2 provides some examples of these metrics. A common metric calculated through CUA permits comparisons to be made among interventions aimed at diseases with diverse outcomes, such as death, acute illness, and prolonged disability. Although common outcome metrics facilitate comparisons of many health interventions, numerous subjective and ethical assumptions underlie their construct, and therefore they are not always easily interpreted (see Table 78.2).

*Although private consumers individually judge the value of prevention, this chapter focuses on the public sector.

TABLE 78.1 Examples of Economic Evaluations of Vaccine Policy and Their Derivation

Term	Definition
Cost analysis (CA)	Summation of vaccine program costs including vaccine, administration, adverse events, public health marketing
Cost-benefit analysis (CBA)	Computed as a benefit-to-cost (B:C) ratio equal to cost of prevented disease/ costs of program *or* net benefit equal to cost of prevented disease minus costs of program where prevented disease equals the difference in costs of disease (direct, indirect, and intangibles) without and with vaccine and costs of program equals the cost analysis
Cost-effectiveness analysis (CEA)	(Cost of program minus cost of prevented disease) divided by prevented health outcome
Cost-utility analysis (CUA)	(Cost of program minus cost of prevented disease) divided by standardized health metric

TABLE 78.2 Examples of Health Outcome Metrics[a]

Metric	Definition
Cases	An outcome may be stated as the number of cases that occur or are prevented by an intervention. It does not necessarily account for the various severity states of sequelae. One could further stratify the total number of cases by minor illness, hospitalizations, and long-term sequelae.
Deaths	Death may be easily quantified but defies an economic valuation. For this reason, CEAs are often used in health outcome assessments with deaths (or some variation) in the denominator.
Years of potential life	YPL is a refinement of the death metric performed by quantifying the total years of life lost or prevented from being lost. It integrates the difference in an expected life span of each person and the age at which a death occurs. YPL may be adjusted by discount and age weights to account for societal preference of time and years of productivity; there are considerable ethical considerations in the choice of a weighting scheme for different ages.
QALY or DALY	QALY and DALY are further refinements of the YPL metric that integrate mortality and morbidity states. Various health metrics have been developed to help quantify disease burden states to compare disparate health outcomes. QALYs and DALYs integrate mortality, the YPL, and a valuation of the disability from morbidity. They are calculated by adjusting the YPL by the time spent with various disabilities. These units may be discounted and contain an age-weighting factor. The weights are systematically defined and then used as standards for subsequent studies. They offer a common metric to compare many health outcomes.

[a]Any type of policy analysis requires a quantification of morbidity and mortality states. Although there is no universal consensus metric, attempts have been made to create a single unit to systematically quantify many diverse possible health outcomes.

CEA, cost-effectiveness analysis; DALY, disability-adjusted life-year; QALY, quality-adjusted life-year; YPL, years of potential life.

IMPORTANT CONSIDERATIONS IN QUANTITATIVE POLICY ANALYSIS

There are many decisions to be made in designing a quantitative policy analysis. Widespread variability in choices of parameters or metrics has made it difficult to make comparisons among many of the published studies. This variability has been criticized.[2] In the 1990s, a task force in the United States drew up suggested guidelines for carrying out cost-effectiveness studies of preventive services.[3] The journal *Vaccine* has published an editorial statement on the submission of economic evaluations of vaccines.[4] The U.S. Centers for Disease Control and Prevention (CDC) has published guidelines that are required to be followed to present any economic analysis to the CDC Advisory Committee on Immunization Practices (ACIP). Additionally, the World Health Organization (WHO) has published a guide for the standardization of economic evaluations of immunization programs.[3,4]

Economic models also may consider the indirect effects of immunization from the decrease of transmission (herd immunity).[5] As an example, the impact of immunization of U.S. infants with pneumococcal conjugate vaccine on the occurrence of pneumococcal disease in older, unvaccinated populations has been quite large.[6] Additionally, there has been increasing interest in providing different modelers with common data to assess the divergence and convergence of results and identify important nuances for consideration. This approach has been taken with respect to research focusing on rotavirus and human papillomavirus (HPV) vaccines and measles eradication.[7]

As a result of the increased levels of attention being given to the evidence base for new vaccine use, analytical tools and initiatives to assess new vaccine cost-effectiveness have been developed. The ProVac Initiative of the Pan American Health Organization is a notable example.[8] The Strategic Multi-Attribute Ranking Tool for Vaccines (SMART-Vaccines) has been developed to capture both quantitative and qualitative explicitly stated values to help make transparent decisions related to vaccine policy.[9] Many attributes, such as impacts on societal disruptions, macroeconomic considerations and development, may be considered. These tools can help National Immunization Technical Advisory Groups (NITAGs) assess available information and make a country-based and evidence-based decision on quantitative issues related to vaccines and immunization programs.[10]

Perspective

Individuals, healthcare systems, commercial vaccine manufacturers, and society at large all benefit from immunizations. Although the societal perspective theoretically captures all costs and benefits, the costs and benefits of a vaccination program clearly accrue to different budget lines. Results from any analysis would vary from each perspective[11,12] and should be explicitly stated.

Time Frame

Vaccines protect against diseases that would otherwise occur at a variable future time. While measles vaccine protects against childhood disease, hepatitis B or HPV vaccines can prevent liver and cervical cancer, respectively, in adults. Because a vaccine investment is made in a time frame different from the eventual benefit, economic analyses frequently discount future effects to take into account the implicit valuation that society has for health and financial costs and benefits over time. Even after accounting for inflation (which affects both costs and potential savings), there is an implicit difference in

the value of an event that occurs today versus one that occurs in the future. For example, it is generally thought that a child's death prevented today is worth more than a child's death prevented 50 years from now. Even though this is an ethical and subjective valuation, there is general agreement among economists to discount both costs and benefits at the same rate, typically from 3% to 10% per year, most often at the lower end of the range. Study results may be presented as both discounted and undiscounted to see the influence of this subjective assumption.

Disease Burden

Estimates of the potential impact of a vaccine on disease may be derived from surveillance data (usually an underestimate), extrapolations from other representative populations, vaccine clinical trials (e.g., vaccine probe studies),[13] or the results of mathematical models that simulate the natural history of microbes, infections, degrees of severity, and population mixing, among other variables. Analyses might need to be specific for age group, population and occupational group, risk of exposure (e.g., healthcare workers), and outcome, including the timing of the outcome (e.g., death in the next year vs death 50 years in the future). An understanding of the different rates of transmission of microbial agents is essential to appreciate the direct and indirect impact that vaccines can have on populations.

Measures Used for Health States

Outcomes (see Table 78.2) may be stated as the number of cases or deaths that occur or are prevented by an intervention. Years of potential life represents a refinement of the death metric that quantifies the total years of life lost or prevented from being lost. It integrates the difference in an expected life span of each individual and the age at which death occurs. QALYs, which measure the number of years of healthy life lived, or the currently used WHO metric, DALYs, which measure the years of healthy life lost, are further refinements of years of potential life that integrate a reduced valuation of various morbid states.

QALYs and DALYs are complementary concepts. Both metrics are derived from the product of the number of years lived and by the quality of those years. QALYs use utility weights of health states; DALYs use disability weights to reflect the burden of the same states. For example, if the utility of blindness is 0.67, the disability weight of blindness is 1–0.67 = 0.33. Disregarding age weighting and discounting, all other things being equal, a person who is blind for 50 years has 0.67 × 50 = 33.5 QALYs compared to 50 QALYs for a person with full sight. The condition of blindness contributed to 50–33.5 = 16.5 DALYs.[14] These metrics defy simplicity, are derived from many subjective inputs, and might not be readily understood by some policymakers; however, they offer a common metric to compare disparate health outcomes.

Economic Valuation of Health Outcome States

Direct and indirect costs and benefits are usually counted. Direct costs include those related to medical treatment and to administering the vaccine, including ancillary costs such as screening for target groups. Indirect costs include lost wages of those ill and/or their caregivers. Intangible costs, such as pain and suffering or death, are difficult to quantify; however, they may be implicitly valued as the denominator of a CEA or CUA (e.g., as cost per death prevented), allowing the reader to apply the reader's own value to the stated health outcome. An alternative valuation tool is the concept of willingness to pay, where individuals are asked what each would be willing to pay to achieve alternative health outcomes given different economic circumstances and probabilities. These collective responses are integrated with actual probabilities of risk to gauge individual or societal valuation of prevention.

Vaccine Program Characteristics

Vaccine effectiveness in the field, coverage, adverse effects, and the potential for indirect benefits to accrue to unvaccinated persons from decreased transmission are all program characteristics that should be accounted when evaluating the cost-effectiveness of vaccine initiatives. Fig. 78.1 shows a schematic model of a vaccine program. Each component has an associated cost or a benefit.

Sensitivity Analysis

Quantitative policy analysis provides the opportunity to evaluate the likely effects of different probabilities of events (or different costs) from those postulated in the base case analysis. This is particularly relevant when the value of a parameter used in an analysis is uncertain and must be estimated. Testing a range of values for an uncertain assumption or group of assumptions can help identify the variables to which the conclusion is most sensitive or robust. In turn, defining those variables' values can be the focus of further research.

EXAMPLES OF USES OF QUANTITATIVE POLICY ANALYSIS

In general, cost-benefit analysis, CEA, and CUA have shown that immunization is an excellent investment (highly cost-effective and often cost-saving) for older vaccines that have historically been recommended for the pediatric population. As Table 78.3, which summarizes selected studies of different vaccines, demonstrates, consideration of particular strategies in certain populations can show considerable variation,. With the advancement of computational capabilities, modeling software, the maturation of health economics applications to public sector health programs, and the expansion of the repertoire of new vaccines in recent years, the number of economic studies to evaluate vaccines has greatly expanded. Given that more than 1000 economic analyses have been published since the 1980s, we only present results of selected studies to highlight the relevant issues for consideration. Unless otherwise indicated, all costs are in U.S. dollars. A summary table of cost-effectiveness studies of other selected health interventions is included for reference (Table 78.4).[15,16]

Use of Models

Economic models are becoming more important to decision-making. A survey of NITAGs published during 2010 found that more than 90% considered financial aspects when issuing recommendations.[17] The United Kingdom and Australia NITAGs use the results of economic analysis as a criteria for public vaccine funding.[18] Vaccine recommendations by the WHO now routinely include data on economic impact. The Global Alliance for Vaccines and Immunization (GAVI) uses four categories of criteria and indicators to determine which vaccines it will support, one of which is cost and value as measured by total procurement costs, incremental in-country operational cost per vaccinated person, and procurement cost per death or case averted. From another perspective, vaccine manufacturers may consider the results of economic analyses when setting vaccine prices.

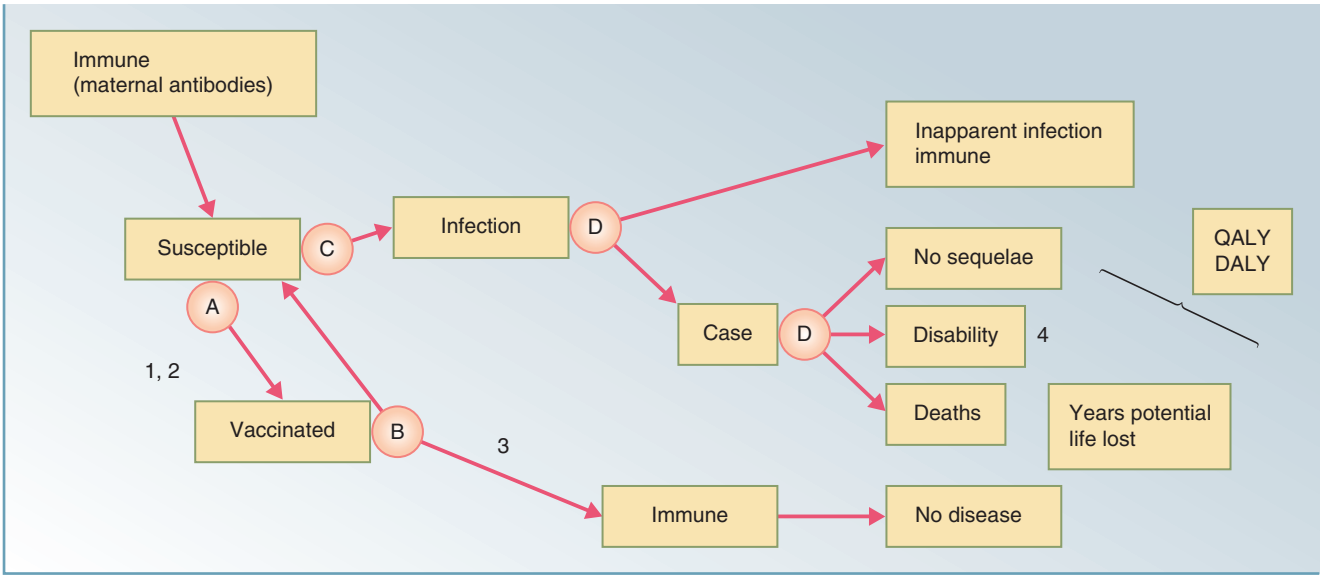


Figure 78.1. Simplified schematic of compartmental model of infectious disease transmission with a vaccine-preventable strategy. Each of the boxes represents a particular state of a vaccine-preventable disease. Assuming all persons are susceptible to infection after birth (following maternal antibody protection), there are variable probabilities for passage into another immunological state throughout the person's lifetime. These probabilities can be quantified to model the impact of a vaccination program on a birth cohort followed throughout life to quantify disease burden. *A,* Vaccination coverage rate (age specific and by dose). *B,* Vaccine efficacy by dose (effectiveness more relevant to account for herd effect). *C,* Age-specific rate of infection. *D,* Probabilities of progression to each outcome state. *1,* A screening program could be added here to help target vaccination efforts. *2,* Adverse event probabilities could be added here. *3,* Dose and possibly time dependent. *4,* Any number of mutually exclusive outcome states could be added here. DALY, disability-adjusted life-year; QALY, quality-adjusted life-year.

TABLE 78.3 Illustrative Examples[a] of Economic Analyses of Selected Childhood Vaccines

Reference	Type	Scope	Result	Comment
GENERAL				
Brenzel (2006)[27]	CEA	Global	Reviewed 30 studies; average cost to fully immunize a child with DPT, BCG, polio and measles vaccines US$22, ranging from US$8 (Tanzania, fixed site) to US$33 (Cameroon, national campaigns)	All costs adjusted to 1992 dollars (equivalents)
Miller (2000)[85]	CEA	Global	Country-specific analysis for vaccination against hepatitis B, *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, and rotavirus. The global cost per life-year saved ranged from US$29 to US$150, with great variation by income and economic groups	Costs of vaccine were based on relative wealth of individual countries
Zhou (2005)[19]	CBA	United States	Benefit-to-cost ratios of 7 routine childhood immunization results in direct and societal benefit-to-cost ratios of 5.3 and 16.5, respectively	
BACILLE CALMETTE-GUÉRIN				
Trunz (2006)[31]	CEA	Global	At US$2–US$3 per dose, BCG vaccination costs US$206 (US$150–US$272) per year of healthy life gained	
CHOLERA				
Schaetti et al (2012)[290]	CEA	Zanzibar	During a mass vaccination campaign, the incremental cost-effectiveness of oral cholera vaccine compared to no vaccine was US$750,000 per death, US$6000 per case, and US$30,000 per DALY averted	Static model used
DENGUE				
Durham et al (2013)[277]	CEA	Brazil	Dengue vaccine estimated to be cost-effective at vaccination costs up to US$534 and cost-saving at costs up to US$207	Base-case assumption of 70% vaccine efficacy against all dengue combined

Continued on following page

TABLE 78.3 Illustrative Examples[a] of Economic Analyses of Selected Childhood Vaccines *(Continued)*

Reference	Type	Scope	Result	Comment
DIPHTHERIA–TETANUS–ACELLULAR PERTUSSIS				
Ekwueme (2000)[305]	CBA	United States	Benefit-to-cost ratios for acellular pertussis vaccine from a societal and healthcare system perspective were 27:1 and 9:1, respectively, but were marginally more expensive than whole-cell vaccine	Additional benefit of acellular vaccine is increased consumer confidence
GROUP B STREPTOCOCCUS				
Oster et al (2014)[297]	CEA	United States	Incremental cost-effectiveness of routine maternal immunization added to screening and intrapartum antibiotic prophylaxis would be US$91,321 per QALY gained	Base case assumed 85% coverage with vaccine cost of US$100 per person
HEPATITIDES A AND B				
CDC (2006)[63]	CEA	United States	Cost-effectiveness was estimated at US$173,000 per life-year gained and US$24,000 per QALY gained	Substantially more cost-effective when decreased transmission through herd effects are included
HUMAN PAPILLOMAVIRUS				
Jit et al (2015)[107]	CEA	Global	HPV vaccine cost-effective in 158 of 179 countries; vaccinating 58 million 12 year-old girls would prevent 690,000 cases of cervical cancer at a cost of US$4 billion	Model: Papillomavirus Rapid Interface for Modelling and Economics (PRIME). Assumed lifelong protection, vaccination presexual debut
Graham et al (2015)[120]	CEA	Canada	US$94.49 saved and 0.04 QALYs gained per vaccinated male age 12 years	Base case vaccine efficacy of 84%, coverage 50%
Laprise et al (2014)[106]	CEA	Canada	All 2- and 3-dose/girls only and girls+boys strategies had a cost-effectiveness ratio of <US$40,000/QALY gained; 2-dose/girls only had the lowest cost/QALY gained at US$7900–US$10,400	Incremental value of 3-dose girls only and 2-dose girls+boys varied depending on parameters; 3-dose girls+boys greater than US$100,000/QALY gained under most scenarios
INFLUENZA				
Skedgel et al (2011)[168]	CEA	Canada	Compared to vaccinating only pregnant women with comorbidities, the incremental cost-effectiveness of vaccinating all pregnant women was <US$40,000 per QALY gained	Net cost of universal vaccination was <US$10 per pregnant woman
MALARIA				
Seo et al (2014)[271]	CEA	Malawi	The incremental cost-effectiveness of adding RTS,S malaria vaccine to existing strategies (primarily bednets) was US$145 per DALY averted	The incremental cost-effectiveness was sensitive to vaccine's duration of protection
MEASLES				
Shepard (1994)[175]	CUA	Global	Vaccination results in US$17 per DALY, one of the most cost-effective health interventions in developing countries	
MEASLES-MUMPS-RUBELLA				
Hatziandreu (1994)[306]	CBA	United States	B:C ratio 16.3 for direct costs; 21.3 for total costs	Also considered B:C of individual components: measles 17.2, 17.2 for direct, total costs; mumps 6.1, 13.0; rubella 4.5, 11.1
NEISSERIA MENINGITIDIS				
Trotter et al (2002)[187]	CEA	England and Wales	The cost per life-year saved for an *N. meningitidis* serogroup C vaccination campaign was £6259; school-based campaigns were more cost-effective than vaccine delivered through physician practices	From healthcare provider perspective
Tu et al (2014)[200]	CEA	Canada	Over the lifetime of an Ontario birth cohort, *N. meningitidis* serogroup B vaccine would cost C$4.8 million per QALY gained	Baseline included 10-year duration of protection, vaccine cost of C$75/dose
PNEUMOCOCCAL (CONJUGATE)				
Ray (2002)[229]	CEA	Australia, Europe, United States	Breakeven vaccine cost from the healthcare system perspective averaged US$17 (range: US$4–US$37)	Reviewed multiple studies and scenarios with consideration of multiple outcomes and herd effects

TABLE 78.3 Illustrative Examples[a] of Economic Analyses of Selected Childhood Vaccines *(Continued)*

Reference	Type	Scope	Result	Comment
RESPIRATORY SYNCYTIAL VIRUS				
Meijboom et al (2012)[301]	CEA	Netherlands	Respiratory syncytial virus immunization with a 3-dose infant schedule would cost €34,142 per QALY gained	Over a full year and with 91% coverage, vaccination would prevent 1.5 deaths and 544 hospitalizations
TYPHOID (POLYSACCHARIDE)				
Carias et al (2015)[294]	CEA	Uganda	Over 3 years following a one-time vaccination campaign, typhoid vaccine would cost US$484 per DALY and US$341 per case averted	Results sensitive primarily to preventable disease burden and vaccine cost
VARICELLA AND ZOSTER				
Damm (2015)[255]	CEA, CBA	Various	Review of multiple studies one-dose varicella vaccination of young children would save 1.61 to 19.33 per unit spent; Zoster vaccine mostly cost-effective	Societal perspective; results dependent on importance of exogenous boosting

[a]These are examples of economic analyses are intended to illustrate the kinds of studies that have been done. The results of economic analyses will vary by methodology, the vaccine being used in a particular population, the characteristics of the population and the burden and epidemiology of vaccine-preventable diseases in that population, vaccine safety, effectiveness, potential for herd immunity, cost, and many other variables.

B:C, benefit-to-cost; BCG, bacille Calmette-Guérin; CBA, cost-benefit analysis; CDC, Centers for Disease Control and Prevention; CEA, cost-effectiveness analysis; CUA, cost-utility analysis; DALY, disability-adjusted life-year; DPT, diphtheria, pertussis, tetanus; HMO, health maintenance organization; HPV, human papillomavirus; QALY, quality-adjusted life-year.

TABLE 78.4 Selected Values of the Cost-Effectiveness of Traditional Clinical Preventive Services

Intervention	Median Cost/QALY (US$)
IMMUNIZATIONS AND CHEMOPROPHYLAXIS	
Immunizations and vaccinations	1500
Pharmaceuticals for asymptomatic persons (e.g., hormone replacement)	13,000
SCREENING TESTS	
Cardiovascular disease	3300
Neoplasms	18,500
Osteoporosis screening and treatment	13,000
Screening blood donors against pathogens	355,000
Other disease screenings	11,500
COUNSELING	
HIV risk behavior	1200
Cardiovascular disease risk	74,000
Presurgical autologous blood donation	730,000

QALY, quality-adjusted life-year.

Adapted from Stone PW, Teutsch S, Chapman RH, et al. Cost utility analyses of clinical preventive services: published ratios, 1976-1997. Am J Prev Med. *2000;19(1):15–23; and Tengs TO, Adams ME, Pliskin JS, et al. Five-hundred life-saving interventions and their cost-effectiveness.* Risk Anal. *1995;15(3):369–390.*

Routine Childhood Immunization

A single study evaluated the economic impact of the routine U.S. childhood immunization schedule, including DTaP (diphtheria and tetanus toxoids and acellular pertussis vaccine), Hib (*Haemophilus influenzae* type b) conjugate vaccine, IPV (inactivated poliovirus vaccine), MMR (measles, mumps, rubella) vaccine, hepatitis B vaccine, and varicella vaccine. Routine childhood immunization with the seven vaccines was evaluated as cost-saving, with a societal B:C ratio of 16.5.[19]

Many studies have addressed immunization in developing countries using the vaccines against six diseases initially used in the WHO's Expanded Programme on Immunization (EPI): bacillus Calmette-Guérin (BCG) for tuberculosis (TB), DTP (diphtheria and tetanus toxoids and whole-cell pertussis vaccine), OPV (oral poliovirus vaccine), and measles. They generally demonstrate low costs per immunized child and favorable B:C ratios.[20–24] Studies published in 2000 in Ethiopia, Morocco, Bangladesh, and Côte d'Ivoire indicated a total cost of less than US$25 to fully immunize a child with basic EPI vaccines.[25,26]

The Disease Control Priorities Project estimates that increased coverage from 70% to 79% with the traditional EPI program in South Asia would cost US$8 per DALY averted and that a second opportunity for measles vaccination would cost US$1 to US$5 per DALY averted in sub-Saharan Africa.[27,28]

Bacillus Calmette-Guérin

BCG vaccine primarily prevents disseminated TB in infants and young children, but has little, if any, impact on disease transmission. Development of highly cost-effective strategies in industrialized countries based on case-finding and treatment have made BCG vaccination less competitive as an effective intervention against TB. Because BCG vaccination complicates identification of TB exposure, many countries have discontinued routine BCG vaccination. A B:C ratio of 0.13 for revaccination of schoolchildren was calculated in Japan and represented a very low level of cost-effectiveness. It was subsequently found that the cost of treating a case of pediatric TB in Japan was much lower than the cost of preventing cases through vaccination.[29,30]

In developing countries with high rates of childhood TB, BCG vaccination of infants might represent a better investment. In 2006, a metaanalysis and assessment of the cost-effectiveness of BCG vaccination to prevent childhood tuberculous meningitis and miliary TB concluded that BCG vaccination of infants in Southeast Asia, sub-Saharan Africa, and the western Pacific would cost US$206 per year of healthy life gained.[31]

Diphtheria, Tetanus, and Pertussis and Components

Although tetanus toxoid may be used as a single antigen, diphtheria toxoid is only used in combination with tetanus toxoid, and pertussis vaccine has only been used in combination with the two toxoids, as DTP. Results from economic analyses of single-antigen tetanus toxoid varied among the administration of toxoid to women who were pregnant (or were of childbearing age) to prevent neonatal tetanus, the mass immunization of the general population, and primary or booster vaccination among the elderly. In Haiti, a B:C ratio of 9 for communitywide tetanus immunization was estimated, with the primary benefit resulting from reductions in the occurrence of neonatal tetanus.[32] By contrast, primary immunization of the elderly in Canada was estimated to cost US$810,000 per year of life gained.[33] In the United States, it was estimated that decennial tetanus toxoid boosters would cost US$143,138 per year of life saved compared to US$4527 per year of life saved by application of a single booster at age 65 years.[34] Still, the decennial booster approach has been estimated to prevent four times as many cases as the single booster.

In the combined DTP vaccine, reductions in the direct costs of diphtheria were essentially equal to reductions in pertussis direct costs; however, diphtheria was the major contributor to total savings in indirect costs.[35] Cost-benefit analyses of pertussis vaccine use in the United States consistently show an excess of benefits over costs, even in the face of a dramatically increasing cost of vaccine.[36,37] Newer studies concentrated on the marginal improvement made by the pertussis component of combination vaccines. In many industrialized countries, acellular pertussis vaccines have replaced whole-cell vaccines, which were known to be the component of the combined vaccine most likely to be associated with adverse events. Vaccines that include acellular components of pertussis (available as DTaP) are more expensive than whole-cell vaccines (DTP) and may have an increased waning immunity.[38] There are, therefore, value trade-offs—increased consumer confidence in safer vaccines with possible higher coverage versus the need for additional doses to address waning immunity. In Germany, vaccination with acellular vaccines would be as cost-saving as the use of whole-cell vaccine if coverage could be raised by 7.5%,[39] but could require increased doses for adolescents and adults.[38] The efficacy and prices of different acellular and whole-cell vaccines vary greatly in many populations, which would require specific cost-effectiveness evaluations.

There is a rising incidence of pertussis in the United States, with a high proportion of cases occurring in adolescents and adults. Because of the lower rates of adverse events associated with acellular pertussis vaccines, ACIP has recommended use of Tdap (tetanus and reduced-dose diphtheria toxoids and acellular pertussis vaccine) in adolescents and adults.[40] A study has found that immunizing adolescents 10 to 19 years of age in the United States would be cost-saving.[41] Similar results were found in Canada.[42] Other studies have not found adolescent vaccination to be cost-saving, but have found it to be a good investment nonetheless. A boosting dose at 8 years or adolescence in The Netherlands[43] or adolescence in the United States would cost approximately US$4000 to US$22,000 per QALY saved, assuming various parameters of vaccine cost, waning immunity and quality of life.[44,45] Other studies have shown cost savings for vaccination in adults[46] including those older than 65 years of age.[47]

As DTP has become the platform for other combinations, numerous studies have been performed on the benefits and costs of the convenience of combinations with other antigens (hepatitis B, Hib, inactivated poliomyelitis vaccines). It was estimated that US$90 million to US$150 million per year could be saved in the United States by using combined whole-cell DTP-Hib or DTP–hepatitis B–Hib vaccines compared to using the vaccines separately.[48]

Haemophilus influenzae Type B

The original Hib vaccines used purified capsular polysaccharides effective in toddlers but not in infants. Nevertheless, their use was shown to be economically justified.[49] These vaccines have been replaced by conjugated polysaccharide vaccines, which have almost eliminated disease incidence in all countries that have introduced them into routine schedules. Owing to the greater cost of these vaccines compared to historically used vaccines, their initial evaluation was based on a single dose at 18 months.[50] In 1991, a breakeven price of US$7 was determined when the Hib vaccine is coadministered with DTP.[51] Numerous other economic studies have shown that a routine pediatric schedule with primary doses and a booster were cost-effective in a number of industrialized countries with higher vaccine costs.[51–60] All these studies justified introducing Hib vaccine without necessarily also accounting for the pain and suffering from sequelae and death from a preventable disease.

Developing countries have usually lagged in the introduction of novel vaccines. However, a study in Chile from 1993 showed that a three-dose regimen without booster at a vaccine cost of US$1 had a B:C ratio of 1.7.[61] After a study in Africa demonstrated the utility of Hib vaccine to prevent acute respiratory infections with radiographic evidence of alveolar consolidation or pleural effusion,[62] greater interest arose for their widespread use in developing countries.

Because of vaccine cost, quantification of disease burden in economic terms may be necessary in middle- and low-income countries to demonstrate the utility of its incorporation into routine schedules. A number of economic studies show Hib vaccine to be cost-effective at a vaccine price under US$2 to US$3,[63–65,66] with the exception in western Asia, where disease burden had not been well defined.[67] For many developing countries, the financial considerations for the national purchase of Hib vaccines had been daunting. However, cost-effectiveness studies helped justify to GAVI the benefits of financial support of Hib vaccine for the poorest countries,[68] with disease reduction results similar to wealthier nations. Donor support and improved communication through international initiatives has stimulated demand for the vaccines and increased the number of competing manufacturers, thus increasing vaccine supply, with a subsequent decline in price, which makes this vaccine more affordable to a wider population.

Hepatitis A

Hepatitis A vaccines were licensed in the late 1990s. At first, the widest use in many industrialized countries was in travelers to countries with high endemicity and in those whose behavior put them at higher risk (e.g., men who have sex with men). One study also found that vaccinating food service workers against hepatitis A could cost the health system US$13,969 per life-year saved.[69] The cost-effectiveness of adolescent hepatitis A vaccination in the 10 states in the United States with the highest incidence rates was also studied, with a cost of US$13,722 per life-year saved.[69] In the United States, routine immunization of 1-year-olds was recommended in the 17 states that had the highest incidence of hepatitis A beginning in 1999. Implementation of these recommendations was associated with dramatic declines in the incidence of hepatitis A in these states. In 2006, ACIP recommended universal immunization of children 12 to 23 months of age throughout the United States.[70] Nationwide immunization of 1-year-old

children in the United States was estimated to cost US$173,000 per life-year gained and US$24,000 per QALY gained.[70] In Australia, universal hepatitis A vaccination for infants and/or adolescents was found to have comparable cost-effectiveness compared with other preventive strategies.[71]

One study analyzed the epidemiologic and economic impact of using combined hepatitides A/B vaccination in all 1- to 15-year-olds or all 11- to 15-year-olds in Germany.[72] Although the strategy of vaccinating 11- to 15-year-olds was more cost-effective, it would only prevent one-third as many cases of hepatitis A and one-half the number of cases of hepatitis B as vaccinating all 1- to 15-year-olds. It was concluded that although vaccination of 1- to 15-year-olds was initially more expensive, it was nonetheless more beneficial in terms of epidemiologic impact.

In Catalonia, it was calculated that universal vaccination of preadolescents with combined hepatitides A/B vaccine had a B:C ratio of 2.58 compared to the previous program using hepatitis B vaccine alone.[73] A study in Canada similarly found cost savings if the current practice of vaccinating 9- and 10-year-olds against hepatitis B alone was replaced with a combined hepatitides A/B vaccine.[74] In Argentina and Chile, hepatitis A vaccination of all children at 12 and 18 months of age was found to be cost-saving.[75,76]

Hepatitis B

Hepatitis B poses a particular issue in economic evaluation because the primary benefits of vaccination are the prevention of chronic liver disease and death from cirrhosis or cancer, all of which typically occur many years after the age of vaccination.

Hepatitis B vaccines were relatively expensive when first licensed in the 1980s, prompting many evaluations to identify their most efficient use. Earlier studies considered screening strategies for targeting vaccine use to subpopulations that were at greatest risk for infection, such as healthcare workers, injection-drug users, and persons who were at risk for sexually transmitted diseases.[77,78] The relative costs of vaccination and screening and the prevalence of hepatitis B virus infection in the specific subpopulations studied were the most important determinants of outcomes. Early studies also addressed prenatal screening to identify the most efficient approaches for screening and passive and active immunization.[79–82]

As the cost of vaccines decreased, studies began to address the costs, benefits, and effectiveness of routine vaccination of various age cohorts, including infants and adolescents. Because vaccination is most easily delivered through an existing infant vaccination infrastructure, universal infant vaccination with hepatitis B vaccine has become a common practice, and the WHO has recommended universal infant immunization against hepatitis B since 1992.[83] Many analyses that take into account variations of vaccine price and prevalence of infection in different countries have been conducted.[84–86] Although a number of initial studies in developed countries in Europe determined that infant vaccination would not be cost-beneficial given the declining incidence,[87] studies in the United States,[88] United Kingdom,[89,90] Canada,[91] Switzerland,[92] Israel,[93,94] and Australia[95] have all found that universal infant vaccination was cost-effective.

In settings with higher incidence and prevalence, hepatitis B vaccination is more valuable. It was estimated that the cost to avert a death from liver cancer in The Gambia would be US$1200 to US$1500, using a 6% discount rate.[96] A B:C ratio greater than 40 from routine infant immunization was calculated in Jinan City, China.[97] In India, the cost-effectiveness results of hepatitis B vaccine use ranged from 1 year of life saved for less than one GDP per capita to 1 QALY saved for two times GDP per capita.[98,99] One study estimated a cost of US$47 per DALY averted as a result of infant immunization in Mozambique.[100] Before GAVI was established, hepatitis B and Hib vaccines were estimated to have a global cost per life-year saved of US$29 to US$150, with great variation by income and economic groups of countries studied.[85]

The most cost-effective strategy is not necessarily the best choice to address a particular health problem if it deals only with a limited part of the problem. Williams and colleagues found that the most cost-efficient use of hepatitis B vaccine in the United Kingdom would be to concentrate on high-risk populations. However, that would have only a small impact on the overall hepatitis B disease burden compared with the impact of a more costly universal infant or adolescent immunization strategy.[82] Additionally, experience indicates that vaccination efforts focused on high-risk persons may be more difficult to implement and achieve lower coverage rates than efforts directed at the entire population.

The economic implications of the use of hepatitis B vaccine changed with its presentation in combination with other vaccines. The incremental cost-effectiveness from combined Hib–hepatitis B vaccines in the United States compared to separate vaccines has been estimated at US$17,700 per life-year saved.[101] One study found that using combined DTP–hepatitis B–Hib vaccines in the EPI would be cost-effective compared to using them separately.[102] Subsequent studies with lower-cost and combination vaccines greatly improved the cost-effectiveness.[86]

Human Papillomavirus

The HPV vaccine, another cancer-prevention vaccine, has a number of similarities to the hepatitis B vaccine. Both involve two types of latency periods, the time between vaccination and potential infection to others and the prolonged interval between infection and severe manifestations of disease. Each prevents a sexually transmitted infection that has the potential to cause malignancy later in life, as well as less-severe events that occur earlier. Because the largest benefits accrue to both the patient and to society decades after vaccine administration, decisions pertaining to the optimal age of vaccination and discount rates of future benefits greatly affect economic analyses.

Quadrivalent and bivalent HPV vaccines protect against serotypes that are associated with 70% of cervical cancers, with an additional 10% to 15% coverage from the more recent nine-valent vaccine. Because HPV vaccine is specific to certain papilloma types, it does not obviate the need for routine cervical cancer screening, which covers a broader range of abnormal cervical pathology and papilloma types. Consequently, HPV vaccination could reduce the overall cost and frequency of screening if disease onset is delayed or terminated prematurely.[103,104] By contrast, if HPV immunization programs reduce screening utilization, it may reduce the impact of that prevention program. Because screening programs are integral to cervical cancer prevention programs regardless of vaccine introduction, the full benefit of these programs for secondary prevention should be included in economic models, although a recent analysis suggests this seldom occurs.[105] Beyond the issue of screening, a recent analysis concluded that most existing economic studies captured only a fraction of generated values, with data frequently lacking for productivity and patient and caretaker time and costs.[106]

A study using the Papillomavirus Rapid Interface for Modeling and Economics (PRIME) assessed cost-effectiveness for prevention of cervical cancer through vaccinating 12-year-old girls across 179 countries.[107] Assuming lifelong protection, the authors reported for a cohort of 58 million children a

reduction globally of 690,000 cancer cases and 420,000 deaths at a cost of US$4 billion. This equated to a cost per DALY averted less than the GDP per person in 87% of countries. A systematic analysis of GAVI-eligible countries found that at a vaccination cost of US$2 per dose, HPV vaccine would be cost-effective for all the poorest nations.[108] Numerous studies at the national level using different models have used favorable economic analyses as an aid to justify the expenditure for HPV vaccination in public programs, most of which have focused on HPV vaccination in combination with screening among 11- to 12-year-old girls.[103,104,109–111,112–116]

While a consensus exists on the efficiency of HPV vaccination in combination with screening for young girls, other issues remain to be resolved. Some studies found that vaccination of males adds little to vaccinating women alone.[109,117,118] However, other studies incorporating all HPV-related diseases found that vaccination of males may be cost-effective even at current vaccine prices, particularly when female vaccination coverage is low.[119–121] The utility of providing catch-up vaccination to older females (such as those <30 years of age) remains unknown, with results dependent on the assumption that vaccine has some effect for nonnaïve women.[122–124] Immunization of nontraditional high-risk populations to prevent anal cancer, such as men who have sex with men, also remains poorly evaluated, with some studies suggesting such an approach may be cost-effective.[125] Lastly, the benefit of more than two doses has been questioned.[106]

Different groups have reached different policy conclusions from the available data. In the United States HPV vaccination is recommended for universal administration to girls and boys at 11 to 12 years of age, with catch-up vaccination recommended for females through age 26 years and males through age 21 years with a permissive recommendation for males through age 26 years.[126–128] The WHO recommends a first priority of achieving high coverage with two doses spaced 6 months apart among females 9 to 13 years of age, with possible extension to older adolescents and young women as a secondary step; the WHO does not consider vaccination of males a priority.[129]

Influenza

Despite the frequently changing antigenic structure of the influenza virus and recent studies that have challenged the overall protective effects of influenza vaccines in the elderly,[130,131] the high annual attack rates of influenza and death rates among the elderly have historically been cited to justify expenditures to provide annual influenza immunization to persons older than 65 years of age and persons who are chronically ill. Newer pharmacoeconomic studies have concentrated on the expansion of vaccination efforts to other target age groups.

One of the earliest influenza cost-analysis studies assessed the cost of annual vaccination.[132] U.S. Congressional interest through the Office of Technology Assessment supported studies to evaluate federal funding of influenza vaccine. Assessments supported vaccine use in persons older than 64 years of age and in high-risk groups,[133] although they called for better epidemiologic data on transmission. In Australia, influenza vaccination was deemed a cost-effective intervention for healthy populations 45 to 64 years of age.[134]

The required annual vaccination needed to control disease caused by the dynamic evolution of influenza viruses has continuously challenged public healthcare systems. Economic analyses have been performed to assess administrative costs of annual programs that target various age groups and working populations. These analyses are undertaken from numerous perspectives, such as that of the healthcare system, society, or the employer. Newer studies have concentrated on recommendations that have expanded target groups to the pediatric population.

Because children are the primary force of transmission of influenza, increased attention has been placed on the desirability of vaccinating children both to protect them and indirectly protect high-risk groups, such as the elderly. Studies have focused on the vaccination of children 6 to 24 months of age. Vaccination of high-risk children was modeled to be cost-saving[135] compared to indiscriminate vaccination.[136,137] Vaccination of other age target groups modeled the cost-benefit analysis at 6 to 23 months, 6 to 59 months, and 5 to 14 years at various proportions of high-risk conditions.[138] The targeted vaccination of high-risk groups was universally cost-saving. Breakeven costs between US$45 and US$48 per administered dose were found for vaccinating the three age cohorts when these groups contained 10% high-risk children. In 2010, ACIP recommended annual influenza immunization of all people in the United States 6 months of age and older. Cost-effectiveness analysis shows this strategy costs twice as much as a targeted program but reduces influenza-related healthcare services by 52%.[139]

Influenza is unique among the vaccine-preventable diseases in that it requires annual revaccination; therefore, administration costs are highly relevant to economic analyses. Programmatic considerations in school-based programs greatly influenced the potential impact of group-based vaccination of healthy children; in school-based settings, US$35 per child was saved versus US$4 for individual-initiated programs from individual office visits.[140] These studies did not account for the indirect effects.

With the advent of health maintenance organizations, there has been a greater demand to justify vaccination programs from the provider's perspective. Although the effectiveness of vaccination programs to prevent hospitalizations for pneumonia and influenza is only 30%, vaccinating elderly persons saved US$1.10 for each person vaccinated. Furthermore, when groups were stratified, the net cost was only US$4.82 per vaccination for non–high-risk elderly persons, a negligible amount when pain and suffering are also taken into account.[141] Influenza vaccination in the elderly was found to be cost-saving when extended to other health outcomes, such as all hospitalized illness and mortality.[142,143] Although vaccination of elderly persons with high-risk conditions was found to be cost-saving, results for groups with fewer chronic conditions were more equivocal.[144,145] The paucity of randomized, controlled trials of vaccine efficacy in the elderly require cautious interpretation of these findings.

Because most of the estimated annual US$71.3 to US$166 billion (2003) in expenditures owing to influenza is attributable to lost productivity,[146] many economic studies on influenza control concentrate on younger, healthy workers. An average number of workdays missed owing to influenza-like illness by workers with work loss was valued at US$137 per person.[147] Algorithms that incorporate disease epidemiology, worker productivity, and economic cost allow the economic evaluation of influenza vaccination.[147] One study found a breakeven vaccination cost of US$16 when time lost from work exceeded 2.4 days per episode of influenza.[148] Some programs found that routine annual vaccination of healthy adults was cost-saving from the employee and employer perspectives,[149,150] but estimates are sensitive to many assumptions[151] as well as to the specificity and sensitivity of measured outcomes.[152] Vaccination was not always cost-saving, especially in seasons where the circulating virus differed from the vaccine strain.[153] In the United Kingdom, annual incidence rates as low as 2% still provided economic justification for influenza vaccination,[154] and in The Netherlands, many modeled scenarios

showed savings.[155] However, reviews of studies highlight the disparities of findings of vaccinating the healthy adult population, demonstrating the sensitivity of results to numerous assumptions.[156]

Influenza vaccine was also found to be cost-saving in a variety of settings in middle-income countries, including South Africa (seniors and healthy working adults),[157] Brazil (workers),[158] Mexico (elderly),[159] and Thailand (elderly),[160,161] but not in Hong Kong.[162]

The licensure of live attenuated influenza vaccines has led to their economic evaluation in children[163] especially as the cost differential with other influenza vaccines decreased.[164] For young healthy adults, the break-even cost of live attenuated influenza vaccines and its administration was between US$25.72 and US$58.92.[165]

The WHO has identified pregnant women as the highest priority target group for influenza immunization, based largely on studies in developed countries demonstrating an increased risk of hospitalization following infection with pandemic A(H1N1).[166] An economic analysis of vaccinating pregnant women against influenza in the United Kingdom showed this strategy would cost approximately US$35,000 per QALY gained with variation depending on whether vaccine provided protection over more than one season, whether vaccine protected infants as well as mothers, vaccine efficacy, and costs of hospitalization, outpatient visits, vaccine, and vaccine administration.[167] A Canadian study found vaccine to be cost-saving when targeted to pregnant women with comorbidities and cost-effective if delivered universally to pregnant women; compared to the UK study, an additional important factor in determining cost-effectiveness was duration of illness.[168]

A randomized clinical trial in Bangladesh found that influenza vaccine of pregnant women prevented a substantial amount of febrile respiratory illness and low birth weight birth among their infants.[169,170] A subsequent study in South Africa found an impact on mild illness, particularly among mother–infant pairs where the mother had HIV infection, but no impact on severe disease or birth outcomes.[171] Using reduction in infant outcomes to drive the economic argument for maternal immunization in developing country settings will depend on impact against severe outcomes rather than solely mild respiratory infection, the programmatic cost of delivering vaccine during pregnancy, and the impact of seasonality and trimester of vaccine receipt on vaccine efficacy.

Measles, Mumps, and Rubella

Measles, mumps, and rubella vaccines as individual antigens have repeatedly been shown to be cost-saving when administered to young children. The B:C ratio rises even higher when the vaccines are administered in combination because the costs of administration are substantially lower. For measles vaccine, the B:C ratio for a single dose is high; of particular interest, however, is whether the additional benefits derived from a second dose also outweigh the additional costs.

A 2004 study of a two-dose MMR program in the United States found it to be highly cost-saving, with B:C ratios of 14.2 and 26.0 for direct cost and societal perspectives, respectively. The incremental B:C ratio of the second dose showed ratios of 0.31 and 0.49, respectively.[172]

Measles is a major cause of death in developing countries, causing an estimated 139,300 deaths globally in 2010, with India accounting for 47% of the estimated mortality and the WHO African region for an estimated 36%.[173] In the Ivory Coast, the cost per death averted in 1986 was found to be US$479.[174] A 1994 study estimated the global cost of US$17 per DALY averted, an excellent buy.[175] Although a single dose of measles vaccine provides protection to approximately 80% to 95% of young children in developing countries, experience has shown that two doses are needed to provide the level of population immunity required to have a dramatic impact on measles mortality.[176] The second dose can be administered either by adding a second dose to the routine immunization schedule or by carrying out periodic campaigns (supplemental immunization activities) that can reach children who received the first dose as well as children who were missed by the routine immunization system. A study comparing the cost-effectiveness of supplementary immunization for measles in India concluded that supplementary immunization activities for measles are cost-effective but could not be considered superior to a second dose of measles in routine immunization.[177]

With the cost-effectiveness of measles, mumps, and rubella vaccines well established, attention is shifting to considerations of the cost-effectiveness of elimination or eradication strategies. In the English-speaking Caribbean, estimates have been made of the B:C ratio for a rubella-elimination initiative on top of the routine MMR immunization of 1 year olds. B:C ratios of 4.7 for Barbados,[178] 38.8 for Guyana,[179] and 13.3 for the entire Caribbean[180] have been calculated, with a cost of US$2900 per case of congenital rubella syndrome prevented. Analyses of the cost-effectiveness of global measles eradication have shown that eradication of measles would be a good economic investment; eradication would be more cost-effective than maintaining a very high level of measles control.[181]

Meningococcal Vaccines

Disease caused by *Neisseria meningitidis* historically has been addressed using polysaccharide vaccines against the four major serogroups A, C, Y, and W. With few exceptions, polysaccharide vaccines have not been used in routine schedules owing to perceived limitations of the duration of effectiveness, especially when administered during infancy, and thus this review will focus on newer polysaccharide-protein conjugate vaccines as well as subcapsular vaccines for serogroup B.

Because most vaccines are serogroup specific, the key epidemiologic feature for meningococcal vaccine is serogroup prevalence, which can differ dramatically. For example, serogroup A infections have vanished from developed countries without routine vaccination and several populations in Asia that have routinely used polysaccharide vaccines in children.[182,183] By contrast, the African meningitis belt has experienced seasonal endemic serogroup A disease combined with massive regional outbreaks every decade or so.[184] Moreover this region of Africa has experienced large outbreaks of X,[185] W,[186] and for the first time during 2015 in Niger serogroup C.

Studies have reviewed the cost-effectiveness of various strategies including routine coverage with monovalent meningococcal C[187–191] or tetravalent meningococcal ACYW conjugate vaccines.[192] In general, analyses were sensitive to age-specific incidence of disease, and therefore the cost-effectiveness is based on assumptions of the duration of immunity from the different formulations of these vaccines and the potential impact on the ecology of the different *N. meningitidis* serogroups. Monovalent serogroup A conjugate vaccine has successfully eliminated serogroup A outbreaks in the meningitis belt[193]; economic studies have not been done based on the assumption that control of massive disruptive epidemics in one of the world's poorest areas justified vaccine use.

The high societal fear of meningococcal meningitis has led to the development of serogroup C–specific conjugate vaccines for use in infancy and a tetravalent conjugate vaccine. Although the incidence of serogroup C disease is relatively

low, a number of nations have adopted routine vaccination, partially based on economic studies.[191] Specific serogroup C conjugate vaccines were introduced into the routine infant immunization scheme in the United Kingdom, with subsequent published analyses of their cost-effectiveness in various epidemiologic scenarios.[187] A review was conducted on the role of economic evaluations on decisions about the introduction of the conjugate vaccines in 21 countries.[191] A positive correlation was found between reported incidence rates of serogroup C meningococcal disease and the performance of economic evaluations. In The Netherlands, routine childhood vaccination at 14 months was estimated to cost €2200 to €2400 per life-year gained, and up to €17,700 per-life-year for catch-up compared to €147,000 for a vaccination program implemented during infancy.[190] In Canada, a three-dose program was modeled as most-effective, and a one-dose program with a vaccine cost of C$50 yielded US$23,000 per life-year gained.[189]

With the advent of the tetravalent meningococcal vaccine against types A, C, Y and W disease, there have been a number of assessments of its utility. Cost-effectiveness depends on local epidemiologic conditions. For the United States, for example, a catch-up and routine vaccination program for adolescents would prevent 48% of disease over a 10-year period at a societal cost of US$223,000 per case averted, approximately US$2.6 million per death prevented, and approximately US$88,000 per QALY at a cost per vaccinee of US$83.[194]

The economic case for use of serogroup B subcapsular vaccines remains controversial. A study from England concluded an infant vaccination program would be cost-effective at a cost of approximately US$15 per dose, rising to US$25 per dose assuming substantial impact on carriage as well as disease.[195] Despite this, the UK Joint Committee on Vaccination and Immunisation originally concluded four-valent serogroup B vaccine would not be cost-effective at any vaccine price.[196] This position was criticized as overemphasizing economic analyses rather than reduction in long-term suffering and death,[197,198] and was subsequently modified based on results using different model assumptions.[199] A later evaluation from Canada concluded an infant serogroup B immunization program was unlikely to be economically attractive.[200]

Pneumococcal Vaccines

As with influenza vaccination, the use of pneumococcal polysaccharide vaccine for the elderly and chronically ill has demonstrated a reasonable cost per QALY gained.[201–212] Additional assessments have also been made for children in developing countries[213] and healthy adults in the military.[214] Given different levels of age-specific incidence in healthy adults, one study found that this vaccine would be cost-effective at the minimal levels of vaccine effectiveness against pneumonia of 30% and 71% for ages 35 and 22 years, respectively.[215] With the advent of conjugate vaccines, more recent studies have concentrated on the cost-effectiveness of routine vaccination of pediatric populations.

A prelicensure study on the cost-effectiveness of the original seven-valent conjugate vaccine established a breakeven vaccine cost of US$46, where the expected treatment savings from routine use would offset the financial commitment to vaccinate the U.S. childhood population.[216] The manufacturer, who had a monopoly at the time, offered this vaccine to the public sector at this price. Although it was cost-effective for the United States at this cost, the vaccine was considered prohibitively expensive for many other populations in need. Likewise, at this cost, vaccination in other developed countries, such as The Netherlands,[217] Australia,[218] and Canada (with a B:C ratio of 0.57), was not cost-effective.[219] Given various economies and incidence rates in different populations, pneumococcal conjugate vaccine was considered to be cost-effective for Switzerland,[220] Spain,[221] the United Kingdom,[222] and Germany,[223] though often at the higher limits. In one review, cost-effectiveness ranged from US$26,000 to US$66,000 per QALY.[224]

Assessments that account for reduced transmission of the pathogen through herd effects provide a more favorable cost-effectiveness ratio.[225] Several reviews assessed the sensitivity of cost-effectiveness, mainly because of vaccine cost and whether societal perspectives were included.[226] The relatively high cost of this vaccine has led to the analysis of alternative strategies, such as reduced number of doses,[227] to justify its use. Nongovernmental organizations and public and private partnerships continue to work on demonstrating the utility of this vaccine for the public sector and to help with tiered pricing mechanisms so that a cost-effective intervention is also financially feasible for lower-income populations.

An example of the differences of the cost-effectiveness of the direct and indirect impact of vaccination was shown in a particular setting assessing the seven-valent pneumococcal conjugate vaccine. The direct cost was estimated at US$201,000 per life-year saved, and the indirect benefit of reduction of invasive pulmonary disease in the older population reduced this cost per life-year saved to US$10,400.[228,229]

The cost-effectiveness for this vaccine for resource-poor countries is more ambiguous. One analysis for The Gambia estimated that at a vaccine price of US$3.50 per dose, seven-valent pneumococcal conjugate vaccine would save a life at approximately threefold the national GDP, with uncertainty about type-specific circulating strains, replacement serotypes, and herd-immunity effects.[230] With the advent of additional valent vaccines and dynamic pricing and serotype distributions, the utility of any of these vaccine formulations should be assessed at the local level.

Poliomyelitis

A number of past analyses have favored the global eradication of polio, but they were based on assumptions that are no longer valid.[231,232] One study models the economic impact of various strategies and concludes that eradication, defined as the interruption of wild poliovirus transmission globally, is less costly and more effective than continued control.[233,234] However, the scenarios favoring eradication, defined as the cessation of wild-type virus transmission, assumed that during the posteradication period, vaccination with OPV could cease without replacement by IPV. This concept has been seriously challenged,[235–239] given the likelihood that poliomyelitis can reemerge.[240]

Taking into account the need for continued use of polio vaccines to ensure long-term control or sustained eradication, the CDC estimates the cost for eradication strategies is on the order of US$20 billion to US$23 billion through 2020 based on eradication of wild poliovirus by 2010.[241] One paper estimates a cost of US$31 billion,[242] given the generally accepted requirement to use currently supply-limited IPV for the foreseeable future,[238] but the cost would be offset by the savings from reducing the incidence of poliomyelitis from 1988 to 2035 compared to the preeradication era. Costs per DALY saved range from US$210 to US$1000 for low-income and low-middle income countries, respectively, to cost savings for upper-middle-income countries.

Economic assessments have been conducted on switching from OPV to IPV as natural disease is eliminated in populations. In the United States, an estimated B:C ratio of 6.1 was found for an OPV-only schedule, and a four-dose sequential IPV-OPV schedule had a ratio of 5.7.[35] One study found that

changing from an OPV-only schedule to an IPV-only schedule or a sequential IPV-OPV schedule would not be cost beneficial; the cost per case of vaccine-associated paralytic poliomyelitis averted was estimated at US$3.0 million and US$3.1 million for these options, respectively. The fact that the United States and other countries opted for IPV use with knowledge of these studies indicates the investment that the U.S. public health programs are willing to expend to avoid preventable adverse events in publicly mandated vaccine programs.[243]

A study found that introducing IPV into the Australian immunization schedule was not likely to be cost-effective unless it came in a combined vaccine with the IPV component price less than US$10.[244] Another analysis compared the cost-effectiveness of maintaining routine and intensive OPV use after eradication with that of switching to IPV use. It found that considering current costs of routine and intensive OPV use, adopting IPV to replace OPV will not increase the total global cost.[245]

As part of the strategy to eradicate polio, the WHO has recommended all countries introduce at least a single dose of IPV in the routine infant EPI schedule, followed by a switch from trivalent, serotype-2 containing OPV to a bivalent vaccine without OPV. Because of the widespread commitment to global eradication, this decision was made in the absence of short-term considerations of cost-effectiveness to individual countries and to the need of continued use of IPV for at least some unspecified period following eradication to guarantee sustained eradication.

Rotavirus

Rotavirus infection is nearly universal in infants, and it is a major cause of diarrhea around the world. Rotavirus vaccines have been licensed and recommended for universal use in the United States.[246] Earlier studies have found that rotavirus immunization in the United States would be a good investment, although not necessarily cost-saving.[247,248] A CDC study found that from a societal perspective, rotavirus vaccination would likely be cost-effective at a total cost per child of up to US$156 (approximately US$42/dose). At a cost of more than US$268 per child (approximately US$79/dose), a vaccination program would most likely have a net cost to society.[247]

A study from Finland found that the break-even cost for vaccine was equivalent to US$19.60.[249] A similar study from Australia estimated that rotavirus vaccination would be cost-neutral to the healthcare system at a vaccine price of US$19 and cost-neutral to society at a vaccine price of US$26.[250] In Vietnam, it was concluded that universal vaccination of infants at a vaccine cost less than US$7.26 per dose would be considered cost-effective by World Bank standards for low-income countries (i.e., <US$140/DALY).[251] The recent licensure of a low cost vaccine from Indian manufacturers holds promise for higher volume uptake at lower costs.[252]

The ubiquitous nature of rotavirus and high rates of medical expenditures for treatment might justify higher premiums for this vaccine where it is affordable and where the public sector is responsible for healthcare costs.[251] Economic considerations for use of this vaccine should take into account the lower efficacy and higher incidence of severe outcomes found in resource-poor settings.

Varicella

Varicella vaccination has generally not been shown to be cost-saving when only direct medical costs are considered. However, it is clearly cost-saving in developed countries when the indirect costs of lost income from caregivers are included in the analysis. Before licensure of the vaccine in the United States, a B:C ratio of 6.9 was estimated with a hypothetical vaccine, with most of the savings from indirect costs averted.[253] Another study estimated a B:C ratio of 2.0 for routine vaccination in the United States.[254] In the United States the B:C ratio was estimated to be 5.0, and the medical cost saved per life-year was around US$2500.[255] Scuffham and colleagues[256] estimated a B:C ratio of 2.8 for routine varicella vaccination in New Zealand; in Australia, routine varicella vaccination was not found to be cost-saving, considering direct medical costs alone. A B:C ratio of 1.6 was calculated for universal immunization of 15 month olds in Spain (including indirect costs).[257]

Although the varicella vaccine is effective, its effectiveness is not as high (or potentially as durable) as desired. Consequently, the ACIP has recommended that the immunization schedule include two doses of varicella-containing vaccine.[258]

Varicella vaccination of healthcare workers has also been studied. One study analyzed the experience with varicella exposures among hospital healthcare workers and found costs associated with furloughing susceptible workers and employing replacement workers were significantly higher than the cost to vaccinate all susceptible workers (B:C ratio = 3.0).[259] Vaccination of potentially susceptible healthcare workers could potentially result in net cost savings of US$59 per person.[260]

The long-term impact of universal varicella vaccination on the subsequent occurrence of zoster (shingles) is not clear. Some estimate an increase in zoster as a result of a decline in exogenous boosting of varicella zoster antibody titers owing to reduced circulation of varicella virus, although there might be a subsequent decrease in incidence of zoster once the vaccinated cohort ages.[261] A higher-titer varicella vaccine to prevent zoster (shingles) is effective,[262] has been licensed, and is now recommended for use in all persons 60 years or older in the United States.[263] A published economic analysis estimated a cost of less than US$100,000 per QALY gained if the unit cost of vaccination is less than US$200, the vaccine is administered before the age of 70 years, and vaccine efficacy lasts at least 30 years.[264]

Varicella vaccine has been combined with MMR vaccine as MMRV, which is widely used in the United States, presumably adding to its cost-effectiveness. The cost-effectiveness of a two-dose schedule of MMRV in young children compared to a single dose of varicella vaccine in adolescents in Germany showed it to be cost-saving.[265] However, evidence has shown that recipients of MMRV have a higher incidence of postvaccination febrile seizures than those who receive MMR and varicella separately (at the same visit) and has led the ACIP to suspend its preference for use of the combined MMRV vaccine for the first dose of vaccines.[266]

Malaria

While not currently licensed, the RTS,S vaccine against *Plasmodium falciparum* has completed Phase III studies, including a 4-year follow-up,[267–269] and as of July 2015 had received a positive scientific opinion from the European Medicines Agency.[270] For clinical malaria, and among children vaccinated at 5 to 17 months of age, these studies demonstrated a 36% overall reduction (range: 22% to 75% by transmission setting) with a booster and 28% without a booster (range: 18% to 66%); this corresponded to a reduction in cases of 1.8 and 1.4 per child vaccinated with and without a booster. Impact on severe disease was less, and overall was 0 at 48 months without a booster. Waning immunity occurred by 4 years in the absence of a booster. The latter issue is particularly problematic: a study from the Malawi RTS,S study site assessed cost-effectiveness relative to long-lasting insecticide-treated nets and concluded that vaccine would be highly cost-effective

across a wide range of assumptions, but was sensitive to immune duration.[271] Other considerations for economic analyses of malaria vaccines will include further assessment of earlier safety signals, optimal age of vaccination, and relative benefit of vaccine when integrated with other interventions with differing levels of coverage.[272]

Dengue

Two Phase III studies of a novel dengue vaccine have been completed, in Asia[273] Latin America.[274] Both of these studies found moderate vaccine efficacy against all virologically confirmed disease that was lowest for serotype 2, a large vaccine preventable disease incidence for mild disease, and a smaller vaccine preventable disease incidence—despite higher vaccine efficacy—for dengue hospitalization as the latter was relatively rare in the study population. Analyses conducted in advance of these pivotal results have shown that vaccine is likely to be cost-effective and in some circumstances cost-saving at vaccine costs up to approximately US$300[275–277] largely as a consequence of the massive costs of outbreaks.[278] Factors found to influence results include immune duration, vaccine cost, number of doses, vaccine efficacy based on serotype distribution in different settings, and the definition and accounting for costs of dengue outbreaks.

Specialized Vaccines

Adenovirus

Oral adenovirus vaccines had been used for 25 years in special populations (e.g., military recruits) before production was discontinued owing to regulatory issues relative to the manufacturer. Adenoviruses have historically contributed to the high burden of respiratory infections among military recruits. The resultant cessation of vaccination and the depletion of vaccine stores in 1999 led to a resurgence of adenovirus types 4 and 7 among military recruits.[279,280] This vaccine is an example of the fragility of vaccine supply and the economic situation of a single large purchaser (the U.S. military) and a single manufacturer. When commitments from the military to help update the manufacturer's production facilities could not be guaranteed, production was suspended, resulting in the need for costly expenditures to relicense the product. Had appropriate economic studies been done before this vaccine was discontinued, capital investments might have been justified to prevent cessation of production.

Studies have set out to examine the costs and benefits associated with reacquiring and using vaccines among military recruits, including the alteration of the vaccination schedule.[281] It was estimated that terminating the program would cost US$26.4 million and result in 12,370 cases of adenoviral acute respiratory disease. It was concluded that a seasonal program would be most cost-effective, preventing 7800 cases of acute respiratory disease and saving US$16.1 million in direct and indirect costs.[281] A similar study found that a seasonal vaccine program among naval recruits could save US$2.8 million and avert 4015 cases compared to a year-round program, which would prevent 4555 cases of acute respiratory disease and save the military US$2.6 million per year.[282] Economic reevaluations have reinforced the utility of adenovirus vaccines despite the increased funding that was needed to resume production.

Anthrax

Anthrax vaccines have long been used among humans and livestock in other countries, and in 1970, the U.S. Food and Drug Administration licensed the anthrax vaccine adsorbed (AVA; trade name BioThrax) for human use. In 1997, all military personnel were scheduled to receive anthrax vaccine. After anthrax bioweapon scares in 2001, ACIP supplemented their previous recommendations on anthrax vaccine use for the U.S. civilian population. Individuals at risk for repeated exposure to *Bacillus anthracis* were recommended to be given priority for preexposure vaccination. Despite being deemed to be safe and efficacious,[283] the use of anthrax vaccines and other prophylaxis and treatment strategies to protect against bioterrorist attacks remains controversial. Because the primary vaccination series entails six doses, postexposure vaccination does not seem feasible.

Two studies have set out to address the cost-effectiveness of various prevention and treatment strategies to reduce the mortality associated with a potential attack of aerosolized anthrax. One study found that in the case of an actual anthrax attack, three postexposure doses of anthrax vaccine and antibiotic therapy is the most effective strategy (0.33 life-year gained per person) and the cheapest strategy (US$355 saved per person) compared to vaccination alone.[284] The study concluded that in a preexposure or preattack scenario, a no-vaccination strategy would be cheaper than a vaccination scheme and would also provide marginally higher QALYs gained per person owing to the potential adverse effects of the vaccine.[284] These estimates are based on the probabilities of attack (1%) and the probability of exposure in the event of an attack (10%). The second study found that if an exposure was unlikely to occur or was small in scale, neither a vaccination nor an emergency surveillance and response program would be cost-effective.[245] Only if an attack was probable would the emergency surveillance and response system be more cost-effective than vaccination (US$73 versus US$29,600 per life-year saved).[285]

Cholera

The earliest economic studies regarding vaccines against cholera were geared toward their use as a preventive measure for travelers.[286] With the rise of endemic areas in Latin America during the 1990s, investigators have tried to justify the utility of routine vaccines against cholera by factoring in the potential prevention of macroeconomic trade disruptions.[287]

As in other vaccines against enteric diseases, the utility of cholera vaccines must be judged relative to other interventions designed to prevent and mitigate the effects of enteric infections, particularly improved water and sanitation and the use of oral rehydration therapy. A comprehensive CEA of cholera vaccination, which provided estimates for study sites in Mozambique, India, Bangladesh, and Indonesia, found that when the known immunological herd effect of cholera vaccination[288] is taken into account, all immunization programs in all four countries would be cost-effective.[289] Cholera outbreaks are difficult to predict and recent results emphasize the importance of incidence—as well as vaccine cost—in determining cost-effectiveness.[290] These results have been used to justify creation of a vaccine stockpile for outbreak control, but this stockpile is hampered by lack of a clear vaccination strategy.

Japanese Encephalitis

Japanese encephalitis is a mosquito-borne viral disease that is endemic throughout Asia and occasionally occurs as an epidemic. The death-to-case ratio in children is estimated at 10%; 37% of survivors can manifest subsequent neurologic disability. A live attenuated vaccine has been developed, and its impact as a routine childhood immunization in Bali, Indonesia, has been assessed. The conclusion was that routine vaccination of children in this setting was highly cost-effective.[291]

The Bill & Melinda Gates Foundation has invested in the development of a number of vaccines against arboviruses including Japanese encephalitis, dengue, and yellow fever. Furthermore, an inactivated Japanese encephalitis vaccine is available for travelers.

Typhoid

Three formulations of typhoid vaccine now exist: a parenteral Vi polysaccharide vaccine, Vi-conjugate, and an attenuated live oral vaccine. None have gained wide acceptance for community-based administration, perhaps owing to the underestimation of the burden of typhoid[292] attributable to factors such as limited healthcare access in high-burden communities, poor laboratory diagnostics, and failure to appreciate long-term sequelae. A cost-effectiveness study of use of Vi vaccination of all children 2 to 15 years of age in Kolkata, India; Karachi, Pakistan; North Jakarta, Indonesia; and Hue, Vietnam, found that the cost per DALY averted would qualify such vaccination as highly cost-effective in Kolkata, Karachi, and North Jakarta, but not in Hue, where the incidence of typhoid is substantially lower.[293] The newest Vi-conjugate holds the promise of offering long-term protection from infancy, and manufacturers are marketing this vaccine in Asia. A study in Uganda reported vaccine would be highly cost-effective when used in a typhoid endemic area.[294]

Yellow Fever

Live attenuated yellow fever vaccine has been available for decades and has been recommended for routine universal vaccination in yellow fever–endemic zones as well as for emergency outbreak control. A CEA for Nigeria indicated that for a single moderate-size epidemic, the cost-effectiveness of emergency mass immunization was twofold higher than that of a cumulative routine immunization (EPI) program. However, the EPI approach was more effective in preventing cases and deaths. In settings of epidemics occurring over successive years, the EPI approach was more cost-effective than emergency mass vaccination.[295]

Vaccines in Development

HIV/AIDS

The HIV/AIDS pandemic has been recognized as one of the largest public health threats over the last 30 years. Behavioral modification approaches and highly active antiretroviral therapies, along with other therapeutic modalities for the treatment of opportunistic infections, have contributed to primary and secondary prevention, but these are suboptimal and relatively costly. Therefore, the value of alternative means of control has become increasingly important, particularly the development of a preventive or therapeutic vaccine. As the quest for a vaccine against HIV/AIDS is pursued, increased attention will be given to the potential cost-effectiveness and financing of vaccines relative to and in conjunction with other control measures.[296] Economic analyses and estimations of effectiveness through modeling efforts are crucial in helping manufacturers and public health policy makers plan for market demand and for increased production capacity early in the development cycle.

Group B Streptococcus

Group B *Streptococcus* is a major cause of neonatal sepsis, pneumonia, and meningitis and thus in principle would be a good candidate for maternal immunization. A cost-effectiveness evaluation of a hypothetical group B *Streptococcus* vaccine in the United States found that immunization added to screening might prevent 899 cases and 35 deaths annually at a cost of US$91,321 per QALY gained.[297] In South Africa, risk factor based intrapartum antibiotic prophylaxis was estimated to prevent only 10% of cases, a figure that rose to 48% with the addition of a hypothetical vaccine.[298] Sensitivity analyses for these studies showed the importance of disease incidence, vaccine efficacy, and vaccine cost in determining the results.

Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) is a major cause of acute respiratory infectious disease and hospitalization in the pediatric and elderly population. However, documented case-fatality ratios are relatively low[299]; this latter feature may occur because critically ill children are less likely to receive diagnostic testing, thus underestimating mortality rates. An economic assessment of a hypothetical RSV vaccine emphasized the importance of mortality rates in determining economic utility.[300] In The Netherlands, and despite an estimation of 1.5 annual deaths averted, RSV vaccination in infants might be cost-effective but will depend on schedules, efficacy, inclusion of out-of-pocket expenses, and vaccine cost.[301] Similarly, two studies evaluated the potential utility of immunization programs targeting the elderly and both found that under some circumstances this might be an economically reasonable approach but depended on model values for RSV-associated mortality, life expectancy, determination of QALYs in the elderly population, and duration of immunity.[302,303]

OTHER ECONOMIC CONSIDERATIONS

Other economic considerations include the price of vaccines, their affordability, and the value placed on them. Traditional EPI vaccines have been relatively inexpensive in developed and in developing countries, reflecting lower prices of research, development, and production in the past and the fact that research and development costs have already been recovered. Newer vaccines are more expensive because of increased costs of development and production and of intellectual property rights. The question of how to make these vaccines available in developing countries has been addressed by a number of strategies, including tiered pricing and the creation of the GAVI, which provides grants to developing countries to purchase and administer newer vaccines. Seventy-two countries were originally eligible for GAVI support based on their annual per capita income of less than US$1000/year. Beginning in 2011, as a result of improvements in income, eligibility criteria were revised to require an annual per capita income of less than US$1500/year; 56 countries are now eligible. As of 2017, GAVI is supporting 12 vaccines.

The ultimate goal is for all countries to place a high enough value on vaccines to give them priority within their national budgets. This is particularly problematic as per capita public expenditure on health may be less than US$20/year, which was true in 13 countries in 2007.[304] Although the cost of fully immunizing all the children in the world will become increasingly difficult as the result of a mounting number of more-expensive vaccines, it remains an excellent investment of funding resources.[68]

CONCLUSION

Quantitative policy analysis techniques can support rational decision making in immunization by explicitly stating assumptions, costs, and benefits of different strategies and allowing, through sensitivity analysis, an indication of the

most important determinants of program outcomes. They are important tools to help evaluate competing options. When conducted appropriately, they help elucidate values, summarize data and knowledge gaps, and indicate the relative importance of epidemiologic and economic assumptions. Nevertheless, they are only tools and should not be the sole basis for making decisions about immunization policies or programs. Still, evidence from economic analyses have demonstrated immunizations as among the best investments in global health.

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79 Regulation and Testing of Vaccines

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Vaccines are one of the most significant achievements of science and public health. As a result of successful vaccination programs and campaigns, many vaccine-preventable diseases are now uncommon in the United States. Vaccines for prevention of infectious diseases are regulated by the U.S. Food and Drug Administration (FDA) and the legal framework for regulation is derived from Section 351 of the Public Health Service Act and from certain sections of the federal Food, Drug, and Cosmetic Act (FD&C Act).[1,2] The FD&C Act defines drugs, in part, by their intended use as "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease."[2] Thus, vaccines are a unique class of pharmaceutical products that meet the statutory definition of both a drug and biological product. Prophylactic vaccines differ from many other drugs and biologicals primarily in how they are administered to a large population, in particular, young healthy people to prevent rather than treat disease, their mechanism of action, and their risk/benefit profile. Although subject to the same regulations as other biological products, vaccines are inherently more difficult to develop, characterize, and manufacture than most pharmaceutical products. Current U.S. licensed vaccines are listed in Tables 79.1 and 79.2.

HISTORICAL PERSPECTIVE

Regulation of biologics has historically been initiated in response to issues of safety. Over time, legislative authorities have evolved to strengthen and modernize the regulation of vaccines and other biologics. Prior to 1902, manufacturing and product standards for biologics were not federally mandated. However, in 1902, the U.S. Congress passed an act to regulate the sale of viruses, serums, toxins, and analogous products (later referred to as the Biologics Control Act) following the deaths of 20 children who received contaminated products.[3] This Act authorized the Hygienic Laboratory of the Public Health and Marine Hospital Service to issue regulations that governed all aspects of commercial production of vaccines, serums, toxin, and antitoxins and similar products with the objective of ensuring their safety and purity. The regulations under this legislation contained the primary concepts for regulation of biologicals, such as labeling, mandatory facility inspections, and batch-certification guidelines. In 1930, the Hygienic Laboratory was reorganized, expanded, and renamed the National Institutes of Health (NIH).

In 1944, Congress recodified the 1902 Biologics Control Act as part of the U.S. Public Health Service Act (PHS Act) of 1944.[4] The PHS Act incorporated the 1902 Biologics Control Act into Section 351 of the PHS Act (42 U.S.C. 262). As with the 1902 Act, the 1944 PHS Act focused primarily on extensive control over manufacturing methods to ensure safety and purity. Unique to the 1944 PHS Act was Congress' explicit addition of the requirement that biologics manufacturers demonstrate potency as a measure of clinical usefulness. The PHS Act created the Laboratory of Biologics Control to facilitate testing and licensure of biologicals products and manufacturing establishments. After 1944, the authority of the Laboratory of Biologics Control was derived from Section 351 of the PHS Act and from certain sections of the 1938 FD&C Act. In 1948, the Laboratory of Biologics Control joined the NIH Division of Infectious Diseases and Division of Tropical Diseases to form the National Microbiological Institute (later renamed the National Institute of Allergy and Infectious Diseases). Administrative authority for regulation of biologics was originally granted to the National Microbiological Institute.

Although important regulations had been enacted to improve product safety, by the 1950s, the only legal requirement for vaccine licensing was submission of written protocols for vaccine production and safety testing to the Laboratory of Biologics Control. Regulations were dramatically expanded in 1955, when more than 200 cases of polio were attributed to incompletely inactivated polio vaccine manufactured by the Cutter Laboratories. As a result of the "Cutter Incident," administrative authority for the regulation of biologicals was transferred by Congress to the Division of Biologics Standards, a newly created division within the NIH. Regulations were strengthened that required more precise experimental testing to assess the safety of vaccines.

Congress passed the Consumer Safety Act of 1972 and transferred regulatory authority from NIH to FDA for the administration of the 1944 PHS Act. In 1972, the Division of Biologics Standards, which was charged with administering and enforcing Section 351 of the PHS Act, was transferred by the Secretary of Health, Education and Welfare to the FDA, and became the Bureau of Biologics. Once administrative responsibility for the regulation of biologicals was transferred from NIH to FDA, the FDA announced its intention to require that all new biologicals satisfy the additional standards of safety and efficacy mandated in the Drug Amendments Act of 1962. This resulted in the transfer of the regulations pertaining to biologics from Part 73 of Chapter I of Title 42 (USC §262) to Chapter I of Title 21 of the CFR. In 1982, the Bureau of Biologics was renamed the Office of Biologics Research and Review and combined with the Office of Drugs Research and Review to form the Center for Drugs and Biologics. In 1987, following a series of organizational changes within the FDA, the Bureau of Biologics was ultimately transformed into the Center for Biologics Evaluation and Research (CBER). A chronology of the development of the U.S. Biologicals Control Authority is summarized in Table 79.3 and outlined in Fig. 79.1.

FEDERAL LAWS AND REGULATIONS

Since its inception, the FD&C Act has been amended by Congress several times including the FDA Modernization Act (FDAMA) of 1997, the FDA Amendments Act (FDAAA) of 2007, and more recently, the FDA Safety and Innovation Act (FDASIA) of 2012.

Food and Drug Administration Modernization Act

Among other things, the FDAMA of 1997 included measures to modernize the regulation of biologics by synchronizing their review process with that of drugs and eliminating the requirement for an establishment license for biologics. Expedited approval mechanisms for life-threatening conditions also were authorized under FDAMA.[5]

TABLE 79.1 Bacterial Vaccines Currently Licensed in the United States

Vaccine	Manufacturer
Anthrax vaccine, adsorbed	Emergent Biodefense Operations Lansing, Inc.
BCG vaccine	Organon Teknika Corporation
Cholera vaccine, live, oral	Pax Vax Bermuda Ltd.
Diphtheria and tetanus toxoids adsorbed	Sanofi Pasteur, Inc.
Diphtheria and tetanus toxoids and acellular pertussis vaccine, adsorbed	Sanofi Pasteur, Inc.,[a] Sanofi Pasteur, Ltd., GlaxoSmithKline Biologicals
Diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed, hepatitis B (recombinant) and inactivated poliovirus vaccine combined	GlaxoSmithKline Biologicals
Diphtheria and tetanus toxoids and acellular pertussis adsorbed and inactivated poliovirus vaccine	Sanofi Pasteur, Ltd., GlaxoSmithKline Biologicals
Diphtheria and tetanus toxoids and acellular pertussis adsorbed, inactivated poliovirus and *Haemophilus influenzae* type b conjugate (tetanus toxoid conjugate) vaccine	Sanofi Pasteur, Ltd.
Tetanus and diphtheria toxoids, adsorbed	Massachusetts Public Health Biological Laboratories
Tetanus and diphtheria toxoids, adsorbed for adult use	Sanofi Pasteur, Inc., Sanofi Pasteur, Ltd.
Tetanus toxoid	Sanofi Pasteur, Inc.
Tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine, adsorbed	Sanofi Pasteur, Ltd., GlaxoSmithKline Biologicals
Haemophilus influenzae type b conjugate vaccine (meningococcal protein conjugate)	Merck Sharp and Dohme Corp.
Haemophilus influenzae type b conjugate vaccine (meningococcal protein conjugate) and hepatitis B (recombinant) vaccine	Merck Co., Inc.
Haemophilus influenzae type b conjugate vaccine (tetanus toxoid conjugate)	GlaxoSmithKline Biologicals, Sanofi Pasteur S.A.
Meningococcal (groups A, C, Y, and W-135), oligosaccharide diphtheria CRM_{197} conjugate vaccine	Novartis Vaccines and Diagnostics, Inc.
Meningococcal group B vaccine	Wyeth Pharmaceuticals Inc., Novartis Vaccines and Diagnostics, Inc.
Meningococcal groups C and Y and *Haemophilus influenzae* type b tetanus toxoid conjugate vaccine	GlaxoSmithKline Biologicals
Meningococcal (groups A, C, Y and W-135) polysaccharide diphtheria toxoid conjugate vaccine	Sanofi Pasteur, Inc.
Meningococcal polysaccharide vaccine, A, C, Y, W135 combined	Sanofi Pasteur, Inc.
Pneumococcal polysaccharide vaccine, polyvalent	Merck Co., Inc.
Pneumococcal 7-valent conjugate vaccine (diphtheria CRM_{197} protein)	Wyeth Pharmaceuticals Inc.
Pneumococcal 13-valent conjugate vaccine (diphtheria CRM_{197} protein)	Wyeth Pharmaceuticals Inc.
Typhoid vaccine, live oral, Ty21a	Berna Biotech
Typhoid Vi polysaccharide vaccine	Sanofi Pasteur S.A.

[a]License for the pertussis component of this product is held by the Research Foundation for Microbial Diseases of Osaka University (Aventis Pasteur Laboratories, Inc.).

Pediatric Research Equity Act

The Pediatric Research Equity Act (PREA) of 2003 amended the FD&C Act by adding Section 505(B) to address product development for pediatric subjects from birth to 16 years of age.[6] It requires that manufacturers submit a pediatric assessment with every application submitted under Section 505 of the FD&C Act or section 351 of the PHS Act for a new active ingredient, new indication, new dosage form, new dosing regimen, or new route of administration unless the applicant has obtained a waiver or deferral from the FDA. The pediatric assessment must contain data adequate to assess the safety and effectiveness of the drug or the biological product for the claimed indications in all relevant pediatric subpopulations and data to support dosing and administration for each pediatric subpopulation for which the product is safe and effective.

Food and Drug Administration Amendments Act

The FDAAA of 2007 includes 11 titles that added many new provisions to the FD&C Act.[7] It reauthorized and amended several drug and medical device provisions, and provided the FDA with additional responsibilities and new authorities. The provisions of FDAAA that have had a significant impact on the regulations of vaccines and the review process are contained in Title IV, the PREA, and Title IX, Enhanced Authorities Regarding Postmarket Safety of Drugs. The FDAAA reauthorized and revised the PREA, primarily to enhance FDA oversight and applicant accountability for the agreed-upon

TABLE 79.2 Viral Vaccines Currently Licensed in the United States

Vaccine	Manufacturer[a]
Adenovirus type 4 and type 7 vaccine, live	Barr Labs Inc.
Diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed, hepatitis B (recombinant) and inactivated poliovirus vaccine combined	GlaxoSmithKline Biologicals
Diphtheria and tetanus toxoids and acellular pertussis adsorbed and inactivated poliovirus vaccine	Sanofi Pasteur, Ltd., GlaxoSmithKline Biologicals
Hepatitis A vaccine, inactivated	Merck & Co., Inc., GlaxoSmithKline Biologicals
Hepatitis B vaccine, recombinant	Merck & Co., Inc., GlaxoSmithKline Biologicals
Hepatitis A vaccine, inactivated and hepatitis B (recombinant) vaccine	GlaxoSmithKline Biologicals
Haemophilus influenzae type b conjugate vaccine (meningococcal protein conjugate) and hepatitis B recombinant vaccine	Merck & Co., Inc.
Human papillomavirus (types 6, 11, 16, 18) recombinant vaccine	Merck & Co., Inc.
Human papillomavirus bivalent (types 16 and 18) vaccine, recombinant	GlaxoSmithKline Biologicals
Human papillomavirus 9-valent vaccine, recombinant	Merck & Co., Inc.
Influenza virus vaccine, trivalent, types A and B	Sanofi Pasteur, Inc., Novartis Vaccines and Diagnostics Ltd., GlaxoSmithKline Biologicals, ID Biomedical Corporation of Quebec, CSL, Ltd., Protein Sciences Corporation
Influenza A (H1N1) 2009 monovalent	Sanofi Pasteur, Inc., Novartis Vaccines and Diagnostics Ltd., ID Biomedical Corporation of Quebec, MedImmune Vaccines, Inc., CSL, Ltd.
Influenza virus vaccine, H5N1 (for national stockpile)	Sanofi Pasteur, Inc.
Influenza A (H5N1) virus monovalent vaccine, adjuvanted	ID Biomedical Corporation of Quebec
Influenza vaccine, adjuvanted	Novartis Vaccines and Diagnostics
Influenza virus vaccine, quadrivalent, types A and B	Sanofi Pasteur, Inc., GlaxoSmithKline Biologicals, ID Biomedical Corporation of Quebec, MedImmune Vaccines, Inc.
Influenza virus vaccine, intranasal	MedImmune Vaccines, Inc.
Japanese encephalitis vaccine, inactivated	The Research Foundation for Microbial Diseases of Osaka University
Japanese encephalitis vaccine, inactivated, adsorbed	Valneva Austria GmbH
Measles, mumps, and rubella virus vaccine, live	Merck & Co., Inc.
Measles, mumps, rubella, and varicella vaccine, live	Merck & Co., Inc.
Poliovirus vaccine inactivated, monkey kidney cell	Sanofi Pasteur, S.A.
Rabies vaccine	Novartis Vaccines and Diagnostics Ltd., Sanofi Pasteur, S.A.
Rotavirus vaccine, live, oral	GlaxoSmithKline Biologicals
Rotavirus vaccine, live, oral, pentavalent	Merck & Co., Inc.
Rubella virus vaccine, live	Merck & Co., Inc.
Smallpox (vaccinia) vaccine, live	Sanofi Pasteur Biologics
Varicella virus vaccine live	Merck & Co., Inc.
Yellow fever vaccine	Sanofi Pasteur, Inc.
Zoster vaccine, live	Merck & Co., Inc.

[a]These are the names of the license holders. Company names may be different.

pediatric assessments.[8] Of note, a new provision directed the FDA to establish the Pediatric Review Committee (PeRC), an internal review committee with pediatric expertise. This committee is required to provide consultation to FDA review divisions on all pediatric plans and assessments and on all deferral and waiver requests. Thus, early in the application review process the review team must assess whether PREA applies. If PREA applies, then a pediatric assessment must be presented to the PeRC.

Section 901 of Title IX of the FDAAA authorizes the FDA to require certain postmarketing studies and clinical trials for prescription drug and biological products approved under Section 505 of the FD&C Act or Section 351 of the PHS Act (42 USC §262). Section 901 of the FDAAA also created new Sections 505–1 and 505(o)(4) of the FD&C Act, authorizing the FDA, under certain circumstances, to require risk evaluation and mitigation strategies and safety-related labeling changes, respectively. The FDAAA also specifies adverse event reporting requirements for products with labeling changes that are a result of a pediatric assessment. Specifically, during the 12 months from the date that such a labeling change is made, all adverse event reports are reviewed by the FDA Pediatric Advisory Committee. Following review, the Pediatric Advisory Committee makes recommendations regarding whether

TABLE 79.3 Chronology of the Development of Biologic Control Authority

Year	Legislation Enacted	Existing Organization
1902	Biologics Control Act (Virus, Serum, Toxin Law) of 1902	Public Health Hygienic Laboratory
1930		Hygienic Laboratory renamed National Institutes of Health (NIH)
1937		Laboratory of Biologics Control (LBC) formed within NIH
1944	Enactment of U.S. Public Health Service Act (42 USC §§262, 263)	
1948		LBC incorporated into the National Microbiological Institute (later renamed the National Institute of Allergy and Infectious Diseases)
1955		Establishment of the Division of Biologics Standards (DBS) by the Surgeon General
1972		DBS transferred to FDA to become Bureau of Biologics (BoB)
1982–1983		BoB renamed Office of Biologics Research and Review (OBRR); joined with Office of Drugs Research and Review (ODRR) to form the Center for Drugs and Biologics (CDB)
1987		OBRR renamed Center for Biologics Evaluation and Research (CBER)
1997	Food and Drug Administration Modernization Act of 1997	
2007	Food and Drug Administration Amendments Act (FDAAA)	
2012	Food and Drug Administration Safety and Innovation Act (FDASIA)	

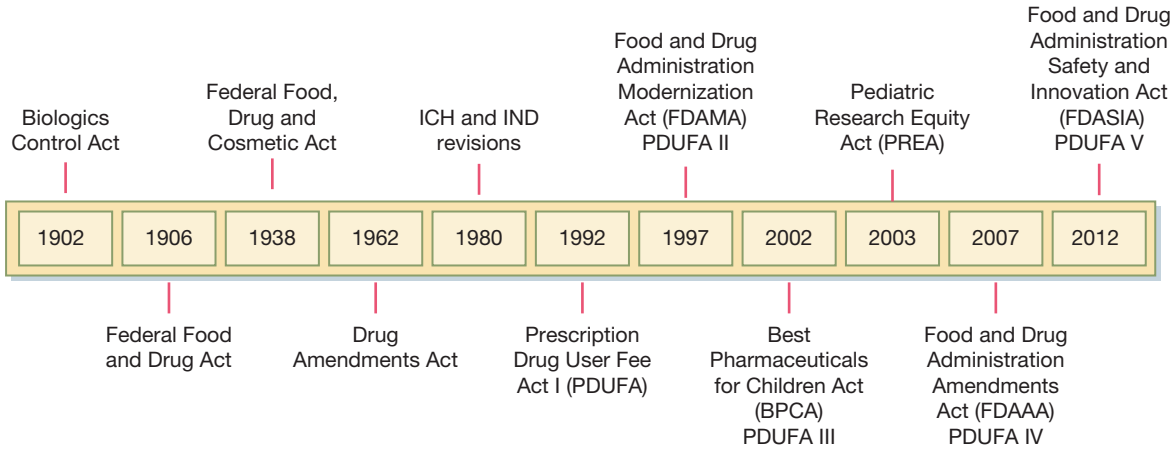


Figure 79.1. History of Food and Drug Law. ICH, International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; IND, investigational new drug.

the FDA should take action in response to such reports and whether the current pharmacovigilance plan is adequate.

Food and Drug Administration Safety and Innovation Act

The FDASIA was signed into law in 2012 and expanded the FDA's authority by strengthening the agency's ability to safeguard and advance public health by promoting innovation, increasing stakeholder involvement in FDA processes, and enhancing the safety of the drug supply chain.[9] In addition to reauthorizing the prescription drug and medical device user fee programs, FDASIA established new user fee programs for generic drugs and biosimilar biological products. The provisions of FDASIA that impact the regulation of vaccines are contained in Pediatric Drugs and Devices (Title V) and Drug Approval and Patient Access (Title IX). Title IX expands the scope of products that qualify for accelerated approval and creates a new "breakthrough therapy" program, among other things (see below). FDASIA also revised PREA to include a provision that requires vaccine manufacturers to submit a Pediatric Study Plan early in the drug development process. This initial Pediatric Study Plan must contain an outline of the pediatric study or studies that the sponsor plans to conduct including, to the extent practicable, study objectives and design, age groups, relevant end points, and statistical approach, as well as any request for a deferral, partial waiver, or waiver. The FDA internal PeRC must be consulted for the review of the initial study plan, the agreed initial pediatric plan, and certain amendments to such plans. Both sponsors and the FDA must comply with prescribed timelines regarding submission, review, responses, and agreements reached

regarding the Pediatric Study Plan, which are also described in applicable FDA guidance.[10]

Prescription Drug User Fee Act

Of note, these amendments to the FD&C Act also renewed the Prescription Drug User Fee Act (PDUFA) that was first enacted in 1992, and authorized the FDA to collect user fees from companies. These fees enabled FDA to hire additional reviewers and support staff and upgrade its information technology systems. In return for these additional resources, the FDA agreed to certain review performance goals, such as completing reviews of new drug applications and biologics license applications (BLAs) and taking regulatory actions on them in predictable timeframes. These changes revolutionized the drug approval process in the United States and enabled FDA to speed the application review process for new drugs and biologics without compromising the FDA's high standards for demonstration of safety, efficacy, and quality. The PDUFA program has been reauthorized every 5 years, 1997 (PDUFA II), 2002 (PDUFA III), 2007 (PDUFA IV), and 2012 (PDUFA V). It includes the 5-year review performance goals for drug and BLAs, supplements and resubmissions, meeting management goals, clinical holds, major dispute resolution, special protocol question assessment and agreement, electronic applications and submissions, discipline review, and complete response letters.

FEDERAL REGULATIONS AND FOOD AND DRUG ADMINISTRATION GUIDANCE

The FDA's CBER is the national regulatory authority in the United States charged with the regulation of biological products including vaccines. The review of vaccine applications occurs among CBER's Office of Vaccines Research and Review, Office of Compliance and Biologics Quality, and Office of Biostatistics and Epidemiology. CBER's current legal authority for the regulation of vaccines derives primarily from Section 351 of the PHS Act and from certain sections of the FD&C Act. The statutes of the PHS Act are implemented through regulations codified in the Code of Federal Regulations (CFR). The CFR is published annually and contains all changes in regulations that have occurred during the previous year that were published in the *Federal Register*. Regulations are adopted in conformity with the Administrative Procedure Act.[11] Thus, before a regulation can be established, repealed, or revised, it must be proposed and published in the *Federal Register* with an invitation to all interested individuals or parties to comment within a prescribed time, commonly a period of 1 to several months. Once comments are received, they are evaluated and considered by the FDA before publication of the final regulation in the *Federal Register*.

Title 21 of the CFR, parts 600 through 680, contains regulations specifically applicable to vaccines and other biologicals. In addition, because vaccines meet the legal definition of a drug under the FD&C Act, manufacturers must comply with regulations for Current Good Manufacturing Practices (CGMPs) (parts 210 and 211). Table 79.4 summarizes the regulations applicable to vaccines and other biological products. These regulations cover not only the methods and establishment standards pertaining to the manufacture of a biological product to assure that the product is safe and meets the quality and purity characteristics that are claimed by the manufacturer, but also requirements for performing clinical trials (i.e., 21 CFR §312).

A single set of basic regulatory requirements applies to all vaccines, regardless of the technology used to produce them. The regulatory approval criteria contained in Title 21 CFR also apply to vaccines, regardless of their indication or intended target population. Section 351 of the PHS Act (42 USC §262) states that a BLA can be approved based on a demonstration that "…(a) the biological product that is the subject of the application is safe, pure, and potent; and (b) the facility in which the biological product is manufactured, processed, packed, or held meets standards designed to assure that the biological product continues to be safe, pure, and potent…"

Some of the more pertinent operational definitions for biologics contained in the statutes and 21 CFR are as follows:

- Section 351 of the PHS Act defines a biological product as any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product applicable to the prevention, treatment, or cure of diseases or conditions of human beings. Thus, vaccines clearly are regulated as biological products.
- Safety is defined as the relative freedom from harmful effect to people affected directly or indirectly by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the

TABLE 79.4 Regulations Applicable to the Development, Manufacture, Licensure, and Use of Vaccines[a]

Title 21, Code of Federal Regulations, Chapter 1: FDA, DHHS[a]	Subject
SUBCHAPTER F—BIOLOGICS[b]	
600	Biologic products, general, definitions Establishment standards Establishment inspection Adverse experience reporting
601	Licensing
610	General biologicals product standards
SUBCHAPTER C—DRUGS: GENERAL	
201	Labeling
202	Prescription drug advertising
210	Current good manufacturing practice in manufacturing, processing, packing, or holding of drugs
211	Current good manufacturing practice for finished pharmaceuticals
SUBCHAPTER D—DRUGS FOR HUMAN USE	
312	New drugs for investigational use
314	Applications for FDA approval to market a new drug or an antibiotic drug
SUBCHAPTER A—GENERAL	
25	Environmental impact considerations
50	Protection of human subjects
56	Institutional review boards
58	Nonclinical laboratory studies, good laboratory practice regulations,[a] FDA, DHHS

[a]Food and Drug Administration, Department of Health and Human Services, Administration Code of Federal Regulations (CFR) Title 21. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm

[b]Consists of Parts 600–680. Parts 606, 607, 640, 660 and 680 apply to blood, blood products, diagnostic test and allergenics.

DHHS, Department of Health and Human Services; FDA, Food and Drug Administration.

recipient at the time. Thus, the property of safety is relative and cannot be ensured in an absolute sense.
- Purity is defined as the relative freedom from extraneous matter, regardless of whether it is harmful to the recipient or deleterious to the product. Usually, the concepts of purity and safety coincide; purity most often relates to freedom from such materials as pyrogens, adventitious agents, and chemicals used in manufacture of the product.
- Potency is defined as the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through administration of the product in the manner intended, to effect a given result. Potency, as thus defined, is equivalent to the concept that the product must be able to perform as claimed, and, if possible, this must correspond with some measurable effect in the recipient or correlate with some quantitative laboratory finding.
- Standards mean specifications and procedures applicable to an establishment or to the manufacture or release of products that are designed to ensure the continued safety, purity, and potency of biological products. The word standard is also used with a secondary meaning, usually in the sense of a reference preparation, such as a bacterial or viral antigen that can be used in evaluating potency or, in some cases, safety and purity.
- The regulations regarding biological products, in addition, define effectiveness as the reasonable expectation that, in a significant proportion of the target population, pharmacologic or other effects of the biological product, when administered under adequate directions for use and warnings against unsafe use, will serve a clinically significant function in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans.
- CGMPs define a quality system that manufacturers use as they build quality into their products. The regulations outline the minimum manufacturing, quality control, and quality assurance requirements for the preparation of a drug or biological product for commercial distribution. For example, approved products developed and produced according to CGMPs are safe, properly identified, of the correct strength, pure and of high quality.

The FDA also periodically publishes various guidelines and guidance documents with regard to the manufacture and clinical evaluation of biologicals. These documents published by the FDA do not have the force of law, but are intended to provide useful and timely recommendations; Table 79.5 lists those applicable to vaccines. Guidance documents are particularly useful as a means for the FDA to provide recommendations that are current with areas of rapidly progressing science, and for specifying a degree of detail beyond what is included in the regulations. In the past few years, several FDA regulations and guidance documents have had a direct impact on the review of vaccines for licensure by the FDA, such as the "Guidance for Industry: Expedited Programs for Serious Conditions—Drugs and Biologics (2014)."[12] Some of these regulations and guidance documents evolved from an effort to streamline the regulatory process, while others—such as "Guidance for Industry: Clinical Data Needed to Support the Licensure of Trivalent Inactivated Influenza Vaccines (2007)", "Guidance for Industry: Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines (2007)," "Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Material Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases (2010)," and "Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications (February 2006)"—were written to facilitate the development of new vaccines with new technologies.[13–16] These documents are available at CBER's website.[17]

COLLABORATIONS BETWEEN THE FOOD AND DRUG ADMINISTRATION AND NATIONAL AND INTERNATIONAL PARTNERS

The FDA's CBER regulatory review staff consists of an internal multidisciplinary team of scientists, medical officers, and regulatory and public health professionals. To stay current on scientific advances and biotechnologies, the team is involved in a dynamic exchange of information with the outside scientific community through laboratory research and collaborations, participation in workshops and seminars, and engagement with national partners. The FDA also relies on the expertise of formal advisory committees, which include experts in the fields of vaccinology, microbiology, infectious diseases, immunology, biostatistics, epidemiology, and clinical trial design. In addition, CBER works closely with its counterparts in other U.S. government agencies within the Department of Health and Human Services and the U.S. Public Health Service (PHS), such as the National Vaccine Program Office, the Centers for Disease Control and Prevention (CDC), the NIH, and the Health Resources and Services Administration. The CDC is responsible, among its other duties, for epidemiologic surveillance of disease and for support of immunization programs. Its Advisory Committee on Immunization Practices makes recommendations for vaccine use. The Director of the National Vaccine Program Office coordinates vaccine efforts throughout the PHS and other governmental agencies. The NIH is responsible for conducting and providing funds for a wide variety of biomedical research. The Health Resources and Services Administration is responsible for managing the National Vaccine Injury Compensation Program. Other important collaborators inside the U.S. government involved in vaccine activities include the U.S. Department of Defense, and the Department of Veterans Affairs. Additionally, CBER works closely with its multilateral partners, notably the Pan American Health Organization and the World Health Organization (WHO), to provide assistance in regulatory capacity building. CBER has been very active in supporting the WHO's Developing Countries' Vaccine Regulators Network and the WHO's African Regional Office-led African Vaccine Regulatory Forum. As a WHO Collaborating Center, CBER contributes to a range of activities, including establishment of physical and written standards, implementation of WHO international standards, strengthening global regulatory systems, and serving as the National Regulatory Authority (NRA) of reference for WHO's vaccine prequalification program. CBER experts also contribute as members of several WHO Advisory Committees including the Global Advisory Committee on Vaccine Safety, the Polio Research Committee, the HIV Vaccine Advisory Committee, and the Expert Committee on Biological Standardization. CBER is an Essential Regulatory Laboratory in WHO's Global Influenza Surveillance and Response System. In addition, the FDA has confidentiality arrangements with many NRAs around the globe, allowing it to share information as part of its regulatory processes. These arrangements have strengthened interactions between the regulatory authorities and have contributed to improving the promotion and protection of public health globally.

MANAGED REVIEW PROCESS

The regulatory review in CBER incorporates a managed and integrated regulatory process that is continuous from

TABLE 79.5 Guidance Documents Applicable to Development, Manufacture, Licensure, and Use of Vaccines[a]

Document	Date
GUIDANCE DOCUMENTS	
Draft Guidance for Industry: Formal Meetings between the FDA [Food and Drug Administration] and Sponsors or Applicants of PDUFA [Prescription Drug User Fee Act] Products	2015
Guidance for Industry: Expedited Programs for Serious Conditions—Drugs and Biologics	2014
Providing Submissions in Electronic Format—Postmarketing Safety Reports for Vaccines: Draft Guidance for Industry 7/2014	2014
Guidance for Industry: General Principles for the Development of Vaccines to Protect Against Global Infectious Diseases	2011
Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications	2010
Draft Guidance for Industry: Clinical Considerations for Therapeutic Cancer Vaccines	2009
Guidance for Industry: Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccine	2007
Guidance for Industry: Clinical Data Needed to Support the Licensure of Trivalent Inactivated Influenza Vaccines	2007
Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials	2007
Draft Guidance: Emergency Use Authorization of Medical Products	2007
Draft Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases	2006
Guidance for Industry: Reports on the Status of Postmarketing Studies—Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997	2006
Guidance for Industry: Clinical Studies Section of Labeling for Prescription Drugs and Biologics—Content and Format	2006
Draft Guidance for Industry: Labeling for Human Prescription Drug and Biological Products: Implementing the New Content and Format Requirements	2006
Guidance for Industry: Adverse Reactions Section of Labeling for Human Prescription Drug and Biological Products—Content and Format	2006
Draft Guidance for Industry: INDs [investigational new drugs]—Approaches to Complying with CGMP [Current Good Manufacturing Practices] During Phase 1	2006
Guidance for Industry: Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications	2006
Guidance for Industry: Fast Track Drug Development Programs—Designation, Development, and Application Review	2006
Guidance for Industry: Quality Systems Approach to Pharmaceutical Current Good Manufacturing Practice Regulations	2006
Guidance for Industry: Providing Regulatory Submissions to the Center for Biologics Evaluation and Research (CBER) in Electronic Format—Lot Release Protocols	2006
Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications	2005
Guidance for Industry: Development and Use of Risk Minimization Action Plans	2005
Draft Guidance for Industry: How to Comply with the Pediatric Research Equity Act	2005
Guidance for Industry: FDA Review of Vaccine Labeling Requirements for Warnings, Use Instructions, and Precautionary Information	2004
Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing Current Good Manufacturing Practice	2004
Draft Guidance for Industry: Vaccinia Virus—Developing Drugs to Mitigate Complications from Smallpox Vaccination	2004
Draft Guidance for Industry: Postmarketing Safety Reporting for Human Drug and Biological Products Including Vaccines	2001
Draft Guidance for Industry on Recommendations for Complying with the Pediatric Rule	2000
Guidance for Industry: Formal Meetings with Sponsors and Applicants for PDUFA Products	2000
Guidance for Industry: Submitting and Reviewing Complete Responses to Clinical Holds	2000
Guidance for Industry: Content and Format of Chemistry, Manufacturing and Controls Information for a Vaccine or Related Product	1999
Guidance for Industry: Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products	1998
Draft Guidance for Industry: Stability Testing of Drug Substances and Drug Products	1998
Guidance for Industry: Implementation of Section 126 of the Food and Drug Administration Modernization Act of 1997—Elimination of Certain Labeling Requirements	1998
Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications	1998
Guidance for Industry for the Evaluation of Combination Vaccines for Preventable Diseases: Production, Testing and Clinical Studies	1997
Guidance for Industry: Changes to an Approved Application: Biological Products	1997
Guidance on Alternatives to Lot Release for Licensed Biological Products	1994

Continued on following page

TABLE 79.5 Guidance Documents Applicable to Development, Manufacture, Licensure, and Use of Vaccines[a] *(Continued)*

Document	Date
GUIDELINES	
Guidance for Industry: Process Validation: General Principles and Practice[a] Revision of the 1987 guidance General Principles of Process Validation.	2011
Guideline on General Principles of Process Validation	1987
Determination of Residual Moisture in Dried Biological Products	1990
POINTS TO CONSIDER	
Supplement: Nucleic Acid Characterization and Genetic Stability	1992
Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology	1985

[a]Guidance documents are available at http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/default.htm. They are also available at no charge from the Office of Communication, Training and Manufacturers Assistance, HFM-40, 1401 Rockville Pike, Rockville, MD 20852–1448.

discovery to postmarketing, and is designated as the Managed Review Process.[18] CBER's managed review process is designed to effectively review all regulatory submissions to reach informed evidence-based regulatory decisions to ensure safe and effective biological products. CBER uses a team-based approach with substantive involvement of discipline team leaders and management.

The Managed Review Process begins when a sponsor requests a pre–investigational new drug (IND) meeting that may result in the submission of an IND and, eventually, a BLA. The review process in CBER begins with an initial review of a submission for scientific content and compliance with the regulations. Members of a multidisciplinary review team are selected based on their expertise with the type of product and its method of manufacture. It is the responsibility of CBER's review component to evaluate submissions and recommend appropriate regulatory action to facilitate the approval of safe and effective biological products. The review includes an evaluation of chemistry, manufacturing, and controls information; the manufacturing facility and equipment; preclinical and clinical data on the safety, efficacy, pharmacology, and toxicology; the suitability of clinical trial design; and analysis of clinical data derived from such trials. In addition, reviewers monitor for conformance with FDA regulations in all phases of biological product development, including postmarketing. CBER scientists also perform research in the areas of statistical and epidemiologic analysis, clinical trial design, and chemistry, manufacturing and control specific to product issues, and contribute to policy development. Surveillance activities are performed to ensure that the safety of biological products is not compromised. These activities ensure the rapid availability and approval of safe and effective biological products.

MEETINGS WITH SPONSORS

The FDA encourages meetings with sponsors to the extent that they aid in the evaluation of the vaccine and in resolving scientific issues concerning the product. The general principle underlying the conduct of such meetings is that there should be free, full, and open communication about any scientific or medical question that may arise. Agreements reached at PDUFA meetings (e.g., pre-IND, IND, pre-BLA, and BLA meetings) are recorded in official minutes taken by FDA personnel and provided to the sponsor. They serve as a permanent record of any agreements reached. Detailed information on the conduct of regulatory meetings is described in 21 CFR §312.47.[19]

STAGES OF THE REGULATORY REVIEW OF VACCINE PRODUCTS

Premarketing Phase

The regulatory requirements for biological products cover the entire life-cycle of the product from the pre-IND stage, through the premarketing (consisting of the various IND phases and prelicensure) and postmarketing stages. The pre-IND stage consists of laboratory development, preclinical testing of candidate vaccines, and development of the manufacturing process. The clinical development of a new drug in the United States usually begins with a sponsor approaching the FDA for permission to conduct a clinical study with an investigational product through submission of an IND application form. These requirements can be found in the IND regulations.[20] Sponsors are encouraged to request a pre-IND meeting with FDA to discuss preclinical studies, clinical study design, and data requirements that require resolution prior to the initiation of clinical trials. In the application, the sponsor (a) describes the composition, source, and method of manufacture of the product and the methods used in testing its safety, purity, and potency; (b) provides a summary of all laboratory and preclinical animal testing; and (c) provides a description of the proposed clinical study and the names and qualifications of each clinical investigator. The FDA has a maximum of 30 days to review the original IND application and determine whether study participants will be exposed to any unacceptable risks. As part of the IND process, each clinical investigator files information describing the investigator's qualifications for performing clinical trials, details of the proposed study, and assurance that a number of conditions specified by the regulations will be met. A signed informed consent must be obtained from each study participant. Approval for the study must be obtained in advance from a local institutional review board. The regulations also cover the evaluation of the preclinical laboratory animal studies undertaken to support the use of the product in humans.

Investigational Phase

Only licensed vaccines may be shipped from one state to another; however, during the premarketing phase, interstate shipment of products for investigational use is allowed under the law and regulations. There are generally three separate phases in the clinical evaluation of experimental biologicals at the premarketing stage (Fig. 79.2). These phases may overlap, and the clinical testing may be highly iterative because

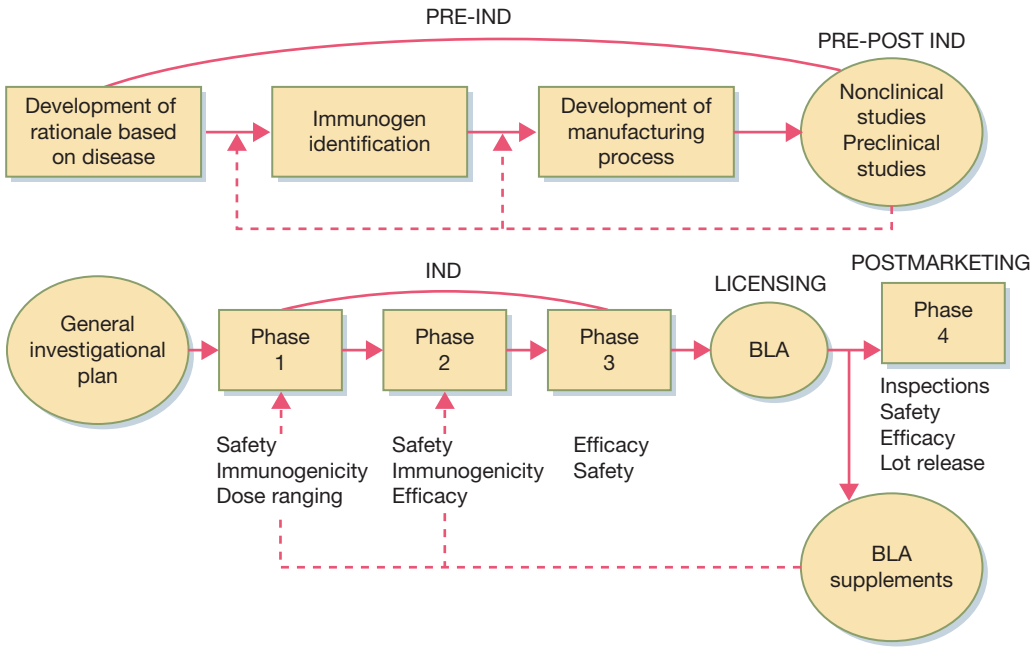


Figure 79.2. Sequence of key events in product development through the premarketing experimental investigational new drug (IND) and licensing phases and the postapproval marketing phase. Dashed lines indicate additional research/development submissions when significant changes are made in the product or its indications. BLA, biologics license application.

multiple Phase I or Phase II trials may be performed as new data are obtained. Phase I trials are intended primarily to provide a preliminary evaluation of safety and immunogenicity. These trials are typically conducted in a small number (e.g., 20 to 80) of closely monitored adult volunteers. If the ultimate target population for the vaccine is infants or young children, as is commonly the case, the product is usually evaluated in a stepwise progression from older to younger age groups down into the first year of life. Phase II studies can involve up to several hundred participants, are often randomized and well-controlled, and provide further information on safety and immunogenicity and optimal dose. In some cases, Phase II studies may provide preliminary data on the vaccine's activity against the infectious disease of interest. Phase III studies are large-scale trials to provide a more thorough assessment of safety as well as a definite assessment of efficacy.

The general considerations for clinical studies to support licensure of a vaccine include demonstration of safety, efficacy (immunogenicity may be sufficient in some cases), and evaluation of simultaneous administration with other licensed vaccines. Vaccine efficacy should be demonstrated, ideally in randomized, double-blind, well-controlled trials. The end points are product specific, and may be clinical disease end points or immune response end points if efficacy against clinical disease had been previously established and there are immune correlates or surrogates of that protection. In recent years, efficacy trials for various vaccines have involved a broad range in the number of study participants, from thousands to tens of thousands. This broad range is related to a number of interconnected variables such as study design and the incidence of the disease to be prevented. For example, clinical disease end point studies that are designed to demonstrate that a new vaccine is noninferior to an already existing product of the same type generally require larger numbers than one in which a new vaccine can be compared with a control that has no activity against the clinical disease. The incidence of the disease to be prevented in the study population is also important. As an example, a trial to show that pneumococcal seven-valent polysaccharide conjugate vaccine (PCV7) was successful in preventing a low incidence of invasive pneumococcal disease caused by the *Streptococcus pneumoniae* capsular serotypes included in the vaccine enrolled close to 40,000 children who were randomized equally to receive the pneumococcal conjugate vaccine or an unrelated control vaccine. In contrast, licensure of the pneumococcal 13-valent polysaccharide conjugate vaccine (PCV13) was based on noninferiority comparative studies to PCV7. Effectiveness of PCV13 was inferred from measuring anti-polysaccharide binding and functional opsonophagocytic antibodies because clinical end point disease efficacy studies were no longer feasible owing to the further decline of invasive pneumococcal disease as a result of introduction of PCV7 in the United States.

In some situations, human challenge studies have been conducted during early clinical development or in lieu of clinical trials in an endemic area. These studies served to demonstrate "proof of concept" of the vaccine early in clinical development (e.g., *Plasmodium falciparum* sporozoite challenge of malaria-naïve U.S. volunteers previously administered a candidate malaria vaccine). Human challenge studies may also be considered to demonstrate the efficacy of the vaccine. For example, the Agency convened the Vaccines and Related Biologics Products Advisory Committee (VRBPAC) to consider whether data from human challenge studies in U.S. subjects could be sufficient to demonstrate efficacy of a cholera vaccine in travelers to endemic areas who are at high risk for contracting the disease. In 1998, the VRBPAC agreed that human challenge studies could suffice to demonstrate efficacy of a cholera vaccine provided that studies were adequate and well-controlled and conducted under the provisions of good clinical practices.[21] In 2016, the FDA approved Vaxchora, a live, attenuated vaccine for the prevention of cholera in adults traveling to cholera-affected areas. Efficacy of Vaxchora was demonstrated in a controlled human challenge study in adult U.S. volunteers.

Safety is one of the most important considerations when evaluating new vaccines and modifications to currently

licensed vaccines. The initial responsibility for determining vaccine safety starts with clinical investigators and vaccine manufacturers. The FDA is responsible for assuring that clinical trials are done under good clinical practices, a requirement essential for the evaluation of safety data intended to support a license application. In general, when evaluating safety, one must compare the risk of the vaccine-preventable disease with the risk of the adverse event(s) potentially associated with the vaccine, and these may change over time. As an example, the reported association between Rotashield (rotavirus vaccine, live, oral, tetravalent, manufactured by Wyeth) and intussusception resulted in the additional requirement for the evaluation of the safety of RotaTeq (live, oral pentavalent human-bovine reassortant rotavirus vaccine, manufactured by Merck) with respect to intussusception. This clinical trial enrolled more than 70,000 infants divided equally between RotaTeq and placebo. The primary safety hypothesis was that the oral RotaTeq would not increase the risk of intussusception relative to placebo within 42 days of any dose. The intended target population should be taken into consideration in assessing the adequacy of the safety database. For routinely administered childhood vaccines in the United States, the target population would be the birth cohort in the United States (approximately 4 million/year). This is generally a healthy population, and a government body (e.g., state or local governments) may mandate vaccination. Common reactions can be studied adequately in hundreds of individuals, but many thousands will be required to define low-incidence adverse reactions.

For vaccines evaluated in clinical end point efficacy trials, a large safety database likely will derive from a double-blind, randomized, well-controlled efficacy study. However, for vaccines evaluated in immunogenicity end point studies, additional studies likely will be needed to obtain an adequate safety database. Additional controlled safety studies are often requested when the numbers of subjects included in the efficacy studies are deemed insufficient to provide adequate safety data. Safety studies may be unblinded if the number of injections, route of administration, or schedule differs between groups, in particular when infants and young children are involved. Phase II safety studies should provide data on common local and systemic reactions to the study vaccine. Phase II clinical development also should include immunogenicity and preliminary safety data on the concurrent administration of the study vaccine with other vaccines, if relevant. Phase III safety studies are designed to evaluate less common reactions, may be unequally randomized, and may have a simplified trial design for assessing less-common adverse events in large trials. If a vaccine is recommended on the same schedule as other routinely recommended vaccines, safety and immunogenicity data should be obtained in prelicensure studies to support simultaneous administration.

Licensing Phase

Following completion of IND studies demonstrating the safety and efficacy of the vaccine for a specific use and population the sponsor can submit a BLA to obtain a license for a new vaccine under section 351 of the PHS Act for commercial manufacture and distribution of the product. Prior to the submission of a BLA, a pre-BLA meeting with the FDA is strongly encouraged to discuss the sponsor's product development plan. For the FDA to provide sponsors with advice regarding the adequacy of information to support a BLA, the following information in advance of the pre-BLA meeting should be submitted, depending on the type of meeting: (a) an executive summary of the clinical studies to be submitted in the application; (b) a proposed format for organizing the submission, including methods for presenting the data; (c) information on the status of needed or ongoing studies; and (d) any other information for discussion at the meeting. The primary purpose of this exchange is to uncover any major unresolved problems; identify those studies that the sponsor is relying on as adequate and well-controlled to establish the product's effectiveness; identify the status of ongoing studies; acquaint FDA reviewers with the general information to be submitted in the BLA (including technical information); review methods used in the statistical analysis of the data; and discuss the best approach for the presentation and formatting of data in the application.

At the time that an applicant submits a BLA to the Director of CBER's Office of Vaccines Research and Review precise production methods and procedures should be defined, and the manufacturing process should be standardized. Critical information to be contained in the BLA include data derived from nonclinical laboratory and clinical studies that demonstrate that the manufactured product meets prescribed requirements for safety, purity, and potency. The BLA should contain information that supports compliance with standards addressing requirements for (a) organization and personnel; (b) buildings and facilities; (c) equipment; (d) control of components, containers, and closures; (e) production and process controls; (f) packaging and labeling controls; (g) holding and distribution; (h) laboratory controls; and (i) records to be maintained. Furthermore, a full description of manufacturing methods; data establishing stability of the product through the dating period; sample(s) representative of the product for introduction or delivery for introduction into interstate commerce; summaries of test results performed on the lot(s) represented by the submitted sample(s); specimens of the labels, enclosures, and containers; and the address of each location involved in the manufacture of the biological product should be included in the BLA.

An application for a biologics license is not considered as filed (or accepted by the agency for review) until CBER determines that it has received all pertinent information and data from the applicant. In this regard, CBER can refuse to file a BLA if it deems the submission to be incomplete. Additionally, the manufacturing facility must be inspection-ready at the time the BLA is submitted. The applicant is also required to include either an environmental assessment or a claim for categorical exclusion from the requirement to submit an environmental assessment or an environmental impact statement. Other components of the BLA review include product labeling, which describes the indications for use, contraindications, dosage and possible adverse effects; protocols for the manufacturing and testing of the number of product lots specified to establish the consistency of the process; and confirmatory testing results within CBER of samples of in-process material or product in final containers and conformance to existing regulations.

An internal CBER multidisciplinary committee performs the scientific review of the BLA. This process occurs for each BLA or supplement to a BLA in which significant changes are proposed. During the review, there are discussions and exchanges of correspondence between the sponsor and the CBER review committee concerning issues that may arise. During the FDA review of the BLA an announced prior approval inspection (PAI) of the manufacturing facility is performed. This inspection is designed as an in-depth review of the facilities, records, total production process, methods, equipment, quality control procedures, and personnel. With the implementation of the BLA process, changes have occurred in the scope of issues reviewed during the PAI. Instead of the manufacturer submitting detailed records with the BLA regarding studies on cleaning validation, monitoring data for

pharmaceutical-grade water, facility support systems (e.g., clean steam, compressed air, and building management systems), and other facility-related systems, a more detailed review of this type of data is done onsite during the PAI. PAIs tend to require longer periods of time for the FDA inspectors to be in the facility because of the increased scope of issues that are reviewed onsite. If licensure is denied following inspection for the original license application, reinspection will occur after receipt of assurance that all deficiencies that were the basis of the denial were corrected.

With the implementation of FDAAA (2007) and FDASIA (2012), in addition to completing the discipline reviews during the PDUFA V mandated timelines, the review committee must complete numerous additional tasks. These include, but are not limited to, review committee assignments, internal information exchange meetings, filing decisions, presentation of the application to the PeRC, midcycle review meetings and midcycle communication with the applicant, late-cycle meetings with the applicant, and presentation of planned or required postmarketing studies to an internal FDA safety committee.

After CBER reviews the entire package of information in the BLA, its advisory committee (the VRBPAC) and consultants, if needed, are asked to review and comment on the adequacy of the data to support safety and efficacy in the target population. The standards for safety and efficacy are relative; that is, the benefit-to-risk ratio of a biological product is considered. The VRBPAC's advice is considered in CBER's decision regarding licensure, and in developing recommendations for use to be given in the package insert. The committee may recommend additional studies to be performed either before or after approval. Once CBER determines that the data and information from the applicant are satisfactory and support the safety and efficacy of the product, the product is licensed.

Postmarketing Phase

If the manufacturer wishes to significantly modify the approved manufacturing process or directions for vaccine use, prior approval must be obtained from the FDA before these changes can be implemented. The applicant is required to submit an account of these changes to the appropriate license applications. Modifications to the manufacturing process may occur post licensure, such as scale-up or change in equipment to optimize the production process. Furthermore, clinical studies with the product also may be performed after licensure as the manufacturer seeks additional indications for product use (e.g., new target populations that would benefit from vaccination). For most new approvals, manufacturers may be asked to commit to completing specific postmarketing or so-called Phase IV studies, for example, to provide additional assessments of less-common or rare adverse events or further assess the duration of vaccine-induced immunity. These studies may also be designed to collect additional safety data in large numbers of vaccine recipients, as well as focus on issues that were identified during the prelicensure testing. Submission of status reports for certain postmarketing studies are required by regulation. In particular, this requirement for status reports pertains to postmarketing studies for clinical safety, efficacy and pharmacokinetics, and nonclinical toxicology to which an applicant committed in writing prior to licensure.[22]

Prior to FDAAA 2007, the FDA required postmarketing studies in the following situations (a) accelerated approvals for products approved under 505(b) of the FD&C Act or Section 351 of the PHS Act, respectively, which require postmarketing studies to demonstrate clinical benefit, (b) deferred pediatric studies, where studies are required under PREA, and (c) Animal Efficacy Rule approvals, where studies to demonstrate safety and efficacy in humans are required at the time of use. Under new Section 505(o) of the FD&C Act, the FDA is authorized to also require postmarketing studies or clinical trials at the time of approval or after approval if the FDA becomes aware of new safety information. Section 505(o)(3)(B) states that postmarketing studies and clinical trials may be required to (a) assess a known serious risk related to the use of the drug involved, (b) assess signals of serious risk related to the use of the drug, and (c) identify an unexpected serious risk when available data indicate the potential for a serious risk and when the adverse event reporting system is not adequate. The FDA has defined a clinical trial as any prospective investigation in which the sponsor or investigator determines the method of assigning treatment or other intervention to one or more human subjects. A study is all other investigations, such as investigations using humans, that are not clinical trials as defined above (e.g., observational epidemiologic studies), animal studies, and laboratory experiments. The FDA has issued guidance for industry to describe the type of studies and clinical trials that are required (Post Marketing Requirement [PMR]) under the FDAAA 2007, and those that will remain agreed-upon postmarketing commitments. A PMR describes all required postmarketing studies or clinical trials including those required under Accelerated Approval, PREA, the Animal Rule, and FDAAA. Examples of required studies are pharmacoepidemiologic studies designed to assess a serious risk, trials with a primary safety end point, preclinical studies investigating specific end organ toxicities, as well as pharmacokinetic studies in the indicated population at potential risk for high drug exposure that could result in toxicity. Studies that generally would not be considered required postmarketing studies or clinical trials are agreed-upon studies (postmarketing commitments) and include biologic quality studies (such as manufacturing, stability, and immunogenicity studies that do not have a primary safety end point), trials in which the primary end point is related to further defining efficacy, and pharmacoepidemiologic studies designed to examine the natural history of disease or background rates for adverse events. Since passage of FDAAA 2007, several new vaccines have been approved with either PMRs or postmarketing commitments. The FDA has the authority to monitor the progress of postmarketing studies or trials by requiring the applicant to submit an annual status report. Applicants are required to provide a timetable for study completion, a periodic status report on the status of the study including whether enrollment has begun, the number of participants enrolled, the expected completion date, and whether any difficulties in completing the study have been encountered.

ADVERSE EVENT MONITORING (POSTLICENSURE FOLLOW-UP)

The FDA is responsible not only for approving vaccines but also for monitoring their safety postlicensure. Because of the relatively small size of most prelicensure trials, rare adverse events are unlikely to be detected. Consequently, postlicensure or postmarketing surveillance (i.e., the continued monitoring of vaccine safety in the general population after licensure) is critical for identifying and evaluating rare or uncommon adverse events.

An adverse event refers to any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. The Vaccine Adverse Event Reporting System (VAERS) is a national system for passive surveillance of adverse events following vaccination. Established in 1990 as a result of the National Childhood Vaccine Injury Act of 1986, VAERS is administered jointly by the FDA and the CDC and, in recent years, has received more than

30,000 reports per year. The purpose of VAERS is to detect possible signals of adverse events associated with vaccines to help ensure the safety of U.S.-licensed vaccines. VAERS collects and analyzes information from reports of adverse events that occur after the administration of U.S.-licensed vaccines. Reports are submitted by healthcare providers, vaccine recipients or their parents or guardians, vaccine manufacturers, and other interested parties. FDA medical officers review all serious reports (defined as events that are fatal, disabling, or life-threatening; require or prolong hospitalization; result in congenital anomalies; require medical intervention to prevent such outcomes; or are deemed to be other medically important conditions). The VAERS system is not limited to routinely recommended pediatric vaccines; voluntary reports of adverse events occurring after administration of any vaccine are also accepted. FDA and CDC continually monitor VAERS reports for any unexpected patterns of adverse events.

Another important mechanism used by CBER to monitor adverse events is the Vaccine Safety Datalink, a collaborative effort between CDC's Immunization Safety Office and nine healthcare organizations. The Vaccine Safety Datalink uses electronic health data from participating sites and conducts vaccine safety studies based on questions or concerns raised from the medical literature and VAERS reports. When there are new vaccines that have been recommended for use in the United States, or if there are changes in how a vaccine is recommended, the Vaccine Safety Datalink will monitor the safety of these vaccines.[23]

INNOVATIVE SYSTEMS FOR EVALUATING VACCINE SAFETY POSTMARKETING

The FDA's Sentinel Initiative was launched in 2008 in response to a Congressional mandate in the FDAAA of 2007. The Sentinel Initiative aims to develop and implement a proactive system that will complement existing systems that the FDA has in place to track reports of adverse events linked to the use of its regulated products. It is a national electronic system that is transforming the FDA's ability to track the safety of drugs, biologics, and medical devices once they reach the market.[24] The Post-Licensure Rapid Immunization Safety Monitoring system (PRISM), a component of the FDA's Sentinel Initiative dedicated to vaccines, uses the FDA's Sentinel Distributed Database, which includes a population exceeding 178 million. PRISM monitors the largest U.S. general population cohort designated for active surveillance of vaccine safety by linking data from health plans with data from state and city immunization registries. The FDA structured PRISM as a program that includes specific vaccine evaluations. For example, several vaccines including a human papillomavirus vaccine, Gardasil, and two rotavirus vaccines, RotaTeq and Rotarix, were chosen for surveillance because their evaluations would benefit most from PRISM's large cohort size.

Postlicensure Manufacturing Changes

In 1997, the FDA published a Final Rule, Changes to an Approved Application, which amended 21 CFR §201.12 and §314.70 to simplify and categorize manufacturing reporting requirements for changes in testing methods, equipment, facilities, or personnel.[25] Proposed changes in manufacturing methods that have a substantial potential to have an adverse effect on the safety or effectiveness of the product may not become effective until notification is given of CBER's approval. The changed created the following categories: (a) those sufficiently significant with regard to safety, purity, potency, and effectiveness of the product to require preapproval of a supplemental application before product distribution; (b) those of lesser importance for which the manufacturer must provide notification 30 days before distribution of product made using the change; and (c) changes for which the manufacturer need only notify the agency by submission of an annual report. The guidance document, "Changes to an Approved Application: Biological Products" (1997), provides examples of changes that fall into these categories.[26]

Following approval, there is continued surveillance of the product and of the manufacturer's production activities. For most licensed vaccines, samples are submitted along with protocols for each lot prepared by the firm that provide the details of production and a summary of test results. Although not required by law or regulation, CBER often performs selected laboratory tests. The type and extent of confirmatory testing performed by CBER depend on several factors, such as the newness of the product or the difficulties that may have arisen with manufacture or use of the product. Release or rejection is based on a review of all test results, including those done by the manufacturer and those performed by CBER. Alternatives to official lot release are allowable under the provisions outlined for extensively characterized products having a track record of continued safety, purity, and potency.[27] A manufacturer must be able to produce a vaccine that repeatedly meets the standards for potency, purity, and stability of bulk and final container material while using a consistent process. Important factors to be considered are the nature of the product with respect to correlation between the measure of potency and biological activity and effectiveness. Surveillance samples and protocols may be required to be submitted to CBER at predetermined intervals.

Licensed establishments are inspected at least every 2 years. The purpose of the inspection is to determine whether licensed products are manufactured and tested as described in the license application and in accordance with applicable regulations. Manufacturers who fail to meet product standards or who are not in compliance with CGMPs may have their licenses suspended or revoked, depending on the nature of the potential health hazards created. The major issues observed during inspections can be categorized in three major areas: (1) process-related issues, (2) quality unit-related issues, and (3) facility- and production environment–related issues. Some examples of process validation issues include lack of documentation of time limits for major steps in the production process, lack of validation of rework or reprocessing steps in the manufacturing process, and lack of data to support in-process specifications. Quality unit-related issues include the appropriate reporting of out-of-specification results and process deviations (including adequate investigations into causes), appropriate documentation of product release, and adequate training of personnel. Facility and production monitoring concerns include controlling production environments by appropriately monitoring heating, ventilation, and air conditioning (HVAC) system performance and microbial quality (e.g., pressure differentials, appropriate sampling sites, and frequency of sampling). Other concerns pertaining to the facility include adequate cleaning, sanitization, storage, and changeover procedures for multiproduct areas and equipment. If the inspection team finds CGMP deficiencies in an already licensed facility, the team may remain in the facility until they have achieved an audit that provides confidence in the ability of the firm to reproducibly manufacture a safe and potent product.

Accelerating Availability of Vaccines and Pathways to Licensure

Mechanisms for providing earlier access to vaccines to prevent or treat severe and life-threatening illness have been developed.

These include fast-track development and the breakthrough therapy designation. The fast track program (Section 506(b) of FD&C Act) was added by FDAMA (1997) and amended by FDASIA (2012). It is designed to facilitate the development and expedite the review of new drugs and biologicals that are intended to treat serious or life-threatening diseases or conditions and for which nonclinical or clinical data are available that demonstrate the *potential* of the product to address unmet medical needs. A designation of a vaccine as fast track program provides the opportunity for frequent interactions of the applicant with the review team. The breakthrough therapy program (Section 506(a) of FD&C Act) was added by FDASIA of 2012. It applies to products for the treatment of serious or life-threatening disease or conditions for which preliminary clinical evidence indicates that the product may demonstrate substantial improvement on a clinically significant end point over available therapies. A sponsor may request a designation as a breakthrough therapy concurrently with, or any time after, submission of an IND. This new designation assists drug developers to expedite the development and review of new drugs with preliminary clinical evidence that indicates the drug may offer a substantial improvement over available therapies for patients with serious or life-threatening diseases. Breakthrough therapy designation provides for increased interaction with FDA to expedite the development and review of the application. In contrast to Fast Track designation, a breakthrough therapy designation requires evidence of substantial improvement over current treatments.

Products regulated by CBER are eligible for priority review if they provide a significant improvement in the safety or effectiveness of the treatment, diagnosis, or prevention of a serious or life-threatening disease. The FDA has 8 months to complete the review of a new BLA it designates as a priority, as opposed to 12 months for the completion of the review of a standard BLA submission.

Under the FDA's traditional approval pathway, a demonstration of vaccine effectiveness is based on a clinical disease end point (e.g., prevention of disease) or, alternatively, an accepted correlate of protection. In addition to these programs, the FDA's regulations provide for expedited pathways for licensure. Accelerated approval, 21 CFR §601.40, may be granted for certain biological products that have been studied for their safety and effectiveness in treating a serious or life-threatening disease or condition and that provide meaningful therapeutic benefit over existing treatments. Such an approval is based on adequate and well-controlled clinical trials establishing that the product has an effect on a surrogate end point that is reasonably likely to predict clinical benefit or on a clinical end point that can be measured earlier than irreversible morbidity or mortality that is reasonably likely to predict an effect on irreversible morbidity or mortality or other clinical benefit. Approval under this pathway is subject to the requirement that the sponsor study the biological product further, to verify and describe its clinical benefit, where there is uncertainty as to the relation of the surrogate end point to clinical benefit. Of note, the FDASIA of 2012 provided that evidence to support an end point is reasonably likely to predict clinical benefit to include "epidemiological, pathophysiological, therapeutic, pharmacologic, or other evidence developed using biomarkers, for example, or other scientific methods or tools." In other words, FDASIA expanded the scope of available end points that can be used to demonstrate that a product qualifies for accelerated approval, but do not affect the quantity and quality of evidence needed to demonstrate substantial evidence of effectiveness or safety.

Two vaccines to protect against meningococcal B diseases were licensed using the accelerated approval provisions and were designated breakthrough therapy. While the incidence of meningococcal serogroup B disease in the United States is low, outbreaks on several college campuses in the United States heightened concerns. Because of the diverse nature of meningococcal group B strains, as well as the low incidence of disease and the sporadic and unpredictable nature of an outbreak, clinical end point efficacy studies to support effectiveness of a serogroup B vaccine are not feasible. Given the public health concerns about meningococcal serogroup B disease in the United States, CBER agreed to provide breakthrough therapy designation and license two meningococcal group B vaccines, Trumenba and Bexsero, manufactured by Pfizer and GSK, respectively, under the accelerated approval regulations, 21 CFR §601 Subpart E. The Agency determined that it would be appropriate to use the accelerated approval pathway, basing approval on the ability of the vaccines to induce bactericidal antibodies, as measured by the human complement serum bactericidal assay, that are able to kill a panel of meningococcal group B strains that are representative of prevalent strains in the United States. The breadth of coverage of Trumenba and Bexsero against diverse meningococcal group B strains will be confirmed in subsequent clinical studies that examine the ability of the vaccine to induce bactericidal antibodies against a larger panel of meningococcal serogroup B strains representative of strains endemic in the United States.

In 2002, the FDA amended the biological products regulations to incorporate 21 CFR §601.90, Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible. This rule, referred to as the "Animal Rule," allows the use of animal efficacy data in lieu of human efficacy data when human challenge studies cannot be conducted ethically and field efficacy studies are not feasible because of infectious disease epidemiology (in the case of vaccines). In these situations, certain drug and biological products (e.g., vaccines) that are intended to reduce or prevent serious or life-threatening conditions caused by lethal or permanently disabling toxic chemical, biological, radiologic, or nuclear substances may be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals and additional supporting data. Safety, pharmacokinetics, and immunogenicity data are still necessary in humans. Under the animal rule, the FDA licensure of a product for which safety has been established and the requirements of 21 CFR §601.60 have been met is based upon adequate and well-controlled animal trials, when results of these animal studies establish that the product is reasonably likely to provide clinical benefit to humans. The FDA can rely on the evidence from animal studies to provide substantial evidence of the efficacy of these products when:

1. There is a reasonably well-understood pathophysiological mechanism for toxicity of the chemical, biological, radiologic, or nuclear substance and its amelioration or prevention by the product.
2. The effect is demonstrated in more than one animal species that is expected to react with a response that is predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model (in other words, the model has been adequately evaluated for its responsiveness) in predicting the response in humans.
3. The animal end point is clearly related to the desired benefit in humans, which is generally the enhancement of survival or prevention of major morbidity.
4. The data or information on the pharmacokinetics and pharmacodynamics of the product or other relevant data or information in animals and humans is sufficiently well understood to allow selection of an effective dose in humans, and it is reasonable to expect the efficacy of the

product in animals to be a reliable indicator of its efficacy in humans.

The animal rule does not apply if the product can be approved based on standards described elsewhere in FDA regulations (e.g., accelerated approval based on surrogate markers or clinical end points other than survival or irreversible morbidity).

Emergency use authorization (EUA) is another regulatory mechanism by which the FDA can accelerate the availability of vaccines and other pharmaceutical products. Under an EUA, the FDA can authorize the use of an unapproved product or the unapproved use of an approved product when an emergency or a potential emergency exists. Section 564(b)(1) of the Federal FD&C Act was amended by the Project BioShield Act of 2004 to allow the Secretary of Health and Human Services (Secretary) to authorize the introduction into interstate commerce of a drug, device, or biological product intended for use in an actual or potential emergency. Before an EUA may be issued by FDA, the Secretary must declare an emergency justifying the authorization based on:

- A determination by the Secretary of Homeland Security that there is a domestic emergency or a significant potential for an emergency that involves a heightened risk of attack with a specified biologic, chemical, radiological, or nuclear agent or agents; or
- A determination by the Secretary of Defense that there is a military emergency or a significant potential for an emergency that involves a heightened risk of attack with a specified biologic, chemical, radiological, or nuclear agent or agents; or
- A determination by the Secretary of Health and Human Services of a public health emergency under section 319 of the PHS Act that affects or has the significant potential to affect national security and that involves a specified biological, chemical, radiological or nuclear agent or agents or a specified disease or condition that may be attributable to such agent(s).

Once the Secretary declares an emergency, the FDA can authorize the emergency use of a particular product if the other statutory criteria and conditions are met. Based on the particular circumstances, the process for authorization can be expected to range in duration from hours to days. The Secretary has delegated the authority to issue an EUA under Section 564 of the FD&C Act to the FDA Commissioner.

Vaccine Testing

Vaccines are tested during both the prelicensure and the postlicensure phases. Testing procedures are developed with the goals of controlling and minimizing the potential for product-related adverse events by taking into consideration the experience gained from the same or related products. As an example, for inactivated vaccines, a clear understanding of the kinetics of inactivation is critical. For live vaccines, attenuation must be stable both to avoid reversion to virulence and to avoid the vaccine becoming over attenuated and, as a consequence, less potent. For example, during the first decade of its widespread use, Yellow Fever 17D vaccine virus was serially propagated in eggs, as required to meet demand. However, it soon became obvious that the level of attenuation of the vaccine from one passage to the next could vary considerably. Some lots were excessively neurovirulent, especially in infants and young children, whereas successive lots might by chance be overattenuated and, consequently, not immunogenic. The WHO formulated a solution to this problem, which was to adopt a "seed-lot system" wherein the vaccine is prepared from a master seed virus at a specified passage number in eggs. Working seed virus is prepared by one passage of the master seed and is, in turn, used to generate all production lots. All 17D vaccines and all other live virus vaccines now adhere to a seed-lot system for manufacture.

The FDA requires that cell substrates and vaccine viral seeds used in production be appropriately selected and tested to ensure that they do not introduce any unintended risks. Current cell substrates used to manufacture licensed vaccines are primary avian cells (embryonated eggs or chick embryo fibroblasts), diploid cells, continuous cell lines (Vero and Madin-Darby canine kidney [MDCK]), as well as yeast and insect cells. Table 79.6 lists the cell substrates used in current U.S.-licensed vaccines. In 2010, the FDA published the guidance, "Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases."[28] This document provides manufacturers of viral vaccines with guidance for the characterization and qualification of cell substrates, viral seeds, and other biological materials used for the production of viral vaccines for human use to assure that they meet the highest safety standards achievable using modern technology. Characterization of cell substrates should address certain general issues that might affect the safety and purity of vaccine products. For example, in the early 1960s, exogenous and endogenous contamination

TABLE 79.6 Cell Substrates Used in Current U.S.-Licensed Vaccines

		Vaccine	
Type	**Substrate**	**Live**	**Inactivated**
Animal tissues	Mouse brain Chicken eggs	 Influenza, yellow fever virus	Japanese encephalitis virus Influenza
Continuous cell lines (non-tumorigenic)	African green monkey cells (Vero)	Smallpox, rotavirus	Poliovirus, Japanese encephalitis virus
Diploid cells	Human MRC-5 cells Human WI-38 cells	Varicella, varicella-zoster Rubella, adenovirus types 4 and 7	Hepatitis A, rabies, poliovirus
Primary cell cultures	Chick embryo fibroblasts (CEFs) Madin-Darby canine kidney (MDCK) cells	Measles, mumps	Rabies Influenza vaccine
Insect cells	*Trichoplusia ni* *Spodoptera frugiperda*		Human papillomavirus Influenza vaccine
Yeast	*Saccharomyces cerevisiae*		Hepatitis B, human papillomavirus

of primary monkey kidney cells with simian virus 40 and chick embryo fibroblasts with avian leukosis virus were reported. Although chick embryo fibroblasts are still used for the production of viral vaccines, these cell substrates must be well-characterized and tested to assure absence of potentially infectious agents. Issues related to cell substrates have been discussed in a variety of forums.[29–31] Furthermore, if a vaccine is manufactured in a cell substrate that is derived from a tumor, or that has developed a tumorigenic phenotype through an unknown mechanism, it was considered to carry a higher theoretical risk of containing oncogenic substances such as oncogenic viruses and cellular DNA, derived from that cell substrate. This was the main reason tumorigenic cells or cells derived from human tumors had been considered unsuitable as cell substrates. However, at a 2012 meeting of the VRBPAC, experts recognized that cell lines derived from human tumor are an important tool for manufacturing of vaccines and could provide a wider repertoire of cell substrates for vaccine production.[30] They indicated that risk-mitigation strategies are the same for vaccines generated using human tumor-derived cell lines as for other cell substrates. A thorough characterization of the cell substrate with respect to adventitious viruses, which includes oncogenic viruses, should be done using new virus-detection technologies such as massively parallel sequencing, virus microarrays and broad-range polymerase chain reaction to complement existing assays. The manufacturing process should lower the amount and reduce the size of the DNA. Residual DNA for continuous nontumorigenic cells, such as low-passage Vero cells, should be limited to less than 10 ng/dose for parenteral inoculation and to less than 100 µg/dose for oral vaccines. Cells with tumorigenic phenotypes or other characteristics that give rise to special concerns may require more stringent limitation of residual DNA quantities and size to assure product safety.

The regulation of biologicals includes requirements for testing of licensed products (21 CFR §610). These tests include those for bacterial and fungal sterility, general safety, purity, identity, suitability of constituent materials and potency, thereby this specific testing performed may vary depending on the vaccine. For example, tests for potency may be based on studies of immunogenicity or, for some vaccines, protection from virulent challenge in laboratory animals. However, in vitro tests, including virus titration (e.g., live vaccines such as polio, measles, mumps, and rubella), antigen content (e.g., influenza and inactivated poliovirus vaccines), and biochemical and biophysical measurements (e.g., meningococcal conjugate vaccines) have been used.

Tests for purity are designed to determine that the product is free of extraneous material, except that which is unavoidable in the manufacturing process described in the approved license application. Tests for residual moisture and pyrogenic substances may also be included. Final-container material must be identified by a test specific for each product (e.g., neutralization of each of the components of live measles, mumps, and rubella vaccine with specific antisera). With regard to constituent materials, the manufacturer must ensure that all ingredients used in the product, such as diluents, preservatives, or adjuvants, meet generally accepted standards of purity. An adjuvant may not be used unless there is adequate proof that it does not adversely affect the safety or potency of the product.

Of note, the FDA periodically evaluates the appropriateness of testing requirements. An example is the general safety test (required under 21 CFR §610.11) used to detect extraneous toxic contaminants that may be present in the product in the final container from every final filling of each lot of the biological product. Technological advances have increased the ability of manufacturers to control and analyze the manufacture of many biotechnology-derived biological products. Thus, the FDA has published a final rule to remove this requirement.

In addition to the tests required by regulation, other tests tailored to the specific product may be required (e.g., neurovirulence testing and cell culture and animal tests for extraneous viruses). Once the product is licensed, the manufacturer's testing must be conducted according to the exact specifications in the manufacturer's license application, and the results of these tests must be within the specified prescribed limits.

Product Labeling and Advertising

Prescription drug labeling, also known as the package insert, package circular, or prescribing information, is the primary mechanism through which the FDA and drug manufacturers communicate essential, science-based prescribing information to healthcare professionals. Labeling provisions contained in 21 CFR §§201.57 and 201.56 require that prescribing information must summarize the essential information on the safe and effective use of the product; that information contained in the labeling must be accurate and not false and misleading; and that there must be no implied claims or suggestions for use if evidence of safety or effectiveness is lacking.[32] Whenever possible, data contained in labeling should be derived from human experience. In the United States, the FDA regulates the format and content of labels for product containers, cartons, and the package insert that accompanies the product. In January 2006, the FDA issued a final drug labeling rule, commonly referred to as the Physicians' Labeling Rule, amending the content and format of prescribing information for human drug and biologic products. The new format is intended to provide healthcare professionals with clear and concise prescribing information by reorganizing critical information into a streamlined format. Moreover, these revisions make it simpler for healthcare professionals to access, read, and use prescribing information, and enhance the safe and effective use of prescription drug products. New sections were added to the label, such as the Highlights section, which contains key benefit and risk information, and a table of contents for the full prescribing information. On December 3, 2014, the FDA issued the Pregnancy Lactation and Labeling Rule, which revises the content and format of the pregnancy subsection of labeling for prescription drugs and biologics (Sections 8.1 to 8.3 of the prescribing information). Previous regulations required that each product be classified under one of five pregnancy categories (A, B, C, D, or X) based on the risk of reproductive and developmental adverse effects or, for certain categories, such risk weighed against potential benefit. The most significant change proposed by the rule is the replacing of these letter risk categories with a narrative summary of the risks and benefits of using a drug during pregnancy, based on the available human and/or animal data and a discussion of the data. Additionally, the rule requires that prescription drug labeling include relevant clinical information to help healthcare providers make prescribing decisions and counsel women about the use of drugs during pregnancy and/or lactation.

The structured product labeling defines the content of human prescription drug labeling in XML format. It is a document markup standard approved by Health Level Seven and adopted by the FDA as a mechanism for exchanging product and facility information.[33] Structured product labeling documents contain both the content of labeling (all text, tables, and figures) for a product and additional machine-readable information (drug listing data elements). Drug listing data elements include information about the product (product and generic names, ingredients, ingredient strengths, dosage forms,

routes of administration, appearance, Drug Enforcement Agency schedule) and the packaging (package quantity and type).

During the BLA review, the agency considers the draft labeling and clinical studies submitted by the manufacturer, and the proposed indication for the licensed product, which is based on clinical data submitted by the sponsor demonstrating the safety and effectiveness of the product for its intended use and target population. Subsequently, significant changes in labeling, including new indications for use, new dosage forms or regimens, expanded patient populations who receive the product and additional information regarding safety and effectiveness, require manufacturers to submit a supplemental filing for review and approval by CBER. Unlike other product labeling, the promotional labeling and advertising are not subject to preclearance; however, they are monitored for misleading claims. These documents must also meet the standard of fair balance, that is, claims of efficacy are balanced with information about the product's safety.

Labeling changes are usually initiated by the manufacturer but may be initiated by CBER. Historically, manufacturers have had to obtain prior approval from CBER before the labeling changes were made. The changes to 21 CFR §601.12, mentioned previously, also apply to labeling changes and allow exceptions for a change that adds or strengthens a contraindication, warning, precaution, or adverse reaction; adds or strengthens instructions about dosage and administration intended to increase safe use; or deletes false, misleading, or unsupported indications for use or effectiveness claims. Under this regulation, a manufacturer could effect such changes and, at the same time, submit them and the supporting data to CBER without preapproval.

As described earlier, the FDAAA amended the FD&C Act by adding new Section 505(o) authorizing FDA to require and, if necessary, *order* labeling changes if the FDA becomes aware of new safety information that the FDA believes should be included in the labeling of the drug. Section 505(o)(4) of the FD&C Act imposes timeframes for application holders to submit and for FDA staff to review such changes, and gives the FDA new enforcement tools to bring about timely and appropriate safety labeling changes.

SPECIAL CONSIDERATIONS

Adjuvants

Strategies and approaches for the development and delivery of vaccine antigens have expanded over the last several decades, leading to a broad range of novel products comprised of purified subunit antigens or subunit proteins. These antigens may require the presence of adjuvants to enhance the immune response to the vaccine antigens, reduce the dosing frequency, induce cross-protective effects, direct the immune response and/or achieve antigen sparing. The number of investigational vaccines containing novel adjuvants evaluated in clinical trials has increased in recent years and some vaccines containing novel adjuvants have been licensed by the FDA. For example, the human papillomavirus vaccine manufactured by GlaxoSmithKline, Cervarix, contains AS04, an adjuvant system comprised of an aluminum hydroxide and monophosphoryl lipid A. GlaxoSmithKline's pandemic influenza vaccine, Q-Pan, contains AS03, an adjuvant system comprised of an oil-in-water emulsion.

The CFR defines adjuvants, together with ingredients, preservatives, and diluents as constituent materials (21 CFR §610.15).[34] These regulations state, "All ingredients…shall meet generally accepted standards of purity and quality" and that, "An adjuvant shall not be introduced into a product unless there is satisfactory evidence that it does not adversely affect the safety or potency of the product."

From a regulatory perspective, adjuvants are not considered active ingredients as defined in 21 CFR §210.3(b)(7) and vaccine adjuvants are not licensed separately.[35] It is the adjuvanted vaccine formulation, in toto, that is tested in nonclinical and clinical trials and licensed. There is a requirement that the adjuvanted vaccine formulation, as with any vaccine, must be both safe and effective, with its benefits outweighing the risks of adverse events that may occur. However, there is no explicit requirement for demonstrating the added safety and effectiveness of the adjuvanted vaccine formulation over that of the unadjuvanted vaccine formulation in comparative clinical trials.

The regulatory considerations for the nonclinical and clinical development of preventive vaccines, as well as pathways to licensure described elsewhere in this chapter, are largely also applicable to vaccines formulated with adjuvants. However, adjuvants can exhibit a range of properties that invoke complex immune responses, the mode of action is not always known or fully understood, and animal models that are relevant for evaluating both the safety and the efficacy of an adjuvant–antigen combination are frequently not available. Thus, there are some unique issues to be addressed during preclinical and clinical development of the adjuvanted vaccine formulation. A WHO guideline published in 2013 describes the nonclinical, quality, pharmacological, toxicological, and other information needed to support initiation of clinical trials with a vaccine combined with a novel adjuvant.[36]

In 2007, CBER published two guidance documents ("Guidance for Industry: Clinical Data Needed to Support Licensure of Pandemic Influenza Vaccines"[37] and "Guidance for Industry: Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines"[38]). Each of these documents discusses the development of adjuvanted inactivated influenza vaccines and notes that "[d]ata to support the safety of the adjuvanted formulation and added benefit over the unadjuvanted formulation must be submitted in the BLA…"[37,38] Sponsors were advised that "at an early stage of development, clinical data supporting the value of adding the adjuvant should be provided…"[37,38] The seasonal influenza vaccine guidance notes further that "[i]f an adjuvant is added to a licensed seasonal vaccine without antigen sparing effects…the immune response elicited by the adjuvanted formulation should be substantially better than that elicited by the unadjuvanted vaccine…"[38] As the FDA's experience with novel adjuvants has grown, the agency continues to reexamine guidance and engages with sponsors and applicants regarding the clinical development of adjuvanted vaccines.

Vaccine manufacturers should provide a rationale for the use of an adjuvant in the vaccine. The "added benefit" of an adjuvant may be defined as evidence of enhanced immune response, antigen-sparing effect, dose sparing, increased breadth of immune response, or superior clinical efficacy. Information to support the "added benefit" of the adjuvant may be derived from preclinical studies, for example, in vitro assays and/or proof-of-concept studies in animal models conducted prior to the initiation of clinical trials or early phase clinical trials. There is no regulatory requirement to demonstrate the "added benefit" of an adjuvant in clinical comparative Phase III effectiveness trials unless the applicant plans to make a claim of superiority of the adjuvanted product over unadjuvanted product.

The benefits from incorporating or adding an adjuvant to any vaccine formulation need to be balanced with the risk of adverse reactions. Adjuvants have their own pharmacological activity, which may affect both the immunogenicity and the safety of vaccines. Adverse reactions may include local

reactions such as pain, swelling, injection site necrosis, and granulomas. Systemic reactions may include nausea, fever, arthritis, as well as potential immunotoxic reactions. Unexpected, rare events may also occur. For example, during the H1N1 influenza pandemic in 2009–10, an increased risk of narcolepsy, a chronic neurological disorder caused by the brain's inability to regulate sleep–wake cycles normally, was observed in several European countries following vaccination with an AS03 adjuvanted, monovalent 2009 H1N1 influenza vaccine, Pandemrix.[39–43] The finding of narcolepsy in several European countries following vaccination with Pandemrix caused the European Medicines Agency to recommend restricting use of Pandemrix.[44]

Pandemrix was not licensed for use in the United States and no adjuvanted influenza vaccines were used in the United States during the influenza pandemic. The CDC published a study on a possible association between U.S.-licensed unadjuvanted 2009 H1N1 influenza vaccines, 2010–11 seasonal influenza vaccines, and narcolepsy.[45] The analysis included more than 650,000 people who received the pandemic influenza vaccine in 2009 and more than 870,000 people who received the seasonal influenza vaccine in 2010–11. The study found that neither vaccine was associated with an increased risk for narcolepsy. Additional studies including those conducted by the vaccine manufacturer will help discern whether this finding is attributable to the adjuvant, the H1N1 vaccine antigen, or both.

The safety of an adjuvanted vaccine formulation has to be demonstrated in adequate and well-controlled prelicensure safety studies. Safety information supporting licensure of an adjuvanted vaccine may include the safety experience obtained from domestic or foreign trials conducted using the adjuvanted vaccine formulation. In addition, safety experience with the same adjuvant formulated with other vaccine antigens may also contribute to the safety evaluation of the adjuvant. Early in clinical development (e.g., Phase I and Phase II clinical trials), supportive safety data may be derived by comparing the adjuvanted vaccine to a placebo or the unadjuvanted vaccine antigen, if feasible. Safety follow-up of human subjects administered vaccine with novel adjuvant is typically longer than for nonadjuvanted vaccines (e.g., 12 months rather than 6 months) and includes specific inquiries regarding symptoms consistent with autoimmune and neuro-inflammatory diseases. Furthermore, the safety database required to support licensure of a vaccine formulated with novel adjuvant may be larger than for unadjuvanted vaccines.

In summary, regulatory pathways supporting development and approval of vaccines formulated with novel adjuvants are similar to those for unadjuvanted vaccines. Efficient planning of the development pathway for any adjuvanted vaccine requires careful attention to preclinical testing, study design, dosing decisions, and safety monitoring. Although manufacturers are not required to demonstrate the "added benefit" of adjuvanted versus unadjuvanted vaccines in clinical comparative Phase III studies, manufacturers should provide a justification for including an adjuvant in the vaccine. Lastly, evaluation of safety of an adjuvanted vaccine needs to include special safety considerations.

Vaccines Against Global Infectious Diseases, Emerging Infectious Diseases, and Biothreat Agents

Immunization programs in the United States have been remarkably effective at reducing morbidity and mortality from the most common, naturally transmitted infectious diseases, such as polio, measles, and diphtheria. However, emerging infectious diseases (EIDs)—from pandemic influenza to novel pathogens like severe acute respiratory syndrome and Ebola, to biological threats that have the potential to be intentionally released into the general population by humans—also pose a threat to global public health. Vaccines will continue to be an important medical countermeasure against a broad range of infectious diseases from anthrax, smallpox, and influenza to newly emerging infectious diseases. Moreover, infectious diseases, such as tuberculosis and malaria present global public health challenges and increasing resistance to currently available treatments by common bacteria such as staphylococci, underscores the importance of the development of safe and effective vaccines.

The development of safe and effective vaccines to protect against global infectious diseases (e.g., tuberculosis, malaria, HIV/ AIDS), enteric diseases, and other neglected diseases of the developing world is of critical public health importance. Development and availability of such vaccines, particularly for use in the developing countries most affected by these diseases, will benefit U.S. and global health. In 2011, the FDA published a revised guidance document to assist sponsors in developing vaccines targeted against infectious diseases or conditions endemic in areas outside the United States. The FDAAA of 2007 revised the FD&C Act by adding Section 524, recognizing the importance of having products to treat and prevent tropical diseases that disproportionately affect poor and marginalized populations and for which there is no significant market in developed nations. Under Section 524, the Agency can grant priority review of applications under Section 505(b)(1) of the FD&C Act or Section 351 of the PHS Act for the treatment and prevention of specified tropical diseases, including tuberculosis, malaria, cholera, and "any other infectious disease for which there is no significant market in developed nations and that disproportionately affects poor and marginalized populations, designated by regulation by the Secretary." Consequently, this guidance provides general recommendations for regulatory pathways to use in the development of vaccines to protect against global infectious diseases for U.S. licensure and clarifies applicable regulations: (a) the FDA can license vaccines to protect against infectious diseases or conditions that are not endemic or have not been reported to occur in the United States; (b) the regulatory pathways to U.S. licensure for the development of vaccines to protect against infectious diseases that are not endemic or have not been reported to occur in the United States are the same as for vaccines to protect against diseases that are endemic in the United States; (c) a sponsor may submit data from clinical trials conducted outside the United States to support product licensure, (d) noting that the accelerated approval regulations may be used in appropriate cases; and (e) that when pivotal studies are conducted outside the United States, in some instances, it may not be necessary to conduct studies in the United States. This guidance also responded to the congressional mandate in Section 740 of the fiscal year 2010 Appropriation Act (Agriculture, Rural Development, Food and Drug Administration, and Related Agencies Appropriations Act, 2010, Public Law 111-80) by requiring the FDA to make recommendations on appropriate preclinical, trial design, and regulatory paradigms to prevent, diagnose, and treat rare diseases and neglected diseases.

The U.S. military has implemented vaccination programs to protect troops against several biological threats; however, the risk-to-benefit ratio for protecting civilians against agents of bioterrorism is more difficult to assess. As of this writing, there is one licensed smallpox vaccine in the United States, Sanofi Pasteur Biologics' ACAM2000. New smallpox vaccines are being developed under IND applications, with the goal to seek licensure, and new vaccinia immunoglobulin

preparations to treat certain complications of smallpox vaccination have been approved. There is one licensed anthrax vaccine in the United States, anthrax vaccine adsorbed (Emergent BioSolutions' BioThrax).

There are significant scientific and regulatory challenges associated with developing and testing new vaccines against EIDs and biothreat agents. Vaccines against EIDs are more likely to use novel technologies, and the science behind these technologies may be more complex; for example, use of novel cell substrates, the need to develop alternative potency assays, and the need to identify surrogate markers in humans or animals that predict vaccine effectiveness. To respond to this challenge, the FDA collaborates with interagency groups within the Department of Health and Human Services, such as the Biomedical Advanced Research and Development Authority, the CDC, and the NIH, as well as the Department of Defense and Department of Homeland Security to prepare for responding to an emergency. A committed, continuous investment in regulatory science is essential to producing medical countermeasures against public health threats. As noted in the *Public Health Emergency Medical Countermeasures Review*,[46] "Enhancement and ultimate application of updated regulatory science and scientific review capacity will help strengthen the MCM [medical countermeasure] regulatory process and thus streamline the MCM development process. [The] FDA will undertake a new initiative designed to focus on augmenting the tools used to assess the safety, efficacy, and quality of medical products, with a particular focus on MCMs, and to get them from concept through the approval process efficiently." An example of how the FDA's regulatory science efforts have assisted the agency in facilitating the licensure of vaccines against emerging diseases and biothreats is the successful public–private partnership during the 2009 H1N1 influenza pandemic, which resulted in the development and approval of safe and effective vaccines against the pandemic in record time. This included creating vaccine strains needed for vaccine manufacturing within weeks of the very first 2009 pandemic H1N1 influenza cases appearing, developing reagents and tests through international collaborations to measure the vaccine's potency, consulting the FDA's expert vaccine advisory committee to review the Agency's approach to approval of the 2009 H1N1 vaccine s as well as extensive in process quality control and product testing. Licensure of vaccines against the 2009 H1N1 influenza virus occurred in September 2009 based on the FDA's determination that standards to ensure the safety and potency of these vaccines had been met. In parallel to these efforts, NIH and vaccine manufacturers initiated clinical trials to determine the optimal vaccine dosage and number of doses needed to induce a protective immune response against pandemic 2009 H1N1 virus.

Another example illustrating collaboration both within the Department of Health and Human Services and between the Department and international partners is the response to the Ebola virus disease outbreak in West Africa in 2014–15 that caused more than 25,000 cases of Ebola virus disease and claimed the lives of more than 10,000 people, representing the most widespread epidemic of Ebola virus disease in history. To address this need, the FDA engaged with federal partners, sponsors of medical products, and international organizations to facilitate development of vaccines. To advance promising vaccine candidates to Phase I clinical trials to obtain preliminary human safety data, the FDA worked closely with manufacturers, clinical trial sponsors, and international regulators to rapidly assess product characterization data and protocols for these first human clinical trials and granted permission for the initiation of these Phase I studies in an expedited manner. Guidance provided by the FDA assured that these studies were conducted according to applicable regulations including the requirement for nonclinical testing and protection of human subjects. These efforts resulted in rapid availability of safety and immunogenicity data for the leading Ebola vaccine candidates, initiation of U.S. government–sponsored Phase III clinical studies to demonstrate the safety and effectiveness of these vaccines in individuals residing in outbreak areas, and collection of data supporting licensure of these products. To enable an initiation of these pivotal studies in an expedited manner, the FDA engaged in joint reviews with vaccine manufacturers, the WHO, regulatory agencies in Europe and Canada, and West African regulators to discuss study designs, ethical considerations, and required product information to reach international regulatory convergence, thus facilitating trial initiation. It is not always possible to test whether a vaccine or treatment will work against a new or emerging infectious disease, or against a biothreat, because the threat may be rare or even nonexistent at the time the vaccine or therapy needs to be developed. Moreover, many vaccines against EIDs and biothreats pose difficult challenges with regard to obtaining clinical efficacy data. For many of these infectious agents or toxins, human efficacy trials are not feasible because natural exposure no longer occurs (e.g., smallpox), occurs at a very low incidence, or occurs in an unpredictable manner. The animal rule described earlier (see "Accelerating Availability of Vaccines and Pathways to Licensure") is one regulatory mechanism that allows the FDA to address the challenges of obtaining clinical efficacy data for these products if the results of adequate and well-controlled animal studies establish that the product is reasonably likely to provide clinical benefits to humans.

Animal testing is often the only available option, but many diseases lack even good animal models, and animal studies are technically difficult to conduct and typically limited in size. Consequently, regulatory science is needed to develop and validate improved predictive models. Regulatory science can also support the identification and validation of surrogate measures of product efficacy. Biomarkers that predict efficacy are not yet available for most terrorism threats, emerging pathogens or major global infectious diseases. Efforts to develop, refine, and validate new biomarkers may lower development costs and improve and speed the development of safe and effective products for unmet public health needs.

In summary, for licensure, an EID vaccine product, just as for any product, must have an acceptable quality, safety, efficacy, and potency profile. Likewise, production and quality control also must be in compliance with CGMPs.

However, vaccines against EIDs and biothreats agents present unique issues for clinical development and evaluation by the FDA. Overall planning and coordination among the FDA and its national and international partners is necessary to move these products toward licensure and into distribution. FDA guidance and engagement with partners is critical to make sure these products can move from the future into the present.

CONCLUSION

The primary responsibility of NRAs is to ensure the quality, safety, and effectiveness of pharmaceutical products. The implementation of a strong regulatory system will facilitate these goals, which are especially critical for vaccines that are inherently more difficult to develop, characterize, and manufacture than most pharmaceutical products. The FDA has developed a managed review process that provides regulatory oversight through all phases of vaccine development. Advances across a wide range of scientific disciplines have enhanced the

prospects of developing new and better vaccines. Novel vaccine approaches such as recombinant vaccines and novel adjuvants and delivery systems pose regulatory challenges for NRAs. However, NRAs should be dynamic and flexible entities, as they strive to develop regulatory requirements to address the evolving science. Further, NRAs must be prepared to address public health emergencies that will require expedited approval mechanisms, such as biological terrorist events, pandemic influenza, and other EIDs.

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80 Regulation of Vaccines in Europe

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Vaccines are complex biological medicinal products developed with increasingly sophisticated technologies. They can contain live infectious agents and many other ingredients. Because of the major individual and population benefits, immunization with certain vaccines is mandatory in many European countries, and any safety hazard could affect a large number of persons. In this context, regulatory authorities have the mandate to promote and protect public health by allowing use of safe and effective vaccines and by continuously assessing their benefit and risk profile after marketing authorization. These obligations are defined in two key European legislative texts, Regulation (EU) No. 726/2004 and Directive 2001/83/EC.

Many actors are involved in these activities: member states and their national competent authorities, public health institutions and national technical institutes including academia, and, at the European level, the European Commission in Brussels and the European Medicines Agency (EMA) in London. Another European agency established in 2004 through Regulation (EC) No. 851/2004, the European Centre for Disease Prevention and Control (ECDC), identifies, assesses, and communicates, in partnership with national health protection bodies across Europe, current and emerging threats to human health posed by infectious diseases. Since 1995, the EMA coordinates the scientific evaluation and supervision of medicines for use in the European Union, including vaccines; fosters scientific excellence in this activity; and makes scientific recommendations for marketing authorization of medicines to the European Commission.

MARKETING AUTHORIZATION

Marketing Authorization Procedures in the European Union

National Procedure

A national procedure applies when the manufacturer of a medicinal product or vaccine submits a marketing authorization application in a single member state where it intends to market the medicinal product or vaccine. This procedure for authorization of the product is the sole responsibility of the member states and is only possible if the concerned medicinal product does not fall within the mandatory scope of the centralized procedure.

A medicinal product may receive a marketing authorization that is valid in more than one member state in the European Union by one of two routes. These are the decentralized and the centralized procedures.

Decentralized Procedures

There are two decentralized procedures. In one, a marketing authorization application is submitted to more than one member state in parallel. In the other, a member state grants the first marketing authorization, which is then mutually recognized in the other concerned member states chosen by the manufacturer of the medicinal product or vaccine; this is the *mutual recognition procedure.* Such procedures are facilitated by the Co-ordination Group for Mutual Recognition and Decentralised Procedures—Human (CMDh), which meets at the EMA monthly.[1] The marketing authorization application submitted via one of these procedures (decentralized or mutual recognition) must be identical to the original marketing authorization application granted in the first member state, called the *reference member state.*

Centralized Procedure

European Union (EU) regulations define which medicinal products must go through the centralized procedure to receive a marketing authorization that is valid for the whole of the EU. In general, vaccines containing a new active substance outside any of the mandatory therapeutic indications defined in the Annex I of Regulation (EU) No. 726/2004[2] may follow the centralized procedure or the decentralized procedure. The centralized procedure is required for all medicinal products (including vaccines) developed by recombinant DNA technology, such as controlled expression of genes coding for biologically active proteins in prokaryotes (e.g., Cervarix) expressed in baculovirus and eukaryotes (e.g., Infanrix hexa), expressed in Vero human cell lines (e.g., Gardasil [human papillomavirus vaccine]), in yeast cell lines, and flu vaccines incubated in fertilized eggs. This includes transformed mammalian cells, or hybridoma and monoclonal antibody methods. This registration procedure must also be used if the concerned vaccine is considered to be an advanced therapy medicinal product or an orphan medicinal product pursuant to Regulation (EC) No. 141/2000.

The EMA and its Committee for Human Medicinal Products (CHMP) is responsible for evaluating the quality, safety, and efficacy of medicinal products submitted through this procedure. The CHMP consists of one member plus an alternate for each EU member state, one member each from Iceland and Norway (European Economic Area countries) plus their alternates, and five coopted members selected based upon their expertise and experience in evaluating medicinal products. The CHMP is assisted by a wide network of experts from all over Europe. The regulation allows the CHMP to establish working parties, such as the Vaccines Working Party and the Biologics Working Party, to draft guidance and advise the CHMP on specific aspects of product evaluation and supervision.

The scientific review of a marketing authorization application is performed to the highest scientific standards following a flexible timetable. The total duration to assess a marketing authorization application, draft and discuss reports, and adopt an opinion should not take more than 210 days. This duration can be shortened under certain conditions. Although the dossier submitted by the marketing authorization holder forms the basis for the evaluation of the benefits and risks of the vaccine, other sources of information, including those from the ECDC, national research institutions, including academia or the World Health Organization (WHO), might, in addition, be consulted to perform a scientific assessment. The result of the evaluation is a positive (favorable) or negative (unfavorable) CHMP opinion to recommend granting a marketing authorization for the medicinal product.

The CHMP may also consult the established vaccines scientific advisory group, possibly including additional ad hoc experts formed by leading experts in the relevant field and chosen based upon the content of the questions referred by the CHMP.[3] Scientific advisory groups assist the CHMP on specific questions, especially in cases where there is a divergence of scientific opinions.

Three types of positive opinions can be recommended by the CHMP: full marketing authorization, conditional marketing authorization, and marketing authorization under exceptional circumstances.

A full marketing authorization is adopted by the CHMP for a medicinal product for which a positive benefit-and-risk assessment has been concluded based on sufficiently comprehensive data.

A conditional marketing authorization may be recommended by the CHMP for certain categories of medicinal products and in certain situations; for example, to meet unmet medical needs and in the interests of public health, and where it is necessary to grant marketing authorizations on the basis of data that are less complete than is normal and therefore subject to specific obligations. The categories of products concerned should be medicinal products that aim to treat, prevent, or diagnose seriously debilitating or life-threatening diseases; medicinal products to be used in emergency situations in response to public health threats recognized either by the WHO or by the EU; or medicinal products designated orphans. Such conditional marketing authorizations are granted for 1 year, renewable until the specific obligations are fulfilled.[4]

The CHMP may recommend granting a marketing authorization under exceptional circumstances, subject to specific defined conditions, if the applicant can show that it is unable to provide comprehensive data on the efficacy and safety under normal conditions of use for one of the following reasons[5]:

- The indications for which the product in question is intended are encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence.
- In the present state of scientific knowledge, comprehensive information cannot be provided.
- It would be contrary to generally accepted principles of medical ethics to collect such information.

In all instances, the final opinion adopted by the CHMP is sent to the European Commission. If its standing committee's opinion is favorable, the commission issues a formal decision that serves legally as the EU marketing authorization, which is valid throughout the EU in all member states. Following the commission's decision, the EMA publishes the European Public Assessment Report after deleting commercially confidential information.[6]

The choice whether to follow the decentralized or centralized procedure lies with the applicant unless the vaccines fall under the mandatory scope of the centralized procedure as described above.

Preauthorization Evaluation of Benefit and Risk

In the context of a new drug application, the assessment of the benefits and risks must objectively reach a sufficient level of confidence that the quality, efficacy, and safety of the new medicinal product have been demonstrated. A marketing authorization may be refused if the therapeutic efficacy is insufficiently demonstrated or if the benefit-to-risk balance is not considered favorable.[7] This assessment should be based on the available tests and trials, which must be designed to determine the efficacy and safety of the product under normal conditions of use. The assessment should consider the perspectives of different stakeholders, in particular those of patients and treating physicians. Authorization decisions under the centralized procedure should be to the exclusion of economic and other considerations such as cost-effectiveness.

Data Requirements for New Vaccines

Efficacy

Protective efficacy studies ideally are presented for the authorization of new vaccines, but in some situations such studies are either not necessary or not feasible, such as in cases where use of immunologic data to predict protection against infection can be justified. Examples include cases such as the following:

- Immunologic correlates of protection against specific infections (e.g., diphtheria, tetanus) have been established.
- Estimation of protective efficacy is not feasible because the potentially preventable infectious disease occurs at a rate too low for a study to be performed in a reasonable period (e.g., brucellosis, Q fever).
- Efficacy studies are not feasible and no immunologic correlate of protection exists, but the likely efficacy of a vaccine may be estimated by comparison of immunologic responses with those seen in past studies of similar vaccines with proven protective efficacy (e.g., acellular pertussis vaccines).
- Efficacy studies are not feasible and there is no established immunologic correlate of protection or previous efficacy studies that might provide immunology data for comparison (e.g., anthrax).

In case a protective efficacy study is not performed, the applicant needs to provide a sound justification for the lack of such data. The protocols used for studies of protective efficacy should include a rationale for the choice of the study population and a detailed description of the methods used for diagnosing infection (e.g., clinically apparent or inapparent infections). In principle, validated methods should be used for diagnosis or for other evaluations (e.g., histology). Immunogenicity data to characterize the immune response to the vaccine and to support the development of specific posology need to be collected under all circumstances.

Guidelines with respect to quality, nonclinical and clinical development of vaccines are available on the EMA's website.[8]

Safety

Safety data should usually be collected after each dose of the vaccine. Because most adverse reactions to vaccines occur within the first few days after each dose, it is generally accepted to focus the collection of information on adverse events that occur within 5 to 7 days after vaccination (longer for live vaccines). In most cases, it is not reasonable to expect that the preauthorization database is sufficiently large to identify rare adverse events. As a minimum, the total data from preauthorization studies is usually sufficient to reliably determine the frequency of uncommon local and systemic adverse events that have an incidence of between one per 100 and one per 1000 vaccinated persons. Unless otherwise justified, the recommended minimum sample size should be at least 3000 subjects for new vaccines. Certain vaccines, such as those containing novel adjuvants, may have to provide additional evidence of their safe use.

Differences in the safety profile might be observed between certain subpopulations and also with consecutive doses during the immunization schedule. In these cases, it may be necessary

to obtain sufficient data to detect at least uncommon adverse events in various subsets before a marketing authorization could be granted, and the applicant should address these matters in adequately powered postmarketing studies.

Authorization Based on Limited Data

Special consideration is needed for clinical development of vaccines when protective efficacy studies are not feasible and when there is no established immunologic correlate of protection. This applies, for example, to vaccines intended to prevent rare infections that carry considerable morbidity and mortality, including by some pathogens that have the potential to cause widespread disruption to humanity in case of an epidemic or deliberate release.

In some cases, it is feasible to obtain some relevant data on protective efficacy from challenge studies in animal models. Immunologic correlates of protection might be established for very similar but not identical antigens that might be used in the meantime as a guide for likely efficacy. If possible, immunologic studies should focus on measuring functional immune responses. Taking the results of these and any other relevant investigations together, a presumptive risk-to-benefit relationship could be derived that might support authorization.

When authorization is based on limited data, it might not be possible to estimate the vaccine's effectiveness in the postauthorization period unless a natural epidemic or deliberate release occurs. In any case, it is likely that reliable data could be obtained only from national surveillance programs operated by public health authorities or those coordinated by the ECDC. Applicants are encouraged to work with public health authorities to develop plans that would allow collection of data on safety and efficacy if the opportunity arises (e.g., a significant outbreak or major epidemic). It is recommended to seek scientific advice when considering a marketing authorization application based on limited data.

SCIENTIFIC ADVICE AND AUTHORIZATION OF CLINICAL TRIALS

The EMA may provide pharmaceutical companies with scientific advice and protocol assistance; protocol assistance consists of scientific advice for companies developing medicines for orphan or rare diseases.[9] Advice may be requested at any stage of development of a medicinal product (e.g., before the submission of a marketing authorization application) or during the postauthorization phase. This is particularly useful when the development used by a company deviates from the available guidance or when relevant EU guidance does not exist. Scientific advice can be sought at any stage during product development and life-cycle and covers quality, nonclinical and clinical as requested.

The EMA scientific advice or protocol assistance procedure accepts questions related to clinical studies performed first in humans and questions related to clinical development plans and addresses them from a scientific point of view, adding a disclaimer clarifying responsibilities. Scientific advice can also relate to postauthorization monitoring of vaccines.

Requests for scientific advice are independent of the type of registration procedure followed for the marketing authorization. It has been shown that companies following EMA's advice are more likely to succeed with the authorization procedure, receive faster evaluation of the marketing authorization application, and have reduced risk of refusal of a marketing authorization.[10] For medicinal products for human use, scientific advice is given by the CHMP upon recommendation of the Scientific Advice Working Party. The advice is a preparatory act of the procedure, and it is not legally binding. The scientific advice can be followed or not by companies and regulators, and it does not ensure a favorable outcome for the marketing authorization application. The scope of this procedure can be very broad and can include questions regarding, for example, legal aspects of orphan medicinal products, a class of medicinal products, the use of scales in a therapeutic indication, or a risk-management plan. Scientific advice may be sought for products intended to be marketed exclusively outside the EU in cooperation with the WHO (see later discussion).

The EMA and the U.S. Food and Drug Administration cooperate to provide coordinated scientific advice. It is expected that this program will contribute to a common understanding of scientific issues, offer opportunities to optimize product development globally, and avoid unnecessary or divergent testing requests.

One goal of EMA's long-term strategy is to foster research and the uptake of innovative methods in the development of medicines. For this purpose, the multidisciplinary EMA Task Force on Innovation has been established as a discussion platform for early dialogue with applicants to proactively identify scientific, legal, and regulatory issues for emerging therapies and technologies and for borderline products that have characteristics belonging to diverse legal frameworks, such as medicines and medical devices.

The authorization of clinical trials is the responsibility of national competent authorities of EU member states in which the sponsor wishes to conduct the trial. The same applies for ethics committees. Guidance and legislation in relation to the conduct of clinical trials in Europe is described in volume 10 on clinical trials of the EudraLex.[11]

REQUIREMENTS FOR PEDIATRIC MEDICINAL PRODUCTS

The EU pediatric regulation (Regulation [EC] No. 1901/2006, as amended) was adopted in Europe to support the development, quality, and availability of medicines for children, promote ethical research in children, and ensure availability of information on the use of pediatric medicines.[12] This regulation also applies to vaccines used in children.

For new medicines (including vaccines) marketing authorization applications must include the results of studies conducted in the pediatric population in compliance with a pediatric investigation plan agreed upon between the EMA Pediatric Committee and the applicant. A pediatric investigation plan is also needed for a new pediatric indication, pharmaceutical form, or route of administration added to an existing authorized medicinal product in the EU. The pediatric investigation plan covers relevant clinical trials in the pediatric population, timelines to perform these, and measures proposed to obtain a pediatric indication; it also may propose an age-appropriate formulation in the target pediatric population. Deferrals to research may be granted when, for instance, development of the pediatric version is postponed until efficacy and safety have been demonstrated in adults. Waivers are granted when the Pediatric Committee does not consider specific pediatric development necessary or appropriate. When a pediatric authorization for a product is obtained for all EU member states and when the study results, whether positive or negative, are included in the product information, the medicine is eligible for a 6-month patent extension. Off-patent medicines developed specifically for pediatric use can also benefit from a marketing authorization for pediatric use with 10 years of data protection.

The EMA and the U.S. Food and Drug Administration cooperate and exchange information to foster the development of medicines for children.

MEDICINAL PRODUCTS INTENDED FOR MARKETS OUTSIDE THE EUROPEAN UNION

The CHMP may give a scientific opinion, in the context of cooperation with WHO, for evaluating certain medicinal products for human use intended exclusively for markets outside the EU. Examples of this so called *Article 58 procedure* include vaccines to be possibly used in the WHO Expanded Programme on Immunization, vaccines for protection against a WHO public health priority disease, and vaccines that are part of a WHO-managed stockpile for emergency response.[13] The procedures and requirements for the centralized procedure are applied in this case. The European Commission does not formulate a decision for a marketing authorization, but the Article 58 procedure does not exclude a future application for a marketing authorization in the EU. The CHMP assesses the quality, safety, and efficacy of the medicinal product applying the same scientific scrutiny and criteria as for an EU marketing authorization application using input from WHO experts and from observers from authorities from developing countries recommended by WHO as needed. This assessment is performed according to guidelines of the EU and International Conference on Harmonization or, if appropriate, to guidelines issued by WHO and other bodies. Different benefit-to-risk profiles of the product for the target population may be considered based on assumptions about possible conditions of use in the targeted country or region.

An Article 58 application may be assessed in parallel with a WHO prequalification procedure, in which case EMA collaborates with the WHO and interacts as necessary with relevant WHO expert advisory committees.

Following the CHMP opinion, the opinion holder is responsible for post opinion activities and life-cycle management as foreseen for products for which there is an EU authorization. A conditional opinion or an opinion under exceptional circumstances is applicable as well. New data are assessed by the CHMP in cooperation with the WHO, as appropriate. The opinion holder also ensures that suspected adverse reactions to a medicinal product that are brought to his attention are recorded and promptly reported to the competent authorities of the countries where the product is marketed according to their pharmacovigilance reporting obligations. Adverse reactions are also reported in the EU in compliance with applicable guidelines and standards. The CHMP may perform a benefit-to-risk review at any time and revise its scientific opinion after consulting the WHO.

RISK MANAGEMENT

Current European legislation requires applicants for a marketing authorization of a medicinal product to include in the application a detailed description of the risk-management system they will introduce. This system is described in Module V of the Good Pharmacovigilance Practices and is defined as "a set of pharmacovigilance activities and interventions designed to identify, characterize, prevent, or minimize risks relating to medicinal products, including the assessment of the effectiveness of those activities or interventions."[14] The description of a risk-management system takes the form of an EU risk-management plan.

An EU risk-management plan has two parts (Box 80.1). Part I includes a safety specification and pharmacovigilance plan. The safety specification summarizes what is known and not known about different aspects of the safety profile of the medicinal product at the time of authorization and thereafter at particular points of its life cycle. The plan helps identify important risks and important missing information that need to be addressed in a pharmacovigilance plan. Part II presents any risk-minimization activities deemed necessary for any of the identified risks. Vaccines are different from most other medicinal products in ways that influence safety considerations. Specific aspects to be addressed in the safety specification and pharmacovigilance plan of EU risk-management plans for vaccines have been provided in a specific good pharmacovigilance practices module focused on vaccine vigilance (Box 80.2).[15]

New EU legislation on pharmacovigilance has been operational since July 2012, and from that time all new EU risk-management plans must include the planning of efficacy and effectiveness studies as needed, as well as safety studies and risk-minimization planning. An important element of the new pharmacovigilance legislation is the possibility for regulatory authorities to impose to marketing authorization holders the conduct of postauthorization safety studies with the aim of identifying, characterizing, or quantifying a safety hazard; confirming the safety profile of the medicinal product; or of measuring the effectiveness of risk-management measures.[16] According to the new pharmacovigilance legislation, postauthorization efficacy studies can also be imposed in specific circumstances to marketing authorization holders.

POSTAUTHORIZATION EVALUATION OF BENEFITS AND RISKS

Postauthorization evaluation of the benefit-to-risk balance of vaccines is an ongoing process under the responsibility of marketing authorization holders and competent authorities (medicine regulatory authorities). The marketing authorization holder has the legal obligation to inform and submit any relevant changes to the data that were the basis for the original marketing authorization, in the form of relevant and, as appropriate, supported variations, in accordance with the EU Variation Regulation. This can lead to actions and communications on changes in the benefit-to-risk profile to public health institutions, healthcare professionals and patients.

Box 80.3 summarizes the roles and responsibilities for the benefit-and-risk evaluation for centrally authorized medicinal products. For the decentralized procedure, this activity is coordinated by the reference member states and the CMDh in case of points of disagreement raised by a member state. The responsibility for purely national procedures lies with member states.

The process of any benefit-to-risk evaluation includes taking the following steps:

- Promptly detect and share signals of possible unexpected adverse reactions.
- Confirm, quantify, and assess potential risks.
- Evaluate or reevaluate the benefits of vaccines, including any available data on effectiveness, duration of immunity, vaccination coverage, and public health impact.
- Improve the benefit-to-risk balance, such as by promoting correct use of the vaccine or close surveillance of vaccinated patients to detect early symptoms of severe adverse reactions.
- Evaluate the effectiveness of measures aiming to improve the benefit-to-risk balance.

A key component in this process is vaccine pharmacovigilance, defined as the set of activities for detecting, assessing, understanding, preventing, and communicating adverse events following immunization or of any other vaccine- or immunization-related issues.[17] To establish and maintain a pharmacovigilance system is a legal obligation of marketing authorization holders and member states, an important element being the recording and reporting by the marketing

BOX 80.1 Structure of the European Union Risk-Management Plan

Part I. Product Overview
Part II. Safety Specification
SI. Epidemiology of the indications and target population
SII. Nonclinical part of the safety specification
- Toxicity
- General pharmacology
- Drug interactions
- Other toxicity-related information or data

SIII. Clinical trial exposure
SIV. Populations not studied in clinical trials
SV. Postauthorization experience
- Action taken by regulatory authorities and/or marketing authorization holders for safety reasons
- Nonstudy postauthorization exposure
- Postauthorization use in populations not studied in clinical trials
- Postauthorization off-label use
- Epidemiological study exposure

SVI. Additional EU requirements for the safety specification
- Potential for harm from overdose
- Potential for transmission of infectious agents
- Potential for misuse for illegal purposes
- Potential for medication errors
- Potential for off-label use
- Specific pediatric issues

SVII. Identified and potential risks
- Newly identified safety concerns
- Recent study reports with implications for safety concerns
- Details of important identified and potential risks from clinical development and postauthorization experience
- Identified and potential interactions including food-drug and drug-drug interactions
- Pharmacological class effects

SVIII. Summary of the safety concerns
Part III. Pharmacovigilance plan
- Routine pharmacovigilance activities
- Additional pharmacovigilance activities
- Action plans for safety concerns with additional pharmacovigilance requirements
- Summary table of additional pharmacovigilance activities

Part IV. Plans for postauthorization efficacy studies
- Summary of existing safety data
- Table of postauthorization efficacy studies

Part V. Risk minimization measures
- Routine risk minimization
- Additional risk minimization activities
- Format of risk minimization plan(s)
- Evaluation of the effectiveness of risk minimization activities
- Summary of risk minimization measures

Part VI. Summary of activities in the risk-management plan by medicinal product
- Format and content of the summary of the risk-management plan
- Overview of diseases epidemiology
- Summary of treatment benefits
- Unknown relating to treatment benefits
- Summary of safety concerns.
- Summary of risk minimization activities by safety concern
- Planned postauthorization development plan
- Summary of changes to the risk-management plan over time

Part VII. Annexes to the risk-management plan

Modified from European Medicines Agency (EMA). Guideline on good pharmacovigilance practices (GVP). Module V–Risk management systems (Rev 1). 15 April 2014. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/06/WC500129134.pdf

authorization holder of suspected adverse reactions. For authorized medicinal products, independent of the authorization procedure, notifications of adverse reactions received from healthcare professionals or patients, either spontaneously or through postauthorization studies, and adverse reactions identified from the worldwide published literature should be reported within defined timelines to Eudravigilance, a European database and data-management system of suspected adverse reactions for products authorized in the European Economic Area. For vaccines, cases of lack of efficacy should also be reported, in particular with a view to possible signals of reduced immunogenicity in a subgroup of vaccinees, waning immunity, and strain replacement.

Suspected adverse reactions should also be reported by marketing authorization holders in periodic safety update reports, which provide competent authorities with an update of the worldwide safety experience of medicinal products at defined points after authorization. This update includes a critical evaluation of the benefit-to-risk balance of the product in the light of new or changing information and ascertains whether further investigations need to be done and whether changes should be made to the marketing authorization and product information. For vaccines, the periodic safety update report also includes data of lack of efficacy. Periodic safety update reports should also be submitted immediately upon request from a competent authority and, for centrally authorized products, from the EMA.

Given the complexity of the benefit-to-risk evaluation of vaccines and the large number of aspects to be considered,[16] a broad range of expertise is needed in this process. In the EU this is made available by the Pharmacovigilance Risk Assessment Committee, the CHMP Vaccine Working Party, other CHMP working parties, vaccines scientific advisory group, and other ad hoc expert groups as needed.

To protect public health, it may become necessary to implement urgent measures, such as to recall or halt the distribution (quarantine) of (a) batch(es) of a vaccine because of a suspected batch-specific safety signal or defect. The principle of public health protection may be particularly relevant in certain situations (e.g., vaccines for healthy children, particularly in case of a localized incident). However, batch recall or quarantine may have a detrimental impact on the vaccination program itself, even if absence of association between the suspected batch(es) and the adverse events is later demonstrated, and thus may cause overall harm to public health. In situations where a batch-specific quality or safety issue has not been confirmed, measures other than recall or quarantine may be warranted initially while an investigation is ongoing (e.g., providing recommendations on patient surveillance and follow-up postvaccination). This may be considered when recall or quarantine may lead to vaccine supply shortages and alternatives are not widely available. Consequently, the Good Pharmacovigilance Practices Module P.I. on vaccine pharmacovigilance also includes recommendations to national

BOX 80.2 Aspects of the European Union Risk-Management Plan to Be Specifically Addressed for Vaccines

PART I. PRODUCT OVERVIEW

Type of vaccine and composition, manufacturing process (e.g., genetically engineered vaccine or novel concept); details of combined vaccines; any new technology or novel delivery systems; any immunogenic adjuvants, stabilizers, preservatives, excipients, and residual material from the manufacturing process.

PART II. SAFETY SPECIFICATION

SI. Epidemiology of the Indications and Target Population

Expected or observed (for vaccines already included into a vaccination program) impact of the vaccine on the epidemiology of the vaccine-preventable condition

SII. Nonclinical Part of the Safety Specification

Findings of preclinical testing related to the antigen, other vaccine constituents and residuals, and to interactions of vaccine components; vaccine-related quality aspects if relevant to safety

SIV. Populations Not Studied in Clinical Trials

Immune response in different age groups; risk to the fetus from vaccination of the mother with an inactivated vaccine; risks and immune response in immunocompromised individuals

SVI. Additional EU Requirements for the Safety Specification

Potential for Transmission of Infectious Agents. Potential for medication errors resulting from inappropriate handling or breakdown of the cold chain, the method of administration, noncompliance with the recommended vaccination schedule, product packaging and branding, circumstances of a mass vaccination, situations where several vaccines are marketed in a same country with the same indications

SVII. Identified and Potential Risks

Potential risks to be considered include waning immunity; those anticipated from experience with similar vaccines and vaccine ingredients; those associated with concomitant administration of several vaccines; potential interactions with medicinal products; syndromes closely resembling wild type disease, caused on rare occasion by some live attenuated vaccines; adverse events of special interest (AESIs).

SVIII. Summary of the Safety Concerns

Missing information to be considered include long-term duration of protection, waning immunity and need for (a) booster dose.

PART III. PHARMACOVIGILANCE PLAN

- Routine pharmacovigilance activities put in place for the surveillance of serious but rare adverse reactions, batch-related adverse reactions, identified and potential interactions, possible safety concerns identified with combined vaccines, AESIs, inappropriate use of vaccines and patterns of errors, cases of breakthrough infections, adverse reactions indicating a possible reversion to virulence. As part of the routine follow-up of adverse reactions, information should be collected on the vaccination schedule and route of administration; the vaccine and the diluent (including the batch number), batch release specifications in case of suspected quality defect, relevant comorbidities in the target population, including autoimmune disorders
- Additional pharmacovigilance activities may be needed to detect strain replacement phenomena; address patterns of shedding, transmissibility to contacts and effectiveness of the vaccine; establish evidence of safety for novel vaccines or for vaccines with a novel adjuvant; investigate clusters of adverse reactions
- Action plans for safety concerns with additional pharmacovigilance requirements
- Summary table of additional pharmacovigilance activities

PART IV. PLANS FOR POSTAUTHORIZATION EFFICACY STUDIES

- Summary of existing safety data
- Table of postauthorization efficacy studies

PART V. RISK MINIMIZATION MEASURES

- Routine risk minimization
- Additional risk minimization activities
- Format of risk minimization plan(s)
- Evaluation of the effectiveness of risk minimization activities
- Summary of risk minimization measures

Modified from European Medicines Agency (EMA). Guideline on good pharmacovigilance practices (GVP). Product- or Population-Specific Considerations I: Vaccines for prophylaxis against infectious diseases. 9 December 2013. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/12/WC500157839.pdf.

regulatory authorities regarding elements to be taken into account when considering the recall or quarantine of vaccine batches for safety reasons.

In Europe, the ECDC may provide scientific advice on vaccine-related issues and measures that can affect vaccination programs and disease epidemiology. It may also help identify data sources for additional investigations based on linkage between vaccine exposure and health outcomes. For specific issues, specific expert groups may be established.

COMMUNICATION OF BENEFITS AND RISKS

Communication of the benefit-to-risk balance of vaccines needs careful consideration. Benefits in terms of protecting the public health may be difficult to convey, particularly if the incidence of the target disease is low. On the other hand, common and nonserious adverse events following immunization, such as fever or injection-site pain, can affect its acceptability. Uncertainty on potential rare and serious adverse reactions can further decrease public confidence in vaccination programs. Key elements of communication on vaccines therefore include factual information on what is known and not known about disease epidemiology, benefits and risks of the vaccines, and activities undertaken to address missing data and to minimize risks.[16]

In the European context, it is essential that consistent information with coordinated timelines be provided in all EU member states. EMA has a lead responsibility in preparing (in collaboration with the member state coordinator) communications on the benefits and risks of vaccines. The ECDC has a role in coordinating national communications regarding vaccination programs, which are the responsibility of member states, and disease epidemiology. Both EMA and ECDC have communication plans and provide information on their websites.

Among the available communication tools, direct communications to healthcare professionals are often used to reinforce safe and effective use of medicinal products, including vaccines, and are delivered directly to healthcare professionals by a marketing authorization holder or a competent authority.

BOX 80.3 Key Roles and Responsibilities of Partners Involved in the Postauthorization Evaluation of Benefit and Risk Evaluation and Decision-Making Process for Centrally Authorized Vaccines

MARKETING AUTHORIZATION HOLDER

- Establish and maintain a system to collect, collate, evaluate, and report pharmacovigilance data, including suspected adverse reactions and periodic safety update reports
- Respond to requests from authorities for additional information necessary for the evaluation of benefits and risks

MEMBER STATES

- Have in place national pharmacovigilance systems, including the provision for serious adverse reactions that have occurred on their territory to the agency and the marketing authorization holder
- Inform other partners of any relevant actions
- Identify and evaluate safety concerns and conduct evaluations of benefits and risks
- Implement European Commission decisions
- In case of urgent action needed to protect public health, suspend the use of the vaccine in the member state's territory and inform the agency and the European Commission of the basis of that action

EUROPEAN MEDICINES AGENCY

- Coordinate the pharmacovigilance system
- Monitor the legal obligations of the marketing authorization holders
- In agreement with the rapporteur, identify signals of possible unexpected adverse reactions or changes in severity, characteristics, or frequency of expected adverse reactions
- In agreement with the rapporteur, inform all involved parties of any safety concerns
- Coordinate the evaluation of data by the rapporteurs
- Communicate Committee for Medicinal Products for Human Use (CHMP) opinions to the European Commission

COMMITTEE FOR HUMAN MEDICINAL PRODUCTS RAPPORTEUR

- Evaluate safety concerns, adverse reactions, and benefit and risk data (e.g., periodic safety update reports and company reports) and obtain additional information as necessary
- Provide assessment reports to agreed-upon timetables for consideration by the Pharmacovigilance Working Party and CHMP, with proposals for appropriate action

PHARMACOVIGILANCE RISK ASSESSMENT COMMITTEE

- Evaluate potential signals arising from spontaneous reporting and other sources
- Evaluate emerging safety concerns at the request of the rapporteur
- Assess study protocols for imposed postauthorization safety studies (PASS) and provide advice to CHMP on nonimposed PASS
- Provide advice to the CHMP on safety, evaluations of benefits and risks, and actions necessary to minimize risk and maximize benefits

COMMITTEE FOR HUMAN MEDICINAL PRODUCTS VACCINE WORKING PARTY

- Provide advice to the CHMP on any vaccine-related issue

COMMITTEE FOR HUMAN MEDICINAL PRODUCTS

- Discuss the benefit-to-risk balance on the basis of the rapporteur's assessment report
- Formulate opinions

EUROPEAN COMMISSION

- Formulate decisions
- Enforce legal requirements and implementation of decisions by member states and marketing authorization holders

Modified from European Medicines Agency (EMA). Good pharmacovigilance practices. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000345.jsp&mid=WC0b01ac058058f32c.

In general, an agreement between the marketing authorization holder and the competent authority is needed on the format and content of the information, the recipients, and the timetable.

CONCLUSION

Historically, many of the established vaccines have been nationally authorized by the EU member states. With the creation of the EMA to evaluate and monitor medicinal products in 1995, European legislation harmonized many aspects of the production, distribution, and use of medicinal products in the EU. This now supports a single EU-wide authorization for vaccines that could be made available for 500 million citizens. The CHMP evaluates new applications for vaccine marketing authorization, determines if the benefit-to-risk balance is positive or negative for the vaccine, and issues an opinion to the European Commission on whether to grant an EU-wide marketing authorization. Recent experience led to the authorization of new innovative vaccines such as pneumococcal, human papillomavirus, and (pandemic) influenza vaccines, resulting in major public health benefits.

One of the ongoing challenges in the regulation of vaccines in the EU is the disharmony of vaccination schedules among the member states. Efforts toward harmonizing vaccination schedules would favorably affect vaccine development and the harmonization of product information of vaccines authorized in the EU.

Information on adverse events that occur rarely, with delay, or in specific population groups can, under certain circumstances, only be detected after authorization. Therefore a European system is in place to detect, evaluate, minimize, and prevent if possible severe adverse events following immunization and any decrease of effectiveness that would affect the benefit-to-risk balance of the vaccine.

The authorization and surveillance of vaccines requires expertise from many actors: healthcare professionals, academic experts, vaccine manufacturers, regulatory authorities, public health institutions, and European agencies. It is through their efficient collaboration that public health benefits of vaccines will be maximized.

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Disclaimer: The views expressed in this chapter are the personal views of the authors and may not be understood or quoted as being made on behalf of the European Medicines Agency or one of its committees or as reflecting the position of the European Medicines Agency, one of its committees, or one of its working parties.

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81 Regulation of Vaccines in Low- and Middle-Income Countries

Lahouari Belgharbi, Alireza Khadem Broojerdi, Carmen A. Rodriguez-Hernandez, and David J. Wood

The largest-ever epidemic of Ebola virus disease in 2014–15 triggered an unprecedented accelerated vaccine development process. Innovative clinical trial designs were successfully used to evaluate complex vaccine constructs[1] in countries with very limited regulatory resources and little or no experience in assessing vaccine clinical trial applications. Nevertheless, appropriate regulatory oversight was exercised in part because of the willingness of better-resourced regulatory agencies to provide peer-to-peer scientific support to the regulatory agencies in the affected countries. A key lesson learned from the 2014–15 Ebola virus disease epidemic is that regulatory preparedness for infectious disease threats needs to be strengthened in all countries, especially in low and middle-income countries (LMICs), and especially for regulation of vaccines.

Vaccines are manufactured in a subset (between 40 and 45) of the 194 Member States of the World Health Organization (WHO). They are used, without exception, in every Member State. A growing proportion of vaccines that are used to immunize the world's population are manufactured in LMICs. Appropriate regulatory oversight of vaccines in the developing world is critical not only to ensure the quality, safety and efficacy of vaccines used in national immunization programs in the country of manufacture but also to protect and enhance investments in the global immunization enterprise.

The WHO establishes international standards for the regulation of vaccines and assesses implementation of these standards. The functionality of National Regulatory Authorities (NRAs) in key vaccine-producing LMICs is improving, but further work is needed (e.g., to strengthen health systems to embed the gains made). The NRAs of LMICs that import vaccines are, in most cases, not resourced to provide specialist regulatory oversight of vaccines. The most appropriate strategy for such countries is to leverage the regulatory evaluations performed elsewhere. To assist these countries, the United Nations procures vaccines that have been prequalified by the WHO to be of assured quality. At the end of 2014, it was estimated that 64% of the global infant population was immunized with WHO prequalified vaccines. The key regulatory functions for this group of countries are to register vaccines; to monitor the safety of vaccines in use in the country; and to provide oversight of clinical trials of candidate vaccines. Networks of regulators are being developed to enhance the capabilities of LMICs to perform regulatory oversight for vaccines.

REGULATION OF VACCINES IN LOW- AND MIDDLE-INCOME COUNTRIES

Safe and effective vaccines are universally recognized as essential public health tools. Consistent production of safe and effective vaccines is difficult, and doing so at an affordable price even more so. Vaccines are regulated by pharmaceutical law and are among the most complex pharmaceuticals available today. Informed decision making for vaccines requires specialist regulatory expertise. Benefit-to-risk regulatory evaluations for vaccines requires an understanding of, first, biological production and quality control processes and, second, the implications (in the case of preventive vaccines) of administering products derived from living systems, often to very young infants, at a population scale. Regulatory authorities must always be ready to evaluate new information on potential or real risks and benefits of vaccines and change decisions as needed. Box 81.1 provides an example of the type of problem confronting regulators and how they have reacted to it.

LMIC vaccine manufacturers are an attractive option to supply the global market, but currently the potential of such suppliers is not fully realized. One obstacle is confidence in the quality of vaccines from such suppliers. The WHO has defined a program to strengthen national regulatory systems to address this concern. The aim is to ensure that the NRA in each producing country is functional against international standards for vaccine regulation. The functionality is assessed by WHO-led audits. Between 1997 and 2014 more than 100 assessments were conducted, including the NRAs of all vaccine-producing countries (Fig. 81.1).

Six functions are defined by the WHO for a NRA to provide comprehensive regulatory oversight of vaccines (Table 81.1).[4–8]

Comprehensive regulatory oversight should be in place in countries that manufacture vaccines. Regulatory systems in these countries should have a legal framework and a mandate to enforce the six regulatory functions recommended by the WHO. These functions are: (a) marketing authorization and facility licensing, (b) pharmacovigilance including adverse events following immunization (AEFI) surveillance, (c) NRA lot release, (d) laboratory access, (e) regulatory inspections, and (f) authorization and monitoring of clinical trials. However, for the majority of countries that import their vaccines, only a subset of regulatory functions need be in place. Stratification of the needed regulatory functions by source of vaccine has proved an effective and pragmatic strategy to guide countries of the level of investment that is appropriate to their context.

A "vaccine of assured quality" is defined as a vaccine that has been produced in a country with a functional NRA and has a positive benefit/risk evaluation for use in the target populations. The WHO recommends that 100% of vaccines that are used in national immunization programs are of assured quality.

VACCINES OF ASSURED QUALITY AND FUNCTIONALITY OF NATIONAL REGULATORY AUTHORITIES: GLOBAL STATUS AND TRENDS

Based on data available to the WHO, the proportion of doses of assured quality vaccines used in national immunization programs as of December 2014, reached 97%, as shown in Fig. 81.2. This contrasts with the situation in early 1990, when it is estimated that less than 50% of vaccines were of assured quality. Sustaining the gains made in vaccine-producing countries is constantly challenging, however. For example, a slight decrease was expected in early 2015 as one NRA was found in 2014 to be nonfunctional. The production from this country accounts for approximately 5% to 8% of the global vaccine

supply. The government of this country is committed to address the issues and the WHO expected the system to become functional again soon. A special capacity building and monitoring program was proposed to address this matter urgently.

Table 81.2 shows the status of NRA functionality in the area of vaccine regulation. Fig. 81.3 shows the trends for the implementation of the six specific regulatory functions for vaccines defined by the WHO. The majority of countries without a functional NRA use WHO prequalified vaccines in their national immunization programs.

BOX 81.1 Porcine Circoviruses and Rotavirus Vaccines as an Example of the Complexity of Vaccine Regulation

Rotavirus gastroenteritis is the most common cause of severe diarrheal disease in young children throughout the world, and rotavirus immunization is recommended by the World Health Organization (WHO). In 2010, researchers discovered porcine circovirus type 1 (PCV1) virus and DNA in one oral rotavirus vaccine (Rotarix) and fragments of DNA from PCV2 virus in another oral rotavirus vaccine (Rotateq). Regulatory authorities, academic virologists, and the vaccine manufacturers conducted investigations to understand this finding. The new data were used to conduct revised benefit-risk evaluations. Consensus emerged that the benefits of rotavirus vaccination for children far outweighed any known risk associated with use of vaccines that contained PCV or PCV DNA. The WHO recommended the continued use of Rotarix and RotaTeq for immunization programs, in particular in those parts of the world with elevated mortality of those younger than age 5 years that is associated with rotaviruses.[2] The lessons learned from this event have been codified by the WHO to provide guidance to regulators and the immunization community[3] should a similar event happen in the future.

WORLD HEALTH ORGANIZATION PREQUALIFICATION PROGRAM FOR VACCINES

Prequalification is a procurement term that refers to limiting a global public tender to fewer than the total universe of possible suppliers. Vaccine prequalification is a WHO-led activity with a primary purpose to ensure that the vaccines purchased by United Nations (UN) procurement agencies will be consistently safe and effective under conditions of use for national immunization programs.

The vaccine prequalification program (vxPQ) was formally put in place within the WHO in 1987 to provide advice to UN procuring agencies on the quality, safety, and efficacy of vaccines for purchase. As of this writing, 124 countries are served by the UN procurement system, 90 through the UNICEF Supply Division and 34 through the Pan American Health Organization's Revolving Fund. This accounts for approximately 64% of the total global infant population, which thus receives vaccines of assured quality. The list of prequalified vaccines published[9] on the WHO website is used also by non-UN agencies, by nongovernmental organizations that purchase vaccines, and by countries that procure their vaccines directly through international bidding processes. Its use is thus going beyond its primary aim of serving UN purchasing agencies having become a widely recognized "seal of quality."

Since its establishment in 1987, the procedure has been revised several times to introduce improvements and to adapt to upcoming needs.[10] The current version of the procedure

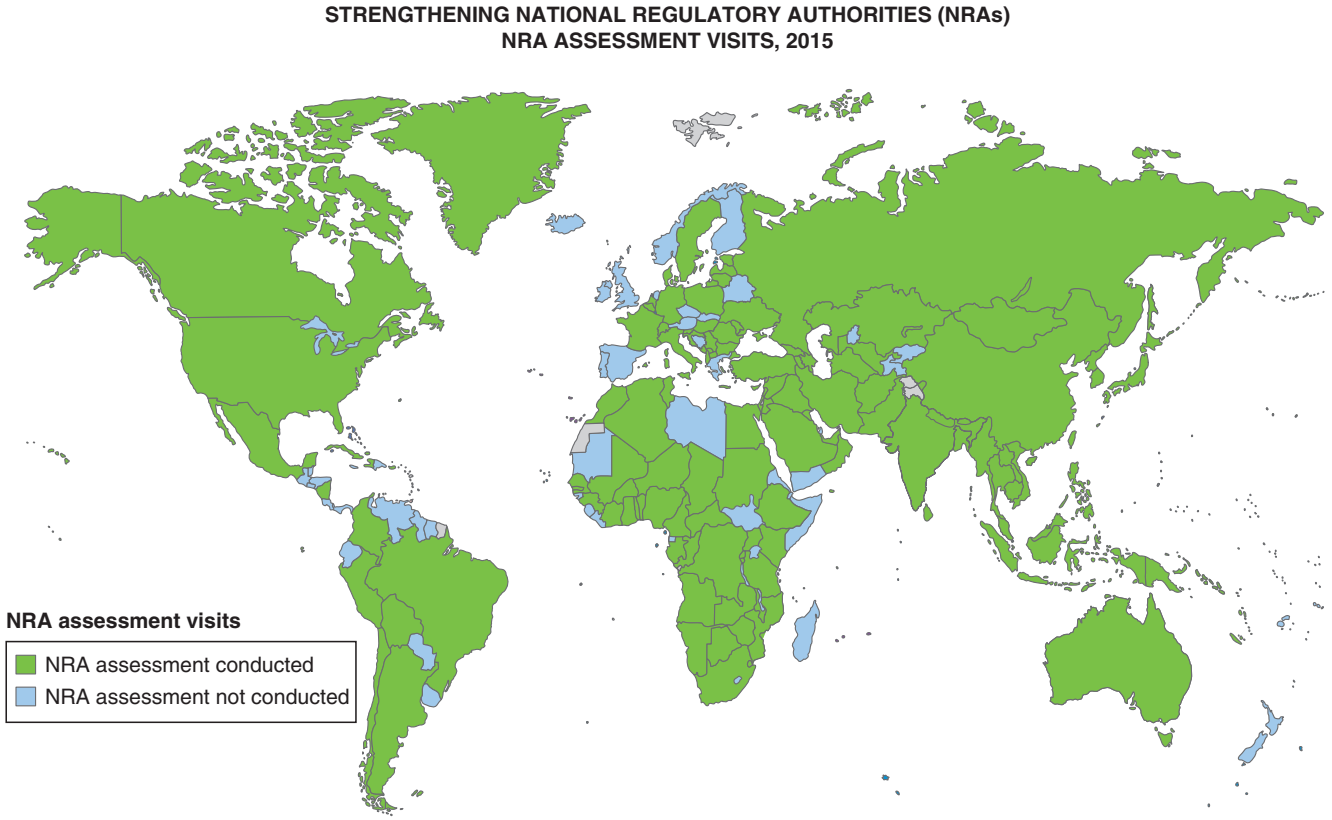


Figure 81.1. Mapping of assessments of national regulatory systems for functionality for vaccines by the World Health Organization. *(Copyright World Health Organization, 2011. All rights reserved.)*

TABLE 81.1 Stratification of National Regulatory Functions According to Source of Vaccines Used in National Immunization Programs Within Countries

Functions	Sources of Vaccines as a Metric to Categorize Countries		
	Vaccines Provided by United Nations Agency	**Vaccines Self-Procured by the Country**	**Vaccines Produced by the Country**
A. National regulatory system[a]	X[b]	X	X
Function 1: Marketing authorization and licensing activities[c]	X	X	X
Function 2: Postmarketing activities, including surveillance of adverse events following immunization (AEFI)[d]	X	X	X
Function 3: Lot release[e]	Functions undertaken by national regulatory agencies (NRAs) in producing countries. The NRAs have been assessed by the WHO and objectively shown to appropriately implement the regulatory functions	X	X
Function 4: Laboratory access[f]		X	X
Function 5: Regulatory inspections[g]			X
Function 6: Authorization and monitoring of clinical trials[h]	X[i]	X[i]	X

[a]A national regulatory system requires a legal framework and a mandate to enforce the regulatory functions recommended by the WHO, plus a quality management system; strategic planning; adequate resources; a recall system; transparency; and conflict of interest provisions.
[b]X indicates a regulatory function that WHO recommends should be implemented by countries in each category.
[c]Marketing authorization and licensing activities includes provisions for registration and evaluation of products and facilities. The function covers import, export, and distribution activities.
[d]The postmarketing function includes capacity to detect and investigate significant vaccine safety issues and to take regulatory actions when needed.
[e]The lot release function requires the NRA to have a system for lot-to-lot review of each vaccine based, as a minimum, on independent review of a lot summary protocol provided by the manufacturer to allow release, against defined standards, of vaccines lots into the market.
[f]The laboratory access function requires that the NRA has a National Control Laboratory established to provide independent testing of vaccine lots submitted by the manufacturer. An operational agreement to use an external quality control laboratory is also acceptable.
[g]The regulatory inspection function means that the NRA has a system to conduct Good Manufacturing Practice (GMP) inspections and has the enforcement power to suspend or stop production in any vaccine manufacturing facility in the country, should the need arise.
[h]The clinical trials function means that there is provision for regulation of clinical trials consistent with WHO Good Clinical Practices (GCP), and enforcement power to take action if standards are broached.
[i]In countries that conduct clinical trials.

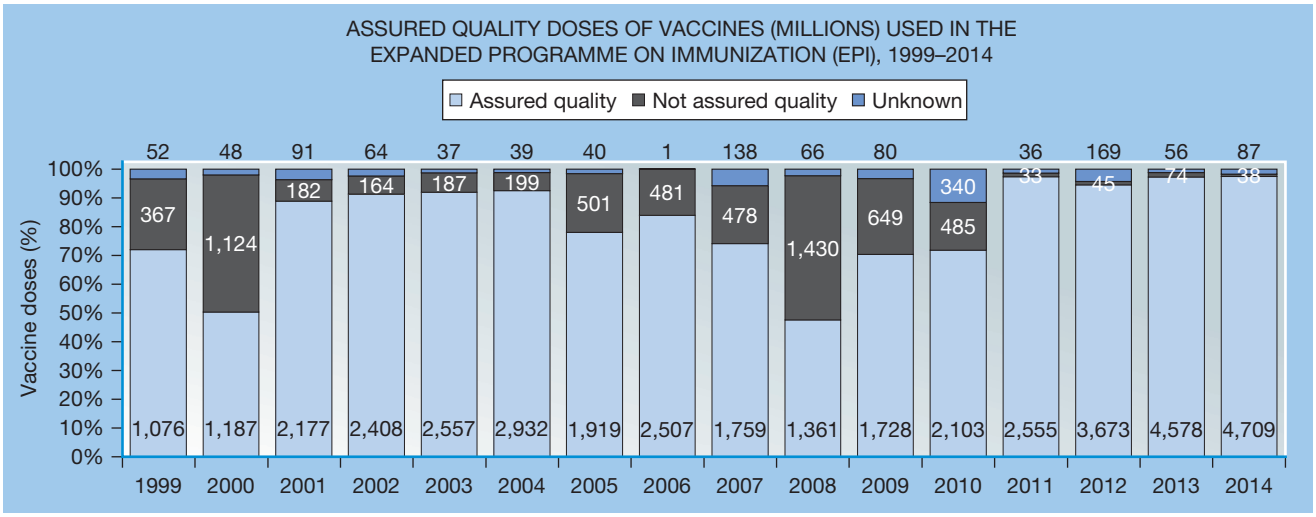


Figure 81.2. Assured quality doses of vaccines (millions) used in the Expanded Programme on Immunization (EPI), 1999 to 2014. *(Data from World Health Organization (WHO)/Essential Medicines and Health Products, as of May 2015. Doses of vaccines reported mainly from WHO/UNICEF Joint Reporting Form and WHO National Regulatory Systems Lot Release Database.)*

was established by the WHO Expert Committee on Biological Standardization.[11] Written standards developed by the WHO Expert Committee on Biological Standardization for individual vaccines define the quality specifications used by the program and are also key to developing technical specifications for the UN procurement tender documents.

The prequalification procedure includes a review of summary documentation on production and control of the specific vaccine under evaluation, proof of consistency of final product characteristics, proof of compliance with Good Manufacturing Practices (GMPs) (assessed through a site audit), and proof of adequate implementation of quality systems by the

TABLE 81.2 Status of National Regulatory Authority Functions in the Area of Vaccine Regulation, 2015[a]

Main Source of Vaccines	Number of Countries That Have Functional or Nonfunctional NRAs		
	Functional, N (%)	Nonfunctional, N (%)	Total
Producing	36 (83%)	7 (17%)	43
Procuring	20 (41%)	28 (59%)	48
United Nations agency	9 (9%)	94 (91%)	103
Total	**65**	**129**	**194**

[a]As of October 2015.
From World Health Organization, Geneva, Switzerland.

manufacturer. An important added value of the prequalification evaluation is its focus on the review of the product characteristics against the WHO-recommended standards as published in the WHO Technical Report Series and compliance with the UN tender specifications. The vxPQ review evaluates the programmatic suitability of product characteristics such as the vaccine presentation, stability profile in view of constraints in the cold chain in target countries, and preservative effectiveness for vaccines filled in multidose vials, among others. It ensures the relevance of the clinical trial data to the UN target population in the recommended schedules including co-administration with vaccines used in the national immunization programs in developing countries.

A vaccine can only be accepted for review for prequalification if the vaccine regulatory system in the country of manufacture has been assessed as functional by the WHO. The role of the national authority in the country of manufacture is critical not only in the stages prior to the submission—that is, marketing authorization for the vaccine—but also, and most importantly, for the ongoing regulatory oversight to ensure continuing quality of the vaccine if and when the vaccine gets prequalified. For example, release of the vaccine on a lot by lot basis by the NRA is an essential requirement of the UN tender, and provides the basis for distribution and use of the vaccine in the receiving countries. The release certificate by the NRA of the manufacturing country is the only evidence that the receiving country authorities have to ensure that the specific lots shipped to their country have been independently reviewed and cleared by the responsible NRA. GMP inspections (in accordance with internationally accepted standards, i.e., the WHO standard) conducted at regular intervals and monitoring of the efficacy and safety profile is also required to ensure the continuing quality of the prequalified vaccine. Similarly important is the regulation of variations by the NRA to ensure that the prequalified vaccine continues to meet the required standards of quality.

The prequalification program establishes collaboration agreements with the NRAs responsible for the national oversight of the prequalified vaccines to ensure that ongoing regulatory oversight is timely and adequately performed and that any important deviations or concerns regarding quality, safety or efficacy of the vaccines are promptly communicated to the WHO.

In the past, vaccines were first introduced in industrialized countries and years later reached less-developed countries as economies of scale allowed for a drop in price. This paradigm has significantly changed in recent years with the increased support from immunization stakeholders, including donors, to accelerate the introduction of priority vaccines in developing countries. This has proved challenging as some characteristics of vaccines designed for industrialized countries are not suitable for the conditions of immunization programs in developing countries. One example was that of a pneumococcal vaccine available in prefilled non-autodisable syringes. Vaccines supplied through UNICEF are required to be filled in vials, ampoules, or in autodisable injection devices.[12] The reason for this requirement is the need to prevent possible "reuse" of syringes in the field on one hand and, on the other hand, to secure effective destruction of syringes in countries without access to high temperature incinerators.

To address this issue a WHO guidance document describes the expected characteristics of vaccines for use in national immunization programs in developing countries, together with a process for the review of vaccine candidates which deviate from the expected characteristics.[13] Specifying programmatic characteristics in this way provides an incentive to vaccine developers and helps shape the market with respect to technical attributes of each vaccine for the benefit of poorer countries.

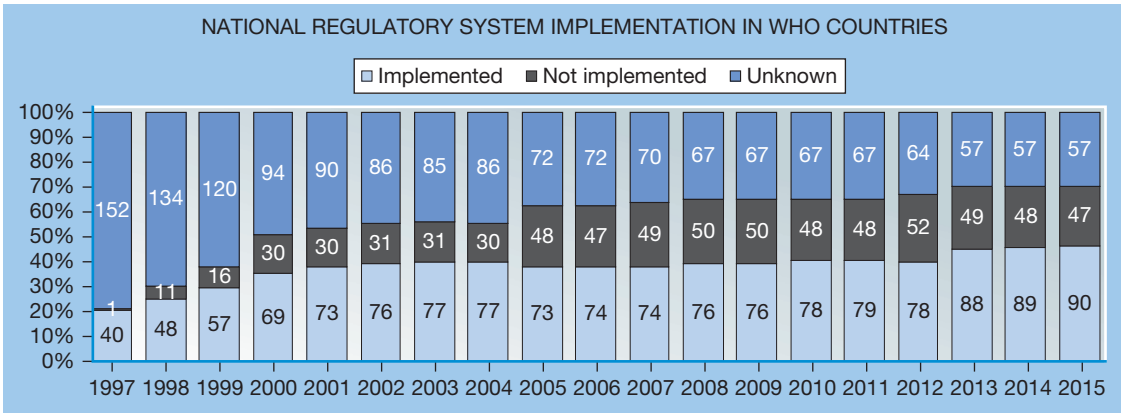
In line with the prequalification of vaccines, the WHO also prequalifies immunization devices relevant to the transport and storage of vaccines through its Performance, Quality, and Safety program. Its main objective is to provide countries with a selected set of products and tools to ensure the quality of vaccines through the supply chain as well as for the administration of vaccines. This work is done in close collaboration with partners through a multipartners' specification working group established in 2014 involving UNICEF, the Global Alliance for Vaccines and Immunization (GAVI), Programs for Appropriate Technology in Health, Clinton Health Access Initiative, and the Solar Electric Light Fund and supported by the Bill and Melinda Gates Foundation. It is recognized that developing countries are encountering a series of challenges in ensuring access to assured quality vaccines to the point of administration. The lack of reliable electricity, the ever increasing volume of vaccines to be transported and stored, the maintaining of storage temperature in the right range are examples of the difficulties countries are facing. To enhance the quality of equipment and facilitate the integration of innovative solutions, the Performance, Quality, and Safety program is developing target product profiles and product specifications against which products are evaluated by independent and accredited laboratories. For example, target product profiles have been published on solar direct-drive refrigerators and solar systems. Target product profiles have also been initiated for waterpack freezers, a freeze-safe cold box, a freeze-safe vaccine carrier, and a remote temperature monitoring system, all of which were expected to be developed in 2015. In 2014, the first long-term passive container was prequalified against new specifications. It can keep vaccines for more than 35 days without being recharged. As a result of this work, countries now have the choice between 258 prequalified products as of December 31, 2014 compared to 163 in 2010, corresponding to a 58% increase between 2010 and 2014.

INITIATIVES TO STRENGTHEN MEDICINES REGULATORY SYSTEMS IN DEVELOPING COUNTRIES FOR REGULATORY OVERSIGHT OF VACCINES

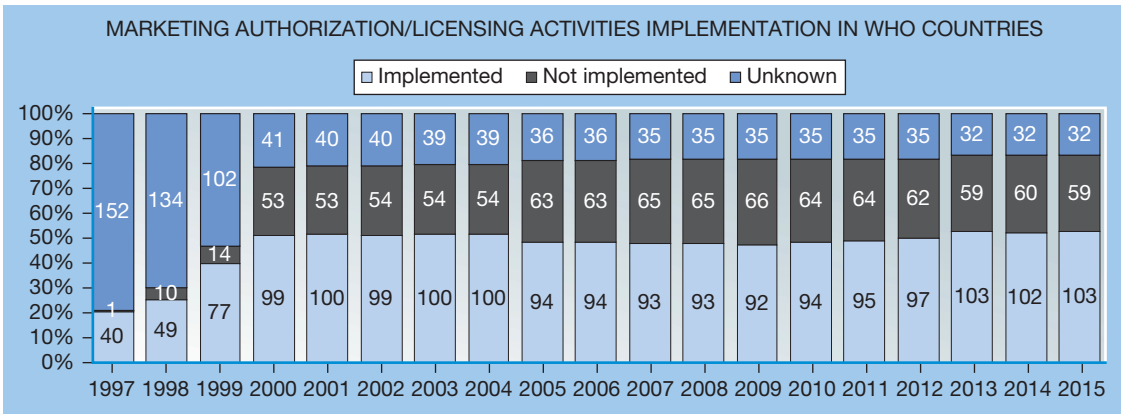
Five Steps for Capacity Building: Assessment and Institutional Development Program

The WHO has developed a five-step capacity-building program that relies on:

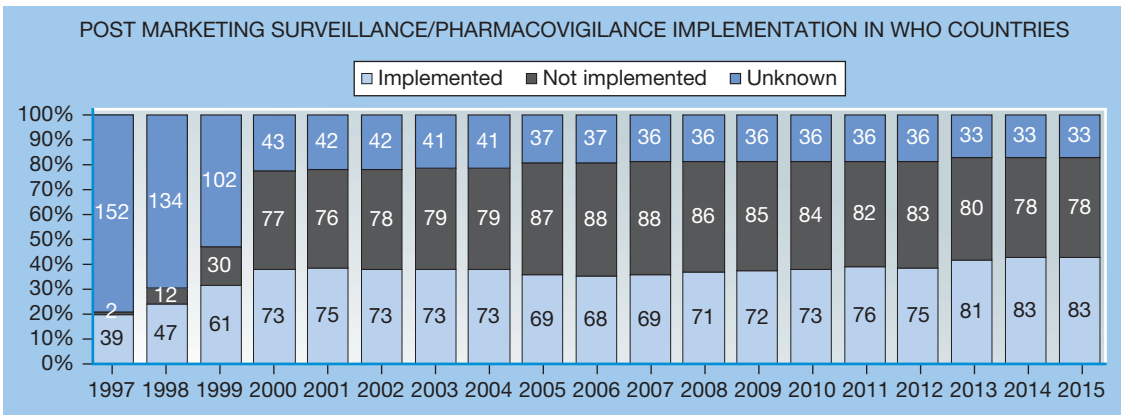
1. Benchmarks to objectively and reliably assess a national regulatory system for vaccines;



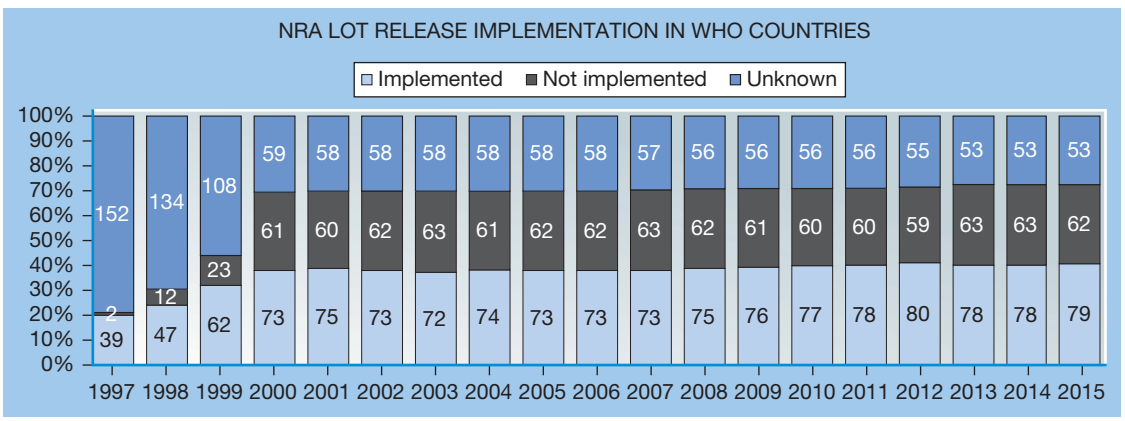
A



B



C



D

Figure 81.3. A, Trends in implementation of national regulatory systems for vaccines. The vertical axis is the percent of countries implementing a national regulatory system for vaccines. The denominator for 1997–99 is 190; for 2000–01 it is 191; for 2002–05 it is 192; and for 2006–10 it is 193. **B,** Trends in implementation of marketing authorizations/licensing activities for vaccines. **C,** Implementation of postmarketing/adverse events following immunization surveillance function for vaccines. **D,** Implementation of National Regulatory Authorities (NRA) lot release for vaccines.

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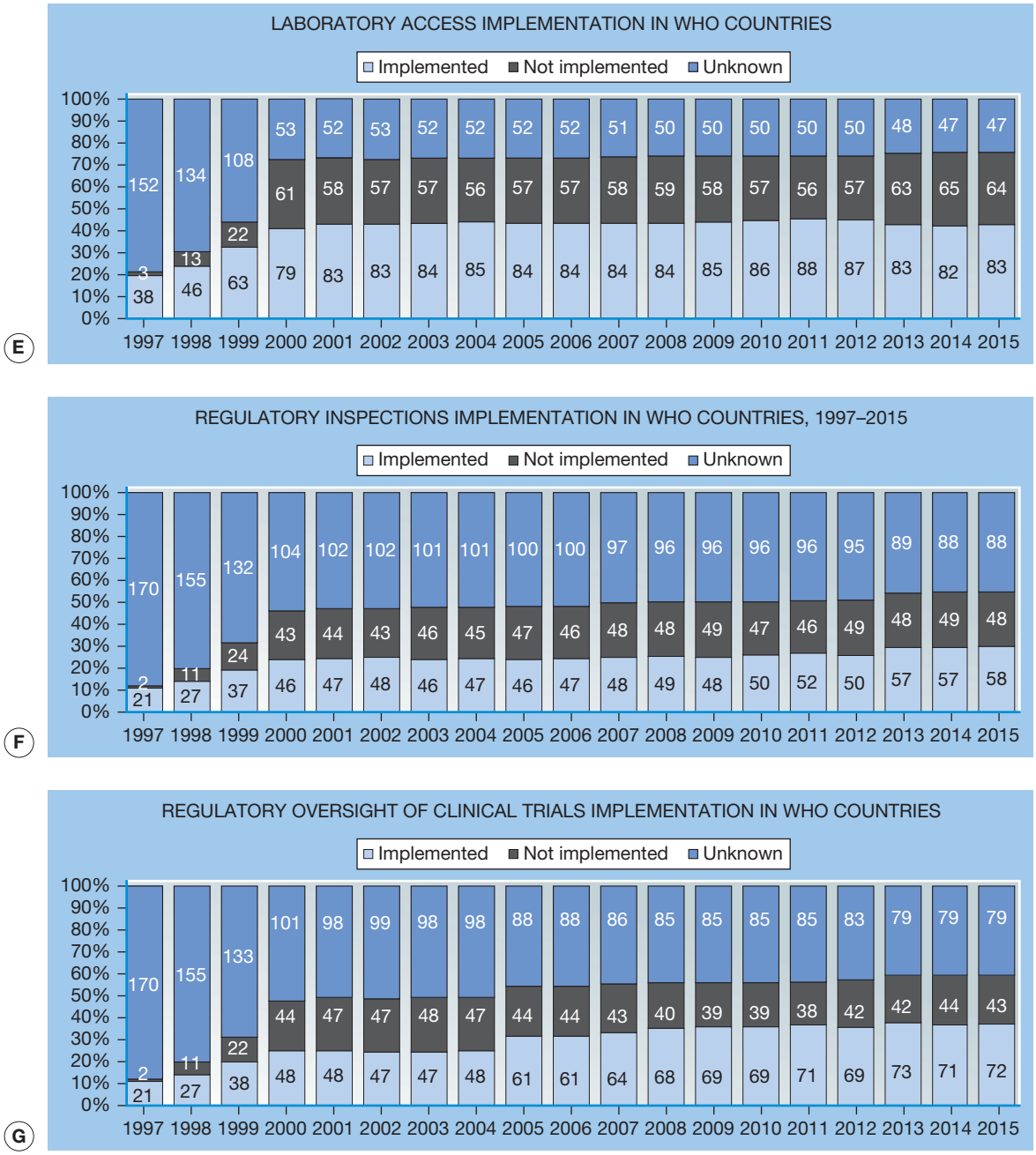


Figure 81.3, cont'd E, Implementation of laboratory access function for regulation of vaccines. **F,** Implementation of regulatory inspections functions for vaccines. **G,** Implementation of oversight of clinical trials for vaccines. *(Data from World Health Organization/Essential Medicines and Health Products, as of August 2015.)*

2. Assessment of a national regulatory system using the benchmarks, via computerized assessment tools for each regulatory function, with indicators and subindicators for each function;
3. Identification of gaps and development of an Institutional Development Plan (IDP) to address the gaps;
4. Provision of technical input and support through learning and training to build capacity in line with the needs identified through the IDP; and
5. Monitoring progress and re-assessment of the performance of the regulatory system.

World Health Organization Observed Audits: Enforcement of Good Manufacturing Practices

Adherence to GMPs during vaccine production is a key principle for consistent production of safe and effective vaccines. Robust regulatory inspections are thus critical. The WHO-observed audits have proved to be powerful tools to raise the competencies and enforcement power of the regulatory system and to guide countries to sustain their regulatory inspections function. In an observed audit, external regulatory experts, appointed by the WHO, monitor the local inspectors as they

perform a routine GMP inspection. Countries that have benefited from this exercise include China, Egypt, India, Indonesia, Iran, Senegal, Thailand, and Vietnam. The local inspectors receive feedback from the external experts on their performance and have an objective benchmark against which they, and the WHO, can measure progress.

Parallel Regulatory Review: Example of Vaccines Against Meningococcal Conjugate A and Japanese Encephalitis Vaccines

Another example of capacity building is the development of collaboration and sharing of information between two NRAs when assessing a vaccine for registration purposes. In this situation one NRA is a mentor that leads the process with its experts, and the second is a mentored NRA that receives advice. One example occurred during evaluation of the meningococcal A conjugate vaccine that was, subsequently, successfully introduced in the African meningitis belt countries.[14] The NRA of India was the recipient of the advice and the Canadian NRA provided its expertise to support this process. In this situation, the product was not intended for registration in Canada, and so the donor of the technical expertise did not derive direct benefit from the process. However, there were multiple indirect benefits. For example, the mentoring process and methods developed to support its implementation were subsequently used as a staff-development tool in the mentor NRA.

In a second example, parallel reviews were conducted for evaluation of a candidate Japanese encephalitis vaccine that had been submitted for registration in both Australia[15] and Thailand. The Thai Food and Drug Administration was technically supported by the Therapeutic Goods Administration from Australia. Each country retained the national prerogative whether to license, or not, the vaccine. The scientific data provided by the sponsor were evaluated by both countries, then discussed and used as a learning opportunity by the less-experienced regulator. In this case, the vaccine was subsequently registered in both countries.

Strengthening Oversight and Monitoring of Clinical Trials for New Vaccines

The responsibilities of the countries that are targets for clinical trials or introduction of new vaccines cannot be replaced by regulatory oversight from the country of manufacture. Although most Phase I clinical trials are done in the country of manufacture of the vaccine, some of the vaccines currently under development target diseases that are endemic in the developing world and Phase II and Phase III clinical trials will have to be conducted in countries where the disease is endemic. Often, these target countries have limited experience in regulatory oversight of vaccine clinical trials. Therefore, regulatory capacity in the areas of nonclinical product review and in protocol review for clinical trial testing needs strengthening.

An efficient approach to build capacity in the regulation of vaccine clinical trials is the use of existing networks of regulators and/or the establishment of new networks. As an example, WHO has worked with the established Association of Southeast Asian Nations (ASEAN) regulatory network to establish a "vaccine chapter," which represents a forum for collaboration in the area of vaccine clinical trial regulation. The WHO has also established two networks: the Developing County Vaccine Regulators Network (DCVRN) and a Regional Network in the African Region, the African Vaccine Regulatory Forum (AVAREF).

The DCVRN was established in September of 2004, its mission being to promote and support the strengthening of the regulatory capacity of NRAs of participating and other developing countries for evaluation of clinical trial proposals (including preclinical data and product development processes) and evaluation of clinical trial data through expertise and exchange of relevant information.[16] Nine countries constitute the DCVRN: Cuba, Brazil, China, India, Indonesia, Iran, Korea, South Africa, and Thailand.

Member countries aim to develop harmonized methodologies for evaluation of clinical trial applications and vaccine dossiers; to strengthen the ability of NRAs to conduct the evaluations; to establish open dialogue with multinational vaccine developers and manufacturers to discuss the development of novel vaccines; to ensure that developing country regulators keep up with internationally accepted requirements; and, to the extent possible, to guide other NRAs through regional initiatives.

The DCVRN operates through meetings twice a year and regular communications between meetings. The WHO's role as the Secretariat is to facilitate the participation of experts, vaccine developers, clinical trial sponsor institutions, and NRAs of manufacturing countries in scientific sessions. Examples of issues considered by the DCVRN include regulatory challenges relevant to the clinical evaluation of new vaccines including tuberculosis vaccines, vaccines against HIV/AIDS, human papillomavirus, rotavirus, typhoid, Japanese encephalitis, and dengue. This networking exercise provides member countries with the opportunity to share experiences and views, to exchange information and knowledge with other NRAs, to provide feedback to vaccine developers/manufacturers and trial sponsors, and to provide support to regional initiatives through sharing of regulatory procedures, funding of expert staff to support other countries, or taking a lead role in the facilitation of capacity building activities relevant to clinical evaluation of vaccines.

For regulatory oversight of vaccine clinical trials, there are challenges specific to certain regions. For Africa, AVAREF was conceived and established in 2006 as a "regional approach" to provide ongoing support for regulators in the region.[17] It is intended to serve as framework to ensure access to expertise for countries that have to make regulatory decisions for which they may not have the capacity/expertise and as a forum where countries can discuss with peers, strengthen the capacity of weaker countries, and identify the need for support and training.

AVAREF gathers representatives from NRAs and Ethics Committees from 23 countries identified as targets for clinical trials and introduction of novel vaccines. The objectives are to facilitate access to information on clinical development plans for priority vaccines in the region; provide a source of expertise to support countries in their regulatory review processes; identify the need for regulatory documents, training, and guidance; and foster collaboration with regulatory agencies of countries that produce vaccines that are clinically tested in Africa.

Some of the achievements of AVAREF include the development of model harmonized procedures for evaluation of clinical trial applications and regulatory monitoring of clinical trials.

AVAREF also established and tested the concept of joint reviews of protocols of clinical trials to be conducted in some member countries. Joint reviews take place between regulators of several countries targeted for trials of the same vaccine, with the agreement of sponsors and with participation of collaborating regulators with extensive experience in the review of clinical trial protocols and clinical trial data. Sponsors make simultaneous submissions to the target countries; these are independently reviewed in each of the countries who then

participate in the joint review exercise. During the joint reviews the assessors from target countries have the opportunity to discuss among themselves and to put forward consolidated questions to the sponsors and to request all the required clarifications or complementary information that may be needed. Regulators go back to their home countries with the necessary feedback to make informed and independent regulatory decisions.

Candidate malaria and meningitis A conjugate vaccines have been reviewed in this way. Experience showed that joint reviews were a very successful way to improve the quality of the review of clinical trials protocols.

AVAREF was particularly important as a platform in the public health emergency of the 2014–15 Ebola virus disease epidemic.[18] The platform was used to achieve progress in three areas: (a) presubmission discussions between the regulators and the clinical trial sponsors and vaccine manufacturers; (b) agreement on clinical trial approval processes; and (c) joint reviews, convened by WHO, of clinical trial applications. The outcomes were that ethical and regulatory approvals, conducted against international standards, were secured within 90 days for key vaccine studies.

The three networks, DCVRN, ASEAN, and AVAREF, have proven to be efficient as opportunities for interaction with advanced NRAs like the European Medicines Agency (EMA) and the U.S. Food and Drug Administration, who support these initiatives by actively participating and sharing their expertise with the network countries.

The role of networking as a platform to promote information exchange between regulators, harmonization of regulatory requirements and procedures, to identify gaps and training needs, to facilitate the development of guidelines as well as to provide technical support can be further exploited.

Technical Support and Learning Opportunities for Regulators From Developing Countries

Technical support for regulators from developing countries may be provided through multilateral or bilateral processes. WHO-led technical support is based on needs objectively identified during an NRA assessment. Using the outcome of the assessment for each of the regulatory functions, the regulators develop jointly with the independent assessment team the IDP. This plan provides detailed information on the gaps identified by the assessors and an initial proposal on ways to address them. The NRA assessment report and the IDP are shared with country authorities and, after endorsement by the authorities, the IDP becomes a tool to develop a targeted technical support plan.

A diversity of approaches can be used to provide technical support depending on the needs, such as participation in established global courses, or performance of tailor-made in country or subregional training activities, placements in centers of excellence for the specific field in question and/or by facilitating on site consulting.

Courses that become part of the set of approved WHO courses go through a proven process of effective course development. The WHO contracts a course development team to draft the course which includes both content and learning methods experts. The draft course is developed in modules concurrently with a manual with required technical information and guidelines for delivery aimed at fostering effective learning, as well as a participant's guide and reference and reading materials. Course materials are peer reviewed and cleared through a formal process.

The courses are selected both on the basis of identified needs and the potential to be developed and run effectively in a cost-effective manner. Some of the courses are conducted at established Global Training Centers or can be conducted as itinerant courses for regulators in one specific country or for a small group of neighboring countries with similar needs.

All WHO-approved courses are constructed in the form of learning modules with stated and measurable learning objectives. The courses include detailed procedures for monitoring progress daily and procedures for correcting identified weaknesses during the course. The modules are each evaluated through defined procedures as part of the course administration and following the course through additional tools and channels.[19] The WHO follows Kirkpatrick's *Four Level Evaluation* scheme.[20]

DCVRN members have developed a curriculum for training on regulatory inspections of clinical trials, to educate NRAs of developing countries. The material was further developed and finalized for delivery by the WHO. This is an example of contribution of one of the networks to the availability of global training materials.

Global learning events may not necessarily be the answer for a defined gap in a particular NRA assessment. In some cases, an in-country learning event designed on a custom made basis is required. However, in all cases, WHO ensures that design principles are properly defined and quality procedures are followed in development of the new material.

It is expected that the implementation of distance based e-learning will broaden the reach of current training activities significantly. The development of relevant e-learning curricula is a priority for the WHO.

Leverage of Regulatory Decisions Made Elsewhere: Expedited Review of Imported Prequalified Vaccines for Use in National Immunization Programs

Countries sourcing vaccines through UN agencies receive vaccines of "assured quality." Such vaccines have been appropriately licensed by the responsible regulatory authority and, in addition, have been reviewed by the WHO in the context of the prequalification evaluation. During the prequalification process, the WHO has specifically ensured that the vaccine meets the WHO recommendations, the UN tender specifications, and has closely reviewed the clinical data to ensure that they are relevant to the target population and compatible with the immunization schedules used in the receiving countries. The WHO has also checked that product characteristics—such as presentation, stability profile, and others—are suitable for use in developing country settings. Ongoing regulatory oversight is ensured by the responsible NRA through release of vaccines on a lot by lot basis, review of variations, regulatory inspections, and by the WHO through targeted testing of lots shipped to countries and monitoring of complaints and reports of AEFIs. In addition, the WHO reassesses all prequalified vaccines at regular intervals.

Under such circumstances, countries may opt to waive an additional comprehensive registration process. It may be more efficient to use available resources to focus on postmarketing activities such as the pharmacovigilance of the vaccines being used. Consequently, the WHO has developed a collaborative procedure for review of imported vaccines that have been prequalified.[21] The procedure allows for facilitated registration of vaccines included in the immunization program. The use of such a procedure allows for registration of all the prequalified vaccines used in the country in a short timeframe without disruption to the vaccine supply. Countries wishing to adopt this procedure are advised to ensure that their legislation includes provisions for waiving and/or accelerating regulatory

procedures. The WHO provides training to authorities of countries wishing to implement the procedure and additional support to ensure its effective implementation.

Consideration of the outcomes of assessments and inspections by trusted authorities substantially contributes to savings in regulatory resources and improvements in the quality of regulatory decisions, while retaining the prerogative of NRAs to conclude their assessment by sovereign decisions, which reflect their own judgement of the benefit-to-risk balance as it relates to their specific country situation and the legislation in place. Taking into consideration the regulatory decisions of other NRAs requires setting up a system that will permit:

- Identification of reference authorities whose regulatory decisions are based on acceptable standards and identification of documents associated with such regulatory decisions, which are relevant to the regulatory environment in the country wishing to rely on such decisions;
- Assurance that the product for which the decision has been taken by the reference NRA is identical to the product being assessed, or, if it is not identical, that a clear understanding exists of the differences between the products subjected to assessment in the two regulatory environments;
- Efficient use of available scientific expertise and human and financial resources to decide, with reasonable certainty, on the benefit-to-risk profile of an evaluated product when used in a given country;
- The choice by each NRA of the approaches that will make best use of the resources, workload, and competence of individual NRAs.

Approaches could range from completely independent data reviews and inspections to adoption of regulatory decisions of trusted authorities without any further scientific review. A pragmatic approach is to verify product identity and assess only those areas which relate to use of the product in the country concerned and where failure to comply with regulatory standards could pose health risks. In the other areas, the outcomes of trusted authorities may be adopted.

Enhanced collaboration and information exchange between NRAs and WHO/vxPQ benefits both partners. Subject to the agreement of the WHO prequalification holders concerned, NRAs have access to assessment outcomes that are not in the public domain and that have been prepared in conformity with the WHO recommended standards on which the "Procedure for prequalification of pharmaceutical products" and the "Procedure for assessing the acceptability in principle of vaccines for purchase by United Nations Agencies" are based. Such reports and other documents help NRAs to make their decisions and also assist in educating national regulatory staff. At the same time, feedback from NRAs on the information and documentation received from WHO/vxPQ under the Procedure allows WHO/vxPQ to improve its work and ensures that the outcomes of prequalification assessments are relevant to NRAs. As a consequence vaccinees benefit from this collaboration by gaining faster access to products that have been found acceptable in principle for procurement by UN agencies. Depending on available resources, participating authorities have the opportunity to participate in the assessment process and in inspections organized by WHO/vxPQ.

This collaborative procedure also benefits manufacturers of prequalified vaccines through faster and better harmonized regulatory approvals in participating countries. This Procedure, when combined with the "Collaboration Procedure with NRAs in inspection activities," alleviates the burden of additional national inspections on manufacturers.

FUTURE DIRECTIONS

Preventing Duplication of Effort

As NRAs become stronger, it is expected that the WHO prequalification program will implement a *risk-based approach* that will allow more reliance on NRAs. The prequalification process will be tailored according to factors such as the experience of the manufacturer and NRA, and to the level of risk that has been seen with similar products–for example linked to incidence of reported AEFIs, or to the experience of the vaccine producer in supplying prequalified products. In this way, the prequalification process will be focused more on novel vaccines. For this purpose products will be classified in one of the following three categories:

- *Category A*. High risk: products submitted by manufacturers with limited or no experience with the prequalification process or with the product in question, and/or the NRA is borderline with respect to functionality.
- *Category B*. Medium risk: manufacturer has at least one other prequalified vaccine and has experience with the product in question (although it is not prequalified), or manufacturer is new to the system but is supported by a joint venture with a well-established manufacturer, and NRA is functional.
- *Category C*. Low risk: Manufacturer is well established, more than one product prequalified, and may or may not have experience with product in question but has good research and development infrastructure. NRA is functional.

Products in categories A and B will be thoroughly assessed and monitored (as per current procedure). Products in category C will be subject to a lighter process with strong reliance on the NRA responsible for the product regulatory oversight.

The prequalification of new vaccines poses additional challenges from the regulatory point of view, in terms of implementation in immunization programs, since some of these vaccines are likely not to be used in the country of manufacture and therefore the responsible NRA would not have firsthand postmarketing surveillance data to monitor the performance of these newly introduced products in terms of safety and efficacy. To address the issue, the WHO is working on the establishment of a global network of sentinel countries, with strong postmarketing surveillance systems that would monitor the safety profile of recently introduced UN-supplied vaccines during a specified period (or significant number of doses administered) to increase the information on the postlicensure real-life safety profile of the product.

Strengthening Vaccine Safety Systems

Objective analyses performed by the WHO show that vaccine pharmacovigilance is one of the weaker regulatory functions (see Fig. 81.3C). The capacity of countries to detect and investigate significant vaccine safety events varies, not unexpectedly, with developmental status.

The relationship between functionality of the vaccine safety surveillance system and health expenditure also applies within the subset of LMICs.

As all countries use vaccines, these data indicate that, from a global strategic perspective, there are risks that vaccine safety events may be missed and thus inappropriately managed. Looking to the future, a global vision has been articulated that all countries are able to ensure effective vaccine safety monitoring and response so that unwanted vaccine reactions are minimized and public confidence in vaccination is maintained.[22] Three levels of engagement have been defined to realize this vision:

- Building capacity for effective vaccine safety monitoring so that all LMICs have at least a minimal capacity.
- Enhancing the level of vaccine safety activity in countries that manufacture vaccines and in early introducers of newly available vaccine so that these countries have the ability to carry out active surveillance and database studies.
- Fostering international collaboration and encouraging global strategic planning so that national systems are adequately supported, all vaccines are under adequate postmarketing surveillance and vaccine safety information is shared internationally.

It is proposed that minimal capacity at the country level encompasses both a vaccine safety monitoring structure and also appropriate managerial requirements embedded with the health system of the country.

The vaccine safety monitoring structure may consist of:

- A national system of spontaneous reporting with a national database of AEFI reports;
- A national AEFI advisory committee;
- A clear communication strategy for routine communication and crisis communication;
- A national vaccine pharmacovigilance center with designated staff collaborating with the WHO Programme for International Drug Monitoring;
- Links to external technical support if required.

The managerial requirements may consist of:

- A proper regulatory framework (with provisions for monitoring and management of AEFI);
- Clear lines of accountability identified for the conduct of vaccine safety work;
- A management plan that is periodically evaluated and revised to ensure continuous quality improvement in the conduct of national vaccine safety activities;
- A commitment to sharing information on vaccine safety with other countries.

Increased international as well as national resources are also required to bolster vaccine safety capacity. The international dimension includes setting standards such as for harmonized methodologies, case definitions and safety updates for prequalified vaccines; capacity building tools, such as a pool of experts to assist countries, international research groups working on vaccine safety issues, global rumor monitoring, and a global database of AEFIs; electronic tools, for example to enable rapid sharing of information on vaccine safety issues, and for case verification; quality improvement tools, such as best practice registry and performance indicators; and expert advice, particularly independent scientific advice on global vaccine safety issues. This latter function is already performed by the WHO Global Advisory Committee on Vaccine Safety.[23]

There will be many challenges to turning the vision into reality that will require sustained support and commitment from a variety of stakeholders.

Increasing and Sustaining Global Supply of Vaccines: Investing in Developing Countries as a Source of Affordable Vaccines of Assured Quality

Developing and emerging economy countries have a vaccine industry capable of manufacturing affordable vaccines of assured quality for global use. Currently, this capability is underused, limiting supply options for global projects. Expanding the supply base in a sustainable manner requires capacity building approaches and solutions that will vary from country to country. The common feature is that functionality of the NRA is the key to unlock the potential. As noted previously, functionality is assessed by WHO-led expert audits of the NRA. The audits result in an IDP to strengthen the NRA in areas where gaps are identified. The IDPs are effective tools to develop a roadmap with the government of the host country to address gaps, and to mobilize resources (internal and external) to support the necessary capacity building initiatives.

The concept of an IDP being used to inform a roadmap of capacity building against defined milestones, with regular review and feedback has been successfully applied in both China and India. In China, the concept enabled the Chinese NRA, the State Food and Drug Authority, to achieve functionality against WHO criteria on March 1, 2011. In India, the concept enabled the Indian NRA, the Central Drugs Standard Control Organisation, to regain functionality against WHO criteria in March 2009. The experience in India shows that functionality of key NRAs can go down as well as up, requiring special efforts to maintain status as functional and to continue to drive improvements.

On the manufacturing side, there are different challenges in each country. In China, there are more than 30 vaccine manufacturers that could apply for vaccine prequalification. This ranges from well-established manufacturers to new companies with research pipeline products. The feasibility of all these manufacturers reaching the standards needed for WHO prequalification remains to be seen. As these companies are new to UN supply, it is anticipated (from previous experience) there will be a steep learning curve. Tools to evaluate the viability of the manufacturers would therefore be useful, both to help prioritize applications to WHO prequalification, and also inform capacity building in the companies.

In India, there is a well-established industry that currently supplies the global market through UN procurement. However, in 2010 one major supplier of pentavalent vaccine was delisted from the WHO list of prequalified products.[24] This was because the manufacturer had introduced a series of changes into the production process, compared to the originally prequalified process, in an attempt to improve yields and to scale-up to meet increasing demand. This was done without commensurate scaling-up the quality management system to cope with the increased demands from increased production. Indicators that would provide early warning that a company is disproportionately scaling-up would provide a useful risk-mitigation tool. This could be implemented for key companies with strategically important contracts as identified by the UN procurement agencies. In addition to the quality management systems, it would also be important to closely monitor the capacity of key companies to respond to global vaccine safety issues, through having an adequately resourced postmarketing surveillance group.

Regulatory Pathways for Vaccines Manufactured in Developed Countries but Intended for Use in Developing Countries—Article 58

Medicinal products that are not intended for marketing in the European Community are not given a European marketing authorization and for those medicines that are no longer marketed in the European Union, the marketing authorization is not renewed. The lack of a marketing authorization in Europe could potentially impair the registration of such medicinal products in countries importing them that require registration in the country of origin. To avoid this European

law from becoming a barrier to access to medicines from European origin not intended for the European population, the EMA developed, in collaboration with the WHO, an alternative regulatory pathway ("Article 58") that allows the EMA to provide a "Scientific Opinion" on candidate medicines through an evaluation process that mimics the centralized procedure for registration of medicines.[25]

The scope of the procedure includes

- Vaccines used or of possible use in the WHO Expanded Programme on Immunization;
- Vaccines for protection against a WHO public health priority disease;
- Vaccines that are part of a WHO managed stockpile for emergency response;
- Medicinal products for WHO target diseases such as HIV/AIDS, malaria, tuberculosis, lymphatic filariasis (elephantiasis), trachoma, leishmaniasis, schistosomiasis, African trypanosomiasis (sleeping sickness), onchocerciasis (river blindness), dengue fever, Chagas disease, and leprosy.

An Article 58 Scientific Opinion follows the same rules and requirements as a marketing authorization for Europe. In addition, the procedure includes participation of observers from the WHO vaccines prequalification program (when the vaccine is expected to be also prequalified) and it includes participation of WHO-designated experts and NRAs from countries targeted for use of the candidate vaccines. EMA will focus the review of clinical data in the target population in the user countries. This focused review by EMA allows the WHO to consider the streamlining of the prequalification evaluation when this is requested by the manufacturer. It is expected that countries procuring vaccines directly through international tenders will also recognize the EMA Scientific Opinion as an equivalent to the registration in Europe.

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82 Vaccine Safety

Frank Destefano, Paul A. Offit, and Allison Fisher

During the past 100 years, pharmaceutical companies have made vaccines against diphtheria, tetanus, pertussis, polio, measles, mumps, rubella, varicella, hepatitis A, hepatitis B, *Haemophilus influenzae* type B (Hib), pneumococcus, meningococcus, rotavirus, and human papillomavirus, among others (Table 82.1). As a consequence, the number of children in the United States killed by pertussis decreased from 8000 each year in the early 20th century to fewer than 20; the number paralyzed by polio decreased from 15,000 to 0; the number killed by measles decreased from 3000 to 0; the number with severe birth defects caused by rubella decreased from 20,000 to 0; and the number with meningitis and bloodstream infections caused by Hib decreased from 25,000 to fewer than 100.

Vaccines have been among the most powerful forces in determining how long we live.[1] However, the landscape of vaccines is also littered with tragedy. In the late 1800s, starting with Louis Pasteur, scientists made rabies vaccines using cells from nervous tissue, including animal brains and spinal cords. Although the vaccine prevented a uniformly fatal infection, it also caused seizures, paralysis, and coma in as many as 1 of every 230 people who used it.[2–5]

In 1942, the military injected hundreds of thousands of American servicemen with a yellow fever vaccine. To stabilize the vaccine virus, scientists added human serum. Unfortunately, some of the serum came from people unknowingly infected with hepatitis B virus. As a consequence, 330,000 soldiers were infected with hepatitis B, severe disease developed in 50,000, and 62 died.[6–9]

In 1955, five companies made Jonas Salk's new formaldehyde-inactivated polio vaccine. However, one company, Cutter Laboratories of Berkeley, California, failed to completely inactivate poliovirus with formaldehyde. Because of this failure, 120,000 children were injected with live poliovirus; 40,000, developed mild polio, 200 were permanently paralyzed, and 10 died. It was one of the worst biological disasters in American history.[10]

Vaccines have also caused uncommon but severe adverse events not associated with production problems. For example, acute encephalopathy after whole-cell pertussis vaccine,[11,12] Guillain-Barré syndrome (GBS) after swine flu vaccine,[13] paralytic polio following live attenuated oral polio vaccine,[14] anaphylaxis following several different vaccines,[15] severe or fatal viscerotropic disease following yellow fever vaccine,[16] and intussusception following rotavirus vaccine,[17–19] are problems associated with the use of vaccines, albeit rarely. As vaccine use increases and the incidence of vaccine-preventable diseases decreases, vaccine-related adverse events become more prominent (Fig. 82.1). Even unfounded safety concerns can lead to decreased vaccine acceptance and resurgence of vaccine-preventable diseases, as occurred in the 1970s and 1980s as a public reaction to allegations that the whole-cell pertussis vaccine caused encephalopathy (Fig. 82.1). Recent outbreaks of measles, mumps, and pertussis in the United States are important reminders of how immunization delays and refusals can result in resurgences of vaccine-preventable diseases.[20–22]

METHODS OF MONITORING IMMUNIZATION SAFETY

Because vaccines are given to healthy children and adults, a higher standard of safety is expected of immunizations compared with other medical interventions. Tolerance of adverse reactions to pharmaceutical products (e.g., vaccines, contraceptives) given to healthy people—especially infants and toddlers—is substantially lower than to therapeutic agents (e.g., antibiotics, insulin) used to treat illness.[23] This translates into a need to investigate the possible causes of much rarer adverse events after vaccinations than would be acceptable for other pharmaceutical products. For example, severe side effects are essentially universal for cancer chemotherapy, and 10% to 30% of people receiving high-dose aspirin therapy experience gastrointestinal symptoms.[24]

Safety monitoring is performed both before and after vaccine licensure, with slightly different goals based on the methodological strengths and weaknesses of each step.[25–28] Although the general principles are similar irrespective of country, the specific approaches may differ because of the organization of immunization services and the level of available resources.[29]

Prelicensure Evaluations of Vaccine Safety

Vaccines, similar to other pharmaceutical products, undergo extensive safety and efficacy evaluations in the laboratory, in animals, and in phased human clinical trials before licensure.[30,31] Phase I trials usually involved smaller numbers of subjects and are able to detect only extremely common adverse events. Phase II trials generally enroll several hundred people in each vaccine arm, as in the comparative infant diphtheria and tetanus toxoids and acellular pertussis (DTaP) vaccine trials,[32] and provide data on the impact of antigen content, number of vaccine components, vaccine formulation, effect of successive doses, and profile of common reactions. These data can inform the choice of the candidate vaccines for Phase III trials.[33,34] Sample sizes for Phase III vaccine trials are often based on efficacy considerations, and resultant safety data are dependent on the sample size (approximately 10^2 to 10^5) and the duration of observation (often <30 days).[33] Typically, only observations of rates of common local and systemic reactions (e.g., injection site swelling, fever, fussiness) are feasible. The experimental design of most clinical trials includes a control group (a placebo or an alternative vaccine) and the detection of adverse events by researchers who are "blinded" to which vaccine the patient received. This allows relatively straightforward inferences on the causal relationship between most adverse events and vaccination.[35]

Several ways of enhancing prelicensure safety assessment of vaccines have been developed. One of these ways includes the Brighton Collaboration (www.brightoncollaboration.org), which was established to develop and implement globally accepted standard case definitions for assessing adverse events following immunizations.[36] Without such defined standards, it was often difficult, if not impossible, to compare and

contrast safety data across trials. The Brighton case definitions for each adverse event are further arrayed by the level of evidence provided (insufficient, low, intermediate, and highest); therefore, they also can be used in settings with fewer resources (e.g., studies in less-developed settings or postlicensure surveillance). For example, in the large multisite Phase III infant DTaP trials, definitions of high fever across trials varied by temperature (39.5°C vs 40.5°C), measurement (oral vs rectal), and time (measured at 48 vs 72 hours).[37] This was unfortunate because standardized case definitions had been developed in these trials for efficacy but not for safety, even though the safety concerns provided the original impetus for the development of DTaP.[38,39]

There has been a greater recognition of the need for much larger safety databases before licensure. Because of pragmatic limits on the sample sizes of prelicensure studies, there are inherent limitations to the extent to which they can detect very rare, yet real, adverse events related to vaccination. Even if no adverse event has been observed in a trial of 10,000 vaccinees, one can only be reasonably certain that the real incidence of the adverse event is no higher than one in 3333 vaccinees.[40]

TABLE 82.1 Maximum and Number of Cases Reported in 2004: Morbidity From Vaccine-Preventable Diseases Events, United States

Disease	Maximum Cases (Year)	Cases Reported in 2014	% Change
Smallpox	206,939 (1921)	0	–100
Diphtheria	894,134 (1941)	1	>–99
Measles	152,209 (1968)	667	>–99
Mumps	265,269 (1934)	1223	–99
Polio (paralytic)	57,686 (1952)	0	–100
Congenital rubella syndrome	20,000[a] (1964–65)	1	>–99
Haemophilus influenzae type b	25,000[a]	306	–98

[a]Estimated because no national reporting existed in prevaccine era.

Thus, to be able to detect an attributable risk of one per 10,000 vaccinees (e.g., such as the approximate risk found for intussusception in the postlicensure evaluation of the simian–human reassortant rotavirus vaccine, RotaShield vaccine), a prelicensure trial of at least 30,000 vaccinees and 30,000 control subjects is needed. Both second-generation rotavirus vaccines (the bovine human reassortant vaccine, RotaTeq, and the attenuated human rotavirus vaccine, Rotarix) were subjected to Phase III trials that included at least 60,000 infants.[41,42] While these trials were adequately powered to detect intussusception at the rate seen with RotaShield, they were not adequately powered to detect the rates ultimately seen with either RotaTeq or Rotarix. In addition, the cost of such large safety trials could limit the number of vaccine candidates that could be studied.[43]

Postlicensure Evaluations of Vaccine Safety

Because reactions that are rare, delayed, or which occur in only certain subpopulations may not be detected before vaccines are licensed, postlicensure evaluation of vaccine safety is critical. Historically, this evaluation has relied on passive surveillance and ad hoc epidemiologic studies, but, more recently, Phase IV trials and preestablished large, linked databases have enhanced the capabilities to study rare adverse events after specific immunizations.[35] Such systems may detect variation in rates of adverse events by manufacturer[44,45] or specific lot.[46] More recently, clinical centers for the study of immunization safety have emerged as another useful infrastructure to advance our knowledge about safety.[47]

In contrast with the methodological strengths of prelicensure randomized trials, however, postlicensure observational studies of vaccine safety pose a formidable set of methodological difficulties.[48] Confounding by contraindication is especially problematic for nonexperimental designs. Specifically, persons who do not receive vaccine (e.g., because of a medical contraindication) might have a different risk for an adverse event than vaccinated persons. Therefore, direct comparisons of vaccinated and unvaccinated children are often inherently confounded, and teasing out these confounding issues requires understanding of the complex interactions of multiple, poorly quantified factors.

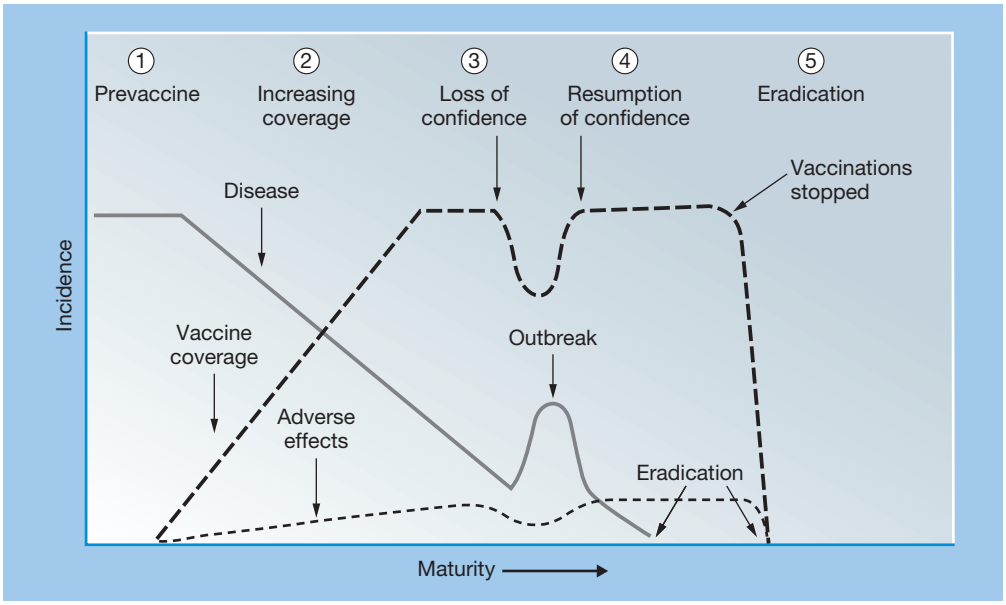


Figure 82.1. Evolution of immunization program and prominence of vaccine safety.

Passive Reporting Systems, Including the Vaccine Adverse Event Reporting System

Informal or formal passive surveillance or spontaneous reporting systems (SRSs) have been the cornerstone of most postlicensure safety monitoring systems because of their relative low cost of operations.[49–51] The national reporting of adverse events following immunizations can be done through the same reporting channels as those used for other adverse drug reactions,[51] as is the practice in France,[52] Japan,[53] New Zealand,[54] Sweden,[55] and the United Kingdom,[56] or with reporting forms or surveillance systems different from the drug safety monitoring systems, as is the practice in Australia,[57] Canada,[58,59] Cuba,[60] Denmark,[61] India,[62] Italy,[63] Germany,[64] Mexico,[65] The Netherlands,[66] Brazil,[67] and the United States.[68] Vaccine manufacturers also maintain SRSs for their products and they forward reports to appropriate national regulatory authorities.[30,65]

In the United States, the National Childhood Vaccine Injury Act of 1986 mandated that healthcare providers report certain adverse events after immunizations.[69] The Vaccine Adverse Events Reporting System (VAERS) was implemented jointly by the Centers for Disease Control and Prevention (CDC) and the U.S. Food and Drug Administration (FDA) in 1990 to provide a unified national focus for collection of all reports of clinically significant adverse events, including, but not limited to, those mandated for reporting.[68]

The VAERS form permits narrative descriptions of adverse events. Patients and their parents—not just healthcare professionals—are permitted to report to VAERS, and there is no restriction on the interval between vaccination and symptoms that can be reported. Report forms, assistance in completing the form, answers to other questions about VAERS, and additional information about the reported reactions, including whether or not the patient recovered, are available on the VAERS website (vaers.hhs.gov). Web-based reporting and simple data analyses are also available.

A contractor, under CDC and FDA supervision, distributes, collects, codes (currently using the Medical Dictionary for Regulatory Activities), and enters VAERS reports into a database. Reporters of selected serious events are contacted by trained clinical staff on report receipt and are sent letters at 1 year after report receipt to provide additional information about the VAERS report, including the patient's recovery. Approximately 30,000 VAERS reports are now received annually, and these data (without personal identifiers) are also available to the public (at vaers.hhs.gov and at wonder.cdc.gov/vaers.html).

Several other countries also have substantial experience with passive surveillance for immunization safety. In 1987, Canada developed the Vaccine Associated Adverse Event reporting system,[59,70] which is supplemented by an active, pediatric hospital–based surveillance system that searches all admissions for possible relationships to immunizations (Immunization Monitoring Program-Active [IMPACT]).[71] Serious Vaccine Associated Adverse Event reports are reviewed by the Advisory Committee on Causality Assessment consisting of a panel of experts.[72] The Netherlands also convenes an annual panel to categorize reports, which are then published.[66] The United Kingdom and most members of the former Commonwealth use the yellow card system, whereby a reporting form is attached to officially issued prescription pads.[50,55] Data on adverse drug events (including vaccine) from several countries are compiled by the World Health Organization (WHO) Collaborating Center for International Drug Monitoring in Uppsala, Sweden.[73]

With so many different passive surveillance systems that collect information on various medical events following vaccination, standardized definitions of vaccine-related adverse events are optimal. Implementation of similar standards across national boundaries has been advanced by the International Conference on Harmonization[74] and the Brighton Collaboration.[36]

Because VAERS is the only surveillance system covering the entire U.S. population with data available on a relatively timely basis, it is the major means available to currently detect possible new, unusual, or extremely rare adverse events. For example, in 1999, passive reports to VAERS of intussusception among children vaccinated with RotaShield was the first postlicensure signal of a problem,[75] leading to epidemiologic studies that verified the association.[17,76] Similarly, initial reports to VAERS of a previously unrecognized serious yellow fever vaccine–associated neurotropic disease[77] and viscerotropic disease[78,79] have since been confirmed elsewhere.[80] Because of the success in detecting these signals, there have been various attempts to automate screening for signals using SRSs reports. New tools developed for pattern recognition in extremely large databases are beginning to be applied.[81] These include empirical Bayesian data mining to identify vaccine-event adverse event combinations that are reported more frequently following a specific vaccine than following all other vaccines combined.[82]

Despite the aforementioned uses, SRSs for drug and vaccine safety have a number of major methodological weaknesses. Underreporting, biased reporting, and incomplete reporting are inherent to all such passive systems, and potential safety concerns might be missed.[83–85] Some increases in adverse events detected by VAERS might not be true increases, but instead might be the result of increases in reporting efficiency or vaccine coverage. For example, an increase in GBS reports was noted in VAERS shortly after the introduction of a new meningococcal conjugate vaccine (MCV4) in 2005, but a larger study conducted in five U.S. health plans with more than 1.4 million MCV4 vaccinations found no increased risk of GBS.[86] As another example, pending litigation resulted in the filing of a large number of VAERS reports claiming that vaccines caused autism.[87,88]

Perhaps the most important methodological weakness of VAERS, however, is that it does not contain the information necessary for formal epidemiologic analyses. Such analyses require calculation of the rate of the adverse event after vaccination and a comparison rate among unvaccinated persons. The VAERS database provides data only for the number of persons who may have experienced an adverse event following immunization and, even then, only in a biased and underreported manner. VAERS lacks data on the denominator of total number of people vaccinated and the corresponding data on number of cases and denominator population of unvaccinated people. Sometimes reporting rates can be calculated by using VAERS case reports for the numerator and, if available, doses of vaccines administered (or, if unavailable, data on vaccine doses distributed or vaccine coverage survey data) for the denominator. These rates can then be compared with the background rate of the same adverse event in the absence of vaccination, if available. Because of underreporting, however, VAERS reporting rates will usually be lower than the actual rates of adverse events following immunization.

A higher proportion of serious events, such as seizures, that follow vaccinations are likely to be reported to VAERS than milder events, such as rash, or delayed events requiring laboratory assessment, such as thrombocytopenic purpura after measles-mumps-rubella (MMR) vaccination.[83] The reporting efficiency or sensitivity of SRSs can sometimes be estimated if an independent source of cases of specific adverse events following immunization is available to conduct capture-recapture analyses.[89]

Formal evaluation has been limited by the quality of diagnostic information on VAERS reports, especially whether a serious event reported to VAERS has been diagnosed accurately. Of 26 cases reported to VAERS in which GBS developed after influenza vaccination during the 1990–91 season, and for which hospital charts were reviewed by an independent panel of neurologists blinded to immunization status, the diagnosis of GBS was confirmed in only 22 (85%).[90] Intussusception was verified in 88% of VAERS reports filed after Rotashield vaccination.[75] Clinical reviews of VAERS reports submitted following 2009 H1N1 influenza vaccine were able to verify 56% of possible GBS reports and 42% of reports of possible anaphylaxis.[91] Clinical review verification rates were similar for VAERS reports following human papillomavirus (HPV) vaccination: 57% for GBS and 38% for anaphylaxis.[92]

These studies highlight the often crude nature of signals generated by VAERS and the difficulty in ascertaining which potential vaccine safety concerns warrant further investigation. The problems with reporting efficiency and potentially biased reporting and the inherent lack of an adequate control group limit the certainty with which conclusions can be drawn. Recognition of these limitations in large part has helped stimulate the creation of more population-based methods of assessing vaccine safety.

Postlicensure Clinical Trials and Phase IV Surveillance Studies

To improve the ability to detect adverse events that are not detected during prelicensure trials, some recently licensed vaccines in developed countries have undergone formal Phase IV surveillance studies on populations with sample sizes that have included as many as 100,000 people. These studies usually have included cohorts in managed care organizations (MCOs) supplemented by diary or phone interviews. These methods were first used extensively after the licensure of polysaccharide and conjugated Hib vaccines.[93–95] Large postlicensure studies on safety and efficacy have also been conducted for several other vaccines, including those for DTaP,[38] varicella, and herpes zoster,[96,97] as well as for less-frequently used vaccines, such as Japanese encephalitis vaccine.[98]

Large Linked Databases, Including the Vaccine Safety Datalink. Historically, ad hoc epidemiologic studies have been used to assess signals of potential adverse events detected by SRSs, the medical literature, or other mechanisms. Some examples of such studies include the investigations of poliomyelitis after inactivated[10,99] and oral[100] polio vaccines, sudden infant death syndrome (SIDS) after diphtheria-tetanus-pertussis (DTP) vaccination,[101–104] encephalopathy after DTP vaccination,[105,106] meningoencephalitis after mumps vaccination,[107] injection-site abscesses after vaccination,[108] and GBS after influenza vaccination.[13,90,109] The Institute of Medicine (IOM) has compiled and reviewed many of these studies.[11,110,111]

Unfortunately, such ad hoc studies are often costly, time-consuming, and limited to assessment of a single event or a few events or outcomes. Given these drawbacks and the methodological limitations of passive surveillance systems (such as described for VAERS), pharmacoepidemiologists turned to large databases linking computerized pharmacy prescriptions (and later immunization records) and medical outcome records.[85] These databases include defined populations such as members of MCOs, single-provider healthcare systems, and Medicaid programs. Such databases cover enrollee populations numbering from thousands to millions, and, because the data are generated from the routine administration of the full range of medical care, underreporting and recall bias are reduced. With denominator data on doses administered and the ready availability of appropriate comparison (i.e., unvaccinated) groups, these large databases provide an economical and rapid means of conducting postlicensure studies of safety of drugs and vaccines.[112–116]

The CDC initiated the Vaccine Safety Datalink (VSD) project in 1990[113] to conduct postmarketing evaluations of vaccine safety and to establish an infrastructure allowing for high-quality research and surveillance. Several MCOs or integrated healthcare systems in the United States, comprising a population of more than 9 million members, participate in the VSD. Each site prepares computerized data files using a standardized data dictionary containing demographic and medical information on their members, such as age and sex, health plan enrollment, vaccinations, hospitalizations, outpatient clinic visits, emergency department visits, urgent care visits, and mortality data, as well as additional birth information (e.g., birth weight) when available. Other information sources, such as medical chart review; member surveys; and pharmacy, laboratory, and radiology data are often used in VSD studies to validate outcomes and vaccination data. There is rigorous attention to the maintenance of patient confidentiality, and each study undergoes institutional review board review.

The VSD project's main priorities include evaluating new vaccine safety concerns that may arise from the medical literature,[11,110] from VAERS,[76,117] from changes in immunization schedules,[118] or from introduction of new vaccines.[94,95] The creation of frequently updated data files has enabled the development of near real-time postmarketing surveillance for newly licensed vaccines and changes in vaccine recommendations. Many studies have been performed within the VSD project,[116] including general screening studies of the safety of inactivated influenza vaccines among children and of thimerosal-containing vaccines; and disease-specific investigations, including studies investigating autism, multiple sclerosis, thyroid disease, acute ataxia, alopecia, rheumatoid arthritis, asthma, diabetes, and idiopathic thrombocytopenic purpura following vaccination.

Among these advantages, a few caveats are appropriate. Although diverse, the population in the MCOs currently in the VSD project is not wholly representative of the United States in terms of geography or socioeconomic status. More important, because of the high coverage attained in the MCOs for most vaccines, few nonvaccinated control subjects are available. Therefore, VSD studies often rely on risk-interval analyses (e.g., to study the question of whether outcome "x" is more common in period "y" following vaccination compared with other periods) (Table 82.2).[119] This approach, although powerful for evaluating acute adverse events, has limited ability to assess associations between vaccination and adverse events with delayed or insidious onset (e.g., autism). The VSD project also cannot easily assess mild adverse events (such as fever) that do not always come to medical attention.[113] Finally, because vaccines are not delivered in the context of randomized, controlled trials, the VSD project may not be able to successfully control for confounding and bias in each analysis,[120] and inferences on causality may be limited.[121]

Despite these potential shortcomings, the VSD project provides an essential, powerful, and cost-effective complement to ongoing evaluations of vaccine safety in the United States.[115,116] In view of the methodological and logistic advantages offered by large-linked databases, the United Kingdom and Canada also have developed systems linking immunization records with medical files.[71,112] Certain countries, such as Sweden and Denmark, have the capability to conduct important

TABLE 82.2 Example of Method for Risk-Interval Analysis of Association Between a Universally Recommended Three-Dose Vaccine and an Adverse Event

1. Define biologically plausible risk interval for adverse event after vaccination (e.g., 30 days after each dose).

2. Partition observation time for each child in the study into periods within and outside of risk intervals, and sum respectively (e.g., for a child observed for 365 days during which 3 doses of vaccine were received, total risk interval time = 3 × 30 person-days = 90 person-days; total non-risk interval time = 365 – 90 = 275 person-days).

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Birth Dose 1 Dose 2 Dose 3 365 days

3. Add up (a) total risk interval and nonrisk interval observation times for each child in the study (Person-Time Observed; for mathematical convenience, example below uses 100 and 1000 person-months of observation), and (b) adverse events occurring in each time period to complete 2 × 2 table (for illustration, example below uses 3 and 10 cases):

Vaccinated in Risk Interval	Adverse Event: Yes	Person-Time Observed (mo)	Incidence Rate
Yes	3	100	0.03
No	10	1000	0.01
TOTAL	13	1100	

Incidence rate adverse event vaccinated = 3/100 = 0.03
Incidence rate adverse event unvaccinated = 10/1,000 = 0.01
Relative rate vaccinated: unvaccinated = 0.03/0.01 = 3.0
Probability finding is the result of chance: <5/100
Conclusion: There is a threefold increase in risk for developing the adverse event within the 30-day interval after vaccination compared to other time periods.

vaccine safety assessments by linking data across national registries.[122–124]

Clinical Centers, Including the Clinical Immunization Safety Assessment Centers. More recently, the immunization safety infrastructure has been augmented by tertiary clinical centers, such as were first developed in certain regions in Italy[47] and Australia.[125,126]

In the United States, the CDC's Clinical Immunization Safety Assessment (CISA) Project was established in 2001 to address unmet vaccine safety clinical research needs. CISA is a national network of vaccine safety experts from the CDC, seven medical research centers, and subject-matter experts. The CISA mission is to improve understanding of adverse events following immunization at the individual patient level. CISA serves as a vaccine safety resource providing expert consultation on clinical vaccine safety issues and with its partners conducts studies to identify risk factors and preventive strategies for vaccine adverse events, particularly in special populations.[28] The CISA investigators bring in-depth clinical, pathophysiological, and vaccinology expertise to assessing causal relationships between vaccines and adverse events,[127] and to understanding the pathogenesis of adverse events following vaccinations. The CISA investigators have published a standardized algorithm for evaluating and managing persons who have suspected or definite immediate hypersensitivity reactions such as urticaria, angioedema, and anaphylaxis following vaccines.[128] Some of the studies undertaken by CISA include an assessment of extensive limb swelling after DTaP,[129] a study of the usefulness of irritant skin test reactions for managing hypersensitivity to vaccines,[130] the clinical evaluation of patients with serious adverse events following yellow fever vaccine administration,[131] and evaluation of vaccine safety among children with inborn errors of metabolism.[132,133] New understanding of the human genome, pharmacogenomics, and immunology hold promise for future CISA studies and may make it possible to elucidate some of the biological mechanisms of vaccine adverse reactions, which, in turn, could lead to the development of safer vaccines and safer vaccination practices, including revaccination when indicated.[134]

SAFETY OF MASS IMMUNIZATION CAMPAIGNS

In mass-immunization campaigns during which many people are vaccinated in a short time, it is critical to have a vaccine safety monitoring system in place that can detect potential safety problems early so that corrective actions can be taken as soon as possible.[135] Mass-immunization campaigns are often conducted in developing countries, which poses a particular challenge of ensuring injection safety.[136] In any setting in which large numbers of immunizations are being administered, more adverse events will coincidentally occur following immunization. Thus, it is important to have background rates available of expected adverse events to allow rapid evaluation of whether reported adverse events are occurring at a rate following immunization that is higher than would be expected by chance alone. The resources devoted to mass vaccination campaigns also provide opportunities to enhance existing immunization safety monitoring systems or to establish a system if none exists and these may lead to long-term improvements in immunization safety monitoring beyond the specific mass immunization campaign.

The response to the 2009 H1N1 influenza pandemic involved probably the largest and most intense immunization safety monitoring effort ever undertaken in the United States and internationally. The emergence of a novel influenza A (H1N1) virus prompted the development of 2009 influenza A (H1N1) monovalent vaccines. The FDA licensed the first 2009-H1N1vaccines in September 2009. With potentially hundreds of millions of people expected to be vaccinated, adverse events were anticipated to occur in some recently vaccinated people. To address the question of whether the vaccine could be causing the adverse events, background rates for several adverse events were developed.[137] To rapidly detect any unforeseen safety problems, the federal government implemented enhanced postlicensure 2009-H1N1 vaccine safety monitoring,[138] including in VAERS and VSD. A new collaboration was initiated that also performed rapid ongoing analyses similar to VSD and included the databases of several large health insurance plans, the Department of Defense, Medicare, and the Veterans Administration. In addition, active case

finding for GBS was conducted in 10 areas of the United States with a combined population of approximately 50 million people. Initial safety data were provided by VAERS, which found that the adverse event profile after 2009-H1N1 vaccine in VAERS (>10,000 reports) was consistent with that of seasonal influenza vaccines.[91,138] Combined results from the U.S. monitoring systems found 1.6 excess cases of GBS per 1 million vaccinations, which is similar to the increased risk found with some seasonal influenza vaccines.[139]

Similar efforts to intensely monitor the safety of influenza A (H1N1) 2009 vaccines occurred in other countries, primarily in North America, Europe, and Australia, but also included the development of new immunization safety monitoring systems in countries such as Taiwan[140] and a multicountry study of GBS.[141] These extensive international safety monitoring activities and collaborations represented an unprecedented commitment to ensuring the safety of influenza A (H1N1) 2009 vaccines, as well as a model for how we might improve tracking of safety for all vaccines going forward.

WEIGHING THE EVIDENCE AND ASSESSING CAUSALITY

A central function of vaccine safety monitoring and assessment activities is to determine if particular adverse events following immunization are caused by a vaccine. This determination is important in guiding immunization policy and individual care and possibly compensation decisions. Causality assessment may be performed at the individual or population level. Epidemiologic studies provide measures of risk on a population level, but they do not provide evidence that a particular vaccine caused an adverse event in a particular individual. It is often not possible to infer causality in individual cases of adverse events following immunization, except in certain special situations. Circumstances in which a causal association in individual cases can reasonably be inferred include: (a) local reactions at a vaccine injection site; (b) immediate hypersensitivity reactions (in absence of other exposures); (c) recurrence–same adverse event recurs in same individual following repeat exposure to same vaccine; (d) isolation of vaccine virus (from typically sterile site), as, for example, Urabe mumps vaccine and aseptic meningitis; and (e) a unique clinical syndrome, such as vaccine-associated paralytic polio.

More general causality assessments rely on weighing different pieces of evidence using criteria such as strength of association, consistency of findings, temporal relationships, potential biases, and possible biological mechanisms. In the United States, the most authoritative assessments of causality have been conducted by the IOM, whose findings have been particularly influential in compiling the vaccine injury table of the National Vaccine Injury Compensation Program. The IOM has conducted three comprehensive reviews of adverse effects of vaccines,[111,142,143] as well as several focused reviews of specific vaccine safety topics. As highlighted in Table 82.3, the adverse reactions for which there is strong evidence of a causal association with recommended childhood vaccines in the United States are few and tend to occur rarely. Newer vaccines and certain other vaccines, however, were not covered in the latest IOM review in 2012. This gap was addressed by a systematic review of the literature commissioned by the Agency for Healthcare Research and Quality.[144] That review identified a few additional adverse events where the evidence favors a causal association, including rotavirus vaccines and intussusception, pneumococcal conjugate vaccine and febrile seizure, hepatitis A vaccine and purpura, and varicella vaccine and thrombocytopenic purpura.

In addition to identifying causal associations, IOM reviews have also been influential in clarifying particularly controversial issues where the evidence does not favor a causal association. These include pertussis vaccines and SIDS,[145] vaccines and autism,[146] hepatitis B vaccine and multiple sclerosis,[147] and vaccines and type 1 diabetes.[148]

TABLE 82.3 Vaccines and Adverse Events for Which Evidence Favors a Causal Association

Vaccine(s)	Adverse Event	Source	Rate Per Million Doses
Tetanus toxoid, pertussis, measles, mumps, rubella, inactivated polio vaccine, hepatitis B, varicella, influenza, meningitis, human papillomavirus	Anaphylaxis	VIT, IOM 2012	1–2[a]
Pertussis (whole cell)	Encephalopathy/encephalitis	VIT	<1[b]
Measles-mumps-rubella (MMR)	Encephalopathy/measles inclusion body encephalitis	VIT, IOM 2012	Case reports only
MMR	Febrile seizures	IOM 2012	333[b]
MMR	Transient arthralgia, women & children	IOM 2012	~5% (postpartum women); <1% (children)
Measles	Thrombocytopenic purpura	VIT	33[b]
Rubella	Chronic arthritis	VIT	Unknown[c]
Varicella	Vaccine strain dissemination	IOM 2012	Case reports
Any vaccine	Injection-related syncope, deltoid bursitis	IOM 2012	Case reports

[a]Data from McNeil MM, Weintraub E, Duffy J, et al. Risk of anaphylaxis after vaccination in children and adults. *J Allergy Clin Immunol*. 2016;137(3):868–178.
[b]Data from World Health Organization (WHO), Department of Vaccines and Biologicals. Supplementary information on vaccine safety, Part 2: Background rates of adverse events following immunization. December 2000. WHO/V&B/00.36. Available at: http://apps.who.int/iris/bitstream/10665/66675/1/WHO_V-B_00.36_eng.pdf
[c]IOM 2012 review determined evidence to be inadequate.
IOM, Institute of Medicine; U.S.; VIT, Vaccine Injury Table, U.S..

VACCINE SAFETY CONTROVERSIES AND MISCONCEPTIONS

Unfortunately, vaccine safety issues have increasingly taken on a life of their own outside of the scientific arena—arguably to society's overall detriment. In particular, various chronic diseases (and their advocates) in search of a simple cause, for which immunizations—as a relatively universal exposure—have made all too convenient a hypothesized link. Case studies of some of these controversies are discussed in the following sections.

Whole-Cell Pertussis Vaccine Causes Permanent Brain Damage

In 1974, Kulenkampff and coworkers[149] reported a series of 22 cases of children with mental retardation and epilepsy following receipt of the whole-cell pertussis vaccine. During the next several years, fear of the pertussis vaccine generated by media coverage of this report caused a decrease in pertussis immunization rates in British children from 81% to 31% and resulted in more than 100,000 cases and 36 deaths from pertussis.[150] Media coverage of the Kulenkampff report also caused decreased immunization rates and increased pertussis deaths in Japan, Sweden, and Wales.[150]

However, many subsequent, excellent, well-controlled studies found that the incidence of mental retardation and epilepsy following whole-cell pertussis vaccine was similar in vaccinated children to that in children who did not receive the vaccine.[151–156]

Deaths Following Vaccination: Temporal Association Versus Causality

When a death occurs shortly following vaccination, there might be a natural tendency to question if the death was caused by the vaccine. Suspected associations of deaths with vaccination can have a negative impact on vaccination programs even when investigations do not find any evidence of a direct causal relationship. For example, when a combination pentavalent vaccine replacing the diphtheria-tetanus-whole-cell pertussis (DTwP) vaccine or DTwP-hepatitis B vaccine was introduced into Sri Lanka, India, and Vietnam in 2008–10, deaths were reported among a small number of vaccine recipients prompting authorities to suspend the use of these vaccines.[157] In 2010, an HPV demonstration project in India was suspended in response to demands from advocacy groups when four deaths occurred following receipt of HPV vaccine.[158] In December 2013, the deaths of 17 infants following hepatitis B virus vaccine made by a manufacturer in China generated widespread media and public interest, leading to a temporary suspension of the vaccine.[159] Investigations in all these examples found that the deaths were not caused by the vaccines.

Historically, SIDS has garnered the greatest concern with respect to a possible association with vaccination. In the mid-1980s, the antivaccine group Dissatisfied Parents Together raised the notion that the whole-cell pertussis vaccine could cause SIDS. In the early 1990s, when the hepatitis B vaccine was recommended for routine use in newborns, a popular television news program raised the question of whether vaccines could cause SIDS. SIDS peaks between 2 and 3 months of age, a time when infants are receiving a relatively large number of recommended vaccinations; thus, it would not be unexpected to observe a coincidental close temporal relationship between vaccination and SIDS. SIDS deaths in the United States have been declining since the early 1990s for a variety of reasons, including recommended changes in sleeping position.[160] This downward trend in SIDS has also been observed in SIDS reports submitted to VAERS.[161] Considerable evidence supports the notion that vaccination is not causally associated with SIDS,[162–165] including an IOM review in 2003 that rejected a causal association between the whole cell pertussis–containing vaccine and SIDS and between exposure to multiple simultaneous vaccines and SIDS.[145]

More recently, the misconception of mistakenly attributing temporally related events as causal was highlighted in the U.S. multistate measles outbreak of 2014–15. Early on, unsubstantiated claims of deaths caused by the MMR vaccine appeared on the Internet.[166–168] These claims were largely based on publicly available VAERS data. VAERS, however, is a voluntary reporting system that accepts any submitted report of an adverse event without judging whether it was caused by a vaccination. Any claims of cause and effect with respect to deaths following vaccination based on VAERS reports should be interpreted with caution. When the complete VAERS reports and accompanying medical records, autopsy reports, and death certificates were reviewed by FDA and CDC physicians, no concerning patterns were detected to suggest a causal relationship with MMR vaccination.[169,170]

With rare exceptions (e.g., anaphylaxis), the evidence does not suggest a causal relationship between vaccination and death. In addition to the 2003 review of SIDS, a previous IOM review of death reports in VAERS concluded that the vast majority of reported deaths were coincidental and not causally related to vaccination.[171] A newer VSD study of more than 13 million vaccinated persons compared rates and causes of death in the vaccinated study population to the general U.S. population. The death rate 1 or 2 months following vaccination was lower than that in the general U.S. population and the causes of death were similar,[172] providing persuasive evidence that vaccinations are not associated with an increased risk of death.

Vaccines Cause Cancer

Simian virus 40 (SV40) was present in monkey kidney cells used to make the inactivated polio vaccine, live attenuated polio vaccine, and inactivated adenovirus vaccines in the late 1950s and early 1960s. Later, investigators found SV40 DNA in biopsy specimens obtained from patients with certain unusual cancers—mesothelioma, osteosarcoma, and non-Hodgkin lymphoma—leading some to hypothesize a link between vaccination and the subsequent development of cancer.[173] However, genetic remnants of SV40 were present in cancers of people who had or had not received contaminated polio vaccines; people with cancers who never received SV40-contaminated vaccines were found to have evidence for SV40 in their cancerous cells; and epidemiologic studies did not show an increased risk of cancers in people who received polio vaccine between 1955 and 1963 compared with people who did not receive these vaccines.[173] Taken together, these findings do not support the hypothesis that the SV40 contained in polio vaccines administered before 1963 caused cancers.

Several studies have evaluated the possible relationship between vaccination and leukemia. The findings from these studies indicate that MMR, DTaP, diphtheria and tetanus toxoids–adult (Td), Hib, hepatitis B, and polio vaccines are not associated with childhood leukemia.[174–177]

Safety of the Immunization Schedule and Simultaneous Vaccinations

One hundred years ago, children received one vaccine—smallpox. Today, children receive 14 vaccines routinely.

Although some vaccines are given in combination, infants and young children could receive more than 20 shots and three oral doses of vaccines by 2 years of age, including as many as five shots at one time.[178–181] A prevalent concern is that little is known about the safety of the recommended immunization schedule as a whole. Parents have expressed the opinion, for example, that too many vaccines are given to children at too young an age and that early childhood immunization overwhelms the immune system. These sentiments reflect concern about the number, frequency, and timing of recommended vaccines rather than about the specific properties of particular vaccines.[178,182–184]

In response to this public concern, the IOM, in 2012, convened a committee to examine stakeholder concerns and scientific evidence regarding the safety of the recommended childhood immunization schedule, and to identify study settings, designs, and methods that would be appropriate to rigorously address this issue.[185] The IOM committee concluded that, although few published investigations had specifically examined the safety of the recommended childhood schedule as a whole, the accumulation of available evidence indicated that the current U.S. immunization schedule is safe. That evidence was based in part on concomitant use studies required by the FDA prior to licensure of a vaccine. Concomitant use studies determine whether new vaccines alter either the safety or immunogenicity profile of existing vaccines that will be given at the same time as the new vaccine and whether existing vaccines alter the safety or immunogenicity profiles of the new vaccine.

Although we have witnessed a dramatic increase in the number of vaccines routinely recommended for infants and young children, the number of immunogenic proteins and polysaccharides contained in vaccines has declined (Table 82.4). The decrease in the number of immunogenic proteins and polysaccharides contained in vaccines is attributable to discontinuation of the smallpox vaccine and advances in the field of protein purification that allowed for a switch from whole-cell to acellular pertussis vaccine, and recombinant DNA technology that allowed for the relatively easy manufacture of single protein vaccines.

A practical way to determine the capacity of the immune system to respond to vaccines is to consider the number of B and T cells required to generate adequate levels of binding antibodies per milliliter of blood.[186] Calculations are based on the following assumptions:

1. Approximately 10 ng/mL is likely to be an effective concentration of antibody directed against a specific epitope.
2. Approximately 10^3 B cells/mL are required to generate 10 ng of antibody/mL.
3. Given a doubling time of approximately 0.75 days for B cells, it would take approximately 7 days to generate 10^3 B cells/mL from a single B-cell clone.
4. Because vaccine-specific humoral immune responses are first detected approximately 7 days after immunization, those responses could initially be generated from a single B-cell clone per milliliter.
5. One vaccine contains approximately 10 immunogenic proteins or polysaccharides (see Table 82.4).
6. Each immunogenic protein or polysaccharide contains approximately 10 epitopes (i.e., 10^2 epitopes per vaccine).
7. Approximately 10^7 B cells are present per milliliter of blood.

Given these assumptions, the number of vaccines to which a person could respond would be determined by dividing the number of circulating B cells ($\approx 10^7$) by the average number of epitopes per vaccine (10^2). Therefore, a person could theoretically respond to approximately 10^5 vaccines at one time. This is probably a conservative estimate for several factors, including consideration of only vaccine-specific B-cell responses and ignoring the dynamic nature of the immune system. For example, a study of T-cell population dynamics in HIV-infected persons found that adults have the capacity to generate approximately 2×10^9 new T lymphocytes each day.[187] Although the quantity of new B and T cells generated each day in healthy people is unknown, studies of HIV-infected persons demonstrate the enormous capacity of the immune system to generate lymphocytes when needed.

TABLE 82.4 Year of Introduction and Number of Immunogenic Proteins and Polysaccharides Contained in Selected Vaccines

Vaccine	Year of Introduction	Number of Proteins or Polysaccharides or Both
Smallpox[a]	1796	198
Rabies	1885	5
Diphtheria[b]	1923	1
Pertussis (whole cell)[a]	1926	~3000
Tetanus[b]	1927	1
Yellow fever	1936	11
Influenza[b]	1945	10
Polio (inactivated)[b]	1955	15
Polio (live, attenuated)[a]	1961	15
Measles[b]	1963	10
Mumps[b]	1967	9
Rubella[b]	1969	5
Hepatitis B[b]	1981	1
Haemophilus influenzae type b (conjugate)[b]	1990	2
Pertussis (acellular)[b]	1991	2–5
Hepatitis A[b]	1995	4
Varicella[b]	1995	69
Pneumococcus (conjugate)[b]	2000	8
Meningococcus (conjugate)[b]	2005	5
Rotavirus[b]	2006	16
Human papillomavirus[b]	2006	4

[a]Formerly in the U.S. routine child and adolescent immunization schedule.
[b]Currently in the U.S. routine child and adolescent immunization schedule.

Vaccines Weaken the Immune System

Infection with wild-type viruses can cause a suppression of specific immunologic functions. For example, infection with wild-type measles virus causes a reduction in the number of circulating B and T cells during the viremic phase of infection and a delay in the development of cell-mediated immunity.[188,189] Similarly, wild-type measles virus infection can cause long-term loss of memory B and T cells, resulting in an increased mortality from other infections.[190] Downregulation of cell-mediated immunity by wild-type measles virus probably results from downregulation of the production of

interleukin-12 by measles-infected macrophages and dendritic cells.[188] Taken together, the immunosuppressive effects of wild-type measles virus account, in part, for the increase in morbidity and mortality from measles infection. Similarly, the immunosuppressive effects of infections with wild-type varicella virus[191] or wild-type influenza virus[192] cause an increase in the incidence of severe invasive bacterial infections.

Live viral vaccines replicate (albeit far less efficiently than wild-type viruses) in the host and, therefore, can mimic events that occur after natural infection. For example, measles, mumps, or rubella vaccines can significantly depress reactivity to the tuberculin skin test,[193–199] measles-containing vaccines can cause a decrease in protective immune responses to varicella vaccine,[200] and high-titered measles vaccine (Edmonston-Zagreb strain) can cause an excess of cases of invasive bacterial infections in developing countries.[201] All these phenomena are explained by the likely immunosuppressive effects of measles vaccine viruses. However, current vaccines (including the highly attenuated Moraten strain of measles vaccine) do not seem to cause clinically relevant immunosuppression in healthy children. Studies have found that the incidence of invasive bacterial infections following immunization with diphtheria, pertussis, tetanus, bacille Calmette-Guérin (BCG), measles, mumps, rubella, or live attenuated poliovirus vaccines was not greater than that found in unimmunized children.[202–206]

Vaccines Cause Autoimmunity

Mechanisms are present at birth to prevent the development of immune responses directed against self-antigens (autoimmunity). T- and B-cell receptors of the fetus and newborn develop with a random repertoire of specificities. In the thymus, T cells that bind strongly to self-peptide–major histocompatibility complexes die, while those that bind with a lesser affinity survive to populate the body. This central selection process eliminates strongly self-reactive T cells, while selecting for T cells that recognize antigens in the context of self–major histocompatibility complex. In the fetal liver, and later in the bone marrow, B-cell receptors (i.e., immunoglobulins) that bind self-antigens strongly are also eliminated. Therefore, the thymus and bone marrow, by expressing antigens from many tissues of the body, enable the removal of the majority of potentially dangerous autoreactive T and B cells before they mature—a process termed *central tolerance*.[207]

It is not simply the presence of autoreactive T and B cells, however, that result in autoimmune disease. Autoreactive T and B cells are present in all people because it is not possible for every antigen from every tissue of the body to participate in the elimination of all potentially autoreactive cells. A process termed *peripheral tolerance* further limits the activation of autoreactive cells.[208,209] Mechanisms of peripheral tolerance include: (a) antigen sequestration (antigens of the central nervous system, eyes, and testes are not regularly exposed to the immune system unless injury or infection occurs); (b) anergy (lymphocytes partially triggered by antigen but without costimulatory signals are unable to respond to subsequent antigen exposure); (c) activation-induced cell death (a self-limiting mechanism involved in terminating immune responses after antigen is cleared); and (d) inhibition of immune responses by specific regulatory cells.[210–213]

Thus, the immune system anticipates that self-reactive T cells will be present and has mechanisms to control them. Any theory of vaccine causation of autoimmune diseases must take into account how these controls are circumvented. As discussed subsequently, epidemiologic studies have not supported the hypothesis that vaccines cause autoimmune diseases. This is consistent with the fact that no mechanisms have been advanced to explain how vaccines could account for all the prerequisites that would be required for the development of autoimmune disease.

At least four key conditions must be met for development of autoimmune disease. First, self-antigen–specific T cells or self-antigen–specific B cells must be present. Second, self-antigens must be presented in sufficient amounts to trigger autoreactive cells. Third, costimulatory signals, cytokines, and other activation signals produced by antigen-presenting cells (such as dendritic cells) must be present during activation of self-reactive T cells. Fourth, peripheral tolerance mechanisms must fail to control destructive autoimmune responses. If all these conditions are not met, the activation of self-reactive lymphocytes and progression to autoimmune disease are not likely.

Evidence That Vaccines Do Not Cause Autoimmunity

Rigorous epidemiologic studies of infant vaccines and type 1 diabetes found that measles vaccine was not associated with an increased risk for diabetes; other investigations found no association between BCG, smallpox, tetanus, pertussis, rubella, or mumps vaccine and diabetes.[214] Specifically, a Canadian study found no increase in risk for diabetes as a result of receipt of BCG vaccine.[215] In a large 10-year follow-up study among Finnish children enrolled in an Hib vaccination trial, no differences in risk for diabetes were found among children vaccinated at 3 months of age (followed later with a booster vaccine) and children vaccinated at 2 years only or with children born before the vaccine trial. The weight of currently available epidemiologic evidence does not support a causal association between currently recommended vaccines and type 1 diabetes in humans.[216–218]

The hypothesis that vaccines might cause multiple sclerosis was fueled by anecdotal reports of multiple sclerosis following hepatitis B immunization and two case-control studies showing a small increase in the incidence of multiple sclerosis in vaccinated persons that was not statistically significant.[219–221] However, the capacity of vaccines to cause or exacerbate multiple sclerosis has been evaluated in several excellent epidemiologic studies.[222–226] Two large case-control studies showed no association between hepatitis B vaccine and multiple sclerosis,[223] and found no evidence that hepatitis B, tetanus, or influenza vaccines exacerbated symptoms of multiple sclerosis.[224] Other well-controlled studies also found that influenza vaccine did not exacerbate symptoms of multiple sclerosis.[225–227] Indeed, in a retrospective study of 180 patients with relapsing multiple sclerosis, infection with influenza virus was more likely than immunization with influenza vaccine to cause an exacerbation of symtpoms.[227]

A 2009 review also showed that, with the possible exception of the swine flu vaccine in 1976, influenza vaccines are not a definite cause of GBS and that any possible increased risk following vaccination is outweighed by a higher risk following natural influenza infection.[228]

Vaccines Cause Allergies and Asthma

Allergic symptoms are caused by soluble factors (e.g., immunoglobulin E) that mediate immediate-type hypersensitivity; production of immunoglobulin E by B cells is dependent on release of cytokines such as interleukin-4 by T-helper type 2 (Th2) cells. Two theories have been advanced to explain how vaccines could enhance immunoglobulin E–mediated, Th2-dependent allergic responses. First, vaccines could shift immune responses to potential allergens from T-helper type 1 (Th1)-like to Th2-like.[229] Second, by preventing common prevalent infections (the "hygiene hypothesis"), vaccines

could prolong the length or increase the frequency of Th2-type responses.[230,231]

Although all factors that cause changes in the balance of Th1 and Th2 responses are not fully known,[232] it is clear that dendritic cells have a critical role. For example, adjuvants (e.g., aluminum hydroxide or aluminum phosphate ["alum"] contained in some vaccines) promote dendritic cells to stimulate Th2-type responses.[233,234] Adjuvants could cause allergies or asthma by stimulating bystander, allergen-specific Th2 cells. However, vaccine surveillance data show no evidence for environmental allergen priming by vaccination.[235] Furthermore, local inoculation of adjuvant does not cause a global shift of immune responses to Th1 or Th2 type.[236,237]

The other hypothesis advanced to explain how vaccines could promote allergies is that by preventing several childhood infections (the hygiene hypothesis), stimuli that evolution has relied on to cause a shift from the neonatal Th2-type immune response to the balanced Th1–Th2 response patterns of adults have been eliminated.[230,231] However, the diseases that are prevented by vaccines constitute only a small fraction of the total number of illnesses to which a child is exposed, and it is unlikely that the immune system would rely on only a few infections for the development of a normal balance between Th1 and Th2 responses. For example, a study of 25,000 illnesses performed in Cleveland, Ohio, in the 1960s found that children experienced six to eight infections per year in the first 6 years of life; most of these infections were caused by viruses such as coronaviruses, rhinoviruses, paramyxoviruses, and myxoviruses—diseases for which children are not routinely immunized.[238] Also at variance with the hygiene hypothesis is the fact that children in developing countries have lower rates of allergies and asthma than children in developed countries even though they are commonly infected with helminths and worms—organisms that induce strong Th2-type responses.[239]

Evidence That Vaccines Do Not Cause Asthma

Although some relatively small early observational studies supported the association between whole-cell pertussis vaccine and development of asthma,[240] newer studies suggest otherwise. A large clinical trial performed in Sweden found no increased risk,[241] and a very large longitudinal study in the United Kingdom found no association between pertussis vaccination and early- or late-onset wheezing or recurrent or intermittent wheezing.[242] Two studies from the VSD project have also lent data to this controversy. In one study of 1366 infants with wheezing during infancy, vaccination with DTP and other vaccines was not related to the risk of wheezing in full-term infants.[243] In another study of more than 165,000 children, childhood vaccinations were not associated with an increased risk for developing asthma.[244] Finally, a study from Finland also suggested that children with a history of natural measles were at increased risk for atopic illness. Such findings run contrary to the hypothesis that the increase in atopic illnesses seen in several countries is a consequence of the reduction in wild measles resulting from immunizations.[245]

A separate concern is whether inactivated influenza vaccination may exacerbate asthma in children with preexisting asthma. Results of studies examining the potential associations between administration of inactivated influenza vaccine and various surrogate measures of asthma exacerbation, including decreased peak expiratory flow rate, increased use of bronchodilating drugs, and increase in asthma symptoms, have yielded mixed results. Most studies, however, have not supported such an association.[246] In fact, after controlling for asthma severity, acute asthma exacerbations were less common after inactivated influenza vaccination than before,[247] and inactivated influenza vaccination seems to be associated with a decreased risk for asthma exacerbations throughout influenza seasons.[248] Several more recent studies have also shown a lack of correlation between receipt of vaccines and the development of asthma.[249–252]

Vaccines Cause Autism and Other Developmental Disabilities

Probably one of the most contentious issues in recent years has been speculation that vaccines cause autism. Autism is a chronic developmental disorder characterized by problems in social interaction, communication, and responsiveness and by repetitive interests and activities. Although the causes of autism are largely unknown, family and twin studies suggest that genetics has a fundamental role.[253] In addition, overexpression of neuropeptides and neurotrophins has been found in the immediate perinatal period among children later diagnosed with autism, suggesting that prenatal or perinatal influences or both have a more important role than postnatal insults.[254] However, because autistic symptoms generally first become apparent in the second year of life, some scientists and parents have focused on the role of MMR vaccine because it is first administered around this time. Concern about the role of MMR vaccine was heightened in 1998 when the *Lancet* published a study based on 12 children that proposed an association between the vaccine and the development of ileonodular lymphoid hyperplasia, nonspecific colitis, and regressive developmental disorders (later termed by some as "autistic enterocolitis").[255] Among the proposed mechanisms was that MMR vaccine present in the intestinal tissues caused bowel problems, leading to the malabsorption of essential vitamins and other nutrients and eventually to autism or other developmental disorders. Concern about this issue led to a decline in measles vaccine coverage in the United Kingdom and elsewhere.[256] Significant concerns about the validity of the study included the lack of an adequate control or comparison group, inconsistent timing to support causality (several of the children had autistic symptoms preceding bowel symptoms), and the lack of an accepted definition of the syndrome.[257] Subsequently, population-based studies of autistic children in the United Kingdom found no association between receipt of MMR vaccine and autism onset or developmental regression.[258,259] Soon after publication of the *Lancet* article that ignited the controversy,[255] two ecologic analyses found no evidence that MMR vaccination was the cause of apparent increased trends in autism over time[260,261]; in addition, two other studies found no evidence of a new variant form of autism associated with bowel disorders secondary to vaccination.[262,263] Several newer studies also refuted the notion that MMR vaccine caused autism.[264–269] Perhaps the most powerful study involved younger siblings where the older child had autism; in this study, receipt of MMR vaccine did not increase the risk of autism in the younger child.[270]

Because of the level of concern surrounding this issue, the CDC and the National Institutes of Health requested an independent review by the IOM.[271] The Immunization Safety Review Committee appointed by the IOM to review this issue was unable to find evidence supporting a causal relationship at the population level between autistic spectrum disorders and MMR vaccination, nor did the committee find any evidence of biological mechanisms that would support or explain such a link. In February 2010, the *Lancet* retracted the 1998 article.[272]

The mercury-containing preservative, thimerosal, has also been suggested to possibly increase the risk of autism. Mercury is a naturally occurring element found in the earth's crust, air, soil, and water. Certain types of bacteria in the environment

can change inorganic mercury to organic mercury (methylmercury). Methylmercury makes its way through the food chain in fish, animals, and humans. At high levels, it can be neurotoxic. Thimerosal, however, contains ethylmercury, not methylmercury. Studies comparing ethylmercury and methylmercury suggest that they are processed differently; ethylmercury is broken down and excreted much more rapidly than methylmercury. Therefore, ethylmercury is much less likely than methylmercury to accumulate in the body and cause harm.

The FDA Modernization Act of 1997 called for the FDA to review and assess the risk of all mercury-containing food and drugs. This led to an examination of mercury content in vaccines. Public health officials found that infants up to 6 months old could receive as much as 187.5 μg of ethylmercury (thimerosal) from vaccines, a level that exceeded recommended safety guidelines for methylmercury from the Environmental Protection Agency, but not levels recommended by the FDA or the Agency for Toxic Substance Disease Registry.[273] Consequently, the United States began transitioning to a childhood vaccine schedule free of thimerosal as a precautionary measure.[274] Currently, only the multidose influenza vaccine contains preservative quantities (i.e., 25 μg per dose) of thimerosal.

Several pieces of biological[275] and epidemiologic evidence indicate that thimerosal does not cause autism. Importantly, several large epidemiologic studies have compared the risk of autism in children who received vaccines containing thimerosal with children who received vaccines without thimerosal or vaccines with lesser quantities of thimerosal; none of the studies found an increased risk of autism associated with thimerosal-containing vaccines.[276–281] The IOM has reviewed these studies and concluded that evidence favored rejection of a causal association between thimerosal in vaccines and autism.[111,282] Denmark, a country that abandoned thimerosal as a preservative in 1991, actually saw an increase in the disorder beginning several years later. In the United States, all childhood vaccines with thimerosal as a preservative (except multidose influenza vaccine) passed their expiration date by 2003 and were no longer available in the United States; nonetheless, autism prevalence increased in the subsequent years.[283]

The hypothesis for why vaccines might cause autism has continued to shift. In 1998, the concern was that the MMR vaccine caused autism. The following year, the concern shifted to include the fear that thimerosal in vaccines caused autism. As data continued to be generated showing that both of these concerns were ill founded, the hypothesis shifted again—this time to include the fear that too many vaccines given too soon caused autism. To address this concern, a CDC study published in 2013 compared the number of antigens from vaccines during the first 2 years of life in a group of children with autism and matched controls without autism. The results showed that the total amount of antigen from vaccines received was the same between children with autism and those who did not have autism.[284]

Related to concerns about autism, the possibility that vaccines could cause other developmental disabilities, such as speech problems or learning difficulties, has also been evaluated. Several studies have evaluated a possible association between exposure to thimerosal in vaccines and neurodevelopmental problems and have found no consistent increased risk.[285–287] The total number of vaccines and vaccine antigens has also not been found to have a relationship with neurodevelopmental problems. Smith and Woods compared children who had received vaccines according to the CDC/American Academy of Pediatrics schedule with children who were not vaccinated, only partially vaccinated, or received vaccines on a delayed schedule, noting no difference between the two groups in neurodevelopmental outcomes.[288] A separate analysis found no association between the number of antigens in vaccines and neurodevelopmental outcomes.[289]

Aluminum in Vaccines Is Harmful

Aluminum salts have safely been used to adjuvant vaccines since the 1930s. However, by the mid-2000s, parents became concerned that aluminum in vaccines might be harmful. Indeed, high levels of aluminum can cause osteomalacia, anemia, or encephalopathy, typically in preterm infants or infants with absent or severely compromised renal function who are also receiving high doses of aluminum from other sources (e.g., antacids).[290] Studies show that children who receive aluminum-containing vaccines have serum levels of aluminum that are well below the toxic range.[291–293]

A specific concern that has been raised about aluminum adjuvants is their possible relationship to macrophagic myofasciitis, a condition consisting of a variety of complaints along with findings in muscle biopsies of minute lesions that contain aluminum salts.[294,295] The WHO Global Advisory Committee on Vaccine Safety has reviewed the evidence regarding macrophagic myofasciitis on several occasions and concluded that aluminum salts can be found in cells at an injection site of an aluminum-containing vaccine, but associated systemic symptoms related to that finding have never been scientifically proven.[296]

Formaldehyde in Vaccines Is Harmful

Formaldehyde has been used in vaccines to detoxify bacterial toxins (i.e., diphtheria toxin, tetanus toxin, pertussis toxins) and to inactivate viruses (i.e., poliovirus). Because formaldehyde at high concentrations can cause mutational changes in cellular DNA in vitro,[297] some parents have become concerned that formaldehyde in vaccines might be dangerous. However, because formaldehyde is a product of single-carbon metabolism, everyone has formaldehyde that is detectable in serum.[298] Indeed, the level of formaldehyde in the circulation is approximately 10-fold more than would be contained in any vaccine.[299] Also, people exposed to high levels of formaldehyde in the workplace (e.g., morticians) are not at greater risk of cancer than people who are not exposed to formaldehyde.[300] Finally, the quantity of formaldehyde present in vaccines is at least 600-fold lower than that necessary to induce toxicity in experimental animals.[301]

Vaccines Contain DNA From Aborted Human Fetuses

Two cell lines, MRC-5 and WI-38, both derived from elective abortions performed in Europe in the early 1960s, have been used as cell substrates in vaccine manufacture. Four vaccines continue to require the use of these cell lines: varicella, rubella, hepatitis A, and one of the rabies vaccines. Human fetal cells were valuable in vaccine research because they support the growth of many human viruses and are sterile; they were first used at around the time that researchers found that primary monkey kidney cells were contaminated with SV40 virus.

Some religious groups have become concerned about the use of cells originally obtained from elective abortions. However, the Catholic Church and other religions have deemed vaccines made using these cells worthy of continued use, despite their origins.[302,303]

SAFETY OF HUMAN PAPILLOMAVIRUS VACCINE

Concerns about safety have been a primary reason for not initiating HPV vaccination.[304] The WHO has noted that a

number of national immunization programs have experienced loss of confidence in their HPV vaccination programs as a result of negative publicity from perceived safety issues.[305] The postulated safety problems have included cerebral vasculitis, complex regional pain syndrome (CRPS), postural orthostatic tachycardia syndrome, and autoimmune diseases.[305–307] The suggestion that HPV vaccine could cause fatal cerebral vasculitis was raised in case reports that hypothesized that inflammation could be related to HPV L1 gene DNA fragments in the vaccine.[308,309] The FDA, however, determined that small amounts of HPV DNA fragments are to be expected as a result of the vaccine manufacturing process and do not represent a safety concern.[310] Moreover, the autopsy findings in the reported cases did not reveal evidence of inflammation and thus do not support a diagnosis of cerebral vasculitis. Concerns about CRPS have been limited to a few case reports in Japan that received considerable media attention.[311] Similar cases have not been reported from other countries. An expert review committee in Japan determined that information was not adequate to make a definitive diagnosis of CRPS in many of the reported cases and a causal relationship to vaccination could not be established.[312] A review by the European Medicines Agency concluded that the evidence does not support a causal association between HPV vaccines and CRPS or postural orthostatic tachycardia syndrome.[313]

Safety monitoring in the United States and internationally support the safety of HPV vaccine. In the United States, a review of VAERS found that reports of syncope and venous thromboembolism (VTE) were higher following HPV4 when compared to other vaccines. Continued monitoring in VAERS has not identified any new or unexpected safety concerns.[314] A population-based study of HPV4 safety from the VSD found no statistically significant increased risks of GBS, stroke, VTE, appendicitis, seizures, syncope, allergic reactions, or anaphylaxis.[315] A manufacturer-sponsored postlicensure safety assessment of HPV4 did not identify any major safety concerns except for an elevated relative risk of Hashimoto thyroiditis; however, further investigation of the temporal relationship and biological plausibility by the safety review committee revealed no consistent evidence for a safety signal for autoimmune thyroid conditions.[316] A national registry linkage study from Denmark found that HPV4 did not increase the risk of VTE.[317] A large population-based cohort study conducted in Denmark and Sweden analyzed more than 696,000 doses of HPV4 among females[318] and found no consistent evidence supporting causal associations with several autoimmune and neurologic conditions or VTE. A case-control study in France found no increased risk of several autoimmune outcomes (idiopathic thrombocytopenic purpura, central demyelination, GBS, connective tissue disorders, type 1 diabetes mellitus, and autoimmune thyroiditis) following HPV vaccination.[319] An analysis of national data from Sweden and Denmark covering 4 million women, including nearly 800,000 who had received HPV4, found no increased risk of multiple sclerosis or other demyelinating diseases following HPV4 vaccination.[124] Arguably, the HPV vaccine has been subjected to greater scrutiny postlicensure than most other vaccines.

NARCOLEPSY ASSOCIATED WITH AS03-ADJUVANTED PANDEMIC H1N1 INFLUENZA VACCINE

During the 2009 H1N1 influenza pandemic, GlaxoSmithKline's AS03-adjuvanted 2009 monovalent H1N1 influenza vaccine Pandemrix was used extensively in Europe and in other countries worldwide, although not in the United States. In August 2010, public health agencies in Sweden and Finland announced that they had received reports of narcolepsy after vaccination with Pandemrix.[320,321] Narcolepsy is a chronic central nervous system disorder characterized by excessive daytime sleepiness.[322] Its incidence is highest during the teen years,[323] but many individuals with narcolepsy are not diagnosed until several years after the onset of symptoms.[324] An increased risk of narcolepsy in Pandemrix-vaccinated compared with unvaccinated children and adolescents was found in Finland,[325] Sweden,[326] Ireland,[327] and Norway,[328] as well as in England [329] and France.[330] A case-control study that pooled data from eight European countries found an overall increased risk, although not in all countries.[331] Some European countries also reported an increased risk after vaccination in adults up to age 40 years, although the risk was smaller than the risk among vaccinees younger than 20 years of age.[326,330] A case-control study from Quebec of the Canadian AS03-adjuvanted vaccine, Arepanrix, found a small increased risk of narcolepsy, mainly in individuals younger than 20 years old, but a possible confounding effect of influenza infection could not be ruled out.[332] No increased risk of narcolepsy was found in a VSD study of the nonadjuvanted 2009 H1N1 pandemic influenza vaccines that were used in the United States.[333] Although no signals have been identified in SRSs,[334] risk of narcolepsy associated with MF59 adjuvanted vaccines has not been evaluated in formal epidemiologic studies. Questions remain about possible biological mechanisms and whether the association between Pandemrix and narcolepsy is confounded by other potential exposures or systematic biases, including publicity around the identified association.[331,335] Newer studies suggest that the issue may be more related to the influenza vaccine and not the adjuvant.[336] This is more fully discussed in Chapter 68.

Vaccine Risk Communication

Disease prevention with vaccination, especially if it requires continuous near-universal compliance, is a formidable task. In the preimmunization era, vaccine-preventable diseases such as measles and pertussis were so prevalent that the risks and benefits of disease versus vaccination were readily evident. As immunization programs successfully reduced the incidence of vaccine-preventable diseases, however, an increasing proportion of healthcare providers and parents have little or no personal experience with vaccine-preventable diseases. In contrast, some degree of personal discomfort, pain, and worry is generally associated with each immunization. In addition, parents searching for information about vaccines on the Internet are likely to encounter sites that encourage vaccine refusal or emphasize the dangers of vaccines alongside information from reputable sources.[337,338] Similarly, the media may sensationalize vaccine safety issues or, in an effort to present "both sides" of an argument, fail to provide perspective.[339,340] For reasons discussed earlier, there may be uncertainty if vaccines are associated with rare or delayed adverse reactions if only because the scientific method does not allow for acceptance of the null hypothesis; consequently, one cannot prove that a vaccine never causes a particular adverse event, only that an adverse event is unlikely to occur by a certain statistical probability.

The combination of these factors may affect parental beliefs about immunizations. Although the majority of parents support immunizations, surveys have found that many parents have concerns or misconceptions[180–182] that could erode their confidence in vaccines.[178] Within this context, the art of addressing vaccine safety concerns through effective risk communication has emerged as an increasingly important skill for managers of mature immunization programs and healthcare providers who administer vaccines.

Risk Perception

The science of risk perceptions and risk communications, which was developed initially for technology and environmental arenas,[341] can also be applied to immunizations.[342] For scientists and other experts, risk tends to be synonymous with the objective probability of morbidity and mortality resulting from exposure to a particular hazard.[343] In contrast, research shows that laypersons may have subjective, multidimensional, and value-laden conceptualizations of risk.[344] Individual people can vary in their perception of the magnitude of risks; studies show that various factors such as sex, race, political worldviews, emotional affect, and trust are associated with risk perception.[345] Risk perception factors such as involuntariness, uncertainty, lack of control, and high level of dread can lead to a heightened perception of risks.[346] All these can be seen as associated with childhood immunizations. Moreover, these factors are referred to as "outrage" factors in the risk communication literature. Outrage can lead to a person responding emotionally and can increase further the level of perceived risk.[346]

People care not only about the magnitude of risks, but also how risks are managed and whether they participate in the risk-management process.[347] In medical decision making, this has resulted in a transition from more paternalistic models to increasing degrees of shared decision making.[348] Some have argued that a similar transition also should occur with immunizations.[349] One study,[350] however, found that physicians who introduced vaccines using a participatory approach were more likely to encounter resistance compared to those who introduced vaccines as a presumptive or default choice. Furthermore, immunization is unlike most other medical procedures in that the consequences of the decision affect not only the individual person, but also others in society. Because of this important distinction, many countries have enacted immunization laws that, in part, are meant to limit an individual person's right to infect others. Without such mandates, persons may attempt to avoid the risks of vaccination while being protected by the herd immunity resulting from others being vaccinated.[351] Unfortunately, the protection provided by herd immunity may disappear if too many people avoid vaccination, resulting in outbreaks of vaccine-preventable diseases.[352,353] Debates in the United States, where such laws are created and enforced at the state level, have focused on whether philosophical (in addition to medical and religious) exemptions to mandatory immunizations for school entry should be more or less restrictive, and what standards for claim of exemption are needed.[349,354,355] For example, in 2015, California removed all nonmedical exemptions to school-entry vaccination requirements beginning in the 2016–17 school year.[356] Thus, vaccine risk communications should not only describe the risks and benefits of vaccines for individuals, but also should include discussion of the impact of individual immunization decisions on the larger community.

Factors That Can Influence Parental Vaccine Acceptance

Vaccination coverage among infants and young children in the United States has been consistently high, suggesting that vaccination as recommended by medical and public health professionals is still the norm.[357] However, high national coverage can mask variation at the local level.[358] Uneven distribution of undervaccinated individuals can mean a greater likelihood of disease outbreaks if exposed. One consequence of the success of vaccines is that an increasing number of parents and clinicians have little or no personal experience with or knowledge of many of the diseases that vaccines prevent. Thus, vaccine-preventable diseases often are not perceived as a real threat by parents.[359,360] Moreover, increasingly parents want to be fully informed about their children's medical care[361]; consequently, merely recommending vaccination may not be sufficient. Stories in the media highlighting adverse events (real or perceived) may also cause some parents to question the safety of vaccines.

Apart from the media attention on vaccine safety issues, several factors can shape parents' vaccine attitudes in the present environment of a low incidence of vaccine-preventable diseases. Some of these factors are: (a) depth of information needed, (b) timing of information given, (c) information sources and social media, (d) personal experience, and (e) general health literacy. An understanding of these factors and a proactive approach to vaccine education may prevent future concerns from escalating into widespread refusal of vaccines, with a consequent increased incidence of vaccine-preventable diseases. Each of these factors is discussed in more detail below.

Depth of Information

There is an association between information and vaccine acceptance. One study found that while 67% of parents agreed that they had access to enough information to make a good decision about immunizing their children, 33% of parents disagreed or were neutral.[362] Parents who disagreed they had enough vaccine information had negative attitudes about immunizations, healthcare providers, immunization requirements and exemptions, and trust in people responsible for immunization policy. Moreover, a larger percentage of parents who reported they did not have access to enough information about vaccines also had several specific vaccine concerns compared with parents who were neutral or agreed they had access to enough information.[362] It may be that when there is a void of accurate, trusted information, doubts about vaccines arise and misinformation is more readily accepted.

By using the principle of audience segmentation (partitioning a population into segments with shared characteristics), a survey study identified five parent groups that varied on health and immunization attitudes, behaviors, information sources, and demographics: Immunization Advocates (33.0%), Go Along to Get Alongs (26.4%), Health Advocates (24.8%), Fencesitters (13.2%), and Worrieds (2.6%).[363] It is important to recognize that parents vary in the amount and depth of information that they need to make decisions about vaccines, and that health communicators should take this into account when developing educational materials.

Timing of Information

Vaccine Information Statements (VISs) are typically given to parents the day their child is scheduled for immunization.[364–366] This often places the parent in a conflict situation of reviewing the VIS or attending to a frightened or upset child. Not surprisingly, studies have shown that parents would rather receive such information in advance of the first vaccination visit.[364,366–368] Suggested earlier times for vaccine education include prenatal clinic visits and just after delivery in a hospital.[369] A national survey indicated that 80% of providers said that a preimmunization booklet for parents would be useful for communicating risks and benefits to parents.[365]

Information Sources and Social Media

The majority of parents (84%) report receiving immunization information from a physician.[178] Furthermore, most parents,

including those who have concerns about vaccines, consider their child's doctor to be an important influence in their vaccination decision and report a high level of trust in their vaccine advice.[370,371] Thus, having a physician who engenders trust providing immunization information and who is available to listen and answer questions is the optimal situation from the public health perspective. If trust in a child's physician is low, parents may be drawn to other, less-credible sources of information.

Even though parents may have a number of sources of vaccine information other than a physician (e.g., family, friends), it is also important to consider the rise in social media use when discussing sources of information and influence regarding vaccine decisions. Online social media platforms, such as Facebook and Twitter, promote the two-way exchange of information and ideas, as opposed to the one-way information presentation of traditional Internet sites.[372] Although it remains to be seen how much of an influence social media interactions will have on parents' vaccine beliefs and behaviors, research to date points to both the potential importance of new technologies for engaging parents with accurate and timely information, as well as the potential for dissemination of myths and misinformation among parents' online social networks.[373]

Personal Experience

When a child experiences an adverse event following receipt of a vaccine, it often raises the question, "Was this vaccine necessary?" To the parent, it may seem that the risks of the vaccine are greater than the risks of not getting the vaccine. Parents who sought medical attention for any of their children owing to an apparent adverse event following immunizations (6.9%) not only expressed more concern about immunizations, but also were more likely to have a child who lacked one or more doses of three high-profile vaccines compared with parents who reported that none of their children had experienced an adverse event following immunization.[374] Two scenarios were seen as plausible. It may be that parents who were already concerned about vaccines before their child began the vaccination schedule sought medical attention for minor side effects (e.g., fever) or nonrelated problems. It is also possible that an apparent adverse event following immunization that resulted in parents seeking medical attention for their child caused the parents' perception of vaccines to become more negative. Both possibilities may result in parents declining future vaccines for their children. It is also important to note, however, that a physician's personal experience, with both vaccinations and vaccine-preventable diseases, can also be influential in helping reluctant parents understand the importance of on-time vaccination.[375] Education and descriptions of vaccine-preventable diseases may also help parents balance the perceived risks of vaccines with the risks of vaccine-preventable diseases.[376]

Health Literacy

There is a wide gap in the level of health literacy across the U.S. population, and this gap emphasizes the need for tailored information. The need for tailored information applies to all areas of health, including childhood immunizations. Immunization educational materials aimed at a "one-size-fits-all" audience are not likely to satisfy all parents' needs.[377] Tailoring should also take into account the need to avoid technical jargon and instead use a plain language approach to communication, regardless of the literacy level of the intended audience. Resources such as CDC's Clear Communication Index[378] can help assess the clarity of educational materials.

Evaluating and Addressing Vaccine Safety Concerns: Role of Healthcare Practitioners and Public Health Professionals

Empathy, patience, scientific curiosity, and substantial resources are needed to address concerns about vaccine safety. Although each evaluation of a vaccine safety concern is in some ways unique, some general principles may apply to most cases. As with all investigations, the first step is objective and comprehensive data gathering.[43] It is also important to gather and weigh evidence for causes other than vaccination. For individual cases or clusters of cases, a field investigation to gather data firsthand may be necessary.[108,379] Advice and review from a panel of independent experts also may be needed.[90,380,381] Causality assessment at the individual level is difficult at best; further evaluation via epidemiologic or laboratory studies may be required.[382] Even if the investigation is inconclusive, such studies can often help maintain public trust in immunization programs.[383]

In the United States, written information about the risks and benefits of immunizations developed by the CDC has been required to be provided to all people vaccinated in the public sector since 1978.[384] The National Childhood Vaccine Injury Act requires every healthcare provider—public or private—who administers a vaccine that is covered by the Act to provide a copy of the most current VIS to the adult vaccinee or, in the case of a minor, to the parent or legal representative, each time a dose of vaccine is administered.[385] Healthcare providers must note in each patient's permanent medical record the date printed on the VIS and the date the VIS was given to the vaccine recipient or the recipient's legal representative. VISs are the cornerstone of provider–patient vaccine risk–benefit communication. Each VIS contains information on the disease(s) that the vaccine prevents, who should receive the vaccine and when, contraindications, vaccine risks, what to do if a side effect occurs, and where to go for more information. Current VISs can be obtained from the CDC's National Center for Immunization and Respiratory Diseases[386] and are available in a number of languages other than English from the Immunization Action Coalition (www.immunize.org). An increasing number of resources that address vaccine safety misconceptions and allegations also have become available, including websites, brochures, resource kits, and videos (Table 82.5). Some studies have been conducted to assess the use and effectiveness of such materials[364,365,387–389]; however, more research in this area is needed.

Immunization programs and healthcare providers should anticipate that some members of the public may have deep concerns about the need for and safety of vaccines. Some may refuse certain vaccines and a few may reject all vaccinations. In the United States, less than 1% of young children have received no vaccines; a number that has been consistent for the past several years according to the National Immunization Survey.[357] However, surveys have estimated that anywhere from 13% to 39%[181,390,391] of parents have intentionally delayed or refused one or more recommended vaccines, depending on the population studied and the definition of delay and refusal used. Acceptance of this practice among physicians is associated with younger physician age, highlighting the importance of physicians' understanding of the risks of vaccine delay and refusal among their patients.[392] An understanding of vaccine risk perceptions and effective vaccine risk communication are essential in responding to misinformation and concerns. Healthcare professionals are also in the best position to help all parents provide evidence-based comfort measures to children to reduce acute pain and anxiety associated with vaccines.[393] It is also important to convey to those who refuse recommended vaccines that their decision is not without risk,

TABLE 82.5 Websites Containing Reliable, Up-to-Date, and Accurate Information About Vaccines

Source	Website
GOVERNMENT	
Centers for Disease Control and Prevention (main)	www.cdc.gov/vaccines
CDC (flu)	www.cdc.gov/flu
CDC (HPV)	cdc.gov/hpv
CDC (childhood vaccines)	www.cdc.gov/vaccines/conversations
Advisory Committee on Immunization Practices	http://www.cdc.gov/vaccines/acip
PROFESSIONAL ASSOCIATIONS	
American Academy of Pediatrics (AAP)	www.aap.org/immunization
American College of Obstetricians and Gynecologists (ACOG)	www.immunizationforwomen.org
SCHOOLS, HOSPITALS, AND EXPERT GROUPS	
The Albert B. Sabin Vaccine Organization	www.sabin.org
Every Child by Two	www.ecbt.org
Immunization Action Coalition	www.immunize.org
Institute for Vaccine Safety	www.vaccinesafety.edu
Parents PACK (provided by the Vaccine Education Center at The Children's Hospital of Philadelphia)	www.vaccine.chop.edu/parents
Vaccine Education Center at The Children's Hospital of Philadelphia	http://www.chop.edu/centers-programs/vaccine-education-center
Immunization Action Coalition	www.vaccineinformation.org
The Vaccine Page	www.vaccines.org
Vaccinate Your Baby	www.vaccinateyourbaby.org
California Immunization Coalition	www.whyichoose.org
National Foundation of Infectious Diseases	www.adultvaccination.com
PATH Vaccine Resource Library	http://www.path.org/vaccineresources/
PARENT AND FAMILY ORGANIZATIONS	
Families Fighting Flu	www.familiesfightingflu.org
The National Meningitis Association	www.nmaus.org
Meningitis Angels	www.meningitis-angels.org
Parents of Kids with Infectious Diseases	www.pkids.org
Voices for Vaccines	www.voicesforvaccines.org

and that they (or their children) are vulnerable to a vaccine-preventable disease and its possible consequences.

Importance of Educating Parents Concerned About Vaccines

It is important not to assume that just because most parents are having their children immunized that they will continue to do so.[394] While the host of factors contributing to parents' decisions to have their children immunized (e.g., need for information, experience with adverse events) might remain stable for some time, it is possible that one or more factors may change so that some parents perceive the risks of vaccines to be greater than the risk of disease. This would then push the parents above a theoretical "unacceptability threshold" in which they would choose not to have their children immunized with one or more vaccines. One study[181] found that many parents whose children were fully vaccinated were "at risk" for vaccine delay or refusal based on their concerns about the safety or necessity of vaccines. Furthermore, vaccination is not a one-time, all-or-nothing decision, but rather an ongoing series of choices over time.

Averting the future possibility of outbreaks of vaccine-preventable diseases will take a concerted effort by healthcare and public health professionals to educate and better communicate with parents concerned about immunizations. In guidance for clinicians, the American Academy of Pediatrics does not recommend physicians remove nonvaccinating families from their practice, but it does give them permission to do so.[394a] Instead, American Academy of Pediatrics suggests that pediatricians should listen carefully and respectfully to parents' immunization concerns, factually communicate the risks and benefits of vaccines, and work with parents who may be concerned about a specific vaccine or having their child receive multiple vaccines in one visit.[395] Providers can make a huge impact on vaccine acceptance, resulting in a cascading effect in which providing information can increase trust and increasing trust can lead to greater acceptance of and confidence in vaccines. For healthcare providers to be able to optimally fill this important role, however, two related issues need to be addressed. The first is the need for quality communication courses and training in medical schools and residencies and training programs for medical and public health professionals regarding vaccine safety.[396–398] The second is for MCOs and medical insurance companies to adequately reimburse physicians for health education. Lack of reimbursement to physicians has been noted as a barrier to implementation of behavioral treatments for health issues such as heart disease[399] and smoking.[400] It is important to note that studies show that education programs can be a cost savings to healthcare systems.[401,402] We live in a world already benefiting from vaccines that exist, and there is the promise of more vaccines to come. The challenge we have now is to make sure that the promise is not lost because we did not present the benefits and risks of vaccines in a meaningful way acceptable to the public.

FUTURE CONSIDERATIONS

An optimal immunization safety system requires rigorous attention to safety during prelicensure research and development; active monitoring for potential safety problems after licensure; and clinical research and risk-management activities, including risk communication, focused on minimizing potential vaccine adverse reactions. Prelicensure activities form the foundation of vaccine safety. Rapid advances in biotechnology are leading to the development of new vaccines,[403] and novel delivery technologies, such as DNA vaccines and new adjuvants, are being developed to permit more antigens to be combined, reducing the number of injections.[404,405]

In the prelicensure evaluation of new vaccines, the trend is likely to continue to conduct larger Phase III trials enrolling tens of thousands of participants. Although such larger trials are helpful in identifying more rare adverse events, even these larger trials may not be large enough to detect increased risks of rare events. For example, the RotaTeq and Rotarix preclinical trials identified no increased risk of intussusception in studies that enrolled more than 60,000 infants.[41,42] Subsequent much larger postlicensure safety monitoring studies, however, identified statistically significant increased risks on the order of approximately one to five per 100,000.[18,19,406,407]

The attributable risks were less than that found for RotaShield (approximately one per 10,000), and, because the benefits of rotavirus vaccines clearly outweighed the small risk of intussusception, no changes were made to the vaccine recommendations.

Although technological advances and more thorough evaluation of safety before vaccines are licensed should lead to the development of safer vaccines, there will continue to be a need for comprehensive postlicensure safety monitoring systems. Combined with the difficulties associated with identifying rare, delayed, or insidious vaccine safety problems in prelicensure studies,[35] the well-organized consumer activist organizations,[408] Internet information of questionable accuracy,[337,338] media eagerness for controversy,[339,409] and relatively rare individual encounters with vaccine-preventable diseases virtually ensure that vaccine safety concerns are unlikely to go away. The existence of a robust vaccine safety monitoring system is essential for providing assurance of the safety of currently marketed vaccines and for rapidly identifying and responding to potential safety problems. Currently, SRSs, such as VAERS, serve as the frontline systems for the early identification of vaccine safety problems. Such systems could be improved if reporting were more complete. Application of web-based and text messaging technologies could make reporting easier and more accurate and also enable more active follow-up of vaccinated persons.[410] Alerts built into electronic medical record systems[411] could also improve reporting to VAERS, as could linkages with immunization registries. Some of these advances will be particularly important to enable monitoring vaccine safety in mass vaccination campaigns during which vaccinations may be administered primarily outside of the traditional healthcare system.

An optimal vaccine safety monitoring system must also include a mechanism or infrastructure to rapidly conduct formal epidemiologic evaluations of potential safety problems identified from SRSs or other sources. In the United States, this function is primarily served by the VSD project. The diffusion of electronic health records and the capability to link records across data systems (such as large health insurance claims databases and immunization registries) may allow the expansion of the population that could be included in postlicensure epidemiologic evaluations of vaccine safety. For example, the FDA Sentinel Initiative has a goal to develop a national electronic system covering 100 million people for monitoring the postlicensure safety of drugs and other medical products, including vaccines.[412]

For adverse reactions that are established as caused by vaccines, clinical and laboratory research is essential for determining the biological mechanisms of the adverse reaction, which, in turn, could lead to the development of safer vaccines. Clinical research is also essential for the development of protocols for safer vaccination, including revaccination of persons who have previously experienced an adverse reaction. Advances in genomics and immunology hold particular promise for elucidating biological mechanisms of vaccine adverse reactions and the development of possible screening strategies for persons who may be at high risk for an adverse reaction.

The need for comprehensive postlicensure monitoring of vaccine safety can be expected to grow with the development of new vaccines and as vaccines are increasingly used in populations that may have different susceptibilities to vaccine adverse events, such as immunocompromised individuals and pregnant women. Vaccination in pregnancy is unique in that it can affect the pregnant woman, the developing fetus, and the newborn infant. Pregnant women, however, are usually excluded from prelicensure clinical trials, making postlicensure monitoring key for evaluating safety of maternal immunizations. Currently in the United States, only two vaccines are routinely recommended for all pregnant women, inactivated influenza and combined tetanus, diphtheria and pertussis (Tdap) vaccines,[413,414] although other vaccines recommended for women of reproductive age are sometimes administered during pregnancy when the need arises. Evidence indicates that influenza and Tdap vaccines are safe when administered during pregnancy.[415–418] Safety data, however, are more limited on vaccination in the first trimester of pregnancy and for repeat Tdap vaccination at short intervals. Safety monitoring will also be important when anticipated new vaccines, such as group B streptococcus, respiratory syncytial virus vaccines,[419,420] and possibly meningococcal vaccines are introduced for use in pregnancy.

Scientific data are essential in the monitoring and evaluation of vaccine safety, but scientific evidence alone often is not sufficient to provide reassurance about the safety of a vaccine. Although immunization levels of U.S. children are high, a sizable fraction of parents do not have their children fully immunized, and concern about vaccine safety is the leading reason for underimmunization. These concerns persist despite the scientific evidence that vaccines do not cause autism or a host of other conditions that have been alleged to be caused by vaccines, such as asthma, diabetes, and autoimmune diseases. Thus, it is critically important that public health agencies, medical organizations, and other influential authorities continue to focus on the safety of vaccines and assure public confidence by providing clear, consistent messages on vaccine safety concerns; supporting effective and transparent vaccine safety monitoring systems and research activities; providing review and recommendations by respected independent expert groups on vaccine safety controversies; and engaging advocacy groups in constructive and open dialogue about their vaccine safety concerns. Although the efforts of government, medical, and other authorities are important, it is healthcare providers who have the greatest influence in determining the acceptance of vaccines by individual people. Even among parents who believe that vaccines may not be safe, most will have their children vaccinated if they have a trusting relationship with an influential healthcare provider. Thus, development of tools and strategies that can assist healthcare providers in effectively communicating with their patients on the risks and benefits of vaccines will continue to be important.

In low- and middle-income countries (LMICs) there is a need to establish minimal vaccine safety monitoring capabilities, such as SRSs, and the capability to rapidly investigate vaccine safety problems and effectively communicate the findings of the investigations. Concerns about vaccine safety can adversely impact immunization efforts, as happened in Vietnam and other countries when newly introduced pentavalent vaccines were falsely believed to have caused infant deaths.[157] An increasing number of vaccines are being introduced and used in LMICs, some are primarily for use in these settings.[421–423] A majority of LMICs, however, have nonexistent or limited vaccine safety monitoring capabilities.[424] In 2012, WHO led the development of the Global Vaccine Safety Blueprint[424] to set forth guidance on establishing and strengthening vaccine safety monitoring systems in LMIC settings. The possibilities for conducting enhanced safety monitoring, particularly with the large-scale introduction of new vaccines, was demonstrated with meningococcal A conjugate vaccine in sub-Saharan Africa.[425] Continued development of LMIC vaccine safety monitoring infrastructures will be needed with the introduction of new vaccine products, such as an adjuvanted malaria vaccine,[426] and viral vector dengue[427] and Ebola virus vaccines.[428] Mass immunization campaigns, during which millions of people receive parenteral immunizations over a period of days,[429] pose substantial challenges to ensuring injection safety,[430] especially given concerns about inadequate

sterilization of reusable syringes and needles, recycling of disposable syringes and needles, and cross-contamination resulting from the current generation of jet injectors.[431] The WHO has promoted the use of safer autodisposable syringes and disposal boxes.[432]

Vaccines are among the most successful and cost-effective public health tools for preventing disease and death. However, like all medical interventions, vaccines are not completely without risk of side effects or other adverse outcomes. A timely, credible, and effective monitoring system, coupled with prompt action in response to identified safety problems, is essential to preventing adverse effects of vaccination and to maintaining public confidence in immunizations. Because immunizations are typically administered to healthy people and are often recommended or mandated to provide societal and individual protection, vaccines must be held to a very high standard of safety. Vaccine safety monitoring and research should optimally be able to detect potentially very small levels of increased risk, especially for adverse events that can result in death or permanent disability. The ultimate goal of such research, including the application of new developments in biotechnology, is to develop safer vaccines and vaccination practices.

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83 Legal Issues

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This chapter reviews liability for vaccine injuries historically; the rationale, development, and implementation of the National Vaccine Injury Compensation Program (VICP); and the program's current status. The first part of the chapter discusses the development of the law in the United States until 1986, the year of the passage of the National Childhood Vaccine Injury Act of 1986 (NCVIA). Later sections discuss the administration of the VICP, reported decisions relating to liability for the production and administration of vaccines after 1986, and current developments in the area of vaccine injury liability and compensation.

VACCINE LIABILITY BEFORE 1986

To understand the decisions that were rendered in cases filed against vaccine manufacturers and administrators before 1986, it is important to understand the nature of products liability law as it was evolving in that era. Before the early 1980s, manufacturers were not held liable for the harm associated with a product unless it failed to comply with the standards of manufacturing or care implemented by similarly situated manufacturers. Usually, vaccine manufacturers sued by consumers would prevail because they used customary practices and complied with statutes and regulations and because of the doctrine of the *learned intermediary,* discussed later.

This standard began to change when, in 1965, the American Law Institute introduced the concept of "strict liability" in its *Restatement (Second) of Torts*. Although the principles embodied in this *Restatement* were not applied uniformly to lawsuits against vaccine manufacturers and administrators, they served as guiding principles in the field and were viewed by many as significant emerging legal doctrines.

Under the *Restatement*'s doctrine of strict liability, a manufacturer who sold a product in a defective condition that made the product unreasonably dangerous was subject to liability for harm caused to the user or consumer even if the manufacturer exercised all possible care in the preparation and sale of the product.[1] A product was deemed defective if it was in a condition not contemplated by the ultimate consumer, which made it unreasonably dangerous to the consumer.[2] Although the doctrine of strict liability lowered the burden required to find manufacturers liable for the harm caused by their products, the authors of the *Restatement* recognized that some products were, by their nature, "unavoidably unsafe," and determined that such products should not be deemed unreasonably dangerous under the doctrine of strict liability.[3] Thus, products such as vaccines, which necessarily entail some risks (risks considered reasonable given their benefits to the community), were not deemed defective or unreasonably dangerous so long as they were properly prepared and accompanied by adequate warnings.[4] Consequently, vaccine manufacturers and administrators were generally not held liable for harm caused by their products before 1986, so long as these requirements of proper preparation and adequate warnings were satisfied.

The risk of liability created by the requirements to properly prepare vaccines and provide adequate warnings is important. If, for instance, a lot of vaccine was defective and caused disease in recipients because the disease-causing agent was not sufficiently inactivated or because of contaminants, the manufacturer could be liable, whether or not the defect could be shown to be the manufacturer's fault. This happened with early lots of Salk poliovirus vaccine, and there were numerous and substantial recoveries by persons who acquired polio from the vaccine.[5] As another example, if a physician administered a vaccine when it was contraindicated (e.g., administered Sabin oral poliovirus vaccine [OPV] to a child known to be immunodeficient or administered a second dose of diphtheria and tetanus toxoids and whole-cell pertussis [DTP] vaccine after a child had a severe adverse reaction to the first), the physician could be liable if the adverse consequence risked by violating the contraindication occurred. Furthermore, if a physician administered a vaccine without warning the patient of the risks, the physician could be liable if the risks occurred.

Despite the limits on liability imposed by general doctrines of products liability law in cases concerning adverse reactions from vaccines, certain judicial decisions in the pre-1986 era imposed liability even when the vaccines were properly made and administered. These cases, which were a significant impetus to the enactment of the NCVIA, can be divided into three categories: the *Reyes* decision, the swine flu litigation against the government, and the 1980s decisions.

Reyes v Wyeth Laboratories,[6] decided in 1974 by the United States Court of Appeals for the Fifth Circuit, held a manufacturer of OPV liable to a child who contracted paralytic poliomyelitis after being administered the vaccine. The decision broadened the potential liability faced by manufacturers in the context of mass vaccination efforts.

This case articulated an exception to the learned intermediary doctrine. Under the general rule, a manufacturer of prescription medicines, including vaccines, is obliged to provide warnings concerning the product to the healthcare provider (the learned intermediary), but has no duty to directly warn the user of the product of its associated risks. This doctrine is based on the assumption that the medical professional makes an individualized medical judgment with respect to the risks and benefits of a particular drug or vaccine and a particular patient. The Reyes decision narrowed this general rule, holding that, when a manufacturer can reasonably be said to be aware that the product (i.e., the vaccine) will be administered in such a way that no personalized medical advice will be provided (e.g., in the context of a public health department's immunization effort in which the patient had no direct contact with a physician), the manufacturer is responsible for providing warnings directly to patients or ensuring that such warnings will be given.

In Reyes, the manufacturer shipped the vaccines to the Texas Department of Public Health, accompanied by the Food and Drug Administration (FDA)-required package inserts containing warnings concerning the possible consequences of the vaccine. The Texas Department of Public Health sent the vaccine to the county health department without ensuring that the warnings would actually be given to vaccine recipients (or their parents). The nurse who administered the vaccine to the child in this case did not warn the parents of the minute risk that a recipient or contact could contract the disease. Although the Reyes Court determined that OPV was not unreasonably dangerous per se, it concluded that the vaccines were

unreasonably dangerous as marketed and that the manufacturer breached its duty because it was aware that the vaccine could result in a particular danger, knew or had reason to know that the vaccine would be administered in circumstances in which vaccine recipients might not be counseled by learned intermediaries (i.e., in mass vaccine clinics), and failed to provide warnings or ensure that such warnings were provided to vaccine recipients.

The next development in the area of vaccine liability involved the swine flu vaccine. In the spring of 1976, leading epidemiologists predicted that the United States could be afflicted the following winter with a strain of influenza that could lead to a pandemic similar to the catastrophic outbreak of 1918.[7] The lack of prior immunity raised fears that many people who contracted the disease would die or have serious illnesses. This prediction led the government to recommend a campaign to immunize almost all American adults.[8,9]

The Reyes decision caused manufacturers to refuse initially to provide the swine flu vaccine to the government. In addition, manufacturers, concerned about other unforeseen risks of liability in a program this large, did not want to accept any liability for the vaccines. As a result, the Swine Flu Act was passed.[10] This law required vaccine recipients who believed they had been injured to sue the federal government, which served as a substitute defendant for vaccine manufacturers. The swine flu program was a considerable success as a matter of effective public health mobilization, insofar as the vaccine was successfully produced, distributed, and administered to more than 45 million people in a few months. However, vaccination was suspended when the feared swine flu epidemic had not yet appeared and suspicions arose that there might be an association between the vaccine and Guillain-Barré syndrome (GBS).[11] Numerous claims concerning GBS were filed under the Swine Flu Act, many of which relied on a Centers for Disease Control and Prevention (CDC)-sponsored study that showed a statistically significant increase in the risk of GBS in the 10 weeks after immunization compared with the risk in unvaccinated people. The government accepted liability for all cases of GBS with onset falling within this period. The government did not agree to accept liability for cases of GBS falling outside this time interval or for other illnesses. The defense of this litigation resulted in more than 100 judicial opinions, which generally shielded manufacturers and administrators from liability by relying on the Swine Flu Act. However, some courts wrote opinions in the *Reyes v Wyeth* tradition that imposed increased liability for the federal government based on a theory of inadequate warning, for example, *Unthank v United States*[12] and *Petty v United States*,[13] in which the federal government was found derivatively liable for a manufacturer's failure to provide plaintiffs with adequate warnings covering the risks of the swine flu vaccine.[14]

The efforts of plaintiffs to establish common law, strict tort liability on the part of vaccine manufacturers (i.e., the manufacturer is liable even if adequate warnings are given and the vaccine is produced and handled in full compliance with FDA regulations) seemed to increase after *Reyes* and the swine flu episode. In the 1980s, plaintiffs achieved some notable successes. For example, in *Johnson v American Cyanamid Co.*,[15] the father of a vaccinated child who claimed to have contracted polio through contact with his child sued the manufacturer and administrator of the OPV. The jury found the vaccine manufacturer liable and imposed a $10 million damages award. The Kansas Supreme Court reversed the jury's verdict and award, determined that there was no manufacturing or design defect, and concluded that the manufacturer's warning was adequate. The court did not resolve whether the vaccine administrator might have been liable for failing to warn the parents of the vaccine's risk had this claim been pursued.

In *Toner v Lederle Laboratories*,[16] a jury returned a verdict of over $1.1 million for a recipient of a DTP vaccine who claimed that the vaccine caused transverse myelitis, a condition that had never been shown scientifically to be caused by any available DTP vaccine. The plaintiff's theory was that the vaccine caused transverse myelitis, that the defendant could have marketed a safer vaccine (a vaccine once marketed by another manufacturer that was withdrawn from the market in the 1970s), and that the manufacturer's failure to make a safer version of the vaccine was negligent. The verdict was appealed to the United States Court of Appeals for the Ninth Circuit, which referred the issues to the Idaho Supreme Court. The Idaho Supreme Court affirmed the jury's verdict that the manufacturer's design of DTP was negligent.

The results reached in these cases are notable and underscore the fear of liability by vaccine manufacturers and healthcare professionals.

NATIONAL CHILDHOOD VACCINE INJURY ACT

The *Reyes v Wyeth* decision and its progeny were followed by the withdrawal of a number of pharmaceutical houses from vaccine production and an increase in the price of vaccines.[14]

The litigious atmosphere of the 1980s and the sharp decrease in the number of vaccine manufacturers had an adverse impact on the research and development of new and safer vaccines. Before a vaccine is marketed and before a manufacturer has a possibility of reaping any profit, the FDA requires the manufacturer to prove that the vaccine is safe and effective. Meeting this burden is costly, and the investment cannot be recovered until the vaccine is marketed. The costs and logistics associated with administering a new vaccine enough times in prelicensure clinical trials to detect rare adverse side effects are high. A manufacturer's inability to accurately estimate its risk of liability and, therefore, the effect of this potential liability on its profit margin translated into limited commercial incentives to develop new vaccines.

During this period, the nation's children were at risk. The American Academy of Pediatrics and the American Medical Association were concerned about the continued availability of vaccines and the possibility that physicians were at risk for liability. At the time, it was common for plaintiffs to join the pharmaceutical company and the administering physician in a complaint, claiming that the physician did not adequately disclose the risk associated with the vaccine. At least one state (North Carolina) enacted legislation protecting participants in the delivery of vaccines from liability and authorizing compensation to injured vaccinees.[17] In the end, the NCVIA of 1986[18] was compromise legislation fought for by parents and physicians with some backing of industry and trial attorneys, but with little support from the Administration.

NATIONAL VACCINE INJURY COMPENSATION PROGRAM

Purpose and Goals

The VICP, established by the NCVIA, was authorized by Congress to address a variety of public policy needs.[19,20] As explained in the NCVIA's legislative history and repeated in many court decisions, two significant concerns led to the enactment of this legislation. First, concerns about the instability and unpredictability of the childhood vaccine market (based on several factors, including litigation and whether affordable product liability insurance was available) led Congress to determine that a new system was required to stabilize the childhood vaccine market, considered essential to the nation's public health. Second, Congress recognized that

persons injured by childhood vaccines should receive fair compensation and determined that the tort system used at the time to provide such compensation was inadequate.[21]

Congress addressed these issues by creating the VICP, a federal "no-fault" system under which awards can be made to vaccine-injured persons quickly, easily, and generously. Persons injured through the receipt of a vaccine after the effective date of the legislation generally are required to file claims with the VICP before they are allowed to bring a civil suit against vaccine manufacturers or administrators. Although the statute is entitled the "National Childhood Vaccine Injury Act," eligibility for compensation extends to children and adults. Rules of evidence, discovery, and other legal procedures are relaxed to accelerate the compensation process. Petitioners in VICP proceedings need not demonstrate negligence on the part of a vaccine manufacturer or administrator, thus the no-fault designation. Petitioners with eligible claims must exhaust their remedies within the VICP before filing lawsuits against vaccine manufacturers or administrators. To do so, a petitioner must withdraw his or her petition from the VICP (if the Court of Federal Claims fails to issue a decision or enter judgment within the time periods provided for in the NCVIA) or reject the judgment entered (whether or not compensation was awarded).

In addition to the establishment of the VICP and the imposition of liability protections, the NCVIA established sweeping vaccine safety provisions and created a more prominent vaccine safety role for the federal government. The NCVIA included a mandate for the reporting of certain adverse events. Healthcare providers and vaccine manufacturers are required to report the occurrence of any event set forth in the Vaccine Injury Table (VIT) (Table 83.1) and any contraindicating reaction to a vaccine that is specified in the manufacturer's package insert.[22] The report must state the symptoms and manifestations of the illness or injury, how long after administration of the vaccine such symptoms occurred, and the manufacturer and lot number of the vaccine administered. These reports are to be made to the Vaccine Adverse Event Reporting System. Other vaccine safety mandates include record keeping by vaccine administrators (documenting the date of vaccine administration, the manufacturer and lot number, and the name and address of the administrator) and development and dissemination of risk-to-benefit information materials (known as Vaccine Information Statements) by the Secretary of Health and Human Services (HHS). The NCVIA also required certain studies, conducted by the Institute of Medicine (IOM), of vaccine adverse events.

The NCVIA also established two advisory panels, the Advisory Commission on Childhood Vaccines (ACCV)[23] and the National Vaccine Advisory Committee (NVAC).[24] The NCVIA mandates that the ACCV include as members equal numbers of health professionals, members of the public (including legal guardians of children injured by vaccines), and attorneys (including a representative of persons injured by vaccines and a representative of vaccine manufacturers). The ACCV makes recommendations to the Secretary of HHS on changes to the VIT and other issues related to the administration of the VICP. The NVAC has much broader responsibilities, including the authority to recommend ways to achieve optimal prevention of disease through changes related to vaccine research, development, delivery, safety, and efficacy. The NVAC makes recommendations to the HHS Assistant Secretary for Health.

Although the NCVIA was landmark in design and scope, numerous amendments to the initial legislation were enacted. Funding of the VICP, not provided for in the original legislation, was authorized by Congress in early 1987.[25] Additional protections for manufacturers defending claims filed by persons after exhausting their remedies under the VICP also were written into the law at this time. These included the statutory bars to actions based on plaintiff allegations of vaccine misdesign or inadequate warning of risk, two common tort theories pursued in the 1980s, and the elimination of punitive damages unless gross negligence in vaccine production was proven.[26] At the same time, the provision requiring claimants to pursue their claim through the VICP before filing a tort claim against manufacturers was expanded to include healthcare providers, a protection that was not offered by the original act. Subsequent legislation in 1993 permanently reauthorized the VICP and provided a mechanism for adding new vaccines and for the Secretary of HHS to modify the VIT.[27] Vaccines recommended by the CDC (usually on recommendation by the Advisory Committee on Immunization Practices) for routine administration to children would be added to the VICP. As described below, a statutory amendment passed in 2016 expanded the VICP's coverage to include vaccines recommended by the CDC for routine administration to pregnant women. Congress also would have to enact an excise tax as a necessary step before coverage could begin.

Text continued on p. 1608

TABLE 83.1 Vaccine Injury Table[a]

In accordance with section 312(b) of the National Childhood Vaccine Injury Act of 1986, title III of Public Law 99-660, 100 Stat. 3779 (42 U.S.C. 300aa-1 note) and section 2114(c) of the Public Health Service Act, as amended (PHS Act) (42 U.S.C. 300aa-14(c)), the following is a table of vaccines, the injuries, disabilities, illnesses, conditions, and deaths resulting from the administration of such vaccines, and the time period in which the first symptom or manifestation of onset or of the significant aggravation of such injuries, disabilities, illnesses, conditions, and deaths is to occur after vaccine administration for purposes of receiving compensation under the Program. Paragraph (b) of this section sets forth additional provisions that are not separately listed in this Table but that constitute part of it. Paragraph (c) of this section sets forth the qualifications and aids to interpretation for the terms used in the Table. Conditions and injuries that do not meet the terms of the qualifications and aids to interpretation are not within the Table. Paragraph (d) of this section sets forth a glossary of terms used in paragraph (c).

Vaccine	Illness, Disability, Injury or Condition Covered	Time Period for First Symptom or Manifestation of Onset or of Significant Aggravation After Vaccine Administration
I. Vaccines containing tetanus toxoid (e.g., DTaP, DTP, DT, Td, or TT)	A. Anaphylaxis	≤4 hours
	B. Brachial neuritis	2-28 days (not less than 2 days and not more than 28 days)
	C. Shoulder injury related to vaccine administration[b]	≤48 hours
	D. Vasovagal syncope[b]	≤1 hour

Continued on following page

TABLE 83.1 Vaccine Injury Table[a] *(Continued)*

Vaccine	Illness, Disability, Injury or Condition Covered	Time Period for First Symptom or Manifestation of Onset or of Significant Aggravation After Vaccine Administration
II. Vaccines containing whole cell pertussis bacteria, extracted or partial cell pertussis bacteria, or specific pertussis antigen(s) (e.g., DTP, DTaP, P, DTP-Hib)	A. Anaphylaxis B. Encephalopathy or encephalitis C. Shoulder injury related to vaccine administration[b] D. Vasovagal syncope[b]	≤4 hours ≤72 hours ≤48 hours ≤1 hour
III. Vaccines containing measles, mumps, and rubella virus or any of its components (e.g., MMR, MM, MMRV)	A. Anaphylaxis B. Encephalopathy or encephalitis C. Shoulder injury related to vaccine administration[b] D. Vasovagal syncope[b]	≤4 hours 5-15 days (not less than 5 days and not more than 15 days) ≤48 hours ≤1 hour
IV. Vaccines containing rubella virus (e.g., MMR, MMRV)	A. Chronic arthritis	7-42 days (not less than 7 days and not more than 42 days)
V. Vaccines containing measles virus (e.g., MMR, MM, MMRV)	A. Thrombocytopenic purpura B. Vaccine-strain measles viral disease in an immunodeficient recipient Vaccine-strain virus identified If strain determination is not done or if laboratory testing is inconclusive[b]	7-30 days (not less than 7 days and not more than 30 days) Not applicable ≤12 months
VI. Vaccines containing polio live virus (OPV)	A. Paralytic polio In a non-immunodeficient recipient In an immunodeficient recipient In a vaccine associated community case B. Vaccine-strain polio viral infection In a non-immunodeficient recipient In an immunodeficient recipient In a vaccine associated community case	 ≤30 days ≤6 months Not applicable ≤30 days ≤6 months Not applicable
VII. Vaccines containing polio inactivated virus (e.g., IPV)	A. Anaphylaxis B. Shoulder injury related to vaccine administration[b] C. Vasovagal syncope[a]	≤4 hours ≤48 hours ≤1 hour
VIII. Hepatitis B vaccines	A. Anaphylaxis B. Shoulder injury related to vaccine administration[b] C. Vasovagal syncope[b]	≤4 hours ≤48 hours ≤1 hour
IX. Haemophilus influenzae type b (Hib) vaccines	A. Shoulder injury related to vaccine administration[b] B. Vasovagal syncope[b]	≤48 hours ≤1 hour
X. Varicella vaccines	A. Anaphylaxis[b] B. Disseminated varicella vaccine-strain viral disease Vaccine-strain virus identified If strain determination is not done or if laboratory testing is inconclusive[b] C. Varicella vaccine-strain viral reactivation[b] D. Shoulder injury related to vaccine administration[b] E. Vasovagal syncope[b]	≤4 hours Not applicable 7-42 days (not less than 7 days and not more than 42 days) Not applicable ≤48 hours ≤1 hour
XI. Rotavirus vaccines	A. Intussusception	1-21 days (not less than 1 day and not more than 21 days)
XII. Pneumococcal conjugate vaccines	A. Shoulder Injury Related to Vaccine Administration[b] B. Vasovagal syncope[b]	≤48 hours ≤1 hour
XIII. Hepatitis A vaccines	A. Shoulder injury related to vaccine administration[b] B. Vasovagal syncope[b]	≤48 hours ≤1 hour
XIV. Seasonal influenza vaccines	A. Anaphylaxis[b] B. Shoulder injury related to vaccine administration[b] C. Vasovagal syncope[b] D. Guillain-Barré syndrome[b]	≤4 hours ≤48 hours ≤1 hour 3-42 days (not less than 3 days and not more than 42 days)
XV. Meningococcal vaccines	A. Anaphylaxis[b] B. Shoulder injury related to vaccine administration[b] C. Vasovagal syncope[b]	≤4 hours ≤48 hours ≤1 hour
XVI. Human papillomavirus (HPV) vaccines	A. Anaphylaxis[b] B. Shoulder injury related to vaccine administration[b] C. Vasovagal syncope[b]	≤4 hours ≤48 hours ≤1 hour
XVII. Any new vaccine recommended by the Centers for Disease Control and Prevention for routine administration to children, after publication by the Secretary of a notice of coverage	A. Shoulder injury related to vaccine administration[b] B. Vasovagal syncope[b]	≤48 hours ≤1hour

TABLE 83.1 Vaccine Injury Table[a] *(Continued)*

(b) Provisions that apply to all conditions listed.
  (1) Any acute complication or sequela, including death, of the illness, disability, injury, or condition listed in paragraph (a) of this section (and defined in paragraphs (c) and (d) of this section) qualifies as a Table injury under paragraph (a) except when the definition in paragraph (c) requires exclusion.
  (2) In determining whether or not an injury is a condition set forth in paragraph (a) of this section, the Court shall consider the entire medical record.
  (3) An idiopathic condition that meets the definition of an illness, disability, injury, or condition set forth in paragraph (c) of this section shall be considered to be a condition set forth in paragraph (a) of this section.

(c) Qualifications and aids to interpretation. The following qualifications and aids to interpretation shall apply to, define and describe the scope of, and be read in conjunction with paragraphs (a), (b), and (d) of this section:
  (1) Anaphylaxis. Anaphylaxis is an acute, severe, and potentially lethal systemic reaction that occurs as a single discrete event with simultaneous involvement of two or more organ systems. Most cases resolve without sequela. Signs and symptoms begin minutes to a few hours after exposure. Death, if it occurs, usually results from airway obstruction caused by laryngeal edema or bronchospasm and may be associated with cardiovascular collapse. Other significant clinical signs and symptoms may include the following: Cyanosis, hypotension, bradycardia, tachycardia, arrhythmia, edema of the pharynx and/or trachea and/or larynx with stridor and dyspnea. There are no specific pathological findings to confirm a diagnosis of anaphylaxis.
  (2) Encephalopathy. A vaccine recipient shall be considered to have suffered an encephalopathy if an injury meeting the description below of an acute encephalopathy occurs within the applicable time period and results in a chronic encephalopathy, as described in paragraph (d) of this section.
    (i) Acute encephalopathy.
      (A) For children less than 18 months of age who present:
        (1) Without a seizure, an acute encephalopathy is indicated by a significantly decreased level of consciousness that lasts at least 24 hours.
        (2) Following a seizure, an acute encephalopathy is demonstrated by a significantly decreased level of consciousness that lasts at least 24 hours and cannot be attributed to a postictal state—from a seizure or a medication.
      (B) For adults and children 18 months of age or older, an acute encephalopathy is one that persists at least 24 hours and is characterized by at least two of the following:
        (1) A significant change in mental status that is not medication related (such as a confusional state, delirium, or psychosis);
        (2) A significantly decreased level of consciousness which is independent of a seizure and cannot be attributed to the effects of medication; and
        (3) A seizure associated with loss of consciousness.
      (C) The following clinical features in themselves do not demonstrate an acute encephalopathy or a significant change in either mental status or level of consciousness: Sleepiness, irritability (fussiness), high-pitched and unusual screaming, poor feeding, persistent inconsolable crying, bulging fontanelle, or symptoms of dementia.
      (D) Seizures in themselves are not sufficient to constitute a diagnosis of encephalopathy and in the absence of other evidence of an acute encephalopathy seizures shall not be viewed as the first symptom or manifestation of an acute encephalopathy.
    (ii) Exclusionary criteria for encephalopathy. Regardless of whether or not the specific cause of the underlying condition, systemic disease, or acute event (including an infectious organism) is known, an encephalopathy shall not be considered to be a condition set forth in the Table if it is shown that the encephalopathy was caused by:
      (A) An underlying condition or systemic disease shown to be unrelated to the vaccine (such as malignancy, structural lesion, psychiatric illness, dementia, genetic disorder, prenatal or perinatal central nervous system (CNS) injury); or
      (B) An acute event shown to be unrelated to the vaccine such as a head trauma, stroke, transient ischemic attack, complicated migraine, drug use (illicit or prescribed) or an infectious disease.
  (3) Encephalitis. A vaccine recipient shall be considered to have suffered encephalitis if an injury meeting the description below of acute encephalitis occurs within the applicable time period and results in a chronic encephalopathy, as described in paragraph (d) of this section.
    (i) Acute encephalitis. Encephalitis is indicated by evidence of neurologic dysfunction, as described in paragraph (c)(3)(i)(A) of this section, plus evidence of an inflammatory process in the brain, as described in paragraph (c)(3)(i)(B) of this section.
      (A) Evidence of neurologic dysfunction consists of either:
        (1) One of the following neurologic findings referable to the CNS: Focal cortical signs (such as aphasia, alexia, agraphia, cortical blindness); cranial nerve abnormalities; visual field defects; abnormal presence of primitive reflexes (such as Babinski's sign or sucking reflex); or cerebellar dysfunction (such as ataxia, dysmetria, or nystagmus); or
        (2) An acute encephalopathy as set forth in paragraph (c)(2)(i) of this section.
      (B) Evidence of an inflammatory process in the brain (central nervous system or CNS inflammation) must include cerebrospinal fluid (CSF) pleocytosis (>5 white blood cells (WBC)/mm[FN3] in children >2 months of age and adults; >15 WBC/mm3 in children <2 months of age); or at least two of the following:
        (1) Fever (temperature >= 100.4 degrees Fahrenheit);
        (2) Electroencephalogram findings consistent with encephalitis, such as diffuse or multifocal nonspecific background slowing and periodic discharges; or
        (3) Neuroimaging findings consistent with encephalitis, which include, but are not limited to brain/spine magnetic resonance imaging (MRI) displaying diffuse or multifocal areas of hyperintense signal on T2-weighted, diffusion-weighted image, or fluid-attenuation inversion recovery sequences.
    (ii) Exclusionary criteria for encephalitis. Regardless of whether or not the specific cause of the underlying condition, systemic disease, or acute event (including an infectious organism) is known, encephalitis shall not be considered to be a condition set forth in the Table if it is shown that the encephalitis was caused by:
      (A) An underlying malignancy that led to a paraneoplastic encephalitis;
      (B) An infectious disease associated with encephalitis, including a bacterial, parasitic, fungal or viral illness (such as herpes viruses, adenovirus, enterovirus, West Nile Virus, or human immunodeficiency virus), which may be demonstrated by clinical signs and symptoms and need not be confirmed by culture or serologic testing; or
      (C) Acute disseminated encephalomyelitis (ADEM). Although early ADEM may have laboratory and clinical characteristics similar to acute encephalitis, findings on MRI are distinct with ADEM displaying evidence of acute demyelination (scattered, focal, or multifocal areas of inflammation and demyelination within cerebral subcortical and deep cortical white matter; gray matter involvement may also be seen but is a minor component); or
      (D) Other conditions or abnormalities that would explain the vaccine recipient's symptoms.

Continued on following page

TABLE 83.1 Vaccine Injury Table[a] *(Continued)*

(4) Intussusception.
  (i) For purposes of paragraph (a) of this section, intussusception means the invagination of a segment of intestine into the next segment of intestine, resulting in bowel obstruction, diminished arterial blood supply, and blockage of the venous blood flow. This is characterized by a sudden onset of abdominal pain that may be manifested by anguished crying, irritability, vomiting, abdominal swelling, and/or passing of stools mixed with blood and mucus.
  (ii) For purposes of paragraph (a) of this section, the following shall not be considered to be a Table intussusception:
    (A) Onset that occurs with or after the third dose of a vaccine containing rotavirus;
    (B) Onset within 14 days after an infectious disease associated with intussusception, including viral disease (such as those secondary to non-enteric or enteric adenovirus, or other enteric viruses such as Enterovirus), enteric bacteria (such as Campylobacter jejuni), or enteric parasites (such as Ascaris lumbricoides), which may be demonstrated by clinical signs and symptoms and need not be confirmed by culture or serologic testing;
    (C) Onset in a person with a preexisting condition identified as the lead point for intussusception such as intestinal masses and cystic structures (such as polyps, tumors, Meckel's diverticulum, lymphoma, or duplication cysts);
    (D) Onset in a person with abnormalities of the bowel, including congenital anatomic abnormalities, anatomic changes after abdominal surgery, and other anatomic bowel abnormalities caused by mucosal hemorrhage, trauma, or abnormal intestinal blood vessels (such as Henoch Scholein purpura, hematoma, or hemangioma); or
    (E) Onset in a person with underlying conditions or systemic diseases associated with intussusception (such as cystic fibrosis, celiac disease, or Kawasaki disease).

(5) Chronic arthritis. Chronic arthritis is defined as persistent joint swelling with at least two additional manifestations of warmth, tenderness, pain with movement, or limited range of motion, lasting for at least 6 months.
  (i) Chronic arthritis may be found in a person with no history in the 3 years prior to vaccination of arthropathy (joint disease) on the basis of:
    (A) Medical documentation recorded within 30 days after the onset of objective signs of acute arthritis (joint swelling) that occurred between 7 and 42 days after a rubella vaccination; and
    (B) Medical documentation (recorded within 3 years after the onset of acute arthritis) of the persistence of objective signs of intermittent or continuous arthritis for more than 6 months following vaccination; and
    (C) Medical documentation of an antibody response to the rubella virus.
  (ii) The following shall not be considered as chronic arthritis: Musculoskeletal disorders such as diffuse connective tissue diseases (including but not limited to rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus erythematosus, systemic sclerosis, mixed connective tissue disease, polymyositis/determatomyositis, fibromyalgia, necrotizing vasculitis and vasculopathies and Sjogren's Syndrome), degenerative joint disease, infectious agents other than rubella (whether by direct invasion or as an immune reaction), metabolic and endocrine diseases, trauma, neoplasms, neuropathic disorders, bone and cartilage disorders, and arthritis associated with ankylosing spondylitis, psoriasis, inflammatory bowel disease, Reiter's Syndrome, blood disorders, or arthralgia (joint pain), or joint stiffness without swelling.

(6) Brachial neuritis. This term is defined as dysfunction limited to the upper extremity nerve plexus (i.e., its trunks, divisions, or cords). A deep, steady, often severe aching pain in the shoulder and upper arm usually heralds onset of the condition. The pain is typically followed in days or weeks by weakness in the affected upper extremity muscle groups. Sensory loss may accompany the motor deficits, but is generally a less notable clinical feature. Atrophy of the affected muscles may occur. The neuritis, or plexopathy, may be present on the same side or on the side opposite the injection. It is sometimes bilateral, affecting both upper extremities. A vaccine recipient shall be considered to have suffered brachial neuritis as a Table injury if such recipient manifests all of the following:
  (i) Pain in the affected arm and shoulder is a presenting symptom and occurs within the specified time-frame;
  (ii) Weakness;
    (A) Clinical diagnosis in the absence of nerve conduction and electromyographic studies requires weakness in muscles supplied by more than one peripheral nerve.
    (B) Nerve conduction studies (NCS) and electromyographic (EMG) studies localizing the injury to the brachial plexus are required before the diagnosis can be made if weakness is limited to muscles supplied by a single peripheral nerve.
  (iii) Motor, sensory, and reflex findings on physical examination and the results of NCS and EMG studies, if performed, must be consistent in confirming that dysfunction is attributable to the brachial plexus; and
  (iv) No other condition or abnormality is present that would explain the vaccine recipient's symptoms.

(7) Thrombocytopenic purpura. This term is defined by the presence of clinical manifestations, such as petechiae, significant bruising, or spontaneous bleeding, and by a serum platelet count less than 50,000/mm[FN3] with normal red and white blood cell indices. Thrombocytopenic purpura does not include cases of thrombocytopenia associated with other causes such as hypersplenism, autoimmune disorders (including alloantibodies from previous transfusions) myelodysplasias, lymphoproliferative disorders, congenital thrombocytopenia or hemolytic uremic syndrome. Thrombocytopenic purpura does not include cases of immune (formerly called idiopathic) thrombocytopenic purpura that are mediated, for example, by viral or fungal infections, toxins or drugs. Thrombocytopenic purpura does not include cases of thrombocytopenia associated with disseminated intravascular coagulation, as observed with bacterial and viral infections. Viral infections include, for example, those infections secondary to Epstein Barr virus, cytomegalovirus, hepatitis A and B, human immunodeficiency virus, adenovirus, and dengue virus. An antecedent viral infection may be demonstrated by clinical signs and symptoms and need not be confirmed by culture or serologic testing. However, if culture or serologic testing is performed, and the viral illness is attributed to the vaccine-strain measles virus, the presumption of causation will remain in effect. Bone marrow examination, if performed, must reveal a normal or an increased number of megakaryocytes in an otherwise normal marrow.

(8) Vaccine-strain measles viral disease. This term is defined as a measles illness that involves the skin and/or another organ (such as the brain or lungs). Measles virus must be isolated from the affected organ or histopathologic findings characteristic for the disease must be present. Measles viral strain determination may be performed by methods such as polymerase chain reaction test and vaccine-specific monoclonal antibody. If strain determination reveals wild-type measles virus or another, non-vaccine-strain virus, the disease shall not be considered to be a condition set forth in the Table. If strain determination is not done or if the strain cannot be identified, onset of illness in any organ must occur within 12 months after vaccination.

TABLE 83.1 Vaccine Injury Table[a] *(Continued)*

(9) Vaccine-strain polio viral infection. This term is defined as a disease caused by poliovirus that is isolated from the affected tissue and should be determined to be the vaccine-strain by oligonucleotide or polymerase chain reaction. Isolation of poliovirus from the stool is not sufficient to establish a tissue specific infection or disease caused by vaccine-strain poliovirus.

(10) Shoulder injury related to vaccine administration (SIRVA). SIRVA manifests as shoulder pain and limited range of motion occurring after the administration of a vaccine intended for intramuscular administration in the upper arm. These symptoms are thought to occur as a result of unintended injection of vaccine antigen or trauma from the needle into and around the underlying bursa of the shoulder resulting in an inflammatory reaction. SIRVA is caused by an injury to the musculoskeletal structures of the shoulder (e.g. tendons, ligaments, bursae, etc.). SIRVA is not a neurological injury and abnormalities on neurological examination or nerve conduction studies (NCS) and/or electromyographic (EMG) studies would not support SIRVA as a diagnosis (even if the condition causing the neurological abnormality is not known). A vaccine recipient shall be considered to have suffered SIRVA if such recipient manifests all of the following:
   (i) No history of pain, inflammation or dysfunction of the affected shoulder prior to intramuscular vaccine administration that would explain the alleged signs, symptoms, examination findings, and/or diagnostic studies occurring after vaccine injection;
   (ii) Pain occurs within the specified time-frame;
   (iii) Pain and reduced range of motion are limited to the shoulder in which the intramuscular vaccine was administered; and
   (iv) No other condition or abnormality is present that would explain the patient's symptoms (e.g. NCS/EMG or clinical evidence of radiculopathy, brachial neuritis, mononeuropathies, or any other neuropathy).

(11) Disseminated varicella vaccine-strain viral disease. Disseminated varicella vaccine-strain viral disease is defined as a varicella illness that involves the skin beyond the dermatome in which the vaccination was given and/or disease caused by vaccine-strain varicella in another organ. For organs other than the skin, the disease must be demonstrated in the involved organ and not just through mildly abnormal laboratory values. If there is involvement of an organ beyond the skin, and no virus was identified in that organ, the involvement of all organs must occur as part of the same, discrete illness. If strain determination reveals wild-type varicella virus or another, non-vaccine-strain virus, the viral disease shall not be considered to be a condition set forth in the Table. If strain determination is not done or if the strain cannot be identified, onset of illness in any organ must occur 7- 42 days after vaccination.

(12) Varicella vaccine-strain viral reactivation disease. Varicella vaccine-strain viral reactivation disease is defined as the presence of the rash of herpes zoster with or without concurrent disease in an organ other than the skin. Zoster, or shingles, is a painful, unilateral, pruritic rash appearing in one or more sensory dermatomes. For organs other than the skin, the disease must be demonstrated in the involved organ and not just through mildly abnormal laboratory values. There must be laboratory confirmation that the vaccine-strain of the varicella virus is present in the skin or in any other involved organ, for example by oligonucleotide or polymerase chain reaction. If strain determination reveals wild-type varicella virus or another, non-vaccine-strain virus, the viral disease shall not be considered to be a condition set forth in the Table.

(13) Vasovagal syncope. Vasovagal syncope (also sometimes called neurocardiogenic syncope) means loss of consciousness (fainting) and postural tone caused by a transient decrease in blood flow to the brain occurring after the administration of an injected vaccine. Vasovagal syncope is usually a benign condition but may result in falling and injury with significant sequela. Vasovagal syncope may be preceded by symptoms such as nausea, lightheadedness, diaphoresis, and/or pallor. Vasovagal syncope may be associated with transient seizure-like activity, but recovery of orientation and consciousness generally occurs simultaneously with vasovagal syncope. Loss of consciousness resulting from the following conditions will not be considered vasovagal syncope: organic heart disease, cardiac arrhythmias, transient ischemic attacks, hyperventilation, metabolic conditions, neurological conditions, and seizures. Episodes of recurrent syncope occurring after the applicable time period are not considered to be sequela of an episode of syncope meeting the Table requirements.

(14) Immunodeficient recipient. Immunodeficient recipient is defined as an individual with an identified defect in the immunological system which impairs the body's ability to fight infections. The identified defect may be due to an inherited disorder (such as severe combined immunodeficiency resulting in absent T lymphocytes), or an acquired disorder (such as acquired immunodeficiency syndrome resulting from decreased CD4 cell counts). The identified defect must be demonstrated in the medical records, either preceding or postdating vaccination.

(15) Guillain-Barré Syndrome (GBS).
   (i) GBS is an acute monophasic peripheral neuropathy that encompasses a spectrum of four clinicopathological subtypes described below. For each subtype of GBS, the interval between the first appearance of symptoms and the nadir of weakness is between 12 hours and 28 days. This is followed in all subtypes by a clinical plateau with stabilization at the nadir of symptoms, or subsequent improvement without significant relapse. Death may occur without a clinical plateau. Treatment related fluctuations in all subtypes of GBS can occur within 9 weeks of GBS symptom onset and recurrence of symptoms after this time-frame would not be consistent with GBS.
   (ii) The most common subtype in North America and Europe, comprising more than 90 percent of cases, is acute inflammatory demyelinating polyneuropathy (AIDP), which has the pathologic and electrodiagnostic features of focal demyelination of motor and sensory peripheral nerves and nerve roots. Another subtype called acute motor axonal neuropathy (AMAN) is generally seen in other parts of the world and is predominated by axonal damage that primarily affects motor nerves. AMAN lacks features of demyelination. Another less common subtype of GBS includes acute motor and sensory neuropathy (AMSAN), which is an axonal form of GBS that is similar to AMAN, but also affects the sensory nerves and roots. AIDP, AMAN, and AMSAN are typically characterized by symmetric motor flaccid weakness, sensory abnormalities, and/or autonomic dysfunction caused by autoimmune damage to peripheral nerves and nerve roots. The diagnosis of AIDP, AMAN, and AMSAN requires:
      (A) Bilateral flaccid limb weakness and decreased or absent deep tendon reflexes in weak limbs;
      (B) A monophasic illness pattern;
      (C) An interval between onset and nadir of weakness between 12 hours and 28 days;
      (D) Subsequent clinical plateau (the clinical plateau leads to either stabilization at the nadir of symptoms, or subsequent improvement without significant relapse; however, death may occur without a clinical plateau); and,
      (E) The absence of an identified more likely alternative diagnosis.

Continued on following page

TABLE 83.1 Vaccine Injury Table[a] *(Continued)*

(iii) Fisher Syndrome (FS), also known as Miller Fisher Syndrome, is a subtype of GBS characterized by ataxia, areflexia, and ophthalmoplegia, and overlap between FS and AIDP may be seen with limb weakness. The diagnosis of FS requires:
(A) Bilateral ophthalmoparesis;
(B) Bilateral reduced or absent tendon reflexes;
(C) Ataxia;
(D) The absence of limb weakness (the presence of limb weakness suggests a diagnosis of AIDP, AMAN, or AMSAN);
(E) A monophasic illness pattern;
(F) An interval between onset and nadir of weakness between 12 hours and 28 days;
(G) Subsequent clinical plateau (the clinical plateau leads to either stabilization at the nadir of symptoms, or subsequent improvement without significant relapse; however, death may occur without a clinical plateau);
(H) No alteration in consciousness;
(I) No corticospinal track signs; and
(J) The absence of an identified more likely alternative diagnosis.

(iv) Evidence that is supportive, but not required, of a diagnosis of all subtypes of GBS includes electrophysiologic findings consistent with GBS or an elevation of cerebral spinal fluid (CSF) protein with a total CSF white blood cell count below 50 cells per microliter. Both CSF and electrophysiologic studies are frequently normal in the first week of illness in otherwise typical cases of GBS.

(v) To qualify as any subtype of GBS, there must not be a more likely alternative diagnosis for the weakness.

(vi) Exclusionary criteria for the diagnosis of all subtypes of GBS include the ultimate diagnosis of any of the following conditions: chronic immune demyelinating polyradiculopathy (CIDP), carcinomatous meningitis, brain stem encephalitis (other than Bickerstaff brainstem encephalitis), myelitis, spinal cord infarct, spinal cord compression, anterior horn cell diseases such as polio or West Nile virus infection, subacute inflammatory demyelinating polyradiculoneuropathy, multiple sclerosis, cauda equina compression, metabolic conditions such as hypermagnesemia or hypophosphatemia, tick paralysis, heavy metal toxicity (such as arsenic, gold, or thallium), drug-induced neuropathy (such as vincristine, platinum compounds, or nitrofurantoin), porphyria, critical illness neuropathy, vasculitis, diphtheria, myasthenia gravis, organophosphate poisoning, botulism, critical illness myopathy, polymyositis, dermatomyositis, hypokalemia, or hyperkalemia. The above list is not exhaustive.

(d) Glossary for purposes of paragraph (c) of this section—

(1) Chronic encephalopathy.

(i) A chronic encephalopathy occurs when a change in mental or neurologic status, first manifested during the applicable Table time period as an acute encephalopathy or encephalitis, persists for at least 6 months from the first symptom or manifestation of onset or of significant aggravation of an acute encephalopathy or encephalitis.

(ii) Individuals who return to their baseline neurologic state, as confirmed by clinical findings, within less than 6 months from the first symptom or manifestation of onset or of significant aggravation of an acute encephalopathy or encephalitis shall not be presumed to have suffered residual neurologic damage from that event; any subsequent chronic encephalopathy shall not be presumed to be a sequela of the acute encephalopathy or encephalitis.

(2) Injected refers to the intramuscular, intradermal, or subcutaneous needle administration of a vaccine.

(3) Sequela means a condition or event which was actually caused by a condition listed in the Vaccine Injury Table.

(4) Significantly decreased level of consciousness is indicated by the presence of one or more of the following clinical signs:
(i) Decreased or absent response to environment (responds, if at all, only to loud voice or painful stimuli);
(ii) Decreased or absent eye contact (does not fix gaze upon family members or other individuals); or
(iii) Inconsistent or absent responses to external stimuli (does not recognize familiar people or things).

(5) Seizure includes myoclonic, generalized tonic-clonic (grand mal), and simple and complex partial seizures, but not absence (petit mal), or pseudo seizures. Jerking movements or staring episodes alone are not necessarily an indication of seizure activity.

(e) Coverage provisions.

(1) Except as provided in paragraph (e)(2), (3), (4), (5), (6), (7), or (8) of this section, this section applies only to petitions for compensation under the program filed with the United States Court of Federal Claims on or after February 21, 2017.

(2) Hepatitis B, Hib, and varicella vaccines (Items VIII, IX, and X of the Table) are included in the Table as of August 6, 1997.

(3) Rotavirus vaccines (Item XI of the Table) are included in the Table as of October 22, 1998.

(4) Pneumococcal conjugate vaccines (Item XII of the Table) are included in the Table as of December 18, 1999.

(5) Hepatitis A vaccines (Item XIII of the Table) are included on the Table as of December 1, 2004.

(6) Trivalent influenza vaccines (Included in item XIV of the Table) are included on the Table as of July 1, 2005. All other seasonal influenza vaccines (Item XIV of the Table) are included on the Table as of November 12, 2013.

(7) Meningococcal vaccines and human papillomavirus vaccines (Items XV and XVI of the Table) are included on the Table as of February 1, 2007.

(8) Other new vaccines (Item XVII of the Table) will be included in the Table as of the effective date of a tax enacted to provide funds for compensation paid with respect to such vaccines. An amendment to this section will be published in the Federal Register to announce the effective date of such a tax.

[a]As of early March 2017, scheduled to be effective March 21, 2017. See 82 Fed. Reg. 6294 (Jan. 19, 2017); 82 Fed. Reg. 11321 (Feb. 22, 2017).
[b]Newly added injury.

In addition to the general statute of limitations, the statute provided for 8 years' retroactive coverage for claimants alleging injuries from a newly added vaccine and for injuries added to the VIT for existing vaccines, with a 2-year window in which to file after addition of coverage. These changes ensured that the Vaccine Act's liability protections and compensation under the VICP would be extended to new categories of vaccines recommended for children.

Structure and Process

The VICP is administered by HHS in conjunction with the U.S. Department of Justice (DOJ), and the Office of the Special Masters, U.S. Court of Federal Claims. Vaccines designated by the CDC for "routine administration to children" and subject to an excise tax are covered by the program. As of March 2017, these included vaccines against 16 diseases: *Haemophilus*

influenzae type b (Hib), diphtheria, tetanus, pertussis, measles, mumps, rubella, polio (inactivated poliovirus vaccine [IPV] and OPV), hepatitis A, hepatitis B, rotavirus, varicella zoster virus (VZV), all seasonal influenza, meningococcal, pneumococcal (pneumococcal conjugate vaccines, e.g., PCV13), and human papillomavirus (HPV). As of December 2016, vaccines recommended by the CDC for routine administration to pregnant women and subject to an excise tax are also covered by the program. As of March 2017, this has not resulted in any additional categories of vaccines under the program.

The VICP created two classes of claims: claims involving vaccinations given before October 1, 1988 ("pre-1988") and claims involving vaccinations administered on or after October 1, 1988 ("post-1988"). Pre-1988 claims were optional but had to be filed by January 31, 1991, and all were fully adjudicated by 2005. Post-1988 injury claims must first be filed under the VICP. Under the general filing deadline, post-1988 injury claims must be filed within 36 months after the first symptom appeared following vaccination. Death claims must be filed within 24 months of the death and within 48 months after the onset of the vaccine-related injury from which the death occurred. The NCVIA provides for up to 14 months from the filing date for the court to issue a decision, subject to extension.

Petitioners, through an attorney or on their own, file a petition with the court, which begins the review and adjudication process. Petitions (claims) are filed against the Secretary of HHS as respondent on behalf of the government. The court assigns the petition to one of eight special masters, who are lawyers handling cases only in the VICP. Supporting documents required by the NCVIA include medical records and affidavits of the parents (or other family members) regarding the vaccination and resulting injury or death. Expert witness reports may also accompany the initial filing. Proceedings are expedited by eliminating formal civil discovery and rules of evidence in favor of a more informal process. Court rules provide for regular telephone status conferences with both parties and informal review and fact determinations by the special master before a hearing, should one be needed. These relaxed rules seem to encourage and facilitate settlements.

Once the claim is filed, HHS assigns it to one of its medical reviewers, who reviews the documents. The VICP medical staff reaches a recommendation on the petitioner's entitlement to compensation, which is then forwarded to the court through the DOJ attorney assigned to the case. Pediatric and adult subspecialists sometimes assist VICP staff in reaching a recommendation on eligibility and testifying about vaccine causation in hearings.

Petitioners can establish medical eligibility for compensation in one of three ways. First, petitioners can prove that a condition listed on the VIT occurred in the prescribed interval meeting the table requirements (and that there is not greater evidence of an alternative cause or "factor unrelated"). Second, petitioners can prove that the vaccine actually caused the injury if the injury does not meet all the requirements (e.g., time frame) for a VIT condition or the injury is not listed on the VIT. A third option is showing that the vaccine significantly aggravated a preexisting medical condition. It is worth noting that a factor unrelated must be a condition of known cause and not "idiopathic." In addition to satisfying one of these three elements, petitioners must also show there were continued effects for longer than 6 months, except in the case of a vaccine-related death, or that the injured person required "inpatient hospitalization and surgical intervention" as a result of the vaccine injury.

VICP medical staff's recommendations are predominantly based on the contemporaneous medical records rather than the affidavits of family members, which are often generated months to years following the alleged injury. Eligibility for compensation is recommended if the VICP staff finds that the records fulfill the requirements of the NCVIA. At the same time, the DOJ determines whether the petition meets the legal requirements for an award. The criteria are detailed and require that each petition contain several elements before the special master can have jurisdiction over the matter (Table 83.2). The court nearly always concurs with a determination by the government that petitioners are entitled to compensation, thereby obviating the need for a hearing. The cases not conceded by HHS often proceed to a hearing before a special master, at which point both sides present testimony, including expert witnesses for each party. In some cases, the court may find the testimony of family members more persuasive than contemporaneously recorded events or give greater weight to the initial diagnosis of the treating physician over determinations made on subsequent clinical evaluations. Therefore, notwithstanding the HHS position, it is not uncommon for the special master to award compensation after an entitlement hearing. In addition, today a significant number (83%) of petitions are resolved between the parties by settlement, based on both parties' perception of their exposure to the risk of an adverse decision. A "litigative risk settlement" is not an admission by the United States or the Secretary of HHS that the vaccine caused the petitioner's alleged injuries, and, in settled cases, the court does not determine that the vaccine caused the injury. A settlement, therefore, cannot be characterized as a decision by HHS or by the court that the vaccine caused an injury. Claims may be resolved by settlement for many reasons, including consideration of prior court decisions; a recognition by both parties that there is a risk of loss in proceeding to a decision by the court making the certainty of settlement more desirable; a desire by both parties to minimize the time and expense associated with litigating a case to conclusion; and a desire by both parties to resolve a case quickly and efficiently.

Once entitlement to compensation is conceded, the special master determines that the petitioner is entitled to compensation, or the parties agree to settle a claim, the DOJ and the petitioner work to reach agreement on the amount of compensation (damages). The level of compensation is often based on life care plans that evaluate the health of the injured person and the person's future needs.

The government and petitioner often use informal negotiations under the guidance of a special master to resolve entitlement and damages issues. Alternative dispute resolution is being increasingly used to avoid holding a hearing. Compensation for injured minors or incompetent persons is usually in the form of a lump sum payment and an annuity designed to provide a lifetime stream of benefits. This includes unreimbursable costs for vaccine-related goods and services from the date of injury, lost wages,[28,29] and pain and suffering (up to $250,000).[30] A benefit of $250,000 is available for eligible death claims. The Court of Appeals for the Federal Circuit has expanded the availability of damages, holding that an eligible petitioner's estate that established vaccine-related injuries and vaccine-related death is entitled to recover a death benefit and provable compensation for medical expenses, lost wages, and pain and suffering of the decedent.[31] "Reasonable" attorneys' fees generally are paid whether or not petitioners are successful in obtaining compensation if the claim was brought in good faith and on a reasonable basis. Punitive damages and awards to others in the family for loss of companionship are not allowed.

Appeals of special master decisions can be made to a judge of the United States Court of Federal Claims and then to a three-judge panel of the United States Court of Appeals for the Federal Circuit. Only twice has a Federal Circuit decision

TABLE 83.2 Legal Requirements Under the National Vaccine Injury Compensation Program

REQUIREMENTS FOR PROPER FILING
Must be properly filed with Department of Health and Human Services and U.S. Court of Federal Claims
Must be filed by proper person as petitioner:
Person who sustained vaccine-related injury; or
Legal representative of minor or individual who sustained vaccine-related injury or death
Must contain:
Affidavit; and
Medical and vaccination records, and, if applicable, death certificate and autopsy (or identification of any unavailable records and reasons for unavailability)
Must be filed within the statute of limitations:
Injuries: within 36 months of first symptom/manifestation of onset (or significant aggravation) of vaccine-related injury; or
Deaths: within 24 months of vaccine-related death and within 48 months of date of first symptom/manifestation of onset (or significant aggravation) of injury from which death resulted; or
Table revisions: if the Vaccine Injury Table revision makes person who was previously ineligible to seek compensation eligible or significantly increases likelihood of person obtaining compensation, such person may file petition within 2 years of effective date of revision so long as vaccine-related injury/death did not occur more than 8 years before date of revision
NATURE OF THE ALLEGATION
Must involve covered vaccine listed in the Vaccine Injury Table or included within the Vaccine Injury Table's general category of vaccines recommended by the CDC for routine administration to children or to pregnant women once excise tax is passed
Must involve proper relationship between injured person and covered vaccine:
Injured person either received covered vaccine;
Or in the case of polio, injured person contracted polio from another person who received oral polio vaccine
Must involve proper place vaccine was administered:
Injured person receiving covered vaccine must have been in United States or its trust territories, unless:
Person was U.S. citizen serving abroad as member of armed forces or employee of United States or dependent of such a citizen
Vaccine manufactured in United States and person returned to United States within 6 months after date of vaccination
Injured did not receive covered vaccine but contracted polio from another person who received oral polio vaccine, and must have been citizen of United States or a dependent of such a citizen
TO OBTAIN COMPENSATION
Must prove compensable injury/death by preponderance of the evidence:
Sustained Table injury or died from covered vaccine and first symptom/manifestation of onset occurred within time period set forth in the Vaccine Injury Table; or
Sustained Table condition or died from covered vaccine and first symptom/manifestation of onset of condition did not occur within time period set forth in the Vaccine Injury Table; or
Sustained any condition not set forth on Table from covered vaccine
Must show residual effects:
Suffered residual effects/complications of vaccine-related injury for more than 6 months after vaccine's administration; or
Died from administration of the vaccine; or
Received inpatient hospitalization and surgery as result of vaccine-related injury
Factor unrelated to administration of the vaccine that caused injury/death must not be shown (by preponderance of evidence)
Must not have previously filed Vaccine Injury Compensation Program claim for same vaccine administration
Must not have previously collected award/settlement of civil action for damages for vaccine-related injury/death
Must elect to accept Court of Federal Claims' judgment to receive compensation awarded

advanced to the next level of appeal, the Supreme Court (see *Shalala v Whitecotton* and *Sebelius v. Cloer* in "Vaccine Injury Compensation Program Cases Since 1986" later).

Implementation and Program Experience

Table 83.3 shows the numbers of claims filed under the VICP by vaccine type and year of administration. Data pertaining to claims filed with the VICP refer to the first vaccine named in each petition. Additional information and data that is updated periodically are available on the webpages for the ACCV[32] and the VICP.[33] This table includes hundreds of vaccines given during the 1950s and 1960s, with the oldest case dating back to 1918 for a death alleged to be associated with the pertussis vaccine. The program processed many claims that were otherwise barred from the tort system by a state statute of limitations. Statutory deadlines have twice brought large numbers of claims during a short period, the first occurring when more than 4000 pre-1988 claims were filed during a 6-month period from 1990 to 1991; in the second, more than 300 hepatitis B virus (HBV) vaccine claims were filed in August 1999 to meet the 2-year deadline for filing of retroactive claims after HBV was added to the VIT (effective August 6, 1997). Given that approximately 118 million doses of seasonal influenza vaccines are distributed annually (personal communication with the Centers for Disease Control and Prevention [CDC], August 4, 2014), it was to be expected that the addition of influenza vaccines in 2005 prompted a large influx of claims. Although fewer than 200 influenza claims were filed by the 2-year deadline for newly added vaccines in July 2007, the number of influenza claims filed annually has increased steadily since that time. Since 2007, more than 3200 influenza claims have been filed.

Starting in late 2001, thousands of claims were filed alleging autism (or autism spectrum disorder) resulting from the thimerosal (a type of mercury) preservative included in certain vaccines or the measles, mumps, and rubella (MMR) vaccine, which has never contained thimerosal. Table 83.4 shows the number of cases submitted to the VICP through February 27, 2017, including the number of cases pending, adjudicated, or dismissed and the dollars awarded.[34] Many pre-1988 cases were dismissed by the court on grounds of

TABLE 83.3 National Vaccine Injury Compensation Program Claims by Year of Administration and Vaccine Type as of February 27, 2017[a,b]

Year of Administration	DTP, DTaP, P, Tdap (and various combinations)	DT, Td, TT	MMR, MMRV, MR, M	Rubella	OPV	IPV	Hep B	VZV	HIB	RV	PCV	Hep A	Influenza	MCV, MPSV	HPV	Total
1910–19	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
1920–29	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
1930–39	1	–	1	–	–	–	–	–	–	–	–	–	–	–	–	2
1940–49	63	1	2	–	1	1	–	–	–	–	–	–	–	–	–	68
1950–59	180	4	3	–	6	190	–	–	–	–	–	–	–	–	–	383
1960–69	377	5	105	6	82	54	–	–	–	–	–	–	–	–	–	629
1970–79	828	6	120	43	55	0	–	–	–	–	–	–	–	–	–	1052
1980–89	1874	35	199	101	77	0	5	–	–	–	–	–	1	–	–	2292
1990–99	699	108	395	35	85	1	421	21	9	31	2	5	17	–	–	1829
2000–09	528	161	278	6	0	11	202	52	15	21	30	41	801	23	200	2369
2010–19	682	75	104	1	0	2	80	25	18	27	62	77	2465	34	144	3796
Total	5234	395	1207	192	306	259	708	98	42	79	94	123	3284	57	344	12422

[a]The total number of claims filed in the VICP was 17,856 as of February 2017. This number reflects the claims represented on this table, in addition to 5,434 claims that were unspecified as to vaccine type (most filed as part of the Omnibus Autism Hearing) or to date of administration, and claims for vaccines not covered by the VICP.

[b]Data pertaining to claims filed with the VICP refer to the first vaccine named in each petition.

DT, diphtheria and tetanus toxoids-pediatric; DTaP, diphtheria, tetanus toxoids, and acellular pertussis; DTP, diphtheria, tetanus, and pertussis; HepA, hepatitis A; Hep B, hepatitis B; HPV, human papillomavirus; IPV, inactivated polio vaccine; M, measles; MCV, meningococcal conjugate vaccine; MMR, measles, mumps, and rubella; MMRV, measles, mumps, rubella and varicella; MPSV, meningococcal polysaccharide vaccine; MR, measles and rubella; OPV, oral polio vaccine; P, pertussis, PCV, pneumococcal conjugate vaccine; RV, rotavirus; Td, diphtheria and tetanus toxoids-indicated for persons 7 years or older; Tdap, tetanus, diphtheria, and acellular pertussis; TT, tetanus toxoid; VICP, National Vaccine Injury Compensation Program; VZV, varicella-zoster virus.

TABLE 83.4 Status of the National Vaccine Injury Compensation Program as of February 27, 2017[a]

	Vaccines Administered Before October 1, 1988	Vaccines Administered on or After October 1, 1988	Total
Claims filed	4,259	13,676	17,935
Claims adjudicated	4,259	11,918	16,177
Compensable	1,187	4,087	5,274
Dismissed	3,072	7,843	10,915
Awards paid	1,187	4,085	5,272
Compensation (millions)	$863	$2,499	$3,362
Attorneys' fees/costs (millions)[b]	$38	$215	$253
Total outlays (millions)	$901	$2,718	$3,619

[a]Compensable cases include cases settled by the parties in which no finding of table injuries or causation was reached.
[b]Includes fees/costs for compensated claims, dismissed claims, and interim fees/costs.

legal or medical insufficiency. Some plaintiffs, given the opportunity to obtain additional records, later refiled. Most of the larger awards include an initial lump sum payment with the remainder going toward purchase of an annuity. Overall, the majority of submissions (30%) allege pertussis-containing vaccines–related effects owing to the large number of pre-1988 filings. The remaining claims break down as follows: 12% from seasonal influenza vaccine; 7% from measles-containing vaccines, given alone or in any combination; 4% from hepatitis B-containing vaccines vaccine; 2% from tetanus-containing vaccines (excluding those containing pertussis); 2% from OPV; 2% from IPV; 2% for HPV vaccines; and 1% or less each for Hib, pneumococcal conjugate, rotavirus, hepatitis A virus (HAV), meningococcal, rubella, and VZV vaccines.

A better barometer of the recent VICP experience is reflected in the percentages by vaccine type filed during the 5-year period from fiscal years 2012 through 2016. A breakdown of 3,449 nonautism claims shows influenza vaccines predominate at 65%, followed by pertussis-containing vaccines (e.g., DTaP alone or in various combinations, Tdap) at 15%, MMR (3%), HPV (5%), HBV (2%), tetanus-containing vaccines (2%), and the remaining vaccines all less than 2%. Although percentages vary by vaccine, overall, injuries account for 93% of claims and deaths the remaining 7%.

As VICP adjudications continued into the mid-1990s, attention focused on the Vaccine Injury Compensation Trust Fund. Excise tax levels for the original seven vaccines covered by the NCVIA were based on the estimated numbers and cost of claims that each would generate. By 1996, the trust fund had exceeded anticipated needs, with $1 billion in holdings and annual receipts totaling $140 million, against peak outlays in the $35 to $42 million range. With passage of the Taxpayer Relief Act of 1997, the vaccine excise structure was revised, setting a 75-cents-per-"dose" (disease prevented) rate on all covered vaccines under the program. This federal excise tax funds the VICP. (At the end of 2014, the trust fund balance was $3.5 billion,[35] with more than $304 million in receipts from excise tax collections and interest on investments against awards totaling $223.4 million.)[34]

VACCINE INJURY COMPENSATION PROGRAM CASES SINCE 1986

Without question, the biggest controversy (and challenge) in adjudicating cases filed in the VICP has been determining vaccine causation. The legislative history underlying the NCVIA noted the lingering controversy about what is and is not vaccine-caused, with a recognition that the scientific understanding on these issues would evolve. For the first 8 years after the VICP was created, the vast majority of petitions filed alleged a VIT injury ("Table claims"), which confers the presumption that the injury, significant aggravation, or death, were vaccine-related. A VIT-based adjudication was straightforward: A petition would usually allege that the injured person experienced the signs and symptoms described in the VIT, as defined by the Qualifications and Aids to Interpretation (QAIs), within a VIT time frame, regardless of whether these allegations were substantiated or contradicted by the medical record. Accordingly, in the earlier years of the VICP, the special masters and the courts concentrated almost exclusively on developing a body of law establishing the quality and quantity of evidence needed to prove an injury included on the VIT. Since then, and partly because of regulatory amendments to the VIT that more precisely reflected the science of vaccine injury causation than did the original statutory VIT, the majority of petitions have shifted to allege injuries not found on the VIT ("off-table claims"), in which petitioners are required to prove that the vaccine actually caused the injury.

Most special masters place more weight on the medical records most contemporaneous to the vaccination than on contradictory evidence assembled later, after memories fade or when litigation is contemplated.[36] In circumstances in which the contemporaneous medical records are contradictory or do not exist, the special masters rely on the credibility of the family and the expert witnesses to determine whether a VIT injury occurred. The appellate courts give deference to special masters' credibility determinations, as long as they were not arbitrary or capricious.[37]

The courts also have addressed questions concerning whether special masters must strictly apply and interpret the definitions in the QAIs, which accompany the VIT. In *Hellebrand v Secretary of Department of Health and Human Services*[38] and *Hodges v Secretary of Department of Health and Human Services,*[39] the U.S. Court of Appeals for the Federal Circuit found that because special masters have wide latitude in adjudicating claims, they are not required to apply the QAIs in a mechanical manner; rather, they have reasonable discretion when applying them.

Shalala v Whitecotton[40] is the landmark Table injury case regarding the meaning of "significant aggravation" under the statute. Significant aggravation is important in VICP cases because if the onset of signs or symptoms following vaccination is not the first evidence of the alleged vaccine-related condition, to receive compensation, the petitioner must prove that the vaccine significantly aggravated a condition present before vaccination. The NCVIA defines significant aggravation as "any change for the worse in a preexisting condition which results in markedly greater disability, pain, or illness accompanied by substantial deterioration of health."[41] According to the legislative history, an example would be a child whose seizure frequency increased from one per month before vaccination to one per day after vaccination.[42] The special master in *Whitecotton* denied compensation on the basis that the child was born with a brain disorder that was responsible for the encephalopathy that appeared after receipt of the third DTP vaccination. The Court of Federal Claims agreed, but the U.S. Court of Appeals for the Federal Circuit reversed the

decision based on a different interpretation of "first symptom or manifestation of onset," writing that "first" did not necessarily mean that other clinical signs could not appear before vaccination.[43] The U.S. Supreme Court unanimously reversed the Federal Circuit's decision, finding that the Federal Circuit misread the NCVIA. However, Justice O'Connor, in a concurring opinion, noted that the Federal Circuit opinion did not address the issue of significant aggravation of a preexisting condition, but instead only addressed the issue of "first symptom or manifestation of onset." The case was remanded to the Federal Circuit, which set forth a test for Table injury-significant aggravation claims, specifying that the special master must (a) assess the person's condition before the administration of the vaccine, (b) assess the person's current condition, and (c) determine if the person's current condition constitutes a significant aggravation of the person's condition before vaccination within the meaning of the statute; and, if the special master determines there has been a significant aggravation, then the special master must (d) determine whether the first symptom or manifestation of the significant aggravation occurred within the period prescribed by the VIT for the injury.[44] Although *Whitecotton* involved a Table injury, the courts have applied the significant aggravation test in off-Table claims.[45,46]

A clarification of the fourth step of the *Whitecotton* test was announced in *Gruber v Secretary of Department of Health and Human Services.*[47] The Court of Federal Claims clarified that the special master, in determining the first symptom or manifestation of onset of the significant aggravation, must take into account the preexisting condition. The court found that the definition of the term *significant aggravation* should more properly be understood as "any change for worse in *[the allegedly aggravated Table injury]* which results in a markedly greater disability, pain, or illness accompanied by substantial deterioration in health." Furthermore, the court indicated that the four-step test articulated in *Whitecotton* does not merge the significant aggravation and sequelae inquiries. Rather, to obtain compensation for sequelae, a petitioner must establish that the aggravation of the Table injury caused them, without reliance on any Table presumption.

The parameters of the *factor unrelated* defense also have been tested. Under the NCVIA, if petitioners show proof of a Table condition occurring within the specified time, they are entitled to a presumption of vaccine causation unless the government can show greater evidence of an alternative cause or factor unrelated. The U.S. Court of Appeals for the Federal Circuit generally used a strict interpretation when it applied the NCVIA's definition of the term: A factor unrelated may not include "any idiopathic, unexplained, unknown, hypothetical, or undocumentable" condition.[48] However, the Federal Circuit has considered whether the government was required to specifically identify a virus it alleged was a factor unrelated. In *Knudsen v Secretary of Department of Health and Human Services,*[49] the Federal Circuit held that so long as the government could prove that the petitioner did in fact have a viral infection, the government need not identify the type of viral infection with specificity. More recently, in the consolidated appeal of *Stone v. Secretary of Department of Health and Human Services* and *Hammitt v. Secretary of Department of Health and Human Services,*[50] the Federal Circuit reiterated prior precedent and found that special masters may consider evidence of a factor unrelated in examining the record as a whole to determine whether or not petitioners have met their burden of proof in establishing a prima facie case.

The original NCVIA allowed the Secretary of HHS to modify or amend injuries listed in the VIT, which was originally included in the statute, and QAIs (see "Modifying the Vaccine Injury Table" later) by regulation.[51] In petitions filed after the first set of VIT changes took effect, the focus of most claims changed from proving a VIT injury to proving that the injury was caused-in-fact by the vaccine. This shift occurred for several reasons. First, HHS removed residual seizure disorder and shock collapse from the VIT. Second, the QAIs for encephalopathy were revised so they more accurately reflected the medical view of significant acute and chronic neurologic injuries, instead of the normal, transient side effects common to some childhood vaccines.[52]

Third, although additional conditions have been added to the "original" vaccines, few of the new vaccines added to the VIT in recent years have had associated VIT injuries (Table 83.5). Since 1997, only three conditions were added for the nine "new" vaccines. Some of these conditions were subsequently removed from the VIT given that they were no longer applicable based upon changed circumstances. Fourth, and arguably most significantly, the licensure of acellular pertussis vaccines for primary use in infants and the transition to an IPV-only schedule all but eliminated DTP vaccine and OPV filings, the former of which comprised a significant percentage of alleged injury and death claims.

Current cases, therefore, establish a challenge for the special masters and the courts to strike an appropriate balance between (a) denying compensation of claims based merely on a temporal relationship between the injury and the vaccine and a lack of apparent alternative causes and (b) the need to fulfill the VICP's mandate to provide compensation "generously" and "fairly," even in the absence of full scientific knowledge about potential vaccine adverse events. Early in the history of the VICP, the court determined in *Grant v Secretary of Department of Health and Human Services* that causation-in-fact was shown when there is "proof of a logical sequence of cause and effect showing that the vaccination was the reason for the injury. A reputable medical or scientific explanation must support this logical sequence of cause and effect."[53] The U.S. Court of Appeals for the Federal Circuit clarified this issue somewhat by holding that the scientific evidence need not rise to the level of being a scientific certainty.[49] Still, there were questions. For example, special masters considered whether animal studies could be used, how much weight should be given to case reports, and whether the petitioner had to rely on any published or peer-reviewed evidence in pursuing a VICP claim.[54,55]

The standard of proof for causation-in-fact has been adjusted and clarified over time. In *Shyface v Secretary of Department of Health and Human Services,*[56] the U.S. Court of Appeals for the Federal Circuit found that the petitioners need not show that the vaccination was the sole or even the predominant cause of the injury or condition. Rather, the petitioner need only show that the vaccination was at least a "substantial factor" in causing the condition and was a "but for" cause. In *Althen v Secretary of Department of Health and Human Services,*[57] the court held that petitioner's burden was to show by preponderant evidence that the vaccine brought about her injury by providing: "(1) a medical theory causally connecting the vaccination and the injury; (2) a logical sequence of cause and effect showing that the vaccination was the reason for the injury; and (3) a showing of proximate temporal relationship between vaccination and injury." The decision held that there is no requirement that petitioners provide scientific literature to support their theory of causation—medical opinion or medical records can suffice under the law. The court stated that while the case involved "a sequence hitherto unproven in medicine, the purpose of the NCVIA's preponderance standard is to allow the finding of causation in a field bereft of complete and direct proof of how vaccines affect the human body." It further stated that, "close calls regarding causation are resolved in favor of injured claimants."

TABLE 83.5 National Vaccine Injury Compensation Program Summary of Modifications to the Vaccine Injury Table and Qualifications and Aids to Interpretation

Effective Coverage Date	Vaccine	Table Changes	Qualifications & Aids to Interpretation Changes	Federal Register Citation
3/10/1995	DTP and DTaP	*Removed*— Hypotonic-hyporesponsive episode *Removed*— Residual seizure disorder *Changed onset interval*— Anaphylaxis and anaphylactic shock (24 hours to 4 hours)	*Added*— Definition for anaphylaxis and anaphylactic shock *Revised*— Definitions of encephalopathy (or encephalitis) and residual seizure disorder	Fed Registr. 1995;60:7678—(FR)
	DT, Td, or TT	*Removed*— Residual seizure disorder *Changed onset interval*— Anaphylaxis and anaphylactic shock (24 hours to 4 hours)	*Added*— Definition for anaphylaxis and anaphylactic shock *Revised*— Definitions of encephalopathy (or encephalitis) and residential seizure disorder	Fed Registr. 1995;60:7678—(FR)
	MMR or any component	*Changed onset interval*— Anaphylaxis (24 hours to 4 hours); Encephalopathy (0–15 days to 5–15 days) Residual seizure disorder (0–15 days to 5–15 days)		Fed Registr. 1995;60:7678—(FR)
	Rubella containing	*Added*— Chronic arthritis, [onset interval: 0–42 days]	*Added*— Criteria for the diagnosis of chronic arthritis	Fed Registr. 1995;60:7678—(FR)
	IPV	*Changed onset interval*— Anaphylaxis and anaphylactic shock (24 hours to 4 hours)		Fed Registr. 1995;60:7678—(FR)
3/24/1997	DT, Td, or TT	*Added*— Brachial neuritis, [onset interval: 2–28 days] *Removed*— Encephalopathy	*Added*— Definition for brachial neuritis	Fed Registr. 1997;62:7685—(FR)
	MMR or any component	*Removed*— Residual seizure disorder		Fed Registr. 1997;62:7685—(FR)
	Rubella containing	*Changed onset interval*— Chronic arthritis (0–42 days to 7–42 days)		Fed Registr. 1997;62:7685—(FR)
	Measles containing	*Added*— Thrombocytopenic purpura, [onset interval: 7–30 days] *Added*— Vaccine strain measles viral infection in an immunodeficient recipient, [onset interval: 0–6 months]	*Added*— Definitions for thrombocytopenia and vaccine strain measles viral infection	Fed Registr. 1997;62:7685—(FR)
	OPV	*Added*— Vaccine strain polio viral infection, [onset interval: 0–30 days/0–6 months]	*Added*— Definition for vaccine strain polio viral infection	Fed Registr. 1997;62:7685—(FR)
	Added— Category for New Vaccines	*Added*— No condition specified, [onset interval: Not applicable]		Fed Registr. 1997;62:7685—(FR)
8/06/1997	*Added*—Hepatitis B (HBV)	*Added*— Anaphylaxis or anaphylactic shock, [onset interval: 0–4 hours]		Fed Registr. 1997;62:7685—(FR) Fed Registr. 1998;63:25777—(FR) Public Law: 105-34
	Added—*Haemophilus influenzae* type–b (Hib) [polysaccharide]	*Added*— Early-onset Hib disease, [onset interval: 0–7 days]	*Added*— Definition for early-onset Hib disease	Fed Registr. 1997;62:7685—(FR) Fed Registr. 1998;63:25777—(FR) Public Law: 105-34
	Added—*Haemophilus influenzae* type–b (Hib) [conjugate]	*Added*— No condition specified, [onset interval: not applicable]		Fed Registr. 1997;62:7685—(FR) Fed Registr. 1998;63:25777—(FR) Public Law: 105-34
	Added—Varicella (VZV)	*Added*— No condition specified, [onset interval: not applicable]		Fed Registr. 1997;62:7685—(FR) Fed Registr. 1998;63:25777—(FR) Public Law: 105-34

TABLE 83.5 National Vaccine Injury Compensation Program Summary of Modifications to the Vaccine Injury Table and Qualifications and Aids to Interpretation *(Continued)*

Effective Coverage Date	Vaccine	Table Changes	Qualifications & Aids to Interpretation Changes	Federal Register Citation
10/22/1998	*Added*—Rotavirus (RV) *to the New Vaccines Category*			Public Law: 105-277
	Added—Rotavirus (RV) *as a distinct vaccine category*	*Added*—No condition specified, [onset interval: not applicable]		Fed Registr. 1999;64:40517—(FR)
12/18/1999	*Added*—Pneumococcal conjugate (PCV-7) *to the New Vaccines Category*			Fed Registr. 2001;66:28166—(FR) Public Law: 106-170
8/26/2002	*Added*—Rotavirus (RV) –rhesus based	*Added*—Intussusception, [onset interval: 0–30 days]		Fed Registr. 2002;67:48558—(FR)
	Removed—Haemophilus influenzae type b (Hib) [polysaccharide]	*Removed*—Early-onset Hib disease	*Removed*— Definition for early-onset Hib disease	Fed Registr. 2002;67:48558—(FR)
	Added—Pneumococcal conjugate (PCV-7) *as a distinct category*	*Added*—No condition specified, [onset interval: not applicable]		Fed Registr. 2002;67:48558—(FR)
			Removed— Definition for residual seizure disorder	Fed Registr. 2002;67:48558—(FR)
12/01/2004	*Added*—Hepatitis A (HAV) *to the New Vaccines Category*	*Added*—No condition specified, [onset interval: not applicable]		Fed Registr. 2004;69:69945—(N) Public Law: 108-357
7/01/2005	*Added*—Trivalent influenza (TIV, LAIV) *to the New Vaccines Category*	*Added*—No condition specified, [onset interval: not applicable]		Fed Registr. 2005;70:19092—(N) Public Law: 108-357
2/1/2007	Added—Meningococcal (MCV4, MPSV4) and human papillomavirus (HPV) to the New Vaccines Category	*Added*— No condition specified, [onset interval: not applicable]		Fed Registr. 2007;72:19937 Public Law: 109-432
11/10/2008	Removed—Rotavirus (RV)–live, oral, rhesus-based	*Removed*—intussusception		Fed Registr. 2008;73:59528—(IFR)
7/22/2011	*Added*—Hepatitis A, trivalent influenza, meningococcal, and human papillomavirus vaccines *as distinct categories*	*Added*—No conditions specified, [onset intervals: not applicable]		Fed Registr. 2011;76:36367—(FR)
11/12/2013	*Added*—All vaccines against seasonal influenza to the New Vaccines Category	*Added*—No condition specified, [onset interval: not applicable]		Fed Registr. 2012;78:67369-01–(N)
7/23/15	*Added*— No new vaccines	*Added*— intussusception	*Added*— intussusception	80 Fed Registr. 35848 (June 33, 2015)—(FR)
3/21/17	*Added*— Seasonal influenza vaccines *as a distinct vaccine category* *Removed*—Trivalent vaccines *as a distinct vaccine category (now included in seasonal influenza vaccines category)* *Modified*— haemophilus influenza type b polysaccharide conjugate vaccines category changed to haemophilus influenza type b vaccines	*Many injuries added*—See Table 83.1	*Multiple definitions changed or added*—See Table 83.1	82 Fed Registr. 6294 (Jan. 19, 2017)—(FR); 82 Fed. Registr. (Feb. 22, 2017)—(FR)

The following parenthetical abbreviations found within the Federal Register Citation column—(FR), (N), and (IFR)—indicate final rule, notice, and interim final rule, respectively.

The Federal Circuit expanded the *Althen* holding in *Capizzano v Secretary of Department of Health and Human Services,*[58] in which it criticized the special master for inadequately considering the opinions of treating physicians. The court held that "medical records and medical opinion testimony are favored in vaccine cases, as treating physicians are likely to be in the best position to determine whether 'a logical sequence of cause and effect show[s] that the vaccination was the reason for the injury.'"

The Federal Circuit continues to refine the body of law regarding causation-in-fact. In *Walther v Secretary of Department of Health and Human Services,*[59] the Federal Circuit clarified that when the petitioner has proven all three *Althen* prongs, the petitioner is not required to eliminate other potential causes to prevail. With regard to the nature of the petitioner's evidence, in *Andreu v Secretary of Department of Health and Human Services,*[60] the Federal Circuit noted that in proving a medical theory of causation, the petitioner is neither required to rely on epidemiologic studies nor to show that the theory has general acceptance in the medical community. When petitioners offer medical literature or epidemiologic evidence, it is not to be evaluated through the lens of a laboratorian, but instead from the vantage point of the preponderance standard.

Most notably in *Andreu*, however, was the decreased level of deference given to the special master's credibility findings. Under prior case law, the Federal Circuit asserted that it is not its role "to reweigh…whether the special master correctly evaluated the evidence" or to "examine the probative value of the evidence or the credibility of the witnesses," because "these are all matters within the purview of the fact finder." The Federal Circuit in *Andreu* rejected the special master's credibility findings of the petitioner's expert witness, remarking that special masters may not cloak their evaluation of the evidence in the guise of a credibility finding. However, in *Moberly v Secretary of Department of Health and Human Services,*[61] the Federal Circuit renewed its deference to special masters and reasserted that special masters are expected and entitled to make reliability and credibility determinations of expert witnesses. Moreover, although petitioners are not required to offer proof in the form of epidemiologic studies or well-established medical experience, it does not mean that special masters are precluded from assessing the reliability of testimony from expert witnesses. This approach was reaffirmed by the Federal Circuit in *Porter v. Secretary of Department of Health and Human Services and Rotoli v. Secretary of Department of Health and Human Services,*[62] in which the Federal Circuit found that a Court of Federal Claims judge incorrectly read *Andreu* to prohibit a special master from using credibility determinations to reject a petitioner's theory of causation. Instead, the court averred, the Federal Circuit has "unambiguously explained" in previous opinions that special masters are expected to consider the credibility of expert witnesses in evaluating Vaccine Act claims. In litigating these difficult, fact-intensive, causation-in-fact cases, several special masters have indicated that a more expeditious resolution could be obtained by combining several cases in a consolidated or "omnibus" proceeding. As of this writing, numerous consolidated and omnibus proceedings have been initiated, and several have concluded. The results of some of these proceedings (e.g., hepatitis B vaccine and demyelinating diseases) have been controversial given the scientific literature.[63–65]

One such omnibus proceeding involved tuberous sclerosis complex (TSC), a genetic disorder characterized in many cases by classical skin lesions, growths (tubers), and other structural changes in the brain, causing seizures and mental retardation. Many of the claims alleging seizure onset within a VIT time frame were litigated as a group to determine whether DTP vaccine significantly aggravated the preexisting tuberous sclerosis complex condition. Based in part upon advances in magnetic resonance imaging in the 1990s,[66,67] the presiding special master ruled that the number and presence of tubers in the cerebral cortex, not the timing of DTP vaccination, ultimately determines clinical outcome. The special master's decision denying compensation was affirmed on appeal in *Hanlon v Secretary of Department of Health and Human Services,*[68]*Turner v Secretary of Department of Health and Human Services,*[69] and *Flanagan v Secretary of Department of Health and Human Services.*[70]

By far the omnibus proceeding that has garnered the most scrutiny is the omnibus autism proceeding. Interest and concern about vaccines and autism started with the publication of a 1998 case series by Wakefield and colleagues[71] in the *Lancet* suggesting that MMR vaccine was associated with autism. Little attention was paid in the United States until the following year when a congressionally mandated FDA review of mercury in biological products showed some vaccinated infants were receiving ethylmercury in excess of one federal safety guideline established for methylmercury, the more extensively studied form of organic mercury. Attention then became focused on thimerosal, an ethylmercury compound used for decades in the formulation of many routinely administered childhood vaccines to prevent bacterial and fungal contamination.[72] In the early 2000s, individual and class action lawsuits were filed in state courts alleging autism or autism spectrum disorder caused by thimerosal-containing vaccines and/or the MMR vaccine (which does not contain the preservative).[73] As noted below, the plaintiffs often argued that they were not required to subject to the NCVIA's exhaustion requirements with respect to claims concerning thimerosal. Multiple large, well-controlled epidemiologic studies all failed to find an association between thimerosal-containing vaccines and autism.[74–77] The IOM concluded in its 2004 report that the body of scientific evidence favors rejection of a causal relationship between MMR and thimerosal-containing vaccines and autism.[78] A 2012 IOM report reiterated the same conclusion for MMR vaccine and autism, and did not again examine thimerosal-containing vaccines as part of its review.[79]

The VICP began receiving claims alleging autism or autism spectrum disorder caused by MMR or thimerosal-containing vaccines in late 2001. As the number of claims grew to nearly 5000, the Office of the Special Masters established the omnibus autism proceeding to adjudicate the cases in a consolidated manner.[80]

The petitioners put forth six test cases to represent two separate theories of causation. Under the first theory ("Theory 1"), petitioners argued that MMR vaccine and thimerosal-containing vaccines cause autism. Under the second theory ("Theory 2"), petitioners argued that thimerosal-containing vaccines alone cause autism. The Office of the Special Masters conducted extensive evidentiary hearings, reviewing 939 medical articles, 50 expert reports, and testimony from 28 experts. The hearings produced more than 5000 pages of transcripts and 7000 pages of posthearing briefs. The three presiding special masters issued decisions in favor of the government in all six cases with respect to both theories.[81–86] Petitioners appealed the three Theory 1 cases to the Court of Federal Claims, without success.[87–89] Petitioners further appealed two of the three Theory 1 cases to the Court of Appeals for the Federal Circuit.[90,91] In both cases, the Federal Circuit ruled in favor of the government, upholding the lower determinations that petitioners had not proven their theory of causation by a preponderance of the evidence. Petitioners did not pursue appeals in the Theory 2 cases.[92]

In 2011, the Federal Circuit issued an *en banc* decision holding that the Vaccine Act's statute of limitations begins to

run on the date of occurrence of the first symptom or manifestation of onset of the injury, reversing a lower court decision that concluded the limitations period does not run until the medical community at large understands and recognizes a causal relationship between the vaccine and the injury.[93] Overruling prior precedent, the Federal Circuit also held that equitable tolling applies to the Vaccine Act. Equitable tolling is a principle of law stating that the statute of limitations shall not bar a claim in cases in which the petitioner, despite due diligence, could not or did not discover the injury until after the expiration of the limitations period. However, finding that equitable tolling under the Vaccine Act because an unawareness of a causal link between an injury and the administration of a vaccine is unavailable, the court declined to apply equitable tolling in the petitioner's case. The issue of whether attorneys' fees and costs were available in this untimely filed case was appealed to the U.S. Supreme Court, which affirmed the judgment of the Federal Circuit, finding that a person whose petition under the VICP is dismissed as untimely may recover an award of attorneys' fees and costs if certain requirements are satisfied.[94]

MODIFYING THE VACCINE INJURY TABLE

The Secretary of HHS has the authority to modify or amend injuries listed in the VIT (and QAIs) in consultation with the ACCV and after opportunity for public comment. Such changes apply only to cases filed after the effective date of the changes. In 1993, Congress provided a mechanism for the Secretary to add to the VIT "new" vaccines recommended by CDC for routine administration to children based on the effective date of the excise tax for that vaccine.[27] Table 83.5 summarizes changes to the VIT through March 2017.

Separate efforts by the VICP to modify the initial statutory VIT and QAIs began with publication of the two congressionally mandated IOM reviews in 1991 and 1994, respectively.[95–98] With a few exceptions, the approach by the VICP was straightforward: If the IOM concluded that there was evidence that a condition was "causally related" to a covered vaccine, it was added to the VIT or left on the VIT. However, if there was no proven evidence of an association, that condition was removed. Publication of final rules in 1995 and 1997 setting forth these modifications was the final step in a 3- to 4-year process for each set of changes involving ad hoc scientific panel reviews by the NVAC, mandatory ACCV reviews, and a 180-day public comment period, including a public hearing.

The first set of changes, effective March 10, 1995, was the most controversial. Hypotonic-hyporesponsive episode and residual seizure disorder were removed as covered conditions under DTP vaccines, and the definitions of encephalopathy and residual seizure disorder were modified in the QAIs.[52] At that time, the only exception in using the IOM conclusions to guide changes to the VIT was encephalopathy/encephalitis under DTP vaccine, which had been proposed for removal but was left in the Table in response to advice from the ACCV. The ACCV recommended that claims of acute encephalopathy of unknown etiology within 3 days of DTP vaccination should continue to receive a presumption of causation but that the definition in the QAIs needed to be more clinically precise. A subsequent 1994 analysis by the IOM[99] of a 10-year follow-up to the British National Childhood Encephalopathy Study tried to answer, but fell short of answering, the ultimate question of whether DTP vaccine causes permanent brain damage,[100] even though many investigators over time had failed to find a link based on epidemiologic and other scientific evidence.[101–107] The changes to the VIT and the QAIs for DTP have proved controversial and have been a topic addressed at congressional oversight hearings.

For the most part, DTP injury claims were filed on behalf of children and adults with chronic encephalopathy of unknown cause manifesting as developmental delay or the onset of seizures during the first year or two of life. Overall, no specific cause is ever determined for as many as 40% of the cases, with most thought to be caused by migrational abnormalities of fetal brain development or metabolic or "genetic" conditions not identifiable by current technology.[108] For petitioners to successfully pursue a VIT claim, the designated condition and time frame must be satisfied, and, if so, there must be insufficient evidence of a factor unrelated to the vaccine, which has to be of known cause and not idiopathic.

As for seizures, nearly half of the early DTP filings involved the initial onset of seizures in an otherwise healthy infant. Significant numbers of seizure claims were being compensated using the statutory version of the VIT originally put into law. Often the fever commonly associated with DTP vaccine would serve as the triggering event for the onset of seizures in a child predestined to have epilepsy. Alternatively, a Table seizure condition would be found in a child with cryptogenic (idiopathic) infantile spasms if the onset of myoclonic seizures was within 3 days of DTP vaccination. This resulted in compensated cases despite controlled epidemiologic studies showing the condition to be non–vaccine-related.[95,96]

Claims alleging death caused by DTP vaccine also proved challenging in the program's early years. Approximately half were attributed to sudden infant death syndrome (SIDS), with compatible histories and forensic findings in accordance with the 1989 National Institutes of Health consensus definition.[109] However, because the cause of SIDS remains unknown, these cases were initially viewed as "idiopathic" by the court and could not be used by the government to prove that a factor unrelated to the vaccine caused the death. Furthermore, based on testimony concerning events preceding the death, the court sometimes concluded that an encephalopathy or hypotonic-hyporesponsive episode was present before death and awarded compensation. More recently, however, the Federal Circuit has said that special masters may consider evidence of SIDS in weighing the strength of the petitioner's case.[110]

The second set of changes to the VIT proved far less controversial and was based, in large part, on the 1994 IOM report covering the five remaining original VICP vaccines, as well as Hib, HBV, and VZV vaccines, which were also added to the VICP because all were recommended by the CDC for routine administration to children. The other modifications, effective March 24, 1997, included the addition to the VIT of thrombocytopenia for measles-containing vaccines and brachial neuritis for tetanus-containing vaccines, the removal of residual seizure disorder under MMR vaccines, and the creation of a provisional or placeholder category on the VIT for vaccines newly taxed and satisfying the CDC recommendation requirement.[111] Only after notice to the public on publication in the *Federal Register* would the newly added vaccine have a separate and distinct listing on the VIT, including the addition of a Table condition when appropriate.

As with the 1995 rulemaking, proposed VIT changes developed by the Secretary paralleled the IOM conclusions for or against causation with two exceptions: the Secretary did not add GBS following OPV and following tetanus-containing vaccines. The IOM's OPV conclusion was based largely on a Finnish study following a national OPV campaign.[112] A subsequent U.S. study (therefore not considered by the IOM) showed no evidence of an increase in GBS following OPV administration.[113] Further doubt was cast when one of the Finnish study's coauthors wrote a letter noting that it was not their intention to claim that OPV was causally related to GBS because the data could be interpreted more than one way.[114]

The question of GBS and tetanus-containing vaccines was even more difficult to decide. The IOM conclusion was based on case reports, particularly of one person who experienced three episodes of GBS, each within weeks following tetanus vaccination. The fact that he had other non–vaccine-related episodes made him immunologically unique. Population studies, in contrast, showed no evidence that GBS incidence is higher in people receiving tetanus vaccine compared with the background rate.[97,98] Because there was no evidence of increased incidence overall, it was thought that petitions involving GBS following tetanus-containing vaccines should continue to require proof of causation, and, therefore, GBS should not be added to the VIT.

Since 1997, the VIT has been modified further (see Table 83.5). The general category of rotavirus vaccines was added effective October 21, 1998,[115] following licensure of RotaShield, a tetravalent rhesus-based rotavirus vaccine. RotaShield was subsequently withdrawn from the market by the manufacturer in October 1999, after epidemiologic studies confirmed an association between the vaccine and cases of intussusception, a potentially life-threatening bowel obstruction that occurs in infants.[116–118] In July 2002, a final rule was published adding intussusception as a listed injury to the VIT under a second category of rotavirus vaccines (i.e., live, oral, rhesus-based).[119] In addition, pneumococcal conjugate vaccines were made a separate and distinct category on the VIT. The second category of rotavirus vaccines (i.e., live, oral, rhesus-based), with the associated injury of intussusception, was removed from the VIT, effective November 10, 2008.[120] This was a technical change, since the 3-year filing deadline for all such claims had long since passed given that the RotaShield vaccine was withdrawn from distribution in the United States in 1999. The general category of rotavirus vaccines with "no condition specified" was not affected.

Beginning in 2004, hepatitis A and trivalent influenza, meningococcal (conjugate and polysaccharide) and HPV vaccines were added to the VIT in the placeholder category for "new" vaccines recommended by CDC for routine administration to children.[121–123] However, the vaccines were subsequently moved to separate and distinct categories in the VIT, effective July 22, 2011.[124] On November 12, 2013, all seasonal influenza vaccines (e.g., quadrivalent influenza vaccines) were added to the VIT in the placeholder category for "new" vaccines.[125] Subsequent to the licensure of two new rotavirus vaccines in 2006 and 2008, new evidence from postmarketing studies suggested intussusception was causally associated with Rotarix and RotaTeq, although much more rarely than had been the case with RotaShield.[154–156] Effective July 23, 2015, intussusception was added to the VIT as an injury for the rotavirus vaccines.[125a]

The 2012 IOM review reported the results of its reviews concerning the evidence with respect to causal associations between 12 VICP-covered vaccines (in various combination) and 158 adverse events.[79] After release of the 2012 IOM report, medical staff at the Health Resources and Services Administration (HRSA) and the CDC reviewed the IOM conclusions as well as other medical and scientific literature not contained in the IOM report. Based primarily on the IOM's review, the work of nine HHS workgroups that reviewed the IOM findings, and consideration of the ACCV's recommendations, a final rule to substantially revise the VIT was published on January 19, 2017.[125b] As of early March 2017, this rule was scheduled to go into effect on March 21, 2017.[125c]

The 2017 final rule included numerous revisions to the VIT. It added two injuries, Shoulder injury-related vaccine administration (SIRVA) and vasovagal syncope, which were found by the IOM to be associated with the injection of a vaccine rather than the components of a particular formulation of vaccine. Thus, the final rule included SIRVA and vasovagal syncope as injuries for all injected vaccines on the VIT. Further, the rule revised the QAI for anaphylaxis and added it as an injury for the varicella, seasonal influenza, meningococcal, and HPV vaccines based on the IOM's findings that the scientific evidence convincingly supports a causal relationship. Additionally, stemming from the IOM's findings, the final rule added vaccine-strain measles viral disease in an immunodeficient recipient to the VIT as an injury for vaccines containing the measles virus, and disseminated vaccine-strain viral disease and varicella vaccine-strain viral reactivation as injuries for varicella vaccines.

Following the recommendation of the ACCV, the final rule also added the injury of GBS to the VIT for seasonal influenza vaccines. The IOM found the evidence inadequate to accept or reject a causal relationship between seasonal influenza vaccines and GBS. However, studies have demonstrated a causal association between some nonseasonal influenza vaccines—the monovalent 2009 H1N1 vaccine and the 1976 swine flu vaccine—and GBS. To date, the H1N1 antigen has been included in all seasonal influenza vaccines beginning with the 2010–11 flu season. Acknowledging that seasonal influenza vaccine formulations, unlike other vaccines, include multiple antigens that change from year to year, and enhanced surveillance activities to detect the incidence of GBS that occurred during the 2009 H1N1 pandemic may not occur with each virus strain change, the ACCV recommended that the Secretary add GBS to the VIT consistent with one of the ACCV's Guiding Principles: where there is credible evidence to both support and reject a change to the VIT, the change should, whenever possible, be made to the benefit of petitioners.

Finally, the final rule included many organizational and structural changes to the VIT and QAI designed to increase clarity and scientific accuracy. For example, it added definitions for terms that had previously been on the VIT or QAIs but were undefined, such as encephalitis and immunodeficient recipient; it updated some of the QAIs to address certain changes in scientific nomenclature; it streamlined the VIT with the deletion of redundant wording; and it changed the category of "trivalent vaccines" to "seasonal influenza vaccines" to cover all seasonal influenza vaccines, including trivalent influenza vaccines, in one category.

MEDICAL REVIEW OF CLAIMS

Most claims filed under the VICP had some clinical outcome in temporal relation to vaccination that varied from the expected side effects of crying, fever, and local swelling to much more serious acute and chronic illness. The claims represent a database of possible vaccine-related events, although only a small percentage of serious outcomes were thought to be caused by vaccines after VICP medical staff review. Some of the more relevant allegations are reviewed here.

Diphtheria, Tetanus, and Pertussis Vaccines

DTP/DTP-Hib Vaccines

Of the thousands of petitions filed for DTP and DTP-Hib vaccines, 84% of them reported outcomes that occurred after the primary series of immunization in children younger than 12 months. In 1997, the CDC recommended that diphtheria, tetanus, and acellular pertussis (DTaP) be used for all doses in the childhood immunization schedule.[126] Prior to that time 33% of all claims associated with the DTP vaccine alleged an injury of seizure disorder (including epilepsy and infantile spasms). Another significant category was the 34% of claimants who reported an injury of encephalopathy. Six percent of claims were related to mental retardation/developmental delay.

Approximately 42% of claims alleging DTP-related death stated that the death was related to SIDS. The next most frequent diagnostic category for alleged death cases involved the 27% of patients with reported encephalopathy followed by the 12% of those who reported the occurrence of a seizure disorder. Approximately 30 deaths were allegedly the result of anaphylaxis.

Approximately 682 DTaP (and any combination of DTaP vaccines) claims had been filed by February 2017, which make up approximately 5% of filings from 2012 to 2017, and 4% of claims overall. While similar types of neurologic conditions have been alleged to be caused by the acellular pertussis vaccine, these are reported in far fewer numbers than the whole-cell DTP product (207 cases of encephalopathy and 17 cases of seizures). Postmarketing surveillance of serious adverse events following DTaP continues to confirm the better safety profile versus the whole-cell product.[127,128] Tetanus, diphtheria, and pertussis (Tdap) claims also comprised 9% during the same 5-year period, with alleged injuries similar to those seen with other tetanus-containing vaccine claims.

Tetanus-Containing Vaccines

Claims involving tetanus-containing vaccine not including a pertussis component (i.e., diphtheria and tetanus toxoids–pediatric [DT], diphtheria and tetanus toxoids–adult [Td], or tetanus toxoid [TT]) comprise 2% of filings overall. Brachial neuritis (17%), a condition listed on the VIT, is the most frequently alleged or documented condition. After that come GBS (16%), encephalopathy (3%), and seizure disorders (2%). Nearly all (90%) are adult vaccine recipients. Litigating GBS claims has been particularly challenging. Few were decided before the 1994 IOM report which concluded that tetanus-containing vaccines could cause GBS if the onset was within 5 days to 6 weeks following immunization.[97,98] In one case in which compensation was not provided, the court found that, although Td can cause GBS, one could not conclude that it did cause GBS in any particular case. The decision was appealed but was affirmed by the U.S. Court of Appeals for the Federal Circuit.[129] In a subsequent report, the IOM concluded that the evidence is inadequate to accept or reject a causal relationship between tetanus containing vaccines and GBS.[79] From 2012–17, approximately 46% of these claims have been compensated on the basis of a litigation risk settlement.

Measles-Mumps-Rubella Vaccines

Overall, MMR-containing vaccines are implicated in 7% of claims, most of which are events associated with immunization during the second year of life. Natural measles infection is known to cause acute encephalopathy/encephalitis and 24% of claims were filed alleging this injury, a VIT condition with a prescribed onset of 5 to 15 days following vaccination. Although the IOM found the evidence to be "inadequate" to determine whether measles vaccine can cause acute encephalopathy, there is some evidence of clustering of encephalopathy/encephalitis cases whose onset is 8 to 10 days following measles-containing immunization, based on an analysis of 48 VICP claims.[130] Only rarely are biological markers demonstrated to support a genetic, metabolic, or vaccine cause. In only one VICP case was cerebrospinal fluid measles antibody, a biological marker for measles causation, found in a previously healthy 2-year-old girl who received the first dose of MMR and varicella vaccine.

There were several other injuries that were alleged in VICP cases following the receipt of MMR-containing vaccines (e.g., seizure disorders). Another common filing alleged that MMR vaccine caused thrombocytopenic purpura (5%), a VIT condition with prescribed onset of 7 to 35 days following vaccination.

Claims alleging injury from rubella vaccine comprise 1% of claims overall. Most (75%) are filed on behalf of adults. Twenty-four percent of claims filed alleged that arthritis or rheumatoid arthritis developed after rubella immunization. Although 13% to 15% of susceptible (serology-negative) women may experience transient arthritis after the currently used rubella vaccine, with a higher percentage (up to 40%) reporting some type of musculoskeletal complaint, it is less clear what role, if any, the vaccine has in causing recurrent or chronic arthropathy (i.e., arthralgia or arthritis).[95,96]

Based on the 1991 IOM finding that chronic arthritis may be caused by rubella vaccine, a special master held a hearing to determine criteria necessary for proof of causation. Following the special master's publication of these guidelines, the VICP added chronic arthritis to the VIT in the 1995 final rule[52] and incorporated several of the court's criteria in the 1997 final rule.[111] The main difference between the criteria adopted by the VICP and those adopted by the court has been the latter's willingness to compensate arthralgia, a subjective symptom that is difficult to assess, unlike observable signs of arthritis. Additional research published since the IOM report, including retrospective case reviews and a prospective double-blind study, has produced mixed results. If rubella vaccine does cause chronic arthropathy, it would seem that the incidence is rare.[131–133] The most recent IOM report also concludes that the evidence supports a causal relationship between MMR vaccines and transient arthralgia in women and children. However, this report also concluded that the evidence is inadequate to support or refute a causal relationship for chronic arthritis in women, chronic arthropathy in women and children, as well as arthropathy in men.[79]

Varicella Vaccine

Varicella vaccine, which was licensed in March 1995, became a covered vaccine in August 1997, after enactment of the excise tax. By February 2017, the VICP had received at least 98 claims in which varicella vaccine was administered alone. The group comprised 38 children between 12 and 18 months of age, 39 older children, and 19 adults. Neurologic conditions were the predominant injury category (e.g., encephalopathy/encephalitis GBS, and seizures), followed by hematologic disorders (e.g., thrombocytopenia), ear, nose, and throat disorders (e.g., hearing loss), and other conditions. Various neurologic disorders have been reported to be associated with primary VZV (wild-type) infections, including meningoencephalitis, encephalitis, transverse myelitis, and acute cerebellar ataxia.[134] Vasculitis and acute cerebellar ataxia also have been reported following receipt of varicella vaccine.[135] Most of these conditions were reflected in the claims filed. The vaccine-strain genotype by polymerase chain reaction was identified in skin lesions of two persons.[136] Since 2012, 17 measles-mumps-rubella-varicella (MMRV) and 26 VZV claims have been filed, reflecting a shift to the combination product following licensure in 2005.[137]

The revised VIT added syncope and SIRVA as well as disseminated varicella vaccine-strain viral disease.

Hepatitis B Virus Vaccine

More than 740 hepatitis B–containing vaccines (e.g., HBV, HAV-HBV, Hib-HBV) vaccine claims have been filed as of February 2017, which represent 2% of all VICP claims. Claims alleged the following categories of injuries: encephalopathy/encephalitis (4%), GBS (4%) and various arthritic conditions

(4%). Vaccine recipients ranged in age from birth to 69 years, with 47% pediatric claims (younger than 18 years), of which 24% were for children younger than 2 years.

In general, there are no serious adverse events linked to the recombinant vaccine other than acute anaphylaxis.[97,98] In April 2002, the IOM Vaccine Safety Review Committee published its findings regarding HBV vaccine and neurologic disorders. The IOM concluded that there is evidence against a causal relation between HBV vaccine and multiple sclerosis and relapse, and insufficient evidence for other central or peripheral nervous system demyelinating conditions.[65]

In 2012, the IOM revised its previous conclusions and stated that the current evidence was inadequate to accept or reject a causal relationship between hepatitis B vaccines and the onset or relapse of multiple sclerosis among children and adults, and affirmed the conclusion that inadequate evidence exists to support or reject a causal relationship for other central or peripheral nervous system demyelinating conditions.[79] Approximately 37% of all the HBV vaccine claims were filed in 1999 before the 2-year window for filing retroactive claims expired. Working with the DOJ and the two petitioners' counsel who filed the majority of these cases, the court agreed to group the claims into diagnostic categories as a means of managing the large volume. After many years of attempting to convene an expert panel to deal with the constellation of cases and matrix of various injuries, the court abandoned the concept, and the cases were reassigned in early 2006 to three newly hired special masters. As a result, the claims are increasingly being assessed individually, not necessarily in relation to other claims involving similar allegations of injury.

In addition to individual hearings, there have been several omnibus proceedings using test cases with similar diagnoses to help move the backlog of claims.

A special master in the Hepatitis B–Neurological Demyelinating Omnibus Proceeding[138] ruled that HBV vaccine can cause transverse myelitis,[138] GBS,[139] multiple sclerosis,[140] and chronic inflammatory demyelinating polyneuropathy.[141] However, another special master ruled differently,[142] consistent with the government's position based on scientific evidence.[143–145] Another consolidated hearing resulted in the special master ruling that the HBV vaccine did not aggravate petitioner's type 1 diabetes mellitus.[146] In another proceeding, the special master ruled that the petitioners never offered a medical theory causally connecting the thimerosal contained in HBV vaccine to sudden death.[147] Finally, a split decision on appeal followed the special master's decision in five cases involving allegations of HBV and autoimmune hepatitis. In this proceeding, the same theory was offered and the same experts testified, but the facts of each of the cases were presented separately, and the special master wrote a separate decision on each of the cases. The special master ruled in favor of HHS in all five cases, but only two were affirmed on appeal by the Court of Federal Claims.[148,149] Ultimately, HHS appealed two of the three cases reversed by the Court of Federal Claims to the U.S. Court of Appeals for the Federal Circuit, which reversed the Claims Court's findings and remanded with instructions that a decision be entered affirming the special master's denial of compensation.[62]

Since 1999, some claims outside the aforementioned groupings have been adjudicated with varied results. Aside from anaphylaxis or anaphylactic shock, the only condition listed on the VIT for HBV vaccine, all HBV claims are being adjudicated on a causation-in-fact basis.

Polio Vaccines

Polio vaccine–related claims totaled approximately 460 of the pre-1988 case filings. IPV is a killed virus vaccine and, if properly manufactured, is not associated with paralytic poliomyelitis. It is reasonable to expect that some persons acquired natural poliomyelitis in temporal relationship to receipt of IPV, particularly if only one or two doses were given. The 1955 Cutter incident, in which an estimated 260 cases of paralytic poliomyelitis were attributed to residual live virus in the vaccine, is well documented.[150] No similar instances of killed vaccine–related poliomyelitis were publicly identified, although there is evidence that at least one lot of Wyeth vaccine given during this same period had infective amounts of live virus.[151] Of 278 IPV claims, nearly all (97%) have been dismissed by the court, with none being found to be secondary to Cutter vaccine administration. Only 13 claims are for vaccines administered since 1979, when the last indigenous case of wild poliovirus transmission occurred in the United States.[152]

OPV claims were approached much differently because paralytic polio is known to be a rare complication in vaccine recipients and in contacts and, therefore, is listed in the VIT. More than half (73%) of the claims alleged an injury of poliomyelitis. Naturally, it is difficult to know which cases were actually vaccine-related when polio was present in the community. Of the 308 claims filed alleging OPV-related injury (or death), 158 were compensated and 150 were dismissed.

Rotavirus Vaccine

Claims alleging intussusception from the rhesus-based rotavirus vaccine (RotaShield) started appearing in 2000. Some claims filed before the final rule adding intussusception as a VIT condition were compensated on a causation-in-fact basis, a burden that was made easier with epidemiologic studies confirming an association.[116–118] Once epidemiologic studies confirmed an association between rotavirus vaccine and intussusception in the first 2 weeks after immunization, HHS began the process for revising the VIT. A 2002 final rule added intussusception to the VIT under a second category of rotavirus vaccines (i.e., live, oral, rhesus-based), with an onset interval of 0 to 30 days following immunization.[119] The wider interval was chosen to provide a generous presumption of causation. Once intussusception was added to the VIT, petitioners otherwise barred under the general statute of limitations benefited from a presumption of causation in claims involving intussusception and had 8 years of retroactive coverage from the effective date of the VIT change, with a 2-year window in which to file their claims.

Ironically, the benign outcome of most cases of intussusception proved challenging for the VICP. Until October 2000, the NCVIA required all claimants to establish that the residual effects of an injury persisted for more than 6 months after vaccination or that a death occurred.

Because most patients with intussusception recover completely within days, some petitioners might be denied compensation under that standard. However, the Children's Health Act of 2000 amended the NCVIA to permit payment of compensation in claims in which the effects of the injury last less than 6 months if the petitioner demonstrates that the vaccine-related illness, disability, injury, or condition "resulted in inpatient hospitalization and surgical intervention."[153] Thus, under current law, infants who experienced intussusception following a rotavirus vaccine and did not have residual effects for more than 6 months qualified for compensation if their injury resulted in inpatient hospitalization and surgery.

With licensure of two new rotavirus vaccines in 2006 and 2008, the program once again began receiving claims alleging intussusception. At the time of publication, new evidence from postmarketing studies suggested intussusception was

causally associated with Rotarix and RotaTeq, although much more rarely than had been the case with RotaShield.[154–156] On July 24, 2013, HHS published a Notice of Proposed Rulemaking seeking comments about its proposal to add the injury of intussusception to the VIT for rotavirus vaccines.[157]

Since the beginning of the program, 79 injury claims had been filed, 43 of those alleged an injury of intussusception. Of all the claims that have been adjudicated, 51 received compensation and were dismissed.

Hepatitis A Virus Vaccines

HAV vaccines were added to the VICP in 2004. Since then, fewer than 1% of nonautism claims filed alleged injury from HAV vaccine. Petitioners ranged in age from 0 to 70 years, with approximately 55% younger than age 18 years. No patterns in claimed injuries emerged, which included liver-related conditions (hepatitis), neurologic disorders (seizure disorder, acute disseminated encephalomyelitis, transverse myelitis, GBS), and various immunologic conditions. The majority of cases heard before the court have been dismissed.

Meningococcal Vaccines

Meningococcal polysaccharide and conjugate vaccines were added to the VIT on February 1, 2007. Fewer than 1% of claims filed since then have alleged injury from these vaccines, and none involved the polysaccharide product. More than one third of the claimants had at least one other vaccine coadministered. The majority of MCV4 claims involved demyelinating conditions, with GBS found in nearly 26% of claims. Other demyelinating disorders found were transverse myelitis, chronic inflammatory demyelinating polyneuropathy, and multiple sclerosis.

Of the 43 claims that have been adjudicated, 84% have received compensation. A report by the CDC in 2006 suggested a small increase in the risk of GBS among meningococcal conjugate vaccine recipients.[159] However, two large unpublished postlicensure safety studies presented to the Advisory Committee on Immunization Practices in June 2010 found no evidence of increased GBS risk following vaccination.[160] The most recent IOM report found that the evidence was inadequate to determine a causal relationship between meningococcal vaccine and GBS.[79]

Influenza Vaccines

Trivalent influenza vaccines (TIV [trivalent inactivated influenza vaccine], LAIV [live attenuated influenza vaccine]) were added to the VICP in July 2005. In 2013 all seasonal influenza vaccines, including the quadrivalent influenza vaccines, were added to the VIT. Since July 2005, approximately 51% of the total VICP claims filed allege an influenza vaccine injury, with increasing numbers being filed each year. Moreover, since 2007, nearly 76% of VICP claims filed are for adults, which is a decided shift from the first 15 years of the program when most claims were filed on behalf of children.

As of February 2017, 3301 petitions had been filed claiming an injury caused by an influenza vaccine. Only 6% were for petitioners younger than 18 years of age. GBS comprised 36% of the claims. In fact, claims alleging GBS are now the most common vaccine injury claim filed annually. Other demyelinating conditions made up a significant portion of influenza claims, which included transverse myelitis, chronic inflammatory demyelinating polyneuropathy, and acute disseminated encephalomyelitis. The remaining claims involved various allegations, such as encephalopathy, seizures, brachial neuritis, and a range of neuromuscular conditions.

After medical review, it was found that more than 380 claims involved a shoulder injury related to vaccination, an injury apparently from the unintentional injection of antigenic material into synovial tissues resulting in an immune-mediated inflammatory reaction. A case series of 13 VICP cases is described in a 2010 report.[161] Most of these claims were conceded by HHS.

No specific injury related to influenza vaccine is listed in the VIT, and HHS has concluded that certain claims have demonstrated injury compensation by a preponderance. Such claims included anaphylaxis,[162] syncopal episodes resulting in severe injuries, and shoulder injury related to vaccine administration. However, it is not uncommon for influenza claims to receive compensation on the basis of a litigation risk settlement (76%) because of the evolved causation standard.

A new challenge faced the VICP after increased rates of GBS were detected following the 2009–10 monovalent influenza pandemic campaign. A metaanalysis of the Vaccine Safety Datalink, Emerging Infections Program (an active population-based surveillance program), and Post-Licensure Rapid Immunization Safety Monitoring programs that analyzed data from 23 million people who were vaccinated with the influenza A (H1N1) 2009 monovalent vaccine found that the 2009 H1N1 inactivated vaccine (a vaccine not included in the VICP) was associated with a small increased risk of GBS within 6 weeks of vaccination.[163] This excess risk is equivalent to 1.6 excess cases in the 6 weeks after vaccination per million people vaccinated. A separate program within HHS is responsible for alleged injuries or deaths related to use of the monovalent product and vaccines against pandemic influenza (see the discussion of the Countermeasures Injury Compensation Program [CICP] in "Related Liability Protection and Compensation Schemes" later). Incorporation of the H1N1 pandemic virus into the seasonal influenza vaccine first occurred with the 2010–11 trivalent vaccine and this strain has been included in seasonal formulations since. Presently, there is no scientific evidence demonstrating that current formulations of the seasonal influenza vaccine, which contain the H1N1 virus, can cause GBS.[79,164,165] However, the degree of surveillance needed to detect an increased risk of approximately one case per million vaccinations, as was seen with the monovalent 2009 H1N1 vaccine, is unlikely to be routinely performed as the strains in the flu vaccines change from year to year. Nonetheless, numerous studies have been conducted to determine whether a possible association between seasonal influenza vaccines and GBS exists, and almost all have not shown any causal relationship.

Human Papillomavirus Vaccines

HPV vaccines were added to the VICP in February 2007. By February 2017, 345 had been filed. The majority of injury claims alleged neurologic injuries, such as GBS, transverse myelitis, and acute disseminated encephalomyelitis. Other neurologic categories included headaches and seizures. There were also significant numbers of claims alleging rheumatologic disorders, including rheumatoid arthritis, systemic lupus erythematosus, and fibromyalgia. One hundred thirteen cases received compensation and of those, 13 injury claims were resolved through a concession of entitlement by HHS. Of the claims that were conceded, the majority of claims (54%) involved injuries resulting from a syncopal episode, one involved complex regional pain syndrome, and one claim involved an injury from the injection process or shoulder injury related to vaccine administration.[161]

There were 14 claims of HPV vaccine–related death. All were adolescents and young adults ages 11 to 21 years. Evidence demonstrating a relationship between the vaccine and

the subsequent death was lacking. Seventy-three percent of claims had been adjudicated by February 2017.

ISSUES OF INTEREST

During the last two decades, the greatest challenge to the VICP has been adjudicating causation-in-fact claims. This depends heavily on the availability of credible and timely scientific evidence. Only through a comprehensive vaccine adverse event surveillance and research program can causal versus coincidental reactions following vaccination be distinguished. Rotavirus vaccine causing intussusception is an example of how epidemiologic research led to expedited compensation once intussusception was found to be vaccine-related. At the same time, safety studies allow for the debunking of erroneous theories of vaccine causation, assisting the court in dismissing claims not shown to be vaccine-related. The creation of IOM committees to perform independent, expedited scientific reviews of current and emerging vaccine safety hypotheses (see discussion of the IOM Immunization Safety Review Committee in Chapter 80) have been an important step in ensuring continued evaluation of the safety of vaccines.

Ten reports issued from 2001–13 have been helpful to HHS and the court in clarifying the current scientific literature on vaccine adverse events.[167] At the same time, securing adequate funding for vaccine safety research overall remains one of the greatest challenges at a time of budget deficits and competing priorities for vaccine program funding. In 2008, the Health Resources and Services Administration engaged the IOM to establish a new IOM committee to review the medical and scientific literature to assess the causal relationship, if any, between 78 adverse events (158 adverse event–vaccine combinations) and 12 VICP-covered vaccines. The consensus report was released in August 2011 and included a lengthy discussion of each adverse event–vaccine combination, as well as a discussion of its approach in assessing vaccine causation generally.[79] The report may result in additional modifications to the VIT by the Secretary.

Criticism by petitioners and their attorneys led to oversight by Congress in the VICP's early years. Government Accountability Office (GAO) reports issued in 1999 and 2000 addressed the VICP process and trust fund, respectively.[168,169] The VICP adjudication process was judged to be easier than the traditional tort system, but not as streamlined as Congress had originally intended. Moreover, although there seemed to be a scientific basis for the VIT changes made by HHS, the GAO found some inconsistencies in applying results of the IOM reviews. The trust fund and its huge, ever-increasing size was the focus of the second effort by GAO. Potential solutions varied depending on stakeholder perspective, with petitioners wanting more claims compensated by decreasing the burden of proof, vaccine companies wanting the federal excise tax reduced to lessen cost, and researchers calling for a portion of incoming revenues annually to pay for vaccine safety research and surveillance. In the end, the GAO's only recommendation was that HHS publish a clear method for future changes to the VIT to help ensure that such changes are perceived as fair.

It should be noted that guidance regarding recommending changes to the VIT has been provided. The HHS National Vaccine Plan recommends updating the VIT in response to evolving science.[170] In 2006, the ACCV adopted principles that should guide their recommendation of changes to the VIT: (a) the VIT should be scientifically and medically credible, and (b) where there is credible scientific and medical evidence to both support and to reject a proposed change to the VIT, the change should, whenever possible, be made to the benefit of the petitioner. [171]

The GAO issued another report in 2014 that examined the adjudication process time, changes to the VIT, and how the balance of and spending from the Trust Fund have changed.[172] It was found that claims filed since 2009 were resolved within 1 to 2 years and that the majority of compensated claims (80%) were resolved through a negotiated settlement. It was also noted that although six new vaccines had been added to the Table since 1999, additional covered injuries associated with these vaccines had not been added, which means that each petitioner must demonstrate that the administered vaccine did, indeed, cause their alleged injury. Finally, it was found that the income to the Trust Fund exceeded the expenditures. The GAO made no recommendations. However, the GAO did conclude that HHS's outreach efforts would be important to ensure that potential petitioners are aware of the program.

Concerns that helped lead to the creation of the VICP (e.g., fears regarding liability and the availability of compensation in relation to adverse events resulting from products) have surfaced with regard to vaccines not covered by the VICP (because they are not routinely administered to children) and to other countermeasures used to prevent and treat particular diseases.

Some interested parties have suggested adding more selective-use vaccines to the VICP, such as pneumococcal polysaccharide, used primarily in adults. An NVAC review in 1996 found little evidence at that time to support the need, based on a lack of liability concerns by manufacturers and healthcare providers.[173]

In more recent years, the issue of maternal immunization (or vaccines administered to pregnant women) has received significant attention. For some time, certain stakeholders have pointed to the need to cover pregnant women for vaccines in development to prevent respiratory syncytial virus, cytomegalovirus, and group B streptococcal infections, which cause significant morbidity and mortality in very young infants.[174–176] A wide variety of parties has cited liability concerns as a key barrier to licensure of new vaccines for maternal immunization Maternal immunization has raised several complex issues under the NVCIA. One concern was that new categories of vaccines intended exclusively for pregnant woman could not be covered by the NVCIA (which extends the statute's liability protections and permits compensation under the VICP). Beyond vaccines included on the original statutory VIT, such coverage could extend only to those recommended by the CDC for routine administration to children and subject to an excise tax. Other concerns were raised with respect to categories of vaccines covered under the NVCIA that are administered to pregnant women (DTaP vaccines and seasonal influenza vaccines), such as the NCVIA's requirement that to be eligible for compensation, a person must have "received" a covered vaccine (putting aside a narrow category for persons who contracted polio from persons who received the OPV) and a requirement that only one petition may be filed per vaccine administration. No binding court decision resolved these issues.

The issue of maternal immunization generated significant interest, partly given the significant public health benefits that could be achieved with new categories of vaccines for pregnant women. In 2013, the ACCV recommended that the Secretary work to expand coverage under the VICP to include vaccines recommended for routine administration to pregnant women (and not specifically recommended for routine administration to children) and support eligibility to pursue compensation for injuries sustained by a liveborn infant whose mother receives a vaccine while the infant is in utero. In 2014, the NVAC released a report, "Reducing Patient and Provider Barriers to Maternal Immunization," recommending that the

Assistant Secretary for Health support efforts to address the issue of in utero injuries allegedly incurred following maternal immunization within the VICP.

On December 2016, the 21st Century Cures Act made changes to the NVCIA in connection with vaccines administered to pregnant women and claims concerning injuries alleged to have occurred in utero.[176a] First, the 21st Century Cures Act added a new category of vaccines to the NVCIA's coverage: vaccines recommended by the CDC for routine administration to pregnant women and subject to an excise tax. While no vaccines fell within this category as of March 2017, this amendment ensures that vaccines designed for administration in pregnant women (perhaps for the protection of their later-born children) could be covered vaccines under the VICP and trigger the NVCIA's related liability protections. Second, the 21st Century Cures Act amended the Vaccine Act to provide that if the vaccination of a pregnant woman leads to injuries both in the woman and in her later, live-born child, claims could be pursued with respect to both alleged injuries in the VICP. Finally, the law now provides that claims may be filed within the VICP on behalf of live-born children alleging injuries alleged to have been sustained in utero as a result of maternal immunization and that vaccine administrators and manufacturers will be afforded the NVCIA's related liability protections, issues that were unresolved prior to this legislation.

RELATED LIABILITY PROTECTION AND COMPENSATION SCHEMES

Despite the successful global eradication of smallpox in 1980, terrorist attacks in 2001 led to concerns that terrorists might have access to the smallpox virus and might attempt to use it against Americans. As part of President Bush's plan to protect the American population from the threat of a smallpox attack, liability protections were enacted to protect covered persons in relation to their involvement in the federal smallpox vaccination campaign. Covered persons included manufacturers of smallpox vaccines, healthcare entities, and healthcare providers under whose auspices the vaccine was administered and state and local governments, including first responders. The liability protections[177,178] were meant to ensure that the smallpox vaccine and other related smallpox countermeasures were available, if necessary, to protect the public health.

Under the liability provisions, no lawsuit for liability for injuries or deaths arising out of the administration of a smallpox vaccine or other smallpox-covered countermeasure named in a declaration could be maintained against covered persons. Instead, such claims had to be pursued against the United States as a substitute defendant. Such claims were to proceed under the Federal Tort Claims Act, which requires plaintiffs to demonstrate negligence or another cognizable tort. Although these liability protections were broad, they were subject to certain limitations. For example, if a covered person failed to cooperate with the government in the defense of a claim, the government would not be liable for any damages resulting from that person's act or omission. Also, if the United States made a payment on a claim that was based on bad acts or omissions by a covered person (e.g., gross negligence), the United States was entitled to recover funds from the covered person.

These liability protections were triggered in January 2003, when the Secretary issued a declaration concluding that a potential bioterrorist incident made advisable the administration of particular smallpox countermeasures (including smallpox vaccines, products designed to prevent or treat smallpox, and products designed to control or treat adverse events from smallpox vaccination).[179] The Secretary recommended that certain categories of persons (including first responders and members of approved smallpox response teams), receive the covered countermeasures.

As a companion to these liability protections and as incentive for the targeted persons to receive smallpox countermeasures, Congress authorized the Secretary to establish the Smallpox Vaccine Injury Compensation Program (SVICP) to provide medical, lost employment income, and death benefits to eligible persons (including smallpox vaccine recipients; persons who came into contact with a vaccinated person or with another person with whom the vaccinated person had contact, known as "vaccinia contacts"; and the estates and survivors of otherwise eligible deceased persons).[180] Among the eligibility requirements, requesters had to demonstrate an injury that resulted from a covered countermeasure. Like the VICP, requesters could demonstrate this by showing that the injured person sustained an injury included in an injury table promulgated by HHS within the table time frame or by demonstrating that the person's injury was actually caused by the vaccine or other covered countermeasure. Unlike the VICP (in which claims are litigated in court), the SVICP was a purely administrative program operated by the HHS. Persons eligible to file SVICP claims had to exhaust their remedies with this program before filing any action against the United States. We are not aware of any post-SVICP claims filed against the United States. A total of 64 persons applied for benefits with the SVICP, and 19 persons received compensation. The SVICP is no longer operational and was terminated in 2008 owing to a lack of funding, among other factors. The smallpox vaccine is now a covered countermeasure under the CICP, discussed below.

More recently, concerns about a potential pandemic and the possibility that the threat of liability would inhibit manufacturers from producing vaccines and other countermeasures needed to prevent or treat a pandemic or other emergency event (and would inhibit healthcare professionals and others from distributing and administering such countermeasures) led to the enactment of the Public Readiness and Emergency Preparedness Act (PREP Act), within the Department of Defense Appropriations Act, in 2005.[181] The PREP Act offers extremely broad liability protections to manufacturers and other persons and entities (e.g., distributors, program planners, administrators) for all claims of loss resulting from the creation, distribution, and administration of certain products if the Secretary issues a declaration determining that a disease or other health condition or threat constitutes a public health emergency or that there is a credible risk that the disease, condition, or threat may in the future constitute such an emergency and recommending that particular countermeasures should be administered or used. In such a declaration, the Secretary must explain his or her findings and the parameters of the attendant liability protections, including the category of diseases or conditions for which the Secretary recommends the administration of the covered countermeasure, the applicable periods, the pertinent population or populations, and the applicable geographic areas.

A very narrow exception to these liability protections allows an exclusive federal lawsuit against a covered party if the plaintiff can prove that the party engaged in willful misconduct and that such misconduct caused death or a serious physical injury. "Willful misconduct" is defined very broadly as an act or omission taken intentionally to achieve a wrongful purpose, knowingly without factual or legal justification, and in disregard of a known or obvious risk that is so great as to make it highly probable that the harm will outweigh the benefit.[182] The PREP Act provides that this standard is more stringent than any form of negligence or recklessness. Other defenses are also available.

The Secretary invoked the liability protections of the PREP Act through a series of declarations published beginning in 2008.[183,184] These declarations extend to several countermeasures meant to prevent or treat pandemic influenza and other unrelated countermeasures (those relating to anthrax, botulism, smallpox, acute radiation syndrome, and Ebola virus). Although vaccines against pandemic influenza A viruses and influenza A viruses with pandemic potential are covered by one of these declarations, seasonal influenza vaccines administered annually are excluded (and are subject to the NCVIA and the VICP). Covered countermeasures include several vaccines and other countermeasures (e.g., antibiotics, antivirals).

The PREP Act provides for the establishment of a program to provide compensation to certain covered persons who sustain serious physical injuries (or death) as a result of a covered countermeasure designated under a declaration of the Secretary. This program, which was established in 2010, is known as the CICP. The structure of and benefits available under this program are very similar to the SVICP. Like the SVICP, the CICP is an exclusively administrative program run by the HHS. In addition, requesters for benefits must satisfy the requirements for a condition listed on a countermeasures injury table or prove that a covered countermeasure caused a specific injury. Under the CICP, the Secretary must rely on compelling, valid, reliable medical and scientific evidence to add injuries to an injury table or to determine that a countermeasure caused a specific injury. The regulations governing the CICP, including any tables of injuries, are codified at 42 CFR part 110.[185] As of February 2017, 475 requests had been filed with the CICP, nearly all (86%) alleging injury from the 2009–10 H1N1 pandemic influenza vaccine. The remaining 14% involved antiviral drugs, anthrax vaccine, and smallpox vaccine, as well as other ineligible items.

We are aware of two cases filed against covered persons under the PREP Act. *Parker v St. Lawrence County Public Health Department*[186] arose from a pandemic influenza vaccine clinic held by a county public health department at a school pursuant to a Secretarial declaration issued under the PREP Act. At the clinic, a kindergartner was administered a vaccine by mistake even though her parents did not consent to the vaccination. The child's mother filed an action alleging that the administration of the vaccine without parental consent was negligence and resulted in a battery of her daughter. A New York appellate court dismissed the claim, concluding that the mother's state law claims for negligence and battery were preempted by the PREP Act's liability protections.

In *Kehler v. Hood, et al.*,[187] the plaintiff alleged that his wife suffered transverse myelitis as the result of a pandemic influenza vaccine administered pursuant to a PREP Act declaration. The plaintiff sued a doctor and her employing hospital, alleging that they were negligent in failing to obtain the patient's informed consent and consult with a specialist prior to the vaccine administration. The defendants brought third-party product liability/failure to warn claims against the vaccine manufacturer. The federal district court dismissed the claims against the manufacturers as barred by the PREP Act (given that willful misconduct was not alleged) and remanded plaintiffs' remaining claims to state court for further consideration.

VACCINE LIABILITY SINCE 1986

The passage of the NCVIA in 1986 did not mark an immediate decline in the number of reported vaccine liability decisions in state and federal courts (largely owing to the length of time until cases reach a final, reported resolution and the fact that retrospective claimants had until January 1991 to file claims with the VICP). However, since 1992, the number of reported vaccine liability cases has declined sharply. This decrease in reported cases suggests that most VICP petitioners have chosen not to pursue traditional tort litigation after the VICP process.

There are few reported federal and state court cases in which a claimant filed a traditional tort claim against a vaccine manufacturer or a vaccine administrator after being denied compensation under the VICP. In many of these cases, the courts concluded that the claims must be dismissed as barred by the applicable statute of limitations (e.g., state statute of limitations) because the underlying VICP claim was time-barred or because the plaintiffs failed to file a timely VICP claim within the limitations period.[188–195] Other reported post-VICP cases address other issues. In one case, a state court dismissed a medical malpractice action filed against a vaccine administrator by a pre-1988 petitioner because of the petitioner's acceptance of a VICP award.[196]

There are very few reported cases of vaccine injury or death lawsuits being pursued outside of the VICP.

As a general rule, as long as petitioners properly exhaust their remedies under the VICP, they may pursue civil actions against vaccine administrators and manufacturers. The NCVIA imposes certain limitations on post-VICP claims that may be filed against vaccine manufacturers. No such protections exist for vaccine administrators. Until recent years, these post-VICP limitations on civil actions filed against vaccine manufacturers attracted little attention.

However, one such limitation was brought into the spotlight as different interpretations of its language led to a post-VICP case, concerning a seizure disorder alleged to be the result of a DTP vaccine that was decided by the U.S. Supreme Court, *Bruesewitz v Wyeth*.[197] The NCVIA provides that "[n]o vaccine manufacturer shall be liable in a civil action for damages arising from a vaccine-related injury or death associated with the administration of a vaccine after the effective date of this part if the injury or death resulted from side effects that were unavoidable even though the vaccine was properly prepared and was accompanied by proper directions and warnings."[198] The issue before the Supreme Court was whether, in post-VICP litigation, this provision prohibits claimants from bringing "design defect" claims against manufacturers in civil court. In these claims, the plaintiff alleges that the design of the vaccine was defective; a safer alternative design was available; the manufacturer failed to design its vaccine in conformity with the safer alternative; and as a result, the claimant was injured.

Before the U.S. Supreme Court's decision, lower courts had differed in their interpretations of this language. The Supreme Court of Georgia concluded in *American Home Products Corp v Ferrari* (a thimerosal–neurologic damage claim) that design defect claims could proceed on a case-by-case basis until a court determined that the particular vaccine was unavoidably unsafe.[199] The U.S. Court of Appeals for the Third Circuit reached a different result, in *Bruesewitz v Wyeth*.[200] In that case, family members filed a petition with the VICP arguing that their child sustained a seizure disorder as the result of a DTP vaccine. After their VICP claim was dismissed, the family filed a design defect claim against the vaccine manufacturer in civil court. On appeal, the Third Circuit dismissed their claim, concluding that the statutory language preempted all post-VICP design defect claims against vaccine manufacturers.

Given the split in judicial interpretations and the significance of the issue, the Supreme Court granted the family's petition for certiorari to clarify whether this provision of the NCVIA preempts all design defect claims in post-VICP litigation filed against vaccine manufacturers. Many stakeholders filed amicus briefs on both sides, emphasizing the significance

of the Supreme Court's anticipated ruling.[201] The federal government filed an amicus brief arguing that the NCVIA preempts all design defect claims against vaccine manufacturers in post-VICP litigation.[202] During oral argument, the federal government emphasized the significant public health implications of the case and the important role of the government in ensuring that licensed vaccines are safe and effective.

In an opinion delivered by Justice Scalia, the U.S. Supreme Court held that the NCVIA preempts design defect claims against manufacturers in civil court.[197] The plain text of the statute suggests that a vaccine's design is not open to question in a tort action. In addition, the structure of the NCVIA reinforces the interpretation that design defect claims are preempted: The NCVIA provides for comprehensive federal agency improvement of vaccine design and for federally prescribed compensation as an alternative means for achieving the aims of design defect tort suits.

Beyond the issue of any limitations on design defect claims, the NCVIA addresses "duty to warn" claims with respect to vaccine manufacturers (but not administrators) by providing that "[n]o vaccine manufacturer shall be liable in a civil action for damages arising from a vaccine-related injury or death associated with the administration of a vaccine after the effective date of this subpart solely due to the manufacturer's failure to provide direct warnings to the injured party."[203]

Before the *Ferrari* and *Bruesewitz* cases arose, the largest development in non-VICP vaccine litigation had been an upsurge in cases, beginning in 2001, in which parents alleged that covered vaccines (the MMR vaccine, which never contained the preservative thimerosal, and/or childhood vaccines containing thimerosal) caused their children's autism. Plaintiffs argued that they were not required to exhaust their remedies within the VICP based on their argument that the preservative thimerosal was an adulterant. This argument hinged on the NCVIA's definition of a "vaccine-related injury or death," which excludes any injury or death associated with an adulterant or contaminant intentionally added to a covered vaccine.[204] The federal government's position, filed in one of these cases, was that thimerosal was not an adulterant because it was part of the vaccine formulation when the products were licensed and approved for use by the FDA.[205] Courts held that such claims must be filed with the VICP and all remedies there exhausted before any such claim may be brought outside the program.[206,207] Thus, unless a claim fits within one of the limited statutory exceptions (see "Other Situations in Which Suits Are Not Barred by the National Childhood Vaccine Injury Act" later), it must be filed with the VICP before tort remedies can be pursued.

Several cases were also filed in state court in New Jersey alleging that a contaminant in the OPV (simian virus 40 [SV40]) caused cancerous tumors.[208] Manufacturers argued that the SV40 was not "intentionally added" to the vaccine and, thus, that jurisdiction should be before the VICP. A decision that the statutory exception did not apply was upheld on appeal.[209]

Risk to Healthcare Professionals

Civil actions against healthcare providers are rare, and few have been pursued since the VICP was modified to cover vaccine administrators, because claimants must go through the VICP before filing most actions against a vaccine administrator. However, if a claimant were to reject the VICP decision, the claimant might file a tort action against the healthcare provider on such grounds as failure to adequately warn, negligent vaccine administration, or negligent postvaccination care.

Other Situations in Which Suits Are Not Barred by the National Childhood Vaccine Injury Act

The NCVIA covers only the losses sustained by the injured person; thus, all loss of companionship claims by the parents, children, or spouse of an injured person are not compensable. Family members may file these so-called derivative claims in state court, if they can be pursued in court under state law, but the claims will be successful only if the plaintiff can prove that the underlying injury or death was related to the vaccine and prove a theory of liability. The NCVIA makes inadmissible in such subsequent litigation any finding of fact or conclusion of law in a VICP case, as well as the VIT.[210] Up until 2001, with the thimerosal litigation, families rarely filed such claims. The issue first came to light in *Schafer v American Cyanamid*.[211] The husband and daughter of a woman who contracted vaccine-related polio filed a claim for loss of consortium and emotional distress in state court after the woman accepted a VICP award. The case was removed to federal court, and a federal appellate court found that the plaintiffs' claim was not precluded by the family member's acceptance of a VICP award. The Court of Federal Claims' interpretation of the NCVIA was consistent with the Schafer holding.

In *Abbott v Secretary of Department of Health and Human Services*,[212] a young man's mother filed a state wrongful death claim against a healthcare provider alleging that the provider negligently left her son unattended in the bathtub. The young man had a seizure in the tub and drowned. Ms. Abbott filed the state claim as mother of the decedent and accepted a settlement from the healthcare provider. The mother later filed a VICP claim as administrator of her son's estate alleging that the death was a sequela of a vaccine-related seizure disorder. The U.S. Court of Appeals for the Federal Circuit concluded that the previous settlement covered only damages for injuries to the son's beneficiaries. The mother received an award under the VICP as a representative of her son's estate. Other civil litigation related to autism claims was filed by plaintiffs. These cases included class action lawsuits filed on behalf of the parents, children, or spouses of the injured person seeking their own damages for loss of companionship or consortium or services and loss of earnings. Other lawsuits sought the costs of medical monitoring to determine whether thimerosal-related injuries would develop in the future, generally alleging $1000 or less in damages.[213] Because the plaintiffs sought $1000 or less per person to pay for "medical monitoring" (i.e., future tests to determine if a child is developing an injury), plaintiffs argued they were not covered by the NCVIA because it exempts claims seeking $1000 or less.[214,215] By 2006, all medical monitoring lawsuits had been dismissed.[216]In *Cook v Children's Medical Group*,[217] parents claimed that a vaccine administrator fraudulently concealed their child's vaccine-related injury, and, as a result of this concealment, the time for filing a VICP claim had passed. The Supreme Court of Mississippi held that the parents' claim against the administrators for fraudulent concealment and breach of fiduciary duty could continue in state court (because it was not the subject of the NCVIA), but that any claim on behalf of the child for his vaccine-related injuries was subject to exhaustion of remedies under the VICP.

INTERNATIONAL COMPENSATION PROGRAMS

Nineteen industrialized countries provide some form of compensation for injuries (or deaths) following vaccination.[218,219] Germany and France were the first to institute programs in the 1960s, followed by Austria, Denmark, Japan, New Zealand, Sweden, Switzerland, United Kingdom, and Northern Ireland in the 1970s; Taiwan, Finland, United States, and Quebec

(Canada) in the 1980s; Italy, Norway, and Republic of Korea in the 1990s; and Hungary, Iceland, and Slovenia since 2000. A variety of factors led to the establishment of such programs, including concerns about liability and vaccine adverse events, concerns about the vaccine supply, and a belief that governments have a responsibility to persons injured by properly manufactured and administered vaccines used in public health programs. Most are managed administratively through the national government, including decisions on eligibility and the amount of compensation.

Eligibility may depend on several factors, including the category of vaccine (e.g., recommended, compulsory) administered. Some programs are part of larger no-fault compensation schemes. Because few vaccine-related injuries have a clinical or laboratory marker, proving actual causation can be difficult. Causation decisions are usually based on the preponderance or "more likely than not" standard. All countries have a threshold severity of injury or disability requirement, and nearly all provide coverage for medical costs, noneconomic damages (pain and suffering), all usually proportional to the injury, and death benefits. No compensation programs outside the United States reimburse attorneys' fees and costs. Funding is generally from the national treasury, with some programs receiving support from other governmental entities or vaccine manufacturers. Two countries fund the programs with a vaccine tax (Taiwan and the United States).

Interest has been expressed in expanding vaccine injury compensation to the developing world (Clements CJ, Oleja S, Fife P. Vaccine injury compensation: an international perspective. Unpublished data, 2006).

THE POWER TO COMPEL VACCINATION

U.S. courts generally have been deferential to public health judgments that mandatory vaccination is required in particular circumstances. In *Jacobson v Massachusetts,*[220] the U.S. Supreme Court upheld a Massachusetts statute authorizing each local board of health to require vaccination of the inhabitants "if, in its opinion, it is necessary for the public health or safety." Under this authority, and responding to a partial outbreak of smallpox, the city of Cambridge required all residents to receive the smallpox vaccination. Although children whose physicians determined that such vaccination was medically contraindicated were exempted from this requirement, such an exemption was not available for adults. Jacobson, who explained that he suffered adverse reactions to the vaccine as a child, refused vaccination and was fined. The Supreme Court rejected Jacobson's argument that the Massachusetts law was constitutionally invalid, holding that the law was a reasonable and proper exercise of the state's police power to protect the public health and safety. The court determined that the Massachusetts legislature was entitled to rely on the commonly held belief (maintained by high medical authority) that vaccination was a preventive of smallpox and was not compelled to commit such a public health and safety matter to the final decision of a court or jury. This case is significant because the Supreme Court recognized the importance of vaccination and deferred to the authority of state lawmakers to develop and impose vaccination requirements on the public. The Supreme Court's 1922 decision in *Zucht v King*[221] had the effect of upholding the authority of the city of San Antonio to issue an ordinance requiring all persons attending public schools or other places of education to first present certificates of vaccination, thereby permitting mandatory vaccination requirements for school attendance. The lawsuit was filed on behalf of a child who refused to be vaccinated and was excluded from a public school and a private school for failure to have the certificate. Although there had been no epidemic of smallpox in San Antonio in *Zucht*, in contrast to the circumstances in *Jacobson*, the Supreme Court in *Zucht* affirmed the validity of a preventive vaccination mandate for a disease not in circulation.[222]

Decisions of contemporary courts continue to be supportive of mandatory vaccination requirements and often cite *Jacobson* in deciding lawsuits challenging immunization mandates. In *Maricopa County Health Department v Harmon,*[223] a health department issued an emergency rule barring unimmunized children from attending school because of an outbreak of measles in the county. The health department sought an injunction excluding unimmunized children from school. The trial court granted the injunction, and the appellate court affirmed. The court rejected the families' argument that the health department had no authority to exclude children unless there had been a confirmed case of measles in the particular school, concluding that the health department had the authority to exclude children even in the absence of a serologically confirmed case.

Contemporary vaccination laws are not as sweeping as the law involved in *Jacobson.* States generally require that children receive certain recommended vaccines to attend schools and childcare centers. There is variation among state laws as to which vaccines are required for school entry. These laws are dynamic and often incorporate new vaccines. The CDC's National Immunization Program maintains a website with current information on state immunization requirements for attendance in schools and childcare centers, together with information on exemptions permitted under these laws.[224] The CDC also maintains websites with data about vaccination coverage.[225,226] Currently, all states allow some form of a medical exemption, approximately one-third of states allow philosophical exemptions, and nearly all allow religious exemptions.[227, 228]

In recent years, particularly in the wake of the measles outbreak in California in 2015 and concerns about the rates of exemptions in certain communities, state legislatures have devoted substantial attention to school immunization requirements, as well as the exemptions available to such requirements. The issue has received particular attention in California, which passed a law in 2012 requiring consultation with a healthcare practitioner about the risks and benefits of immunization as a requirement for personal belief exemptions.[229] In a signing statement, the then governor of California directed the Department of Public Health to allow for a separate religious exemption on the form, even though it was not provided for by law, so that "people whose religious beliefs preclude vaccinations will not be required to seek a healthcare practitioner's signature."[230] The governor's authority to unilaterally change the bill in this manner has been questioned.[231] More recently, on June 30, 2015, the governor of California signed a controversial law eliminating personal and religious exemptions to the State's vaccination requirements.[232] The law applies to pupils in public or private schools and daycare centers, but not to children who are home-schooled. The law retains a single exemption that permits a parent or guardian to file a written statement by a licensed physician "to the effect that the physical condition of the child is such, or medical circumstances relating to the child are such, that immunization is not considered safe, indicating the specific nature and probable duration of the medical condition or circumstances, including, but not limited to, family medical history, for which the physician does not recommend immunization" and exempting such child from the vaccination requirements "to the extent indicated by the physician's statement." In a signing statement, the governor of California noted scientific evidence concerning the benefits of vaccination and that the law, after considerable debates and amendments, "explicitly provides an

exception when a physician believes that circumstances—in the judgment and sound discretion of the physician—so warrant."[233] Students already enrolled in schools or facilities whose families previously objected to immunizations may continue to attend covered schools and facilities until they enter covered grade spans (i.e., kindergarten, seventh grade), at which time they must satisfy the law's immunization requirements. The law permits the State Department of Public Health to subject vaccines against additional diseases beyond those already listed by statute to the law's immunization requirements, but only if exemptions are allowed for both medical and personal beliefs. The law permits a child to be excluded from a school or daycare center if there is good cause to believe that the child has been exposed to a covered disease and documentation proving immunization against such disease is not submitted. Oregon adopted a law permitting religious or philosophical exemptions only after parents either reviewed the risks and benefits of immunization with a physician or completed a vaccine education module.[234]

In 2016, many bills were pending in state legislatures proposing removing existing exemptions, proposing altering existing exemptions (e.g., requiring physician counseling about the risks and benefits of vaccination for exemptions), or proposing new exemptions.

The laws that require vaccination before school entry are congruent with the grant program operated by the U.S. Public Health Service.[235] If a state or local government participates in that federally funded immunization program, it must, among other requirements, have a "plan to assure that children begin and complete their immunizations on schedule" and a "plan to systematically immunize susceptible children at school entry through vigorous enforcement of school immunization laws."[236]

Although all states allow medical exemptions to school immunization laws, the scope and application of these laws vary by state.[224,237, 238] In a case concerning medical exemptions, the Wyoming Supreme Court held that the state of Wyoming's Health Department could waive state immunization requirements for a particular child if such vaccination was medically contraindicated for that child but that the Department exceeded its authority in requiring a child or the child's physician to provide a reason for such a contraindication.[239]

Many states have granted persons exemptions from mandated vaccination requirements based on religious or personal beliefs. Parents have filed numerous lawsuits challenging the fact that states did not allow religious and/or personal exemptions or challenging the scope or application of the specific exemptions. In a case in which a mother challenged the constitutionality of a West Virginia law that allowed no religious exemptions, a federal judge concluded that states need not provide religious exclusions from their compulsory immunization laws.[240] Although the court in *Brown v Stone*[241] struck a religious exemption from a state statute on the grounds that it violated the Equal Protection Clause of the Fourteenth Amendment (by requiring most school children to be vaccinated while exposing them to the hazards of associating with nonvaccinated children subject to a religious exemption), most courts have recognized that states may allow religious exemptions. The courts have focused on the scope of such exemptions. For example, in *McCarthy v Boozman*, a federal district court judge found unconstitutional an Arkansas law that granted religious exemptions to the state's school immunization requirements only to persons who were members or adherents of a church or religious denomination recognized by the state but not to others whose objections to immunization were also grounded in sincere religious beliefs.[242] The judge found that the religious exemption section of the Arkansas law violated the U.S. Constitution, noting that the primary effect of this exemption provision was to "inhibit the earnest beliefs and practices of those individuals who oppose immunization on religious grounds but are not members of an officially recognized religious organization." An appeal was mooted when the Arkansas legislature broadened the law to encompass philosophical and religious exemptions.[243]

In another case, a New York agency sought a court order to immunize foster children over the objections of their foster mother. Applying New York law, the judge considered the nature of the foster mother's objections to determine whether they were religious or philosophical. Although the foster mother conceded that the tenets of her religion did not necessarily preclude vaccination, she argued that her interpretation and strict adherence thereto did. Finding that the foster mother's opposition to immunization was rooted in genuine and sincerely held religious beliefs, the judge determined that she qualified for New York's religious exemption and denied the agency's request for a court order directing the immunization of the foster children.[244]

In another case, the U.S. Court of Appeals for the Second Circuit upheld a lower court's determination that the constitutional rights of a mother, who failed to demonstrate that she sincerely held religious objections to immunizations, were not violated by the school district policy requiring persons requesting religious exemptions to complete individualized questionnaires concerning the nature of their objections.[245] Based upon the language in state law, the Wyoming Supreme Court held that the state was not authorized to hold hearings to judge the sincerity of a parent's religious beliefs before a religious waiver to state immunization requirements would be granted.[246] In a recent case, a federal appellate court dismissed the plaintiffs' claims that a New York immunization law requiring vaccination for school entry (subject to medical and religious exemptions) was unconstitutional. The court also dismissed the parents' challenge to a state regulation allowing unvaccinated children to be excluded from public school based on outbreaks of vaccine-preventable diseases.[247]

In another case, a federal trial court denied a mother's application for a preliminary injunction against a local department of education to admit her unvaccinated child to school under a religious exemption, finding that the objections articulated by the mother had a medical, and not a religious, basis.[248] In a similar case, a federal appellate court upheld a decision that parents failed credibly to demonstrate that they held genuine and sincere religious beliefs that prohibited vaccinations for their child, as required to establish religious-based exemption from the state's immunization requirement.[249]

In another case, a federal appellate court held that West Virginia has a compelling interest in requiring children to be vaccinated before allowing them to attend public school and that a child's rights were not violated when her request for a medical exemption was denied. The court concluded that the state's immunization statute, which did not include a religious exemption, was not facially invalid under the federal Constitution.[250]

Another set of cases concerns the rights of parents and vaccination requirements in the context of guardianship issues. In one case, the Supreme Court of Oregon concluded that the Department of Human Services, which had been named the legal custodian and guardian of eight siblings, held the power to approve their immunization. The parents of the eight children raised religious objections. The court concluded that the ability to assert a religious exemption did not apply, given that the Department had acquired custody and guardianship over the children.[251] A federal district court reached a similar conclusion. In that case, custody of a plaintiff's minor children had been awarded to the local county. While in the county's

care, the children were vaccinated by order of a juvenile court. Plaintiff alleged that this was a violation of her First Amendment rights, as she objected to the vaccinations on religious grounds. The court concluded that such vaccinations did not violate plaintiff's constitutional rights, noting that local authorities may constitutionally mandate vaccinations, that the right to practice religion does not include parental liberty to expose the community or a child to communicable disease, and that states are not required to provide a religious exemption from compulsory immunization.[252]

In another case arising from a child protective proceeding, the plaintiff appealed from a ruling that she had neglected her child and that the Administration for Children's Services could direct that her child be immunized. A New York appellate court upheld a lower ruling that the plaintiff was not entitled to a religious exemption from the law's vaccination requirements because she failed to prove that her opposition to immunization stemmed from genuinely held religious beliefs.[253]

In the *Matter of Christine M.*,[254] a state court held that it was child neglect for an otherwise responsible parent to refuse to permit his 2-year-old child to be vaccinated for measles during a measles outbreak in New York City absent a demonstration of sincere grounds for a religious exemption. However, the court refrained from ordering immediate vaccination on the grounds that the vaccination would be required when the child entered school and that the measles outbreak had ended by the time of the decision. Another court case arose from a disagreement between the parents of a child whether the child should be immunized. The mother did not want her child to be immunized (based on her family's medical history and her research about the safety of vaccines) and sought the exclusive authority to make this determination for the child. After obtaining the input of a physician, the trial court ruled that the child should be immunized, consistent with the father's desire. The Texas appellate court affirmed this ruling, concluding that the trial court did not violate the mother's constitutional and statutory rights to raise her child and ruling that the physician's opinion constituted sufficient evidence to support the trial court's order.[255]

There are no federal governmental vaccination requirements for the general adult population in the United States. However, certain segments of the adult population (e.g., college students and healthcare professionals) may be subject to particular vaccination requirements imposed by schools or employers. Members of the armed forces are also required to receive vaccines in certain circumstances. In one case, military personnel subject to orders to receive the anthrax vaccine brought an action against the FDA, the HHS, and the Department of Defense, challenging the FDA's determination that the anthrax vaccine was effective against inhalation anthrax and seeking temporary and permanent relief from Department of Defense's anthrax vaccination program. A federal court granted the defendants' motion to dismiss, finding in part that the FDA did not act arbitrarily or capriciously, that the agency applied its expertise and found that the vaccine was effective for immunization against anthrax, and that service members challenging the Department of Defense's anthrax vaccine requirements lacked standing to bring that claim.[256]

State and employer vaccination mandates for healthcare workers have garnered significant attention in recent years.[257] In *Ritterband v Axelrod*,[258] a New York trial court upheld health department regulations that required hospital employees and medical staff to have current rubella immunizations, finding unpersuasive a staff physician's argument that the vaccination requirement was a violation of the Fourth Amendment protection against unreasonable searches and seizures and a violation of his privacy. More recently, several provider groups sought a temporary restraining order to prevent the enforcement of a New York State regulation requiring healthcare workers with direct patient contact to get the pandemic H1N1 and seasonal influenza vaccines. The regulations permitted medical exemptions only for medical contraindications.[259] A state court judge granted a temporary restraining order against the application of the regulation to New York State healthcare workers, and the governor announced the suspension of the influenza shot mandate for healthcare workers because of vaccination shortages before the order could go into effect. Thus, the lawsuit was dismissed as moot by the New York Supreme Court.[257]

In another case, a hospital required all employees to receive the influenza vaccine (unless the hospital granted an exemption based upon CDC guidance). A benefits coordinator refused vaccination based on her medical and family history and the recommendation of a physician. Because she was not granted an exemption, the employee was fired and applied for unemployment. Such benefits were only available if the coordinator was not fired for cause. The court determined that the hospital's policy was reasonable, but that the employee's refusal to comply under the circumstances was also reasonable. Thus, the former coordinator was entitled to unemployment benefits.[260]

In another case, a hospital required all healthcare workers to receive the influenza vaccine, but permitted exceptions for medical or religious reasons (and persons receiving such exemptions were required to wear a face mask). A nurse who refused the vaccination on secular philosophical grounds (but wore a face mask) was fired. She challenged a decision denying her unemployment benefits and an appellate court in New Jersey found that she was entitled to such benefits because she did not commit misconduct. The court based its finding in part upon the fact that the employer's policy was not based on solely public health grounds, given that it permitted religious exemptions and concluded that her behavior did not undermine her ability to perform her job as a nurse.[261]

SUMMARY, CONCLUSIONS, AND FUTURE IMPLICATIONS

Our nation's experience with the public's perception concerning immunization and other countries' experiences with the same issue make clear that uncertainty and fear often come together when information is lacking. Although most parents seem to believe in the wisdom of vaccination and use their healthcare providers as a key source of guidance in decision making, immunization must never be taken for granted. Although the federal and state immunization programs, together with the VICP, have done a remarkable job of ensuring that our nation's children will be vaccinated against dangerous diseases and although a national surveillance system and several IOM reports confirm the general safety of immunizations given routinely in this country, public perceptions concerning the risks of vaccines remain an important challenge for vaccine administrators to address. Given the outcome of the omnibus autism proceedings, the VICP was shown to have been able to adequately address this phenomenon. Finally, the ability of the VICP to successfully fulfill its mandate and to further its statutory purposes seems strong, particularly given the near-universal consensus among the VICP's stakeholders (i.e., physicians, manufacturers, lawmakers, attorneys, and members of the public, including parents of injured children) that a successful national immunization program can be achieved only with a compensation program and liability safeguards in place. Recent experiences with legislation enacted in preparation for a potential smallpox attack or the emergence of a pandemic flu or other public health emergencies

have emphasized the necessity of providing clear liability protections and fair compensation to ensure the success of vaccination programs across the United States.

The VICP has largely satisfied the public policy imperatives driving passage of the NCVIA in 1986. First, persons found to have sustained vaccine-related injuries (through a presumption of causation under the Table or by demonstrating causation for off-table claims) receive generous compensation in a streamlined process. Second, the availability of compensation under the NCVIA has generally given plaintiffs a sufficient incentive to abandon the pursuit of tort remedies against vaccine manufacturers and administrators.

Claims against vaccine manufacturers are significantly reduced compared with the litigation manufacturers faced at the inception of the VICP. Although claims against healthcare providers are more difficult to track, there is no indication that their liability experience is any different from that of manufacturers. Until the new wave of court cases pertaining to autism, few cases alleging vaccine-related injuries were filed outside the VICP in recent years. The reduction in the number of such civil actions can largely be explained by the fact that the NCVIA requires petitioners to file for compensation under the NCVIA before pursuing outside litigation for alleged vaccine-related injuries or deaths. With regard to post-1988 claims, petitioners have two options once the VICP process is concluded: (a) accept the court's judgment and the compensation awarded (if any); or (b) reject the court's judgment, with the option of pursuing a civil action in state or federal court (under limitations set forth in the NCVIA). most VICP petitioners, even petitioners who were not awarded compensation under the VICP, have chosen the former option. Even among the small number of VICP petitioners who have chosen the latter option (a group consisting almost entirely of persons who were not awarded compensation under the VICP), most have chosen not to pursue further civil remedies. The fact that most VICP petitioners choose to accept the court's judgment and forgo additional civil litigation can largely be explained by the VICP's generous compensation awards, which are devised in an effort to create a lifetime stream of benefits for the injured party, and the general understanding that the VICP imposes a lesser burden on litigants than that imposed in traditional civil litigation. One remarkable feature of the VICP is that all petitioners, including petitioners for whom compensation is denied, receive attorneys' fees and costs so long as their claims were brought in good faith and on a reasonable basis. A new challenge facing the VICP is the proper standard for judging causation-in-fact claims, an issue that is certain to generate a lively debate among the VICP's stakeholders and to result in an increasing number of judicial opinions as new alleged vaccine-injury relationships are examined.

The reported decisions are consistent with the conclusion that vaccine manufacturers and administrators are generally shielded from any significant liability risk. However, the protection is not absolute. Because the NCVIA does not preclude a person who is otherwise ineligible to file a claim under the VICP (e.g., family members of injured persons) from pursuing civil litigation, liability exposure remains a possibility in states where such suits may be brought. However, the Supreme Court decision in *Bruesewitz v Wyeth*, holding that injured claimants are prohibited from bringing design defect claims against vaccine manufacturers in civil court, clarified the scope of the Vaccine Act's liability protections.

A measure of the success of the VICP is the development and administration to children of new vaccines to prevent childhood diseases. Since the creation of the VICP, the following vaccines have been recommended for routine administration to children by the CDC and included in the VICP's coverage: HBV, HAV, Hib, varicella, rotavirus, pneumococcal conjugate, seasonal influenza, meningococcal, and HPV. That since creation of the VICP such vaccines have successfully traversed the long, arduous licensing process to become routinely administered vaccines demonstrates that the scientific community is able to develop innovative vaccine products to combat childhood diseases under the current system. Moreover, recent statutory changes that resolved issues of liability protections and compensation with respect to adverse events and vaccines administered to pregnant women, and expanded the coverage of the NVCIA to vaccines recommended by the CDC for routine administration in pregnant women, but not children, show that the VICP continues to be seen as a successful means of resolving thorny liability issues while making compensation available to those who sustained adverse events.

Another benefit of the VICP has been that liability concerns no longer lead to destabilization of the marketplace. The market experienced some vaccine supply shortages from 2000 to 2015,[262–264] but such shortages did not seem attributable to liability factors.[265] Although many new vaccines are relatively expensive (reflecting the manufacturers' need to recoup the costs of research and development), vaccine prices today seem to reflect public and private sector purchase trends and the effects of inflation, rather than including significant funds to address liability concerns or repercussions. Thus, the existence and general success of the VICP in meeting the public policy objectives underlying the NCVIA have led to a current system in which innovative vaccine products and technologies are developed, the vast majority of American children are immunized against a growing number of diseases that pose a threat to their health, and the small number of persons who sustain adverse events as the result of such immunizations are able to receive appropriate compensation through the VICP.

Despite its successes, the VICP has been criticized for several perceived weaknesses. For example, one criticism is that the liability protections provided by the NCVIA are not absolute (e.g., persons with claims of $1000 or less need not pursue claims under the VICP, vaccine manufacturers and administrators may be liable if a person exhausts his or her remedies in the VICP and then opts to pursue civil litigation outside of the VICP).[265] Another criticism is that the VICP extends only to certain vaccines, rather than to all vaccines. Thus, for vaccines that are not covered by the VICP (e.g., vaccines against Lyme disease, vaccines recommended only for adults), the NCVIA's expedited no-fault process and attendant liability protections are unavailable. The NCVIA's statute of limitations (for injuries, 3 years after the date of the first symptom or manifestation of onset of the injury) also has been criticized. Because this period begins running from the first symptom rather than from diagnosis or any attribution to vaccines, some believe the NCVIA's statute of limitations is particularly unfair as applied to certain illnesses with early manifestations that may be subtle. The fact that claims for damages sustained by persons close to an injured person (e.g., loss of consortium claims by an injured party's spouse) cannot be pursued in the VICP has been criticized.

The compensation limits for pain and suffering as well as for death have also been criticized. In the original Vaccine Act enacted in 1986, the benefit cap for pain and suffering ($250,000) and the death benefit cap ($250,000) were established and have remained unchanged. It has been recommended that these caps be increased to account for inflation.[266] The conflict inherent in litigation may also be seen as a weakness of the VICP when compared with other compensation programs that are purely administrative. Depending on one's vantage point, some of these perceived weaknesses may be seen as strengths (e.g., that the liability protections afforded by the NCVIA are not absolute may be seen as a weakness by persons who see vaccines as an important but vulnerable part

of the public's health but as a strength by injured parties who want to file suit against persons who they believe are responsible for the damages sustained).

Finally, a judicial program such as the VICP is not an ideal system for reaching complicated decisions about whether vaccines are causing particular injuries. Petitioners alleging injuries that meet the requirements of the VIT need not demonstrate to the court that a VICP-covered vaccine caused their injuries to receive compensation. Instead, it is presumed that the vaccine was the cause of the injury unless another cause is proven. Moreover, the majority of VICP cases today are resolved by settlement, in which the court does not determine that the vaccine caused the injury. Despite this, opponents of vaccines may erroneously refer to the number of cases compensated and the amount of money awarded under the VICP as proof that vaccines cause harm.

An increasing focus has been given to the issue of vaccine safety during recent years. Effective vaccine risk communication is imperative and must include a recognition of the difficult and confusing nature of risk assessment and decision making for some vaccines.[267] Such communication is particularly critical now, at a time when most parents do not have an independent recollection of the devastation caused by the infectious diseases prevented by childhood vaccines. Interwoven into vaccine safety awareness are the issues of state mandates for immunization and state-provided exemptions. Vaccination levels among American children remain remarkably high and of vaccine-preventable diseases, generally low. Courts generally continue to uphold the states' police powers to mandate immunization for school entry, while allowing them to provide exemptions for medical, religious, or philosophical reasons. However, the measles outbreak of 2015[268] has contributed to ongoing discussions regarding the value of public health to reduce/eliminate preventable disease versus the rights of individuals to refuse vaccines.[269] In fact, since the outbreak began, at least 16 states have introduced legislation address exemptions to immunizations.[270]

Immunizations have been hailed as one the 10 greatest public health achievements of the 20th century because of their success in eradicating or substantially minimizing the diseases they are targeting within the American population.[271] It has been estimated that between 1924 and 2012, more than 100 million cases of serious diseases were prevented by childhood vaccinations.[272] With waning vaccine-preventable disease, vaccines may be viewed as a routine nuisance procedure to prevent diseases that few, including most young physicians, have actually experienced. In this setting, it is only natural that people will choose to avoid risk when the benefit is not readily apparent and to demand safer products. However, outbreaks of measles and pertussis in 2012 to 2015 have reignited the debate between the value of immunizations versus personal choice.[269,273,274] Medical experts and public health officials must remember that an effective vaccination program can be successful over the long term only if its purposes and methods are well understood by both healthcare personnel and society. The importance of transparency in explaining the vaccination process, risks, and benefits cannot be overstated in helping to maintain an effective vaccination program.

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84 Ethics

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Vaccines present a spectrum of novel ethical considerations compared with those associated with other medical interventions. Central to these differences are the role of vaccines in disease prevention rather than treatment, the concurrent interest of vaccination policy in improving the health of both communities and individuals, and the focus on children in many vaccination programs. Formal discussions of ethical issues related to vaccination once concentrated principally on aspects of clinical research and specific topics regarding vaccine safety and financing. Recent events, such as outbreaks of vaccine-preventable diseases, increases in parental vaccine hesitancy and refusal, and the 2014–15 Ebola outbreak, demonstrate the value and importance of a more comprehensive and sustained examination of vaccine ethics. Such an approach offers important insights into all aspects of the vaccine life cycle, from the earliest stages of research through deployment in national and global immunization programs.

ETHICAL ISSUES UNIQUE TO VACCINATION

Ethics of Prevention Versus Ethics of Treatment

Unlike pharmaceuticals and most other medical interventions, vaccines are intended to prevent disease rather than to treat it. The ethics of prevention differ greatly from the ethics of treatment. Chief among these differences are the disparate meanings and interpretations of "risk" in each context.

When treating disease, risks are largely confined to two broad categories: the risks associated with a particular intervention and the risks of doing nothing. These risks are weighed against the potential benefits of a specific treatment and compared with the risk-to-benefit profiles of potential alternatives. Decision making is invariably complicated by the uncertainty associated with any assessment of risks and benefits. Nevertheless, every treatment decision is made based on the evaluation of all options in light of the risks and potential benefits of each, compared with the consequences of doing nothing.

When aiming to prevent disease by means of vaccination, assessing the risk of inaction is more challenging. Here, the physiological consequences of a disease in an individual person must be considered *in addition to* the specific likelihood of acquiring that disease. As a result of successful vaccination programs, the incidence of many vaccine-preventable diseases is extremely low in the United States and other wealthy countries. This complicates efforts to convey the continued necessity of vaccines to parents, many of whom may have never seen or experienced the diseases being prevented. Globally, the risk of diseases is subject to wide geographical, national, and occupational variability. In all cases, high rates of vaccination among communities are widely credited with preserving the low incidence of many vaccine-preventable diseases.[1]

The benefit of high vaccination rates highlights a key difference between the ethics of treatment and the ethics of prevention. Prevention, specifically the prevention of infectious diseases through vaccination, has important implications for individual people and communities. While considerations relative to justice and the allocation of scarce resources have ethical relevance to some treatment decisions, rarely are they primary factors in how care is delivered.

As a form of prevention, vaccination requires juxtaposing individual autonomy against societal best interests. These values are often in alignment, but for some, they present competing arguments regarding the decision to vaccinate. Central to this debate is herd protection, the indirect benefits that result from high vaccination rates in a community and provide additional protection to vaccinated and unvaccinated individuals alike. For some parents or patients, the benefits of vaccination may be seen as being outweighed by the associated risks and costs, however small or unlikely they may be. This is a particularly possible scenario for vaccines against diseases that have virtually disappeared in wealthy countries partly as a result of successful vaccination programs. Those choosing not to be vaccinated would still benefit at least somewhat through herd protection, creating the potential ethical problem of benefiting as a "free rider."

Free riders are those who knowingly share in the benefits of social programs such as vaccination without personally assuming any risks, burdens, or costs, such as the risk of adverse events or the time and expense required to receive vaccines. Numerous vaccine-preventable disease outbreaks among unvaccinated persons suggest that personal reliance on herd protection is often inadequate.[2,3] Such decisions also increase the overall risk of vaccine-preventable disease in communities, particularly for their members too young to receive a vaccine or unable to do so because of medical contraindications. Those who knowingly benefit from the actions of others without sharing in the burden or cost of providing that benefit act in an unethical manner.

Target Populations and the Routinization of Vaccines

The significance of healthy children as the largest target population for vaccines cannot be overstated. Young children are ethically vulnerable because they cannot exercise their own autonomy to mediate issues of risk and benefit. With a majority of vaccinations in the United States and other countries recommended for children in the first 24 months of life, vaccine recipients are typically among the most ethically vulnerable of populations. Attention by vaccine stakeholders to vaccine-related risks, whether confirmed or alleged, is understandably heightened with respect to children, as is the importance of the potential benefits of routine, on-time vaccination. While striving to make decisions that are in a child's best interests, parents and guardians must navigate a sea of at times conflicting information related not only to vaccines, but also to all aspects of their child's medical care.[4–6] Widely publicized claims by critics of vaccines questioning the safety and value of vaccinations have added to the challenges faced by parents striving to make responsible and informed decisions about their children's health.

Ironically, communication efforts may be hampered as a result of how routine childhood vaccination has become in large parts of the world. Amid competing demands on healthcare providers' time, efforts to explain the continued importance of vaccination to parents may suffer. Absent unique

concerns raised by parents or providers, communication about vaccines may be left instead to a few short questions and answers or reliance on government-required printed information statements.[6] The routinization of vaccines is amplified by state laws in the United States requiring vaccination against many diseases as a condition of school or daycare attendance. This atmosphere of familiarity partially explains the widespread alarm generated by reports of vaccine-related safety concerns, regardless of whether the concerns are confirmed, alleged, or simply a matter of rumor.

ETHICAL CONSIDERATIONS IN THE VACCINE LIFE CYCLE: AN OVERVIEW

While the preceding discussion highlights some of the unique attributes of vaccines and vaccine decision making, their research, development, and regulation are similar in form and function to those of pharmaceuticals and other medical interventions. The following sections provide an overview of relevant ethical considerations at various stages of the vaccine life cycle, a period that begins with the earliest basic research and extends through licensure and all dimensions of national and international vaccine production and distribution programs.

Research and Development

The successful development of new discoveries increasingly relies on collaborations among academic scientists, government researchers, small biotechnology firms, and large, multinational vaccine manufacturers. Although financial support for this work has traditionally come from a combination of commercial investment and government-sponsored research awards, philanthropic groups and public–private partnerships have become increasingly active supporters of vaccine development, particularly for disease targets that lack large potential markets (and thus profitability) in wealthy countries.

Such a diverse group of research entities and funders all but assures a collection of differing research priorities, objectives, and measures of success. While all contributors share the general aim of developing safe and effective vaccines, conflicts may arise regarding how best to achieve this goal.[7] If unchecked, such conflicts could impede progress toward novel vaccines, waste limited financial resources, and fail to respect the contributions of human research subjects. Large research partnerships have the potential to advance public health in ways that would occur much more slowly, if at all, if attempted by individual entities. While respecting their obligations to shareholders, boards, or other overseers, all contributors to vaccine research and those who invest in their work should remain keenly aware of the morally distinguishing ability of their work to save lives, prevent suffering, and greatly improve global health.

Research partnerships become even more ethically complex when they include clinical research in developing countries. This is especially important as vaccine development efforts increasingly target diseases common in developing countries, often through collaborations in which Western researchers have a leading role. These activities bring needed expertise and capabilities to countries often lacking robust medical research infrastructures and stable health systems, even before considering the potential direct benefits successful new vaccines may bring. During this research and development process, local researchers and health ministries ought to be involved in a meaningful way in all aspects of clinical research taking place in their countries. The benefits of such relationships are many. For example, they may provide additional knowledge and training for local health officials, expertise that could benefit communities long after research has concluded. Meaningful contributions from local officials also create an additional layer of protection to ensure that the generosity of volunteers is not exploited by research that very often would have been deemed unethical had it been proposed for populations in wealthy countries.

No topic related to vaccine research has generated more controversy on ethical grounds than the designs of clinical trials, particularly those in developing countries.[8–12] As an example, one prominent and ongoing debate for nearly 20 years has centered on HIV vaccine trials in developing countries, particularly the level and duration of care that ought to be provided to research subjects who become infected during trials.[13–18] Options range from lifelong treatment with the latest in antiretroviral medications—the norm in many developed countries but highly uncommon elsewhere—to whatever the typical treatment is in the country where the trial took place. Such a level of care is often well below that of the research sponsors' home countries. It may be nothing. Because of the significant consequences of this debate to the feasibility of future research, attempts at reaching consensus on the question of which standards should prevail have largely failed.[13,15] This longstanding and still unresolved debate provides a useful example of the value of robust discussions of ethical considerations *before* controversies develop and decisions cannot be undone.

Vaccine development can never be immune from the twin pressures of personal advancement and corporate profitability. Nevertheless, the world community is best served by vaccine research programs that match these concerns with a continued acknowledgment of the enormous suffering that can be averted by vaccines and respect for the individual people and communities volunteering to assist in clinical research.

Finally, concern for justice requires that the populations in which a vaccine candidate is studied mirror as closely as possible the groups expected to receive the licensed product. The use of mentally handicapped children as primary sources of vaccine research subjects in the 1950s and 1960s exemplifies how this principle was violated in the past.[19] These children often lived in overcrowded institutions with sanitary conditions that placed them at increased risk for the diseases for which potential vaccines were being tested. New vaccines would have been particularly valuable to these children, who also provided a convenient, accessible study population for researchers.

However, mentally handicapped children in state institutions are among the most vulnerable of populations, for whom clinical research is ethically appropriate only in extremely limited circumstances and always with strict oversight. In pursuit of vaccines that would benefit society broadly, the past use of these children as a primary study population often violated this fundamental and timeless tenet of research ethics.

Today, the opposite extreme has become common. Manufacturers have a strong disincentive to include among their research subjects members of potentially high-risk populations, such as pregnant women or children with intellectual disabilities. Without complete data on safety and efficacy in all populations to whom a vaccine will be administered, patients, parents, and policymaking bodies have inadequate information on which to base their decisions regarding vaccination in these groups. The vaccine research and regulatory communities should remain aware of past exploitation of vulnerable research subjects but strive to conduct clinical research in ways that protect subjects while providing as complete a picture as possible of a vaccine's safety and efficacy profile.

Licensure and Safety Monitoring

Vaccines are subject to oversight and regulation by a variety of entities in every country where they are available.[20] It is initially determined whether a new vaccine should be licensed, and if so, the populations for whom it should be recommended. In the United States, these responsibilities belong to groups within the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC), respectively. For the life of the vaccine thereafter, activities are undertaken by these groups in collaboration with its manufacturer to monitor its safety and efficacy.[20,21] These processes have generated considerable controversy in recent years, threatening public confidence in vaccination.[22] As the success of vaccination programs depends on earning and maintaining public trust, there are several points in vaccine regulation at which ethical considerations are relevant to public policy.[9,23]

A primary concern of critics of vaccine policy in the United States is the potential for conflicts of interest among government advisors and researchers who have ties, financial or otherwise, to vaccine manufacturers. The very nature of vaccine development presents unique challenges in avoiding even the appearance of a conflict. Any researcher working on novel vaccine candidates *must* eventually partner at some point with industry owing to the infrastructure needed for large-scale clinical testing and development. To exclude all such researchers for this reason would be to forfeit a wealth of expertise and wisdom on vaccine science and policy, ignoring internationally respected leaders in vaccine science.

However, the importance to vaccination programs of maintaining public trust demands that those contributing to policy exercise particular caution and care regarding their professional and financial relationships. Most advisory bodies have clear policies regarding the disclosure of potential conflicts of interest.[24,25] Even when confident that financial relationships would have no impact on their actions, individuals should remain highly attentive to how such interests might affect the manner in which the decisions to which they contribute may be perceived. Transparency, minimization of personal gain, divestiture, and disclosure are crucial principles that work to counteract the perception of conflict of interest influencing decision making.

Public attention to conflicts of interest among policymakers and their expert advisors is often linked to reports of vaccine safety concerns. The public health and regulatory communities should respond vigorously to reports of vaccine-associated adverse events, even if they initially seem unlikely. Passive surveillance programs, such as the Vaccine Adverse Event Reporting System in the United States, are valuable tools in this effort, but their limitations as hypothesis-generating mechanisms should be clearly and repeatedly conveyed to the public and the media.[26] Emerging patterns of possible safety problems should be explored thoroughly, with the results of these analyses promptly communicated to the public. Even when evidence suggests that a reported safety concern is unfounded, experience shows it is unlikely that any such assurance will allay the worries of all. Nevertheless, open, expeditious, and objective examinations of possible vaccine-associated safety concerns are essential to maintaining overall confidence in vaccination programs and their oversight.[22,27]

It is known that a small number of persons will genuinely be harmed as a result of vaccines. Because vaccination benefits communities, victims are entitled to compensation for their suffering in these rare cases. In the United States, the Vaccine Injury Compensation Program is the principal mechanism for resolving such issues.[28] By means of a no-fault system with funds collected via a tax on every vaccine dose, the system provides compensation without exposing manufacturers to substantial legal and financial risk.

This program has faced scrutiny in recent years regarding how claims are resolved and the scope of its coverage. The Omnibus Autism Proceeding and the case of *Bruesewitz v Wyeth* heard by the U.S. Supreme Court in 2010 represented challenges to aspects of the federal government's system for identifying and compensating vaccine-related injuries.[29,30] In the Omnibus Autism Proceeding, theories that the measles-mumps-rubella vaccine and the vaccine preservative thimerosal could, individually or in tandem, cause autism in vaccine recipients were extensively examined and unequivocally rejected by the special masters who evaluate claims for compensation. *Bruesewitz v Wyeth* affirmed the primary role of the federal Vaccine Injury Compensation Program, not state laws or civil proceedings, in assessing vaccine-related adverse events. Given the concomitant challenges of compensating victims fairly, accurately distinguishing correlation from causation regarding adverse events, and ensuring that manufacturers remain committed to developing and manufacturing vaccines, the current design of the Vaccine Injury Compensation Program is a generally fair way of resolving these ethical obligations.[31]

Vaccine Supply, Access, and Financing

The highly publicized influenza vaccine shortage in 2004 and many other shortages for recommended childhood vaccines underscore the vulnerability of vaccine supply worldwide.[32,33] With limited manufacturers and often only a single licensed product available in a country, vaccines are highly susceptible to large fluctuations in supply and availability owing to unforeseen events.[34–36] In the United States, CDC stockpiles of recommended vaccines are maintained to provide a temporary buffer in case of production or supply problems, but these efforts provide only limited protection.[37,38]

A spectrum of economic and business considerations helps explain why many manufacturers have left the vaccine market and why the remaining manufacturers are not eager to develop new products to compete with older vaccines that, in general, are able to meet demand.[34,35] Finding incentives to increase the number of vaccine manufacturers and to encourage development of new vaccines for common diseases would help ensure a more resilient vaccine supply landscape that is better insulated against the shortages that have become increasingly common.

Even when vaccine supplies are adequate, vaccination rates reflect many of the same racial and ethnic disparities in access present throughout medicine.[39,40] While the underlying causes of these conditions are debated, several programs seek to eliminate vaccine cost as an obstacle to childhood vaccination in the United States, most notably the Vaccines for Children program for uninsured and underinsured children.[41] Established in 1994, this entitlement program makes vaccines recommended by the CDC's Advisory Committee on Immunization Practices available free of charge to children in these groups. The size and cost of the program has grown with additions to the recommended vaccination schedule, especially since the 1990s; in 2016, its annual budget surpassed $4 billion.[42]

With respect to private health insurance in the United States, under the Affordable Care Act, new insurance plans must provide coverage for any vaccine recommended for routine use in children or adults by the Advisory Committee on Immunization Practices.[42] No copayment, coinsurance, or deductible can be required from the insured patient. This provision for vaccines is part of a spectrum of preventive services required of new private insurance plans. According to the

Institute of Medicine, an estimated 11 million children and 59 million adults had private insurance that did not adequately cover costs related to immunization prior to the passage of the Affordable Care Act, a shortcoming that the law is quickly correcting.[43]

In comparison with children and insured adults, uninsured adults in the United States face far greater financial obstacles to vaccination. With an increasing number of vaccines recommended for adults but no program similar to Vaccines for Children, the affordability of vaccines for uninsured adults remains a substantial challenge. Federal funding through the Section 317 program to state and municipal grantees has long provided some assistance in this area, but the available funds have long been outstripped by the needs in this area, all the more so as the adult vaccination schedule has also expanded in recent years.[41] Federal and state governments, in collaboration with vaccine manufacturers, should identify strategies that would reduce these financial obstacles to adult vaccination. The laudable patient assistance programs organized by several vaccine manufacturers may provide a foundation for more expansive efforts in this area.

Vaccination Requirements, Hesitancy, and Refusal

Governments worldwide use a variety of approaches to promote high vaccination rates among their citizens.[41,44–46] The United States is generally unique in its reliance on federal recommendations coupled with state school-entry vaccination requirements as central contributors to the success of vaccination efforts. While specific requirements vary among states, all require that children receive a series of vaccinations as a condition of attending public schools or state-licensed daycare facilities.[47] Every state allows for exemptions based on medical grounds, and nearly all also accept religious or philosophical reasons, although not every state includes all three types of exemptions.[48]

No topic related to vaccine ethics in the United States is the subject of more public scrutiny and, at times, controversy than state vaccination requirements. The debate reflects not only the common tension in public health policy between individual (or parental) autonomy and the public good, but also questions over the role and extent of government intervention to protect the well-being of children. Requirements promote vaccination for individual children, while also striving to limit the potential transmission of diseases among communities to the greatest extent possible. School-entry requirements have long been seen by public health officials as essential to maintaining vaccination rates sufficiently high to preserve herd protection, particularly when other vaccine education and promotion efforts have failed.[49] The high vaccination rates associated with herd protection offer benefits against vaccine-preventable diseases to all members of a community, including those too young to receive vaccines, those unable to do so because of medical contraindications, and those who were vaccinated but did not generate the typical, adequate immune response needed for personal protection.

For those whose views on medical ethics are guided by the primacy of patient autonomy, it is understandable why U.S. vaccination requirements are so contentious. However, few contemporary ethical models place autonomy absolutely above all other considerations. Instead, respect for autonomy is typically one of several factors that should be examined in light of other relevant considerations as part of ethical deliberation and decision making. There is a compelling argument that the lives saved and suffering prevented by vaccination outweigh the potential infringement on personal autonomy created by school mandates. While one may be free to make medical decisions that place one's own health at risk, one may not jeopardize the health of others, a consequence of low vaccination rates within a community. Even in an autonomy-oriented culture such as the United States, there are ethical reasons to place some limits on individual choice.

An ethically preferable scenario would be maintaining current high rates of vaccination without needing the force of mandates to do so. Absent evidence that this is attainable in the United States, the current policy is sound. Mandates serve as a "safety net," a valuable tool to call attention to the importance of vaccines and help direct government and public health resources to vaccination efforts.[47] Exemption policies provide a ready alternative in nearly all states for persons whose personal beliefs do not coincide with protecting their child against vaccine-preventable disease to the greatest extent possible or with promoting public health in their communities. Combined with incomplete enforcement by state health departments or local school districts, current policies fall far short of true compulsion. They are, instead, best understood as presumptive or default approaches to vaccination.

Even though national rates of nonmedical exemptions remain quite low, recent trends—particularly clusters of voluntarily unvaccinated individuals in some communities—are of deep concern to advocates of vaccination.[50] Recent outbreaks of vaccine-preventable diseases, particularly the 2015 measles outbreaks in the United States, prompted calls to reevaluate the compromise reflected in the current approach to vaccination requirements and exemptions.[51] Proposals to eliminate nonmedical exemptions entirely—as has been the case for many years in West Virginia and Mississippi—have been debated in numerous states, and passed in California in 2015. Other proposals have sought to preserve such exemptions but to impose additional administrative burdens to make them more difficult to obtain, an approach shown in states with similarly rigorous procedures to successfully reduce the rate of nonmedical exemptions.

Although a principled and persuasive argument can be made for the elimination of all nonmedical exemptions, some advocates of vaccination worry that a potential public backlash and resulting challenges in enforcement might lead to such reforms actually doing more harm than good to vaccination rates and vaccination programs overall. The experiences of states like California that move to eliminate exemptions should be watched closely to assess whether these hypothesized concerns are or are not realized as new policies are approved and implemented.

These various proposals to restrict or eliminate exemptions all reflect the increasingly clear and ethically defensible assessment that current policies in this area place unvaccinated children and their communities at an unacceptable, elevated risk of preventable disease. At the same time, these legislative approaches to ensuring high vaccination rates must be viewed as part of a comprehensive education and promotional effort that aims to grow and sustain the widespread foundation of public support that remains in the United States. This confidence in the value of vaccination is reflected in high vaccination rates in young children long before they are likely to encounter school or daycare requirements, and it persists despite discouraging trends in vaccine hesitancy and refusal among a small, but growing minority.

A related issue is how physicians and other healthcare providers should respond to parents who desire an alternate approach to vaccination than the evidence-based recommendations developed by public health and medical authorities, such as the schedule in the United States developed by the CDC, the American Academy of Pediatrics, and other medical professional organizations.[4,52] At issue has been whether physicians should decline to care for children whose parents wish

to delay or omit some or all recommended vaccines. Advocates of this view believe it would clearly signal physicians' unambiguous position on the importance of on-time vaccination, and it would reduce the theoretical risk unvaccinated children would pose to other patients in waiting rooms. Critics, including the American Academy of Pediatrics in a longstanding policy statement, believe dismissal of patients in this context should be reserved for the very rare circumstance when conflicts between the views of the physician and parents present a profound impediment to the provision of care to the child.[52] In nearly all other circumstances, the American Academy of Pediatrics recommends continuing to provide care, in hopes that a growing relationship of dialogue and trust between physicians and parents may lead to a changed position over time. Otherwise, children of parents who delay or decline vaccines may be left without advocacy and find themselves cared for by physicians who are willing to accept any (or no) approach to vaccination, a harmful outcome for those children and their communities alike.[4]

SPECIAL TOPICS IN VACCINE ETHICS

Healthcare Providers

Healthcare providers have a twofold role in the success of disease-prevention efforts through vaccination. Considerable evidence points to the importance of recommendations from physicians and other providers as being among the most effective ways to influence the perceptions and decisions of parents regarding vaccines, particularly parents with reservations or questions.[53] Amid conflicting information and contentious debates about the safety, effectiveness, and value of vaccines, healthcare providers can help patients (or parents or guardians) make sense of the science and evidence so often obscured by unproven allegations and anecdotal evidence. Doing so effectively requires sustained attention by providers to new information about vaccines—particularly vaccine safety—and the time and willingness to engage parents with concerns or uncertainty.

Healthcare providers can also demonstrate the value of vaccination by ensuring that they are personally up-to-date on recommended vaccines. Beyond the symbolic value of this action, vaccination of providers protects patients, particularly in hospital settings where diseases like influenza are easily transmitted.

Maintaining high vaccination rates against seasonal influenza among healthcare providers has proven to be a considerable challenge, despite the vaccine being recommended for this group since 1981.[54] A variety of programs, including those involving incentives for compliance and appeals to professional duty, have generally failed to yield adequate vaccination rates, prompting an increasing number of healthcare facilities to mandate annual influenza vaccination as a condition of employment.[55] In light of the consistent failure of voluntary approaches to improve influenza vaccination rates among healthcare workers, mandatory vaccination policies, which now exist in more than 600 hospitals and nursing homes throughout North America, are an appropriate response.[56] This is particularly true in light of the increased susceptibility to infection among many patients in hospitals and related settings.

Vaccination in the Developing World

Special ethical considerations related to vaccines in the developing world extend beyond the research issues discussed previously. A particular challenge is ensuring that new vaccines are introduced against diseases that are most prevalent or most severe in those nations but uncommon or mild in developed countries. Owing to the limited profitability of such vaccines, manufacturers are often reluctant to invest in these efforts, with occasional exceptions for diseases relevant to national security or biodefense.[57,58] Much of the remaining work, at both early and late stages of development, is therefore supported by private philanthropies, nonprofit entities, and public–private partnerships. These efforts should continue to be encouraged so that the benefits of vaccination may be more equitably distributed among all populations. For vaccines developed by corporate manufacturers, work should continue to develop financing arrangements that ensure that existing products are available to populations in developing countries that in many cases stand to benefit most from them.

When organizing vaccine distribution programs, particular respect should be paid to cultural traditions and social customs specific to communities in the developing world. In locations where healthcare infrastructures are radically different from those of wealthy countries, successful vaccination efforts depend on embracing these differences, valuing the input of community leaders, and striving to develop programs that gain widespread support.

Finally, efforts should be undertaken to better understand the concept of consent in the context of developing world vaccination programs. Informed consent in the strict Western sense may not always be attainable, nor may it be a reasonable expectation owing to the varying structures of communities and families present throughout the world. However, vaccination efforts should remain faithful to the spirit of informed consent, with those administering vaccines taking steps, as appropriate, to ensure that surrogates offer consent for recipients and that local and international ethics committees closely supervise research when individual informed consent is weak.

Eradication Campaigns

The global eradication of smallpox, certified by the World Health Organization in 1980, remains one of the foremost achievements in the history of public health.[59,60] By means of a coordinated, extensive vaccination campaign, a disease that had been the cause of untold suffering and death for centuries was effectively eliminated. The successful eradication of smallpox added to the enthusiasm for other eradication campaigns against vaccine-preventable diseases, including those then already underway and others hypothesized at the time. This enthusiasm has continued, despite as yet insurmountable challenges in adding additional diseases to the list of those eradicated.

Measles and polio have long been among the most prominent targets of eradication efforts, and eradication is now mentioned as a target for malaria, a disease for which a partially effective vaccine recently has been developed.[61,62] In recent years, most attention has been directed toward the potential eradication of polio, a goal that seems tantalizingly close in light of the limited number of identified cases (considerably below 500/year for several years) and the three countries where the disease remains endemic—Afghanistan, Pakistan, and Nigeria.[63] The unique characteristics of poliovirus have presented significant challenges for the potential eradication of the disease, despite laudable attention and enormous investment in the effort, much of it supported by philanthropies including The Bill and Melinda Gates Foundation and Rotary International.[64] Horrific attacks on polio vaccination workers in both Pakistan and Nigeria have added a human toll to the already considerable costs and challenges of the continued pursuit of polio eradication.[65] A rare but very serious adverse event, vaccine-associated paralytic poliomyelitis, associated with the oral polio vaccine that has long been central to

polio eradication efforts in developing countries presents still further questions regarding the ethics of eradication.

The obstacles and risks related to the continued pursuit of polio eradication have prompted some observers to suggest that a better overall strategy for global health is to maintain current levels of control while redirecting the sizable resources (both financial and personnel) currently going toward polio eradication campaigns to the many other causes of preventable morbidity and mortality worldwide.[66] A necessary and important debate has ensued among scientists, ethicists, and global health scholars.[67,68] The symbolic significance of disease eradication carries an allure that may not necessarily coincide with evidence-based approaches to global health policy. Because global health resources are limited, policymakers and funding sources should ensure that attention is directed to the prevention and treatment strategies that will prevent the most suffering and thereby do the most good.

Vaccines and Public Health Emergencies

Concerns about anthrax or smallpox bioterror attacks in the aftermath of September 11, 2001; preparedness efforts in the early 2000s for an avian influenza pandemic and the subsequent pandemic caused by a novel strain of H1N1 influenza in 2009–10; and a series of emerging or reemerging infectious diseases including severe acute respiratory syndrome and Ebola have all called attention to the critical role that the development and deployment of vaccines can play in responding to public health and global health emergencies.

The 2009–10 H1N1 influenza pandemic provided a real-time test of global preparedness and planning for a potential influenza pandemic. While much of this planning had focused on the threat of H5N1 (avian) influenza, those preliminary activities served as a valuable foundation for the response to H1N1 influenza. Vaccines were central to those planning and response efforts.[69–72] Within weeks of the identification of a potential pandemic strain of influenza in April 2009, the process of developing seed stocks for an eventual vaccine began.[73] The total time required to produce the first doses of vaccine adhered fairly closely to the long-predicted estimate of 6 months. By the time vaccine was widely available in late 2009, public interest had waned, in part a result of a growing understanding of the relatively modest severity of the virus.

Despite having overall impacts well below some of the most dire forecasts, the 2009–10 H1N1 influenza pandemic was still a significant cause of infection, illness, and death worldwide, particularly among children. It thereby provided valuable knowledge and experience that will inform subsequent planning and response activities for public health emergencies for which vaccines may be available, including potential pandemics and acts of bioterrorism. Among the topics that have received scrutiny are the communication of accurate information about the severity of threats amid uncertainty, the efficiency of vaccine development and the need for greater production capacity and novel technologies, the adequacy of testing and postlicensure safety surveillance, the development of appropriate prioritization strategies, the structure of financing and distribution systems, and the allocation of vaccine to countries in the developing world. As these retrospective analyses are translated into plans for subsequent public health emergencies, public health officials should aim above all to ensure that vaccination programs are designed to maximize benefits and minimize risks fairly among communities, populations, and nations.

As demonstrated in 2009, the urgency that follows the arrival of public health threats makes thoughtful discussion of these topics in real-time all but impossible, making it all the more important to engage in public deliberation and planning well in advance of public health emergencies. In particular, it is necessary to achieve and maintain public consensus on the rules that will govern allocation and rationing. Unless the rules are widely perceived as fair—arrived at by reasonable, open procedures—and just—helping persons at greatest risk while maximizing the public good—response plans are unlikely to gain acceptance.

Similar evidence of the challenges of debating complex scientific, public health, and ethical aspects in the midst of an unfolding health emergency was shown in the response to the 2014–15 Ebola outbreak concentrated in West Africa. Many of these issues pertained to aspects of the response unrelated to vaccines, such as the quarantines and travel bans endorsed by some elected officials in the United States despite a consensus among the scientific community that they were at best unnecessary, if not outright harmful to the response.[74] However, questions also arose regarding how to strike the appropriate balance between rigorously evaluating the safety and effectiveness of potential Ebola vaccines and urgently responding to what, in late 2014, was an unprecedented and out-of-control health crisis in the affected countries. At issue was how to design trials that would provide valid data regarding those vaccines without imposing undue delays on the availability of vaccines (or, similarly, pharmaceuticals) that had at least some potential to aid in the response.

With valuable coordination from the World Health Organization, the global community quickly debated the relative merits—both scientific and ethical—of randomized controlled trials versus other designs, as Ebola vaccine candidates that had received limited attention and investment for years were quickly accelerated into clinical testing.[75,76] Proponents of randomized controlled trials noted that those methods would provide the strongest, clearest evidence of the safety and effectiveness of any vaccine.[75] Advocates of alternative designs—such as nonrandomized approaches, stepped-wedge designs, and others—believed that the magnitude of an unfolding public health emergency necessitated novel approaches that would make potentially valuable interventions available as quickly as possible to as many people as possible, goals to which randomized designs may not be best suited.[76,77]

By early 2015, large-scale clinical trials of multiple vaccines were ready to commence, incorporating both randomized trials and alternative approaches. However, the apparent waning of the outbreak in the countries most severely affected by Ebola by spring 2015 made it likely that the trials might not provide clear evidence of the effectiveness of the vaccines. Such evidence, invaluable to the use of Ebola vaccines to prevent and respond to future outbreaks, may now have to be inferred from other sources such as animal studies or immune response analyses.

While the global community should be commended for the frank and highly engaged discussion regarding the testing and use of unlicensed interventions as part of the Ebola response, the experience again underscores the value of proactive deliberation on issues related to research methodology and ethics in response to public health emergencies. Also, that Ebola vaccine development languished for years because of a lack of investment from public or private sources and the absence of an obvious commercial market provides further evidence for the need to pursue novel strategies to accelerate vaccine research for diseases that have been neglected by the traditional development pathways unless and until a crisis emerges.[78]

THE FUTURE OF VACCINE ETHICS

The study of ethical issues in vaccination has received increasing attention in recent years, but there still have been relatively

few concentrated attempts at broad exploration. Those attempts to examine vaccine ethics at a level larger than a single topic or debate have produced valuable results and can serve as models for future efforts.[79,80] A need continues to exist, however, for the creation of frameworks and key principles for ethical decision making throughout the vaccine life cycle. Such work would move us closer to solutions or consensus for many of the questions raised in this overview. These efforts would do far more good if they occur proactively, long before controversies or crises surface.

Advances in vaccination are attributable to gains in scientific knowledge, breakthroughs in research, and the creation of sound public health policy, among many other factors. Ultimately, however, the success of vaccination depends on maintaining widespread public trust, without which vaccination programs cannot succeed.[23] Preserving trust requires an unwavering awareness that the remarkable societal benefits of vaccination ultimately involve individual people who are entitled to respect, in a broad sense, that manifests itself at every point in the vaccine life cycle. By remaining sensitive and responsive to the consideration of ethical challenges, vaccination is best positioned to add to its history of public health triumphs.

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APPENDIX Websites and Applications for Mobile Devices With Information About Immunization

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Note: Since the sixth edition of *Vaccines* was published, many organizations have begun offering social media features and applications (apps) for mobile devices in addition to their websites. This edition of the appendix includes some of these resources.

CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC) WEBSITES

National Center for Immunization and Respiratory Diseases (NCIRD)

- www.cdc.gov/vaccines

NCIRD is an interdisciplinary immunization program that brings together vaccine-preventable disease science and research with immunization program activities. Its website provides information about vaccine-preventable diseases, the benefits and risks of immunization, vaccine licensures, Advisory Committee on Immunization Practices (ACIP) recommendations, Vaccine Information Statements (VISs), and vaccine supply issues, as well as educational resources for the public and healthcare professionals.

ACIP is composed of 15 experts in fields associated with immunization who provide guidance on the control of vaccine-preventable diseases. ACIP recommendations are available at: www.cdc.gov/vaccines/acip.

- CDC vaccine schedules app: https://itunes.apple.com/us/app/cdc-vaccine-schedules/id875273858?mt=8

Morbidity and Mortality Weekly Report (Weekly Publication)

- www.cdc.gov/mmwr
- Twitter: https://twitter.com/DrNancyM_CDC
- *MMWR Express* app: https://itunes.apple.com/us/app/mmwr-express/id868245971?mt=8

Influenza

- www.cdc.gov/flu

CDC's influenza website includes information for healthcare professionals and the public on influenza prevention, clinical and laboratory diagnosis, treatment, surveillance, and issues for specific groups as well as educational tools.

- Twitter: https://twitter.com/CDCFlu
- CDC Influenza app: https://itunes.apple.com/us/app/cdc-influenza-flu/id577782055?mt=8
- CDC FluView app: https://itunes.apple.com/us/app/fluview/id507807044?mt=8

Immunization Safety Office (ISO)

- https://www.cdc.gov/vaccinesafety/iso.html

ISO identifies possible vaccine side effects, conducts epidemiological studies to determine whether an adverse event is caused by a vaccine, helps determine the appropriate public health response to vaccine safety concerns, and communicates the benefits and risks of vaccines.

National Center for HIV/AIDS, Hepatitis, STD, and Tuberculosis Prevention (NCHHSTP)

- www.cdc.gov/nchhstp

NCHHSTP is responsible for public health surveillance, prevention research, and programs to prevent and control HIV, hepatitis, other sexually transmitted diseases, and tuberculosis.

- Viral hepatitis information: www.cdc.gov/hepatitis
- Human papillomavirus virus (HPV) information: www.cdc.gov/hpv
- Twitter: https://twitter.com/DrMerminCDC and https://twitter.com/DrDeanCDC

Traveler's Health

- wwwnc.cdc.gov/travel

This website provides health information on specific travel destinations, information on disease outbreaks, and ACIP vaccination recommendations for travelers of all ages.

- Twitter: https://twitter.com/cdctravel
- CDC's TravWell app: https://itunes.apple.com/us/app/cdc-travwell/id969599694

OTHER U.S. GOVERNMENT IMMUNIZATION-RELATED WEBSITES

Centers for Medicare & Medicaid Services (CMS)

- www.cms.gov

CMS is the agency responsible for administering programs such as Medicare, Medicaid, and the State Children's Health Insurance Program. A key section is "Immunizations" (www.cms.gov/Medicare/Prevention/Immunizations/index.html), which includes links to pay rates, billing instructions, FAQs for healthcare professionals, and best practices.

- Twitter: https://twitter.com/cmsgov

Dale and Betty Bumpers Vaccine Research Center (VRC)

- https://www.niaid.nih.gov/about/vrc

VRC, part of the National Institute of Allergy and Infectious Diseases, was established to facilitate vaccine development research.

Department of Defense: Defense Health Agency Immunization Healthcare Branch

- www.health.mil/vaccines

This website provides access to current immunization program information for the U.S. Department of Defense

and the military services as well as disease and vaccine information.
- Twitter: https://twitter.com/dha_ihb

Department of Health and Human Services (HHS)

- www.vaccines.gov

HHS maintains this website for the public; it includes information about how to find and pay for vaccination services.
- HHS influenza public website: https://www.vaccines.gov/diseases/flu/

Food and Drug Administration (FDA), Center for Biologics Evaluation and Research (CBER)

- www.fda.gov/BiologicsBloodVaccines

CBER is the part of the FDA responsible for ensuring the safety, efficacy, purity, and potency of vaccine products. Information on currently licensed vaccines and their manufacturers is available at: www.fda.gov/BiologicsBloodVaccines/ucm121134.htm.
- Twitter: https://twitter.com/fdacber

National Vaccine Injury Compensation Program

- www.hrsa.gov/vaccinecompensation

This program, enacted by Congress, is a no-fault alternative to the tort system for resolving claims resulting from adverse reactions to recommended childhood vaccines. The site includes information on how to make a claim.

National Vaccine Program Office (NVPO)

- www.hhs.gov/nvpo

NVPO, which resides within HHS, has responsibility for coordinating and ensuring collaboration among the many federal agencies involved in vaccine and immunization activities. The website provides publications and reports on vaccine preventable diseases and information on vaccine safety, vaccine coverage, immunization laws, immunization registries, and reports by the National Vaccine Advisory Committee (NVAC).

Vaccine Adverse Event Reporting System (VAERS)

- http://vaers.hhs.gov

VAERS is a postmarketing safety surveillance program that collects information about adverse events that occur after the administration of U.S.-licensed vaccines. This website provides a nationwide mechanism by which adverse events following immunization can be reported, analyzed, and made available to the public.

STATE AGENCIES AND PROGRAMS

State Immunization Websites

- www.immunize.org/states

This Immunization Action Coalition (IAC) website provides access to all state immunization program websites.

State Immunization Coordinators

- www.immunize.org/coordinators

This IAC website provides access to the names and phone numbers of state and territory immunization program managers, hepatitis B coordinators, adult viral hepatitis prevention coordinators, and Vaccines for Children coordinators.

State Mandates on Immunization and Vaccine-Preventable Diseases

- www.immunize.org/laws

This IAC website provides vaccination mandate information for child care facilities, schools, colleges, and long-term care facilities. CDC also maintains a website related to requirements and laws at: www.cdc.gov/vaccines/imz-managers/laws/index.html.

INTERNATIONAL IMMUNIZATION-RELATED WEBSITES

Bill & Melinda Gates Foundation

- www.gatesfoundation.org

The Bill & Melinda Gates Foundation is dedicated to improving the quality of life for individuals around the world by partnering with organizations to identify issues, find answers, and drive change in the areas of poverty, health, and education. Its Vaccine Delivery Program works to ensure that existing life-saving vaccines are introduced into countries where people need them most and supports innovation to develop new vaccines, as well as new delivery technologies and approaches.
- Vaccine Delivery Program: www.gatesfoundation.org/What-We-Do/Global-Development/Vaccine-Delivery
- Twitter: https://twitter.com/gatesfoundation

Edward Jenner Society

- www.edwardjennersociety.org

The mission of the society is to engage, support, and sustain the professional goals and interests of vaccinologists around the world and to perpetuate the memory of Dr. Edward Jenner.

End Polio Now

- www.endpolio.org

This website documents Rotary International's work in the area of global polio eradication.
- Twitter: https://twitter.com/EndPolioNow

Gavi, the Vaccine Alliance

- www.gavi.org

Gavi is an international organization created in 2000 to improve access to new and underused vaccines for children living in the world's poorest countries. Gavi brings together the public and private sectors with the shared goal of creating equal access to vaccines for children.
- Twitter: https://twitter.com/gavi

Global Polio Eradication Initiative

- www.polioeradication.org

The fight to end polio is led by this organization, which includes Rotary International, UNICEF, CDC, WHO, the Bill & Melinda Gates Foundation, and governments of the world.

International Vaccine Access Center (IVAC)

- www.jhsph.edu/research/centers-and-institutes/ivac

The Johns Hopkins Bloomberg School of Public Health established IVAC to increase access to life-saving vaccines and serve as a source of international vaccine policy information and analysis.

Measles & Rubella Initiative

- www.measlesrubellainitiative.org

The Measles & Rubella Initiative is a global partnership committed to ensuring no child dies from measles or is born with congenital rubella syndrome.

- Twitter: https://twitter.com/MeaslesRubella

Pan American Health Organization (PAHO)

- www.paho.org/english/ad/fch/im/Vaccines.htm

PAHO is an international public health agency working to improve health and living standards of the countries of the Americas.

- Twitter: https://twitter.com/pahowho

PATH

- www.path.org

PATH is a nonprofit organization that works to enable communities to break long-standing cycles of poor health throughout the world. Its Vaccine Resource Library (www.path.org/vaccineresources) offers a variety of documents and links on vaccine-preventable diseases and topics in immunization, including training materials in multiple languages.

- Twitter: https://twitter.com/VaxResources

Safe Injection Global Network (SIGN)

- www.who.int/injection_safety/sign/en

SIGN is a voluntary coalition of stakeholders aiming to achieve safe and appropriate use of injections throughout the world. The website includes resources to assist in developing and managing national injection policies.

Shot@Life

- http://shotatlife.org

Shot@Life, a project of the United Nations Foundation, empowers American citizens to champion vaccines as one of the most cost-effective ways to save the lives of children in developing countries.

- Twitter: https://twitter.com/shotatlife

Viral Hepatitis Prevention Board (VHPB)

- www.vhpb.org

This independent, international, multidisciplinary group of experts works to focus attention on the importance of viral hepatitis and on how to prevent it. Access fact sheets, reports, and articles from the website.

World Health Organization (WHO)

- www.who.int

WHO is the policy vehicle for global public health recommendations. Some of WHO's vaccine-related websites include:

- Index of WHO's vaccine and immunization information pages: www.who.int/topics/vaccines/en
- WHO Department of Immunization, Vaccines and Biologicals: www.who.int/immunization/en
- WHO position papers on vaccines: www.who.int/immunization/policy/position_papers/en

Global Advisory Committee on Vaccine Safety (GACVS)

- www.who.int/vaccine_safety/committee/en

GACVS consists of international vaccine experts who investigate vaccine safety issues of potential global importance. GACVS reports are available at: www.who.int/vaccine_safety/committee/reports/en.

Strategic Advisory Group of Experts on Immunization (SAGE)

- www.who.int/immunization/policy/sage/en

SAGE was established by the director-general of the World Health Organization in 1999 to provide guidance on the work of WHO. SAGE is the principal advisory group to WHO for vaccines and immunization.

Vaccine Safety Net (VSN)

- www.who.int/vaccine_safety/initiative/communication/network/vaccine_safety_websites/en/

VSN is a project aimed at improving global dissemination of reliable web-based vaccine safety information. The VSN newsletter is available at: www.who.int/vaccine_safety/initiative/communication/network/vaccine_safety_websites/en/index3.html.

Immunization Surveillance, Assessment and Monitoring

- www.who.int/vaccines/globalsummary/immunization/scheduleselect.cfm

This web page provides immunization statistics and schedules for individual nations.

- WHO position papers on vaccines: www.who.int/immunization/documents/positionpapers/en

ENGLISH LANGUAGE RESOURCES FROM NON-U.S. ORGANIZATIONS

Immunize Canada

- www.immunize.cpha.ca

This national nonprofit program offers vaccination information and resources for parents and patients.

Canadian Paediatric Society's "Caring for Kids"

- www.caringforkids.cps.ca/handouts/immunization-index

This section of this professional society's website provides information for parents about immunization.

Department of Health, United Kingdom of Great Britain and Northern Ireland

- www.gov.uk/government/collections/immunisation

This website is the official source of immunization information for health and social care professionals in the United Kingdom from the National Health Service. The following National Health Service website is targeted toward parents and patients: www.nhs.uk/Conditions/vaccinations/Pages/vaccination-schedule-age-checklist.aspx.

Health Canada, Immunization & Respiratory Infections Division

- www.phac-aspc.gc.ca/im

This website includes general information on immunization and vaccine safety as well as Canadian-specific material.

Immunise Australia Program

- www.immunise.health.gov.au

The website of the Australian government's immunization program provides information for healthcare professionals, patients, and parents.

ORGANIZATIONS WITH IMMUNIZATION-RELATED WEBSITES

317Coalition

- www.317coalition.org

The 317 Coalition, an alliance of more than 100 U.S. organizations, advocates for increased 317 funding from Congress to ensure that underinsured children and uninsured adults have access to recommended vaccines.

Alliance for Immunization in Michigan (AIM)

- www.aimtoolkit.org

The AIM website provides a toolkit of practical immunization resources for healthcare professionals and the public.

American Academy of Family Physicians (AAFP)

- www.aafp.org

AAFP provides immunization information to providers at: www.aafp.org/online/en/home/clinical/immunizationres.html.

American Academy of Pediatrics (AAP)

- www.aap.org

AAP provides immunization information to pediatricians and parents at www2.aap.org/immunization.

AAP has several apps, including the AAP *Red Book* app (included with a subscription) and *AAP News*: https://www.aap.org/en-us/Pages/Get-the-AAP-Mobile-App.aspx.

American College of Obstetricians and Gynecologists (ACOG)

- www.acog.org

ACOG's Immunization for Women website provides information for obstetricians and gynecologists and their patients at: www.immunizationforwomen.org.

- ACOG app: www.acog.org/ACOGapp

American College of Physicians (ACP)

- www.acponline.org

ACP provides information related to adult immunization at: https://www.acponline.org/clinical-information/clinical-resources-products/adult-immunization.

American Nurses Association (ANA)

- www.nursingworld.org

ANA maintains a website devoted to immunization topics, including a newsletter, at www.anaimmunize.org.

American Pharmacists Association (APhA)

- www.pharmacist.com

APhA's online Pharmacist Immunization Center (www.pharmacist.com/immunization-center) is a key resource for pharmacist immunizers that delivers news, tools, and resources.

Association of Immunization Managers (AIM)

- www.immunizationmanagers.org

AIM, a national organization that represents state and territory immunization managers, was created to enable them to work together to effectively prevent and control vaccine-preventable diseases and improve immunization coverage.

- Twitter: https://twitter.com/aim_iz

Association for Prevention Teaching and Research (APTR)

- www.aptrweb.org

APTR is an interprofessional association of health promotion and disease prevention educators and researchers. The website includes a number of online immunization training programs.

Center for Vaccine Awareness and Research, Texas Children's Hospital

- www.texaschildrens.org/vaccine

This organization provides online vaccine education resources for patients and healthcare professionals and is currently conducting National Institutes of Health-funded research on vaccines for pregnant women.

Every Child by Two (ECBT) and Vaccinate Your Baby

- www.ecbt.org and www.vaccinateyourbaby.org

ECBT works on reducing morbidity and mortality in children through timely immunization. The Vaccinate Your Baby website features information for parents. A related ECBT blog (Shot of Prevention) is updated for providers and parents: www.shotofprevention.org.

- Twitter: https://twitter.com/everychildby2

EZIZ Training, California Department of Public Health

- www.eziz.org

EZIZ is the e-learning website for the California Vaccines for Children Program. EZIZ offers unique resources and free online training related to vaccine storage and handling and administration.

Group on Immunization Education (GIE)

- www.immunizationed.org

GIE is part of the Society of Teachers of Family Medicine. The group's website provides immunization information for healthcare professionals and educators, including free smartphone apps and computer software for the childhood and adult immunization schedules.

HealthMap Vaccine Finder

- http://vaccine.healthmap.org

HealthMap Vaccine Finder is a free, online service where users can search by zip code for locations that offer immunizations. This service is maintained by a team of researchers, epidemiologists, and software developers at Boston Children's Hospital.

- Twitter: https://twitter.com/healthmap

History of Vaccines

- www.historyofvaccines.org

The History of Vaccines website was created by The College of Physicians of Philadelphia and explores the role of immunization in the human experience and examines its continuing contributions to public health.

- Blog: www.historyofvaccines.org/blog
- Twitter: https://twitter.com/historyvaccines

Immunization Action Coalition (IAC)

- www.immunize.org, www.vaccineinformation.org, and www.immunizationcoalitions.org

IAC publishes a wide array of immunization information for healthcare professionals and their patients at www.immunize.org, including hundreds of free downloadable CDC-reviewed print materials at www.immunize.org/handouts. IAC offers free subscriptions to two quarterly periodicals, *Needle Tips* and *Vaccinate Adults*, and a weekly email news service, *IAC Express*, at: www.immunize.org/subscribe. IAC provides translations of CDC's Vaccine Information Statements (VISs) in more than 30 languages at: www.immunize.org/vis. A second website, www.vaccineinformation.org, provides reliable immunization information to patients and parents and includes an extensive video library. IAC's third website, www.immunizationcoalitions.org, provides resources and support for immunization coalitions.

Ask the Experts, a collection of more than 1000 Q&As answered by CDC vaccine experts, can be found at: www.immunize.org/askexperts.

- Twitter: https://twitter.com/ImmunizeAction

National Academy of Medicine (NAM)

- www.nationalacademies.org/hmd

The mission of the IOM is to advance and disseminate scientific knowledge to improve human health. The Institute provides objective, timely, authoritative information and advice concerning health and science policy.

Institute for Vaccine Safety (IVS)

- www.vaccinesafety.edu

Based at the Johns Hopkins University, IVS is committed to investigating vaccine safety issues and providing timely and objective information on vaccine safety to healthcare professionals, journalists, and parents.

Institute for Safe Medication Practices National Vaccine Errors Reporting Program (VERP)

- http://verp.ismp.org

The Institute for Safe Medication Practices, a nonprofit national patient safety organization, launched VERP in collaboration with the California Department of Public Health. VERP is a place where healthcare professionals and patients can report vaccine errors confidentially. By collecting and quantifying information about these errors, ISMP will be better able to advocate for changes that could reduce the likelihood of vaccine errors in the future.

- Twitter: https://twitter.com/ismp1

Medscape

- www.medscape.org/infectiousdiseases

Medscape offers free continuing education activities, including vaccine topics, for physicians and other healthcare professionals. There is no charge for this service, but you must register.

- Twitter: https://twitter.com/medscape
- Medscape app: https://itunes.apple.com/app/medscape/id321367289

Medical Assistants Resources and Training on Immunization (MARTi)

- www.marti-us.org

MARTi provides medical assistants with access to practical immunization information as well as training opportunities.

- Twitter: https://twitter.com/martidotorg

National Adult and Influenza Immunization Summit (NAIIS)

- www.izsummitpartners.org

NAIIS, dedicated to addressing and resolving adult and influenza immunization challenges, is a national network of more than 130 public and private sector organizations that is cosponsored by CDC, NVPO, and IAC. NAIIS sponsors an annual meeting and ongoing work groups that operate throughout the year.

National Association of County and City Health Officials (NACCHO)

- www.naccho.org

NACCHO is the national organization representing local health departments. Its website offers a number of toolkits related to immunization.

National Association of School Nurses (NASN)

- www.nasn.org

NASN's website includes immunization resources, including position papers and continuing education opportunities: www.nasn.org/ToolsResources/Immunizations.

National Conference of State Legislatures

- www.ncsl.org/research/health/school-immunization-exemption-state-laws.aspx

NCSL collects data from all 50 states about religious and philosophical exemptions from school immunization requirements and provides links to specific statutes (if available).

National Foundation for Infectious Diseases (NFID)

- www.nfid.org

NFID is a nonprofit organization dedicated to research, education, prevention, and treatment related to infectious diseases. The website features sections on many vaccine-preventable diseases, including resources for healthcare professionals and patients.

- Twitter: https://twitter.com/nfidvaccines

PKIDs (Parents of Kids With Infectious Diseases)

- www.pkids.org

PKIDs offers information and resources for parents looking for support services, counseling, referrals, and information concerning infectious diseases in children.

- Twitter: https://twitter.com/pkids

Sabin Vaccine Institute

- www.sabin.org

The Institute promotes rapid scientific advances in vaccine development, delivery, and distribution worldwide via vaccine research and development, academic support, and public awareness. The Institute houses the International Association of Immunization Mangers (IAIM).

IAIM provides a forum where immunization managers can discuss and exchange best practices, build and support international and regional networks of immunization managers, and improve their technical and leadership capacity.

- IAIM website: www.sabin.org/programs/vaccine-advocacy-education/international-association-immunization-managers
- Twitter: https://twitter.com/sabinvaccine

ShotbyShot

- http://shotbyshot.org

A collection of stories from people who have been affected by vaccine-preventable diseases, sponsored by the California Immunization Coalition.

- Twitter: https://twitter.com/ShotbyShotorg

Vaccine Education Center at The Children's Hospital of Philadelphia (VEC)

- http://vec.chop.edu/service/vaccine-education-center

VEC provides reliable, up-to-date information about vaccines, including videos and tear sheets on vaccines and vaccine concerns for healthcare professionals to use with patients and parents. The Center also sponsors the Parents PACK program, where parents can get answers to questions or subscribe to an email newsletter: www.chop.edu/centers-programs/parents-pack. Healthcare professionals can sign up for updates here: vec.chop.edu/professionals/vaccine-healthcare-providers/home.html.

- "Vaccines on the Go: What You Should Know" app for parents: https://itunes.apple.com/us/app/vaccines-on-go-what-you-should/id692759940?mt=8

Vaccine Facts and Policy (VFAP)

- www.vaccinefactsandpolicy.org

VFAP is a partnership of the Association of Immunization Managers, George Washington University, and the Immunization Action Coalition. The website includes extensive information about many major topic areas related to state immunization law and policy: demographics and rates, fiscal environment, law and policy, strategies and initiatives, and the structure of immunization programs. The site is searchable by state and by topic.

Vaccine Investigation and Online Information Network (VIOLIN)

- www.violinet.org

VIOLIN is a searchable online database of vaccine-related research data collected and curated at the University of Michigan. The website launched in 2007 and now contains information on more than 3000 vaccines/vaccine candidates.

Voices for Vaccines (VFV)

- www.voicesforvaccines.org

VFV is a parent-run member organization that provides clear, science-based information about vaccines and vaccine-preventable diseases. It provides the public with opportunities to join regional and national discussions about the importance of vaccination. VFV is a project of the Task Force for Global Health.

- Twitter: https://twitter.com/voices4vaccines

ORGANIZATIONS WITH DISEASE-SPECIFIC WEBSITES

Hepatitis

Asian Liver Center (ALC)

- http://liver.stanford.edu

ALC at Stanford University addresses the high incidence of hepatitis B and liver cancer in Asians and Asian Americans through outreach and education, advocacy, and research. ALC's website includes resources for healthcare professionals and patients, including brochures in many Asian languages.

CDC's Viral Hepatitis website

- www.cdc.gov/hepatitis

Hepatitis B Foundation (HBF)

- www.hepb.org

HBF is a national nonprofit organization dedicated to the global problem of hepatitis B and to finding a cure and improving the quality of life for people affected by hepatitis B. The website content is provided in 10 languages and also features an expert speakers forum.

- Twitter: https://twitter.com/hepbfoundation

National Task Force on Hepatitis B, Focus on Asian and Pacific Islander Americans

- www.hepbtaskforce.org

The Task Force brings together scientists, healthcare professionals, and nonprofit organizations in an effort to eliminate hepatitis B–related mortality and morbidity.

National Viral Hepatitis Roundtable (NVHR)

- www.nvhr.org

NVHR is a coalition of many public, private, and voluntary organizations dedicated to reducing the incidence of infection, morbidity, and mortality from viral hepatitis in the United States through strategic planning, leadership, coordination, advocacy, and research.

Human Papillomavirus (HPV)

American Sexual Health Association's National HPV & Cervical Cancer Prevention Resource Center

- www.ashasexualhealth.org/stdsstis/hpv

This website includes information about HPV and personal stories and support for infected persons. The National Cervical Cancer Coalition (NCCC), part of ASHA, is dedicated to helping women who are affected by HPV and cervical cancer. It advocates for cervical health by promoting prevention through early vaccination and testing. NCCC's website is www.nccc-online.org.

CDC's HPV website

- www.cdc.gov/hpv

Influenza

Childhood Influenza Immunization Coalition (CIIC)

- www.preventchildhoodinfluenza.org

CIIC seeks to address and improve the low influenza immunization rates among children.

Families Fighting Flu (FFF)

- www.familiesfightingflu.org

FFF is a nonprofit organization made up of families who have experienced the loss of a child to influenza or who have had a child experience severe medical complications from influenza. The organization's mission is to reduce pediatric morbidity and mortality due to influenza by raising awareness about the importance of getting all children vaccinated against this disease.

- Twitter: https://twitter.com/famfightflu

Meningitis

Meningitis Angels

- www.meningitis-angels.org

Meningitis Angels is a nonprofit organization of survivors and families of meningitis victims. It offers information and support to families affected by meningitis.

- Twitter: https://twitter.com/MeningitisAngel

National Meningitis Association (NMA)

- www.nmaus.org

NMA is a nonprofit organization founded by five parents whose children died or have long-term disabilities from meningococcal meningitis. NMA advocates for meningitis disease awareness and prevention.

Rotavirus

Rotavirus Vaccine Program

- www.path.org/projects/rvp.php

A PATH affiliate, the Rotavirus Vaccine Program's mission is to reduce child morbidity and mortality from diarrheal disease by accelerating the availability of rotavirus vaccines appropriate for use in developing countries.

Pharmaceutical Company Websites

- Emergent Biosolutions: www.emergentbiosolutions.com
- European Vaccine Manufacturers: www.evm-vaccines.org
- GlaxoSmithKline: www.gskvaccines.com
- MedImmune, Inc. (parent company AstraZeneca): www.medimmune.com
- Merck & Co, Inc: www.merckvaccines.com
- PaxVax: www.paxvax.com
- Pfizer: www.pfizer.com
- Protein Sciences Corporation: www.proteinsciences.com
- Sanofi Pasteur: www.sanofipasteur.us
- Seqirus: www.seqirus.com
- Valneva (Intercell USA): www.valneva.com